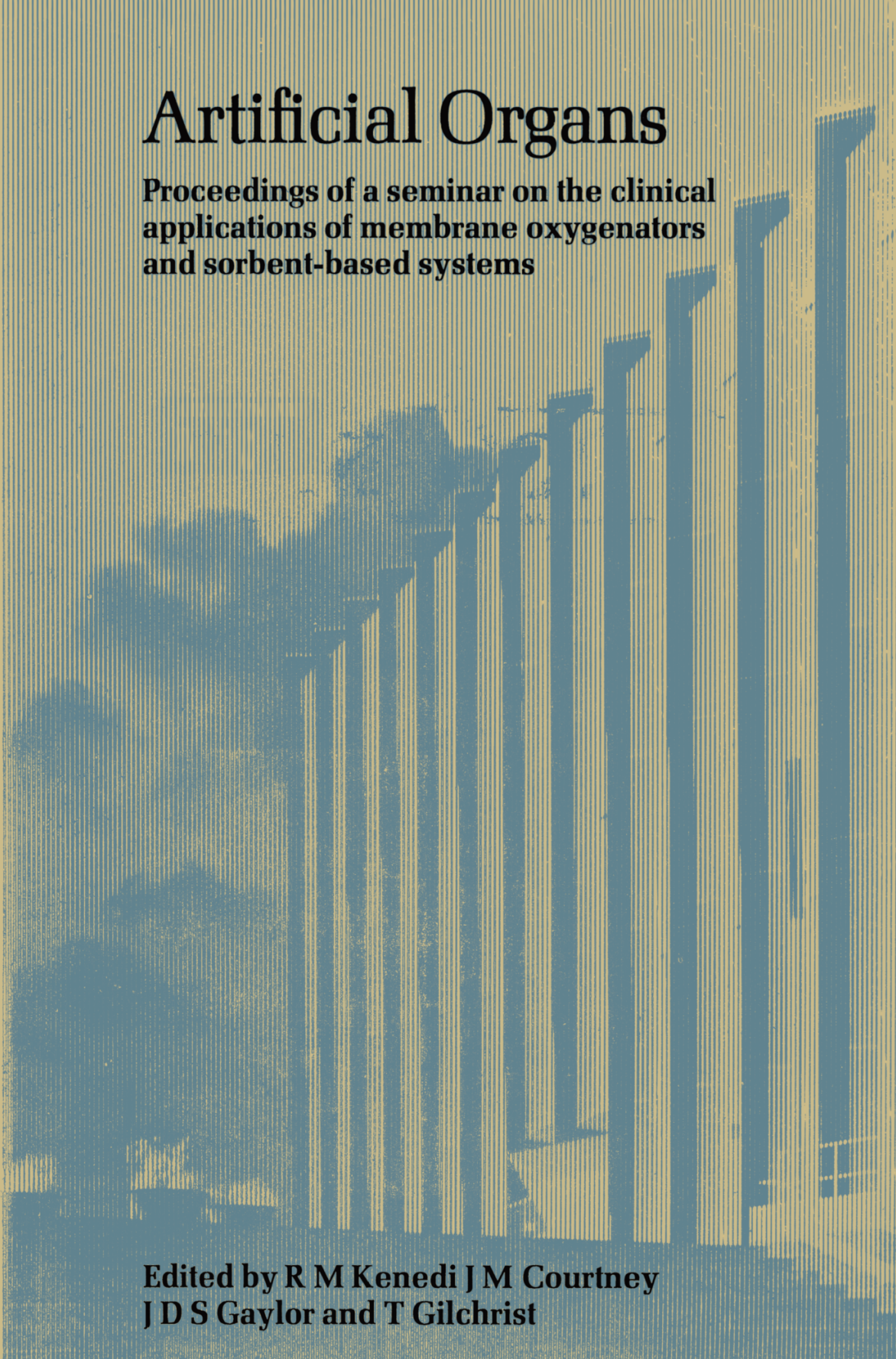
# Artificial Organs

## Proceedings of a seminar on the clinical applications of membrane oxygenators and sorbent-based systems



Edited by R M Kenedi J M Courtney
J D S Gaylor and T Gilchrist

# ARTIFICIAL ORGANS

STRATHCLYDE BIOENGINEERING SEMINARS

# ARTIFICIAL ORGANS

*Proceedings of a seminar on the clinical applications of membrane oxygenators and sorbent-based systems in kidney and liver failure and drug overdose, held at the University of Strathclyde, Glasgow, in August, 1976*

*Edited by*
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*University of Strathclyde*

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# PREFACE

The Strathclyde Bioengineering Seminars are held annually and explore in depth a clinically significant problem area within the research activities of the University of Strathclyde's Bioengineering Unit.

The series, which is a successor to successful broad-spectrum symposia held in 1964 and 1972, opened in August 1975 with consideration of the biomechanics of tissue viability and its clinical applications. The proceedings of this first seminar have been published by The Macmillan Press under the title of *Bed Sore Biomechanics* in July, 1976.

This present seminar, the second in the series, was held in August, 1976 and considered research, development and clinical applications in the areas of membrane oxygenation and sorbent-based systems. The therapeutic role of the latter was explored in kidney and liver failure and drug overdose. There were two hundred and forty-nine participants from Belgium, Canada, England, France, W. Germany, Italy, Israel, the Netherlands, Norway, Scotland, Spain, Sweden and the U.S.A.

The details of the Scientific, Technical and Working Group sessions are given in the Contents List. There was also an Exhibition of Related Medical Engineering Equipment in which 20 firms and organisations participated (see page xxiii).

All the papers presented, together with additional contributions and the discussions are printed complete in this volume, grouped in the six sessions shown in the Contents List.

The organisers acknowledge with gratitude their sense of indebtedness to the City of Glasgow District Council for the very enjoyable civic reception given by them for Seminar participants and guests. The first of the newly instituted *Adam Thomson Lectures* was delivered at this reception by Professor Robert E. Sparks of Washington University, St Louis, USA.

In these difficult economic times the continued support of the Seminar programme by the Greater Glasgow Area Board is particularly appreciated.

Additionally the organisers are very pleased to acknowledge the contributions of AB Gambro; Becton, Dickinson Ltd.; Extracorporeal; I.C.I.; Norit-Clydesdale Co. Ltd.; Organon Teknika Ltd.; Sandev Ltd.; Sandoz Products Ltd., Medical Equipment Division; and Travenol Laboratories Ltd., towards travelling expenses of invited lecturers.

As in previous symposia, banking and travel-agent facilities were made available to participants by courtesy of the Bank of Scotland and Nairn Travel Co. Ltd. and Co.

All the work connected with the seminar was carried out by the staff of the Bioengineering Unit with the unstinted co-operation of the University Administration. The organisers have pleasure in offering for the second year running, and if possible with increased warmth, their best thanks to all concerned.

*Glasgow, 1976* R. M. Kenedi

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# OPENING REMARKS AND WELCOME TO PARTICIPANTS

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Ladies and Gentlemen, my first duty - and my pleasure - is to welcome you most warmly to the University of Starthclyde and to this seminar.

As you may perhaps already know, our University, although relatively new in name, can in its constituent colleges already look back on 180 years of active life and development.

Founded in 1796 under the will of Professor John Anderson M.A., F.R.S. (sometime Professor of Oriental Languages and subsequently Professor of Natural Philosophy in the University of Glasgow), in the days when James Watt's invention of the separate condenser for the steam engine was making possible the Industrial Revolution, our University has, since then, been at the very centre of technological education and development. Its students have made outstanding contributions to industrial, commercial and economic progress throughout the world. For those of you who are interested in statistics, in recent years Strathclyde supplied more than half of all University trained technologists in Scotland and more than one eighth of these in the United Kingdom.

During my years of postwar service in Strathclyde I was privileged to have the responsibility for guiding the development of what is now known as the Mechanical Engineering Group of Departments. This covers a wide field ranging from all aspects of mechanical, engineering design, and production engineering to bio-engineering.

The last mentioned began in the Engineering Group of Departments in the early 1960s as a closely collaborative venture with surgeons and physicians in Glasgow's teaching hospitals. As Head of the Group, I was only too happy to give every support to this new development of applying engineering in medicine, firstly because of the promise which such collaborative fields held of easing human suffering, and in particular of prolonging active life, and secondly because I was convinced that in view of the sense of commitment of all concerned outstanding success would be achieved.

Since then I think it is fair to say that outstanding success *has* in fact been achieved. The original relatively small Medical Research Council Biomechanics

Group of 1962 has grown into the present Bioengineering Unit, a fully established research and postgraduate teaching Department of the University, housed in its own fine new building, the Wolfson Centre. It also has associated with it the National Centre for Training and Education in Prosthetics and Orthotics. This is truly 'national' in character in that it is a United Kingdom centre in the training of paramedical professionals in this field.

The success of broadly based international meetings held at Strathclyde in 1964 and 1972 has led to the establishment of these annual Strathclyde Bioengineering Seminars, the proceedings of which are published by The Macmillan Press. Each seminar explores in depth a clinically significant problem area within the Unit's activities.

The first seminar in the series, which was held last year, dealt with Tissue Mechanics with particular reference to the important clinical topic of pressure sores. The present seminar, the second in the series, has as its subject *Artificial Organs.*

Artificial organs are becoming increasingly important in our times. In Europe for example, the annual increase of new dialysis patients is of the order of 14 per million. In broad figures something like 65 to 127 per million of population are under treatment in European countries. There is no question that treatment availability falls far short of treatment requirement. This is why this seminar is so important. I am sure that your discussions in the various sessions concerned with assistive devices in the case of lung, kidney and liver failure will materially assist advancement in this area of activity.

You have a very full programme indeed, covering subjects of major importance. May I wish you successful and fruitful discussions and finally may I also hope that in addition to your scientific activities you will also have a most enjoyable time socially while you are with us.

Summary of
The 1976 Adam Thomson Lecture

# PROGRESS AND PROSPECTS IN BIOENGINEERING

ROBERT E. SPARKS

## Preamble

Professor Adam S. T. Thomson was appointed Head of the Department of Mechanical and Chemical Engineering and Applied Mechanics of the then Royal College of Science and Technology, Glasgow, in 1947. Under his guidance the department evolved into the Mechanical Engineering Group of departments of the University of Strathclyde, comprising Bioengineering, Dynamics and Control, Engineering Design and Drawing, Mechanics of Materials, Production Management and Manufacturing Technology, and Thermodynamics and Mechanics of Fluids.

Professor Thomson was elected Strathclyde University's first Vice-Principal (1964 - 68) and was appointed its first Deputy Principal (1968 - 1973). He retired in 1973 and was created an honorary LL.D. of the University in 1976.

To commemorate his outstanding contribution to Strathclyde's development the University instituted the *Adam Thomson Lecture in Bioengineering.* This is to be delivered each year in association with and relevant to the annual Strathclyde Bioengineering Seminar of that year, by an invited lecturer of international eminence in the field.

The 1976 and first Adam Thomson lecturer was Robert E. Sparks, Ph.D., Professor of Chemical Engineering, Washington University, St. Louis, Missouri, USA.

On the evening of Wednesday 18 August 1976 the City of Glasgow gave a Civic Reception to participants and invited guests at which the Right Honourable the Lord Provost of Glasgow Peter T. McCann, J.P., B.L., welcomed those attending. The Adam Thomson lecture was delivered by Dr Sparks to an audience of some 500 in the Banqueting Hall of the City Chambers as part of the proceedings of the Civic Reception.

## 1. Introduction

I would like to discuss bioengineering in somewhat broad terms, to highlight some specific accomplishments, problems and possibilities for the future.

From magazines, newspapers and our own experience, we are familiar with the fact that many synthetic materials and devices are available to supplant or supplement various functions or organs of the body. We are all familiar with hearing aids, artificial teeth and artificial limbs. We are becoming accustomed to hearing about 'artificial' heart valves, hip joints, finger joints and blood vessels. The

support of malfunctioning organs is also becoming commonplace with the use of complex apparatus such as artificial kidneys, heart-lung machines, ventilators and defibrillators. In addition, we know that it is possible in certain cases to transplant an organ from a donor to a recipient. Since transplants are of limited availability, and the major problems associated with their use are not engineering problems, I will exclude them from the discussion.

Looking at this list, one might be fooled into thinking that many of the problems were solved. However, the people who have benefited from these devices and the physicians and researchers familiar with them are painfully aware of how far short they fall of the original organs and materials.

It is true, of course, that some artificial organs provide their critical function rather well, even though they may be expensive and cumbersome. For example, there may now be as many as 75 000 people in the world, at a rough estimate, whose lives are being sustained through the use of the artificial kidney. Even here, there are many problems in maintaining the patients in reasonable health for an extended period. In many of the artificial organ problems, we have taken only the first tentative steps and are just beginning to understand the nature of the problems.

I would like to illustrate the progress which has occurred in some areas with an overview of the development of the artificial kidney.

## 2. The Artificial Kidney

In 1913, over lunch one day, Professor John Abel of Johns Hopkins University proposed to his colleagues the concept of removing diffusible species from the blood by allowing them to pass through membranes arranged as tubes. He used cylinders of bread to illustrate how the apparatus might be constructed. Professor Abel referred to the device as an *artificial kidney* and foresaw its use in aiding malfunctioning kidneys. The device was first used on rabbits, clearing the blood of salicylic acid as efficiently as the natural kidney The next major breakthrough came in 1943, in Holland, where Dr Willem Kolff developed the first artificial kidney which could be used on patients. However, there was no convenient way to attach the patient to the device until the development in 1958 by Scribner and his colleagues of the plastic shunt, which permitted repeated access to the blood stream. Chronic haemodialysis then became possible.

The heart of the artificial kidney is the dialyser, where the uraemic wastes pass through the membrane into the receiving fluid. The two standard dialysers were the flat-plate Kiil dialyser which was large and had to be reassembled after each use, and the coil dialyser, which was light and pre-sterilised, but which usually had to be discarded after each use. Within a few years, the Dow capillary dialyser appeared, and it has been followed by more efficient coils and recently small disposable flat-plate dialysers.

In the last few years sorbents have been studied to augment the removal of the uraemic waste metabolites. One use of sorbents (exemplified by CCI Life Systems Inc. Redy dialyser) in the regeneration of the dialysate permits the volume of

dialysate to be reduced to only 5 litres thus making possible a portable artificial kidney. The direct perfusion of sorbent cartridges is now receiving considerable attention for use not only in uraemia, but also in the rapid removal of specific poisons from the body. This is an area in which the Bioengineering Unit at Strathclyde University has developed considerable expertise.

Further reduction in the size and conceptual changes in the design of the artificial kidney will probably have to await development of new methods of urea removal.

### 3. Population Control

I would like to direct your attention to a different area in which bioengineers will possibly play an important role in the near future. This is the critical problem of controlling the world's population. In 1960 the pill was introduced. This was the first major advance in contraception for many years. Although the pill is widely used, its contraceptive effectiveness depends on taking it on a rigorous schedule, and it also causes some abnormalities in a small percentage of women. Hence, there is need for a more foolproof technique which will also have fewer side effects. In the last few years this problem has begun to receive the attention of chemists and bioengineers. One attractive goal would be the release of contraceptive agents near the reproductive tract, where they would have maximum effect, and also be metabolised locally, minimising systemic drug levels. The first result of this recent flurry of activity is the 'Progestasert' developed by the Alza Corporation in Palo Alto, California. In this a T-shaped intrauterine device (IUD) is modified such that progesterone is released slowly from a reservoir around the stem. This combines the contraceptive effects of the IUD and the progestational agent. Presently under way are many studies on the release of contraceptive agents from biodegradable polymers. I expect to see new and exciting ideas in this general area in the next few years.

### 4. Biocompatibility

Another disease in which there is a specific bodily malfunction is diabetes, in which the beta cells of the pancreas do not properly secrete insulin in response to changes in blood sugar concentration. Although present methods of administering insulin are effective, they are inconvenient, and occasionally there are medical complications. One of the bioengineering approaches to this problem being studied is the development of a small glucose sensor which could be implanted in the blood stream. The signals would be fed to a controller which is connected to a small insulin infusion pump. One of the long-term problems in using such apparatus is the need for permanent implantation of the glucose sensor. This is an example of a ubiquitous problem in many areas of bioengineering: biocompatibility. Long-term contact of synthetic materials with the blood or tissues can cause thrombosis and also brings the body's immune responses into play. These are important considerations with artificial hearts and valves, arteries, cannulae,

catheters, membranes and many other devices. One of the keys to future developments in many areas of bioengineering is the solution to this general problem of matching synthetic materials to biological materials.

## 5. The Biological Interface and Data Handling

I want to mention briefly two further problems which will bring us to our last technical consideration of the evening. Present artificial limbs, although of considerable help to those who need them, fall far short of the performance of human limbs. Important work is now being done in describing carefully the tasks which must be handled by artificial limbs, and learning how to fabricate them well and accommodate them to patients. The Strathclyde Bioengineering Unit is a recognised leader in this area.

One of the important considerations in developing more complex limbs is the problem of control. This is particularly true in the case of the hand. One attractive approach to this problem is to pick up the tiny myoelectric signals generated, for example, in the muscles in a remaining arm stump, magnify them and use them to control the movements of an artificial hand. Researchers in the United Kingdom, led by the late Dr Bottomley, have been doing exciting work in this area for some years. However, the problems of sorting the myoelectric signals, presenting them to the artificial hand and fingers and powering the many sensitive movements which are part of even simple manipulations are staggering.

Another problem, in which processing and presenting a large amount of information is even more critical, is that of aiding the blind. In recent work by Dobelle and Mladejovsky at the University of Utah, stimulated by the pioneering work of Brindley and his co-workers in England, arrays of electrodes have been placed on a portion of the surface of the brain which is responsible for visualisation. When an electrode is stimulated, the patient experiences a sensation of a spot of light or phosphene, in his 'visual' field. Immediately the problem becomes complex, because the arrangement of the phosphenes bears no relation to the arrangement of the electrodes. The brightness of the phosphenes also is a nonlinear function of the stimulus strength. Hence, even the presentation of simple geometrical patterns requires a computer.

Obviously, one of the serious problems in these studies is the biological interface between the electrodes and the brain. Another problem is the sheer volume of information which would have to be processed nearly instantly and transferred from our information handling system, the computer, to the body's information handling system, the brain. The staggering amount of information which must be handled can perhaps be illustrated by thinking of how much data with instant interpretation we obtain by simply scanning our eyes across a room. This would seem to be an impossible data handling task, but I would like to finish by discussing the trends of computers which might in the future be asked to perform this job.

## 6. Data Processing and Computer Development

In 1944, the first IBM Mark I computer was developed. It contained 3300 electro-mechanical relays, weighed five tons, and could multiply two 10-digit numbers in approximately a second. The relays were too slow, so the IBM 604, which used vacuum tubes, was developed in the next two years. It could perform the same multiplication in one-fortieth of a second. By 1953, vacuum tubes computers were capable of this multiplication in only 500 microseconds. In the late fifties, transistors not only permitted a drastic decrease in the size of computers but were more rugged and required dissipation of much less heat. They could carry out the multiplication of two 10-digit numbers in 10 microseconds, 100 000 times faster than the original Mark I.

Solid logic circuits followed, in which transistors only 1/32 inch were placed on half-inch ceramic modules. The typical time for switching an element on and off was only six billionths of a second. By 1972 large scale integration of micro-miniature circuits had arrived, with hundreds of chips being made on the surface of a slice of silicon. Photographic etching and plating technologies permitted many tiny circuits to be placed on the tiny chips, which were then mounted on modules for use. The techniques used to form these circuits can draw lines so fine that they would permit the entire Bible, 1250 pages, to be reproduced on a $1\frac{1}{2}$ inch square. To further increase the speed of computers, IBM are working on the Josephson tunnelling junction, which has an on-off switching time of 10 trillionths ($10^{-11}$) of a second. In this period of time, light (or electrical current) can travel only 3 mm! Hence, the miniaturisation which can now be obtained may itself be a limiting factor in the ultimate speed of computation.

As a practical matter, it is also noteworthy that the cost of data processing has also decreased drastically. In 1960 it cost a dollar to obtain $10^{12}$ state changes of a flip-flop. In 1975 that cost had fallen 10 000 fold, to 0.01 cent, and is still falling.

Hence, the size and cost of computing equipment are decreasing, while the speed of computation is increasing rapidly. It is possible that within 10 years, the sophistication of data handling equipment will be sufficiently advanced to permit artificial organ designers to consider complex designs which presently would be considered impossible. This is particularly exciting in view of the recent work at the University of California, Los Angeles, in which electro encephalographic signals and eye position alone were used to control a computer solving a simple maze.

## 7. Summary

All the work described takes a great deal of hard work by many talented people. The necessary factors can only be properly brought to bear on the problems with stable laboratory financial support. The advances which have been made thus far owe much to the financial support of government agencies, philanthropic organisations and industrial companies. A present danger is the increasing difficulty of

obtaining long-term funding of laboratories studying these problems in the early stages.

However, the problems are important and seem to attract people who become committed to their solution. We have made large first steps, but the problems are very difficult. Hence, this is only the beginning. If stable funding can be obtained those of us in this room will see great strides in the next ten years.

For the researchers, this is a most exciting time. The growth of information is rapid, and new possibilities are continually surfacing. I wish all of you in the field the greatest success in your endeavours to provide the medical profession with more powerful and sophisticated tools with which to attack man's age-old medical problems.

Thank you.

SESSION A

# THE INTERNATIONAL STATE OF THE ART OF ORGAN FUNCTION REPLACEMENT

*Chairman*
Professor A. C. Kennedy, M.D., F.R.C.P.(Ed.), F.R.C.P.(Glas.)

# 1

# MEMBRANE OXYGENATION: AN OVERVIEW

PIERRE M. GALLETTI

**Summary**

Membrane oxygenation has recently become a reliable tool for routine cardiac surgery and an exciting instrument for investigating the concept of long-term respiratory assistance in man. Yet the technology underlying this modality of care is of relatively long standing. Indeed it was in the mid 1950s that Clowes and his team in Cleveland (Clowes *et al.*, 1956) demonstrated the clinical feasibility of membrane oxygenation. Why then did it take 20 years to reach the stage of widespread clinical application? What were the obstacles on the road? What has finally made the difference? And where do we go from here? These are the questions to be considered in this overview of membrane oxygenation.

## 1.1 Early History of Membrane Oxygenation

The concept of membrane oxygenation started with the clinical observation that during the course of haemodialysis using the rotating drum kidney, venous blood channelled through cellulosic tubing would turn red upon prolonged exposure to an aerated dialysis medium. Investigators soon realised that gas exchange between watery media separated by a hydrophilic membrane was quite inefficient. Furthermore, it would present many other problems such as ultrafiltration control and electrolyte balance. Thus the search turned toward relatively hydrophobic plastics: polyethylene and ethylcellulose were tried without much success because they proved to be too impermeable to gases. However, when Teflon became available in the form of thin sheets, reasonably pinhole free because of the industrial application for which they were designed (capacitors), a major step forward resulted with the first operational clinical membrane oxygenator. The design of the original Clowes oxygenator, which dates back to 1955, and its successful application in over 100 clinical cases, are tributes to the creativity and perseverance of cardiovascular surgeons. However, the cumbersomeness of the equipment and its cost were such that it could not realistically compete with the sturdy, reliable bubble or disc oxygenators which were just then coming into favour (see table 1.1).

Throughout the sixties, this set of circumstances led to a long period of incubation of membrane lung technology. Important conceptual and experimental advances were made, without however reaching a stage of practicality that would

**Table 1.1** Milestones in membrane oxygenator development. 1950s: clinical observations and fundamental concepts

| | | |
|---|---|---|
| Oxygenation of venous blood through dialysis | 1944 | Kolff |
| Evaluation of cellulosic membrane oxygenator | 1951 | Karlson |
| Large scale, sandwich type of membrane oxygenator | 1955 | Clowes |
| Clinical validation in open-heart surgery | 1957 | Clowes |
| The reinforced (coated fabric) silicone resin membrane | 1958 | Thomas |
| The $CO_2$ bottleneck with Teflon membranes | 1958 | Melrose, Peirce |
| Silicone rubber membranes | 1960 | Dow Corning |
| Oxygen transport limitation by fluid films | 1960 | Peirce, Marx |
| Microporous membrane materials | 1960 | McCaughan |

permit the membrane oxygenator to compete with the standard surgical instruments, which in the interval had all turned into sleek, see-through, disposable devices. The milestones of membrane oxygenator development during that decade are worth remembering, because the knowledge gathered by a coterie of tenacious investigators is at the root of the major practical advances which have been made in the seventies.

## 1.2 A Decade of Transition

The recognition (around 1958 - 59) of the favourable gas permeability properties of silicone polymers, coupled with technological advances which permitted manufacturing this material in the form of thin sheets, was the key element in further progress of membrane oxygenation. Now investigators could compare not only various thicknesses of the same Teflon membrane but also another material with quite different transport properties. Even before permeability had been measured in recognisable standard units, Peirce and Marx had independently made the fundamental observation that given membrane materials with permeability properties equal to, or better than, those of silicone rubber and Teflon, the oxygen transfer capacity of a membrane lung would not be limited by the nature of the plastic membrane itself, but by the thickness of the blood boundary layer adjacent to it (Peirce and Peirce, 1963; Marx *et al.*, 1962). Hence the generalisation which dominated membrane lung design until recently: $CO_2$ transfer is limited by the chemical nature and the thickness of the polymer membrane, but $O_2$ transfer is primarily limited by the geometry of the blood path. This view encouraged developmental work not solely toward thinner membranes (typically obtained by coating a silicone polymer suspension over a textile cloth), but also toward uneven blood membrane interfaces, which could be expected to induce some degree of convective mixing ('turbulence') in the blood layer immediately adjacent to the membrane. Such effect could be obtained, with various degrees of success, by proper selection of the mesh size and orientation in textile-reinforced membranes, by using knit rather than single strand fabrics, by embossing or engraving a particular pattern on the membrane sheet itself, or by the simple expedient of sandwiching a wide-mesh screen within the blood layer between adjacent plastic membranes. The dilemma of membrane lung design

became increasingly clear as membrane materials could be experimentally characterised for their permeability properties and design problems could be expressed in terms of mass transfer theory derived from chemical engineering. As the influence of blood flow pattern and blood flow distribution began to be quantitatively analysed, a step forward in performance and reliability could be observed, recruiting a new group of believers in the clinical effectiveness of membrane oxygenation (table 1.2).

**Table 1.2** Early 1960s: a period of transition

| | | |
|---|---|---|
| Logistics of multiple layer, parallel flow devices | 1961 | Peirce |
| Multiple point support for flat membrane sheets | 1961 | Galletti, Peirce |
| Pressurised 'shim' to stabilise blood flow distribution | 1962 | Harvey |
| The combined artificial lung-kidney machine (Klung) | 1962 | Hopf, Galletti |
| The screen-in-blood concept of blood stream mixing | 1965 | Bramson |
| The concept of implantable lung | 1965 | Bodell |
| Pattern definition on membrane surfaces | 1966 | Peirce |
| The ultra-thin silicone rubber membrane | 1966 | Burns, Melrose |
| The silicone-polycarbonate copolymer | 1966 | General Electric |

Better flow designs led to a renewed demand for improved membranes, and stimulated three major technical advances: the synthesis and fabrication of the General Electric polycarbonate-silicone copolymer, which could be made into thinner sheets than the parent silicone because it was mechanically stronger, the unravelling (in a London hospital) of a new technology for the fabrication of silicone rubber sheets, leading to ultrathin, pinhole-free, unsupported membranes (Burns, 1969); and, finally, the industrial production of very small, very thin-walled tubes of silicone copolymer, known in the trade as hollow fibres. Hollow fibres, assembled in a bundle and potted together at both ends, strike the clinician's imagination because their parallel arrangement so closely resembles our intuitive image of a capillary bed. For a while it seemed that the hollow fibre approach would be the ultimate answer to membrane lung design, until recurring problems of the thrombus formation on the 'face sheet' of the fibre bundle identified the Achilles' heel of such devices. Because hollow fibres claimed an ancestry in textile technology, it was not long before someone thought of weaving them as a cloth, and of designing a filter-pack type of oxygenator, with the gas circulating through the lumen of the fibres and blood in the interstices between them. In short, a reversed capillary bed.

New concepts arose in parallel with technological advances, two of which at least seem to have risen again to the forefront of progress today: the demonstration, in the Klung design (Galletti *et al,* 1962) that blood - gas exchange and haemodialysis could be combined in a single artificial lung - kidney, and the very first prototype for an implantable artificial lung, based on parallel bundles of thin-walled silicone rubber tubing inserted in Teflon envelopes within the chest.

## 1.3 The Encounter of Science, Technology and Industry

Several unrelated factors combined in the late sixties and early seventies to plant the seeds of major progress. Biomaterials science finally emerged as a respectable academic endeavour, and attracted a group of distinguished physical scientists who made some order of what had been heretofore empirical knowledge. The biocompatibility of plastic materials was systematically investigated. Advances in plastics technology were applied to fabrication problems such as casting, moulding, glueing and sealing of membrane oxygenator parts, leading to great simplification in design and assembly of the newer prototypes. Industry became involved, and with the commercialisation of the Landé-Edwards oxygenator (Edwards Laboratories, Santa Ana, California), problems of large scale production, reliability, quality control, sterilisation, shelf-life and shipping were for the first time confronted and resolved. The distribution of that standardised, ready to use, disposable membrane oxygenator, which soon elicited industrial competition, did much to broaden the ranks of those who could advance the field in the laboratory or in the clinic. This led in turn to a greater degree of standardisation in reporting oxygenator performance data, and in time to a more general consensus about the advantages and drawbacks of membrane oxygenation. By 1974, oxygenator modules had reached such a level of performance and reliability that they could seriously be considered for total body perfusion as required in cardiac surgery (see table 1.3).

**Table 1.3** Late 1960s: beginning of the industrial era.

| | | |
|---|---|---|
| Industrial development of a disposable oxygenator | 1965 | Landé-Edwards |
| Physical characterisation of membrane permeability | 1966 | Galletti |
| Liquid - liquid (fluorocarbon) oxygenators | 1968 | Clark |
| Gas permeable hollow fibres | 1969 | Dow Chemical |
| Hydrophobic microporous membrane materials | 1969 | Dantowitz, Lautier |
| N.I.H. Medical Device Application Program | 1970 | Hastings, Dennis |

## 1.4 The Value of Membrane Oxygenation for Clinical Perfusion

Before considering the present status of membrane lung technology and the trends for the future, it may be worthwhile pausing and reflecting on the attractive features, as well as the pitfalls, of this particular approach to extracorporeal gas exchange.

Modern membrane lungs are effective inasmuch as they can easily cover the gas exchange needs of a normothermic adult. Since anaesthetised, haemodiluted, moderately hypothermic patients under perfusion have a reduced metabolism, membrane lungs, without necessarily reaching the peak performance of an efficient bubble oxygenator, do nevertheless provide enough of a safety margin for the perfusionist to feel secure under any clinical situation. Possibly the most

important aspect of effectiveness of membrane lungs is their unflinching performance regardless of the duration of perfusion. Properly employed, and certainly within the most generous time boundaries of current cardiac surgery, membrane lungs display a constant performance. Thus there is no decay in gas exchange capability to compound the biological problems of an unexpectedly long operation. A point of caution, however: some membrane lungs are limited in terms of carbon dioxide elimination capacity, which depends upon the nature of the membrane used as well as the parameters of oxygen supply to the artificial lung. The operator of a heart-lung machine must be aware of the limitations of his equipment.

Membrane lungs are said to be safe, meaning that the trauma imposed on blood by continuous recirculation outside the body is less than that observed with the more traditional bubbling or filming systems. The scientific evidence for this is not entirely unequivocal. The ambiguity stems in part from the fact that the design of coronary suction systems, and the manner in which such systems are used in the operating room, are much more important as potential sources of blood trauma than the blood oxygenator itself. Thus changing the gas exchange device alone is not a recipe for trouble-free, post-perfusion periods. Nevertheless, several surgeons have reported an easier blood pressure control, less post-operative confusion, and earlier awakening and recovery in patients perfused with membrane oxygenators, compared to older equipment.

Membrane lungs are reliable because they are geometrically much better defined than their competitors of the bubble or disc type. The blood compartment of a membrane lung is closed to atmospheric pressure, and its content is almost constant (excluding fluctuations associated with the compliance of the membranes). This, however, is a mixed blessing. On the one hand, a fixed blood volume in the extracorporeal circuit eliminates ambiguities in the patient's blood pressure and volume control associated with fluctuations in blood level of the oxygenator. On the other hand, it is not possible to conduct a surgical perfusion without a blood reserve in the circuit, and thus distensible reservoirs must be included for safety's sake. Because the blood volume in the oxygenator is fixed, blood flow in and blood flow out of the device must be accurately matched, otherwise the gas exchange unit would collapse or explode. Blood circuits and pumping mechanisms must include safety features to prevent such accidents. In some designs of membrane lung, the problem cannot be visually anticipated in as direct a manner as through the change in blood level in a disposable bubble oxygenator, and the perfusion team must have other warning systems at hand.

The last criterion for the modern membrane lung is that it should be a disposable item of moderate cost. Because membrane lungs rely on narrow, geometrically well-defined blood paths, arranged in a complex manner within a sealed box, it is practically impossible to clean them sufficiently after use so that they can be reused clinically. This brings into consideration the issue of cost. One of the most positive results of the increasing competition among membrane lung manufacturers for the cardiac surgery market is the progressive lowering of prices. This is associa-

ted in part with volume of production, labour-saving assembly techniques and, in recent years, the advent of new membrane materials inherently less expensive than the classical silicone polymers or Teflon.

### 1.5 Recent Trends in Membrane Oxygenation

Four main lines of research have dominated the membrane lung field since 1970. Some investigators have attempted to revive the concept of liquid-to-liquid exchangers through sophisticated designs; others have pursued various fluid dynamic approaches to minimise the obstacle to oxygen transport resulting from the fluid boundary layer adjacent to the exchange membrane; still others have violated the taboo against direct blood-gas interface and experimented with microporous membranes; finally a last group has concentrated its efforts toward the elimination of membrane surface imperfections thought to be responsible for platelet deposition and eventually thrombosis in the extracorporeal circuit.

Liquid-liquid exchangers are still in the experimental stage. For a while the accent was placed on liquid fluorocarbons, which are transparent organic liquids with a very high coefficient of solubility for oxygen and carbon dioxide. These liquids are immiscible with water or blood, and because of their high specific gravity, can be made to flow in direct contact with blood without mixing, the interface between the two liquids acting as a virtual membrane across which oxygen and carbon dioxide can be traded from one fluid to the other. Elegant designs have been proposed to accomplish this in a continuous manner, and the efficiency of the process has been well established. Two factors, however, have discouraged clinical application: the mechanical complexity of the systems (which must be backed by a bubble oxygenator to load the fluorocarbon with oxygen and remove its $CO_2$), and the danger of fluorocarbon embolism into the blood stream. The pharmacology and toxicology of these complex organic liquids are far from being elucidated, and thus liquid fluorocarbon oxygenators have remained heretofore a laboratory curiosity.

An equally intriguing approach is that of blood exchange via haemodialysis through catalytic membranes. Here the concept is to expose blood to water soluble precursors or derivatives of the respiratory gases. This means, in practice, haemodialysis against a liquid containing a large source of oxygen (hydrogen peroxide solution) and a sink for $CO_2$ (chemical binding of bicarbonate with a strong base). A transition metal oxide (ruthenium oxide has been found to be the most effective) is deposited within the mesh of cellulosic membranes otherwise similar to those used in haemodialysis. The catalyst is fixed and does not leach out into the blood or the hydrogen peroxide solution. A chemical reaction occurs within the fluid-filled cellulosic matrix and oxygen is liberated in immediate contact with the blood which flows adjacent to the membrane. The effectiveness of this system has been well established with laboratory prototypes. However, two main difficulties remain: one is the 'leak-through' into blood of minute quantities of hydrogen peroxide, which at least in a catalase-poor animal such as the dog, leads to progressive methaemoglobinaemia. The other problem is that ultimate binding of $CO_2$ in

the form of carbonates also leads to a loss of calcium, magnesium and phosphates, which must then be compensated by intravenous infusion of appropriate salt solutions to the perfused subject.

A number of approaches have been proposed to control and minimise the blood boundary layer which effectively limits oxygen transport in laminar flow systems. They all rely on the promotion of convective transport within the blood phase in order to mix and homogenise the blood as it flows through the exchange device. The term 'secondary flow' is used by fluid dynamicists to designate convective transport within a moving fluid at an angle or in a plane different from that of the main through-flow. Such mechanisms have been empirically used since the beginning of membrane oxygenation, but it is only in recent years that detailed engineering analysis has led to artificial lung configurations which take maximum advantage of these phenomena.

It would not be possible to review here all systems which have been proposed. The oscillating toroidal chamber of Drinker and Bartlett (Bartlett *et al,* 1972),, the grooved plate and highly pulsatile flow of the Oxford-Bellhouse oxygenator (Bellhouse *et al*, 1973), and the rotating microporous disc-membrane oxygenator of Hill (Hill *et al*, 1974), are the best known of these systems. All have demonstrated a remarkable gas transport efficiency in laboratory prototypes, and the last named at least has been successfully employed in cardiac surgery. Nevertheless, many surgeons find these oxygenators still mechanically too complex and delicate to be used without afterthought in the operating room (table 1.4).

**Table 1.4** Early 1970s: the encounter of science, technology and industry.

| | | |
|---|---|---|
| Clinical, long-term respiratory support | 1970 | Hill |
| Progress with the spiral coil, membrane oxygenator | 1970 | Kolobow, Zapol |
| Standardisation of gas transfer performance analysis | 1972 | Galletti, Richardson |
| Secondary flows generated by external forces | 1972 | Drinker, Melrose |
| Secondary flows inherent to blood flow path geometry | 1972 | Mockros, Dorson |
| Secondary flows associated with blood pulsation | 1973 | Bellhouse |

The concept of using microporous materials to eliminate the membrane resistance factor in gas transport between oxygen and blood was promulgated simultaneously in the United States and in France around 1969. Against the conventional wisdom of the time, experimentalists on both sides of the Atlantic surmised that through a microporous hydrophobic material, oxygen and blood could be brought into direct contact without actually mixing, because surface tension would keep both phases separated. Prototypes demonstrated the feasibility of such systems, and also their remarkable gas exchange efficiency. Then followed a phase of industrial research and development which took advantage of the availability and relatively low cost of microporous materials (initially Teflon in the USA, silicone-coated paper in France). The net result has been the design

**Table 1.5** Mid 1970s: the outlook.

| | | |
|---|---|---|
| Enhancement of gas transport through catalytic membranes | 1972 | Updike |
| Clinical use of microporous membrane oxygenator | 1973 | Murphy |
| Rotating microporous membrane oxygenator | 1974 | Hill |
| Silica-free silicone rubber membranes | 1974 | Kolobow |
| Perfusion with minimal anticoagulation | 1975 | Zapol |
| Ethylcellulose perfluorobutyrate and polyalkylsulphone membranes | 1975 | Ketteringham |
| Permanently implanted booster lung | ? | |

and distribution at a competititive price of compact, disposable membrane lungs fully capable of meeting the needs of cardiac surgery.

The last area of development in recent years has been the recognition that surface irregularities such as protruding silica crystals on the exposed surface of silicone rubber membranes were indeed responsible to a major extent for platelet deposition on blood-exposed membranes, and could be incriminated in the development of thromboembolic phenomena in the extracorporeal circuit. Conversely, membranes prepared in a manner to minimise such irregularities, such as silica-free silicone rubber, would allow perfusion with minimal doses of anticoagulants. Several membrane materials are currently under investigation from the standpoint of improved platelet compatibility combined with adequate permeability and carbon dioxide. Carbon may replace silica as the filler in silicone elastomers. Ethylcellulose perfluorobutyrate and polyalkylsulphones seem to be at the present time at the forefront of the future competitors with more traditional membrane materials. Progress in material formulation, the use of clean room fabrication and assembly techniques in the manufacturing of membrane lungs, and a better appreciation of the membrane - blood interactions probably all play a role in the success reported by several investigators with the use of minimal doses of heparin (see table 1.5).

## 1.6 Outlook for the Future

With the increasing acceptance of membrane lungs for cardiac surgery, and the technical progress associated with keen competition between manufacturers, the field of membrane oxygenation could well be considered as provisionally settled. New major challenges, however, seem to fascinate investigators and are likely to occupy their attention in the years to come.

One such field is the tailoring of microporous membranes toward the specific function of gas exchange. Much remains unexplained as to the exact physical nature of blood - gas interaction at the microporous interface. The possibility of membrane polarisation, the value of specific chemicals, the detailed formulation of the microporous polymer and their lining with live cells or haemocompatible materials, may well lead to new modalities of application of microporous membrane devices.

A major challenge is the design of nonthrombogenic membrane oxygenators which would not require the use of any anticoagulant. This requires consideration not solely of the membrane oxygenator itself, but of the entire extracorporeal circuit. There is increasing evidence that circuits coated with a single surface material, whatever that material may be, fare much better with minimal doses of heparin than circuits assembled from different materials, however well selected. The clinical value of preserving the normal clotting mechanism during the course of long-term perfusions is readily apparent.

The ultimate challenge is to develop a membrane lung that could be permanently implanted in the body. Such a prosthesis could function as a 'booster lung' to supplement inadequate respiratory function over prolonged periods of failure, or it could fully replace the natural organ. However formidable this problem might appear, however remote a clinically acceptable solution, the technical challenge of an implantable lung is probably no greater than that of an implantable heart, and the medical need is just as great. History of artificial organs suggests that it takes 15 to 20 years to pass from a working laboratory prototype to a device acceptable for pilot clinical evaluation. If this should be the case with the implantable lung, a clinically acceptable device should be available before the turn of the century.

## 1.7 References

Bartlett, R. H., Drinker, P. A., Burns, N. E., Fong, S. W. and Hyans, T. (1972). The toroidal membrane oxygenator: design, performance and prolonged by-pass testing of a clinical model. *Trans. Amer. Soc. Artif. Int. Organs,* **18**, 369.

Bellhouse, B. J., Bellhouse, F. H., Carl, C. M., MacMillan, T. I., Gunning, A. J., Spratt, E. H., MacMurray, S. B. and Nelems, J. M. (1973). A high efficiency membrane oxygenator and pulsatile pumping system, and its application to animal trials. *Trans. Amer. Soc. Artif. Int. Organs,* **19**, 72.

Burns, N. (1969). Production of a silicone rubber film for the membrane lung. *Biomed. Eng.*, **4**, 356.

Clowes, G. H. A., Jr., Hopkins, A. L. and Neville, W. E. (1956). An artificial lung dependent upon diffusion of oxygen and carbon dioxide through plastic membranes. *J. Thorac. Surg.*, **32**, 630.

Galletti, P. M., Hopf, M. A. and Peirce, E. C. II. (1962). A membrane lung-kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **8**, 47.

Hill, J. D., Iatridis, A., O'Keefe, R. and Kitrilakis, S. (1974). A technique for achieving high gas exchange rates in membrane oxygenation. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 249.

Marx, T. I., Baldwin, B. R. and Miller, D. R., (1962). Factors influencing oxygen uptake by blood in membrane oxygenators: report of a study. *Ann. Surg.*, **156**, 201.

Peirce, E. C., II, and Peirce, G. (1963). The membrane lung: the influence of membrane characteristics and lung design on gas exchange. *J. Surg. Res.*, **3**, 67.

# 2

# SORBENTS IN THERAPEUTIC MEDICINE

Y. NOSÉ AND P. S. MALCHESKY

**Summary**

The use of bulky equipment in renal support has been unavoidable in the past because large volumes of dialysate fluid were required. In order to make the devices smaller and portable, the amount of dialysate fluid has to be reduced and used in conjunction with a sorbent or eliminated altogether.

The use of sorbents in dialysate regeneration, their use in haemoperfusion and their potential in hepatic assist are outlined. The problems of sorbent particle release and blood trauma remain as deterrents to their widespread chronic application. Systems are proposed which may combine haemodialysis and haemoperfusion in the treatment of renal and hepatic failure. It is suggested that future development in this area will be characterised by the advent of sorbent systems exhibiting characteristics more specific to the biological needs of particular applications.

## 2.1 The Use of Sorbents

Sorbents have been employed in the past in many ways - one of the simplest being ingestion. However, eating the quantities of sorbents required is neither feasible nor pleasant; and so, a more acceptable method of ingestion is being explored. This consists of the surgical construction of an isolated intestinal loop through which the sorbents (such as activated charcoal) can be administered (Nosé *et al.*, 1972). By this method the sorbent and the gut fluid are mixed in the jejunum enhancing the removal of toxic substances. However, a more practical system is that of adding sorbents to dialysate fluid for dialysate regeneration.

## 2.2 Sorbents and Dialysate Regeneration

Several years ago, a Japanese group (Maeda *et al.*, 1973) developed an artificial kidney which uses a small amount of dialysate (30 l), which is regenerated by activated charcoal and aluminium oxide. This system proved to be effective even although the amount of urea removed is less than that in a conventional dialysis. It is less complex than the Redy system, which employs a number of sorbents and the enzyme urease, but can operate with a dialysate volume of about 5 l (Lewin *et al.*, 1972).

The Japanese company, Takeda, has commercialised this development in a system which consists of a cartridge containing approximately 600 g of activated

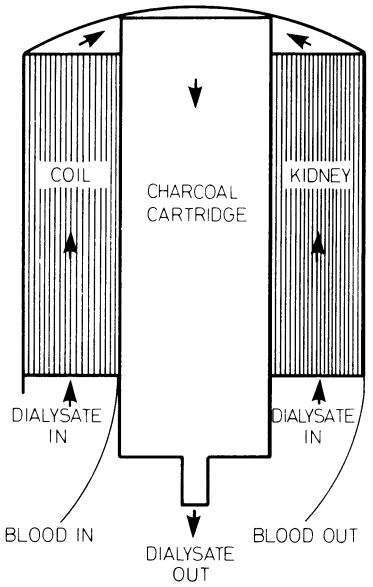


Figure 2.1 Diagram of a sorbent cartridge used in conjunction with a dialysis membrane

charcoal and 200 g of aluminium oxide. Additional equipment is required in order to use this device with a conventional coil artificial kidney and, for this reason, the cartridge has been modified (Surovy *et al.*, 1974). The tubular dialysis membrane along with its support was simply wound around the cartridge, as shown in figure 2.1, so that the dialysate fluid would be regenerated after passing through the cartridge. This system requires only 30 to 40 l of dialysate for removal rates comparable to those of a conventional dialysis. The exact volume of dialysate required is a function of the patient's body pool volume for those solutes not removed by the sorbents (Malchesky *et al.*, 1976b). In this connection, one area that specifically needs more study is that of urea removal. In consideration of the work by Maeda *et al.* (1974), it is still debatable whether or not it is necessary to remove as much urea as in the conventional dialysis procedure to assure patient well-being.

## 2.3 Problems of Haemoperfusion

One of the most popular areas of interest is that of haemoperfusion. Various devices have been produced commercially for this purpose and are being used clinically. However, almost all these devices have inherent problems, in particular that of particle release (Hagstam *et al.*, 1966) and (since blood is exposed to a large sorbent area) blood trauma. In order to alleviate these problems many blood compatible coatings have been developed for sorbents (Andrade *et al.*, 1971) and sorbents in such microencapsulated form are now used routinely (Chang, 1972).

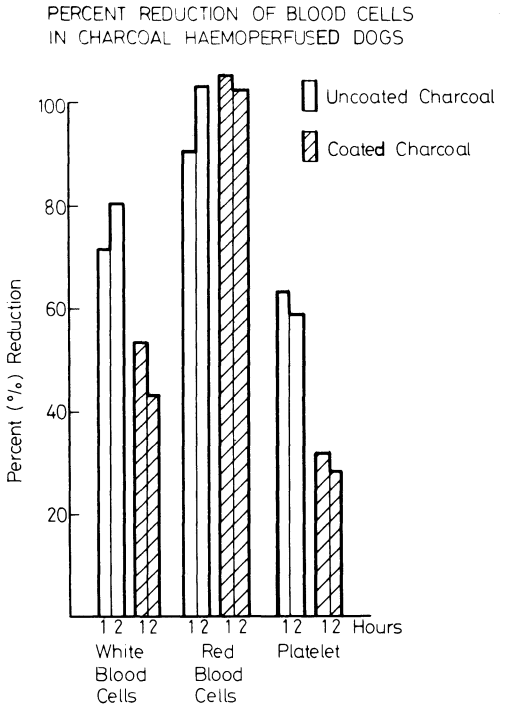


Figure 2.2 Changes in numbers of blood cells and platelets in dogs haemoperfused with coated and uncoated charcoal

However, a dilemma occurs in that to prevent particle release the sorbent must be coated; but the coating itself is a limitation to solute removal. Whereas a thin coat is susceptible to fracture, increasing the coating thickness reduces the efficiency of the system. The majority of devices incorporate a 0.2 to 3% coating, which, although reducing the efficiency of the system, helps to prevent particle release. Figure 2.2 shows the results of a study performed with dogs in which commercially available haemoperfusion systems were used - one with microencapsulated activated charcoal and the other with uncoated activated charcoal. These results show that coated charcoal does not always prevent white cell or platelet reduction, although this effect appears more dependent on the method and type of coating. Therefore, although coating generally enchances blood compatibility, it does not always follow that superior results are obtained in using coated (rather than uncoated) sorbents. The problem of particle release has repeatedly been seen to be present in that after haemoperfusion such microparticles anchor in the lung tissues and increase after each perfusion. As the lung is a good 'garbage collector', this effect can probably be tolerated in acute application where the need for perfusion often outweighs the possible risk to the patient. Thus, while haemoperfusion can probably be justified for those indicated acute applications, there is doubt as to application of currently available systems for chronic patient maintenance.

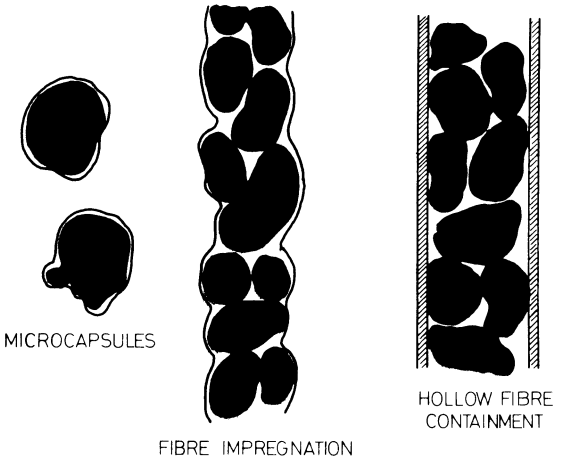


Figure 2.3 Diagram showing three forms of charcoal encapsulation

Particle release remains a problem in long-term treatment. It is especially evident when a device has been transported after assembly and portions of thinly coated sorbent have broken loose. In order to prevent this occurrence and the subsequent leaching of particles into the blood stream, new and better methods of sorbent encapsulation and immobilisation must be pursued.

## 2.4 Novel Haemoperfusion Systems

One such method of immobilisation tried was that of assembling the sorbent in a fibre fashion (figure 2.3). However, this technique does not solve the problem of particle release, since the surface of the fibre is similar to that of a microencapsulated particle in that it has a thin coating which is susceptible to fracture.

During the last two years, work has been carried out on a system which will *not* release particles (Malchesky *et al.*, 1976a), involving the use of sorbents contained within hollow fibres of commercially available Cuprophane. The present fibres have a 10 $\mu$m wall thickness and a diameter of about 300 $\mu$m (figure 2.3). The particles of sorbent within the fibres are very small and because of their large surface area can remove quantities of solutes (expressed per gram of sorbent weight) comparable to those removed by commercial units, including uncoated charcoals. The fibres are assembled in rectangular configuration in much the same fashion as hollow fibre artificial kidneys. This geometry was decided on after studying the inefficiencies of commercially available hollow fibre systems (Agishi *et al.*, 1975) and appears optimal from the point of view of efficiency. The specifications of a typical module are: channel thickness 0.7 cm, length 11 cm, width 24 cm, membrane surface area 1.1 $m^2$, charcoal content 35 g, and priming volume 50 ml. For effective haemoperfusion, three of these units comprise the system used giving a priming volume of 150 ml and a charcoal content of ~ 100 g.

The authors felt that since the efficiency of this system was found to decrease

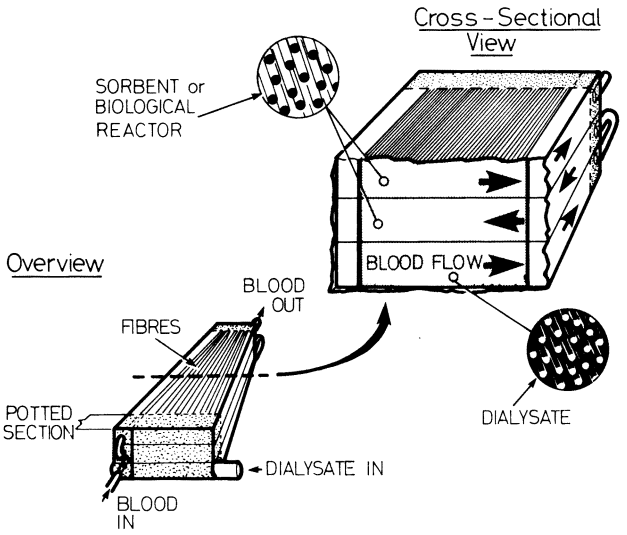


Figure 2.4 Diagram of sorbent fibre utilisation

with time, it would be better to employ four rather than three units in the one system. With such a device, clearances of 20 mg creatinine/g charcoal and 20 mg uric acid/g charcoal were obtained, which compare well with the value of 18 mg creatinine/g for uncoated activated charcoal systems. The commercially available microencapsulated system has a somewhat lower efficiency per gram of charcoal; but, of course, more charcoal (300 g) is used. The design of the capillary fibre system is such that other sorbent fibres, biological reactor systems and dialysis and ultrafiltration type fibres may be conveniently incorporated into single units, as shown in figure 2.4. When dialysate is employed, it flows in the lumen of the fibres and blood flows over the fibres. It is particularly noteworthy that no particle release has been found with these hollow fibre systems. The advantages of this system are therefore the good, proven blood compatibility of the membrane, the possibility of urea removal and water ultrafiltration by applying negative pressure to the inside of the fibres and the possibility of using different sorbents appropriate to the function desired. One system studied, for example, has a combination of activated charcoal and aluminium oxide in a single unit. Such a device can, therefore, be tailored to meet the specific demands of a given patient since the various sorbents can be chosen according to requirements.

## 2.5 Microporous Membrane Systems for Hepatic Assist

The limitation of the fibre system is the permeability of the encapsulating membrane. Although Cuprophane is highly permeable to solutes encountered in renal failure and drug overdose, protein bound molecules and molecules associated with hepatic failure would be excluded. However, the fibre system could be applied to

hepatic assist with a membrane more porous than that used in renal support. The system envisaged would utilise a porous hydrophilic membrane through which plasma components could pass. To study such a system, a flat sheet polycarbonate membrane of pore size 0.4 μm and thickness of 10μm (Nosé *et al.*, see p. 372, this volume), which gives a sieving coefficient for albumin of approximately 90%, has been employed. The plasma components are exposed to the uncoated sorbent, which removes specific toxic materials, and are then returned to the main blood stream. Because plasma filtration is so important to solute removal rates, the system is limited by the filtration rate and surface area of the membrane material used. With the membranes employed, particle release is prevented. In early studies, after 5 h perfusions with blood, the membranes appeared clean, but on closer examination by scanning electron microscopy, cellular deposits were apparent. It has been predicted by Forstrom *et al.* (1975) that if the cellular deposition parameter is less than 0.15, red cell deposition is prevented. The latest systems studied were designed to have deposition parameters of less than 0.1, and show that after 5 h perfusions the membranes appear clean and free of cellular deposition.

## 2.6 Conclusions

New concepts have been discussed which are being studied to overcome some of the inherent problems of haemoperfusion and, to some extent, the therapeutic utilisation of sorbents. Although, at present, conventional haemodialysis is still the major form of treatment in renal failure, it has been shown that the transition to a sorbent-based device can be made successfully if haemodialysis is used *in conjunction* with dialysate regeneration. Extensions of the systems discussed utilise hollow fibre membranes containing sorbents for blood purification and highly permeable microporous membranes in combination with specific sorbents, as required by the disease state.

## 2.7 References

Agishi, T., Ohta, K., and Nosé, Y. (1975). Is hollow fibre occlusion due to maldistribution of blood? *Proc. Eur. Dial. Transpl. Assoc.*, **12**, 519.

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood haemoperfusion HEMA/activated carbon. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 222.

Chang, T. M. S. (1972). *Artificial Cells*, Thomas, Springfield, Illinois.

Forstrom, R. J., Bartelt, K., Blackshear, P. L. and Wood, T. (1975). Formed element deposition onto filtering walls. *Trans. Amer. Soc. Artif. Int. Organs*, **21**, 602.

Hagstam, K. E., Larsson, L. E. and Tyssel, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital intoxication and uraemia, including histopathological findings. *Acta. Med. Scand.*, **180**, 593.

Lewin, A. J., Greenbaum, M. A., Gordon, A. and Maxwell, M. H. (1972). Current status of the clinical application of the Redy dialysate delivery system. *Proc. Dial. Transpl.*, **2**, 53.

Maeda, K., Kawaguchi, S., Manji, T., Kobayashi, K., Ohta, K., Saito, A., Shimoji, T., Yui, T. and Hori, M. (1973). Portable artificial kidney with adsorbents. *Proc. Europ. Dial. Transplant. Assoc.*, **10**, 293.

Maeda, K., Kawaguchi, S., Shimizu, K., Manji, T., Kobayashi, K., Ohta, K., Saito, A., Amano, I., Yoshiyama, N., Nakogawa, S. and Koshikawa, S. (1974). Ten-litre dialysate supply system with adsorbents. *Proc. Eur. Dial. Transplant. Assoc.*, **11**, 180.

Malchesky, P. S., Nokoff, R., Varnes, and Nosé, Y. (1976a). The charcoal capillary haemoperfusion system. *Proc. Eur. Dial. Transplant. Assoc.*, **13** 242.

Malchesky, P. S., Surovy, R. M. and Nosé, Y. (1976b). The charcoal-coil: A dialysis-dialysate regeneration system. Presented at the *Symposium on Sorbents in Uraemia and Hepatic Failure*, Downstate Medical Centre, Brooklyn, New York, November 3 - 4 (in press).

Nosé, Y., Tajima, K. and Nakazona, M. (1972). The intracorporeal artificial kidney. *Proceedings of 5th Annual Contractors Conference*, p. 45.

Surovy, R. M., Malchesky, P. S., Sato, H. and Nosé, Y. (1974). Charcoal-coil: A dialysis-dialysate regeneration system. *Proceedings of 27th Annual Conference on Engineering in Medicine and Biology*, p. 3.

# 3

# PLATELET BEHAVIOUR IN EXTRACORPOREAL CIRCULATION (ECC)

J. C. F. DE JONG, C. TH. SMIT SIBINGA AND
CH. R. H. WILDEVUUR

**Summary**

Haemocompatibility of nonphysiological materials is of increasing importance. The basis of assessing such compatibility has shifted from the quantification of erythrocyte damage to estimation of clotting capacity. Specified in terms of the number of circulatory thrombocytes and the bleeding time, this yields significantly more relevant information for ranking materials in relation to their blood compatibility than does assessment of haemolysis alone.

## 3.1 Introduction

During the last decade there has been an evolution in the parameters for measuring the haemocompatibility of nonphysiological materials. In the early attempts compatibility has been described in terms of damage to erythrocytes or haemolysis; the estimation of free haemoglobin was used as a quantitative indication. However, haemolysis as an expression of erythrocyte damage only yields limited information, since the measured free haemoglobin levels are not only the result of damage to the erythrocytes, but also of the processes of binding to plasma proteins and elimination. Because of the extensive binding capacity of the plasma proteins, certain degrees of erythrocyte destruction cannot be detected. Therefore the measurement of free haemoglobin is not suitable to test haemocompatibility.

The frequently encountered complication of bleeding in open heart surgery shifted attention from the erythrocytes to the phenomenon of clotting. To estimate the clotting capacity the counting of circulating thrombocytes was generally accepted. This approach seemed to yield more relevant information for differentiating materials in their properties of compatibility. Yet, there are restrictions to this also. As in an army on the battlefield the number of soldiers present does not give information about the condition of each individual soldier - healthy, wounded or dead - so the knowledge of the number of circulating thrombocytes does not mean that all cells counted are in optimal condition. Therefore thrombocyte function tests are also required. Such function assays are usually performed by means of ADP or collagen induced aggregation tests, the results of which together with platelet counts give detailed information of haemocompatibility. However, we do not know whether the unphysiological, *in vitro* aggregation test is a reliable

expression of the *in vivo* haemostatic capacity of the thrombocyte. An alternative way of assessing the overall strength of the thrombocyte 'army' is by measuring the bleeding time; since decreases both in number and function of the thrombocytes may result in a lengthening of this time, it seems to yield in an integrated fashion more relevant information than the separate parameters.

It is naturally accepted that from a theoretical point of view the bleeding time can be influenced by disturbed fibrin formation or fibrinolysis; however, both phenomena only contribute to haemostasis to a minor extent, as compared to the role of the thrombocytes. Consequently, a critical and comparative look at the thrombocytes and the bleeding time appears to make it possible to define haemocompatibility quantitatively.

### 3.2 The Thrombocyte Function Normogram

Laboratory procedures for measuring ADP-aggregation curves under standardised conditions of thrombocyte numbers involve manipulations in order to concentrate or to dilute the platelet rich plasma samples in an attempt to equalise numbers. In our laboratory we have chosen a different approach: aggregation tests were performed on pure platelet-rich plasma (PRP). As shown in figure 3.1, the measured parameters are the maximal optical density loss ($OD_{max}$) and the cotangent of the initial slope ($\alpha$). In a serial dilution system $OD_{max}$ and the cotangent of the initial slope ($\alpha$), expressed as a proportion of their pre-dilution values, plotted against the corresponding number of thrombocytes, create the normogram shown in figure 3.2. This normogram consists of two closely placed parallel straight lines for thrombocyte counts under 82 000/mm$^3$. Since for practical purposes the two lines are virtually coincident they are shown as *one* straight line. Thus, for low thrombocyte concentrations there is a linear relationship between thrombocyte number and function; it is also seen that for concentrations over 82 000 thrombocytes/mm$^3$, the aggregation parameters are not influenced by increasing numbers. This thrombocyte numbers versus function normogram therefore makes it possible to compare aggregation curves from samples with different thrombocyte concen-

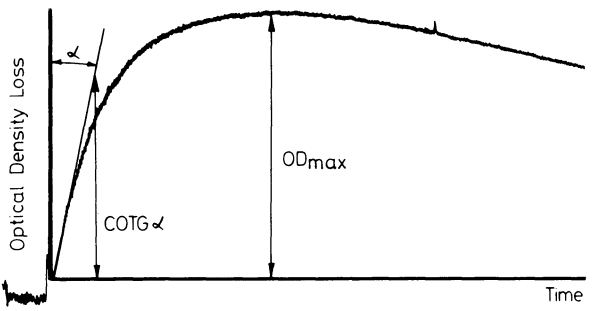


Figure 3.1 ADP induced platelet aggregation: parameters 1.0 ml PRP + 0.2 ml ADP 3 mg/ml

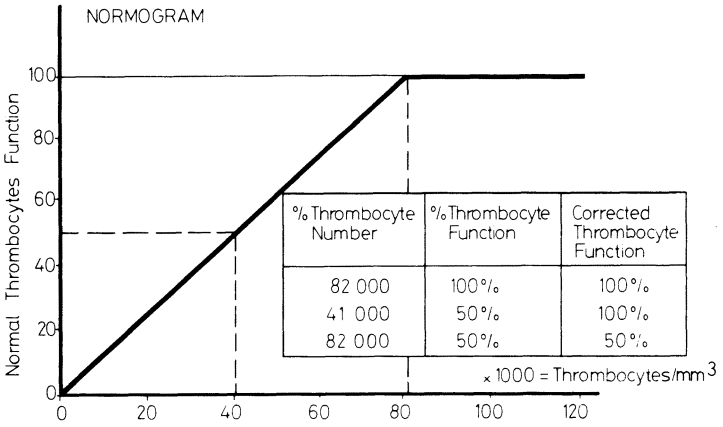


Figure 3.2 Normogram of thrombocyte function

trations without any manipulation. To give an example: samples with 82 000 or more thrombocytes need no correction; PRP in a concentration under 82 000, for example 41 000/mm$^3$ and a measured $OD_{max}$ percentage of 50, actually has a 100% function.

The parameters discussed have been utilised in a series of experiments to establish platelet behaviour in extracorporeal circulation.

### 3.3 Platelet Behaviour in Extracorporeal Circulation (ECC)

Mongrel dogs, weighing 28 to 35 kg were cannulated and connected in a veno-venous or veno-arterial shunt to a standardised extracorporeal circuit, consisting of tubing, blood pump, artificial organ, heat exchanger and flow probe (figure 3.3). Blood flow rate was maintained at 4000 or 3000 ml/min and total heparinisation was employed. Blood samples for haematological studies were taken from an in-dwelling catheter in the femoral artery. The ECC was primed with equivalent quantities of heparinised donor blood and a gelatine plasma expander. Each series consisted of at least six experiments. The time scheme for all experiments was uniform and consisted of an initial period of ECC, lasting for 30 min, followed by a period of stabilisation up to a total of 2 h from commencement. In the subsequent recovery period the dog has been assessed for three weeks. In this recovery period the first week after ECC appears to be the most important one.

Figure 3.4 presents the typical pattern of thrombocyte numbers observed in all ECC experiments during the initial 30 min. As early as *one min after starting the bypass the number of circulating thrombocytes has decreased by about 90%*; a minimum level of 5% is reached after 3 min only. Thereafter a constant but slow increase is observed.

The momentarily massive disappearance of thrombocytes from the circulation can only be explained by an acute aggregation phenomenon. Others have suggested that this aggregation occurs in the ECC but this appears to be impossible. As

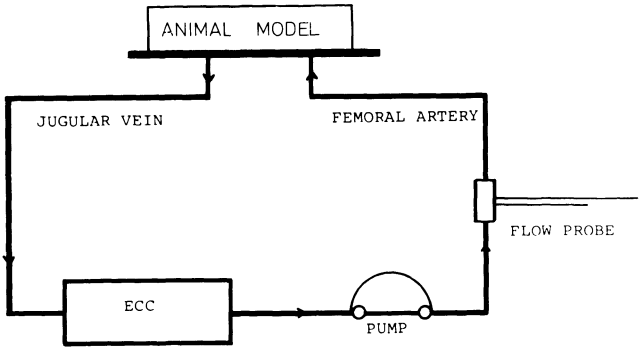


Figure 3.3 Experimental set up

shown in figure 3.4 the phenomenon takes place within minutes, during which only a part of the total blood volume could have passed the circuit. It is more likely that at the first contact of blood with the material of the ECC device bioamines like ADP or serotonin are released from damaged red blood cells. This could initiate the observed massive intravascular aggregation. The gradual increase after this initial dip points to a partly reversible process.

This hypothesis is substantiated by the following experiment, shown in figure 3.5. An analogous extracorporeal system was connected at the outflow side to a blood bag and not to the dog. The blood thus collected from the extracorporeal system was not immediately reinfused, instead simultaneous infusion of

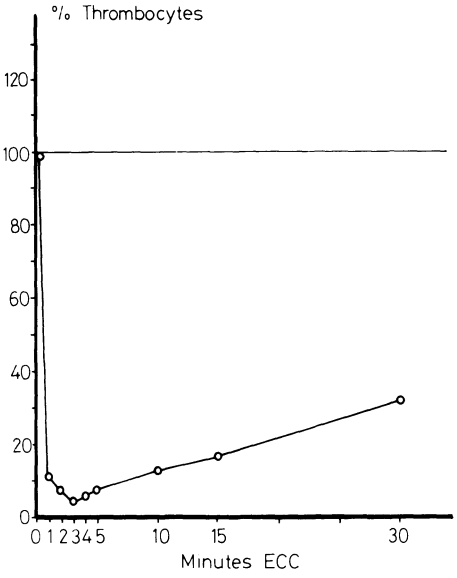


Figure 3.4 Thrombocyte time variation in initial period of ECC

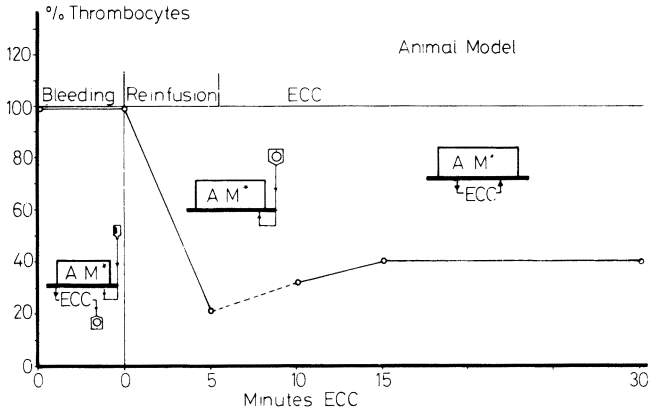


Figure 3.5 Experimental set up for 'initial platelet dip' hypothesis

saline was given. As can be seen no changes in platelet numbers occurred during this procedure. However, when the collected blood was reinfused, the same immediate dip was seen, followed by a degree of platelet restoration in the further course of this ECC experiment. It is evident that some active substance, released from the blood cells consequent to their passage through the ECC, appears responsible for the severe initial thrombocyte dip. Identical dips in the number of thrombocytes in circulation have also been observed as a result of intravenous ADP injection.

The differences in reactions of thrombocytes during and after ECC between a bubble oxygenator (BO) (lower curve) and a membrane oxygenator (MO) (upper curve) in otherwise identical circuits are shown in figure 3.6. In the BO experiments, the initial dip is more pronounced and thereafter only a minor restoration was seen. This is in contrast to the thrombocyte behaviour in the MO experiments, where the initial dip is less severe and a distinct restoration can be observed. (In

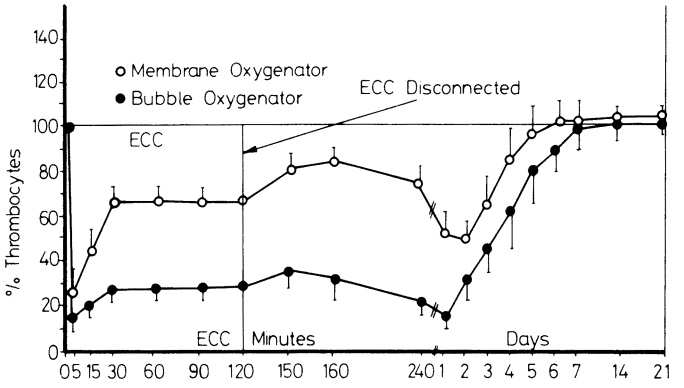


Figure 3.6 Thrombocyte time variation during and post ECC

case the initial dip is dependent on the amount of released bioamines from damaged cells, the question arises whether the initial dip could be a measure for haemocompatibility. This will be discussed later.)

The restored numbers, after 30 min, appeared to be stable as shown by the plateaux. Apparently there is an equilibrium between aggregation and consequent disaggregation of the thrombocytes. The highly significant differences between the plateaux of MO as compared to BO suggest differences in aggressiveness of the ECC used. These plateaux have generally been used as indicators for haemocompatibility of the systems employed.

After disconnection of the ECC a slight increase in thrombocyte numbers is observed initially, with decreases thereafter. These decreases culminate in a clear secondary dip on the first or second day post ECC; hereafter a gradual restoration towards normal values occurs.

After disconnecting the ECC, platelet aggregation is not stimulated further. Thus the immediate post ECC increase of thrombocytes is explained as release of platelets from formed aggregates through disaggregation. The decrease thereafter is most logically explained by the elimination of the thrombocytes affected by the ECC procedure. 'After the war is over and the dead and wounded are removed', the final outcome of the real strength and condition of the original thrombocyte 'army' is reflected in the secondary dip.

After the secondary dip, all curves show identical and parallel patterns of increasing thrombocyte numbers until normal values are reached. This recovery is determined by the thrombopoietic capacity of the animals which explains the parallel course of the curves. The very large differences between the BO and MO experiments emphasise the possible importance of the secondary dip as a measure for haemocompatibility. Apart from a difference of about 35% in thrombocyte numbers, the recovery to equal values showed a delay of 1 to 2 days in the BO as

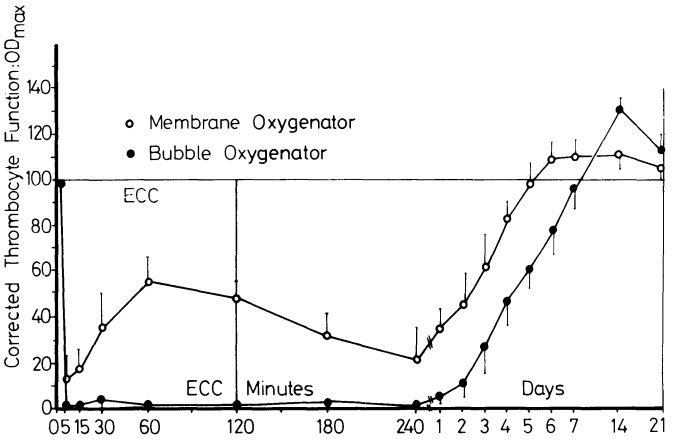


Figure 3.7 Thrombocyte time variation during and post ECC corrected for thrombocyte function

compared with the MO experiments.

Figure 3.7 shows the curves of the thrombocytes' function, as expressed by the normogram corrected $OD_{max}$'s. A similar general pattern as for the thrombocyte numbers was seen; most impressive is the complete loss of function in the BO experiments during ECC and lasting for a considerable time after ECC. A clear commencement to restoration of function is observed only after the second day.

The results of the consequent bleeding times in the BO and the MO experiments are given in figure 3.8. They substantiate the expected differences for thrombocyte numbers and functions. At the end of ECC and the following day the bleeding times in the BO group were severely lengthened to over 15 min, whereas in the MO group bleeding times were about normal (2 min ± 1). The observed lengthening of the bleeding time in the BO group extended to day 5. This observation seems to be of great practical importance, because an increased bleeding tendency is a common complication after open heart surgery, in which bubble oxygenators are generally used. This figure indicates that with the use of a membrane oxygenator improvements may be obtained.

From the results presented it is clear that detailed description of platelet behaviour in relation to extracorporeal circulation gives the tools to discriminate between the haemocompatibility of the systems used. Differences in thrombocyte numbers in the initial dip, the plateau reached during ECC and the secondary dip in the recovery period are the significant indicators. Of the parameters used, the behaviour of thrombocyte numbers and functions showed similar patterns. Actually, little additional information of thrombocyte behaviour was obtained from the *in vitro* aggregation function test, apart from information on platelet numbers. The bleeding time, however, as a complement of thrombocyte number and function, seems to be of major practical importance.

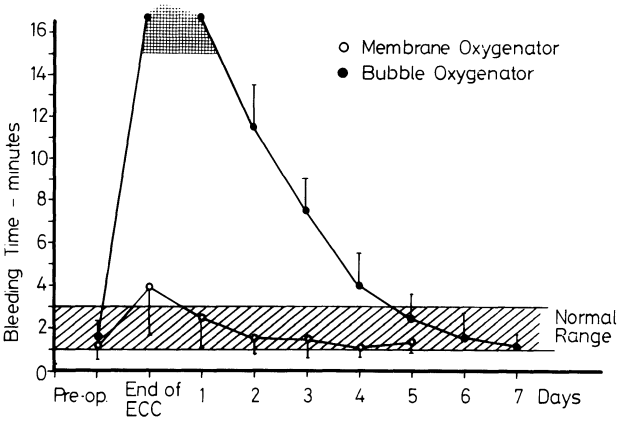


Figure 3.8 Bleeding times in BO and MO ECC

### 3.4 Haemocompatibility of ECC System Components in Open Heart Surgery

The components of commonly used standard equipment for open heart surgery are the circuit material: PVC versus silica-free silicone rubber; the blood pump: standard two head roller pump versus a low rotation speed pump (developed by Rhône-Poulenc) and the oxygenator: bubble versus microporous membrane. The testing of the suction and the commonly used arterial filters has not been included in our present study. This will ultimately complete the picture when it is clear to what extent haemocompatibility improvements can be obtained for the complete equipment system necessary for open heart surgery procedures.

Figure 3.9 presents the differences in thrombocyte numbers at the moment of the *initial dip*. No significant differences are shown between PVC and silica-free silicone rubber. When in a silica-free silicone rubber circuit the Rhône-Poulenc pump was used, a less severe, although not significantly different, initial dip was seen. The interconnection of an oxygenator results in a more pronounced initial dip. Although not significant, the dip for the MO tended to be less severe than for the BO. No improvement was seen using silica-free silicone rubber and the Rhône-Poulenc pump. Finally the indications obtained from the *initial dip* do not correspond to the major differences already clearly established between the BO and the MO groups. Thus we do not feel that the *initial dip* is a reliable indicator for haemocompatibility.

Figure 3.10 shows the plateau reached in the period of equilibrium during ECC, a measure that is most commonly used to express differences in haemocompatibility. No significant differences are seen due to the materials or different pump systems used in the circuit. Major differences, however, do obtain between the oxygenator and the bubble oxygenator and the plateau does seem to be indicative therefore of haemocompatibility of the system. It is probably noteworthy that no differences were seen in the plateau values when the MO was

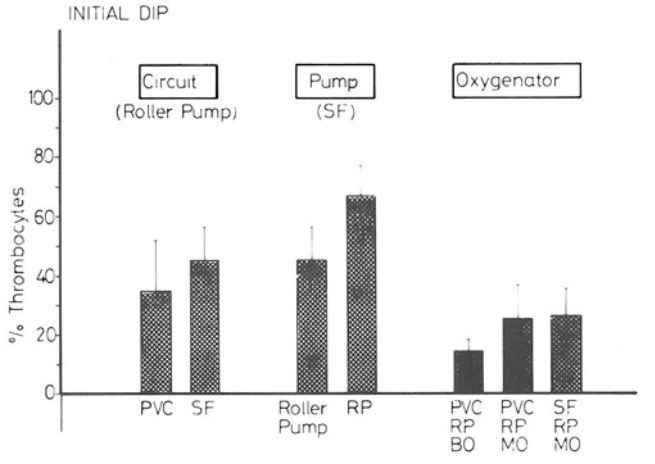


Figure 3.9 Haemocompatibility of ECC components – indicator initial dip

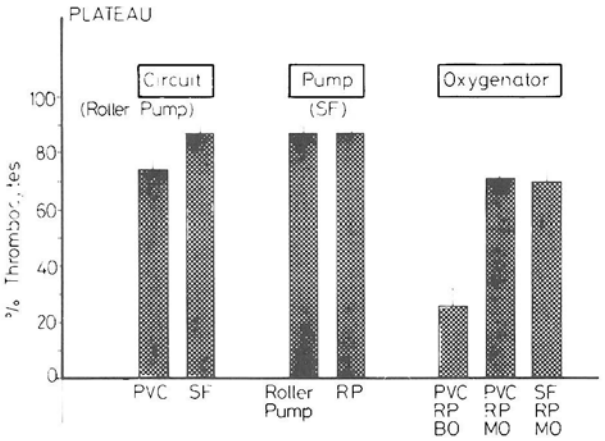


Figure 3.10 Haemocompatibility of ECC components – indicator plateau

applied in the circuit with either PVC/roller pump or silica-free silicone rubber/ Rhône-Poulenc pump.

Exploration of the secondary dip in the recovery period as a haemocompatibility indicator is shown in figure 3.11. As indicated before, this measure is, in our opinion, the best overall assessment of the damage to the thrombocytes, caused by the ECC. It is evident that at this moment of maximal decrease in the number of circulating thrombocytes after ECC, all affected cells have been cleared from the circulation. So, the remaining number of thrombocytes at the moment of the secondary dip represents the percentage of cells not affected by the ECC. The information is similar to that obtained from the value of the plateau post ECC: no significant differences between the circuit materials and the pump systems,

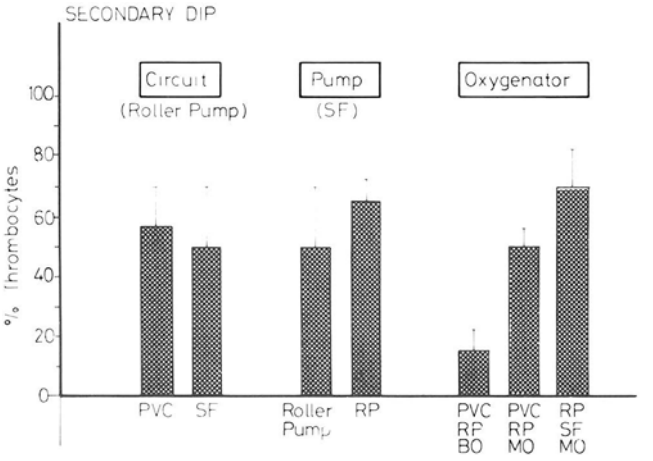


Figure 3.11 Haemocompatibility of ECC components – indicator secondary dip

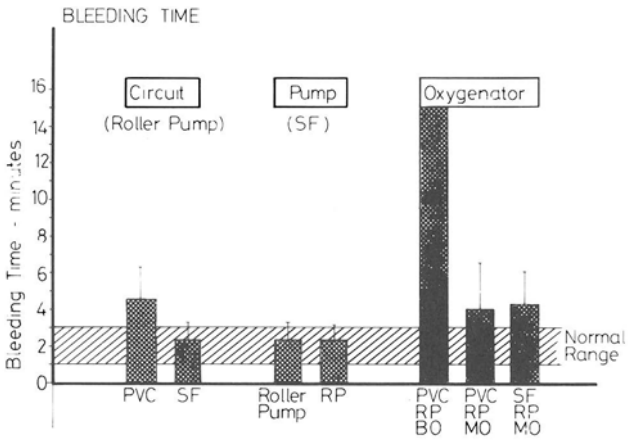


Figure 3.12 Haemocompatibility of ECC components – indicator bleeding time

but major differences between MO and BO. One exception: in contrast to the information obtained from the plateaux, it may be of interest that a significant improvement in haemocompatibility was obtained when an optimal material and pump were used with the membrane oxygenator. This might indicate that the cumulative effects of relatively small improvements in each component can ultimately result in an important contribution to the overall haemocompatibility of the system. The clinical relevance of our results is demonstrated by figure 3.12, in which the bleeding time after ECC is presented. The only significant lengthening of the bleeding time was encountered when a bubble oxygenator was used in the circuit. Completely normal bleeding times were only obtained when silica-free silicone rubber material was employed in the circuit.

## 3.5 Conclusions

In any assay of platelet behaviour, as an indication of haemocompatibility, it is important to use methods which express platelet function in relation to their numbers. The *normogram* concept enables such an assay to be made with minimal manipulation of the thrombocytes.

The *bleeding time* has been introduced as a relevant parameter complementing thrombocyte number and function impairment. However, because of the difficulties implicit in standardisation of the method, the bleeding time should not be regarded as a very sensitive parameter for haemocompatibility. Since, as has been shown, small improvements in haemocompatibility of the ECC components might be important in their cumulatively beneficial effect on the total ECC system, a more sensitive parameter is needed. At this stage of the art, the *secondary dip* in thrombocyte number and function during the recovery phase appears to be the most sensitive of such haemocompatibility indicators.

SESSION B

# MEMBRANE OXYGENATORS

*Chairman*
P. M. Galletti, M.D., Ph.D.

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# 4

# MASS TRANSFER MODELLING FOR MEMBRANE OXYGENATORS

W. J. DORSON, JR.

**Summary**

Theoretical analysis of $O_2$ and $CO_2$ transfer in membrane lung channels with streamline flow has been sufficiently developed to adequately predict experimental results. These methods are of great utility both for data interpretation, since generalised coordinates are used, and determining either deficiencies or benefits of a particular membrane lung design. Total bypass units, of necessity, must contain large permeable membrane surface areas unless a technique to augment respiratory gas transfer is employed. The verification and advantages of theoretical methods will be considered along with their extension to geometries which use secondary flows to increase the efficiency of $O_2$ and $CO_2$ exchange.

## 4.1 Primary Flows

The convective-diffusive analysis of respiratory gas transfer in membrane lung channels can be greatly simplified due to the low mole fraction content of $CO_2$ and $O_2$ constituents. Also, in considering the large artificial lung channel dimensions possible with present technology compared to the red blood cell (RBC) dimensions, a continuum approach assuming all chemical species to be in equilibrium with the dissolved component is appropriate.

The governing equations at steady state for dilute species can be expressed as

$$\mathrm{div}(\nu C_T) = \sum_i \nabla \cdot D_i \nabla C_i \tag{4.1}$$

where local equilibrium and a continuum including RBC and plasma interactions are assumed. This approach has often been taken for $O_2$ and $CO_2$ transfer in macroscopic flow channels (Drinker, 1972; Dorson *et al.*, 1971, 1974 and 1976; Voorhees, 1976). The physical interpretation is that the axial convection of content (left side of equation 4.1) is balanced by the diffusion of important dissolved species (right side of equation 4.1). This total concept has proved acceptable for membrane lung design purposes in describing both $O_2$ and $CO_2$ transfer in streamline flows (Cartesian and cylindrical geometries), as well as with well-defined secondary flows when appropriate boundary conditions are incorporated into the analytical solutions. The boundary conditions for membrane artificial lungs include a uniform inlet content and partial pressure, symmetry at the centreline for dual permeable membrane Cartesian channels and the tubular

geometry, and a wall flux-matching expression between the blood mass transport and membrane transfer at the containing but permeable boundary. The case of a single permeable wall in Cartesian coordinates (all for semi-infinite channel dimensions where width/height $\gg 1$) becomes a simple extension of the same approach (Dorson *et al.*, 1976; Voorhees, 1976).

Specific results of this methodology take advantage of the unique characteristics of blood. For $O_2$ transport, film theory concepts can be employed due to the extremely high Schmidt number ($\nu/D_e$). Thus, the exact nonlinear content relation with dissolved $O_2$ can be grossly simplified when large $pO_2$ gradients exist, as outlined in the exposition of the Advancing Front Theory (AFT) for a flowing blood stream with both appreciable haematocrit and inlet 'desaturation' (Lightfoot, 1968). A number of studies based on computational comparisons infer that the AFT approach should overestimate the $O_2$ transfer to blood. In fact, a logical extension of the original AFT approach to include wall resistance and redistribution of dissolved $O_2$ at the exit cannot be distinguished from carefully obtained data (Dorson *et al.*, 1971; Voorhees, 1976) as will be shown..

For laminar flow in a tube with the transfer of only dissolved gases (non-reactive) and a constant diffusion coefficient, equation 4.1 becomes

$$2\bar{u}\left(1 - \frac{r^2}{a_i^2}\right)\frac{\partial C_1}{\partial z} = \frac{D_1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_1}{\partial r}\right) \tag{4.2}$$

considering only axial convection and radial diffusion to be important. With the same restrictions, mass transfer with laminar flow in a semi-infinite ($W/h \gg 1$) Cartesian channel can be described by

$$\frac{3}{2}\bar{u}\left(1 - \frac{y^2}{a^2}\right)\frac{\partial C_1}{\partial z} = D_1\frac{\partial^2 C_1}{\partial y^2} \tag{4.3}$$

Numerical and restricted analytical solutions to these equations exist which provide the opportunity to compare theory with data and verify experimental procedures. In addition, for multiple reacting and diffusing species which can be linearly related to the dissolved gas at equilibrium a similar set of equations has been derived by Dorson *et al.* (1974) with $D_1$ replaced by $D_e/F$ (the slope of total content with respect to dissolved concentration is designated as $F$). Based on this approach, a design procedure for $CO_2$ removal from blood in membrane lungs was developed which compared favourably with single channel data for low total content removal, zero base excess, and normal pH buffer power (Dorson *et al.*, 1974; Dorson *et al.*, 1976). The dimensionless corrdinates which correlate the data are

$$\text{Tubes: } Z^* = \frac{\pi D_e L N}{2QF},\ \theta = \frac{\bar{P} - P_o}{P_g - P_o},\ \gamma = \frac{\alpha_b D_e \ln\frac{a_o}{a_i}}{\mu} \tag{4.4}$$

Dual membrane flat channels: $$Z^* = \frac{4WNLD_e}{3aQF}, \quad \theta = \frac{\bar{P} - P_o}{P_g - P_o}, \quad \gamma = \frac{\alpha_b D_e t}{\mu a} \tag{4.5}$$

In order to provide more accurate predictions over a wider range of $CO_2$ removal, geometric and membrane similarity conditions, and inlet blood variations, numerical schemes which account for blood chemistry changes must be utilised (Benn, 1974; Voorhees, 1976). However, a loss of generality accompanies the more accurate analysis and the three coordinates given by either equations 4.4 or 4.5 are not sufficient to uniquely correlate the data. A series of experiments was performed by Voorhees (1976) at low $\gamma$ values to maximise the observation of blood side transfer control. The fractional content removal from a single permeable tube is shown in figure 4.1 for three different inlet $CO_2$ contents, along with numerical predictions based on a two species model. The equilibrium chemical relations were defined by

$$C_T = C_1 + C_2, C_1 = \frac{C_2}{K'} 10^{C_2/\beta}, \frac{1}{\beta} = \frac{d\text{pH}}{dC_{\text{base}}} \tag{4.6}$$

This representation allows the generation of a sample specific $CO_2$ dissociation curve knowing only the inlet total content, $pCO_2$, haemoglobin concentration, and haematocrit. Where the experimental accuracy is greatest, $\theta_c > 0.1$, the comparison of theory and data is excellent. Similarly, nonreactive theoretical predictions and data for $O_2$ transfer to or from flowing water in both permeable and microporous tubes, single membrane flat channels, and dual membrane flat channels were statistically indistinguishable.

Content measurements in this study were determined using a chromatographic method which is superior to oximetric techniques for haemoglobin saturations exceeding 95%. Thus, a more extensive comparison of data with AFT predictions was possible. The dimensionless AFT coordinates which correlate the data are

$$\text{Tubes: } \xi_g = \left(\frac{P_g - P_o}{C_{bo}}\right)\left(\frac{\alpha_b \pi NL}{4Q}\right) D_{o_2} f = \frac{s - s_o}{1 - s_o}, \gamma = \frac{\alpha_b D_{o_2} \ln \frac{a_o}{a_i}}{\mu} \tag{4.7}$$

Dual membrane flat channels: $$\xi_g = \left(\frac{P_g - P_o}{C_{bo}}\right)\left(\frac{\alpha_b NWL}{2Qa}\right) D_{o_2} f = \frac{s - s_o}{1 - s_o}, \gamma = \frac{\alpha_b D_{o_2} t}{\mu a} \tag{4.8}$$

Data from the latter geometry are shown in figure 4.2 where different $\gamma$ values can be compared with a single AFT prediction by correcting $\xi_g$ by $\gamma f/4$. The inlet saturations were held between 68 and 77% during testing, and the data are well described by the AFT with the dissolved correction (Voorhees, 1976).

Recently, microporous materials have been incorporated into commercial

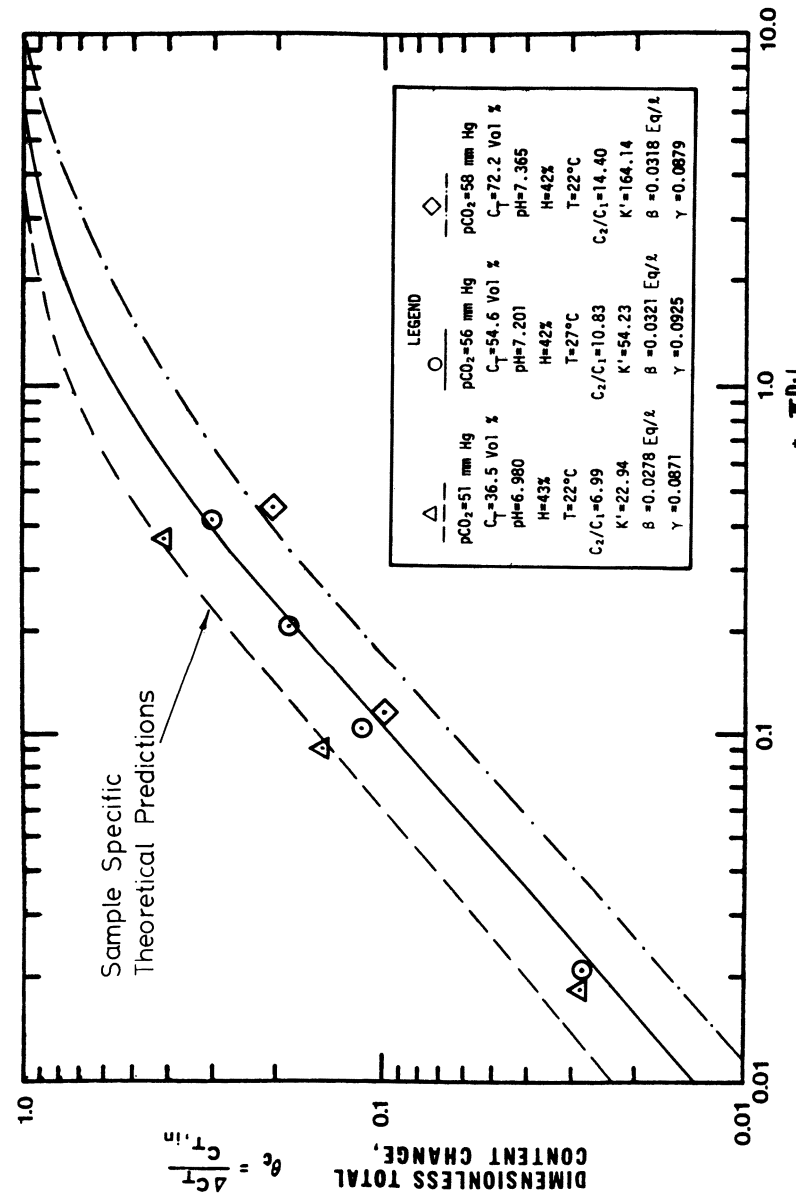


Figure 4.1 Effect of varying inlet $CO_2$ contents on removal from blood flowing in a single permeable tube, comparison of theory with data

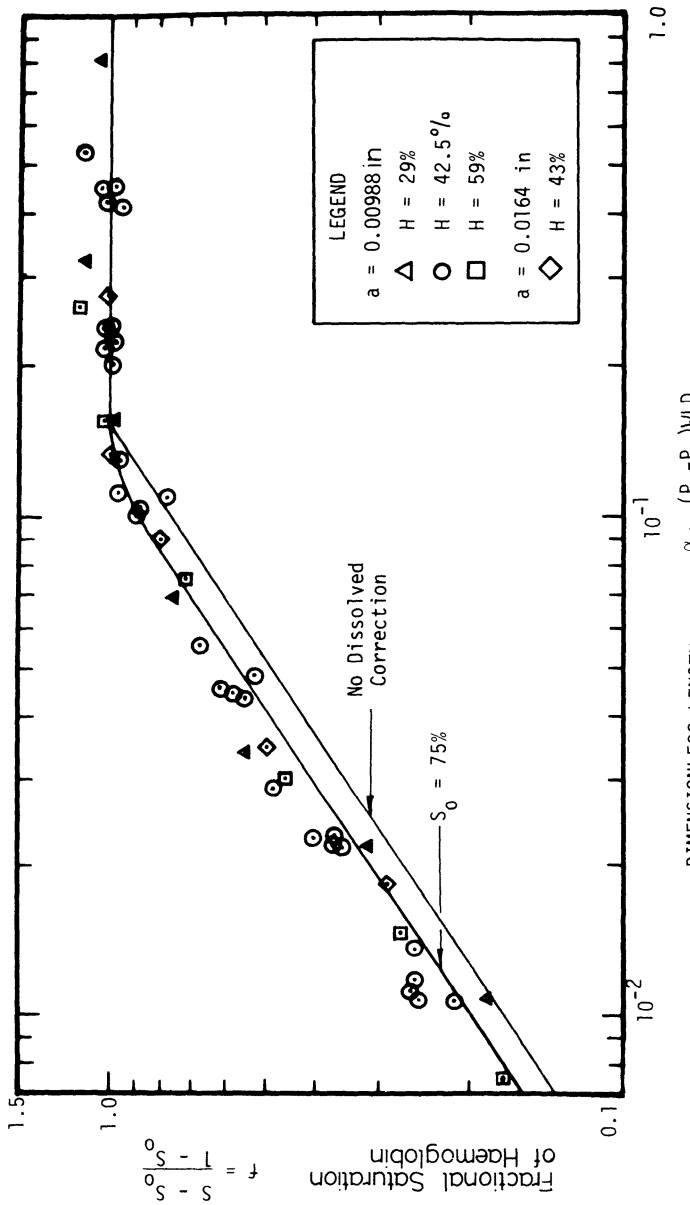


Figure 4.2 Comparison of advancing front theory with data for oxygenation of blood flowing between two permeable membranes (flat channel geometry, $W/h \gg 1$)

membrane oxygenators replacing solution-diffusion controlled homogeneous materials such as silicone. Oxygen transfer measurements in a single microporous tube were made with the same inlet saturation limits (Voorhees, 1976) and are shown in figure 4.3 using AFT coordinates with $\gamma$ taken to be zero. Again, excellent prediction is afforded by the AFT incorporating the dissolved $O_2$ correction. Based on these comparisons, where data were obtained with freshly drawn human blood and tonometered in a membrane lung system to avoid blood gas interfaces, no overprediction of the AFT can be substantiated.

## 4.2 Secondary Flows

The success of the extended AFT concept in streamline Cartesian and cylindrical geometries leads to the question of its utility as applied to different flow geometries. Artificial lungs can be made significantly more efficient by employing secondary flows to exchange oxygen-rich blood near the membrane surface with inlet blood located far from the surface where the axial (primary) flow is greatest. The potential limits to the applicability of the AFT are twofold. Firstly, for gentle secondary flows a geometric analogue for the effect of the convective augmentation of diffusion must be readily visualised. This limit applies to such methods as the toroidal oxygenator (Drinker *et al.*, 1969), coiled permeable tubes (Weissman et al., 1968; Dorson *et al.*, 1971), and rotating parallel permeable boundaries (Hill *et al.*, 1974) as long as the locally defined flow is streamline (laminar). The second limitation applies to more aggressive methods of blood oxygenation. For example, if the flow is made to be pseudoturbulent, transport within the plasma phase can be forced to be extremely rapid. Discounting the potential damage to formed elements, two phenomena can be seen to prevent the direct application of AFT to this flow situation. The stroma within the RBC is extremely viscous while the 'separating' membrane easily transmits shear stress. Thus, the combined reactive-convective-diffusive (or electrolyte transport) phenomena inside the RBC could easily limit the total transfer of $O_2$. This limitation to AFT concepts is still readily analysed by more exact theoretical methods. The second effect would be the diminution of local shear stress by the compliant or absorbent qualities of the RBCs, reducing the eddy diffusion of $O_2$ through the flowing blood stream.

As an example of the AFT extension to secondary flows, coiled silicone tube oxygenators were studied. An AFT analysis is possible for the established helical flow effect at low Dean values. Under these conditions, the primary effect of the induced angular and radial velocities is to bring saturated blood from the wall region to the centre of the tube. For straight tubes the saturated zone is visualised as an annular region bounded by the tube wall and inlet blood conditions, whereas for oxygenation in coiled tubes the saturated region can be visualised as two symmetric protractor shapes with dimensions dependent on the position at which the angular velocity is zero ($v_\theta = 0$). Therefore, not only is saturated blood convected to the tube centre where the axial velocity is maximum, but a second diffusional front is established to help dispense dissolved $O_2$ towards the $v_\theta = 0$ point. For low $Dn$ numbers the $v_\theta = 0$ position is stable at an approximate value of $r/a_i = 0.429$ as

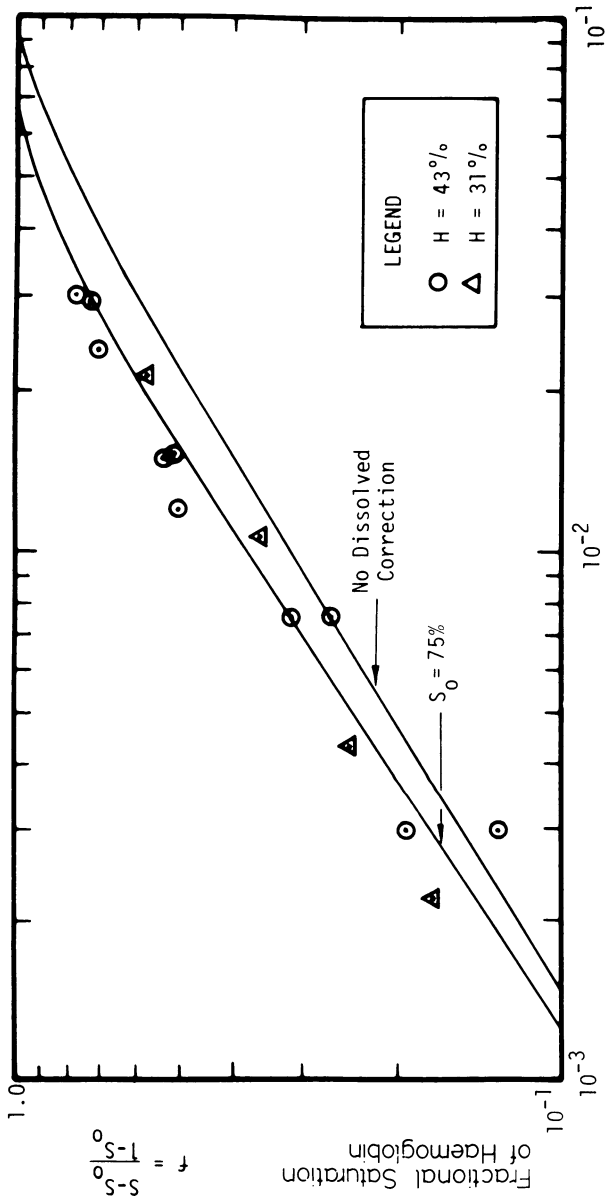


Figure 4.3 Comparison of advancing front theory with data for oxygenation of blood flowing in a single microporous PTFE tube

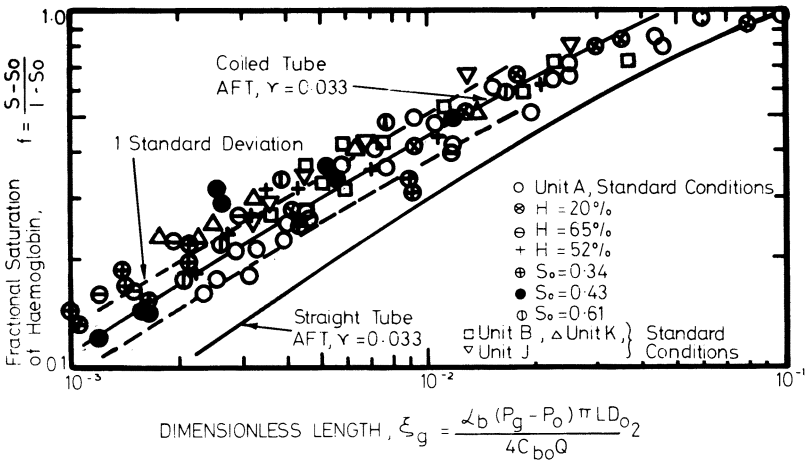


Figure 4.4 Comparison of coiled advancing front theory with data for oxygenation of blood flowing in single coiled tubes at low Dean numbers ($Dn \leqslant 12$). (Standard conditions were $S_0 = 0.75$, $pCO_2$ of 35 to 40 mm Hg, H of 43% to 48%, $T = 25°C$, pH of 7.1 to 7.4)

predicted by Dean (1927). The corresponding AFT equations have been presented by Dorson and Larsen (1971) and Larsen (1971) with generalisation accounting for a variable $v_\theta = 0$ position also available (Larsen, 1971; Christianson, 1976). The limited coiled tube blood oxygenation data presented for comparison in Dorson and Larsen (1971) are thought to be in error while subsequent data comparisons (Dorson *et al.*, 1971; Larsen, 1971) were in excellent agreement with the low *Dn* AFT predictions.

Of particular interest is the fact that the calculated dissolved $O_2$ correction for coiled tubes was insignificant. This conclusion was verified by coiled tube data (highest *Dn* number less than 12), as shown in figure 4.4. The data agree quite well with the coiled tube AFT predictions for $f \leqslant 0.7$ where the coiled tube secondary flows are well established. In this series of experiments, as the fractional saturation approached unity ($f \rightarrow 1$) the *Dn* value fell below unity. Thus, the data approached straight tube predictions as $f \rightarrow 1$. In addition to a wide variation of haematocrit and fractional inlet saturation, $s_0$, accomplished during this experimental series, the test unit geometries were as shown in table 4.1, with the pitch ratio, $\pi_0$, ranging between 1.22 for unit J to 2.0 for units A and B. The significance of these results includes the applicability of AFT generalised coordinates to gentle secondary flows, elimination of the need to precisely control experimental blood conditions, and a further verification of the AFT approach within the outlined limitations.

The two questions yet to be answered involve the transition criteria between straight and coiled tube oxygenation of blood and the departure of coiled tube oxygenation from the low *Dn* AFT predictions. Note that this AFT prediction

**Table 4.1**

Test unit geometries

| *Test unit* | *L(cm)* | $d_i$*(cm)* | $d_o$*(cm)* | $\lambda$ |
|---|---|---|---|---|
| A* | 146 | 0.198 | 0.318 | 0.092 |
| B | 291 | 0.198 | 0.318 | 0.092 |
| K | 91.5 | 0.08 | 0.13 | 0.08 |
| J | 150 | 0.147 | 0.195 | 0.085 |

*Standard unit on figure 4.4

is independent of the helix ratio, and this result was also verified in test units with $\lambda$ ranging from 0.013 to 0.092 (Dorson *et al.*, 1971; Larsen, 1971).ı. Christianson (1976) attacked the transition problems and established criteria such that no significant deviation from straight tube oxygenation was observed until the similarity parameter *ReSc* exceeded $10^4$ based on both water and blood data.

Experiments with *Dn* numbers up to 100 demonstrated substantial further increases in the $O_2$ transfer efficiency approaching the membrane wall limit at low $f$ values (Dorson *et al.*, 1976). However, as the flow rate was decreased the data again correlated with either the low *Dn* coiled or straight tube theories ($f \rightarrow 1$). This behaviour results from the interdependence of $\xi_g$ and *Dn* given by

$$\xi_g = \alpha_b \left( \frac{P_g - P_o}{C_{bo}} \right) \frac{\sqrt{\lambda} L}{DnSc\, d_i}$$

Thus, for a fixed geometry and inlet blood condition, increasing $\xi_g$ to achieve full saturation automatically decreases the *Dn* number and diminishes the augmentation. At high *Dn* low $f$ conditions, accounting for the movement of the $v_\theta = 0$ position close to the permeable wall is not sufficient to fully explain the observed augmentation. As the $v_\theta = 0$ point approaches the membrane, convective removal in the wall region significantly alters the dissolved oxygen concentration profile. An increased flux is obtained, but the utility of the AFT approach is questionable due to the complexity of the convective-diffusive equations. Christianson (1976) used a two term series representation for $v_\theta$ near the wall to extend the AFT approach to high *Dn* values. Due to the added dependence of the flow field on the *Dn* number a series of graphical predictions is necessarily dependent on the inlet conditions. Thus, the generality achieved by using AFT to describe blood oxygenation in streamline flows with and without gentle secondary mixing does not hold for vigorous augmentation in coiled tubes.

## 4.3 References

Benn, J. A. (1974). Carbon dioxide transfer from weak acids and blood: The effects of carbonic anhydrase and oxygen uptake on carbon dioxide transfer in an oxygenator. Ph.D. Thesis, Massachusetts Institute of Technology.

Christianson, H. B. (1976). Secondary velocity effects relating to gas exchange with fluids flowing in helical tubular membranes. Ph.D.Thesis, Arizona State University.

Dean, W. R. (1927). Note on the motion of fluid in a curved pipe. *Phil. Mag.*, **4**, 208.

Dorson, W. J., Jr. and Larsen, K. G. (1971). Secondary flows in membrane oxygenators. *Adv. Cardiol.*, **6**, 17.

Dorson, W. J., Jr., Larsen, K. G., Elgas, R. J. and Voorhees, M. E. (1971). Oxygen transfer to blood: Data and theory. *Trans Amer. Soc. Artif. Int. Organs,* **17**, 309.

Dorson, W. J., Jr. and Voorhees, M. (1974). Limiting models for the transfer of $CO_2$ and $O_2$ in membrane oxygenators. *Trans. Amer. Soc. Artif. Organs,* **20**, 219.

Dorson, W. J., Jr. and Voorhees, M. E. (1976). Analysis of oxygen and carbon dioxide transfer in membrane lungs. In W. M. Zapol and J. Qvist (Eds.), *Artificial Lungs for Acute Respiratory Failure,* Hemisphere, Washington, D.C.

Drinker, P. A., Bartlett, R. H., Bialer, R. M. and Noyes, B. S., Jr. (1969). Augmentation of membrane gas transfer by induced secondary flows. *Surgery,* **66**, 775.

Drinker, P. A. (1972). Progress in membrane oxygenator design. *Anesthesiology,* **37**, 242.

Hill, J. D., Iatridis, A., O'Keefe, R. and Kitrilakis, S. (1974). Technique for achieving high gas exchange rates in membrane oxygenation. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 249.

Larsen, K. G. (1971). Oxygen transfer to water and blood flowing in permeable helical tubes. Ph.D. Thesis, Arizona State University.

Lightfoot, E. N. (1968). Low-order approximations for membrane blood oxygenators. *Amer. Inst. Chem. Eng. J.*, **14**, 669.

Voorhees, M. E. (1976). Mutual transfer of carbon dioxide and oxygen to and from blood flowing in macrochannel devices. Ph.D. Thesis, Arizona State University.

Weissman, M. H. and Mockros, L. F. (1968). Gas transfer to blood flowing in coiled circular tubes. *J. Engng. Mech. Div. Amer. Soc. Civ. Engrs.*, **94**, 857.

## 4.4 Notation

$a$ = half channel height ($h/2$)
$a_i$ = inner radius of tube
$a_o$ = outer radius of tube
$C_1$ = concentration of dissolved gas
$C_2$ = concentration of a second reacted species
$C_{bo}$ = concentration of unoxygenated haemoglobin
$C_i$ = concentration of the $i$th species
$C_T$ = total content of all species
$d_i$ = inner diameter of tube
$d_o$ = outer diameter of tube
$D_1$ = effective diffusion coefficient of the dissolved species
$D_e$ = effective diffusion coefficient

$D_i$ = diffusion coefficient of the $i$th species
$D_{O_2}$ = effective diffusion coefficient of oxygen
$Dn$ = Dean number ($Re\sqrt{\lambda}$)
$f$ = fractional saturation
$F$ = linearised slope of $C_T$ versus $C_1$
$h$ = channel height
$K'$ = sample specific constant
$L$ = tube or channel length
$N$ = number of parallel tubes or channels
$\bar{P}$ = average (mixing cup) partial pressure
$P_g$ = gas phase partial pressure
$P_o$ = inlet partial pressure
$Q$ = total volumetric flow rate
$r$ = radial position
$Re$ = Reynolds number $\dfrac{\bar{u}d_i}{\nu}$
$s$ = fractional blood saturation
$s_o$ = inlet fractional blood saturation
$Sc$ = Schmidt number ($\nu/D_e$)
$t$ = membrane thickness in flat channel
$\bar{u}$ = average channel velocity
$v_\theta$ = angular velocity within the tube cross-section
$\bar{v}$ = generalised vectorial velocity
$W$ = channel width
$y$ = variable position within the channel ($y < a$)
$z$ = axial position
$Z^*$ = generalised length coordinate
$\alpha_b$ = effective Bunsen solubility
$\beta$ = buffer index
$\gamma$ = dimensionless wall resistance
$\lambda$ = helix ratio ($d_i$/helix diameter)
$\mu$ = membrane permeability for a given species
$\nu$ = kinematic viscosity
$\pi_o$ = helix pitch ($d_i$/tube spacing)
$\theta_c$ = dimensionless content change ($\Delta C_T/C_{T,in}$)
$\theta$ = dimensionless partial pressure change
$\xi_g$ = dimensionless length for blood oxygenation

# 5

# EXPERIMENTAL MEMBRANE OXYGENATORS WITH CONVECTIVE MIXING FOR GAS TRANSFER AUGMENTATION

J. D. S. GAYLOR, K. GILROY, R. MAINI, A. McNAIR and H. Y. TON

**Summary**

Principle of operation, design and oxygenation performance of three membrane oxygenators employing convective mixing for enhanced gas transfer are described. Two oxygenators are of curved rectangular channel geometry, one with vortex formation induced by pressure driven flow and the second with vortices generated by torsional oscillation of the channel. The third oxygenator is of the capillary membrane type incorporating a chamber which functions as a mixing stage and a blood pump. The efficiencies of the units are compared with existing membrane oxygenators and design optimisation of the most promising system is presented.

## 5.1 Introduction

With the introduction of highly permeable membranes (e.g. ultra-thin silicone rubber and microporous films) the major challenge in membrane oxygenator design has been to reduce the rate limiting diffusion resistance of the blood phase. High efficiency units with small membrane areas are desirable for reasons of lower blood trauma, priming volume, cost and ease of operation. One method of increasing performance is to flow the blood in thin films (e.g. in membrane capillaries) thereby decreasing the effective diffusion distance. Technical difficulties apart, clogging of the flow passages and high pressure loss may occur in these systems.

A more fruitful approach is to create convective mixing, distributing the hyperoxic layers adjacent to the membrane to regions of oxygen-poor blood. Devices in which this is carried out can be classified as passive or active types. In passive units the energy of the blood flow coupled with flow path geometry is used to generate mixing. Examples are eddy mixing with surface perturbations, screens and secondary flows in coiled tubes (Mockros *et al.*, 1971). In active systems secondary flows and shear thinning of the boundary layer are achieved by external energy, e.g. oscillation of curved tubes (Drinker *et al.*, 1969), rotating discs (Hill *et al.*, 1974), flow pulsation across grooved walls (Bellhouse *et al.*, 1973) and rotation of concentric cylinders (Gaylor *et al.*, 1973). Generally the active devices

possess stronger mixing and higher efficiency than the passive devices but are more complex. In this paper the development by the authors of membrane oxygenators with convective mixing is reported. The systems that have been investigated are now listed.

*Passive type*
Generation of Dean vortices in a curved rectangular channel with tangential pressure driven flow.

*Active types*
(1) Torsional oscillation of curved rectangular channel.
(2) Pulsed membrane tubes producing boundary layer breakup and integral pumping.
(3) Formation of Taylor vortices in annulus of coaxial cylinder arrangement with inner rotating cylinder.

The principles of operation, design and oxygenation performance of experimental units for all these systems apart from the Taylor-vortex type are presented in the following section. The Taylor-vortex oxygenator is considered separately by Smeby (see p. 70).

## 5.2 Experimental Membrane Oxygenators

*Curved channel oxygenator with passive induced Dean vortices*
Analogous to flow in a coiled tube, an imbalance of centrifugal forces in the radial direction occurs for tangential fluid flow in a curved rectangular channel (Dean, 1928; Reid, 1958). A series of asymmetric counter-rotating vortices are formed in the axial direction (figure 5.1) if the flow rate exceeds a value determined by the critical Dean number, $Dn$. If the inner radius of curvature, $R_i$, is much greater than the channel gap, $d$, theory predicts vortex formation for

$$Dn = Re\,(d/R_i)^{\frac{1}{2}} \geqslant 36$$

where $Re$ = Reynolds number, $\bar{U}d/\nu$, $\bar{U}$ = mean tangential fluid velocity and $\nu$ = kinematic viscosity. Chang *et al.* (1971) obtained numerical solutions to the convective diffusion equations describing transfer in a curved channel membrane oxygenator. The results indicated that $O_2$ transfer with vortices was three orders of magnitude greater than that for laminar flow alone. An experimental assessment of this theory was carried out (Gilroy, 1976) and is summarised below.

Vortex formation was studied in acrylic channels of similar dimensions to the mass transfer device using aqueous suspensions of aluminium flakes. A typical set of vortices is shown in figure 5.2 for $R_i$ = 69.8 mm, $d$ = 3.18 mm, width = 100 mm and $Dn$ = 44. Vortex motion was established at $Dn \simeq 36$ as predicted. Entry effects varied with inlet manifold design but stability occurred after 90° of travel. Alteration of suspension viscosity to that approximating normal human blood did not affect the critical Dean number.

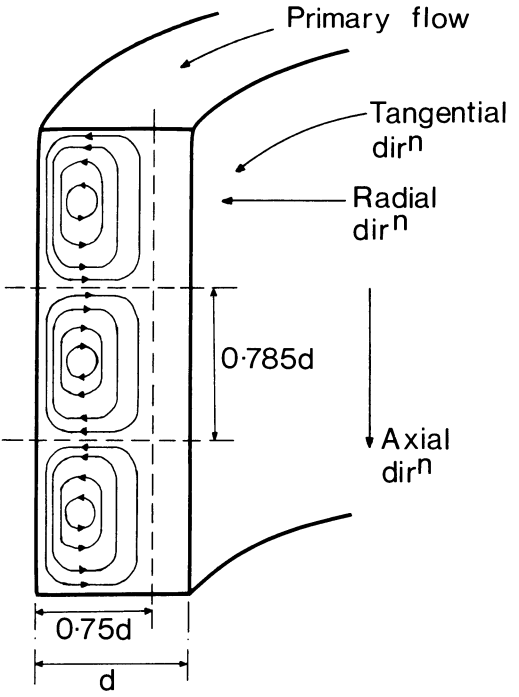


Figure 5.1 Vortex formation in curved channel

A layout of the model oxygenator is shown in figure 5.3, the membrane (microporous polypropylene, 0.025 mm thick, Celgard 2400, Celanese Plastics Co.) being supported by resin-strengthened open pore foam. Ventilating gas (100% $O_2$) was ducted through the support via an inlet manifold and holes in the cylinder flanges. Dimensions are $R_i$ = 66.5 mm, $d$ = 2.6 mm, width = 57 mm and effective length = 400 mm.

Tap water at room temperature was fed to the oxygenator and discharged to drain at outlet. Changes in $pO_2$ were measured across the oxygenator as a function of flow rate. For $O_2$ transfer to blood, the oxygenator was operated in closed loop mode with a deoxygenator and blood reservoir in series. By varying the $O_2$, $N_2$ and $CO_2$ gas mixture to the deoxygenator a range of inlet conditions could be obtained. Heparinised, fresh, bovine blood with haemoglobin concentration of 15.5 ± 0.3 g% and at 37°C was used.

No augmentation of the magnitude predicted was found for either $O_2$-water or $O_2$-blood transfer at onset of vortex formation or at superciritical *Dn*. To reduce errors due to entry or exit effects an oxygenator of similar $R_i$, $d$ and width as above but with a 2 m channel length (achieved by helical winding of membrane) was tested. For this unit, as shown in figure 5.4, the $O_2$ concentration increase versus *Dn* follows closely the prediction for laminar flow alone. The

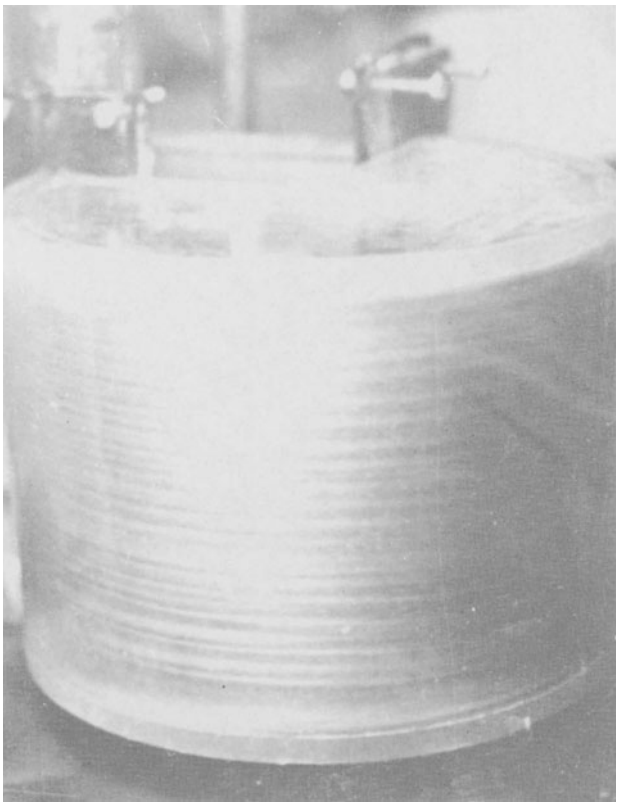

Figure 5.2 Visualisation of vortices for $Dn$ = 44, $Re$ = 207, $d$ = 3.18 mm, $R_i$ = 69.8 mm

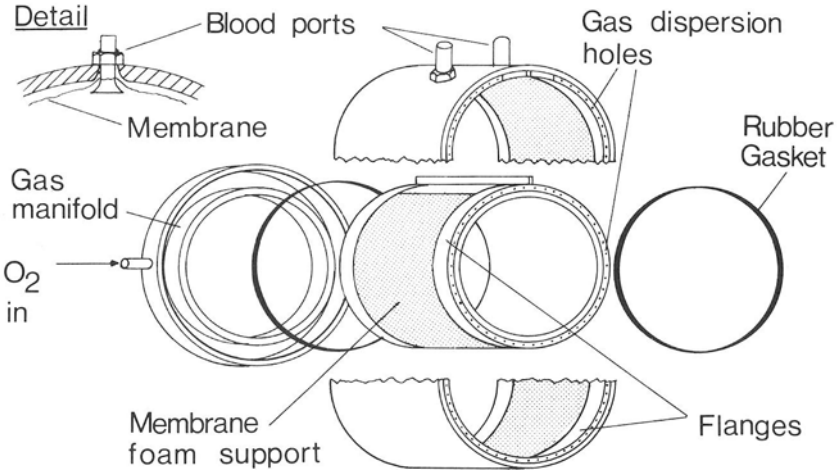


Figure 5.3 Exploded view of curved channel oxygenator

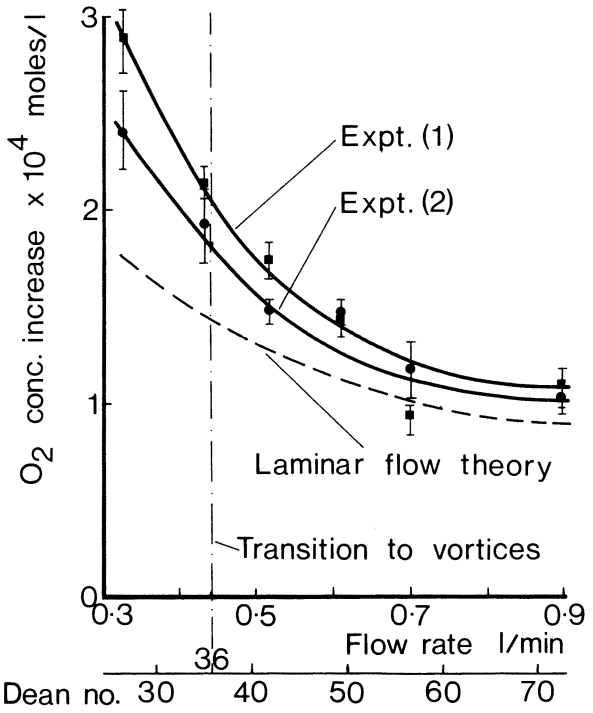


Figure 5.4 $O_2$ concentration increase versus water flow rate

failure of the system to improve efficiency is attributed to overestimation in the theoretical analysis of the secondary flow amplitude which greatly influences the transfer rate. A nonlinear stability analysis of the flow performed by Brighton (Gilroy *et al.*, 1977) provided an amplitude factor about one tenth that used by Chang and Mockros (1971).

*Torsionally oscillated curved channel oxygenator*

Drinker *et al.* (1969) have shown that the vortices formed by flow in a static coiled tube are replaced by similar vortices but with reversed motion when the tube is torsionally oscillated about its axis. These investigators have utilised the stronger nature of the oscillatory-induced vortices to develop a highly efficient membrane oxygenator. It seemed reasonable to expect that a similar phenomenon would occur for torsional oscillation of the curved channel system described above and preliminary studies (Gilroy, 1976) are now presented.

The oxygenator tested was similar in construction to that in figure 5.3 but with dimensions $R_i$ = 130 mm, $d$ = 4 mm, width = 30 mm and effective area = 0.044 $m^2$. The oxygenator assembly was mounted axially on a platen which could be oscillated through an arc of 40° at frequencies of 0 - 400 oscillations/min (1 oscillation ≡ 80° total angular travel).

An *in vitro* blood circuit similar to that described above was employed with blood inlet conditions: haemoglobin = 13.8 – 14.6 g%; haematocrit = 35 - 40% and $O_2$ saturation = 24 - 70%. Oxygenator inlet and outlet samples were measured for $O_2$ saturation, pH and $pO_2$ for various combinations of flow rate and oscillation frequency.

An empirical correlation was sought between the dimensionless groups characterising the system. This approach is suitable when, as in this case, a rigorous mass transfer theory is not available and can also provide a basis for optimisation of the system. For the oscillating oxygenator the dimensionless groups are:

$$\text{overall Sherwood number, } Sh = hd/D$$
$$\text{oscillatory Dean number, } Dn_o = (d/R_i^{\frac{1}{2}})(f\phi R_i d/\nu)$$
$$\text{Reynolds number, } Re = \bar{U}d/\nu$$
$$\text{Schmidt number, } Sc = \nu/D$$

where $f$ = frequency of oscillation, $\phi$ = angular displacement/oscillation, $D$ = effective $O_2$ diffusivity in whole blood. The overall mass transfer coefficient, $h$, is based on the log mean total $O_2$ concentration driving force as defined by Smeby and Grimsrud (1974).

The $O_2$ transfer was found to be strongly dependent on the oscillation rate (Dean number) but was essentially independent of $Re$ over the range tested ($18 < Re < 140$). Thus, for the small variation in haematocrit, $Sc$ can be considered constant ($2750 < Sc < 3100$) and the experimental data could be represented by the following equation:

$$Sh = 0.00155 Dn_o^{2.19} \quad 54 < Dn_o < 227$$

The correlation is applicable for rated flow blood conditions (Galletti *et al.*, 1972) and for 'rated' $O_2$ transfer the blood flow rate for the model oxygenator is 3.21 l/min/m$^2$. Optimisation of the design on the basis of the empirical correlation is discussed on p. 59.

*Capillary membrane oxygenator with integral pump*

The performance of capillary membrane oxygenators can be increased by about 40% by incorporating a blood mixing stage which disrupts the hyperoxic boundary layer (Mockros and Weissman, 1971). Because a mixing chamber in the blood path increases the complexity of fabrication and provides additional sites for thrombus formation other designs have used high pressure oxygen to pulse the capillaries augmenting transfer and achieving a pumping effect. However the risk of gas embolism is introduced. To avoid these drawbacks the device reported here possesses a hydraulic pump chamber which is constructed around a section of the capillary bundle during the fabrication process.

The construction method is reported in detail elsewhere (Ton, 1975). The oxygenator (figure 5.5) consists of parallel-connected thin-wall silicone rubber tubing. In addition to the end tube sheets and associated blood chambers a

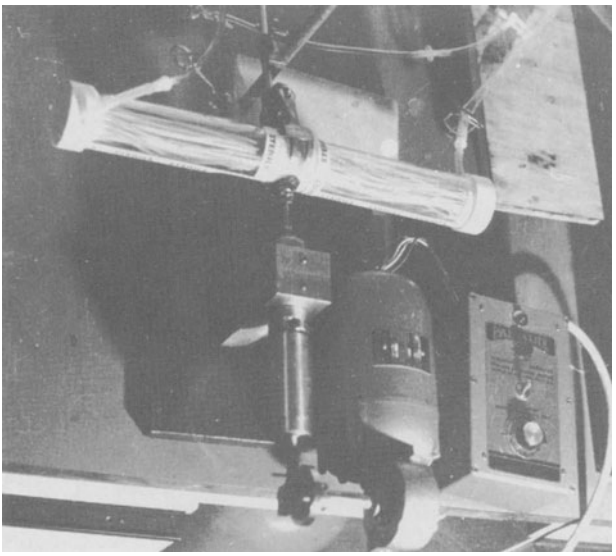

Figure 5.5 Capillary pump/oxygenator with hydraulic pump circuit

further two tube sheets are located midway along the capillary length. An acrylic jacket encloses the tube sheets forming two gas chambers and a central pump chamber connected to a reciprocating piston. The pump circuit is saline filled. Oxygen is introduced to the tube bundle via an axial manifold and is vented at the periphery of the jacket. Unidirectional blood flow is attained by means of one way valves in the blood circuit. Oxygenator dimensions are given in table 5.1.

Transfer rates were obtained for no-pumping and pumping modes. A single pass, gravity fed, blood circuit was employed with average inlet conditions: haemoglobin = 14.7 g %, haematocrit = 38% and $O_2$ saturation = 30%.

The $O_2$ transfer rate as a function of blood flow rate is shown in table 5.2 for both operational modes. It is evident that pumping enhances transfer. From

**Table 5.1**

Specifications for experimental capillary pump/oxygenator

| | |
|---|---|
| Capillary internal diameter | 1.016 mm |
| Capillary external diameter | 1.168 mm |
| Number of capillaries | 220 |
| Active capillary length | 320 mm |
| Capillary pump length | 20 mm |
| Total capillary length including tube sheets | 380 mm |
| Priming volume | 67 ml |
| Effective transfer area | 0.22 $m^2$ |

Table 5.2

$O_2$ transfer rates with and without pumping for capillary pump/oxygenator or versus blood flow rate. (37 °C; haemoglobin = 14.7g%; inlet $O_2$ saturation ≃ 30%)

| *No pumping* | | *Pumping* | |
|---|---|---|---|
| *Blood flow rate ml/min* | *$O_2$ transfer rate ml $O_2$/min* | *Blood flow rate ml/min* | *$O_2$ transfer rate ml $O_2$/min* |
| 68 | 4.04 | 68 | 7.72 |
| 80 | 4.6 | 77 | 7.11 |
| 114 | 5.22 | 88 | 6.71 |
| 128 | 6.71 | 118 | 12.25 |
| 150 | 7.10 | | |

other data the 'rated' blood flow with pumping was found to be 0.69 l/min/m$^2$ which compares favourably with 0.64 l/min/m$^2$ as predicted for an ideal mixing stage and with 0.45 l/min/m$^2$ for no mixing.

## 5.3 Discussion

Ideally, a membrane lung should possess high gas transfer efficiency, low priming volume, low pressure loss and minimal blood trauma, and should be easy to operate. Rarely are such properties found in a single device and in practice a compromise must be struck by trading off one parameter against another. Thus it is usual to find a unit with a low priming volume and high pressure loss or vice versa. In table 5.3 rated flow rate, priming volume and pressure loss are given for several commercial membrane oxygenators and for the oscillating channel and capillary pump/oxygenator systems described here. Also a projected design for the oscillating channel is included.

Whilst the rated flow of the experimental oscillating channel compares favourably with the Dow, Landé-Edwards and Travenol units, a modest increase in gap and inner radius increases the performance considerably as shown by the projected design value. The performance is similar to that of the oscillating torus of Bartlett *et al.* (1972) with a lower pressure loss but higher priming volume.

The capillary pump/oxygenator has a similar rated flow to the capillary Dow unit which does not possess a mixing stage. This is due to the smaller diameter (190 μm) of capillaries in the Dow oxygenator compared to that (1016 μm) in the experimental unit. Mockros and Weissman (1971) have shown that $O_2$ transfer is independent of tube radius for a given saturation increase, tube length and flow rate. Thus if the rated flow is expressed per unit area then the smaller diameter tubes will be more efficient. By adopting a tube diameter similar to that of the Dow device the rated flow of the pump/oxygenator will be about 40% greater than the Dow. However, the frequency of pumping to achieve a given flow rate would have to be increased to compensate for the reduced stroke volume of the smaller capillaries.

**Table 5.3**

Comparison between experimental and commercial membrane oxygenators

| *Oxygenator* | *Principle* | *Area* $m^2$ | *Rated flow / Area* $l/min/m^2$ | *Priming volume / Rated flow* $l/l/min$ | *Pressure loss at rated flow* mm Hg | *Comments* |
|---|---|---|---|---|---|---|
| Tecna/Searle | Rotating disc | 0.65 | 9.2 | 0.117 | pump action | 200 rpm |
| Drinker | Oscillating torus | 0.43 | 5.8 | 0.069 | 350 | 400 oscill/min |
| Authors' projected | Oscillating channel | 0.43 | 5.8 | 0.362 | 34.8 | 400 oscill/min $d = 4.2$ mm, $R_i = 200$ mm |
| Authors' experimental | Oscillating channel | 0.044 | 3.21 | 0.58 | 4.0 | 400 oscill/min $d = 4.0$ mm, $R_i = 130$ mm |
| Travenol-Teflo | Parallel plate | 2.25 | 2.67 | 0.067 | 120 | |
| Sci-Med | Spiral coil | 2.5 | 1.0 | 0.135 | 160 | |
| Authors' Experimental | Capillary-pump | 0.22 | 0.69 | 0.44 | integral pump | 1016 $\mu$m i.d. capillaries |
| Dow | Capillary | 7.0 | 0.66 | 0.113 | 133 | 190 $\mu$m i.d. capillaries |

Of the four oxygenator systems described in the introduction the most promising would appear to be the oscillating channel, notwithstanding the difficulties in design of an oscillatory drive for an adult capacity unit. For the projected design in table 5.3 the required membrane area for 5 l/min rated flow could be attained with two concentric channels of width 17 cm and mean radii of 19 cm and 21 cm. As the membrane, support and clamping structure are located at the periphery the oxygenator mass will be less than that of the equivalent oscillatory torus design which requires rigid support plates 61 cm in diameter. Continuing studies of the oscillating channel oxygenator include flow visualisation of vortex formation, *in vitro* haemolysis, $CO_2$ exchange and effect of various gap/radius ratios on performance. The information gained from these studies will facilitate the design of a practical oxygenator system.

## 5.4 Acknowledgement

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## 5.5 References

Bartlett, R. H., Drinker, P. A., Burns, N. E., Fong, S. W. and Hyans, T. (1972). The toroidal membrane oxygenator: Design, performance and prolonged bypass testing of a clinical model. *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 369.

Bellhouse, B. J., Bellhouse, F. H., Curl, C. M., MacMillan, T. I., Gunning, A. J., Spratt, E. H., MacMurray, S. B. and Nelems, J. M. (1973). A high efficiency membrane oxygenator and pulsatile pumping system, and its application to animal trials. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 72.

Chang, H. K. and Mockros, L. F. (1971). Convective dispersion of blood gases in curved channel exchangers. *Amer. Inst. Chem. Eng. J.*, **17**, 541.

Dean, W. R. (1928). Fluid motion in a curved channel. *Proc. Roy. Soc. (London), A*, **121**, 402.

Drinker, P. A., Bartlett, R. H., Bialer, R. M. and Noyes, B. S. (1969). Augmentation of membrane gas transfer by induced secondary flows. *Surgery*, **66**, 775.

Galletti, P. M., Richardson, P. D., Snider, M. T. and Friedman, L. I. (1972). A standardised method for defining the overall gas transfer performance of artificial lungs. *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 359.

Gaylor, J. D. S., Murphy, J. F., Caprini, J. A., Zuckerman, L. and Mockros, L. F. (1973). Gas transfer and thrombogenesis in an annular membrane oxygenator with active blood mixing. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 516.

Gilroy, K. (1976). Secondary flow augmented mass transfer in annular membrane oxygenators. Ph.D. Thesis, University of Strathclyde, Glasgow.

Gilroy, K., Brighton, E. and Gaylor, J. D. S. (1977). The influence of fluid vortices on mass transfer in a curved channel artificial membrane lung. *Amer. Inst. Chem. Eng. J.*, **23**, 106.

Hill, J. D., Iatridis, A., O'Keefe, R. and Kitrilakis, S. (1974). Technique for achieving high gas exchange rates in membrane oxygenation. *Trans. Amer, Soc. Artif. Int. Organs,* **20**, 249.

Mockros, L. F. and Weissman, M. H. (1971). The artificial lung, in J. H. U. Brown, J. E. Jacobs, and L. Stark, (Eds.), *Biomedical Engineering,* Davis Company, California, p. 325.Reid, W. H. (1958). On the stability of viscous flow in a curved channel. *Proc. Roy. Soc. (London), A* **244**, 186.

Smeby, L. C. and Grimsrud, L. (1974). Theoretical investigation of mass transfer in membrane oxygenators. *Med. Biol. Engng.*, **12**, 698.

Ton, H. Y. (1975). Design of a tubular membrane oxygenator with integral pumping. M.Sc. Thesis, University of Strathclyde, Glasgow.

## 5.6 Notation

$d$ = radial gap in curved channel
$D$ = effective $O_2$ diffusivity in whole blood
$Dn$ = Dean number for static curved channel $[= Re\,(d/R_i)^{1/2}]$
$Dn_o$ = oscillatory Dean number $[= (d/R_i)^{1/2}\,(f\phi R_i d/\nu)]$
$f$ = frequency of oscillations
$h$ = overall mass transfer coefficient
$Re$ = Reynolds number $[= \bar{U}d/\nu]$
$R_i$ = inner radius of curved channel
$Sc$ = Schmidt number $[= \nu/D]$
$Sh$ = Sherwood number $[= hd/D]$
$\bar{U}$ = mean tangential fluid velocity
$\nu$ = kinematic viscosity
$\phi$ = total angular displacement/oscillation

# 6

# A HIGH-PERFORMANCE MEMBRANE LUNG

B. J. BELLHOUSE

**Summary**

A method of improving gas transfer in membrane lungs is described. Blood is pumped back and forth across furrowed membranes forming the blood channels, and vortices are formed in the hollows. These vortices mix the blood, cause negligible blood trauma and enhance gas transfer. When compared with capillary-type membrane lungs, oxygen transfer is improved by a factor of about five when silicone membranes are used, but when microporous membranes are used performance can increase by a factor of 15.

Animal experiments, in which awake, newborn lambs were perfused for periods up to 48 h, using veno-venous bypass, are reported. The merits of various types of solid and microporous membranes are discussed.

## 6.1 Introduction

Simple and efficient bubble oxygenators are widely used in open heart surgery, but they cause significant blood trauma if perfusions last for more than a few hours. When a thin, permeable membrane is used to separate blood from gaseous oxygen, blood trauma can be reduced greatly, but the early designs of membrane oxygenator were bulky and inefficient. These oxygenators had long, narrow blood passages, and since oxygen diffuses only slowly in blood, boundary layers formed on the membranes and limited oxygen transfer.

Efficiency can be improved by having very narrow blood channels, so that diffusion paths are short. However, this requires a large number of parallel paths, if hydraulic resistance is to be kept acceptably small, and uneven perfusion and subsequent capillary occlusion are frequent complications of these designs.

An alternative method of enhancing oxygen transfer is to use wide blood channels ($\sim$ 1 mm) and to stir the blood, transporting oxygen-poor red blood cells from the centre of the channel to the membrane and displacing oxygen-rich cells from the membrane surface into the mainstream. The first practical designs which accomplished this were reported by Drinker *et al.* (1969) and Melrose *et al.* (1972). Although construction of these oxygenators differed, they worked in a similar way. The blood channels took the form of concentric circles which were oscillated in their own plane about an axis through the circles' centre. The primary flow was directed along the centre lines of the channels, and channel curvature and oscilla-

tory motion of the channel walls induced centrifugal forces in the viscous boundary layers at the membrane surface. These forces produced twin, contra-rotating, helical vortices superimposed on the primary flow. Both these designs were effective in transferring oxygen and caused as little blood trauma as steady-flow membrane oxygenators, although they required fairly cumbersome machinery to drive them.

Another practical secondary-flow membrane lung was reported by Hill *et al.* (1974). The device consisted of a thin rotor, with microporous membranes on both circular faces, and two stators, each clad with a single membrane. They were assembled to form two blood channels, one on each side of the rotor. The rotor was driven at constant speed, which generated high shear rates at the membrane surface and induced fluid mixing. The mixing mechanism has not been described in detail, but is probably associated with transition from laminar to turbulent boundary layers in the outer part of the annulus (Kitrilakis, 1975).

Taylor vortices have also been used to mix the blood in a membrane lung (Gaylor *et al.*, 1973).

Instead of moving the membrane surface to induce high shear rates and secondary flows, it is possible to achieve the same effect by pulsating blood back and forth over a furrowed membrane surface. In both forward and reversed flow, vortices are formed in the hollows of the blood channels; the vortices have sufficient strength to scour the membrane surface and to mix the blood within the hollows. In accelerating or decelerating flow considerable mass exchange occurs between the mainstream and fluid within the hollows, completing the mixing process. The Oxford membrane lung utilises these principles.

### 6.2 Fluid Mechanics of the Furrowed Membrane Lung

The dimensions of the furrowed blood channels were chosen on the basis of flow visualisation experiments using water flow between a pair of 'Perspex' plates. The plates had furrows machined in them, with their axes perpendicular to the direction of blood flow. The furrows were 2 mm from ridge to ridge (chord) and had cross sections as arcs of circles. The furrows on opposing sides of the plates were aligned in the first experiments, and the effect of misalignment was studied in subsequent experiments.

The channel was perfused with steady flow and dye was made to seep into the bottom of one furrow so that flow patterns could be made visible. There was negligible fluid exchange between the hollow and the mainstream unless the flow became unstable and this occurred only at unrealistically high rates of perfusion. The flow patterns within the hollows consisted of slowly rotating, closed streamline vortices, generated by the shearing action of the mainstream on the fluid trapped within the hollows.

When the channel was perfused with reversing, pulsatile flow, the flow patterns were altered radically. As the flow accelerated (figure 6.1a) the mainstream expanded into the hollows, displacing fluid from within them. As peak velocity was approached (figure 6.1b), strong vortices formed in all the hollows, of strength considerably greater than could be attained at comparable steady flow rates. When

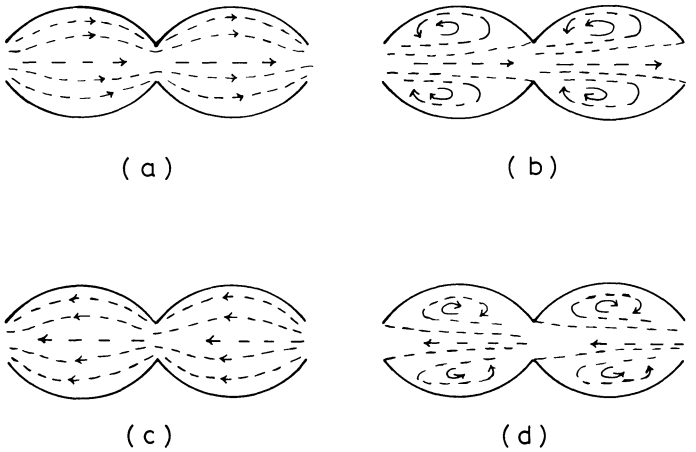



Figure 6.1 Flow patterns in a furrowed blood channel in pulsatile flow. (a) Flow accelerating from left to right, with mainstream fluid displacing fluid in the hollows. (b) Peak forward flow, with vortices formed in the hollows. (c) Flow accelerating from right to left, similar to (a). (d) Peak reverse flow, with vortices formed in the hollows, but in the opposite direction to the vortices in (b)

the flow reversed, fluid was again ejected from the hollows (figure 6.1c), then another set of vortices was established in the hollows (figure 6.1d), of opposite direction to the first set.

High-speed ciné film was taken of the flow patterns in the model furrowed channel, and was analysed frame by frame to determine the best channel configuration and operating conditions for a prototype membrane lung. The high efficiency of the resulting oxygenators encouraged a more careful study of the underlying fluid mechanics.

The second rigid-walled Perspex model of a blood channel in the membrane lung is shown in figure 6.2a. The channel was 140 mm long and 100 mm wide; it had furrows of 4 mm chord and 1 mm depth machined in both walls of the channel. Mean flow through the channel could be varied independently of pulsatile flow superimposed on it. In addition to steady flow, produced with a constant-head inlet tank, two other flows were investigated. They were: quasi-steady flow produced by a roller pump, and interrupted flow, generated by a solenoid-operated valve in the inlet pipe to the channel.

Dye was introduced into a single hollow of the model channel (figure 6.2a) and the time for the dye to be washed out of the hollow, under the various flow conditions, was recorded.

In steady flow, vortices with closed streamlines formed in the hollows, and dye cleared from them only very slowly, by diffusion. When fluid velocities within the channel reached about 25 cm/s, fluid from the mainstream would enter the hollows intermittently, the vortices became stronger and dye cleared from the hollows

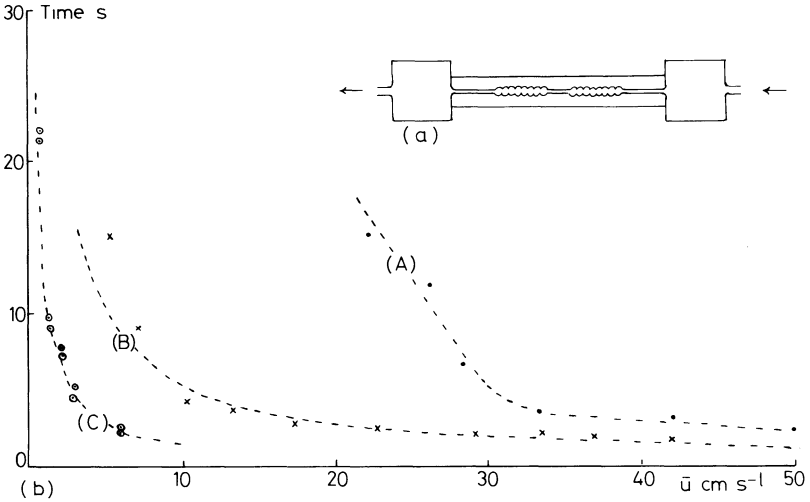



Figure 6.2 Dye clearance times from a single hollow in (A) steady flow, (B) roller pump flow and (C) interrupted flow. Mean velocity, $\bar{u}$, is plotted along the abscissa, and clearance time along the ordinate

more rapidly. As the mainstream velocity increased, dye clearance times became shorter. This is shown by curve (A) in figure 6.2b, where mainstream velocity is plotted along the abscissa and dye clearance time along the ordinate. Channel gap was varied with shims and it was clear that mainstream velocity, and not mainstream flow, defined the transition point between diffusive and convective mixing in steady flow. The transition Reynolds number, $(\bar{u}c)/\nu$, where $\bar{u}$ is mainstream velocity, $c$ hollow chord and $\nu$ kinematic viscosity was about 1000 in steady flow.

To investigate the effect of low-amplitude oscillations, the header tank was replaced with a roller pump. The roller pump produced regular pulsations in velocity, at a frequency double the rate of rotation of the roller shaft, since there were two rollers. The effect of the pulsations was to replace closed-streamline vortices with vortices fed convectively from the mainstream, and to produce substantial reductions in dye clearance times (curve (B) in figure 6.2b). The transition from diffusive to convective mixing for roller pump flow was about 240.

To increase the amplitude of the velocity fluctuations, a solenoid-operated on/off valve was introduced into the water circuit between the roller pump and the furrowed channel. The valve abruptly halted or restarted the flow, and dye clearance times were again reduced substantially (curve (C) in figure 6.2b). (In this experiment the valve was operated with equal on and off times at frequencies of 30/min and 50/min). Convective mixing was evident above Reynolds numbers of about 100.

These simple experiments were useful in quantifying mixing efficiency for a range of hollow dimensions and flow parameters, but were not easily adapted to large-amplitude reversing flow. They also indicated the need for detailed measure-

ments of particle paths, both inside the furrows and in the mainstream, under controlled unsteady-flow conditions.

A water tunnel was built with provision for uniform acceleration or deceleration between chosen mainstream velocities. Instead of attempting to make detailed measurements in very small hollows (2 mm chord in the oxygenator), hollows of 15 mm chord, and semicircular in cross section, were used.

The hollow was cut in a flat plate which spanned the unsteady-flow water tunnel, 5.7 cm from the leading edge. The tunnel had a working section of cross section 9.5 cm by 15.2 cm and was 71.0 cm long. Polystyrene particles were suspended in water and introduced into the test furrow; they were illuminated by a narrow, parallel beam of light obtained from a mercury vapour lamp. Particle paths were photographed using both still and ciné cameras (Bellhouse *et al.*, 1975).

The sequence of events in accelerating flow from rest to 7.5 cm/s in 600 ms was as follows. Initially the streak lines followed the contours of the hollow, and particles were displaced from the hollow by the mainstream. Then the flow separated from the upstream lip to form a starting vortex which grew in size until it occupied all the furrow; about half the particles originally in the furrow were ejected. Vortex strength at the end of the acceleration phase was more than double the vortex strength in equivalent steady flow.

When the flow was decelerated to rest in the same time the pattern was different. Clear fluid from the mainstream entered the hollow at the downstream lip and displaced fluid from the hollow at the upstream lip. Half way through the deceleration phase the region of clear fluid in the furrow had grown, the vortex centre had moved up into the mainstream and velocities in the hollow matched those in the mainstream. At the end of the deceleration phase, all the particles originally in the hollow had been ejected.

In steady flows, where diffusive and convective processes are in balance, Reynolds numbers are valuable parameters since they scale geometrically similar flows. In unsteady flows the balance is upset by inertial terms, and the inviscid frequency parameter, $(c)/\bar{u}\tau$, where $c$ is hollow chord, $\bar{u}$ mainstream peak velocity and $\tau$ a characteristic time, is of prime importance. The parameter may be seen as the ratio of hollow chord to the distance traversed by fluid in the mainstream of each pulse. For vortices to form convectively in the furrows, this parameter must be less than unity.

### 6.3 Design of the Membrane Lung

As a result of the flow visualisation experiments, the furrows in the blood channels of the membrane lung were chosen to be of wavelength 2 mm, depth 0.5 mm and the channel spacing was chosen to be 0.4 mm. This geometry combined good fluid mixing with low hydraulic resistance. Several single-channel prototypes were built and tested before a three-channel oxygenator was built, with blood channels 20 cm long, 11.5 cm wide and membrane area of 0. 125 $m^2$. The membranes were silicone rubber about 50 $\mu$m thick, and were mounted on folded stainless-steel supports, stiffened with stainless-steel strips glued at each edge.

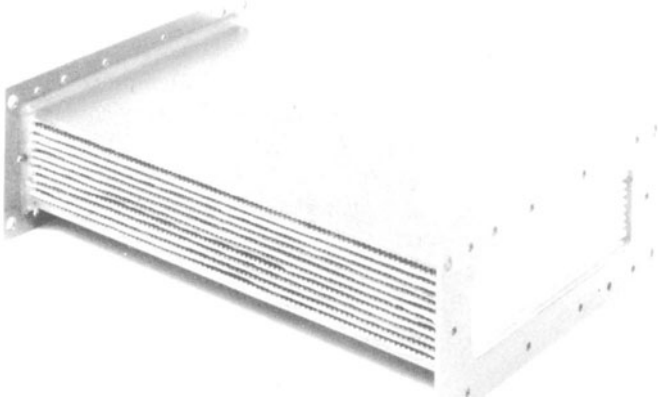


Figure 6.3 Photograph of a nine-channel membrane lung, of length 20 cm and surface area 0.382 $m^2$

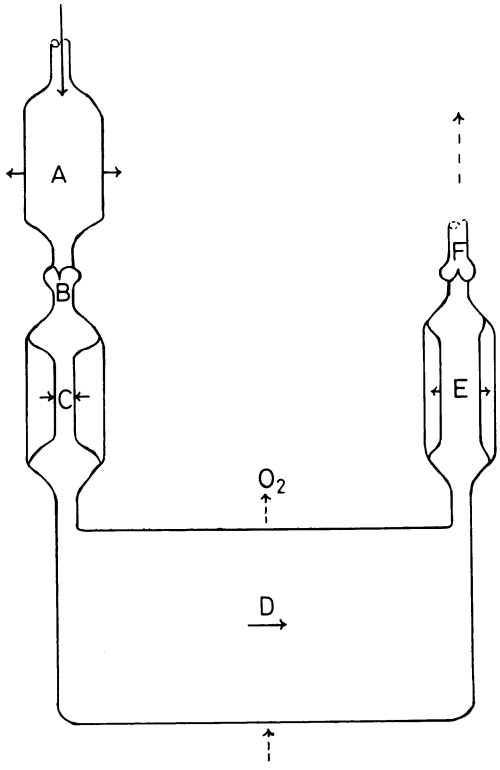



Figure 6.4 Diagram of the membrane lung and pulsatile blood pumps

The membranes were glued to the support plates and then assembled into a stack. A photograph of a nine-channel oxygenator, of surface area 0.382 $m^2$, is shown in figure 6.3. Oxygen paths are between the corrugations in the membrane support plates, in the direction of the furrows, and perpendicular to blood flow.

The pumping system was designed for veno-venous bypass in newborn lambs. Venous blood from the lamb entered a flexible atrium of volume 30 ml (A in figure 6.4), passed through a trileaflet nonreturn valve (B) into the inlet pump (C). The inlet pump was coupled to the three-channel membrane oxygenator (D), through which blood was pumped to the outlet pump (E), through a second trileaflet valve (F) back into the venous pool of the lamb. The pulsatile pumps were operated in anti-phase at a pulse rate of about 100/min, and each had a maximum stroke volume of 18 ml. The pumps consisted of flexible sacs, made of knitted polyester fabric coated with silicone rubber, and supported along three generators of a rigid, airtight, outer case. They were operated by filling the region between the flexible sac and the outer case with compressed air in systole, and venting to the atmosphere in diastole. The design of the pumps and valves ensured laminar blood flow. The prime volume of the system, including a heat exchanger and cannulae, was 350 ml.

A nine-channel membrane lung (figure 6.3), with 0.382 $m^2$ membrane area, was built and operated in similar manner, although the stroke volumes of the pulsatile pumps were increased to 36 ml, and the prime volume of the system was increased to about 600 ml.

### 6.4 Prolonged Perfusions of Newborn Lambs

Two series of perfusions in newborn lambs were undertaken. In the first series (Bellhouse *et al.*, 1973) three-channel membrane lungs, of 0.125 $m^2$ surface area, were used to partially support six newborn lambs, ranging in age from one to ten days and in weight from 3 - 5.5 kg. The lambs were cannulated under local anaesthetic using the technique of Kolobow *et al.* (1969), and were put on bypass immediately after cannulation. The lambs were housed in a Perspex box, were awake, and free to move. Blood samples were drawn at hourly intervals from the lambs' venous and arterial lines, and from both sides of the oxygenator. These samples were used to measure pH, $pO_2$, $pCO_2$, oxygen contents, plasma haemoglobin, haematocrit, platelet number and function, and other biochemical parameters.

Four of the six lambs survived 44, 48, 41 and 30 h of bypass. Two animals died on bypass after 21 h (sepsis) and 42 h (cerebral haemorrhage). In all the experiments oxygen and carbon dioxide transfer rates were high (up to 160 ml/min/$m^2$) and haemolysis low (plasma haemoglobin $< 25$ mg %), but the loss of platelets was sufficient to cause bleeding from the neck incisions in all experiments.

In the second series of experiments greater attention was paid to oxygenator cleaning and sterilisation, and rheomacrodex was used to wet the oxygenator prior to priming it with blood (Nelems *et al.*, 1974).

Four newborn lambs, between two and five days old (3 - 4.5 kg) were perfused

for periods of 29, 42, 42 and 43 h, using veno-venous bypass. All the lambs recovered uneventfully when taken off bypass, there were no bleeding problems and platelet function was preserved, although platelets were reduced in number. The animals' breathing was controlled and periods of total pulmonary support were attained with the larger oxygenator.

Oxygen transfer rates of up to 160 ml/min/$m^2$ were achieved consistently, a figure four or five times as high as that achieved by simple membrane oxygenators using similar membranes. However, oxygen transfer rates were still only 33% of the measured gas-to-gas membrane limit. Maximum carbon dioxide transfer rates were also 160 ml/min/$min^2$, about 74% of the membrane limit.

## 6.5 Discussion

The silicone rubber membranes in our membrane lung distorted during pulsatile blood flow to such an extent that the pulse was negligible over a substantial part of the lung during each forward and backward stroke of the pumps. This could explain why oxygen transfer in the membrane lung was only a third of the membrane limit, despite the excellent mixing produced in rigid-walled models of the oxygenator. There are two obvious ways of improving still further the performance of this membrane lung.

(1) To use a stiffer 'solid' membrane consisting of a stiff backing coated with silicone rubber, or similar material.

(2) To use a microporous, hydrophobic membrane, much stiffer than silicone rubber.

Silicone rubber is more permeable to $O_2$ and $CO_2$ than other solid membranes, and is about six times as permeable to $CO_2$ as to $O_2$. This is a useful characteristic since the partial pressure difference of oxygen, between gas and blood compartments, is about 680 mm Hg (90.6 kN/$m^2$) if atmospheric pressure oxygen is used in the gas channels. The partial pressure difference of $CO_2$ between the blood and gas channels is only about 50 mm Hg (6.67 kN/$m^2$). The filler-free silicone rubbers described by Kolobow *et al.*, (1974) are reported to have excellent blood compatibility, and would be a good coating material. An attractive alternative is a very thin coating of polyalklsulphone on microporous substrates, as reported by Ketteringham *et al.* (1975).

Microporous membranes made from hydrophobic materials have been used with success in membrane lungs. Both polytetrafluorethylene (PTFE) (Murphy *et al.*, 1974) and silicone-coated porous materials (Hill *et al.*, 1974) have been used in this way. Pore sizes were of the order of 1 $\mu$m, and the gas to gas transfer of these membranes was much higher than for solid membranes. Blood is retained within the blood channels by surface tension at the pores, since those membranes are hydrophobic. Microporous membranes permit high water-vapour transfer rates, especially if blood is maintained at 37 °C. Water condenses in the gas channels and can cause progressive deterioration in gas transfer, particularly of carbon dioxide.

Experiments with the Oxford membrane lung, using a microporous membrane, have shown that $O_2$ and $CO_2$ transfer rates of more than 500 ml/min/$m^2$ can be achieved and maintained for prolonged periods. Animal experiments, similar to those described in this paper, but using veno-arterial bypass, are in progress. These experiments should cast light on the controversy over whether or not microporous membrane lungs are true membrane lungs, or just sophisticated micro-bubble oxygenators.

## 6.6 References

Bellhouse, B. J., Bellhouse, F. H., Curl, C. M., MacMillan, T. I., Gunning, A. J., Spratt, E. H., MacMurray, S. B. and Nelems, J. M. (1973). A high efficiency membrane oxygenator and pulsatile pumping system, and its application to animal trials. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 72.

Bellhouse, B. J., Bellhouse, F. H., Snuggs, T. A. and Aggarwal, J. K. (1975). Fluid mechanics of the Oxford membrane oxygenator and its evaluation in animal experiments. *Seminar on Advances in Oxygenator Design,* Copenhagen.

Drinker, P. A., Bartlett, R. H., Bialer, R. M. and Noyes, B. S. (1969). Augmentation of membrane gas transfer by induced secondary flows. *Surgery*, **66**, 775.

Gaylor, J. D. S., Murphy, J. F., Caprini, J. A., Zuckermann, L. and Mockros, L. F. (1973). Gas transfer and thrombogenesis in an annular membrane oxygenator with active blood mixing. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 516.

Hill, J. D., Iatridis, A., O'Keefe, R. and Kitrilakis, S. (1974). Technique for achieving high gas exchange rates in membrane oxygenation. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 249.

Ketteringham, J. M., Zapol, W. B., Birkett, J. D., Nelson, L., Massucco, A. and Raith, C. (1975). A high permeability nonporous blood compatible membrane for membrane lungs: *in vivo* and *in vitro* performance. *Trans. Amer. Soc. Artif. Int. Organs*, **21**, 224.

Kitrilakis, S. (1975). Theory, design and testing of a rotation membrane blood oxygenator. Presented at the *International Conference on Membrane Lung Technology and Prolonged Extracorporeal Perfusion,* Copenhagen.

Kolobow, T., Zapol, W. and Pierce, J. (1969). High survival and minimal damage in lambs exposed to long-term (1 week) veno-venous pumping with a polyurethane chamber roller pump with and without a membrane oxygenator. *Trans. Amer. Soc. Artif. Int. Organs*, **15**, 172.

Kolobow, T., Stool, E. W., Weathersby, P. K., Pierce, J., Hayano, F. and Suandean, J. (1974). Superior blood compatibility of silicone rubber free of silica filler in the membrane lung. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 269.

Melrose, D. G., Burns, N., Singh, M. P., Elliot, R. L., Read, R., Williams, F. E. Becket, J., Lamb, M. P. and Adams, J. S. (1972). Oscillating silicone membrane tubes: a new principle of extracorporeal respiration. *Biomed. Eng.*, **1**, no. 2, 60.

Murphy, W., Trudell, L. A., Friedman, L. I., Karkvan, M., Richardson, P. D., Karlson, K. and Galletti, P. M. (1974). Laboratory and clinical experience with a microporous membrane oxygenator. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 278.

Nelems, J. M. Bellhouse, B. J., Curl, C. M., MacMillan, T. I. and MacMurray, S. B. (1974). Prolonged pulmonary support of newborn lambs with the Oxford membrane oxygenator. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 293.

# 7

# THE TAYLOR-VORTEX MEMBRANE OXYGENATOR

L. C. SMEBY

**Summary**

Experiments on the oxygen transport in a Taylor-vortex membrane oxygenator have shown that it is a highly efficient mass transfer device. A theoretical estimate of the haemolysis rate, based on a mathematical model for flow in a Taylor-vortex system, has shown that high shear stresses will be the main limitation in the design of this oxygenator. Setting an upper limit to the average wall shear stresses, a design analysis indicates that highest possible rotational speed and long cylinders give the best overall performance. Experimental experience suggests that practical difficulties limit rotational speed to 600 - 800 rev/min and the cylinder length to 40 - 60 cm, which results in a mean diameter of 30 - 35 cm and an annular gap of 3 - 5 mm for a full size oxygenator. The clinical acceptance of the Taylor-vortex oxygenator will depend upon whether a possible reduction in haemolysis can outweigh the drawbacks of large priming volume and overall size.

## 7.1 Introduction

A major developmental problem with membrane oxygenators was the construction of a device with sufficiently large oxygen and carbon dioxide exchange. The appearance of new 'artificial lungs' with improved membranes and 'secondary flows' has reduced this problem, but the methods used to increase gas transport could pose new questions about the advantages of membrane over conventional bubble or disc oxygenators. As stated by Peirce (1973), the most significant advantage of membrane compared to nonmembrane devices is the diminution of blood damage.

The main factors regarding blood damage are the residence time ($V/\dot{Q}$), the shear stresses at the blood-membrane interface and the total membrane area (or $\dot{Q}/A$). With increased gas transfer, the 'efficiency' ($\dot{Q}/A$) can be increased, but shear stresses will also increase and this could in some cases limit the feasibility of the oxygenator.

The aim of this chapter is to give a summary of previously reported work with the Taylor-vortex oxygenator (Smeby and Gaylor, 1974; Gaylor and Smeby, 1975), and to examine the flow and blood damage in such a device. In the Taylor-vortex oxygenator, blood flows axially along a membrane lined ($2.54 \times 10^{-2}$ mm thick, microporous polypropylene film, Celgard ®2400, Celanese Plastics Co)

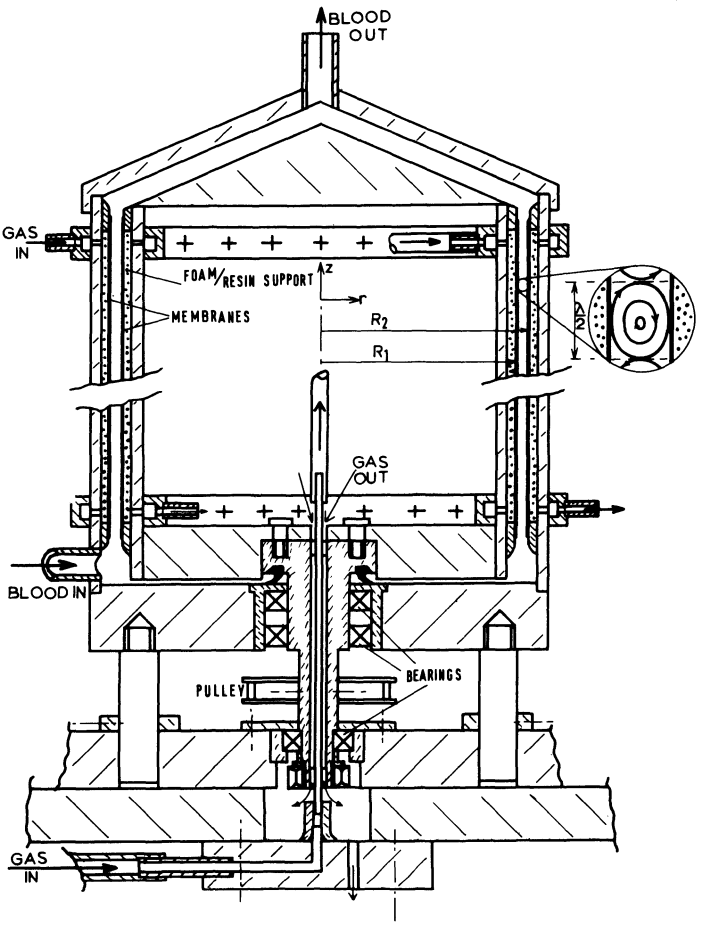



Figure 7.1 Cross-section of a Taylor-vortex membrane oxygenator

annulus formed by two concentric cylinders. When the inner cylinder rotates, toroidal vortices as shown in figure 7.1 can be induced. A cross section of one of the early prototypes is shown in the same figure; more detailed description of the apparatus has been given elsewhere (Smeby and Gaylor, 1974).

## 7.2 Experimental Results

*Mass transfer correlations*

Three different prototype units with membrane areas ranging from 0.2 $m^2$ to 0.3 $m^2$ have been tested *in vitro*. The tests were performed in a closed circuit consisting of 4 - 5 l of fluid which were pumped from a reservoir through the oxygenator and back to the reservoir via a deoxygenator (bubble oxygenator supplied with

a balanced mixture of $CO_2$, $N_2$ and $O_2$). Most of the blood experiments (freshly drawn bovine blood) were performed near 'rated flow' conditions (Galletti *et al.*, 1972) at rotational speeds ranging from 100 rev/min to 900 rev/min. Mean radius ($R_m$) varied from 6.5 cm to 6.7 cm and annular gaps ($d$) of 3 and 6 mm were tested.

Using water as the fluid medium, the experimental correlation between Sherwood number ($Sh_w$) and Taylor number ($T^*$) could be written as

$$Sh_w = 1.4 \times T^{*0.74} \qquad (7.1)$$

$$500 < T^* < 8000$$

The influence of axial Reynolds number ($R_z$) was small and could therefore be neglected from the mass transfer correlation.

Defining mass transfer coefficients for blood with 'total oxygen concentration differences' (Smeby and Grimsrud, 1974), most of the experimental results could be correlated by

$$Sh_B = 0.135 \times T^{*1.1} \qquad (7.2)$$

$$200 < T^* < 3000$$

The difference between equations 7.1 and 7.2 could possibly be explained by the higher Schmidt number for blood, but preliminary experimental results with a sucrose solution ($Sc = 1700$) do not support this theory. Keller (1971) suggested that shear deformation rate induced red blood cell rotation might augment oxygen transport if these rates are as high as 1000 - 2000/s. However, experiments with fully saturated whole blood gave the same correlation coefficient as water ($Sh \equiv T^{*0.74}$). A more plausible explanation could be that some form of 'tank-treading' (Sutera and Mehrjardi, 1975) has a local effect in breaking down boundary layers around the cell wall and thus enhancing the local transfer through the cell wall and inside the red blood cell.

The connection between oxygen transfer to blood and water could be a useful tool in testing production units. Combining equations 7.1 and 7.2 gives

$$\frac{Sh_B}{Sh_w} = 0.0964 \times T^{*0.36} \qquad (7.3)$$

This expression is obviously not valid when $T^* \rightarrow 0$, but could be used with reasonable accuracy for Taylor numbers in the range given by equation 7.2.

*Flow observations*

Several investigators (Coles, 1965; Snyder, 1961; Burkhalter and Koschmieder, 1973) have examined how different flow parameters influence the basic Taylor-vortex motion, and our observations correlate well with these results. However, one feature which (to the author's knowledge) has not been examined previously is the relation between axial pressure drop and rotational speed. Figure 7.2 shows

total axial pressure drop as a function of rotational speed for different flow rates and fluid media. For low flow rates (or low Reynolds numbers) the pressure loss increased monotonically with increased rotational speed, but for higher flow rates the axial pressure drop reached a turning point at a given rotational speed as shown in figure 7.2. This indicated some form of flow transition in the region near the 'turning point', and one possible explanation for this phenomenon could be that the flow pattern changes from 'wavy' to 'axisymmetric' flow when the rotational speed becomes large compared to axial flow.

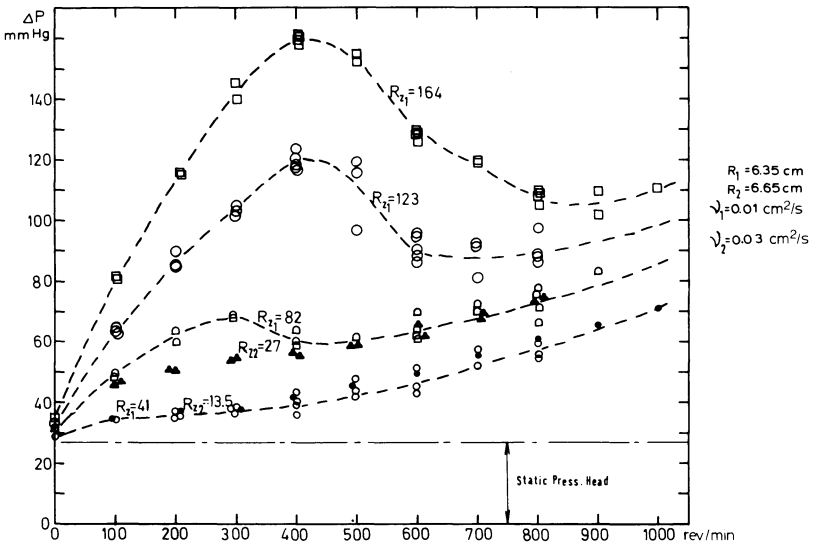



Figure 7.2 Axial pressure drop as a function of angular velocity for different flow rates and fluid media. Open circles refer to water experiments, filled circles refer to blood results. $R_m$ = 6.5 cm, $d$ = 3 mm, $L$ = 27.5 cm

Another remarkable feature about the pressure drop measurements was that at a flow rate of 1 l/min the experimental points for water (open circles) and blood (filled circles) fell along the same line, as shown by the lower curve in figure 7.2. For an axial flow of 2 l/min ($R_{z1}$ (water) $\approx 82, R_{z2}$ (blood) $\approx 27$), the results for blood and water were different up to a rotational speed of 500 rev/min above which they fell roughly along the same curve. If there was some form of 'transition' of the type previously mentioned, these pressure observations could imply that axial momentum transfer in the lower flow regime (and the 'transition' itself) was mainly dependent upon viscous stresses ($\tau_{zr} \equiv \mu(\partial w/\partial r + \partial u/\partial z)$) while pressure losses in the flow regime characterised by high rotational speed (compared to axial flow) were dominated by Reynolds stresses ($\tau_{zr} \equiv -\rho wu$).

This does not necessarily mean that the flow was fully turbulent in this regime, but it indicated that the mechanism by which momentum was transferred was similar to that of turbulent flow.

## 7.3 Theoretical Deductions

*Basic flow equations*

Assuming 'axisymmetric' flow ($\partial\vec{q}/\partial\theta = 0$) and steady state ($\partial/\partial t = 0$), the flow between concentric rotating cylinders can be described by the following equations:

$$u^* \frac{\partial v^*}{\partial r^*} + \frac{a}{\pi}(\bar{w}^* + w^*)\frac{\partial v^*}{\partial z^*} + \frac{v^*}{r^*}\left(\frac{1}{r^*} + u^*\right) = \nabla^2 v^* \qquad (7.4)$$

$$u^* \frac{\partial \xi^*}{\partial r^*} + \frac{a}{\pi}(\bar{w}^* + w^*)\frac{\partial \xi^*}{\partial z^*} + \frac{\xi^*}{r^*}\left(\frac{1}{r^*} - u^*\right) =$$

$$\nabla^2 \xi^* + \left[u^*\left(\frac{\partial^2 - w^*}{\partial r^{*2}} - \frac{1}{r^*}\frac{\partial \bar{w}^*}{\partial r^*}\right) + \frac{2av^*}{\pi r^*}\frac{\partial v^*}{\partial z^*}\right] \qquad (7.5)$$

$$\xi^* = \frac{1}{r^*}\left(\frac{\partial^2 \psi^*}{\partial r^{*2}} - \frac{1}{r^*}\frac{\partial \psi^*}{\partial r^*} + \left(\frac{a}{\pi}\right)^2 \frac{\partial^2 \psi^*}{\partial z^{*2}}\right) \qquad (7.6)$$

where

$$\nabla^2 = \frac{\partial^2}{\partial r^{*2}} + \frac{1}{r^*}\frac{\partial}{\partial r^*} + \left(\frac{a}{\pi}\right)^2 \frac{\partial^2}{\partial z^{*2}}$$

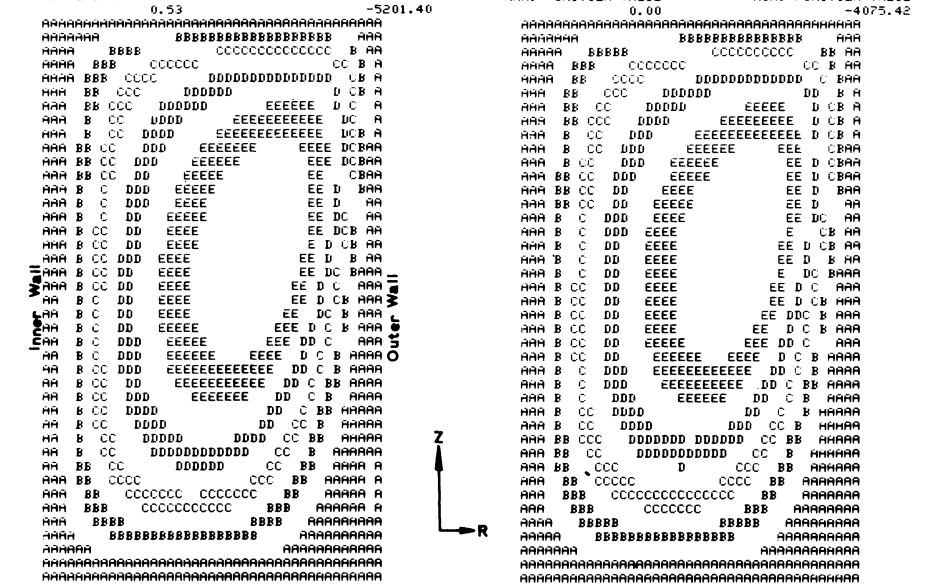
$$u^* = \frac{a}{\pi r^*}\frac{\partial \psi^*}{\partial z^*} \qquad w^* = -\frac{1}{r^*}\frac{\partial \psi^*}{\partial r^*}$$

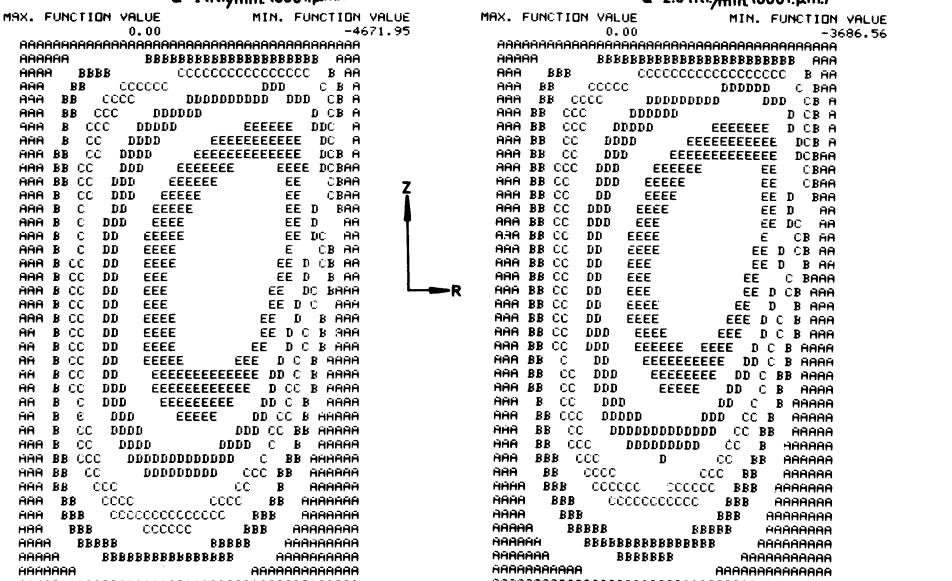
The deduction of the above equations, and the method of solution is described elsewhere (Smeby, 1976). It is sufficient here to mention that the distribution of mean axial velocity ($\bar{w}$) across the gap can cause some problems in the solution of equations 7.4 to 7.6.

Figure 7.3 shows 'contour plots' of the streamlines in a Taylor-vortex oxygenator with a mean radius ($R_m$) of 15.35 cm and a gap ($d$) of 3 mm, at rotational speeds of 600 and 800 rev/min. As seen from the 'minimum function values', the strength of the circulation (clockwise in this figure, which covers only one half wave length) decreased with increasing axial flow and this can be of importance regarding blood damage in a Taylor-vortex oxygenator.

*Shear stresses and blood damage*

From basic fluid dynamics, shearing stresses in a Taylor-vortex system are given by


CONTOUR LINES FOR STREAM FUNCTION
Re=800 r.p.m. Q=0
MAX. FUNCTION VALUE 0.53
MIN. FUNCTION VALUE -5201.40
Inner Wall
Outer Wall
Z
R
CONTOUR LINES FOR STREAM FUNCTION
Re=600 r.p.m. Q=0
MAX. FUNCTION VALUE 0.00
MIN. FUNCTION VALUE -4075.42


CONTOUR LINES FOR STREAM FUNCTION
Q=1 litr/min. (800 r.p.m.)
MAX. FUNCTION VALUE 0.00
MIN. FUNCTION VALUE -4671.95
Z
R
CONTOUR LINES FOR STREAM FUNCTION
Q=2.5 litr/min. (800 r.p.m.)
MAX. FUNCTION VALUE 0.00
MIN. FUNCTION VALUE -3686.56

$$\tau_{r\theta} = \mu\left(\frac{\partial v}{\partial r} - \frac{v}{r} + \frac{1}{r}\frac{\partial u}{\partial \theta}\right) = \mu\dot{\gamma}_{r\theta}$$

$$\tau_{\theta z} = \mu\left(\frac{1}{r}\frac{\partial w}{\partial \theta} + \frac{\partial v}{\partial z}\right) = \mu\dot{\gamma}_{\theta z} \qquad (7.7)$$

$$\tau_{zr} = \mu\left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial r}\right) = \mu\dot{\gamma}_{zr}$$

Calculations based on equations 7.4, 7.5, 7.6 and 7.7 show that the $r\theta$ component is by far the largest, and therefore the main factor to watch regarding blood destruction. One should also note that the largest shear stresses only occur over limited areas near the inner and outer wall, the main portion of the fluid being subjected to stresses considerably lower than these values. Referring to shear stress data discussed by Blackshear (1972), these calculations indicate that wall-induced blood cell damage will be the dominant factor regarding haemolysis in a Taylor-vortex oxygenator.

Based on experimental findings that haemolysis is proportional to the square of the wall shear rate (Keller, 1971; Blackshear, 1972) one can deduce the following formula (Smeby, 1976)

$$\frac{d(\mathrm{Hb})}{dt} = \frac{K}{R_1^* + R_2^*}\int_0^1 (\dot{\gamma}_1^2 R_1^* + \dot{\gamma}_2^2 R_2^*)\,dz^* \qquad (7.8)$$

where

$\frac{d(\mathrm{Hb})}{dt}$ gives the rate of increase of free haemoglobin in plasma per unit area

$R_1^*$ and $R_2^*$ are the dimensionless inner and outer radii respectively

$\dot{\gamma}_1$ and $\dot{\gamma}_2$ are the dimensionless ($\dot{\gamma}^*$) shear rates at the inner and outer wall respectively

$K = k_1\left(\frac{\nu}{d^2}\right)^2$ where $k_1$ is a constant dependent upon haematocrit.

Figure 7.4 shows how the rate of haemolysis, as calculated from equation 7.8, increases with rotational speed for a given gap and mean radius. Sutera and Mehrjardi (1975) calculated average values of the $r\theta$ stress component at the walls

Figure 7.3 Streamfunctions in a Taylor-vortex oxygenator for different angular velocities and axial flow rates. Each plot represents one vortex (half the wavelength). A covers values which are within 10% of the largest (negative) streamfunction values; B covers values between 20% and 30%; C, 40% - 50%; D, 60% - 70%; E, 80% - 90%. Main oxygenator dimensions: $R_m$ = 15.35 cm, $d$ = 3 mm

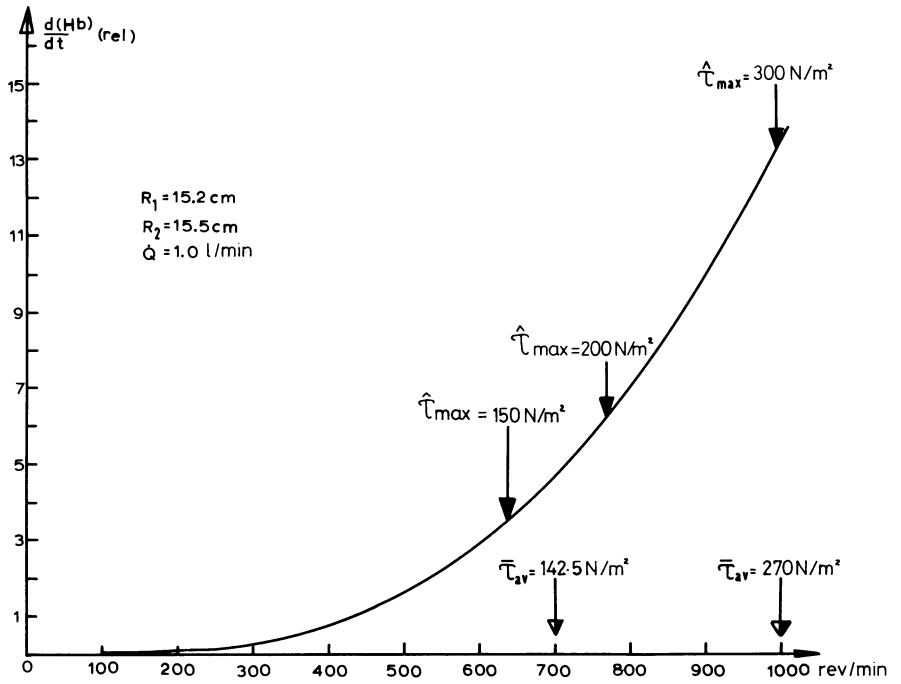



Figure 7.4 Calculated rate of haemolysis per unit membrane area as a function of angular velocity for an oxygenator with a gap of 3 mm and a mean radius of 15.35 cm. The upper arrows indicate maximum shear stress values, while lower arrows indicate the average wall shear stresses

($\bar{\tau}_{av}$) using torque data from Schlichting (1968), and with experimental equipment similar to the Taylor-vortex oxygenator, they found only small increases in haemolysis for $\bar{\tau}_{av}$ below 150 N/m$^2$. According to their data, the rate of haemolysis for $\bar{\tau}_{av}$ equal to 142.5 N/m$^2$ was less than 5%, while an increase to 270 N/m$^2$ resulted roughly in a threefold increase in $d(\mathrm{Hb})/dt$. This agrees well with the calculated values in figure 7.4.

## 7.4 Design Parameters

The most critical design parameter in a Taylor-vortex oxygenator appears to be the shear stresses. From figure 7.5 it can be seen that when the gap($d$) becomes smaller than 3 mm, the average wall shear stress ($\bar{\tau}_{av}$) increases rapidly. From the calculations in section 7.3, and the results of Sutera and Mehrjardi (1975) it seems reasonable to choose a maximum value of $\bar{\tau}_{av}$ = 150 N/m$^2$. However, it should be noted that significantly higher local wall shear stresses will occur at certain positions along the oxygenator. This means that it can be dangerous to base haemolysis estimates on $\bar{\tau}_{av}$ only. To illustrate this point, it can be shown that the total haemolysis rate for a 'rated flow' oxygenator with parameters of

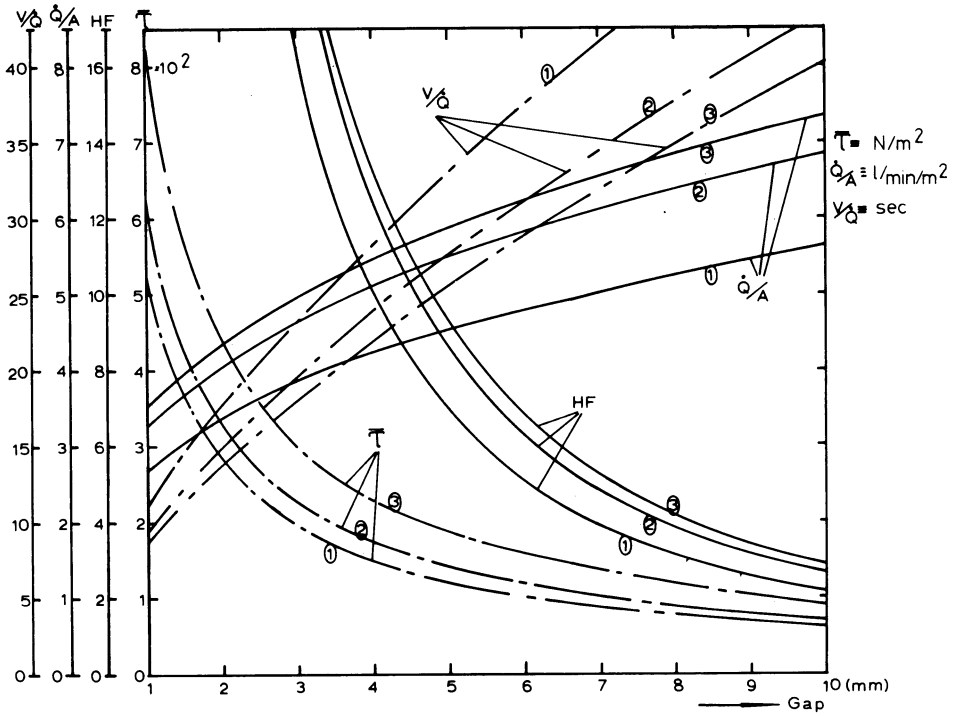



Figure 7.5 Design guidelines for a full size (5 l/min), 'rated flow' T-V oxygenator. Curves (1) are calculated for a rotational speed of 600 rev/min and a cylinder length of 50 cm (2) are calculated for 800 rev/min and the same cylinder length. Curves (3) are the result of calculations based on 800 rev/min and a length of 40 cm. Calculations are based on the Sherwood number correlation given in equation 7.2 and torque data from Schlichting (1968)

$R_m$ = 16.75 cm, $d$ = 4.5 mm and $\Omega_1$ = 73.3 rad/s (700 rev/min) will be roughly half as high as one with $R_m$ = 15.35 cm, $d$ = 3 mm and $\Omega_1$ = 73.3 rad/s, although the value of $\bar{\tau}_{av}$, is about the same for both these oxygenators. Total haemolysis rate calculated as

$$HF = \frac{\partial(Hb)}{\partial t} \times \left(\frac{V}{\dot{Q}}\right) \times A.$$

Another design parameter of practical importance is the cylinder length ($L$). If the cylinders become too long, this will demand an expensive and complicated bearing system to hold the inner cylinder. Our experience with the oxygenator indicates that a cylinder length of 40 - 60 cm is a reasonable choice. Shorter cylinders will result in larger 'residence time' $V/\dot{Q}$ (for a given rotational speed), and as the priming volume should be kept relatively low, this is a significant drawback. Figure 7.5 shows that for a rotational speed of 800 rev/min, a decrease in

cylinder length from 50 cm (curve 2) to 40 cm (curve 3) will require a 1 mm larger gap, resulting in an increase in $V/\dot{Q}$ from 20 to 21.5 s.

The choice of rotational speed is mainly a technical problem. A decrease in rotational speed leads to a larger priming volume and a decreased efficiency as shown by the $V/\dot{Q}$ and $\dot{Q}/A$ curves (figure 7.5). This is important because haemolysis is thought to be proportional to membrane area (for a given wall shear stress) and hence a decreased rotational speed does not necessarily mean lower haemolysis. For $\bar{\tau}_{av} = 150$ N/m$^2$, a decrease from 800 rev/min to 600 rev/min is expected to result in an increased total haemolysis rate. The upper limit on rotational speed could be determined by a transition into fully turbulent flow in which the shear stresses are too high. However, experimental results (Schlichting, 1968) indicate that the relationship between $T^*$ and $\bar{\tau}_{av}$ is the same at least up to a value of $T^* = 5000$. For this $T^*$, a 'rated flow' device (40 cm high, with $\bar{\tau}_{av} = 150$ N/m$^2$) would operate at about 1100 rev/min, and this appears to be above the practical limit which is thought to be in the range 600 rev/min - 800 rev/min.

Figure 7.5 gives only a selection of a few design variables, but it demonstrates the trend when the design parameters for a full size ($\dot{Q} = 5$ l/min), 'rated flow' oxygenator are changed. The main guide-lines can be summarised as follows:

(1) The rotational speed should be as high as possible (mainly practical limitations).
(2) The cylinder length must be determined from practical considerations, and a compromise between the fact that decreased cylinder length increases transport efficiency ($\dot{Q}/A$), but also increases priming volume (or 'residence time' $V/\dot{Q}$).
(3) The priming volume is a limiting factor in reducing average wall shear stresses. If a larger 'residence time' can be tolerated, this will give a more efficient mass transfer device and reduce $\bar{\tau}_{av}$. This means that any haemolytic effects can be lowered if the priming volume is increased.

## 7.5 Conclusions

The Taylor-vortex oxygenator is a very efficient mass-transfer device. Oxygen transport of more than 300 ml/min/m$^2$ appears to be possible without producing excessive haemolysis. Carbon dioxide removal is mainly dependent upon the choice of membranes, but in our prototypes, employing the microporous membrane types, this posed no problems.

The main drawback with the oxygenator seems to be the relatively high priming volume which is needed to keep the haemolytic effects below a reasonable level. It is obvious that this is a significant factor as long as the extracorporeal circuit has to be primed with whole blood. However, recent experiences with total heart-lung bypass procedures (Lilleåsen, 1976) indicate that up to 50% haemodilution can in fact improve the general quality of the treatment. Hence a priming volume as high as 2 l in a membrane oxygenator may not necessarily be such a serious drawback.

On the basis of the experimental oxygen transfer results summarised in equation 7.2, the shear stress calculations based on the model indicated in section 7.3, and our practical experience with the prototypes, an optimal full size (5 l/min) 'rated flow' oxygenator would be characterised by

| | |
|---|---|
| Mean diameter | 31.5 cm |
| Annular gap | 4.5 mm |
| Transport efficiency ($\dot{Q}/A$) | 5.0 l/min/m$^2$ |
| Residence time ($V/\dot{Q}$) | 23 s |
| Rotational speed | 700 rev/min |
| Cylinder height | 50 cm |

As previously discussed the relatively high priming volume (2.25 l) of this device could be somewhat reduced either by increasing the rotational speed or by increasing the cylinder length, but that is mainly a technological problem which will not be further discussed here.

Compared to similar membrane oxygenators such as the oscillating torus (Drinker, 1969) and the rotating disc (Hill *et al.*, 1974) the main drawback of this device is the priming volume, a possible advantage could be a reduced haemolysis rate. However, this can only be determined by further experimental work, and until such evidence is available, the future of the Taylor-vortex oxygenator remains open.

## 7.6 References

Blackshear, P. L. (1972). In Y. C. Fung, N. Perrone and M. Anliker (Eds.), *Biomechanics*, Prentice-Hall, Englewood Cliffs, New Jersey, pp. 501 - 528.

Burkhalter, J. E. and Koschmieder, E. L. (1973). Steady supercritical Taylor-vortex flow. *J. Fluid Mech.*, **58**, no. 3, 547 - 560.

Coles, D. (1965). Transition in circular Couette flow. *J. Fluid Mech.*, **21**, no. 3, 385 - 425.

Drinker, P. A., Bartlett, R. H., Bialer, R. M. and Noyes, B. S. (1969). Augmentation of membrane gas transfer by induced secondary flows. *Surgery*, **66**, 775 - 781.

Galletti, P. M., Richardson, P. D., Snider, M. T. and Friedman, L. I. (1972). A standardized method for defining the overall gas transfer performance of artificial lungs. *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 350 - 368.

Gaylor, J. D. S. and Smeby, L. C. (1975). The Taylor-vortex membrane oxygenator : Design analysis based on a predictive correlation for oxygen transfer. *Advances in Oxygenator Design*, Seminar in Copenhagen.

Hill, J. D., Iatridis, A., O'Keefe, R. and Kitrilakis, S. (1974). Technique for achieving high gas exchange rates in membrane oxygenation. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 249.

Keller, K. H. (1971). Effect of fluid shear on mass transport in flowing blood. *Fed. Proc.*, **30**, no. 5, 1591 - 1599.

Lilleåsen, P. (1976). *Scand. J. Thorac. Cardiovasc. Surg.* (in press).

Peirce, E. C. (1973). *The Mount Sinai J. of Med.*, **40**, 2, 119 - 134.
Schlichting, H. (1968). *Boundary Layer Theory*, 6th ed., McGraw-Hill, New York, pp. 500 - 508.
Smeby, L. C. (1976). Ph.D. Thesis, Bioeng. Unit, Univ. of Strathclyde.
Smeby, L. C. and Gaylor, J. D. S.(1974). Further development of the Taylor-vortex membrane oxygenator. *Eur. Soc. Artif. Organs*, **1**, 62 - 66.
Smeby, L. C. and Grimsrud, L. (1974). Theoretical investigation of mass transfer in membrane oxygenators. *Med. Biol. Engng.*, **12**, 698 - 706.
Snyder, H. A. (1961). Experiments on the stability of spiral flow at low axial Reynolds numbers. *Proc. Roy. Soc. London, A*, **265**, 198 - 214.
Sutera, S. P. and Mehrjardi, M. H.(1975). Deformation and fragmentation of human red blood cells in turbulent shear flow. *Biophys. J.*, **15**, 1 - 10.

## 7.7 Notation

$A$ = membrane area
$a$ = axial wave-number ($=2\pi d/\lambda$)
$d$ = annular gap ($=R_2 - R_1$)
$D_B$ = diffusivity in whole blood ($\approx 2.25 \times 10^{-5}$ cm$^2$/s)
$D_w$ = diffusivity in water ($\approx 2.25 \times 10^{-5}$ cm$^2$/s)
Hct = haematocrit
Hb = haemoglobin concentration
HF = total haemolysis rate
$h_o$ = overall mass transfer coefficient
$K$ = a constant ($=k_1(\nu/d)^2$)
$k_1$ = a constant ($\equiv (Hct)^2/(1 - Hct)$)
$L$ = cylinder length
$\dot{Q}$ = flow rate
$\vec{q}$ = velocity vector
$r$ = radial distance
$r^*$ = dimensionless radial distance ($=r/d$)
$R_1$ = outside radius of inner cylinder
$R_1^*$ = $R_1/d$
$R_2$ = inside radius of outer cylinder
$R_2^*$ = $R_2/d$
$R_m$ = mean radius ($= (R_1 + R_2)/2$)
$R_z$ = axial Reynolds number ($=\dot{Q}/(\pi R_m \nu)$)
$Sc$ = Schmidt number ($=\nu/D$)
$Sh_B$ = Sherwood number for blood ($=2h_o d/D_B$)
$Sh_w$ = Sherwood number for water ($=2h_o d/D_w$)
$T^*$ = Taylor number ($=(\Omega_1 d/\nu)\sqrt{R_m d}$)
$u$ = radial velocity component
$u^*$ = dimensionless radial velocity ($=ud/\nu$)
$v$ = peripheral velocity component
$v^*$ = dimensionless peripheral velocity ($=vd/\nu$)
$V$ = priming volume

$w$ = axial velocity component due to the vortex motion
$w^*$ = dimensionless axial velocity component due to the vortex motion ($=wd/\nu$)
$\bar{w}^*$ = dimensionless axial velocity component due to main flow ($\equiv R_z$)
$z$ = axial distance
$z^*$ = dimensionless axial distance ($=za/\pi$d)
$\xi$ = vorticity about the $\theta$-axes ($=\partial u/\partial z - \partial w/\partial r$)
$\xi^*$ = dimensionless vorticity ($=\xi d^2/\nu$)
$\dot{\gamma}$ = shear rate
$\dot{\gamma}_1$ = dimensionless shear rate in the $r$-$\theta$ plane at the inner cylinder wall ($=(\dot{\gamma}_{r\theta})_{r=R_1} \cdot d^2/\nu$)
$\dot{\gamma}_2$ = $(\dot{\gamma}_{r\theta})_{r=R_2} \cdot d^2/\nu$
$\theta$ = peripheral distance
$\psi$ = streamfunction
$\psi^*$ = dimensionless streamfunction ($=\psi/(\nu d)$)
$\Omega_1$ = angular velocity of inner cylinder
$\lambda$ = axial wavelength
$\rho$ = density
$\nu$ = kinematic viscosity
$\tau$ = shear stresses
$\hat{\tau}$ = highest value of the $r\theta$ shear-stress component at the walls
$\bar{\tau}_{av}$ = average value of the $r\theta$ shear-stress component along the walls
$\mu$ = dynamic viscosity

# 8

# TOTAL LONG-TERM VENO-ARTERIAL BYPASS BY VENTRICLE PUMP AND MEMBRANE OXYGENATOR

K. HANKELN, B. HASPER, N. SAUTTER, N. PROSS, S. SCHNEIDER, A. TANZEEM and H. H. STORCH

**Summary**

The necessity for prolonging the time a patient is sustained by extracorporeal circulation has encouraged, particularly during the past decade, the development of improved blood pumps (Klimeš *et al.*, 1975; Granger *et al.*, 1974 and Kletschka *et al.*, 1975). During the time spent by the authors in Heidelberg, they were instrumental in the development of electronically controlled systems for prolonged oxygenation as well as of diastolic cardiac arrest devices for patients, following open heart surgery (Hankeln *et al.*, 1975).

## 8.1 Introduction

Patients treated by the (standard) extracorporeal circulation (ECC) systems may encounter difficulties primarily on two counts: firstly high haemolysis rates (figure 8.1) consequent on the use of modified standard roller pumps and secondly side effects of ECC in the form of kidney problems.

The authors have been encouraged by the results of several research groups in the United States of America and the Federal Republic of Germany to develop a

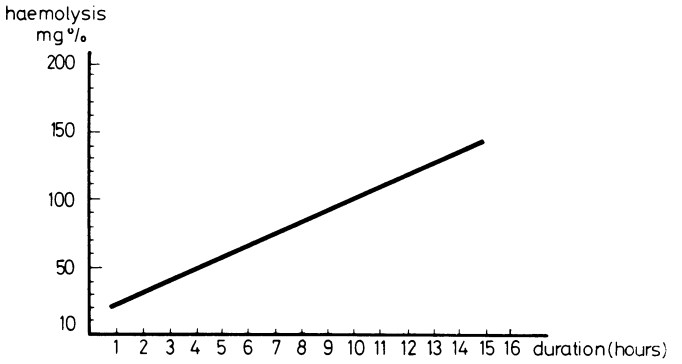



Figure 8.1 Haemolysis rates by standard roller pumps during prolonged extracorporeal support

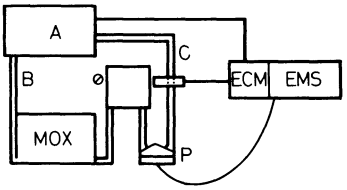



Figure 8.2 Schematic representation of new bypass system for long-term support. A experimental animal, MOX membrane oxygenator, B venous line, C arterial line, ECM and EMS electronic monitoring system and driving unit, P membrane pump

new bypass system for long-term support. This consists of a membrane oxygenator and a membrane pump as well as an electronic control system, designed in particular to eliminate the danger of air embolism. The assembly is shown schematically in figure 8.2.

## 8.2 Method

Experiments using the arrangement shown in figure 8.2 were carried out on mongrel dogs of 20 to 30 kg body weight. Venous blood drainage by gravity was passed through a membrane oxygenator (Landé-Edwards, Travenol Modulung) with low gradient into an arterial reservoir. From here the blood was returned to the ascending aorta via the arterial line. The blood pump used was a newly developed membrane ventricle type (Hankeln and Wagenbrett, 1975), driven either by the Datascope 80 system or the AEG - Telefunken unit. The parameters monitored were: lifetime of the animal, tolerance of bypass (as indicated by cardiac and kidney function and haemolysis), haematocrit, oxygenator function, and coagulation time together with several other factors to be reported elsewhere.

## 8.3 Oxygenator Function

The 3 $m^2$ Modulung and the 3 $m^2$ Landé-Edwards devices showed themselves to be efficient for flow rates of up to 2 l/min of bypassed blood volume. In some experiments the $CO_2$ exchange capacity of the oxygenators was unsatisfactory. Cardiac and kidney functions were maintained without difficulty for periods in excess of 10 hours.

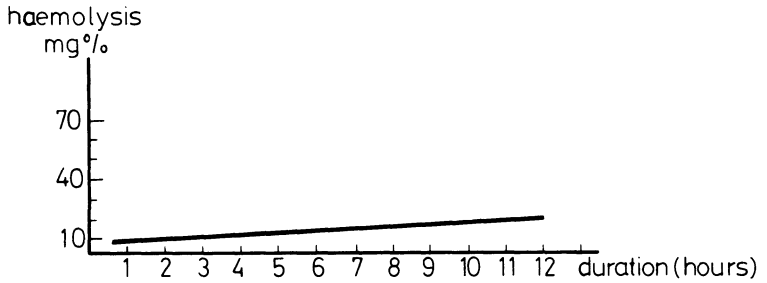



Figure 8.3 Membrane pump haemolysis in *in vivo* experiment. Flow rate 2 - 2.5 l/min

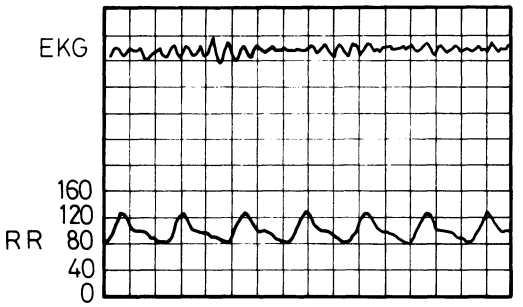



Figure 8.4 Pressure recorded in the femoral artery of the experimental animal

## 8.4 Haemolysis

Using the most suitable type of membrane pumps haemolysis rates could be kept below 20 mg per cent (figure 8.3).

The flow profile of the system, recorded in the femoral artery of the experimental animal is shown in figure 8.4. The heart of the test animal was artifically fibrillated to eliminate artefacts by ventricular ejection.

## 8.5 Conclusions

Membrane pumps (figure 8.5) may well be able to replace roller pumps for extracorporeal circulation. Appropriate layout of valves and pump configuration can be helpful in reducing haemolysis and kidney disorders during the following prolonged bypass.

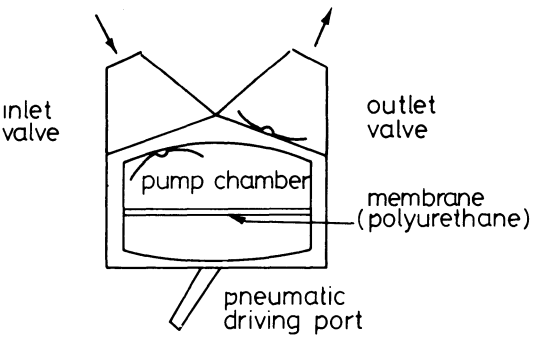



Figure 8.5 Membrane pump (schematic)

## 8.6 References

Granger A., Butruille, Y., Lissot J., Chevallet, J., Christen, G. and Sausse A. (1974). An artificial organ: the heart-lung R. P. machine for long lasting cardiopulmonary support. *Eur. Soc. Artif. Organs.*

Hankeln K., Harper B. and Storch H. H. (1975). Automated heart-lung machine for long term oxygenation. International Cardiovascular Society, Edinburgh.

Hankeln K. and Wagenbrett C. (1974). Membrane pump data (unpublished) from Medizin technik und Feinmechanik, Sonnebergstrasse 12, 28 Bremen 41, Federal Republic of Germany.

Kletschka H. D., Rafferty E. H. and Alson D. (1975) Artificial heart. *Minnesota Medicine,* **58**, 756 - 781.

Klimeš F., Vašku J., Darěna F. and Andreovosky, F. (1975). Czechoslovak devices to drive, control and monitor assisted blood pumps and total replacement of the heart. *Proc. Eur. Soc. Artif. Organs,* **1**, 170.

# 9

# CLINICAL REFLECTIONS ON EXTRACORPOREAL MEMBRANE OXYGENATION

P. E. GHADIALI

An increasing number of patients in the prime of their lives, holding responsible positions in society to which they would return, undergo open heart surgery with extracorporeal circulation. Perhaps the time has come to consider the effects of heart-lung bypass in greater depth not only from the short-term but also the long-term point of view. There is increasing experimental and clinical evidence that during perfusion there are changes at cellular level which may or may not cause permanent damage to vital organs. This damage, although undetectable by tests available to most of us, may in the long run lead to such conditions as premature deterioration of mental faculties and dimished cardio-pulmonary reserves resulting in a poorer quality of life for the patient.

Although considerable advances have been made in the invention and development of surgical techniques to deal with a variety of lesions, comparable progress has not perhaps been achieved in the sphere of heart-lung bypass. The most important factor responsible for changes in the body must be the inevitable mechanical and biological damage to blood in the extracorporeal circuit. To minimise such damage certain technical refinements in the apparatus have been made over recent years - the most notable of which have been the introduction and availability of membrane oxygenators suitable for routine use and micropore filtration.

Membrane oxygenators have been used at the Brompton Hospital for the last five years: the Landé-Edwards membrane oxygenators for perfusions in neonates, infants and children and the microporous Teflo Modulung in adults. Well over one thousand perfusions have been performed. During this period plastic disc and bubble oxygenators have also been used. Our findings can be summarised as follows:

(1) Perfusions have become more physiological, for example, changes in the peripheral vascular resistance were much less.
(2) Tests for blood damage showed less reduction in platelet counts, less haemolysis and better preservation of clotting factors.
(3) Ultrasonic bubble detector tracings from the arterial line were relatively free from signals as compared to those from bubble and disc oxygenators.

(4) Particulate microembolisation was reduced as the oxygenators acted as additional filters.
(5) Post-operatively, there was less bleeding and the general condition of the patients from all aspects appeared to be much better.

Membrane oxygenators have enabled us to improve the quality of our perfusions and to use circulatory support for prolonged periods with confidence in the treatment of intra-operative heart failure.

The disadvantages have been the increased cost and the management of the more complex technical matters.

Although currently available membrane oxygenators have incorporated considerable technical improvements, there are a number of problem areas which require clearing up with further resources of expert technology. The characteristics of an ideal membrane lung from a purely clinical point of view are many and one hopes that they would be embodied in future developments in this field. After all, our ultimate objective should be that a patient suffers no harm from being on cardio-pulmonary bypass; in fact, in certain cases, he should benefit from it.

# 10

# THE CORPUSCULAR VOLUME DISTRIBUTION OF ERYTHROCYTES DURING ECMO AND AFTER EXPOSURE TO SHEAR FORCES

D. BIRNBAUM, R. THOM and E. S. BÜCHERL

**Summary**

The volume of red blood corpuscles is constant under normal conditions and follows the law of Gauss's distribution of a population. Thus an alteration of the volume of the erythrocytes is of pathophysiological significance.

Severely damaged blood cells undergo lysis, which is detectable by an increase of the plasma haemoglobin concentration. It may occur in patients who were subjected to cardiac surgery and treated with heart-lung machines. By use of membrane oxygenators instead of bubble oxygenators haemolysis is avoidable (Brinsfield *et al.*, 1962). With regard to intravascular cell lysis there is obviously no need for further investigation for evaluation of devices replacing conventional blood oxygenators. However, patients developed severe anaemia, without the signs of late intravascular lysis of blood cells, whenever they were subjected to extracorporeal membrane oxygenation (ECMO), especially if this treatment lasted for longer periods.

Many investigations were undertaken to illuminate this postsurgical complication and to look for methods to indicate cause and extent of subhaemolytic damage of red blood cells. Alterations of the osmotic fragility (Bernstein *et al.*, 1967), of the survival time (Douglas *et al.*, 1971), of electron and scanning electron microscopic morphology (Hochmuth *et al.*, 1972; Sutera *et al.*, 1975), of enzyme release (Bernstein *et al.*, 1967), of glucose metabolism (Bernstein, 1971) and other functions (Nanjappa *et al.*, 1973) of cells after exposure to shear stress supported the idea of the occurrence of minor wall lesions.

If the integrity of the cell wall is in disorder after intimate contact with foreign surfaces one should expect alterations of the volume of the single cells caused by a disturbance of the water and electrolyte balance. This study was undertaken to answer the following questions:

(1) Is there any alteration of the volume distribution of red blood cells caused by shearing?

(2) Will those cells which have been stressed recover after their volumes have changed?

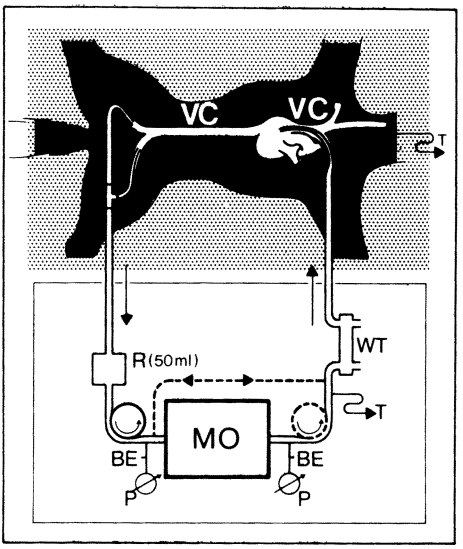



Figure 10.1 Membrane oxygenator in veno-venous bypass. R reservoir, WT heat exchanger, MO membrane oxygenator, P pressure transducer, BE blood sampling, T temperature probe, VC vena cava

## 10.1 Materials and Methods

*In vivo experiments*

Anaesthetised hypoventilated dogs were connected to an extracorporeal circulatory system shown schematically in figure 10.1. The venous blood of the animal drained via a cannula in the inferior vena cava into a small reservoir and was pumped by an ordinary roller pump through the membrane oxygenator and the heat exchanger. The oxygenated blood returned by the superior vena cava to the animal's blood circulation. In some cases two pumps had to be used in combination with the recirculation line in the system if required by the oxygenator's flow and pressure characteristics. Such a two-pump system was also used for the control experiments, which had no oxygenator in the system at all. ECMO was maintained for at least 7 h. Handling and installation of the membrane oxygenator followed exactly the manufacturer's instructions. The following oxygenator types were used: Dua - Lung (General Electric), Modu - Lung (Travenol), Spiral-Coil-Kolobow (Scientific Medical), Teflo-Lung (Travenol). The system was primed with glucose-electrolyte solution. The dogs were heparinised (Birnbaum *et al.*, 1976). Blood samples taken from the animals were immediately analysed for the mean cellular volume of erythrocytes ($MCV_e$) and for the volume distribution respectively.

*In vitro experiments*

Freshly obtained human blood was stabilised with EDTA and exposed to shear forces of 10 $N/m^2$ (100 $dyn/cm^2$) for different periods. This was performed in a concentric cylinder viscometer with a rotating inner steel cylinder of 60 mm length (Rotovisco RV2, Haake, Berlin) with its outer and inner surfaces opposed by stationary cylinders. The gap width was 0.35 mm at the inner and 0.4 mm at the outer space. The priming volume was 8 ml blood with haematocrit of 48%. A built-in temperature control maintained a constant temperature of 37°C throughout the shear procedure.

*Analysis of the erythrocyte volume*

This was carried out using a particle analyser MS PD1 of Telefunken, Germany which utilises the Coulter principle. The data output provided particle volume and relative frequency of occurrence in digital form.

## 10.2 Results

Our analysis of particle volumes such as that of red blood cells using this method had an error below 1%.

**Table 10.1**

Mean erythrocyte volume ($\mu m^3$) during ECMO in dogs.
(Mean values of three experiments for each system)

| *System* | *Before EMCO* | *1 h ECMO* | *6.5 h ECMO* |
|---|---|---|---|
| Control group | 70.2 | 69.8 | 69.9 |
| Dua-Lung | 70.7 | 73.3 | 76.3 |
| Modu-Lung | 71.3 | 71.9 | 70.6 |
| Spiral-Lung | 68.9 | 68.5 | 68.0 |
| Teflo-Lung | 68.4 | 68.7 | 68.8 |

Table 10.1 shows the mean erythrocyte volume ($MCV_e$) in dogs from three experimental runs for each oxygenator and for an unoxygenated control group. In the case of the control and of the Modu, Spiral and Teflo-Lung no alteration of the $MCV_e$ was seen after 6.5 h of ECMO. A severe swelling of the red blood cells for almost 9% of the initial volume was detected, however, with the Dua-Lung installed in the ECMO system.

Mechanical traumatisation of blood as caused by a rotoviscometer led to drastic changes of the $MCV_e$. This phenomenon was highly dependent on the duration of the influence of the shear forces. After a short period (15 min) slight diminution of the volume was found. Later, however, a definite swelling occurred of all the erythrocytes of the tested population. The volume distribution

curves are shown in figure 10.2. The most vigorous swelling occurred after two hours of stressing.

If such mechanically traumatised blood was stored at room temperature for 18 h there was a tendency for normalisation of the swollen status (figure 10.3). However, the samples were not stressed for more than 30 min. Otherwise this reversibility failed to appear. For example in those samples which were stressed for 2 h there was even a continuation of the swelling tendency during the period allowed for 'recovery' (figure 10.4).

If the stressed blood was stored at + 4 °C for 18 h analysis of the $MCV_e$ showed a closer reversion of the swelling phenomenon to almost initial values (figure 10.5). Again, if the period of shearing traumatisation was beyond a certain limit the swollen status was shown to be irreversible.

## 10.3 Discussion

The understanding of the effects of sublethal damage to blood cells has become important in medicine because of the many applications in which foreign surfaces contact biological structures. While absence of haemolysis recently allowed long-term (several weeks) pulmonary support of patients with ECMO (International conference, 1975), anaemia and renal insufficiency still remain positive indicators of injured erythrocytes (Brinsfield *et al.*, 1962). Abnormal behaviour of altered red cells from metabolic, functional and morphological aspects is being increasingly investigated. Of special interest are studies about changes of the intracellular sodium concentration (Lubowitz *et al.*, 1974), extra-erythrocytic presence of cell wall molecules (lipids) (Langley and Axell, 1968) and intra-erythrocytic enzymes (Bernstein *et al.*, 1967) following defined traumatisation of red blood cell suspensions. ADP-release (Bernstein and Marzec, 1974) and serotonin liberation (Hochmuth *et al.*, 1972) have also been explained.

Our findings on sheared erythrocytes are explained in terms of membrane wall damage incidence. This results in a major function of the cell membrane (maintenance of ion transportation and gradients) deteriorating by either derangement of the metabolic activity or break off (or regroupment) of structural units of the wall molecular complexes, accompanied finally by an increase of the membrane porosity. In all cases a passive influx of sodium and water may occur leading to intracellular oedema. We consider that shrinkage of the cell at the initial phase of traumatisation may be caused by an efflux of intracellular potassium (figure 10.6). According to our observations this early phase of the cell volume alteration was not always detectable.

Evidently this kind of cell wall damage, with its consequent volume change, is reversible, unless the trauma is severe enough to impede regaining of the proper membrane function. Incidentally, temperature did influence this recovery potential. The appearance of ADP, occasionally described as originating from damaged erythrocytes (Bernstein and Marzec, 1974), supports the assumed frag-

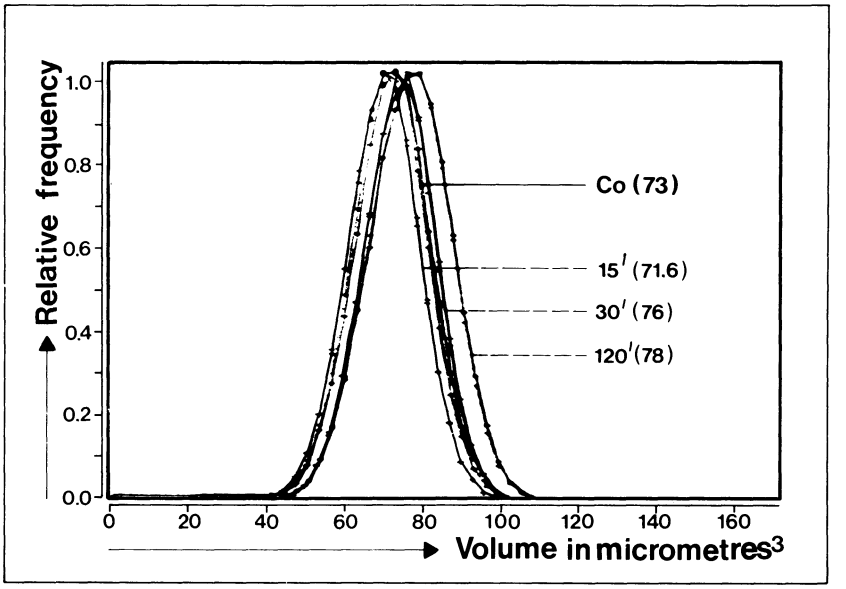



Figure 10.2 Volume distribution of human erythrocytes. Co control; period in min of shear action followed in brackets by $MCV_e$ in $\mu m^3$, e.g. 15′ (71.6). ($\tau$ = 10 N/m² (100 dyn/cm²), $T$ = 37°C, variation in coefficient = 12.4 - 13.5%.) Reproduction of actual record

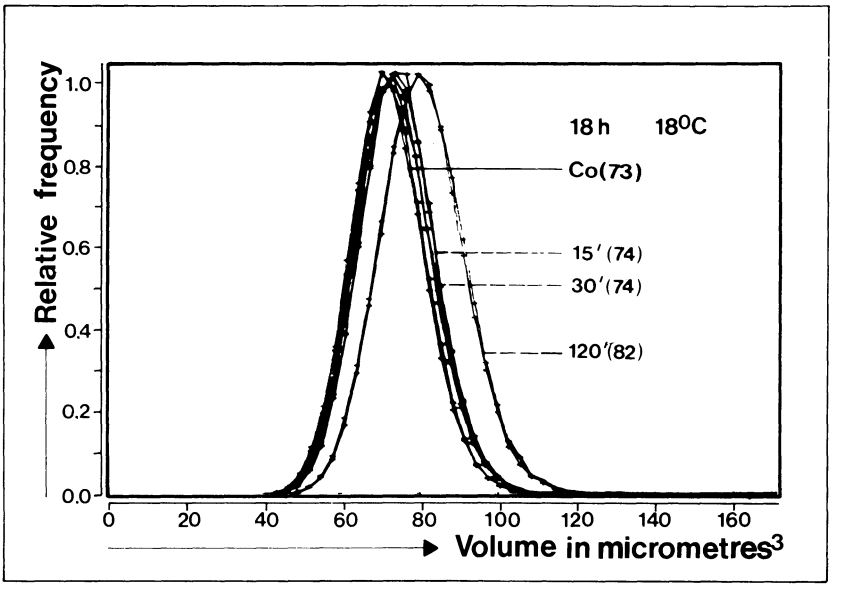



Figure 10.3 Volume distribution of sheared human erythrocytes after storage at room temperature for 18 h. Co control; period in min of shear action followed in brackets by $MCV_e$ in $\mu m^3$. ($\tau$ = 10 N/m², variation in coefficient = 13.0 - 13.8%.) Reproduction of actual record

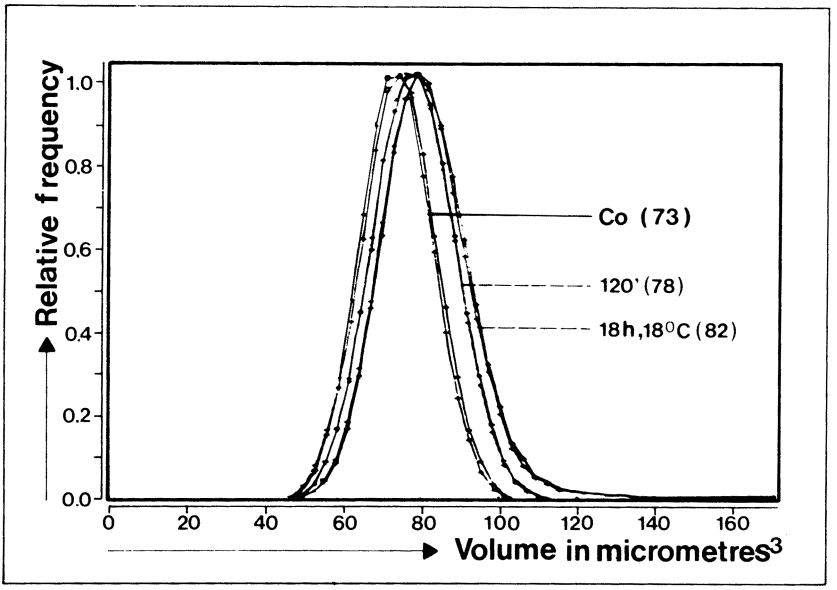



Figure 10.4 Volume distribution of erythrocytes sheared for 2 h. No 'recovery' after 18 h. Co control; period in min of shear action followed in brackets by $MCV_e$ in $\mu m^3$. ($\tau$ = 10 N/m$^2$, variation in coefficient = 12.4 – 13.4%.) Reproduction of actual record

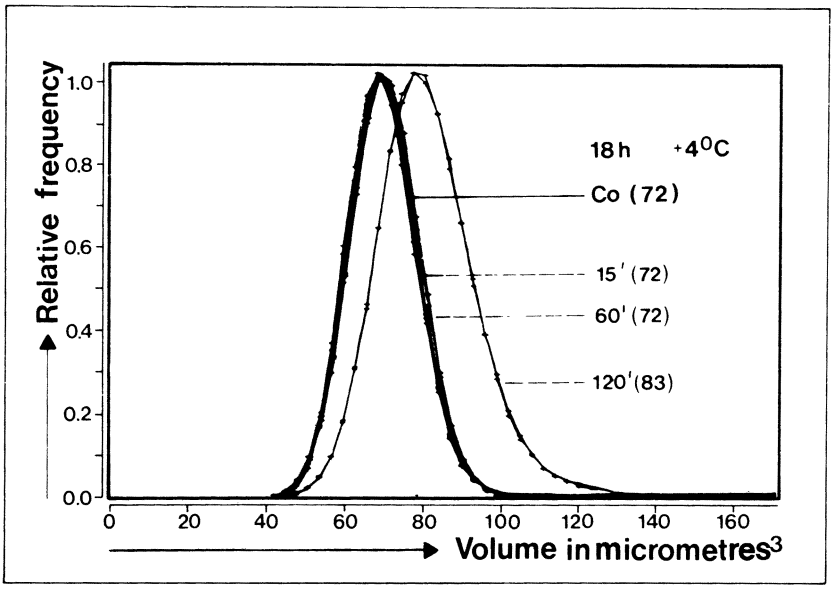



Figure 10.5 Volume distribution of sheared human erythrocytes after storage at +4°C for 18 h. Co control; period in minutes of shear action followed in brackets by $MCV_e$ in $\mu m^3$. ($\tau$ = 10 N/m$^2$, variation in coefficient = 12.2 – 14.0%.) Reproduction of actual record

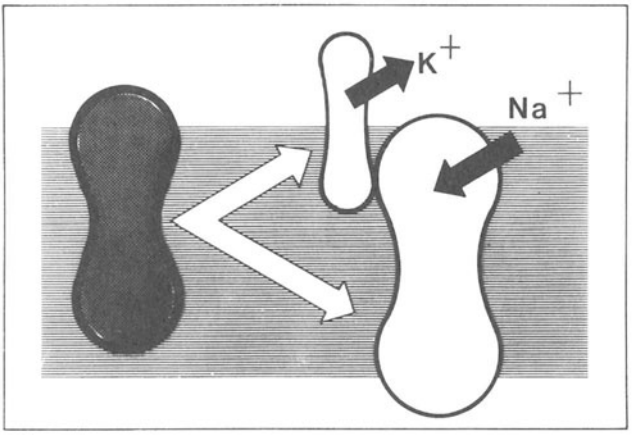



Figure 10.6 Cell volume alteration by transmembrane electrolyte passage

mentation of cell wall structures accompanied by failure of the ion pump mechanisms. This is of pathophysiological importance because of the considerable effects of free ADP upon platelet function and clot formation (Born, 1976).

It is well known that within an erythrocyte population the larger individuals are the older ones. It is supposed therefore that mechanical traumatisation may lead to accelerated aging, manifested by swelling, and to an enhanced sequestration of those cells. Sublethal damage therefore does not necessarily raise a detectable increase of free plasma haemoglobin but might be responsible for late post-cardiosurgical anaemia.

**Table 10.2**

Methods to overcome boundary layer effects

| |
|---|
| 'Multi point support' of the membrane (Drinker *et al.*, 1969). |
| Coiled pattern of flow (Kolobow and Bowman, 1963). |
| Screen within blood passage (Birnbaum and Eiseman, 1972). |
| Induction of secondary flow by oscillation of the device (Drinker *et al.*, 1969). |
| 'Massage' of the blood by oscillating gas flow (Rush *et al.*, 1969). |
| Taylor-vortex formation (Gaylor *et al.*, 1973). |

The gas exchange capacity of a membrane oxygenator is significantly dependent on the extent of the break up of the plasma film, which forms a boundary layer between the red cell mass and the gas transferring membrane. The plasma film in laminar flowing blood is the primary limiting factor for the $O_2$ passage because of the relatively low physical solubility of oxygen in plasma. Many ingenious ideas were developed for oxygenator devices to overcome this difficulty. All the proposals are principally based upon the generation of turbulence (table 10.2). Increasing turbulence of the blood within its pathway promotes

gas exchange but creates shear actions and thus presumably accounts for increasing blood damage. The blood path in our experiments with oxygenator devices (table 10.1) which did not show significant alteration of the $MCV_e$ allowed just one passage for blood to completely exchange gases. In the oxygenator with $MCV_e$ alteration there is a to and fro movement of the blood within the gas exchanging elements. It is obvious that in this case a more intimate and longer lasting contact occurred between the blood and the oxygenator elements. This also produced longer periods of shearing and we feel this to be responsible for the alteration of the erythrocytes.

The detection of the volume distribution of the actual erythrocyte population by the Coulter method is simple and quickly performed. There is no other method available with an accuracy within 1%, with a sensitivity sufficient to detect volume changes of 1 - 2 $\mu m^3$. Conventional methods which calculate the *MCV* by erythrocyte number and haematocrit are inexact and do not allow for variation in the size of the cell population.

## 10.4 Conclusions

One out of four membrane oxygenator devices produced an alteration of the erythrocyte volume distribution. Red blood cells subjected to shear stress of $10N/m^2$ ($100dyn/cm^2$) for 2 h showed an increase in the mean erythrocyte volume of about 10%. Increasing erythrocyte volume following traumatisation indicates subhaemolytic damage.

The erythrocyte volume is proposed as a subtle indicator to signal the onset of undesirable effects in extracorporeal circulatory systems.

## 10.5 Acknowledgement

The authors wish to thank Mrs Ingeborg Runge for excellent technical assistance.

## 10.6 References

Bernstein, E. F., Indeglia, R. A., Shea, M. A. and Varco. R. L. (1967). Sublethal damage to the red blood cell from pumping. *Circulation Suppl. I*, **35**, 226.

Bernstein, E. F. (1971). Erythrocyte metabolism following surface induced injury. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 386.

Bernstein, E. F. and Marzec, U. M. (1974). Effect of wall interaction, shear stress and osmotic injury on erythrocyte adenosine triphosphate concentration, 2, 3 - diphosphoglycerate concentration and the oxyhemoglobin dissociation curve. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 47.

Birnbaum, D. and Eiseman, B. (1972). Laboratory evaluation of a new silicone membrane oxygenator. *J. Thorac. Cardiovasc. Surg.*, **64**, 441.

Birnbaum, D., Eisele, R. and Thom, R. (1976). Kriterien zur Auswahl eines Membran-Oxygenators für die Langzeitoxygenierung. *Langenbecks Arch. Chir. Suppl. Chir. Forum*, 152.

Born, G. (1976). Personal communication.

Brinsfield, D. E., Hopf, M. A., Geering, R. B. and Galletti, P. M. (1962). Haematological changes in long-term perfusion. *J. Appl. Physiol.*, **17**, 531.

Douglas, M., Birnbaum, D. and Eiseman, B. (1971). Biological evaluation of a disposable membrane oxygenator. *Arch. Surg.*, **103**, 89.

Drinker, P. A., Bartlett, R. H., Bialer, R. M. and Noyes, S. B. (1969). Augmentation of membrane gas transfer by induced secondary flows. *Surgery*, **66**, 775.

Gaylor, J. D. S., Murphy, J. F., Caprini, J. A., Zuckermann, L. and Mockros, L. F. (1973). Gas transfer and thrombogenesis in an annular membrane oxygenator with active blood mixing. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 516.

Goldsmith, H. L. (1974). The effects of flow and fluid mechanical stress on red cells and platelets. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 21.

Hochmuth, R. M., Mohandas, N., Spaeth, E. E., Williamson, J. R., Blackshear, P. L., Jr. and Johnson, D. W. (1972). Surface adhesion, deformation and detachment at low shear of red cells and white cells. *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 325.

Kolobow, T. and Bowman R. L. (1963). Construction and evaluation of an alveolar membrane artificial heart - lung. *Trans. Amer. Soc. Artif. Int. Organs*, **9**, 238.

Langley, G. R. and Axell, M. (1968). Changes in erythrocyte membrane and autohaemolysis during *in vitro* incubation. *Brit. J. Haemat.*, **14**, 593.

Lubowitz, H., Harris, F., Mehrjardi, M. H. and Sutera, S. P. (1974). Shear-induced changes in permeability of human RBC to sodium. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 470.

Nanjappa, B. N., Chang, H.-K. and Glomski, Ch. A. (1973). Trauma of the erythrocyte membrane associated with low shear stress. *Biophys. J.*, **13**, 1212.

Peirce, E. C. and Dibelius, R. R. (1968). The membrane lung: Studies with a high permeability copolymer membrane. *Trans. Amer. Soc. Artif. Int. Organs*, **14**, 220.

Rush, B. F., Boone, R. and Malette, W. (1969). A pulsatile flow membrane oxygenator. *Trans. Amer. Soc. Artif. Int. Organs*, **15**, 178.

Sutera, S. P. and Mehrjardi, M. H. (1975). Deformation and fragmentation of human red blood cells in turbulent shear flow. *Biophys. J.*, **15**, 1.

Thom, R. (1972). Vergleichende Untersuchungen zur elektronischen Zellvolumenanalyse. Hochfrequenztechnik.

The International Conference on Membrane Lung Technology and Prolonged Extracorporeal Perfusion (1975). Copenhagen.

# 11

# SUPPLEMENTARY EXTRACORPOREAL OXYGENATION(SECO): EXPERIMENTAL MODELS

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AND CH. R. H. WILDEVUUR

**Summary**

General methods for obtaining steady low levels of arterial oxygen saturation as models for supplementary extracorporeal oxygenation (SECO) treatable respiratory insufficiency (RI) were tested. Table 11.1 lists the criteria applied. Ventilation with low oxygen was deemed the preferred method for quantitative evaluation of the oximetric effect of SECO.

## 11.1 Introduction

Acute respiratory failure (ARF) can be defined as 'a state in which the partial arterial oxygen pressure ($PaO_2$) is below the predicted normal range for the patient's age at the prevalent barometric pressure (in the absence of intracardiac right-to-left shunting) or the $PaCO_2$ above 50 mm of mercury, not due to compensation for metabolic alkalaemia' (Pontoppidan *et al.*, 1972). Pathophysiologically, the two most important conditions in ARF are an increase in pulmonary extravascular water leading to interstitial and alveolar oedema and abnormal gas distribution (Pontoppidan *et al.*, 1972; Staub, 1970). The result is a ventilation-perfusion imbalance, an intrapulmonary shunt, a reduction in functional residual capacity (FRC) and a decrease in pulmonary compliance (Pontoppidan *et al.*, 1972). Successful treatment of ARF is reported by several authors using continuous positive pressure breathing (Uzawa and Ashbaugh, 1969), but recently interest is growing for the use of supplementary extracorporeal oxygenation (SECO), in an attempt to treat patients with severe hypoxia and lung oedema. To test the potentials of SECO treatment for patients with ARF, animal experiments have been performed. For quantitative oximetric studies of SECO an animal model, simulating clinical ARF, should meet the requirements now listed.

(1) Constancy: for the quantification of SECO effects on arterial blood haemoglobin $O_2$ saturation ($SaO_2$), the induced respiratory insufficiency (RI) should not change during bypass.

(2) Predictability: the desired level of RI ($SaO_2$) should be reached without the need for time-consuming adjustments.
(3) Reproducibility: to compare the effect of variables, the same starting conditions are necessary.
(4) No side effects: major circulatory disturbances, other than those related to the state of RI, should be prevented.
(5) Simplicity: the extensive set-up of SECO experiments is not compatible with complicated models of RI.

In a series of 42 dogs, five models of inducing artificial respiratory insufficiency were evaluated. Each model was assessed according to the above criteria.

## 11.2 Materials and Methods

In all the experiments the dogs were anaesthetised with intravenous sodium thiopental, paralysed with suxamethonium chloride and intubated with a cuffed endotracheal tube. Pethidine was given intravenously for analgesia. These pharmaca were given repeatedly as needed during the experiments. The dogs were placed in the supine position and artifically ventilated with room air (Engström model 311). Catheters were placed in the femoral artery and pulmonary artery for continuous pressure recording (Statham P23BB and Statham P23DB) and for blood gas sampling (AVL Blood gas analyser system Harnoncourt). Cardiac output was measured by thermodilution with the injection catheter in the right atrium and the thermistor catheter in the pulmonary artery (positions checked by pressure tracings). Arterial saturation was continuously monitored using an intravascular fiberoptic $SaO_2$ oximeter (Schwartzer Fiberoptic Oximeter IV H - 3). Tracheal pressure, temperature and urine production were also constantly monitored while haemoglobin and haematocrit were measured at intervals.

## 11.3 Results and Discussion

*Alloxan-induced respiratory insufficiency*

Injection of alloxan (an antineoplastic agent), causing capillary endothelial lesions in the lung, results in a severe pulmonary oedema resembling clinical ARF (Aviado and Schmidt, 1957; Gruhzit *et al.*, 1951. Hyman *et al*, 1972; Staub *et al.*, 1967. The effect of alloxan on the $SaO_2$ is given in figure 11.1. With a dose of 25 mg/kg a major circulatory effect was already seen, with only a slight decrease in $SaO_2$. When larger amounts of alloxan were given, severe changes of the electrocardiogram were observed simultaneously with a fall in cardiac output and blood pressure. Furthermore, because alloxan is an unstable solution, no predictable nor reproducible results could be obtained.

It was concluded that alloxan-induced RI does not meet the requirements listed (table 11.1).

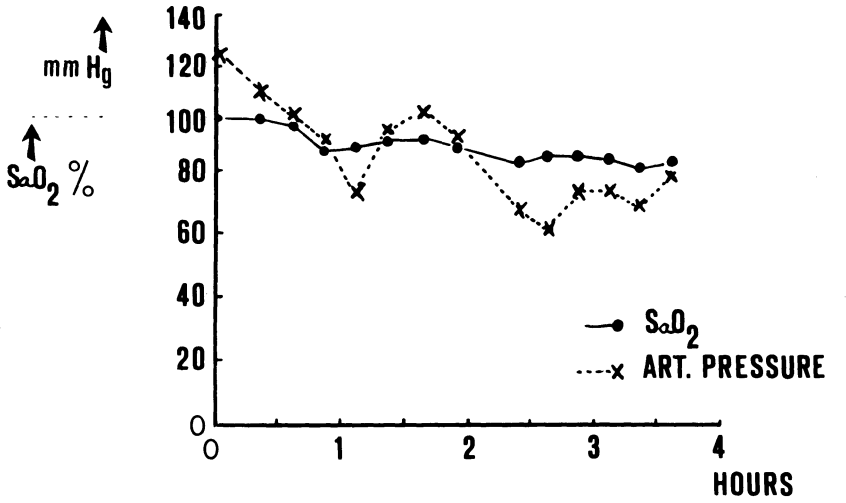


Figure 11.1 Injection of 25 mg/kg alloxan in the pulmonary artery. Small decrease of $SaO_2$ with major decrease in systemic blood pressure

**Table 11.1**
Criteria applied to experimental models

| | *Constant* | *Predictable* | *Reproducible* | *Circulatory side effects* | *Simplicity* | *Clinical ARF* |
|---|---|---|---|---|---|---|
| Alloxan | – | – | – | ++ | + | ++ |
| Oleic acid | ± | ± | + | + | + | ++ |
| Right-to-left shunt | – | ± | ± | ± | --- | ± |
| Hypoventilation | – | – | ± | — | ± | ± |
| Low $O_2$ mixtures | ++ | ++ | ++ | — | + | – |

*Right-to-left shunt*

One of the hallmarks of clinical acute respiratory failure is an imbalance between the alveolar ventilation ($\dot{V}/A$) and the pulmonary blood flow rate ($Q$) and an intrapulmonary right-to-left shunt (Pontoppiddan *et al.*, 1972) resulting in a decrease in $SaO_2$. In the animal experiments this pathophysiological mechanism was simulated by creating an intrathoracic noncardiac right-to-left shunt.

After inducing anaesthesia, a left-sided thoracotomy was performed. The left lung was taken out and the left pulmonary artery directly anastomosed to the left atrium. A snare ligature was placed around the pulmonary artery to control the degree of occlusion, so that any desired level of $SaO_2$ could be obtained (figure 11.2). However, the $SaO_2$ initially obtained tended to decrease. This decrease was probably caused by an increase of vascular resistance in the right lung due to the general hypoxia, while the snare ligature around the left pulmonary artery gave a fixed resistance. This would increase the rate of shunt to total flow

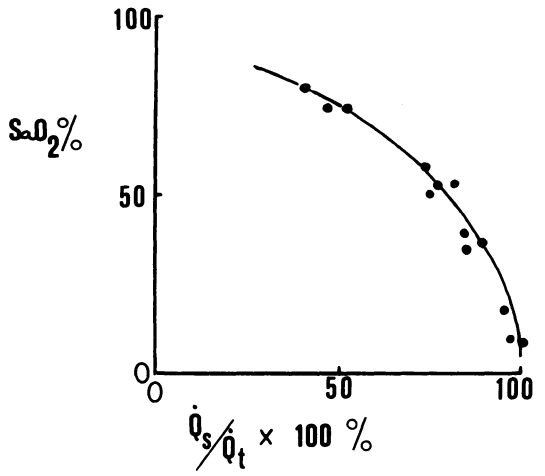


Figure 11.2 Right-to-left shunt by anastomosing the left pulmonary artery to the left atrium. $SaO_2$ related to the ratio of shunt rate of flow ($\dot{Q}_S$) to total rate of flow ($\dot{Q}_t$)

($\dot{Q}_s/\dot{Q}_t$) and consequently decrease $SaO_2$.

Therefore, quite apart from the very laborious operative procedure, this RI model was not judged to be suitable for SECO experiments (table 11.2).

*Oleic acid-induced respiratory insufficiency*

Injection of oleic acid into the right heart or pulmonary artery is known to cause haemorrhagic pulmonary oedema (Ashbaugh and Uzawa, 1968; Gemer *et al.*, 1975), resulting in an intrapulmonary shunt with progressive hypoxaemia. Oleic acid was injected via a catheter into the pulmonary artery in 2 injections of respectively 0.250 and 0.125 ml/kg with an interval of 1 - 2 h. The course of the $SaO_2$ of a typical experiment is given in figure 11.3. After the first injection of oleic acid there was a gradual decrease of $SaO_2$. Following the second dose a more rapid and substantial decrease was seen. The degree of saturation decrease was, to a certain extent, related to the dosage given. For the same dosages reproducible results could be obtained.

In all experiments a rise in haematocrit combined with a decrease in arterial blood pressure was seen, due to loss of intravascular fluid into the lung parenchyma. This loss had to be replaced by dextran solution in order to maintain systemic blood pressure. With this correction of fluid balance, no major circulatory side effects were observed during the experiments.

This model of RI, which resembles the clinical picture of fat embolism, is simple to institute and well controllable in its haemodynamic side effects. As such it is in general a useful model of RI. However, the inadequate predictability of the effect of dosage and the short-lasting constancy of $SaO_2$ prevent its use in specific oximetric SECO studies.

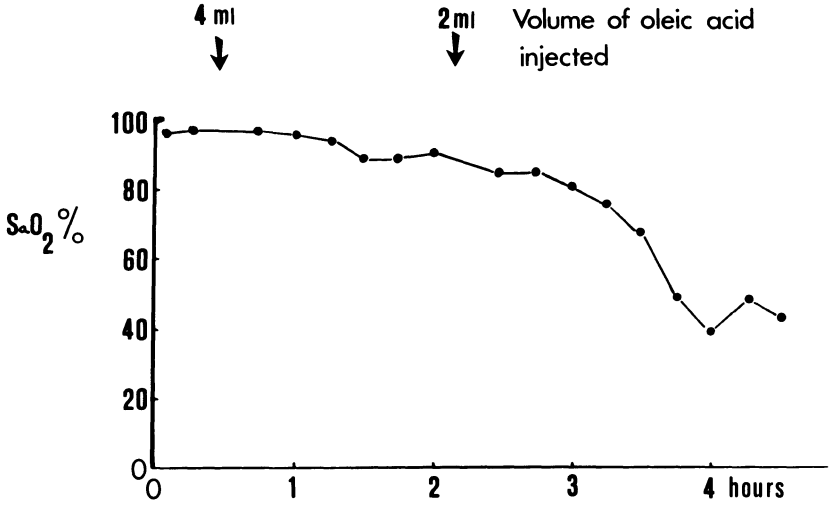


Figure 11.3 Injections of oleic acid in the pulmonary artery. Progressive decrease of $SaO_2$ after the second injection

*Hypoventilation with or without positive end-expiratory pressure (PEEP)*
Hypoventilation disturbs the ventilation-perfusion ratio, resulting in a decrease in $SaO_2$. In this respect hypoventilation resembles ARF. Hypoventilation was instituted in dog experiments by decreasing tidal volume, while respiratory frequency was kept constant. A moderate intra-individual reproducibility could be obtained, but there was a poor relationship between the proportionate

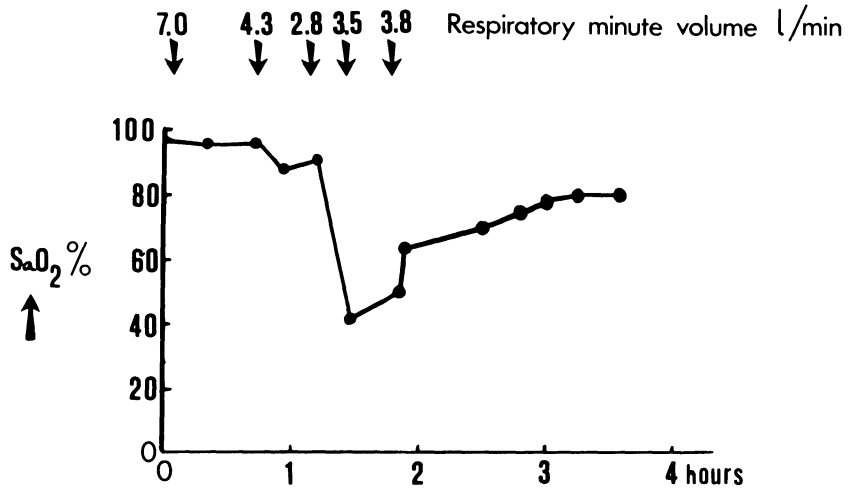


Figure 11.4 Hypoventilation with PEEP (+50 mm $H_2O$): relation of $SaO_2$ to respiratory minute volume

decrease of tidal volume and that of $SaO_2$. Moreover no constancy of $SaO_2$ could be obtained. This might be partly caused by the development of micro-atelectases as observed in longer lasting experiments. In an attempt to prevent these micro-atelectases, positive end-expiratory pressure was added to the hypoventilation, but no substantial improvements were obtained.

The large responses in $SaO_2$ for small tidal volume changes and the slow establishment of a new steady state made the procedure of adjusting tidal volumes difficult and time-consuming (figure 11.4). For this reason hypoventilation is not considered a practicable model of respiratory insufficiency for SECO studies.

*Ventilation with low oxygen mixtures*

The ventilation with gases, containing less than 20.9% oxygen, only simulates the effect of ARF on the blood-oxygen content (hypoxia) without any resemblance to its pathophysiology. Normoventilation with room air was instituted, before the effects of various inspired fractional $O_2$ concentrations ($FiO_2$) were

**Table 11.2**

Mean values and curves of three series

| $FiO_2$ | $SaO_2$ % | S.D. | n |
|---|---|---|---|
| 0.123 | 66 | 4.0 | 9 |
| 0.086 | 49 | 2.4 | 13 |
| 0.079 | 45 | 0.9 | 4 |

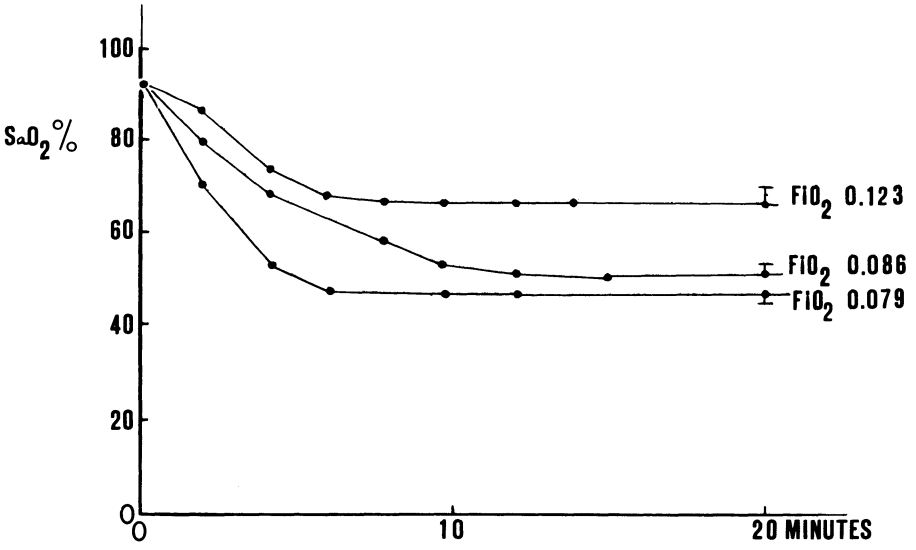


Figure 11.5 Mechanical ventilation with low-oxygen mixtures, containing 5% $CO_2$. Rapid development of steady $SaO_2$ levels

tested in combination with $FiCO_2$ of 0.05. The mean values and curves of three series are given in table 11.2 and figure 11.5. A stable constant level of $SaO_2$ was always reached within 15 min and lasted over 1h. The level of $SaO_2$ reached was $FiO_2$ dependent and predictable. A high intra-individual and inter-individual reproducibility was obtained.

Apart from the poor resemblance to clinical ARF, the hypoxic effect of the method meets the requirements needed for reliable quantitative oximetric SECO studies.

## 11.4 References

Ashbaugh, D. G. and Uzawa, T. (1968). Respiratory and haemodynamic changes after injection of free fatty acids. *J. Surg. Res.*, **8**, 417.

Aviado, D. and Schmidt, C. F. (1957). Pathogenesis of pulmonary oedema by alloxan. *Circ. Res.*, V, March.

Gemer, M., Dunegan, L. J., Lehr, J. L., Bruner, J. D., Stets, C. W., Don, H. F., Hayes, J. A. and Drinker, P. A. (1975). Pulmonary responses induced by oleic acid in the sheep. *J. Thorac. Cardiovasc. Surg.*, **69**, 793.

Gruhzit, C. C., Peralta, B. and Moe, G. K. (1951). The pulmonary arterial pressor effect of certain sulfhydryl inhibitors. *J. Pharm. Exp. Ther.*, **101**, 107.

Hyman, A. L., Pennington, D. G. and Jaques, W. E. (1972). Pulmonary responses to alloxan. *J. Pharm. Exp. Ther.*, **181**, no. 1.

Pontoppidan, H., Geffin, B. and Lowenstein, E. (1972). Acute respiratory failure in the adult. *New Engl. J. Med.*, **287**, 690.

Staub, N. C. (1970). The pathophysiology of pulmonary oedema. *Hum. Path.*, **1**, 419.

Staub, N. C., Nagona, H. and Pearce, M. L. (1967). Pulmonary oedema in dogs, especially the sequence of fluid accumulations in lungs. *J. Appl. Physiol.*, **22**, 227.

Uzawa, T. and Ashbaugh, D. G. (1969). Continuous positive-pressure breathing in acute haemorrhagic pulmonary oedema. *J. Appl. Physiol.*, **26**, 427.

# 12

# ROLE OF VENO-ARTERIAL BYPASS ON NATURAL LUNG OXYGEN TRANSFER DURING LONG-TERM RESPIRATORY ASSISTANCE WITH MEMBRANE LUNG OXYGENATOR

F. LEMAIRE, T. DARRAGON, F. JARDIN, B. TEISSEIRE, D. LAURENT and M. RAPIN

**Summary**

Clinical investigations of ten cases of acute respiratory failure who were given long-term respiratory assistance are presented. In these investigations left ventricular blood sampling was used for easier assessment of the natural lung oxygen transfer and pulmonary haemodynamics.

Improvements of pulmonary oxygen transfer efficiency as shown by the increased left ventricular arterial oxygen partial pressure and by the reduced shunting may be due to the shift of oedema or to the increased ventilation – perfusion ratio ($\dot{V}/\dot{Q}$) related to pulmonary blood flow reduction.

Our data emphasise the inadequacy of arterial oxygen measurements as an indicator of pulmonary improvement without the concomitant measurement of pulmonary blood flow.

## 12.1 Introduction

During the past five years several teams have successfully used extracorporeal perfusion with a membrane oxygenator to treat patients with overwhelming acute pulmonary failure considered irreversible by current medical treatment (Hill *et al.*, 1973; Bartlett *et al.*, 1974).

The two aims of extracorporeal membrane oxygenation (ECMO) are to maintain life by preventing hypoxia and hypercapnia and to provide a better a better opportunity for healing of the pulmonary injury (Zapol *et al.*, 1975).

The increase in arterial oxygen content with initiation of bypass is the easiest result to observe but the mechanisms of this improvement are far from being unequivocal. Furthermore, the prognostic significance of such improvement must be challenged (Lemaire *et al.*, 1974).

ECMO may contribute to an increase in systemic arterial oxygen tension by directly supplying oxygenated blood to systemic arteries and/or by increasing the

mixed venous oxygen content (Corso *et al.*, 1974; Hanson *et al.*, 1974; Soeter *et al.*, 1973).

Another effect of ECMO could be an improvement of pulmonary $O_2$ transfer efficiency by lung water shift or an improved ventilation perfusion relationship induced by acute reduction of pulmonary perfusion (Geelhoed *et al.*, 1974; Hill *et al.*, 1973; Zapol *et al.*, 1975).

Retrograde flow of the oxygenator-return up the aorta during veno-arterial bypass and increased venous content had been documented elsewhere (Hanson *et al.*, 1974). On the other hand changes in $\dot{V}_A/\dot{Q}$ and the ratio of pulmonary shunt to total flow ($\dot{Q}_s/\dot{Q}_t$) are poorly documented in man. Accurate measurement of these parameters requires sampling from the left side of the heart since aortic sampling may be contaminated by the retrograde extracorporeal blood flow (ECBF).

The purpose of the clinical study presented was to evaluate the pulmonary $O_2$ transfer during veno-arterial bypass (using a catheter positioned in the left ventricle) especially in relation to changes in extracorporeal blood flow rate.

## 12.2 Material and Methods

*Patients* Ten cases of acute respiratory failure were selected for ECMO using the protocol for extracorporeal support of the US National Heart and Lung Institute (1974).

The age of the patients ranged from 22 to 57 years with an average of 37 years. The clinical diagnosis and course during ECMO are summarised in table 12.1. Prior to the decision to use ECMO, patients were treated with maximum conventional respiratory care including volume mechanical ventilation (MV) (Engström) with positive end expiratory pressure (PEEP) and increasing oxygen concentration.

Despite immediate clinical improvement on commencement of ECMO death occurred in every case during or after ECMO.

*ECMO* was carried out in the two intensive care units of the Ambroise Paré and Henri Mondor Hospitals. The circuit was veno-arterial in every case and the membrane oxygenator was that manufactured by Rhone-Poulenc with pressure servocontrolled pumps and silicone rubber circuitry. Venous blood was drained from cannulae inserted through femoral and internal jugular veins into both vena cavae. Femoral arterial bidirectional perfusion was used with additional right axillary artery perfusion. The duration of bypass ranged from 12 h to 144 h (mean 80 h). ECBF rate was $2.7 \pm 0.5$ l/min. Membrane lung transfer was about 30 ml/min/$m^2$.

*Measurements* Right atrial, pulmonary arterial and wedge pressures were monitored from a 7 F Swan Ganz catheter. Left radial arterial pressure was registered through a 5 F Teflon needle. A catheter (6 F pigtail Cordis) was positioned in the left ventricle (Nitemberg *et al.*, 1974) in order to determine the afterload effect due to arterial injection and to sample blood processed by the lung without direct

**Table 12.1**

Summary of clinical data for ten patients treated with extracorporeal membrane oxygenation (ECMO)

| *Case number* | *Patient (age, sex)* | *Diagnosis* | *Associated disease* | *Duration of MV before ECMO* | *Duration of ECMO* | *Outcome* |
|---|---|---|---|---|---|---|
| 1 | D (35, F) | Influenza Pneumonia | – | 1 day | 72 hours | Died, 7 days after ECMO |
| 2 | G (38, F) | Gram negative Septicaemia | – | 10 days | 12 hours | Died |
| 3 | B (45, F) | Shock lung | – | 15 days | 105 hours | Died |
| 4 | N.P. (67, M) | Influenza Pneumonia | Ischaemic Heart Disease | 2 days | 144 hours | Died (infection) |
| 5 | Y (25, M) | Busulphan Overdose | – | 3 days | 96 hours | Died, 1 day after ECMO |
| 6 | G (45, M) | Barbiturate Overdose | – | 4 days | 96 hours | Died, 4 days after ECMO |
| 7 | H (23, M) | Shock lung | – | 12 days | 48 hours | Died |
| 8 | D (22, M) | Influenza Pneumonia | Hodgkin | 5 days | 48 hours | Died |
| 9 | T (37, M) | Influenza | – | 1 day | 96 hours | Died, 5 days after ECMO |
| 10 | G (32, F) | Aspiration Pneumonia | – | 4 days | 88 hours | Died |

contamination by ECBF. Pressures were measured with Statham P 23 DB transducers.

Pulmonary blood flow (PBF) was determined using injection of indocyanine green into the pulmonary artery with sampling from the left ventricle (the thermodilution technique was not used because of the venous cannula suction). ECBF was measured with an ultrasonic Delalande directional flowmeter.

Left radial arterial $O_2$ tension was used as an index of the mixing of blood flowing from the left ventricular and femoral artery cannula.

Left ventricular arterial blood oxygen partial pressure ($LVPaO_2$) was used as an index of natural lung $O_2$ exchange. $PaO_2$, pH, $PaCO_2$ were measured with a Radiometer BMS 3 and $O_2$ saturation with OSM 1.Blood contents were determined by the Van Slyke method or by calculation.

The $\dot{Q}_s/\dot{Q}_t$ ratio was calculated from the shunt equation, using left ventricular and pulmonary artery blood samples after 20 minutes of ventilation with fractional inspired $O_2$ concentration ($FiO_2$) equal to 1.0. Oxygen uptake from the natural lung ($L\dot{V}O_2$) was calculated as the product of PBF value and the difference between arterial and mixed venous $O_2$ content. Extracorporeal $O_2$ transfer ($EC\dot{V}O_2$) was assessed as the product of the ECBF by the oxygenator and the difference between the oxygenator outlet and inlet $O_2$ content. It was assumed that the total $O_2$ consumption ($T\dot{V}O_2$) was equal to $L\dot{V}O_2 + EC\dot{V}O_2$.

In order to evaluate the precise mechanism of improvement in systemic oxygenation which occurred during veno-arterial bypass $L\dot{V}PaO_2$ and $\dot{Q}_s/\dot{Q}_t$ measurements were performed with rapid ECBF variations being induced.

## 12.3 Results

*Haemodynamic data* Table 12.2 summarises the haemodynamic data we obtained before, during and after ECMO. Pulmonary artery pressure and flow rate

**Table 12.2**
Haemodynamic data before, during and after ECMO

| | *ECMO (veno-arterial bypass)* | | |
|---|---|---|---|
| | *Before* | *During* | *After* |
| Mean aortic pressure (PAO) mm Hg | 46.3 ± 24 | 67.7 ± 18 | 62.8 ± 28 |
| Pulmonary capillary (wedge) pressure (PCP) mm Hg | 8.6 ± 3.4 | 6.3 ± 3 | 4.7 ± 0.6 |
| Pulmonary artery pressure (PAP) mm Hg | 22 ± 7 | 17 ± 5 | 19 ± 7 |
| Left ventricular end diastolic pressure (POD) mm Hg | 6.6 ± 3 | 5.4 ± 4 | 4.7 ± 1 |
| Pulmonary blood flow rate $\dot{Q}_L$ l/min | 5.8 ± 1 | 3 ± 1 | 6.2 ± 1.4 |

were markedly reduced by veno-arterial bypass. High bypass flow did not increase the left ventricular end diastolic and wedge pressure. Mean aortic pressure increased during veno-arterial bypass.

*Gas exchange data* Total $O_2$ uptake during ECMO did not change markedly from the pre-bypass value (table 12.3). For an average flow rate of 2.7 l/min the $E\dot{C}VO_2$ was about half the $T\dot{V}O_2$.

The blood gas data course is summarised in table 12.4. During ECMO, increased $L\dot{V}PaO_2$ and decreased $\dot{Q}_s/\dot{Q}_t$ were observed. Mixed venous blood $O_2$ saturation was not substantially increased.

During rapid ECBF rate variation $L\dot{V}PaO_2$ increased immediately with acute reduction of $\dot{Q}_s/\dot{Q}_t$ as PBF decreased.

In some cases (Hanson *et al.*, 1974) increase of $L\dot{V}PaO_2$ and decrease of $\dot{Q}_s/\dot{Q}_t$ were concomitant with a chest radiography. Clearly this longer-term effect suggested the disappearance of alveolar oedema.

## 12.4 Discussion

Several authors have shown that ECMO, by supplying $O_2$ exchange, may provide life support for patients with severe hypoxaemia.

In this work oxygen exchange measurements were performed to elucidate the

**Table 12.3**

Oxygen uptake and pulmonary blood flow before, during and after bypass

| | | *ECMO* | | |
|---|---|---|---|---|
| | | *Before* $n = 9$ | *During* $n = 9$ | *After* $n = 5$ |
| $\dot{Q}_L$ | l/min | 5.8 ± 1 | 3 ± 1 | 6.2 ± 1.4 |
| $\dot{Q}_{EC}$ | l/min | – | 2.7 ± 0.5 | – |
| $\dot{Q}_{L+EC}$ | l/min | – | 5.7 ± 0.7 | – |
| $\dot{V}O_2L$ | ml $O_2$/min | 278 ± 72 | 149 ± 45 | 352 ± 56 |
| $\dot{V}O_2$ EC | ml $O_2$/min | – | 131 ± 34 | – |
| $\dot{V}O_2$ EC + L | ml $O_2$/min | – | 280 | – |

**Table 12.4**

Blood gas data

| | *ECMO* | | |
|---|---|---|---|
| | *Before* $n = 9$ | *During* $n = 9$ | *After* $n = 5$ |
| $L\dot{V}PaO_2$ mm Hg ($FiO_2 = 1$) | 56.7 ± 19.4 | 70.5 ± 26.6 | 129 ± 80 |
| $SvO_2$ % | 53.3 ± 14 | 56 ± 9 | 51.6 ± 18.3 |
| $\dot{Q}_s/\dot{Q}_t$ % | 42 ± 11.5 | 36.5 ± 11.3 | 33 ± 13.6 |

mechanisms of systemic arterial $O_2$ tension improvement during veno-arterial bypass. This may include the following techniques (Hanson *et al.*, 1974).

(1) Direct perfusion by ECMO returning blood into the systemic arteries.
(2) The increase of the $O_2$ saturation of mixed venous blood.
(3) Improved relationship between ventilation and perfusion.

Left ventricular sampling permits elimination of the direct perfusion contribution. In our cases $L\dot{V}O_2$ increased slightly but for a given shunting there is a close relationship between $PaO_2$ and $L\dot{V}O_2$. Since $L\dot{V}O_2$ is only slightly increased, the $L\dot{V}PaO_2$ increase and the $\dot{Q}_s/\dot{Q}_t$ reduction suggest improvement of lung performance. Reduction of alveolar oedema is most likely the cause of decreased shunting as indicated by the observed progressive clearing of the chest X-rays. When ECBF is rapidly increased instantaneous reduction of $\dot{Q}_s/\dot{Q}_t$ cannot be explained by the shifting alveolar oedema which is a longer-term effect.

The relationship between PBF and $\dot{Q}_s/\dot{Q}_t$ suggests that the acute reduction of perfusion excludes from the pulmonary circulation the poorly ventilated areas of the lung (derecruitment phenomenon). Our data suggest that improvement in $\dot{Q}_s/\dot{Q}_t$ should not be used as a pulmonary performance index without concomitant measurement of PBF (Nitemberg *et al.*, 1974).

## 12.5 References

Bartlett, R. H., Cazzaniga, A. B., Fong, S. W. and Burns, N. E. (1974). Prolonged extracorporeal cardiopulmonary support in man. *J. Thorac. Cardiovasc. Surg.*, **68**, 918 - 932.

Corso, P. J., Geelhoed, G. W. and Joseph, W. L. (1974). Cardiopulmonary changes with venoarterial bypass in the hypoxic primate. *J. Surg. Res.*, **16**, 318 - 323.

Geelhoed, G. W., Corso, P. and Joseph, W. L. (1974). The role of membrane lung support in transient acute respiratory insufficiency of *Pneumocystis carini* pneumonia. *J. Thorac. Cardiovasc. Surg.*, **68**, 802 - 809.

Hanson, E. L., Drinker, P. A., Don, H. F., Van Devanter, S. H., Bonnet Eymard, J. and Moore, F. D. (1974). Veno-arterial bypass with a membrane oxygenator: successful respiratory support in a woman following pulmonary haemorrhage secondary to renal failure. *Surgery*, **75**, 557 - 565.

Hill, J. D., O'Brien, T. G., Murray, J. J., Dontignez, O. O., Bramson, M. L., Osborn, J. J. and Gerbode, F. (1973). Extracorporeal oxygenation for post traumatic respiratory failure (shock-lung syndrome). Use of the Bramson membrane lung. *New Engl. J. Med.*, **286**, 629 - 634.

Lemaire, F., Teisseire, B., Harari, A. and Loisance, D. (1974). La $PaO_2$ en oxygène pur est-elle un critère fiable du transfert pulmonaire d'oxygène au cours des assistances respiratoires par poumon à membrane? *Nouv. Presse Med.*, **3**, 1815.

National Heart and Lung Institute (1974). Division of lung diseases. Protocol for extracorporeal support for respiratory insufficiency. Collaborative Program, May.

Nitemberg, A., Lemaire, F., Lellouche, D. and Laurent, D. (1974). Hemodynamique gauche au lit du malade. *Nouv. Presse Med.*, **3**, 2028.

Soeter, J. R., Smith, G. T., Anema, R. J., Suehiro, G. T., and McNamara, J. J. (1973). Distribution of oxygenated blood in femoral and brachial arterv perfusion during veno arterial bypass in primates. *J. Thorac. Cardiovasc. Surg.*, **65**, 825 - 828.

Zapol, W. M., Quist, J., Pontoppidan, H., Liland, A., McEnamy, T. and Laver, M. B. (1975). Extracorporeal perfusion for acute respiratory failure. *J. Thorac. Cardiovasc. Surg.*, **69**, 439 - 449.

# 13

# AN EXPERIMENTAL MODEL OF RESPIRATORY INSUFFICIENCY FOR SUPPLEMENTARY EXTRACORPOREAL OXYGENATION

H. C. SCHIPPERS, E. T. HULL, P. H. MOOK,
H. HEEMSTRA and Ch. R. H. WILDEVUUR

**Summary**

Acute respiratory failure (ARF) is mostly a nonspecific reaction resulting in an increase in pulmonary extravascular water (PEVW), culminating in alveolar oedema (Pontoppidan *et al.*, 1972) leading to a decrease in arterial oxygen haemoglobin saturation ($SaO_2$). Therefore optimal treatment of ARF should supply the additional oxygen requirements to provide life support and should result in a decrease of PEVW allowing the lung to recover. Supplementary extracorporeal oxygenation (SECO) is a new treatment of ARF which offers these potentials. To experimentally study the therapeutic effects of SECO an analogous clinical picture must be induced with an increase in PEVW and a reproducible decrease of $SaO_2$. For this purpose we have studied the effects of alloxan, which has been described as mimicking septic shock (Pontoppidan *et al.*, 1972), and oleic acid which mimics fat embolism (King *et al.*, 1971). In these experiments emphasis was placed upon continuous measurement of the development of respiratory insufficiency to enable accurate assessment of effects..

## 13.1 Methods

Ten mongrel dogs weighing from 18 - 26 kg were used for the experiments. Anaesthesia was induced with 30 mg/kg thiopental sodium, additionally 50 mg pethidine were administered intramuscularly. The dogs were placed in the supine position. Muscle relaxation was induced and maintained with succinylcholine and the dogs were artificially ventilated (Engström model 311) through a standard cuffed endotracheal tube. A catheter was placed in the pulmonary artery via the right jugular vein for measurement of pulmonary artery pressure (PAP) (Statham P23BB) and for injection of tricarbocyaninum for cardiac output (CO) measurements. A catheter was placed in the left femoral artery for arterial pressure (FAP) measurements (Statham P23DB) and blood withdrawal for dye dilution curves (Waters densitometer D401; Howard withdrawal pump 2603). A fiberoptic catheter (Schwartzer F7) was placed in the thoracic aorta via the right femoral artery for continuous measurement of arterial oxygen saturation (Schwartzer

Fiberoptic Oximeter IV H - 3). Electrodes (Hull *et al.*, 1976) were glued bilaterally to the skin over the 6th ribs for continuous measurement of transthoracic electrical impedance (TEI) (Minnesota Impedance Cardiograph model 302). Lead II ECG was continuously recorded (Hewlett Packard 8811A). Rectal temperature was monitored and maintained with a heated mattress.

Respiratory insufficiency was induced with either alloxan or oleic acid injected intravenously as follows:

alloxan or oleic acid in- mg/kg, 100 mg/kg, and two sequential injections of 50 mg/kg separated by 30 min.

Oleic acid: four dogs with doses of 0.25 ml/kg, 0.44 ml/kg, 0.50 kl/kg and 0.56 ml/kg. The injections were slowly administered over a 10 min period.

Oleic acid: three dogs receiving a dose of 0.30 ml/kg over a 10 min period.

In the oleic acid experiments $CaCl_2$ was given additionally because of the binding capacity of oleic acid for calcium. During development of pulmonary oedema about 400 ml of a gelatine plasma expander (haemacel) was infused to prevent hypovolaemia.

## 13.2 Results

*Alloxan experiment*

Respiratory insufficiency did not develop with a dose of 50 mg/kg. A dose of 100 mg/kg resulted in immediate ECG changes followed by cardiac failure and death of the dog within 30 min. There were only slight decreases in $SaO_2$ and TEI. Two doses of 50 mg/kg 30 min apart resulted in a gradual decrease in $SaO_2$ to 55%, within 65 min after the second injection, and a decrease of 16 ohms in the TEI. However, these effects were accompanied by ECG changes and a steady fall in cardiac output to less than 0.6 l/min.

*Oleic acid experiment: dose dependency*

The response of $SaO_2$ and TEI after doses of 0.25, 0.44, 0.50 and 0.56 ml/kg appear in figure 13.1. The larger doses resulted in more rapid decreases in both $SaO_2$ and TEI. An initial increase of TEI was observed in the two dogs which received 0.50 and 0.56 ml/kg. This coincided with a sharp increase in PAP and concomitant decreases of FAP and CO.

In the dogs receiving 0.25 and 0.44 ml/kg oleic acid PAP increased only slightly and FAP with CO initially decreased but recovered to subnormal levels. With these doses ECG changes were only seen secondary to low $SaO_2$. The dosage of 0.30 ml/kg in the following experiments was chosen to cause a more rapid development of respiratory insufficiency than that achieved with 0.25 ml/kg, and avoid severe haemodynamic changes as observed with doses of 0.50 and 0.56 ml/kg.

*Reproducibility of 0.30 ml/kg oleic acid*

The responses of $SaO_2$ and TEI in these three dogs appear in figure 13.2. $SaO_2$ decreased to a value of 50% within 40, 55 and 65 min after the start of injections of

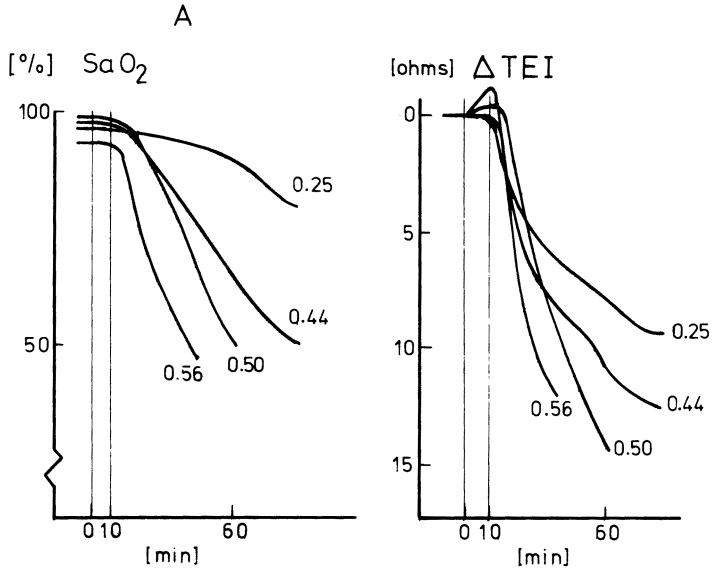


Figure 13.1 The response of arterial oxygen saturation ($SaO_2$) and the decrease from control value of transthoracic electrical impedance (Δ TEI) after oleic acid injection in 4 dogs. Oleic acid in the indicated dosage (ml/kg) was slowly injected over a 10 min period

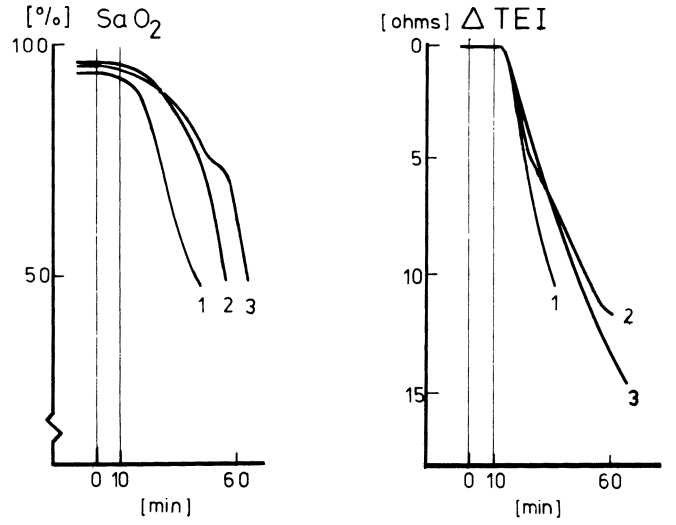


Figure 13.2 The response of arterial oxygen saturation ($SaO_2$) and the decrease from control value of transthoracic electrical impedance (Δ TEI) after 0.30 ml/kg oleic acid injections in 3 dogs. The injections were administered slowly over a 10 min period

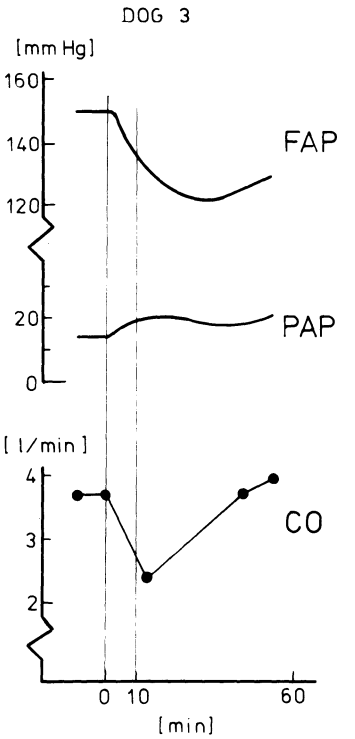


Figure 13.3 The responses of femoral artery pressure (FAP), pulmonary artery pressure (PAP) and cardiac output (CP) of dog 3 (see figure 13.2) after a 0.30 ml/kg injection of oleic acid administered slowly over a 10 min period

oleic acid, and the corresponding decreases in TEI were 10, 12 and 14 ohms. Initial increases in TEI were not observed and the haemodynamic condition of these dogs remained good as typically illustrated for dog 3 of this series (figure 13.3). PAP increased from 14 to 22 mm Hg, FAP decreased from 150 to 120 mm Hg and then recovered to 130 mm Hg with the CO decreasing from 3.7 to 2.4 l/min and then increasing to 4 l/min.

## 13.3 Discussion

Maintenance of satisfactory $SaO_2$ and a decrease of pulmonary extravascular water should result from any long-term treatment of ARF patients. Only continuous measurement of these two parameters allows immediate and accurate assessments of the course of ARF and the efficacy of the initiated therapy.

Changes in the value of transthoracic electrical impedance measurements have been shown to correlate with changes in intrathoracic fluid volume (Luepker *et al.*, 1973; Pomerantz *et al.*, 1970). We have previously demonstrated that through the use of specially constructed electrodes movement artifacts in TEI can be largely eliminated (Hull *et al.*, 1976), allowing accurate long-term monitoring.

The fiberoptic oximeter gives continuous reading of $SaO_2$ (Krauss *et al.*, 1972). In the range of 40 to 100% $O_2$ saturation the standard error of *in vivo* fiberoptic measurements as compared with *in vitro* spectrophotometric determination was 2.7 (author's unpublished data). In the experiments reported here the continuous registration of TEI and $SaO_2$ gave an instantaneous picture of the development of respiratory insufficiency. This allows comparison of the effects of different SECO circuits, having the same predetermined starting condition of $SaO_2$ or TEI.

Alloxan, in doses large enough to cause respiratory insufficiency (RI), had a direct toxic effect on the heart as seen by early changes in ECG. This side effect was considered to be unacceptable in a model of ARF for studying the therapeutic effects of SECO.

The effect of oleic acid observed in our experiments can be summarised as follows:

(1) The initial dose-dependent effect, causing immediate increases in PAP and TEI and decreases in FAP and CO, can be explained by severe lung embolism. The initial increase in TEI is probably due to a decrease in pulmonary venous blood volume. With doses of 0.30 ml/kg or less these acute haemodynamic effects were easily tolerated by the dogs.
(2) With a dose of 0.30 ml/kg the interindividual variability of the response of TEI and $SaO_2$ is considered to be acceptable. Decreases in $SaO_2$ to 50% were achieved within a reasonable length of time (40 - 65 min).

In conclusion: oleic acid-induced RI presents an experimental model of ARF which is predictable and reproducible without major side effects. The pathophysiology, analogous to clinical pulmonary fat embolism, makes this model suitable for SECO studies.

## 13.4 References

Hull, E. T., Irie, T., Heemstra, H. and Wildevuur, Ch. R. H. (1976). Transthoracic electrical impedance - artifacts associated with electrode movement. *J. Appl. Physiol.*, (in press).

King, E. G., Wagner, W. W. Jr., Ashbaugh, D. G., Latham, L. P. and Halsey, D. R. (1971). Alterations in pulmonary micro-anatomy after fat embolism. *Chest*, **59**, May.

Krauss, X. H., Verdouw, P. D., Hugenholtz, P. G., Hagemeijer, F. and Polanyi, M. R. (1972). Continuous monitoring of $O_2$ saturation in the intensive care unit. *Circulation*, **46**, Suppl. II, 178.

Luepker, R. V., Michael, J. R. and Warbasse, J. R. (1973). Transthoracic electrical impedance: quantitative evaluation of a noninvasive measure of thoracic fluid volume. *Amer. Heart J.*, **85**, 83.

Pomerantz, M., Delgado, F. and Eiseman, B. (1970). Clinical evaluation of transthoracic electrical impedance as a guide to intrathoracic fluid volume. *Ann. Surg.*, **171**, 686.

Pontoppidan, H., Geffin, B. and Lowenstein, E. (1972). Acute respiratory failure in the adult (part 1). *New Engl. J. Med.*, **287**, 690.

# SESSION B DISCUSSION

*Galletti* commented that Dorson's experimental data regarding $CO_2$ transfer through microporous membranes indicated lower transport values than those predicted from membrane and fluid film characteristics. He asked if there was any experimental evidence for Dorson's suggestion that this was due to the incorporation of blood into the void space of the membrane material. He felt that other mechanisms might equally well be involved, such as the deposition of a protein film at the blood - gas interface, and/or condensation of water vapour on the gas side of the membrane. *Dorson* responding stated that all three mechanisms mentioned by Galletti had been investigated and that the blood incorporation hypothesis was the only one which matched the data. This implied the deposition of an extremely thin film of relatively stagnant material in the inter-nodule space and although the film resistance accounted for only 2% of the overall resistance to flow, it had a significant effect on the mass transfer. He stated that if membranes from the same batch were washed, dried and retested, reproducible data were obtained.

The topic of the effect of shear stresses on platelets was raised by *Feijen*. He asked Bellhouse what the range of shear stresses was in his oxygenator and were the shear stress effects the main cause of the platelet drop. *Bellhouse* said that some calculations had been made but that these were not carried to the level which would permit a definitive answer to Feijen's question. The large platelet drop that occurred within the first five minutes of the early animal experiments had nothing to do with shear stress or in general with any fluid mechanic effect, but could be attributed to poor priming of the system. He said that in subsequent experiments the system was primed with rheomacrodex which not only reduced the platelet drop but avoided it unless fluid stagnation occurred on first introduction of blood to the oxygenator.

*Feijen* then raised the topic of platelet damage as opposed to haemolysis. He pointed out that platelet damage was to be expected at shear stresses higher than 15 $N/m^2$. Yet the oxygenator design put forward by Smeby was designed for shear stress values some ten times this threshold. Would this not mean that platelet damage of a significant kind would occur well before serious haemolysis? *Smeby* agreed that the Taylor-vortex oxygenator was based on 150 $N/m^2$ as an upper limit for wall shear stress induced haemolysis. This limit was based on the observations of Sutera and Mehrjardi 1975 (p. 81) on the incidence of shear stress induced haemolysis. *Maini* supporting Smeby suggested that the order of magnitude of shear stresses in virtually every membrane oxygenator with blood flowing through small channels at a rate of 5 l/min would be likely to exceed 15 $N/m^2$.

*Galletti* wished to emphasise two points on the relationship between platelet damage and haemolysis. As demonstrated by de Jong there is a very early loss of platelets in any extracorporeal circuit which significantly occurs *before* the entire volume of blood in the body has come into contact with the unphysiological materials of the ECC. The phenomenon, therefore, must be related to the early exposure of the first platelets to the ECC. The second point related to the interaction between platelets and red blood cells under conditions of high stress. From the literature it would appear that at stresses below that inducing haemoglobin release (which requires red cell membrane rupture) ADP and other inducers of platelet aggregation are released. He therefore urged that the effects on platelets and red cells be considered as interrelated. Although haemolysis was originally taken as a primary indicator of blood damage, it now seems to be an upper limit. Sensitive systems capable, for example, of detecting platelet damage and thus identifying early signs of 'blood damage' should therefore be used. *Wildevuur* commented on the initial platelet drop aspect by re-emphasising de Jong's hypothesis that the release of bioamines was due to erythrocyte rather than platelet damage. The amount of bioamine needed to produce the massive platelet aggregations encountered could only be provided in the main by erythrocytes since these are present in the blood in far greater numbers than platelets. He suggested that with this in mind the use of drugs to reduce platelet drop should be directed to the protection of erythrocytes. *Galletti* added that there was evidence that if erythrocyte membranes were stabilised by drugs (such as chlorpromazine) there was indeed less platelet loss.

*Galletti* wished to know the diameter of the tubes in the Gaylor oxygenator and asked if smaller tubes of about 250 $\mu$m diameter would be more advantageous. *Gaylor* gave the inner diameter of the tubes of his oxygenator as 1 mm and pointed out that if smaller diameters were used the pumping frequency or the pump chamber length would have to be increased in order to achieve the same stroke volume or flow rate. He agreed that the performance of the oxygenator expressed per unit membrane area would in fact be increased for smaller diameter tubes. He then went on to put the following two questions to Bellhouse. What percentage of the membrane limitation was the $O_2$ transfer rate when using microporous membranes? Was an increase in pump rate (flow rate) achieved by changing the pump frequency? *Bellhouse* had no information on the first question and in answer to the second said that increase in pump rate was originally achieved by increasing the pumping frequency. The later system used a roller pump (without the non-return valve) which controlled the mean flow rate.

*Galletti* commented on a new point. Ten or fifteen years ago $O_2$ transport was the only consideration in membrane lung design whereas now, as demonstrated by Dorson, $CO_2$ has become its bottleneck. Similarly the problem of water vapour has been given little attention even although 25 - 30 g of water/h is collectable from the expelled gases. This could be of the order of 500 ml/min of water vapour, i.e. the same amount as the $O_2$ and $CO_2$ transport, yet it is mentioned only as a secondary complication caused by condensation in the cooler parts of the oxygen-

ator devices. There might therefore be a place in microporous membrane systems for a composite membrane incorporating a thin film coating which would act as a barrier and limit water vapour transport.

*Falke* asked the Groningen group: Did the administration of heparin produce endobronchial bleeding in oleic acid-induced pulmonary oedema? *Wildevuur* agreed that the use of heparin did produce bleeding problems. *Hull* added that severe pulmonary oedema does occur after injection of oleic acid and that the fluid lost must be replaced. *Kim* asked what their rationale was for using oleic acid and had other fatty acids been tried? Many investigators had observed that the introduction of fatty acids into animals caused death through mass emboli occluding the aorta. Had they experienced such fatalities? *Wildevuur* replied that there had been no such death. Further his group had found that when using an ultrasonic probe to attempt to detect microemboli, they found with bubble oxygenators that the probe responded to an increase in the oxygen input. This suggested that the increase in signal was due to dissolved oxygen rather than microemboli. *Ghadiali* agreed that oxygen bubbles could cause a false signal. This, however, did not seem to occur with membrane oxygenators.

A new topic was then raised by *Wildevuur*: the use of membrane rather than bubble oxygenation was without doubt an improvement, yet this to an extent was negated in cardiac surgery, for example by the problem of thrombogenesis which occurs due to the use of the suction techniques employed. He emphasised that the detrimental effect of the continued use of such surgical 'barbarities' must be kept in mind when improvements in the oxygenator components of the systems were being considered. He pointed out that Hill (Hill *et al.*, 1974, p. 69) had already shown that improvements to suction devices were possible and he hoped that this matter would be further explored. *Ghadiali* commented that new developments in the use of oxygenators would always be necessary and he thought that the major development in the future would be improvements in the membrane part of the oxygenator. He did not think that the blood damage caused by suction devices was any reason for not using membrane oxygenators. *Wyatt* pointed out that one advantage of membrane over bubble oxygenators was that the former did not increase peripheral resistance and that this benefit was not negated by the suction techniques. Experience with a TEFLO (membrane) oxygenator indicated that one out of thirty-five patients required vasodilation whereas with bubble oxygenation all the patients required this therapy in order to reduce the perfusion pressure. *Dorson* recalled that while seeking a protocol for comparison of different oxygenators it was discovered that the blood in the vascular system was patent during extracorporeal membrane oxygenation but that once it came in contact with other components of the extracorporeal circuit it became haemolysed. For this reason, Hill (Hill *et al.*, 1974, see p. 69) now operates a policy of replacing haemolysed blood from the suction component of the extracorporeal circulation system with fresh blood. Because of this filters are only used by him in the return line. *Edwards* supported Ghadiali on the need to demonstrate the differences between bubble and membrane oxygenation. His

group at the University of Surrey, UK, had been studying erythrocyte shape change during extracorporeal circulation by means of scanning and transmission electron microscopy. The tests were carried out on blood samples taken during heart - lung bypass operations in Ghadiali's department. In general progressive changes from the normal erythrocyte biconcave disc shape were observed. Firstly, the cell surface roughened, then surface bumps appeared until finally the whole cell changed to a star shape. The rate at which this deterioration occurred was dependent upon the type of oxygenation used as shown below.

| | *Type of oxygenation* | | |
|---|---|---|---|
| *Shape changes* | *Bubble* | *TEFLO* | *Silicone (Landé-Edwards)* |
| Roughened surface | $1\frac{1}{2}$ h | $\frac{3}{4}$ h | 1 h |
| Bumps | 1 h | $1\frac{1}{4}$ h | $1\frac{1}{2}$ h |
| Star shaped | $1\frac{1}{4}$ h | $1\frac{1}{2}$ h | 2 h |

*Gibbons* asked Birnbaum if he had attempted to correlate the volume changes that he observed with the type of shape changes mentioned by Edwards. ***Birnbaum*** had not done this and added that one should be wary of drawing definite conclusions from shape changes as observed by scanning electron microscopy since it was possible that many of these changes were artefacts. *Gibbons* agreed that there were many problems with such techniques but added that by using critical point drying his group had demonstrated that the general shape changes were not artefactual. ***Birnbaum*** felt it was very difficult to prepare slides of membranes with loosely attached deposits for scanning electron microscopy observation. *Edwards* emphasised the need for using control specimens which enhanced the certainty that the observations made were not artefactual.

Session C

# ADSORBENTS IN THERAPEUTIC MEDICINE AND ASSOCIATED TOPICS

*Chairman*
Professor T. M. S. Chang, M.D., Ph.D., F.R.C.P.(C.)

*Associate*
Dr E. Denti, Ph. D.

# 14

# THE DESIGN OF A CHARCOAL HAEMOPERFUSION SYSTEM

J. B. HILL, F. L. PALAIA and C. R. HORRES

**Summary**

The Becton, Dickinson (B - D) charcoal haemodetoxification system was designed for the rapid removal of poisons from the blood of overdosed patients. Owing to the interference with the rate of solute adsorption, coupled with a lack of evidence for protection against fragmentation and platelet loss, coating of the charcoal with polymer films was not considered as a part of this system. Instead, an immobilisation process was developed which allows a maximum of surface contact between the blood and the adsorbing surface. This immobilisation gives good protection from fragmentation of the charcoal by pulsating fluid waves and fluid flows. Resistance to fluid flow is small and the loss of adsorption rate owing to the immobilisation process is minimal. Devices using this process have saved experimental animals from lethal doses of phenobarbital and have been used in man to treat drug overdose.

## 14.1 Introduction

The logical design of a system requires knowledge of what the system is meant to do. This seemingly obvious and elementary statement may have little relevance when developing a system to deal with complex medical problems. For example, what should a haemodialyser do? Beyond saying that it should remove water and certain solutes in order to maintain homeostasis, no one really knows. As a result, there are several types of dialyser with many proponents of one type or another, but no really clear-cut choices to be made in terms of the patient well-being and eventual outcome. The chemical engineer can design all sorts of membranes for dialysis with interesting properties but the physician cannot tell him what is needed. It is difficult for the engineer to comprehend how little the physician really knows of the conditions he must treat.

The charcoal haemoadsorption field is relatively new. Yatzidis (1964) first reported the use of a loose-bed of granular charcoal in an extracorporeal blood stream. Dunea *et al.* (1965) used the technique and reported on it in the following year. Reports of finding charcoal fragments in the tissues of animals so treated (Hagstam *et al.*, 1966; Rosenbaum *et al.*, 1968) led rapidly to the disuse of uncoated charcoal for haemoperfusion. Accounts of damage to the formed elements coupled with fragmentation sent many investigators scurrying in search of a polymer in which they could encase the charcoal granule to prevent its contact with

the blood, in the hopes of preventing fragmentation and platelet loss. We are now in a somewhat similar situation to dialysis in that we have at least four groups of investigators with four types of coated charcoals.

### 14.2 Coating, Fragmentation and Solute Removal

The charcoal haemoadsorption system to be described in this communication was designed for the rapid removal of poisons from the blood of patients. Since we felt the speed of drug removal was important, we could see little justification in our design for a membrane surrounding the charcoal. If the membrane was to be of sufficient substance to prevent micron-sized fragments being broken when granules collided within the column bed, it would probably have to approach the thickness of present dialysis membranes. If so, what advantage would the polymer-encased charcoal have over a dialyser? Such a membrane would slow the rate of solute uptake. Despite some early claims to the contrary, most workers will now agree that this is so. Denti *et al.* (1975) have clearly shown that the degree of adsorption rate inhibition is proportional to the size of the molecule studied. This could prove a serious disadvantage in the removal of drugs with larger molecules or in situations where the size of the molecules to be removed is unknown as in liver failure and the end stage of renal disease.

In studies in our laboratory with charcoal coating techniques, we could not show a platelet sparing effect in our dog experiments. Figure 14.1 shows the

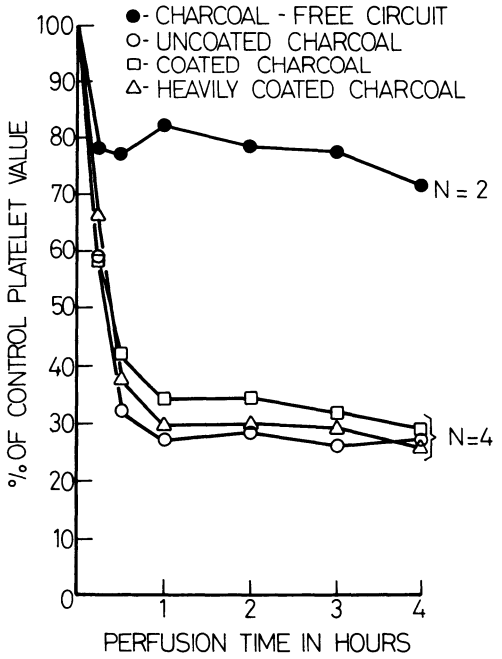


Figure 14.1 A comparison of the effect on dog platelet levels of 4 h of haemoperfusion with uncoated and coated charcoals

fall in platelet level during a 4 h haemoperfusion. Each curve is the average of four separate male Beagle dog experiments in which blood was circulated through a 100 g loose-bed of charcoal at 150 ml/min. The charcoal and the coating procedure corresponded to that outlined by Chang (1972). One curve represents results with the uncoated charcoal, one with results of charcoal coated 'after' Chang, and one presents the results obtained when applying twice the coating material recommended by Chang. The results are also given for a charcoal free circuit. In all cases the charcoal containing devices were filled with 1% albumin refrigerated prior to use. In no case did the charcoal containing circuit results differ significantly from one another.

Each charcoal bed was rinsed with 3 l of saline just prior to use. Table 14.1 shows the estimates of charcoal fragments contained in these rinses which were filtered through 0.8 μm Millipore filters and compared with the Millipore filters with known amounts of fragments on their surfaces. Again no significant differences were obse ved. There was no difference in the extraction of creatinine (mol. wt. 113) from the blood of the dogs by these devices and this essentially confirms earlier studies of Chang and Malave (1970). When the extraction of pentobarbital (mol. wt. 225) was studied, however, a statistically significant decrease in extraction was found for the doubly coated devices. This shown that a coating which interferes with solute removal rates does not necessarily spare platelets or prevent fragmentation. This conclusion on fragmentation is shared by Andrade *et al.* (1972). Experience of this sort led us to search for techniques other than coating in an attempt to solve the fragmentation problem.

A very important factor in rapid solute removal is the surface of the adsorbent available for contact with the blood stream. The smaller the size of the charcoal granule the greater will be this surface. Phenobarbital removal rate from dogs was

**Table 14.1**

Estimates of the weight (μg) of charcoal fragments on Millipore filters from 1 l of sterile saline rinses through the devices

| *Experiment number* | *Type of device* | *Weight of charcoal fragments (μg)* | | |
|---|---|---|---|---|
| | | *First rinse* | *Second rinse* | *Final rinse* |
| 262 | uncoated | 10 | 2 | 2 - 5 |
| 264 | uncoated | 50 - 100 | 50 - 100 | 50 |
| 269 | uncoated | 20 | 10 - 20 | 10 |
| 275 | uncoated | 5 | 20 | 20 |
| 261 | coated | 20 | 2 - 5 | - 20 |
| 266 | coated | 20 - 50 | 20 | 20 - 50 |
| 268 | coated | 10 - 20 | 10 - 20 | 20 |
| 274 | coated | 20 - 50 | 20 - 50 | 20 |
| 263 | double coating | 200 | 50 | 100 |
| 267 | double coating | 10 - 20 | 5 - 10 | 20 |
| 271 | double coating | 20 | 10 | 5 |
| 276 | double coating | 5 | 5 | 2 - 5 |

increased simply by using smaller charcoal granules (Hill *et al.*, 1976a). In the usual loose-bed granular charcoal column, the spaces between the granules become smaller as the granules are decreased in size, and compaction and packing are more likely to occur. For this reason most investigators have chosen charcoal granules of a fairly large size (typical diameters: Chang 1.4 - 3.4 mm, Andrade 0.7 - 1.7 mm, Vale 2 - 4 mm). The large sized particle offers less surface exposed to the blood per gram of charcoal in the device.

## 14.3 Haemodetoxifier Specification and Design

It was necessary to find a way of presenting small particles of charcoal to the flowing blood stream in such a manner that fragmentation was minimised, flow spaces were not excessively encroached upon, and no barrier was placed between the blood and the outer adsorptive surface of the charcoal to inhibit the rate of solute adsorption. Previous experience had shown us that there was a relationship between the degree of motion of the charcoal granules within the adsorbent bed and the number of fragments in effluent solutions.

At this point luck played a role in our design. In studying various charcoals, samples for viewing had been kept under the microscope stuck to the surface of pieces of transparent adhesive tape of the type found on most office desks. At the same time other members of our Research Centre were working with spiral coil oxygenators. As we were a relatively new addition to the Company, we would often be called upon to tell visitors what we were doing. In one of these sessions one of us (Hill) was thinking about the samples on tape and listening to a colleague speak about the spiral coil oxygenator. Why not a spiral coil charcoal device? An adhesive tape with a limited depth of adhesive on a base sheet would give a controllable means of fixing the charcoal granules with a minimum of surface inaccessible to the blood stream. At the same time the granules would be less likely to rub together and the flow spaces between the granules would remain fixed.

The first coils were made from a small strip of transparent adhesive tape from a desktop dispenser sprinkled with charcoal, wound in a coil, pressure fitted within a plastic 20 ml syringe barrel, and connected to a rat extracorporeal circuit. The coils adsorbed solute from the rat's blood stream beautifully and resistance to the blood flow was minimal. Larger tape was obtained and devices to be tested in dogs were made. Those performed in a manner similar to loose-bed devices and showed a very low propensity toward fragmentation.

## 14.4 Device Development and Evaluation

We now produce a charcoal tape coil, the Becton, Dickinson (B – D) Haemodetoxifier which is fitted in a rigid polycarbonate housing in such a manner as to ensure the flow of blood through the charcoal. The individual granules vary from 0.3 - 0.8 mm in size. More complete descriptions of the device may be found elsewhere (Hill *et al.*,

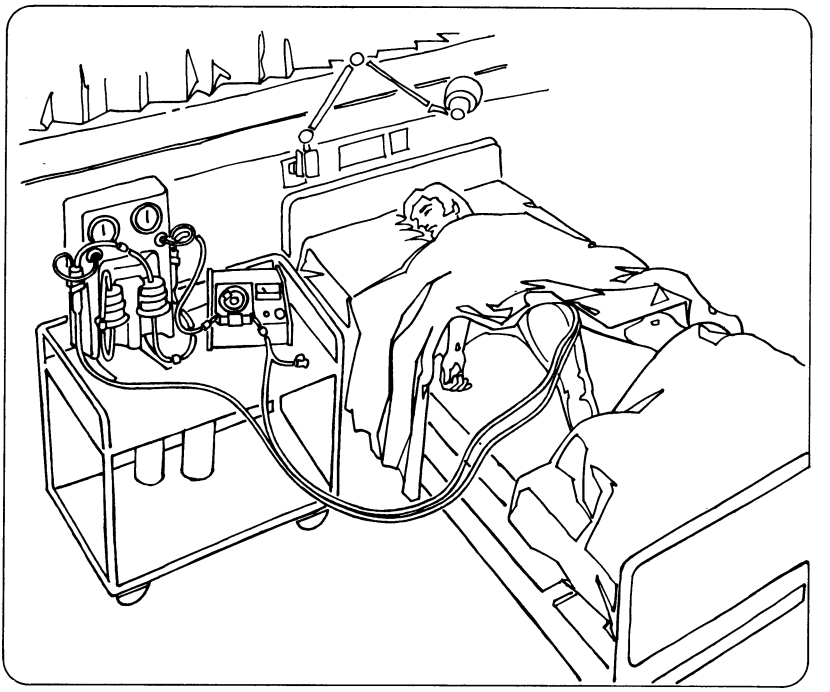

Figure 14.2 An artist's concept of the B-D Haemodetoxifier in use at a patient's bedside

1976a, b). Figure 14.2 shows a drawing of a device being used at a patient's bedside to remove drug from the blood. Plastic catheters are placed in the patient's femoral vein and heparinised blood is recirculated through the charcoal coil by means of a roller pump. Pressures are monitored by aneroid gauges attached to bubble traps on each side of the charcoal coil. This is done to warn of any clotting which might occur in the charcoal coil during the procedure. Over 35 such patients have now been successfully treated for drug overdose. Barbour *et al.* (1976) have reported results on some of these.

Figure 14.3 is an enlarged photograph of a portion of one end of a fixed-bed charcoal coil. In it may be seen the charcoal granules sandwiched between layers of the adhesive coated polyester base sheet. This arrangement results in a charcoal cartridge in which particles are restricted in motion and as a result a pulsating stream of liquid produces relatively few fragments in effluent solutions. Figure 14.4 shows enlarged photographs of portions of Millipore filters used to filter aqueous effluents from equivalent loose and fixed-bed devices challenged with the same pulsating stream. Similar results have been reported previously (Hill *et al.*, 1976a).

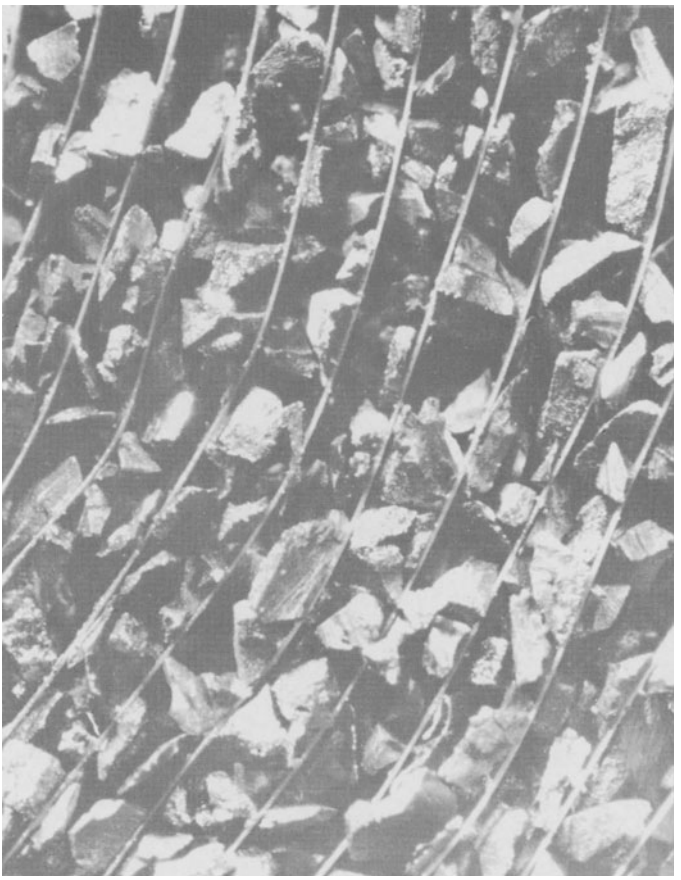

Figure 14.3 An enlarged photograph of one end of a fixed-bed charcoal tape coil

Table 14.2 shows quantitative estimates of charcoal particulates which resulted from pulsating fluid challenges and two subsequent rinses on eight charcoal devices. Four were coated charcoal devices (Chang, 1972) and four were uncoated fixed-bed charcoal devices. It is clear that this coating procedure did not protect the particles from fragmenting nearly as well as did the fixation process. The fragments decrease in the effluent solution from both sets of devices as the pulsations are decreased, but even in the second litre rinse after the 'bump' test they are significantly higher for the coated charcoal devices. This is so despite the fact that the fixed-bed devices contain smaller granules (0.3 - 0.8 mm as against 1.4 - 3.4 mm). Barakat and MacPhee (1970) have reported less fragment production with larger charcoal granules.

Despite the small size of the charcoal granules used in this device, none of the 'packing, sludging and channelling' described by Andrade *et al.* (1972) with

**Table 14.2**

Estimates of quantity (μg) of fragments in effluent solutions from eight devices challenged with liquid pulsation followed by rinses

| *Material filtered* | *Four coated charcoal devices* | | | | *Four fixed-bed charcoal devices* | | | |
|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *1* | *2* | *3* | *4* |
| First 500 ml bump test* | > 500 | > 500 | 200 | 200 - 500 | 5 | 5 | 5 | 5 |
| Second 500 ml bump test | > 500 | 500 | 10 | 100 - 200 | 1 | 1 - 2 | 1 | 1 - 2 |
| First litre rinse after bump | 100 | 50 - 100 | 100 | 10 | < 1 | < 1 | < 1 | 0 |
| Second litre rinse after bump | 5 | 1 | 1 - 2 | 1 | < 1 | < 1 | < 1 | 0 |

*Hill *et al.*, 1976a.

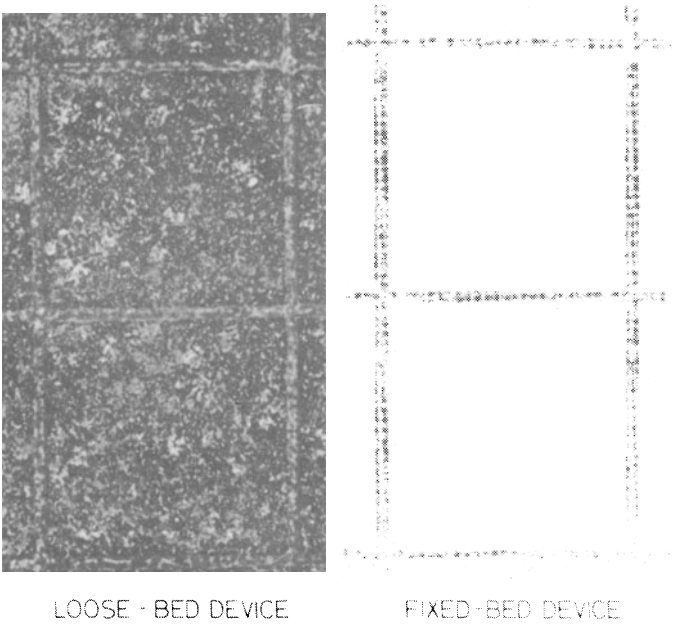


Figure 14.4 An enlarged photograph of a section of two Millipore filters. The one on the left was obtained from a bump test performed on an 80 g loose-bed charcoal device. The one on the right is from the same bump test performed on a 95 g fixed-bed charcoal device. Both devices were made with 0.3 - 0.8 mm coconut shell charcoal

uncoated charcoal was seen. Previously described work (Hill *et al* ., 1976a) clearly demonstrates a minimal resistance to blood flow by these devices, e.g. 35± 4mm Hg at a flow rate of 500 ml/min.

The fixation process must of necessity effectively remove some surface of the charcoal granule from contact with the flowing blood. Figure 14.5 shows results in which the adsorption of salicylate from 4 l of solution by equivalent amounts of equal-sized charcoal granules are compared. In one case the charcoal was in a large flask and the solution was constantly stirred to make a slurry in which the charcoal granules remained fully suspended and in constant motion. In the second case the 4 l of salicylate solution were contained in a recirculation system with a reservoir in which the liquid was pumped through a fixed-bed device at 200 ml/min. The stirred flask was considered an ideal system for contact between the adsorbent and the solution. Despite the fact that more than 90% of the solution was in the reservoir away from the charcoal at any given time and that it took 20 min to pump 4 l through the device, adsorption kinetics were strikingly similar. Recent *in vivo* studies (Hill *et al.*, 1976a) of salicylate clearance from the blood of dogs suggest a 20% loss of effective surface contact when comparing a

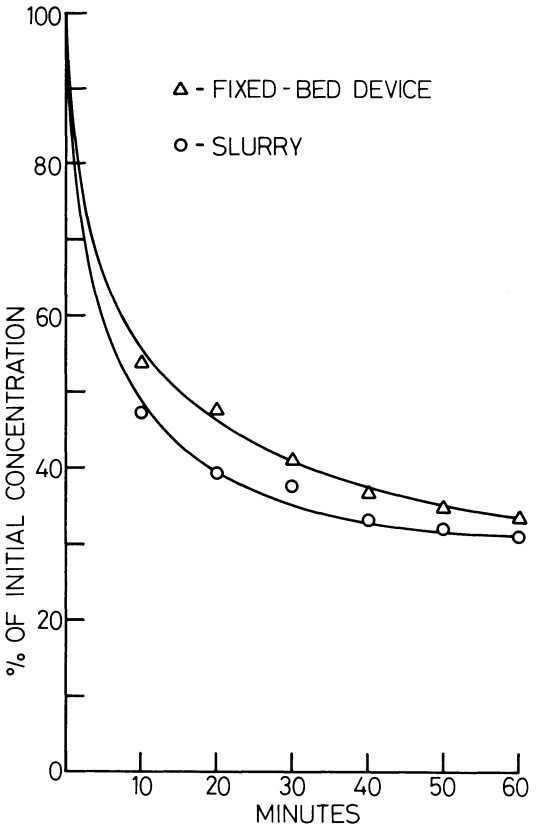


Figure 14.5 The adsorption of salicylate from 4 l of solution by 100 g of charcoal. A 4 l slurry was compared with a fixed-bed device with the 4 l recirculated through it at 200 ml/min from a reservoir

loose-bed charcoal device with a fixed-bed device. This small loss of efficiency seems a small price to pay for protection against fragmentation.

That these devices may indeed be efficacious is demonstrated by the fact that in dog experiments with a 100% lethal dose of phenobarbital use of the device was associated with only a 15% mortality (Hill *et al.*, 1976b).

We have not found any influence of the fixation process on the removal of platelets by the device in our dog experiments. Both loose and fixed-bed devices cause platelet levels to fall. As mentioned previously, we have not found that coating the charcoal with polymer membranes influences the platelet losses. Clinical reports of drug overdosed patients treated with coated charcoal devices (Vále *et al.*, 1975) and fixed-bed charcoal devices (Barbour *et al.*, 1976) have not shown either type of device to be preferable in regard to platelet sparing.

## 14.5 Conclusions

A charcoal haemoadsorption system for the rapid removal of poisons from the blood of patients suffering from drug overdose has been designed and developed. This system uses an immobilisation process which allows a maximum of surface contact between the flowing blood and the adsorptive surface while producing a minimal amount of charcoal fragmentation.

## 14.6 Acknowledgements

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## 14.7 References

Andrade, J. D., Van Wagenen, C., Ghavamian, M., Volder, J., Kirkham, R. and Kolff, W. J., (1972). *Trans. Amer. Soc. Artif. Int. Organs,* **18**, 473.

Barakat, T. and MacPhee, I. W. (1970). *Brit. J. Surg.,* **57**, 580.

Barbour, B. H., LaSette, A. M. and Koffler, A. (1976). *Kidney International* (in press).

Chang, T. M. S. and Malave, N. (1970). *Trans. Amer. Soc. Artif. Int. Organs,* **16**, 141.

Chang, T. M. S. (1972). *Artificial Cells,* Thomas, Springfield, Illinois, p. 137.

Denti, E., Luboz, M. P. and Tessore, V. (1975). *J. Biomed. Mater. Res.,* **9**, 143.

Dunea, G. and Kolff, W. J. (1965). *Trans. Amer. Soc. Artif. Int. Organs,* **11**, 178.

Hagstam, K., Larsson, L. and Thysell, H. (1966). *Acta. Med. Scand.,* **180**, 593.

Hill, J. B. Palaia, F. L., McAdams, J. L., Palmer, P. J., Skinner, J. T. and Maret S. M. (1976a). *Kidney International* (in press).

Hill, J. B., Palaia, F. L., McAdams, J. L., Palmer, P. J. and Maret, S. M. (1976b). *Clin. Chem.,* **22**, 754.

Rosenbaum, J. L., Ronquillo, E. and Argyres, S. N. (1968). *J. Albert Einstein Med. Centre,* **16**, 67.

Vale, J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975). *Brit. Med. J.,* **1**, 5.

Yatzidis, H. (1964). *Proc. Eur. Dial. Transpl. Assoc.,* **1**, 83.

# 15

# THE DESIGN OF A POLYMER COATING FOR ACTIVATED CARBON

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**Summary**

The selection and preparation of a polymer coating for use in perfusion over activated carbon are described. The coating selected is that of a copolymer of methyl methacrylate and dimethylaminoethyl methacrylate. Reasons influencing the choice of monomers and their proportions in the optimum synthesis are discussed. Coating performance has been assessed in terms of creatinine removal and the ability to prevent unacceptable generation of fine carbon particles.

## 15.1 Introduction

Since the original haemoperfusion studies by Yatzidis (1964), the majority opinion has been that coating of activated carbon is necessary to achieve an acceptable clinical performance. The approaches to coating selection can broadly be classified into two areas: the application of commercially available polymers and the use of polymers which have already been shown to be acceptable in other applications. Thus, Yatzidis (1966) and Rosenbaum *et al.* (1968) suggested a coating of cellulose acetate. Chang (1972) pioneered clinical haemoperfusion over activated carbon by coating the adsorbent with collodion followed by treatment with albumin. Andrade *et al.* (1971) evaluated coatings based on hydroxyethyl methacrylate and this has been followed by the use of Hydron (Fennimore *et al.*, 1975). A variation on the original suggestion by Yatzidis has been the introduction of coatings based on deacetylated cellulose acetate (Denti *et al.*, 1975; Thysell *et al.*, 1976).

The approach adopted by the Strathclyde Bioengineering Unit has been different. From a survey of the published information, three areas were chosen for detailed study, namely, selection of a suitable grade of carbon, design of a coating procedure, followed by selection and design of the polymer coating itself.

An evaluation of peat-based activated carbon was conducted in association with Norit-Clydesdale Ltd and an appropriate grade of extruded granules was selected (Cameron *et al.*, 1974). This grade of carbon has now found general acceptability and is designated Norit RBX-1, haemoperfusion grade. A procedure for coating the

carbon granules with polymer was devised and developed by Hood (Hood *et al.*, 1974; Courtney *et al.*, 1976).

A discussion of the last area of study, that of the selection and design of the polymer coating, follows in the subsequent sections.

## 15.2 Polymer Selection

Previous evaluations of haemodialysis membranes (Courtney, 1969) have demonstrated the advantages of certain copolymer systems, in which one monomer contributes to film strength and the other to film sensitivity or reactivity. Two successful membranes were tried out as coatings. These were copolymers of acrylic acid (AA) and *n*-butyl methacrylate (NBMA) (Courtney *et al.*, 1973) and dimethylaminoethyl methacrylate (DMAEMA) and acrylonitrile (AN) (Gilchrist *et al.*, 1975). The final choice of the copolymer as best suited to the role of coating was that of DMAEMA and methyl methacrylate (MMA).

Polymerisation of DMAEMA, a water-soluble aminoester of methacrylic acid, confers water sensitivity and cationic properties. DMAEMA copolymers have been proposed for medical adhesives (Gander, 1967) and pharmaceutical coatings (Volker and Wenzel, 1962; Tuji, 1969). The incorporation of DMAEMA into polymers provides a means of promoting the ionic attachment of heparin (Falb *et al.*, 1966; Idezuki *et al.*, 1975). The suitability of DMAEMA for heparin attachment has been confirmed by an evaluation of AN - DMAEMA films (Lindsay *et al.*, 1976).

Polymers of MMA are very widely used, the most common medical application being that of bone cements (Charnley, 1970). The coating properties of MMA-based polymers are well established (Gusman, 1960) and of particular relevance is the use of such polymers in the coating of medicaments (Utsumi *et al.*, 1963).

## 15.3 Experimental Work

Two methods of polymerisation were evaluated, solution and emulsion, and a range of monomer proportions was studied for each method.

*Solution polymerisation* was carried out in acetone under reflux for 5 h with azobisisobutyronitrile (AIBN) as initiator. On completion of the reaction, the liquid product was cast into a film and dried at 60 °C for 24 h.

*Emulsion polymerisation* was carried out under nitrogen with potassium persulphate (PPS) as initiator. Reaction temperature was 50 °C and reaction time was 4 h. The emulsifying agent used was the nonionic surfactant Pluronic F68, a copolymer of ethylene oxide and propylene oxide. After polymerisation, the emulsion product was broken with methanol. Solid polymer was obtained by filtration followed by drying in an airstream at 50 °C.

Coating of carbon granules, washed with distilled water and dried in an oven at 60 °C, was achieved by rotating the granules in a controlled manner (Courtney *et al.*, 1976) and introducing a solution of the copolymer in acetone/ethanol. During the coating procedure, the carbon particles lose electrons, become positively

charged and contact is hindered while the particles are turned over in a controlled manner and thus free-falling, nonadhering particles are obtained.

The coated granules were then used to pack a polypropylene column and were washed *in situ* with double distilled water. Samples were taken for estimation of monomer and solvent levels by ultraviolet (UV) spectroscopy. The column was then finally flushed with 2 l of saline with samples taken for fine carbon particle estimation using a Coulter counter.

To estimate solute removal, columns containing 130 g of coated carbon were utilised. The ability of the coated carbon to remove solutes was assessed by passing a solution of creatinine, concentration 20 mg/100 ml, from a reservoir at 37 °C through a column at a rate of 200 ml/min. Samples were taken at the column inlet and outlet.

## 15.4 Results and Discussion

The levels of residue of DMAEMA, MMA or acetone could not be detected by UV spectroscopy. This implies residual monomer levels of less than 1 ppm and a residual acetone level of less than 5 ppm.

All samples for fine particle estimation conformed to the British Pharmacopoeia Standard for infusion liquids and had particle levels for 2 $\mu$ and 5 $\mu$ below that permitted, namely $< 1000$ in 1 ml for size $\geqslant 2\mu$ and $< 100$ in 1 ml for size $\geqslant 5\mu$.

The tests have shown that creatinine removal rates increase with increase in DMAEMA content of the coating and with decreasing coating weight. These features are illustrated in figure 15.1, which shows the relationship between DMAEMA

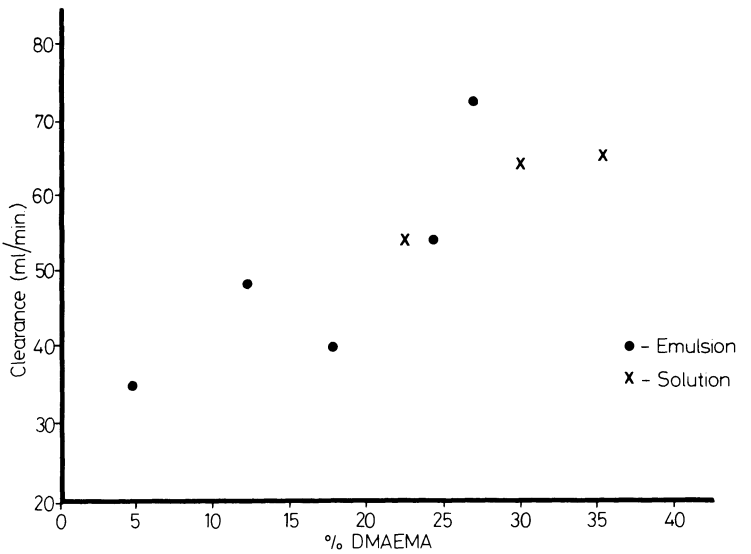


Figure 15.1 MMA - DMAEMA coated carbon. Effect of DMAEMA content on creatinine clearance at 60 minutes (1% coating).

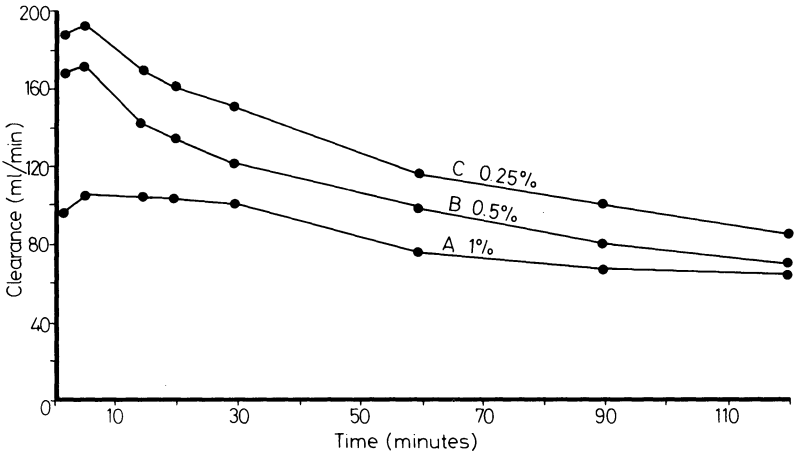


Figure 15.2 MMA - DMAEMA coated carbon. Effect of coating weight on creatinine clearance at 60 minutes (1% coating).

percentage in the copolymer and creatinine clearance for coating weights of 1.0%, and in figure 15.2 which compares with clearance of creatinine for coating weights of 1.0%, 0.5% and 0.25% of the same copolymer (designated A, B and C respectively).

## 15.5 Conclusions

The *in vitro* evaluation of the DMAEMA - MMA coatings demonstrated the possibility of selecting and preparing a polymer system for a particular medical purpose. The results obtained, together with those of a concurrent animal evaluation (Park *et al.*, 1976), led to the selection of the monomer proportion and coating weight to be used in the preparation of coatings for clinical studies. The proportion was MMA: DMAEMA of 50: 50 and the coating weight was 0.25%.

The MMA - DMAEMA coating was designed to operate in conjunction with the extruded carbon granules. However, it is also suitable for use with other forms of carbon and preliminary experiments have been conducted with a form of carbon cloth (Gaylor *et al.*, 1976). This cloth (Bailey *et al.*, 1973) is available in a variety of forms, combines high adsorption with mechanical strength and offers the possibility of alternative haemoperfusion device designs.

## 15.6 Acknowledgements

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## 15.7 References

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood perfusion: HEMA/activated carbon. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 222.

Bailey, A., Maggs, F. A. P. and Williams, J. H. (1973). Carbonising process and apparatus therefor. British Patent No. 1 310 011.

Cameron, A., Courtney, J. M., Gilchrist, T., MacDowall, J. and Walker, J. M. (1974). The selection and use of activated carbon to treat kidney diseases and drug overdose. *Proceedings of 4th London International Carbon and Graphite Conference*, Pennard Press, London.

Chang, T. M. S. (1972). *Artificial Cells*, Thomas, Springfield, Illinois.

Charnley, J. (1970). *Acrylic Cement in Orthopaedic Surgery,* Livingstone, Edinburgh and London.

Courtney, J. M. (1969). New synthetic polymer membranes for extracorporeal haemodialysis. Ph. D. Thesis, University of Strathclyde.

Courtney, J. M. Gilchrist, T., Walker, J. M. and Edwards, R. O. (1973). A new coated adsorbent for blood perfusion. *Digest of 10th International Conference on Medical and Biological Engineering,* Dresden, August 1973.

Courtney, J. M., Gilchrist, T., Hood, R. G. and Townsend, W. B. (1976). A novel method for preparing coated carbon granules. *Proc. Eur. Soc. Artif. Organs,* 2, 210.

Denti, E., Ruboz, M. P. and Tessore, V. (1975). Adsorption characteristics of cellulose acetate coated charcoals. *J. Biomed. Mater. Res.*, **9**, 43.

Falb, R. D., Grode, G. A., Luttinger, M., Epstein, M. M., Drake, B. and Leininger, R. I. (1966). Development of blood-compatible polymeric materials. U. S. Nat. Tech. Inform. Serv., P. B. Report No. 173, 053.

Fennimore, J., Watson, P. A., Munro, G. D. and Kolthammer, J. C. (1975). The design and evaluation of a convenient carbon haemoperfusion system. *Proc. Eur. Soc. Artif. Organs,* **1**, 90.

Gander, R. J. (1967). Pressure-sprayable water-soluble alkyl methacrylate polymer solutions. United States Patent 3 305 513.

Gaylor, J. D. S., Maggs, F. P., Park, G. B. and Smith, E. M. (1976). Activated carbon cloth - a novel material for haemoperfusion. *29th Annual Conference on Engineering in Medicine and Biology, Boston, Massachusetts*, **18**, 171.

Gilchrist, T., Jonsson, E., Martin, A. M., Naucler, L., Cameron A. and Courtney, J. M. (1975). Development of the Strathclyde haemoperfusion system, in R. Williams and I. M. Murray-Lyon (Eds.) *Artificial Liver Support*, Pitman Medical, London, p. 319.

Gusman, S. (1960). Blended methacrylate coating compositions and articles coated therewith. United States Patent 2 940 950.

Hood, R. G., Courtney, J. M., Gilchrist, T. and Gaylor, J. D. S. (1974). A method of coating granules. British Patent Application 3068/74.

Idezuki, Y.,Watanabe, H., Hagiurara, M., Kanasugi, K., Mori, Y., Nagapla. S., Hagio, M., Yamamoto, K. and Tanzawa, H. (1975). Mechanism of antithrombogenicity of a new heparinised hydrophilic polymer: Chronic *in vivo* studies and clinical application. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 436.

Lindsay, R. M., Rourke, J., Reid, B., Friesen, M., Linton, A. L., Courtney, J. M. and Gilchrist, T. (1976). Platelets, foreign surfaces and heparin. *Abstracts Amer. Soc. Artif. Int. Organs,* **5**, 49.

Park, G. B., Apiliga, M. T. and Fairweather, I. A. (1977. Preliminary animal studies on The Strathclyde haemoperfusion device. The I.R.C.S. *Med. Sci. J.* (in press).

Rosenbaum, J. R., Ronquillo, E. and Argyres, E. S. (1968). Column haemoperfusion and haemodialysis techniques to treat barbiturate intoxication in dogs. *J. Albert Einstein Med. Centre,* **16**, 67.

Thysell, H. Lindholm, R., Heinegard, D., Henriksson, H. Jonsson, E., Nylen, U.,., Svensson, T., Bergkvist, G. and Gullberg, C. A. (1976). A haemoperfusion column using cellophane coated charcoal. *Proc. Eur. Soc. Artif. Organs,* 2, 212.

Tuji, Y. (1969). Process for coating pharmaceutical preparations with a hydroxypropylmethyl cellulose-sealing agent moisture-preventing film. United States Patent 3 477 864.

Utsumi, I., Ida, T., Kishi, S. and Hashimoto, T. (1963). Coated medicaments. United States Patent 3 073 748.

Volker, T. and Wenzel, F. (1962). Process for coating medicaments. United States Patent 3 070 509.

Yatzidis, H. (1964). A convenient haemoperfusion micro-apparatus for the treatment of endogenous and exogenous intoxications. 1. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.,* **1**, 83.

Yatzidis, H. (1966). Paper presented at Cleveland Clinic, Cleveland, Ohio, USA.

# 16

# THE ROLE OF ADSORBENTS IN THE WEARABLE ARTIFICIAL KIDNEY

J. M. WALKER, S. C. JACOBSEN, R. L. STEPHEN, W. J. KOLFF and D. ROSE

**Summary**

Conventional haemodialysis systems are large, complex, costly to buy and operate, require numerous adjustments of operational parameters during dialysis resulting in the patient having little mobility. The artificial kidney which has been developed at the University of Utah is fully portable and only requires intermittent connection to a 20 l dialysate tank during two-thirds of the dialysis period. This paper will briefly describe the operation of the 'wearable' artificial kidney and will discuss the present method of removing uraemic toxins from the dialysate side using activated carbon and a 20 l dialysate tank. The research in progress to find alternative methods of urea and potassium removal in order to dispense with the 20 l tank will also be discussed.

## 16.1 Introduction

The hardware components of the 'wearable' artificial kidney (WAK) have been discussed in detail by Jacobsen *et al.*, (1975). Figure 16.1 shows a close up of the wearable module and figure 16.2 illustrates the flow paths for the blood and dialysate solutions.

Blood is withdrawn from and returned to the patient via a single needle catheter and an anti-regurgitation valve. A 1.4 $m^2$ Cordis Dow (model 4) hollow fibre dialyser is used and a bubble trap is inserted in the blood line for safety reasons. The dialysate is produced by forming a negative pressure in the dialysate compartment and a positive pressure in the blood compartment. The ultrafiltrate can be controlled by the ultrafiltration valve and vented as required for the water balance of the patient. The dialysate volume is designed at about 500 ml. The dialysate is regenerated via a 300 g activated carbon cartridge and, until a urea removal module has been developed, a 20 l dialysate tank is used during two-thirds of the treatment.

The Jacobsen design is not a miniaturisation of existing systems, but utilises a unique, pulsatile blood and dialysate fluid pump which operates in synchrony with two passive valves. The advantages of this system include small size and convenient portable operation. The wearable module is uncomplicated and powered by rechargeable nickel-cadmium batteries. The only system adjustments required are controlling the blood flow rate and ultrafiltration rate.

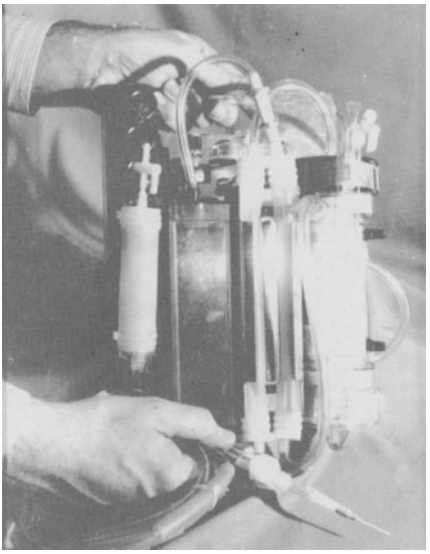

Figure 16.1 Wearable artificial kidney

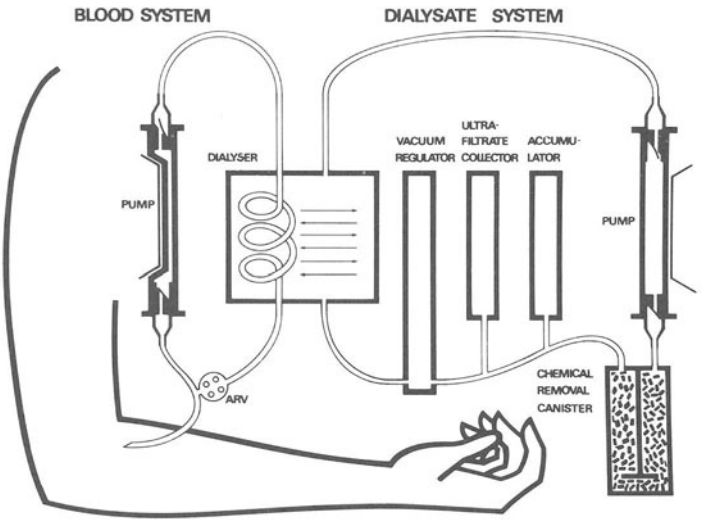


Figure 16.2 Schematic flow diagram of wearable artificial kidney. Blood handling components: single needle catheter, blood pump, dialyser, antirecirculation valve (ARV). Dialysate system: dialysate pump, ultrafiltration rate regulation valve, carbon module, excess ultrafiltrate collection bag, accumulator

## 16.2 Mass Balance Analysis

Assuming that the uraemic patient must vent 1.5 l of fluid per day via the WAK ultrafiltrate and that the ultrafiltrate is in concentration equilibrium with the plasma concentrations of the constituents of interest (this is not entirely true because of protein binding, Donnan equilibria, hollow fibre membrane resistances, etc), table 16.1 lists the amounts vented by 1500 ml of ultrafiltrate compared with normal urine excretion in 24 h. The last column lists the amount in excess which must be removed by the dialysate regeneration system and the amount deficient which must be supplemented or otherwise controlled.

**Table 16.1**

Adult normal values in urine and plasma and the amount of toxins removed by the discharge of 1500 ml ultrafiltrate/day

| *Constituent* | *Amount excreted in urine/24 h 70 kg human* | *Approximate plasma value* | *Amount removed in 1500 ml ultrafiltrate* | *Excess (+) or deficiency (−) compared to normal urine excretion* |
|---|---|---|---|---|
| | g | mg/100 ml | g | g |
| Calcium | 0.24 | 5 | 0.07 | +0.17 |
| Chloride | 7.1 | 355 | 5.3 | +1.8 |
| Creatinine | 1.5 | 1 | 0.02 | +1.5 |
| Glucose | 0 | 90 | 1.35 | −1.35 |
| Magnesium | 0.02 | 2.4 | 0.03 | −0.01 |
| Phosphorus | 0.6 | 3.6 | 0.05 | +0.55 |
| Potassium | 3.1 | 17.6 | 0.3 | +2.8 |
| Sodium | 3.0 | 334 | 4.95 | −1.95 |
| Urea nitrogen | 10.0 | 15 | 0.2 | +9.8 |
| Uric acid | 0.5 | 5 | 0.07 | +0.43 |

Considering the normal ranges for electrolytes, it appears that chloride, magnesium and sodium can be controlled by ultrafiltrate excretion alone. The glucose deficiency can be controlled by a slightly increased caloric intake. This leaves an excess of calcium, phosphate and potassium ions which must be controlled by appropriate binders or resins.

Creatinine and uric acid can be removed effectively by adsorption on activated carbon. However, other techniques must be found for urea removal.

## 16.3 Present Removal Technique

At present the uraemic toxins are removed from the dialysate solution by 300 g of activated carbon (Witco 517) and a 20 l dialysate tank. During approximately one third of the cumulative dialysis time, the patient may be disconnected from the tank and be fully mobile. The ultrafiltration valve regulates the suction. Ultrafiltration rates from 0 to 700 ml/h can be obtained.

Witco 517 was selected as this was one of the better carbons, having high

**Table 16.2**

Ion elution from Witco 517 activated carbon in distilled and tap water.

| | *Time* *h* | *Ca* *mg/l* | *Mn* *mg/l* | *Mg* *mg/l* | *$SO_4$* *mg/l* | *$PO_4$* *mg/l* | *Fe* *mg/l* | *pH* |
|---|---|---|---|---|---|---|---|---|
| Distilled water | 1 | 0.10 | 0.05 | <0.01 | 70.0 | 1.00 | <0.01 | 7.30 |
| | 5 | <0.01 | 0.103 | <0.01 | 0.10 | 0.50 | <0.01 | 6.90 |
| | 12 | <0.01 | 0.120 | <0.01 | 5.00 | 0.44 | 0.20 | 6.80 |
| | Control | <0.01 | 0.020 | <0.01 | 5.00 | 42.5 | 2.22 | 7.90 |
| Tap water | 1 | <0.01 | 0.010 | 4.80 | 85.00 | 3.50 | 0.04 | 7.50 |
| | 5 | <0.01 | 0.001 | 2.40 | 10.00 | 0.62 | 0.15 | 7.20 |
| | 12 | 8.0 | 0.004 | 4.80 | 25.0 | 0.38 | 0.26 | 7.50 |
| | Control | 55.60 | 0.081 | 12.90 | 45.0 | 0.45 | 0.09 | 7.40 |
| Physiological normal adult values for unbound serum or plasma | | 80 | 0.002 | 24 | 30 | 135 | 1 | 7.40 |

adsorption capacity and low microparticle generation rate (Van Wagenen *et al.*, 1975; Walker *et al.*, 1975). In dialysate regeneration using uncoated activated carbon, the main concern is not blood compatibility or microparticle generation but adsorption capacity and ion elution. Adsorption capacity has been well documented by Van Wagenen *et al.* (1975).

Ion elution experiments were conducted in the following manner. An amount of Witco activated carbon, fresh from the drum, was soaked in distilled water and another batch soaked in tap water, both for 3 h. Samples were extracted in distilled water at 60 °C for 1 h or 5 h with changes every hour and overnight with no water changes after 5 h. The analysis is shown in table 16.2. Comparing the changes in distilled water extracted from the carbon samples and the control, the differences observed in the ions are minimal with the exception of phosphate and iron which the carbon appears to be adsorbing. Similarly with tap water, the activated carbon is adsorbing calcium, manganese and magnesium.

Nearly all of the extracted values are well within the physiological range with the possible exception of manganese. At present, the activated carbon as used in the WAK is washed with tap water for an average time of 5 h before use.

## 16.4 Clinical Evaluation

Details of the clinical trials have been reported by Jacobsen *et al.* (1975 a and b); Stephen *et al.* (1975) and Kolff *et al.* (1975). The wearable artificial kidney has been in clinical use for over one year with satisfactory results. For patient dialysis six times per week, 20 l of dialysate fluid are used per treatment and if a patient dialyses three times per week, the 20 l tank is refreshed once during each treatment.

Ultrafiltration could be regulated from 8 ml/h to 700 ml/h depending on the ultrafiltration valve setting. Urea removal varies between 30 and 40 g and potassium removal between 50 - 60 mEq per 5 h dialysis.

## 16.5 Future Development in Removal Systems

The constituents which cannot be removed by activated carbon or controlled effectively by diet are calcium, potassium, phosphate and urea. Calcium and potassium may be controlled by ion exchange for sodium with cation exchange resins. For the 84 mEq of calcium and potassium consumed, 84 mEq of sodium would be released, compensating for the 85 mEq sodium deficiency. Unfortunately, the cation exchange resin would not be specific for the potassium ion and would remove the other cations according to their concentration in solution, valence and molecular weight in the following order of decreasing affinity: calcium, magnesium, potassium and sodium. To minimise the unwanted removal of essential ions, the cation exchange resin could be used in the $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ form. This would increase the effect of the concentration driving force and compensate for the resin's low potassium exchange selectivity. The mixed bed resin would also act as a source and a sink for dialysate cation correction. Sargent *et al.* (1972) reported that a mixed cation resin bed equivalent to 1140 ml of Dowex 50 would be required, assuming potassium ion transport is not membrane limited. As the dialysate volume is small in the wearable artificial kidney, the amount of ions diffusing across the membrane would also be small.

Phosphate levels can be controlled either orally (Alu-Tab, Riker Laboratories, Inc) or by adsorption onto sorbents in the dialysate. Rutherford *et al.* (1973) have evaluated more effective phosphorus binding agents with promising results.

## 16.6 Urea Removal

The urea module should be inexpensive, small, light (100 g) and effective. As seen from table 16.1, 20 g of urea must be removed per day. Dietary techniques to decrease the urea burden have been evaluated by various investigators. Some of these are the use of forced high caloric, low protein diet (Kolff, 1952), keto acids (Walser *et al.*, 1973), a diet sufficient in calories and essential amino acids (Giordano, 1972) and oral ingestion of sorbents such as oxystarch. Although dietary methods are unsatisfactory from the aesthetic point of view and for the patient's palate, they nevertheless decrease the amount of urea being formed and should not be overlooked.

In the category of simple techniques there is direct combination of compounds with urea. This includes compounds such as oxystarch, oxycellulose, polyacrolein, clays and activated carbon. Gregor *et al.* (1969) reported that negative results were obtained in attempts to employ aliphatic clathrates, xanthydrols and metal chelates as urea adsorbents. An appreciable adsorption of urea by resins of the $R\text{-}Hg^+$ type for urea was observed, with capacities as high as 0.5 mM/g. The disadvantages were the high cost of the metal and the problems of its possible toxicity. Mehall *et al.* (1969) evaluated a large number of activated carbons, carbon blacks,

ion exchange resins and inorganic adsorbents with no success for effective urea removal. The most efficient was a carbonised poly(vinylidene chloride) which was capable of removing 8.4 g urea/kg carbon. Walitt and Jones (1969) evaluated montmorillonite, and found the clay unusable because it formed an aqueous gel at moderate concentrations. They also reported that Dowex-50 removed 3.2 mg urea/g resin.

Recently, there has been an expanding interest in the use of oxystarch and oxycellulose (Giordano *et al.*, 1975). It is well known that aldehydes form Schiff base complexes with ammonia, urea and other primary and secondary amines and amides in the pH range 7 - 9. Meriwether and Kramer (1975) found little difference between Sumstar-190 (Miles Laboratories) and Giordano oxystarch.. They also reported that oxystarch binds 120 - 187 mg urea/g and oxycellulose binds 140 mg urea/g at an initial urea concentration of 0.1 M, pH 7.4, 0.1 M phosphate buffered for 24 h at 37 °C. Unfortunately we have not managed to reproduce these results and found a urea removal of 22.2 mg urea/g oxystarch under similar conditions.

Another method is to convert the urea enzymatically to ammonium ion and remove the latter. This procedure is utilised in the Redy dialysate regeneration system (Greenbaum and Gordon, 1972). The disadvantages of this are the large amount of zirconium phosphate required (1600 g) and its nonspecificity. A solution of potassium, magnesium and calcium ions must be reinfused into the system.

## 16.7 Complex Urea Removal Techniques

To prevent competition of other cations in an ion exchange resin, a logical solution would be to use a positively charged membrane or desalination membrane in conjunction with urease and a cationic exchange resin. Research is in progress to develop this at the Weizmann Institute of Science in Israel (Dr. O. Kedem, Dr. G. Tanny, Dr. Z. Bohak) and at the Division of Artificial Organs, University of Utah, Salt Lake City. The original idea was to use a composite device containing the positively charged membrane, urease and ion exchange resin in either a coil or sandwich arrangement. Because of the formation of a negatively charged intermediate, the carbamate ion, this composite model was not feasible and was discontinued. In its place a hollow fibre device with a secondary circuit containing urease and ion exchange resin is envisaged. This idea has also been proposed by Sargent *et al.* (1972). This would necessitate augmenting the existing pump or using another type, perhaps a small roller pump.

Electrochemical techniques have been investigated, namely anodic oxidation (Yao and Wolfson, 1972) and electrosorption (Doniat *et al.*, 1974). In the first process the urea is anodically oxidised to ammonia and carbon dioxide with ammonia undergoing further anodic oxidation to nitrogen and water. Although positive results were obtained, the research was discontinued at the University of Utah (Walker, 1973) because of the difficulty in preventing side reactions occurring with the danger of possible toxic nitrogenous substances being formed. The second process, electrosorption, has been investigated by Doniat *et al.* (1974).

This uses an adsorbent material as an electrode in an attempt to enhance its adsorptive properties. Dr A. Soffer at the Ben Gurion University in Israel (personal communication) found difficulties enhancing urea adsorption by electrosorptive techniques and reported that there was no potential at which urea could adsorb and desorb successfully. If the dialysate is passed through a urease cartridge, the urea is converted to ammonia or ammonium depending on the pH. At a pH of 7.4 practically all the $NH_3/NH_4^+$ is present as the ammonium ion whereas at a pH of 11.4 or higher, most of it is present as dissolved ammonia. The techniques available for $NH_3/NH_4^+$ removal are as follows.

(1) Treatment with ion exchange resins. This is complicated by the presence of other cations, namely the divalent ions.
(2) Vapour stripping. This is not really feasible owing to water evaporation and the concentration of electrolytes in the dialysis bath (Setala, 1970).
(3) Distillation. Denti, Iwamoto and Lentz at the University of Utah (personal communication) researched into the possibility of distilling water and ammonia from the dialysate, removing the ammonia by a strong cationic exchange resin and returning the condensed water to the dialysate. Their results show that 380.6 ml or 140.7 g of resin would be required to remove 10 g ammonia. The disadvantage would be in power requirements; 2.5 Kwh is required for an 80% efficient system.

Enzyme pathways have been discussed by Kolff (1975). *Selenomonas ruminantium* from the rumen is able to degrade urea and synthesise amino acids (John *et al.*, 1974). The drawback is that it is an anaerobic process which may not be feasible for dialysate regeneration. Another enzyme system would be the conversion of the ammonium ion into carbamoyl phosphate which could be induced to form a pyrimidine ring and be removed by adsorption onto activated carbon. The disadvantages are the expense of enzymes and that the process requires several enzyme steps. Setala *et al.* (1972) also suggested the use of bacteria to remove the urea.

Other approaches being investigated are liquid membrane capsules (Asher *et al.* 1975). More research is required to increase the stability and strength of the microcapsules.

## 16.8 Discussion and Conclusions

At present the dialysate regeneration system of the wearable artificial kidney comprises 300 g of Witco 517 activated carbon and a 20 l dialysate tank. Clinical evaluations have shown its effectiveness and the favourable comparison with standard haemodialysis removal rates.

The most studied urea adsorbent is oxystarch/oxycellulose but its effectiveness is still in doubt. Of the more complex urea removal systems, further investigations and evaluation are required, particularly with enzymatic systems. Further evaluation is also required to select and optimise the cation exchange resin to remove the potassium ion.

## 16.9 Acknowledgements

The authors wish to thank Dr Joe Andrade, Dennis Coleman, Dr Ennio Denti, Dr David Lentz, and Gary Iwamoto for their work and helpful advice in the wearable artificial kidney dialysate regeneration area. The work was supported by the Development Fund of the Division of Artificial Organs, University of Utah, Salt Lake City to which contributions were made by Sandoz, Mr Ben Matthews, Mr Lawrence Harvey and many others. The contribution by Mr David Rose of New York City, New York is particularly acknowledged. This consisted not only of benefaction but also of a vivid personal interest in the wearable artificial kidney. Through his world wide connections and travels he has made resources available that would otherwise have been untapped. Mr Rose is also supporting the joint research programme between the Weizmann Institute in Israel and the Division of Artificial Organs, University of Utah, Salt Lake City, USA.

## 16.10 References

Asher, W. J., Bovee, K. C., Hamilton, R. W., Holtzapple, P. G., Frankenfeld, J. W. and Volger, T. C. (1975). Liquid Membrane capsule system for treatment of chronic uremia. NIH-NIAMDD-NO1-AM-3-2224.

Doniat, D., Porta, A. and Mosetti, J. (1974). Method for purifying ionically conducting solutions. United States Patent Office 3 827 961, patented August 6, 1974.

Giordano, C. (1972). Diet and amino acids in uremia. *Proc. Eur. Dial. Transpl. Assoc.*, **9**, 419-435.

Giordano, C., Esposito, R. and Pluvio, M. (1975). Oxycellulose and ammonia treated oxystarch as insoluble polyaldehydes in uraemia. *Proc. Eur. Dial. Transpl.* **7**, S 380-382.

Greenbaum, M. A. and Gordon A. (1972). A regenerative dialysis supply system. *Dial. Transpl.*, **1**, 18

Gregor, H. D., Stamberg, J. S., Brennen, K. R., Chlanda, F. C., Gryte, C., Danitz, M. and Hohrer, E. (1969). Specific extracorporeal absorbents. NIAMDD contract no. PH-43-68-708.

Jacobsen, S. C., Stephen, R. L., Bulloch, E. C., Smith, H. R. and Kolff, W. J. (1975a). A wearable artificial kidney, design and application. *Abstract to the Sixth International Congress of Nephrology*, Florence, Italy, June 1975.

Jacobsen, S. C., Stephen, R. L., Bulloch, E. C., Luntz, R. D. and Kolff, W. J. (1975b). A wearable artificial kidney: functional description of hardware and clinical results. *Clinical Dialysis and Transplant Forum of the National Kidney Foundation*, New York, November 22-23, 1975.

John, A., Isaacson, H. R. and Bryant, M. P. (1974). Isolation and characteristics of a urealytic strain of *Selenomonas ruminantium*. *J. Dairy Science*, **57**,, 1003-1014. 1974.

Kolff, W. J. (1952). Forced high caloric, low protein diet and the treatment of uremia. *Amer. J. Med.*, **12** (6), 667-679.

Kolff, W. J., Jacobsen, S. C., Stephen, R. L. and Rose, D. (1975). Towards a wearable artificial kidney (WAK). *Conference on Sorbents in Uraemia,*

Downstate Medical Centre, Brooklyn, New York, November 3 - 4, 1975. (To be published in *Kidney International.*)

Kolff, W. J. (1975). Longitudinal perspectives on sorbents in uraemia. *Conference on Sorbents in Uraemia,* Downstate Medical Centre, Brooklyn, New York, November 3 - 4, 1975. (To be published in *Kidney International.*)

Meriwether, L. S. and Kramer, H. M. (1975). *In vitro* activity of oxystarch and oxycellulose. *Conference on Sorbents in Uraemia,* Downstate Medical Centre, Brooklyn, New York, November 3 - 4, 1975. (To be published in *Kidney International.*)

Mehall, J. R., Koenig, K. L., Lindan, O. and Sparks, R. E. (1969). Screening Study of adsorbents for urea removal from artificial kidney dialysing fluid. *J. Biomed. Mater. Res.,* **3**, 529 - 543.

Rutherford, E., Mercado. A., Hruska, E., Harter, H., Mason, N., Sparks, R., Klahr, S. and Slatopolsky, E. (1973). An evaluation of a new and effective phosphorus binding agent. *Trans. Amer. Soc. Artif. Int. Organs,* **19**, 446.

Sargent, J. A., Gotch, F. A., Olson, R. S., Evano, M. C., Sturkey, L., Herr, J. W., Keen, M. and Seid, M. (1972). Research on improved hollow fibre artificial kidney. NIAMDD Second Annual Progress Report, December 31, 1972. AK - 2 - 70 - 2302.

Setala, K. (1970). A haemodialyser. *Proc. Eur. Dial. Transpl. Assoc.,* **7**, 419.

Setala, K., Heinonen, H. and Schreck-Purola, I. (1972). Uraemic waste recovery II: *In vitro* studies. *Proc. Eur. Dial. Transpl. Assoc.,* **9**, 514 - 520.

Stephen, R. L., Jacobsen, S. C., Atkin-Thor, E. and Kolff, W. J. (1975). Portable/wearable artificial kidney (WAK) - initial evaluation. *12th Congress, European Dialysis and Transplant Association*, Copenhagen, Denmark, August 26 - 29, 1975.

Van Wagenen, R., Steggall, M., Lentz, D. J. and Andrade, J. E. (1975). Activated carbons for medical applications. *In vitro* microparticle characterisation and solute adsorption. *Biomat. Med. Dev. Artif. Organs,* **3**, (3), 319 - 364.

Walitt, A. L. and Jones, H. L. (1969). Development of urea adsorbents for use in artificial kidneys. NIAMDD Contract no. PH - 43 - 68 - 1016.

Walker, J. M. (1973). Feasibility study of electrochemical deureation. Institute for Biomedical Engineering, University of Utah, Internal report August 1973.

Walker, J. M., Denti, E., Van Wagenen, R. and Andrade, J. D. (1975). Evaluation and selection of activated carbon for haemoperfusion. *Conference on Sorbents in Uraemia,* Downstate Medical Centre, Brooklyn, New York, November 3 - 4, 1975. (To be published in *Kidney International.*)

Walser, M., Coulter, A. W., Dighe, S. and Crantz, F. R. (1973). The effect of keto analogues of essential amino acids in chronic uremia. *J. Clin. Invest,* **52**, 3, 678 - 690.

Yao, S. J. and Wolfson, S. K. (1972). Detoxification by electrochemico-auto oxidation. 1: Deammonation. *Trans. Amer. Soc. Artif. Int. Organs,* **18**, 60.

# 17

# EVALUATION OF HAEMOPERFUSION SYSTEMS: *IN VITRO* METHODS RELATED TO PERFORMANCE AND SAFETY

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**Summary**

The widespread use of a haemoperfusion device (Haemocol™) containing coated granules in drug intoxication has led to its use in other clinical applications. The relative simplicity of the system, together with the ability of coated charcoal to adsorb a wide range of molecular species has encouraged clinical evaluation in acute hepatic failure and uraemia.

This use of adsorbents in extracorporeal circuits (ECC) is of particular significance because it represents the introduction of solid/liquid (i.e. blood) mass transfer systems to ECC and it offers the potential to remove specific toxins in varied clinical situations. This significant advance, however, has necessitated the development of test methods particularly relevant to this type of physical system and to the varied clinical uses.

Amongst the many *in vitro* and *in vivo* techniques used to characterise and evaluate adsorbent systems two will be described which are related particularly to performance and safety.

(1) *In vitro* simulation of haemoperfusion. The factors considered here include the adsorption/ desorption behaviour of the system and its effect upon rate of drug removal, total drug capacity of the system and duration of use.

(2) *In vitro* measurement of particle elution using simulated haemoperfusion.

## 17.1 Introduction

The use of adsorbent materials in extracorporeal haemoperfusion was first reported by Yatzidis in 1964. The problems relating to physical stability, blood compatibility and the unselective nature of adsorption when using relatively fragile microporous materials in direct contact with blood have long been recognised by many workers in the field and various solutions proposed (Andrade *et al.*, 1971; Chang and Malave, 1970; Gilchrist *et al.*, 1975; Hill *et al.*, 1976, Rosenbaum, 1972).

The first commercially available haemoperfusion device (Haemocol™) was developed in the laboratories of Smith and Nephew Research Ltd, in order to make available to clinicians an effective means of removing selected drugs in

cases of severe acute drug intoxication. The development of this device, containing polymer coated activated charcoal granules, has been described elsewhere (Fennimore *et al.*, 1975). The clinical use of the device has extended beyond the acute drug intoxication application (Bartels *et al.*, 1975; Grabensee *et al.*, 1976; Hennemann *et al.*, 1976; Vale *et al.*, 1975a,b,) to clinical trial in fulminant hepatic failure (Editorial, 1976; Gazzard *et al.*, 1974), and chronic renal failure Winchester *et al.*, 1975, 1976). Thus the device has been used to adsorb a wide range of toxic (or potentially toxic) solutes using a variety of treatment times and schedules.

The design and manufacture of any extracorporeal device involve meeting many, and often conflicting, criteria, ranging from performance and safety to production and quality control. However, the actual performance of the device is determined not only by the basic design characteristics but also by the operating conditions which can vary considerably. This situation is well typified by the methods necessary to evaluate haemodialysers (National Institute of Arthritis and Metabolic Diseases, 1972). It is therefore particularly important that an *in vitro* study of the performance of adsorbent systems should be able to simulate as realistically as possible the range of *in vivo* operating conditions and at the same time take account of the specific properties of adsorbent materials. In addition, due recognition must be given to any relevant official standards of regulating authorities.

From the wide range of *in vitro* and *in vivo* test methods that is required to evaluate the safety and performance of adsorbent haemoperfusion systems, one of each has been selected to illustrate the application of the above principles.

The first *in vitro* method to be illustrated concerns the measurement of any particulate contamination which may be removed by the blood flowing through the device, while the *in vivo* technique is used to measure the rate of removal of any chosen solute by the device. Additionally it is used to evaluate the adsorptive capacity of the device; this being of importance to the clinician who may wish to leave a single device in circuit for an extended treatment period.

*Particle measurement in adsorbent haemoperfusion systems*

Adsorbent materials, either natural or synthetic, possess a microporous structure which presents a very large surface area to the solute. As a result of this essentially microporous structure, most adsorbents with an adequate solute capacity, i.e. adequate internal surface area, are relatively soft, fragile materials. A typical example of such a material is granulated charcoal.

The elution of particulate carbon from the surface of microporous charcoal has long been recognised as a potential hazard (Hagstam *et al.*, 1966; Rosenbaum *et al.* 1968) and methods based upon visual examination and weighing of eluted carbon particles have been used in order to select the cleanest grade of activated carbon (Van Wagenen *et al.*, 1976). Further studies using instrumented particle counting techniques on both coated and uncoated carbon have indicated the importance of washing and coating techniques upon the particle elution properties of activated

charcoal granules (Andrade *et al.*, 1971; Fennimore and Munro, 1975). The last mentioned studies enable infusion fluid particulate standards, British Pharmacopoeia Appendix 123, to be applied during manufacturing processes.

A further development of these procedures is described in the text. The method enables the particle elution behaviour of an adsorbent haemoperfusion device to be measured *in vitro* during the simulation of an *in vivo* perfusion situation.

*Measurement of adsorption capacity and rate of solute removal*

In extracorporeal devices such as membrane oxygenators and artificial kidneys, the rate of transfer to or from the blood of either oxygen (in the case of the oxygenator) or, say, creatinine (in the case of a dialyser) is governed by the basic design of the device and by the operating conditions either side of the membrane. In both instances the variable factors affecting the rate of solute transfer across the membrane include the fluid dynamics and solute concentration on both sides of the membrane. Within certain limits, therefore, the operating performance of the device can be varied by changing blood flow and solute concentration on the other side of the membrane.

However, in adsorbent haemoperfusion devices the operating performance of the device is much more dependent upon the basic design of the device since the operating characteristics of the adsorbent are fixed by properties specific to the chosen adsorbent. In the case of carbon the attractive forces which hold any given solute to the internal surfaces, although generally weaker than chemical bonds, are effective over a wider range of solutes. In devices, such as Haemocol, which use a membrane coated adsorbent, the properties of the adsorbent can be modified to retard the adsorption of essential blood constituents (e.g. glucose, calcium) but with uncoated carbon systems, the performance of the device depends entirely upon the basic properties of the adsorbent. It is therefore of particular importance when evaluating the *in vitro* performance of haemoperfusion systems to ensure that the test method is related to both the in-use conditions (which can vary considerably in relation to drug concentration and length of treatment) and basic adsorbent properties.

The property of an adsorbent material which governs the in-use performance can be defined by an adsorption isotherm (Perry, 1973). This defines the equilibrium relationship between the amount of solute adsorbed by the material and a solution of known concentration. It should be noted that this is an equilibrium relationship and represents the maximum quantity of solute that can be adsorbed. Thus, once the weight of adsorbent is chosen for any given device, the maximum adsorption capacity of that device is also fixed. What can be varied, however, is the rate at which this equilibrium condition is approached. For example, a properly applied coating on a charcoal granule does not alter the adsorption capacity of the system but does affect the rate of solute transfer into the granule (Kolthammer, 1975; Langley *et al.*, 1975). Similarly, increasing the surface area of the granules by using smaller granules, increases the rate of solute transfer but not the equilibrium capacity.

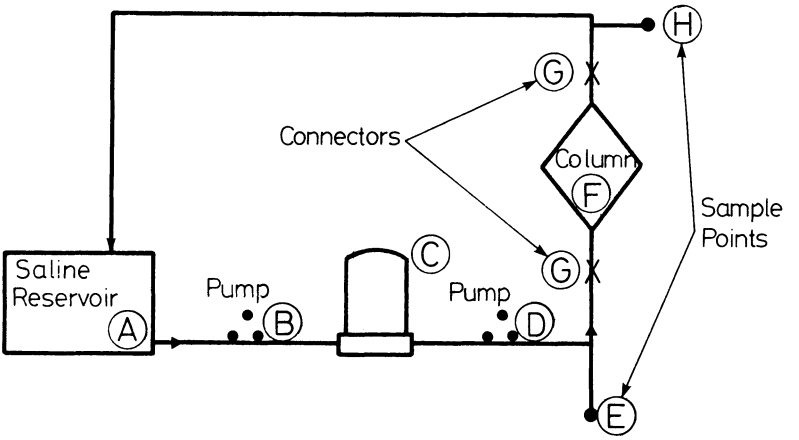



Figure 17.1 Circuit for the measurement of particle elution

A further implication of the equilibrium relationship of an adsorbent is that desorption can occur under certain conditions. Such conditions can obtain if an adsorbent which has been saturated with a solute is exposed to a solution environment which is of a solute concentration lower than that on the adsorbent. This may occur in the clinical situation where the blood levels of a particular toxin falls with time as it is removed. Theoretical considerations of this condition have been published (Cooney and Shieh, 1972). However, in practice, providing the device is correctly designed with regard to adsorption capacity and flow distribution, this should not occur within the usual in-use period. However, it indicates again the significance of the *in vitro* performance testing of adsorbent devices under simulated in-use conditions, i.e. initial solute concentration at clinical levels and falling with time. Such a method is described and discussed in the following sections.

## 17.2 Methods

Figure 17.1 shows schematically an apparatus for the measurement of particle elution. The whole apparatus is situated under a laminar flow hood to minimise the airborne particulate contamination during sampling and insertion of the column into the test circuit. Reservoir A contains 3 - 4 l of saline, 0.9% w/v, which are initially pumped around the circuit with no test column and connectors G - G interlinked. Filter assembly C (Gelman Industries - 0.45 'Acrowflow' cartridge) ensures that the particle concentration in the circulating saline, as measured at sampling point E, is less than 20 particles/ml at $\geqslant 2\mu m$ and less than 2 particles/ml at $\geqslant 5\mu m$ particle size before the column under test is connected into the circuit. Since in the case of Haemocol, the column is supplied sterilised and saline primed, it can easily be introduced into the circuit without the introduction of air or other contamination.

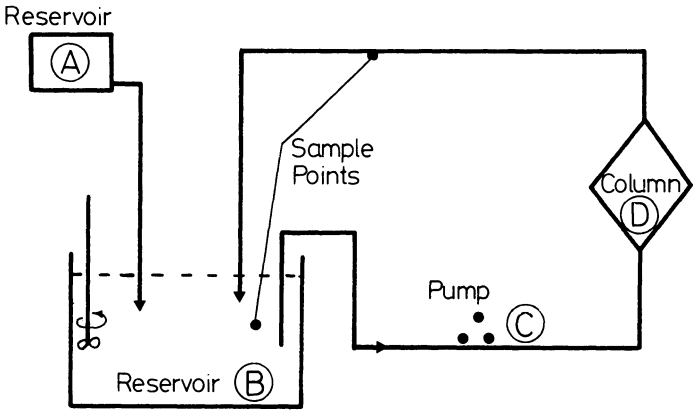



Figure 17.2 Circuit for the measurement of column performance.

Peristaltic blood pumps B and D are set to operate at 200 ml/min. Particulate concentrations are measured via sampling points E and H during a simulated perfusion. Particles are measured at 2, 5, 10 and 25$\mu$m levels (Coulter counter model ZB) which correspond to the particle sizes measured in the British and American Standards for infusion fluids (British Pharmacopoeia 1974, Appendix 123; United States Pharmacopoeia 1975). The adsorbent device (column) performance is assessed by means of the *in vitro* test system shown schematically in figure 17.2.

The well-stirred reservoir, B, contains 40 l of 0.1 M phosphate buffered (pH 7.4) saline (0.9% w/v) with the chosen solute at a typical clinical concentration, with its temperature maintained at 37°C.

Reservoir A contains a concentrated solution of the chosen solute which enables a slow infusion of this solute to be added to reservoir B during the experiment, to ensure maintenance of a realistic solute concentration in reservoir B. The solution is then pumped from reservoir B via the peristaltic pump C at the chosen flow rate through the column D under test and returned to the reservoir. Fluid samples are removed at intervals from reservoir B and the column outlet line for determination of drug concentration.

## 17.3 Results

Typical results for the elution of particles from Haemocol are shown in figure 17.3 in which the difference between the counts at the outlet and inlet of the column is plotted (± S. D.) for $\geqslant 2\mu$m and 5$\mu$m particles (the mean inlet concentration is 13.5 ± 6.3 for $\geqslant 2\mu$m and 0.82 ± 0.7 for $\geqslant 5\mu$m particles). Accurate measurement of counts at $\geqslant 10$ and $\geqslant 25\mu$m was not possible and thus not shown in figure 17.3. Counts that were obtained were very low. These low counts were confirmed by measurements made during manufacture using a light scattering technique on 100 ml samples which indicated that the relevant elution using saline at a flow rate of

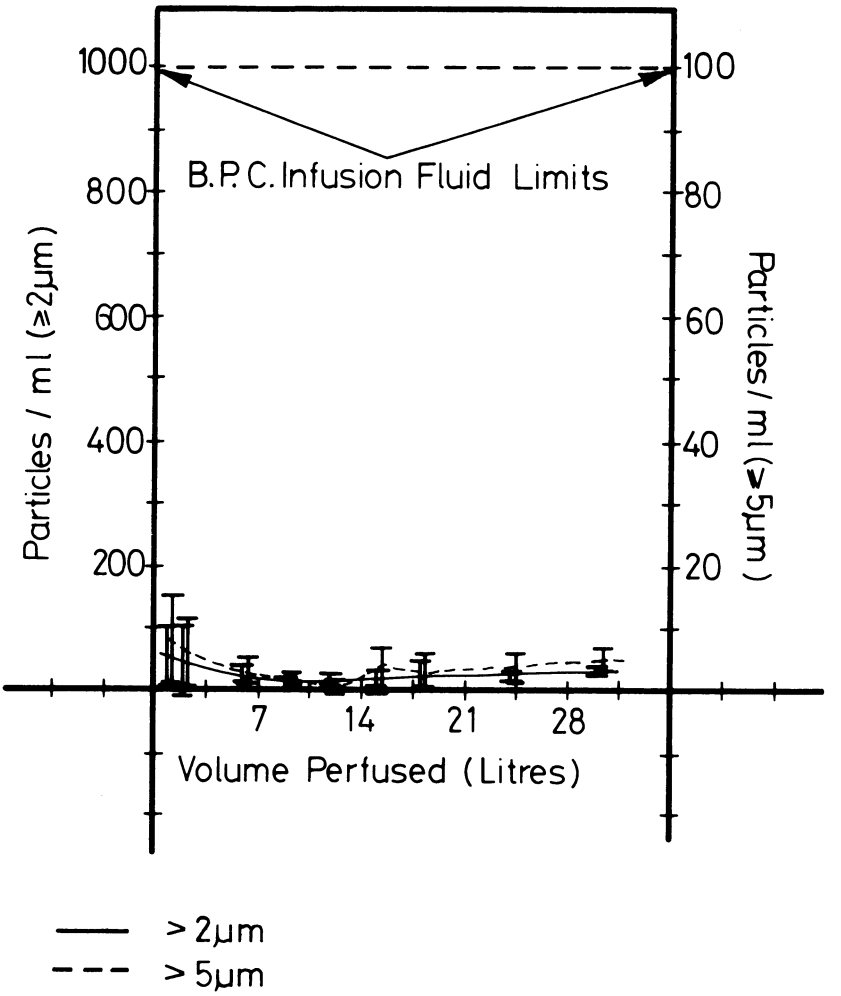



Figure 17.3 *In vitro* particle release by Haemocol.

41/min was 3.74 ± 0.22 particles/ml at ⩾ 10$\mu$m and 1.29 ± 0.12 particles/ml at ⩾ 25$\mu$m.

Although the test circuit (figure 17.1) can be operated using a single peristaltic pump (B) the other one in the circuit (D) is also used. This ensures that the pressure transients normally caused by peristaltic blood pumps are not damped out by the filter but passed on by pump (D) to the column under test. The system has the added advantage that the generation of particles by the elastomeric tubing insert distal to the pump, as it ages with time, can also be studied.

Adsorbent column performance during a simulated haemoperfusion can be

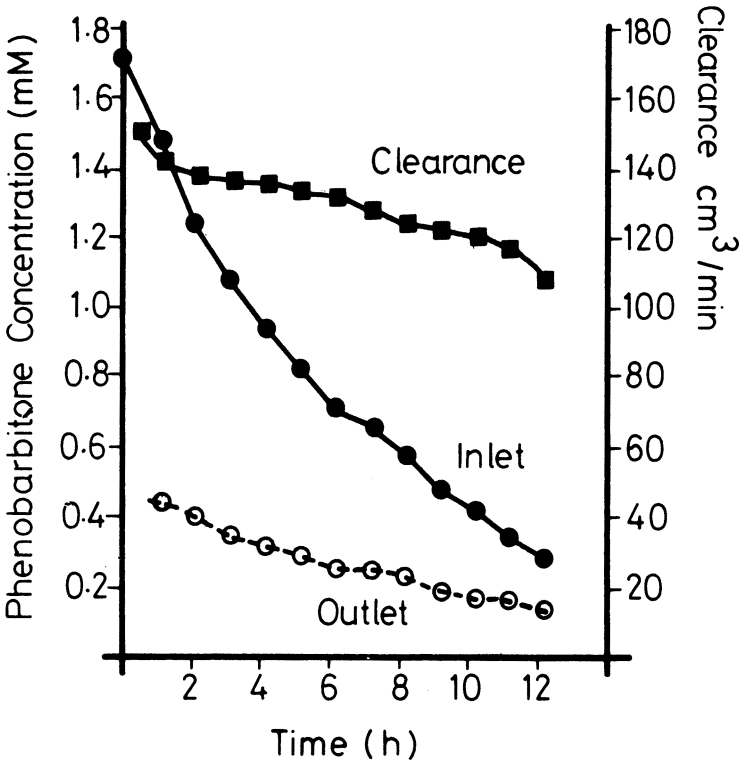



Figure 17.4 *In vitro* removal of phenobarbitone by Haemocol (recirculating method) at a flow rate of 200 ml/min

seen by reference to figure 17.4. This shows the removal of phenobarbitone by Haemocol from which the quantity of drug removed by one column in a given time can be computed. The results for three different drugs are given in table 17.1.

**Table 17.1**

*In vitro* drug removal by Haemocol

| *Drug* | *mol. wt. 225 Pentobarbitone* | *mol. wt. 232 Phenobarbitone* | *mol. wt. 138 Salicylate* |
|---|---|---|---|
| Initial concentration | 0.531 m.mol/l | 1.724 m.mol/l | 10.87 m.mol/l |
| Amount removed at 1 h | 4.76 m.mol | 12.54 m.mol | 114.36 m.mol |
| Amount removed at 4 h | 15.19 m.mol | 41.52 m.mol | 253.75 m. mol |
| Amount removed at 12 h | 30.43 m.mol | 77.28 m.mol | 317.68 m.mol |

This may be compared with the *in vivo* removal of 23.71 m.mol of phenobarbitone in a 6 h perfusion (Vale *et al.*, 1975a and b), which was also computed using a trapezoidal rule. Recently, however, a method has been developed (Bartels *et al.*, 1975) for the elution of drug from a sample of the column contents and subsequent estimation of total quantity adsorbed by the column.

It should emphasised in this connection that the sampling of carbon from the column, must be representative. Obviously, possible nonuniformity of drug distribution in a not fully saturated column must be taken into account.

## 17.4 Conclusion

The clinical use of adsorbent haemoperfusion is growing in regard to both the range of solutes being removed and the in-use conditions applied. This is due to the relative simplicity of the technique and the ability of adsorbents such as charcoal to remove a wide range of drugs (Kolthammer, 1975) and endogenous toxins more effectively than conventional techniques such as forced diuresis and dialysis (Bloomer, 1965; Medd *et al.*, 1973).

The use of solid adsorbents does, however, involve the careful choice of *in vitro* test methods and procedures in order to simulate as far as possible in-use conditions and at the same time measure parameters which relate to the basic properties of adsorbent materials. Such procedures are well illustrated by the measurement of particle contamination and adsorption performance of a device such as Haemocol during simulated haemoperfusion. Regarding performance, comparisons may be made with the *in vivo* situation, but in the case of particulate contamination (in common with other extracorporeal systems where particle concentrations are low) no such *in vivo* comparisons are yet possible.

It is recognised that the use of saline for the study of particle elution is not ideal with respect to viscosity and may therefore lack correspondence with the magnitude of the drag forces imposed upon the carbon by the flow of liquid. However, preliminary work in our laboratories using a more viscous medium (3.5 c.P.) has shown that the elution of particles under these conditions is not significantly different from that obtained using saline as the perfusion medium.

Such simulated techniques, in conjunction with other process and quality control procedures, enable manufacturers to continually improve haemoperfusion devices and also to widen the range of clinical application of adsorbent technology.

## 17.5 References

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood perfusion: Hema/activated carbon. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 222.

Bartels, O., Grumeth, M., Daniel, V., Haas, T., Kramer, G. and Tympner, T. (1975) Early charcoal haemoperfusion for treating *Amanita* poisoning. *Dtsch. Med. Wschr.*, **100**, 2509.

Bloomer, H. A. (1965). Limited usefulness of alkaline diuresis and peritoneal dialysis in pentobarbital intoxication. *New Engl. J. Med.*, **272**, 1309.

British Pharmacopoeia (1974). Appendix 123, Her Majesty's Stationery Office, London.

Chang, T. M. S. and Malave, N. (1970). The development and first clinical use of semipermeable microcapsules (artificial cells) as a compact artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **16**, 141.

Cooney, D. O. and Shieh, D. F. (1972). Fixed bed sorption with recycle. *Amer. Inst. Chem. Eng. J.*, **18**, 245.

Davis T. A., Cowsar, D. R., Harrison, S. D. Jr. and Tanquary, A. C. (1974). Activated carbon fibres for haemoperfusion. *Trans. Amer. Soc. Artif. Int. Organs.*, **20**, 353.

Editorial (1976). Haemoperfusion aids in hepatic coma. *J. Amer. Med. Assoc.*, **235**, 12.

Fennimore J., Watson P. A., Munro, G. D. and Kolthammer, J. C. (1975). The design and evaluation of a convenient carbon haemoperfusion system. *Eur. Soc. Artif. Organs*, **1**, 90.

Fennimore, J. and Munro, G. D. (1975). Design problems, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London, p. 330.

Gazzard B. G., Portmann, B. A., Weston, M. J., Langley, P. J., Murray-Lyon, I. M., Dunlop, E. H., Flax, H., Melton, P. H., Record, C. O., Ward, M. B. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet,* **2**, 1301.

Gilchrist, T., Jonsson, E., Martin, A. M., Naucler, L., Cameron, A. and Courtney, J. M. (1975). Development of the Strathclyde haemoperfusion system, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London, p. 319.

Grabensee, B. Konigsha, T. and Schnurr, E. (1976). The treatment of severe hypnotic poisoning with extracorporeal haemoperfusion. *Dtsch. Med. Wschr.*, **101**(5), 158.

Hagstam, K. E. Larsson, L. E. and Thysell, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital intoxication and uraemia, including histopathological findings. *Acta Med. Scand.*, **180**, 593.

Hennemann H., Nanjoks, R., Gattenlo, W., Rockel, A., Kassler, G., Reiners, W. and Heidland, A. (1976). Haemoperfusion in the treatment of hypnotic poisoning. *Dtsch. Med. Wschr.*, **101**(5), 155.

Hill, J. B., Palaia, F. L., McAdams, J. L. Palmer, P. J., Skinner, J. T. and Maret, S. M. (1976). *Kidney International* (in press).

Kolthammer J. C. (1975). The *in vitro* adsorption of drugs from horse serum onto carbon coated with an acrylic hydrogel. *J. Pharm. Pharmac.*, **21**, 801.

Langley, P. G., Fennimore, J., Munro, G. D. and Hodgson, M. E. (1975). The effect of coating on adsorption, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London p. 337.

Medd, R. K. Widdop, B., Braithwaite, R. A., Rees, A. J and Goulding, R. (1973). Comparison of haemoperfusion and haemodialysis in the therapy of barbiturate intoxication in dogs. *Arch. Toxicol.*, **31**, 163.

National Institute of Arthritis and Metabolic Diseases (1972). *Evaluation of haemodialysers,* Department of Health, Education and Welfare, publication no. (NIH) 72 - 103.

Parry, R. H. and Chilton, C. H. (1973). *Chemical Engineers Handbook,* 5th edition, McGraw-Hill, Maidenhead.

Rosenbaum J. L. (1972). The use of resin haemoperfusion in the treatment of acute drug intoxication, *Clin. Toxicol.,* **5**, 331.

Rosenbaum, J., Ronquillo, E. and Argyres, S. N. (1968). Column haemoperfusion and haemodialysis techniques to treat barbiturate intoxication in dogs. *J. Albert Einstein Med. Centre,* **16**, 67.

United States Pharmacopoeia (1975). Volume XIX, 1st supplement.

Vale J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975a). Use of charcoal haemoperfusion in the management of severely poisoned patients, *Brit. Med. J.,* **1**, 5.

Vale J. A., Thomas, T., Widdop, B. and Goulding, R. (1975b). Use of charcoal haemoperfusion in poisoned patients: a review. *Proc. Eur. Soc. Artif. Organs,* **2**, 244.

Van Wagenen, R. A., Coleman, D. L. and Andrade, J. D. (1975). Adsorbent haemoperfusion: nonbiological particulate matter. *Kidney International,* **7**, S. 397.

Van Wagenen, R. A., Stegal, M., Lentz, D. J. and Andrade, J. D. (1976). Activated carbons for medical applications. *In vitro* microparticle characterisation and solute adsorption. *Biomat. Med. Dev. Artif. Organs,* **3**, 319.

Widdop, B., Medd, R. K., Braithwaite, R. A., Rees, A. G. and Goulding, R. (1975). Experimental drug intoxication: treatment with charcoal haemoperfusion. *Arch. Toxicol,* **34**, 27.

Winchester, J. F., Apiliga, M. T., Mackay, J. M. and Kennedy, A. C. (1975). Haemodyalisis with charcoal haemoperfusion. *Proc. Eur. Dial. Transpl. Assoc.,* **12**, 526.

Winchester, J. F., Apiliga, M. T. and Kennedy, A. C. (1976). Short-term evaluation of charcoal haemoperfusion combined with dialysis in uraemic patients. *Kidney International* (in press).

Yatzidis, H. (1964). A convenient haemoperfusion micro-apparatus over charcoal for the treatment of endogenous and exogenous intoxication. *Proc. Eur. Dial. Transpl. Assoc.,* **1**, 83.

# 18

# CONVERSION OF CREATININE IN THE PRESENCE OF ACTIVATED CARBON

J. TIJSSEN, M. J. F. M. KAPTEIN, J. FEIJEN, A. BANTJES and A. W. J. VAN DOORN

**Summary**

Activated carbon (a.c.) coated with different types of polymer has been used as a sorbent in haemoperfusion techniques for the removal of toxic products (drug overdose, metabolites, etc). The adsorption capacity of different types of a.c. for creatinine, uric acid, etc, has to be determined to select the types of a.c. best suited for specific purposes. Dedrick *et al.* (1967) have studied the adsorption of uric acid onto the a.c. Columbia NXC (Union Carbide Corporation). They reported that uric acid in contact with a.c. was converted at least partially into yet unknown products. We report on the conversion of creatinine in the presence of a.c. (Norit RBX1) and oxygen.

## 18.1 Experimental Work

Ultraviolet (u.v.) spectra were measured in aqueous solutions using a Unicam S.P. 800, and n.m.r. spectra were recorded on a Varian XL-100, using tetramethylsilane (TMS, $\delta$ 0.0) as internal standard and $D_2O$ as solvent. Elemental analyses were carried out by the Organisch Chemisch Instituut TNO under the supervision of W. J. Buis.

*Adsorption experiments*

Aqueous creatinine solutions were contacted with a.c. using *in vitro* recirculation and stirred-batch type of experiments.

Creatinine adsorption experiments in recirculation were carried out in the system shown in figure 18.1. Here creatinine is dissolved in 11 l of buffered saline (0.02 M $KH_2PO_4$, pH 7.4, creatinine concentration 15 mg%). The solution was then circulated at 70 ml/min through a glass column, containing 40 g of Norit RBX1 a.c. The creatinine concentration in vessel V (figure 18.1) was continuously measured by an u.v. spectrophotometer (Zeiss PM 6). The variation of absorption with time of recirculation was followed at a wavelength of $\lambda$ = 260 nm ($\lambda_{max}$ creatinine = 234 nm) because only flow cells with a pathlength of 10 mm were available.

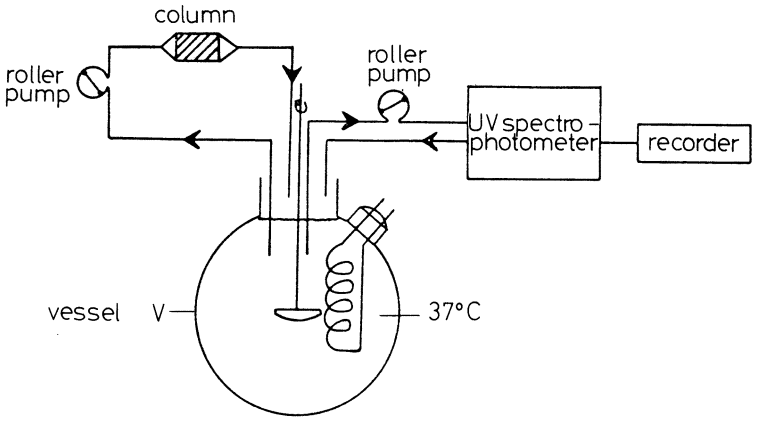



Figure 18.1 Recirculation system

Figure 18.2 shows the results of a typical experiment: the absorption decreases during 3 h of recirculation. This is followed by an unexpected *increase* in the observed absorption. To determine the effect of various factors on the course of the absorption at $\lambda = 260$ nm a number of recirculation experiments have been performed (table 18.1). Additionally complete u.v. spectra of the solution in vessel V (see figure 18.1) were recorded for different recirculation times (figure 18.3).

Similar experiments with Fisher activated coconut carbon gave u.v. absorption results fully comparable with those shown in figures 18.2 and 18.3.

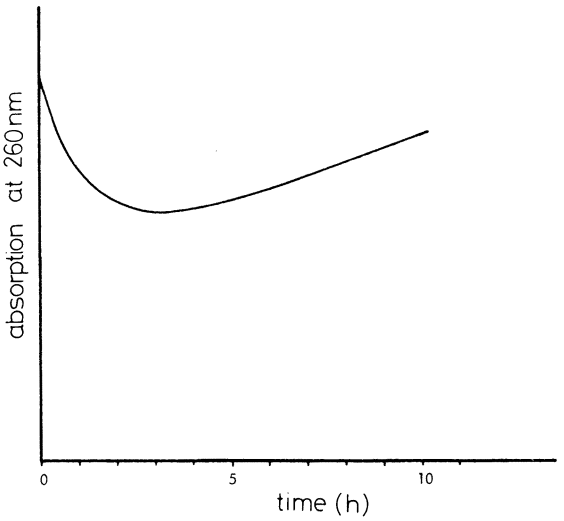



Figure 18.2 Absorption at 260 nm of the solution in vessel V (shown in figure 18.1) versus time. (Reference: phosphate buffered saline solution.)

**Table 18.1**

The course of absorption ($\lambda$ = 260 nm) versus recirculation time in different circumstances. (+ = factor present, – = factor absent)

| *Grouping of factors* | *NaCl 9g/l* | *Phosphate buffer 0.02 M $KH_2PO_4$* | *Creatinine 15 mg%* | *Norit RBX 1 40 g* | *($\lambda$ = 260 nm) versus time during 20 h recirculation* |
|---|---|---|---|---|---|
| I | – | – | – | – | constant |
| II | + | – | – | – | constant |
| III | + | + | – | – | constant |
| IV | + | + | + | – | constant |
| V | + | + | – | + | constant |
| VI | + | + | + | + | as shown in figure 18.2 |

To carry out stirred-batch experiments a.c. (2.9 g Norit RBX1) was suspended in 800 ml creatinine solution (creatinine dissolved in redistilled water at 15 mg%). When the suspension was kept under a nitrogen atmosphere the absorption at $\lambda$ = 260 nm reached a steady state value, whereas the wavelength at maximum absorption remained constant. In the case of an oxygen or air interface, the course of the absorption as a function of time agreed with the results of the recirculation experiments. These experiments therefore show that oxygen is necessary for the conversion of creatinine.

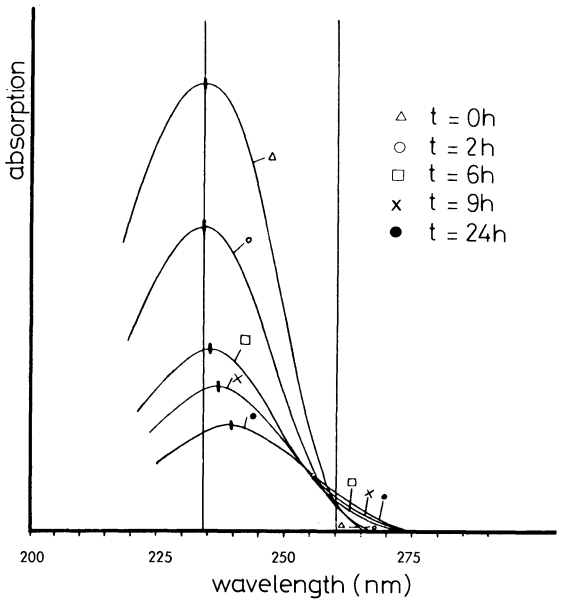



Figure 18.3 Ultra violet spectra of the solution in vessel V (shown in figure 18.1) at different recirculation times. (Reference: phosphate buffered saline solution.)

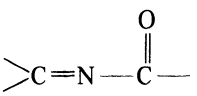
*Conversion products*

To explore these products a creatinine solution (1.65 g creatinine in 11 1 redistilled water, without phosphate buffer and NaCl) was recirculated through a Norit RBX1 a.c. packed column for 24 h. After evaporation of the solution at 40°C, under reduced pressure, a solid material (0.83 g) was obtained. Thin layer chromatography (TLC) of this product (stationary phase: silica gel H according to Stahl: Merck/eluent: water) indicated the presence of at least two components ($R_f$ 0; $R_f$ 0.79) which differ from creatinine ($R_f$ 0.52), and the disappearance of creatinine itself. The conversion product with $R_f$ 0.79 (about 50% of the total amount of solid) was isolated (solvent: water) using a silica gel packed column (silica gel H according to Stahl: Merck) with water as the solvent.

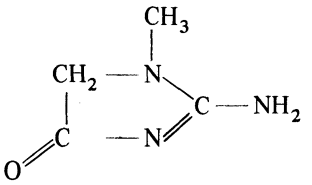
## 18.2 Results And Discussion

From table 18.1, it can be concluded that the combination a.c. - creatinine causes the change of absorption with time. The stirred-batch experiments show that oxygen must be present to give the unexpected increase in absorption at $\lambda$ = 260 nm with time. The graphs given in figure 18.3 show that when the measurements are performed at 234 nm, the increase in absorption with time, as shown in figure 18.2, no longer obtains and that the wavelength corresponding to maximum absorption (initially 234 nm) shifts to higher values as the time of recirculation increases.

TCL shows that creatinine is almost completely converted into at least two components of which product A ($R_f$ 0.79) has been isolated and analysed. Matsumoto and Rapoport (1968) have reported that the u.v. absorption of creatinine at $\lambda$ = 234 nm is caused by the conjugated system

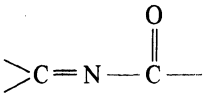


They suggest the following structure for creatinine



2-amino, 5-dihydro-1-methyl -imidazol-4-one

Product A has a maximum u.v. absorption at $\lambda$ = 240 nm (in redistilled water). This wavelength at maximum absorption differs only slightly from that of creatinine ($\lambda_{max}$ = 234 nm), indicating that the same chromophore



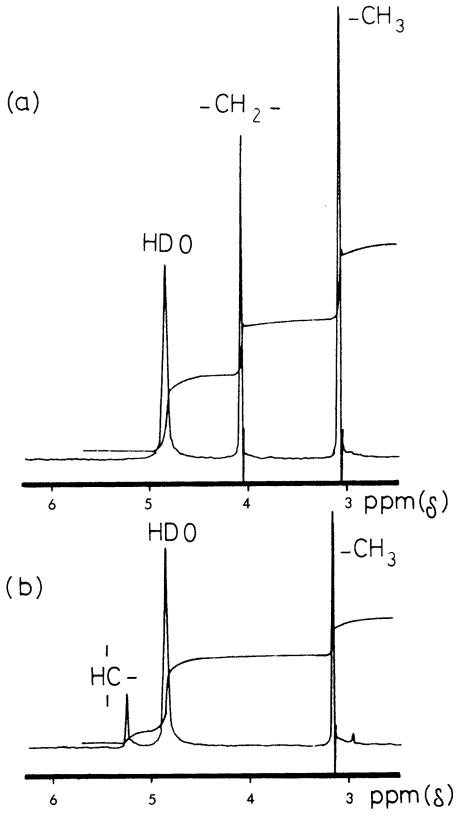



Figure 18.4 n.m.r. spectra at 100 MHz (sweep width kHz) of (a) creatinine and (b) product A

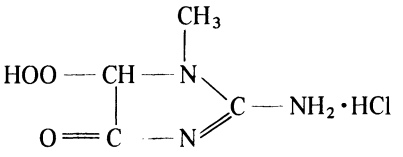
is probably also present in product A. The n.m.r. spectra of creatinine and of product A (figure 18.4) show the following results.

(1) In the spectrum of product A, the absorption of the two protons on C — 5 in creatinine ($\delta = 4.06$) has disappeared.
(2) Product A has a peak at $\delta = 5.24$ (1H) which is not present in the spectrum of creatinine.
(3) In product A and creatinine the chemical shift of the methyl group (N-1) exhibits little difference.

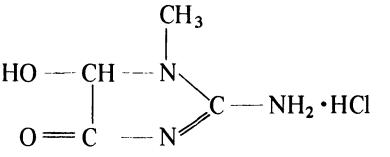
From figures 18.4a and b it can be concluded that during the conversion of creatinine a substitution of one of the protons on C — 5 has occurred. The chemical shift of the single proton at $\delta = 5.24$ of product A can possibly be explained by the introduction of a substituent on C — 5 having an oxygen atom attached to the carbon atom. From (3) it is most likely that N — 1 is not converted into a N-oxide (Feijen and Wijnberg, 1970).

The initial results of the elemental analysis were: C 17.63%, H 2.91%, N 15.29%, O 15.84%, ash 35.77%, unknown 12.56%. Product A still seems to contain some silica gel (ash) and also some chlorine can be established by a test with aqueous silver nitrate solution and ammonia. An explanation of the occurrence of chlorine in product A probably lies in the washing procedure for Norit RBX1 a.c. which uses a hydrochloride solution. Both the presence of chlorine and the slight difference in chemical shift of the protons of the methyl group of product A, as compared to creatinine, point to the formation of a hydrochloride salt. After correcting for the assumed silica gel ash content, and further assuming that the unknown part is chlorine, the following values for the elemental analysis of product A become: C 27.45%, H 4.53%, N 23.81%, O 24.66%, Cl 19.55%. Finally, product A may be shown to be characterised by the formula $(C_4H_8N_3O_2\frac{3}{4}\ Cl)_n$.

It may also be noted that the results of the u.v., n.m.r. and elemental analyses of product A indicate that oxidation of the $-CH_2-$ group of creatinine takes place and that one or a mixture of the following two compounds is formed:



$C_4H_7N_3O_3 \cdot HCl$



$C_4H_7N_3O_2 \cdot HCl$

Analyses of a more purified conversion product are planned and hopefully will lead to a definitive conclusion.

Future research will be carried out to determine other conversion products as well as the possible conversion of other metabolites (such as uric acid) and drugs. The properties of these conversion products (especially toxicity and pyrogenicity) and the kinetics of the conversion reactions are considered important in the application of a.c. in haemoperfusion systems.

## 18.3 Acknowledgement

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## 18.4 References

Dedrick R. L., Vantoch, P., Gombos, E. A. and Moore, R. (1967). *Trans. Amer. Soc. Artif. Int. Organs*, **13**, 236.

Feijen, J. and Wijnberg, H. (1970). *Rec. Trav. Chim.*, **89**, 639.

Matsumoto, K. and Rapoport, H. (1968). *J. Org. Chem.*, **33**, 552.

# 19

# PROTECTIVE EFFECTS OF MICROENCAPSULATION (COATING) ON PLATELET DEPLETION AND PARTICULATE EMBOLISM IN THE CLINICAL APPLICATIONS OF CHARCOAL HAEMOPERFUSION

T. M. S. CHANG

**Summary**

A review of present day applications of the principle of artificial cells commences with a description of the development of the albumin-collodion coated activated charcoal (ACAC) system for haemoperfusion. This is followed by studies of patient safety relating to particulate matter released during perfusion and the consideration of the associated problem of platelet depletion. The review concludes with a discussion of the use of haemoperfusion in chronic renal and liver failure and severe acute drug intoxication.

## 19.1 Introduction

*Artificial cells*

The principle of artificial cells was originally proposed and demonstrated using semipermeable microcapsules (Chang, 1964, 1966, 1972a). Semipermeable microcapsules are ultrathin polymer membrane envelopes, either in the form of spherical membranes containing solutions or suspensions or in the form of a membrane coating on individual solid granules of adsorbent. The large surface to volume relationship (e.g. 2.5 $m^2$ surface area in 10 ml of 20 $\mu$m diameter or 300 ml of 2 - 5 mm diameter microcapsules) and the ultrathin membrane of microcapsules (for example, less than 0.05 micron) is such that the transport of permeant molecules is many times higher than standard haemodialysers. For instance, 10 ml of 2 mm diameter microcapsules have a transport rate which is twice that of a standard haemodialysis machine, and 10 ml of smaller microcapsules of 20 $\mu$m diameter have a transport capacity of more than 200 times that of standard haemodialysers. This incredible potential rate of molecular transport is such that when enzymes or adsorbents are microencapsulated within the membrane, they convert or entrap permeant metabolites entering the semipermeable microcapsule. With the com-

bination of semipermeable microcapsules and either enzyme, adsorbent or enzyme plus adsorbent, we have the basis of a miniature, effective system for the removal of waste metabolites, toxins or drugs (Chang, 1964, 1966, 1972a). These have been used locally at sites where they can have free access to metabolites from body fluids in the form of blood, peritoneal fluid, or intestinal fluid (Chang, 1964, 1966, 1972a). The present paper is a discussion of the use of microencapsulated (coated) charcoal for use in haemoperfusion.

*Microencapsulation (coating) of activated charcoal*

It has been demonstrated that free activated charcoal granules can effectively remove many uraemic metabolites and drugs from the perfusing blood (Yatzidis, 1964, Dunea and Kolff, 1965; Hagstam *et al.*, 1966; Demyttenaere *et al.*, 1967). Unfortunately, with the discovery that they adversely depleted platelets and released embolising particles, clinical use of charcoal haemoperfusion in patients was discontinued around 1966 (Dunea and Kolff, 1965; Hagstam *et al.*, 1966; Demyttenaere *et al.*, 1967). We first proposed the combination of the principle of artificial cells with the activated charcoal by immobilising charcoal within semipermeable microcapsules (Chang, 1966). This way, it was demonstrated that the microencapsulated charcoal, either in the form of spherical ultrathin membranes containing charcoal suspensions or ultrathin membrane coated individual solid granules of adsorbent, would prevent free charcoal from passing out of the membrane and going into the circulation, therefore preventing any free embolising of charcoal powder. When the enveloping membrane is made biocompatible, the platelets in the circulating blood are prevented from coming into direct contact with charcoal or being removed by the polymer membrane. As a result there is no depletion of circulating platelets. For a number of years since this was proposed, this was the only laboratory which was publishing detailed reports on this approach of microencapsulated charcoal. With the demonstration from this laboratory of the safe and effective use of an albumin-collodion coated activated charcoal system for haemoperfusion in patients, an increasing number of other laboratories have more recently started to extend and modify this approach. As a result, there has been a rapid escalation in the number of detailed papers published on the subject of coated charcoal haemoperfusion. This paper describes in detail the studies on platelets and particulates, and discusses the use of ACAC for uraemia, intoxication and liver failure.

## 19.2 Methods and Materials

After testing a large number of membrane materials for microencapsulation of charcoal, including nylon, cellulose acetate, heparin-complexed materials, collodion, albumin-collodion coating, and others (Chang, 1966, 1969, 1972a; Chang *et al.*, 1968) we have arrived at an effective membrane system which is prepared in the following way. After careful washing and sterilisation, each coconut charcoal granule is coated with an ultrathin membrane of collodion (0.05 $\mu$m thick cellulose nitrate). This is placed in a specially prepared silicone coated shunt chamber, and

then carefully steam-autoclaved. Steam-autoclaving does not cause melting or destruction of the membrane material if carried out correctly. After autoclaving and cooling, a second coating consisting of human albumin is then adsorbed to collodion. Albumin adsorbs tightly to collodion and some polymers. However, it should be emphasised that albumin coating is only effective when adsorbed onto polymers like collodion and it does not adsorb as tightly as required to certain other types of polymers like hydrogel. The exact technique for the preparation of the albumin-collodion microencapsulated charcoal system has been described (Chang, 1969) and updated in detail (Chang, 1972a, 1976a). It should be noted that before any coating procedure is carried out, very extensive washing and sterilisation of charcoal granules are required to ensure freedom from contamination or presence of too much charcoal powder on the surface (Chang, 1972a). Furthermore, users should be aware that activated charcoal available commercially varies to some extent in strength, adsorbing capacity, and amount of embolising powder produced. This was discussed in detail in a recent review (Van Wagenen *et al.*, 1975).

The microcapsules used in the present studies had a diameter ranging from 2 - 5 mm. A total of 300 g was used and the total membrane area calculated from the mean diameter was 2.5 $m^2$ with a membrane thickness of 0.05 $\mu$m. This has a total theoretical transport rate of more than 200 times that of a 'standard' artificial kidney. At a blood flow rate of 200 - 300 ml/min, the pressure drop across the microcapsule artificial kidney is less than 10 mm Hg, and in patients using Scribner shunts, a blood flow rate of between 200 - 300 ml/min is possible without the use of blood pumps. A blood pump is required for those with an arterial venous fistula or in those with cutaneous puncture of femoral veins.

### 19.3 Safety Analysis for Particulate Matters

Detailed *in vitro* and *in vivo* studies showed that, unlike uncoated charcoal, there were no microscopically visible particles in the recirculating solution or efferent blood of properly prepared albumin-collodion coated activated charcoal. Detailed histological examination of the lung of dogs showed no visible embolising particles when well prepared albumin-collodion coated activated charcoal was used for haemoperfusion. In consequence more than 160 clinical procedures have been carried out safely by this Unit in the past six years with no clinical evidence of particulate embolism.

In more recent years, interest has been increasing in coated charcoal haemoperfusion. The question of particulate matters has been examined using a variety of approaches with no absolute standard or methodology. The British Pharmacopoeia standard for measuring particulate matters of intravenous solutions using the Coulter counter, as adapted by Smith and Nephew for analysing their charcoal column, appears to be a method which is most suitable for comparative studies of different coating procedures. We have therefore analysed the ACAC haemoperfusion system as follows.

ACAC was prepared as described (Chang, 1969, 1972a, 1976a) including exten-

sive washing before coating, after coating, after autoclaving and 3 l rinsing before use. This is compared to charcoal which has also been washed extensively as described (Chang, 1969, 1972a, 1976a), except that no coating was applied. In the standard analysis, a Travenol roller pump was used to circulate clinical intravenous sodium chloride solution (Baxter) through each 300 g column at 300 ml/min. The pulse pressure at the afferent tubing was measured as 30/0 mm Hg. After 3 l of washing passed through the shunt chamber, samples were taken at the efferent end of the chamber and counted in the Coulter counter for particles of greater than 2 μm and greater than 5 μm. Solution obtained directly from the intravenous solution bag being used at any one time was taken simultaneously for comparison. This was used to simulate the effects of normal haemoperfusion. In simulation tests for possible high pulsatile flow due to partial obstructions of arterial blood flow, the Travenol pump dial was left at the setting for 300 ml/min. The line before its entry into the roller pump was then occluded by a clamp in order to nearly occlude the flow for 2 min. During this time, a pulse pressure of +50/−50(100 mm Hg pulse pressure) was obtained. After 2 min, the occlusion was removed and efferent fluid from the ACAC column analysed for particulate matters. It is felt that 2 min is sufficient time since safety pressure monitors will have warned of arterial line occlusions before this time. The results are shown in tables 19.1 and 19.2. It is clear that albumin-collodion coated activated charcoal prepared in this laboratory did not release a significant amount of particulate matter. On the other hand, significant particulate matter was released in the case of the uncoated activated charcoal column.

In view of this finding, it is interesting to observe the results obtained with fixed-bed charcoal, and coated and uncoated charcoal columns, reported elsewhere in these proceedings (Hill *et al.*, p. 123). Firstly, concerning the fixed-bed procedure, when fine particles of activated charcoal are sprayed onto the adhesive polymer solution of the coil for fixation, it is possible that immediately on contact a film of the wet solution could have spread around each fine particle resulting in an ultrathin polymer coating. This type of coating, like the ultrathin collodion coating in ACAC charcoal, cannot be observed even under the scanning electron

**Table 19.1**

Standard analysis of ACAC haemoperfusion system

*After 3 l of continuous Travenol roller pump (300 ml/min) (particles/ml)*

| | *Uncoated* | | *Coated* | |
|---|---|---|---|---|
| | $> 2$ μm | $> 5$ μm | $> 2$ μm | $> 5$ μm |
| Original intravenous solution | 136.8 ± 14.2 | 21.6 ± 2.2 | 145.2 ± 12.0 | 26.4 ± 8.0 |
| Efferent from chamber | 318.8 ± 36.4 | 36.4 ± 8.2 | 133.6 ± 17.2 | 26.0 ± 8.0 |

**Table 19.2**

Simulation tests for possible high pulsatile flow due to partial obstructions of arterial bloodflow

*Partial inflow occlusion for 2 min (pulse +50/−50 mm Hg) (particles/ml)*

| | *Uncoated* | | *Coated* | |
|---|---|---|---|---|
| | *> 2 μm* | *> 5 μm* | *> 2 μm* | *> 5 μm* |
| Original intravenous solution | 136.8 ± 14.2 | 21.6 ± 2.2 | 207.6 ± 24.2 | 31.6 ± 5.6 |
| Efferent from chamber | 798.4 ± 33.0 | 96.0 ± 13.6 | 187.6 ± 16.6 | 28.8 ± 7.6 |

microscope. Hill and his coworkers have not yet demonstrated that the fine charcoal particles in their fixed bed system are uncoated. Secondly, insofar as their fragmentation studies on uncoated and collodion coated charcoal granule columns are concerned, it is likely that the charcoal granules are not properly coated. On autoclaving their collodion coated charcoal, they have observed that much of the coating membrane material comes off into the washing solution or adheres to the walls of the shunt chamber. We have not found this when the ACAC is prepared according to the procedures described (Chang, 1969, 1972a, 1976a). It is possible therefore that part of the increase in weight of the Millipore filter in Hill's particulate test is due to membrane material which was improperly coated. Otherwise, it would be difficult to explain the larger increase in weight in the coated system when compared to the uncoated during their rinsing experiments. In the 'bumping' test they carried out no details have been given of how severe and prolonged the 'bumping' was.

In conclusion, it is considered that the properly prepared albumin-collodion coated activated charcoal haemoperfusion system, when used under normal conditions, does not release any detectable amount of particulate matter.

### 19.4 Prevention of Severe Platelet Depletion

Platelet depletion is one of the two major problems which have to be solved. Very detailed studies on this aspect have been carried out on a number of coated activated charcoal systems in dogs (Chang, 1969, 1972a, 1974; Chang and Malave, 1970; Watson and Chang, 1975; Chang *et al.*, 1968). The effects of albumin-collodion coated activated charcoal on platelets were also studied in patients (Chang and Malave, 1970; Chang *et al.*, 1971).

Figure 19.1 represents a summary of the results obtained in testing uncoated, collodion coated, and albumin-collodion coated activated charcoal in dogs. Platelet studies carried out in dogs are very severe tests since dog platelets are known to be very sensitive to extracorporeal surfaces. As a result, very careful control studies were carried out, including the control testing of silicone coated shunt chambers and

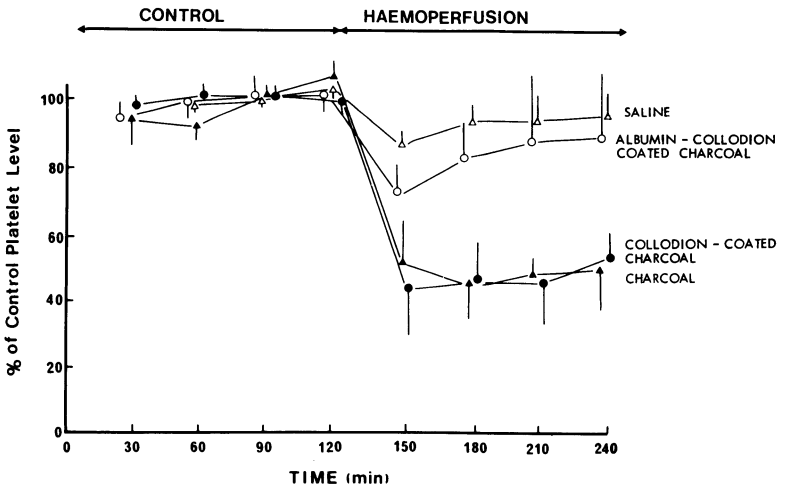


Figure 19.1 Comparison of albumin-collodion coated activated charcoal with collodion coated activated charcoal

tubings containing priming saline but no activated charcoal. Furthermore, no blood pumps were used. The arterial pressure of the dog was sufficient to recirculate blood through the shunt chamber at 100 - 150 ml/min. In these studies, both uncoated and collodion coated activated charcoal systems caused the same degree of platelet depletion with a post-haemoperfusion platelet level of respectively: 54 ± 15% and 56 ± 7%. However, albumin-collodion coated activated charcoal has a very significant protective effect on platelet depletion with a post-haemoperfusion level of 90 ± 10%. In the dog studies of Hill *et al.*, (see p. 123) both uncoated and collodion coated activated charcoal also caused marked platelet depletion with a post-haemoperfusion level of about 30% in both cases. However, they did not report the use of albumin-collodion coated activated charcoal. Furthermore, they did not siliconise the shunt chamber and screen.

Heparin-benzalkonium-complexed collodion membrane coated on activated charcoal also protected platelet depletion in dogs (Chang *et al.*, 1968; Chang, 1969, 1974). Prior infusion of dextran 70 in dogs followed by collodion coated activated charcoal haemoperfusion also prevented platelet depletion (Watson and Chang, 1975).

In clinical trials of patients with chronic renal failure, blood samples were taken immediately before ACAC haemoperfusion. After the completion of haemoperfusion, the blood remaining in the extracorporeal circuit was returned to the patient. Fifteen minutes later, post-haemoperfusion blood samples were taken. The change in systemic platelet level was from the 100% pre-haemoperfusion level to 91.8 ± 11.8% in the post-haemoperfusion sample (Chang *et al.*, 1971). The patients concerned were uraemic with Scribner shunts for blood access, and the procedure did not require blood pumping. Scanning electron microscopic analysis of the surface of the microcapsule after haemoperfusion in patients showed that

on albumin-collodion coated systems there was no significant fibrin entrapment of platelets or of cellular elements (Chang, 1974). On the other hand, on those that were not coated with albumin, there was fibrin deposition and entrapment of cellular elements of blood (Chang, 1974). Albumin coating has also been found to effectively prevent severe depletion of platelets in the adhesive fixed-bed type of haemoperfusion system (Hill, 1975; Dunea *et al.*, 1976). However, it should be pointed out again that albumin does not adsorb adequately to some polymers like hydrogels, and therefore may not be as effective when applied to this system.

One of the most important considerations of the albumin-collodion coated activated charcoal system is that unlike all other charcoal haemoperfusion devices now being used, the albumin-collodion coated system is most effective in preventing platelet depletion.

## 19.5 Chronic Renal Failure

*Clearance*

*In vitro* analysis was carried out to study the clearance of the ACAC microcapsule system for molecules of different molecular weight (Chang and Migchelsen, 1973). At a flow rate of 200 ml/min, the clearance was about 180 ml/min for creatinine and uric acid, and when the flow rate was increased to 300 ml/min the clearance was 230 ml/min and 235 ml/min respectively. For molecules in the 300 - 1200 molecular weight range, the clearance falls to about 120 ml/min and for molecules as large as 6000 molecular weight, the clearance is of the order of 40 ml/min. Unlike the 'standard' haemodialysis machine, the clearance for the middle molecules increases with increasing flow rates. Direct measurement of middle molecules (300 - 1500 molecular weight) has also been carried out in serum of patients with chronic renal failure who have been treated by haemoperfusion (Chang *et al.*, 1974). This was found to be 120 ml/min. The arterial middle molecule level fell to 45 ± 10% standard deviation of the pre-treated level (a decrease of 55%) after 2 h of haemoperfusion. The same degree of fall would require 8 - 12 h using 'standard' haemodialysis machines. Furthermore, the clearance for guanidines is also high. Thus, 2 h of haemoperfusion decreased the arterial guanidine levels to about 45% of the pre-haemoperfusion level, a decrease of 55% (Chang *et al.*, 1971). It is interesting also to note that many protein-bound substances are removed much more effectively by haemoperfusion when compared to 'standard' haemodialysis (Chang *et al.*, 1975). In the combined haemoperfusion-ultrafiltration approach, a significant additional clearance of large molecules appears as a further bonus (Chang *et al.*, 1976).

*Haemoperfusion*

Up to now, a total of about 140 procedures have been carried out by our unit in 14 patients with chronic renal failure (Chang, 1976b). The results show that 2 h of haemoperfusion is as effective as 6 - 8 h of standard haemodialysis in maintaining the patient symptom-free and preventing deterioration of nerve conduction

velocity (Chang *et al.*, 1971, 1972, 1974, 1975, 1976; Chang, 1975a, 1976b). Although the reason for this is not clear at present since the exact uraemic toxins are not known, it is not unreasonable to postulate that this may be related to the more effective removal of some toxic uraemic metabolites by the haemoperfusion system. For example, as discussed earlier, the ACAC haemoperfusion system is very effective in removing the middle molecules both when tested *in vitro* and when analysed in actual clinical trials conducted in patients. Another possibility is the increased effectiveness in the removal of guanidines as described earlier. The other possibility is the much more effective removal of protein-bound uraemic toxins or substances.

Although the ACAC haemoperfusion system constitutes a miniaturised, compact, and inexpensive treatment device for chronic renal failure, it cannot by itself remove water, electrolytes or urea. In patients with a fair amount of residual renal function, especially with a good output of water and electrolytes, the haemoperfusion approach has been used alternately with standard haemodialysis (Chang *et al.*, 1971, 1972, 1974; Chang and Migchelsen, 1973). For the majority of chronic renal failure patients with low residual renal function, the haemoperfusion device by itself would not suffice. Two approaches have been used to solve these problems.

*Haemoperfusion in series with dialysis*

In some of the studies we have carried out, the haemoperfusion devices were connected in series with the 'standard' haemodialysis machines (Chang *et al.*, 1975). This way, the 'standard' haemodialysis machine functions well in removing urea, water and electrolytes, while the haemoperfusion device carried out its function in removing middle molecules, guanidines, and protein-bound molecules. Using the Smith and Nephew haemoperfusion device combined in series with haemodialysers another group has obtained similar results (Winchester *et al.*, 1975). This combined system in series, although cutting down the time of treatment, does not make the haemodialysis machine simpler or less expensive.

*Haemoperfusion in series with ultrafiltration*

The original proposal of combining the ACAC haemoperfusion in series with an ultrafiltrator demonstrated in animals (Chang *et al.*, 1974), has now been demonstrated in patients (Chang *et al.*, 1975, 1976; Chang, 1976b). In the studies with patients, the ACAC haemoperfusion system is combined in series with a small and efficient Amicon ultrafiltrator (Chang *et al.*, 1975; Chang, 1976b). This was originally used for the removal of fluid and electrolytes from overhydrated patients (Silverstein *et al.*, 1974). Such a combined system can effectively remove up to 5 lb (2.27 kg) of fluid without any untoward effect in the patient (Chang *et al.*, 1975, 1976; Chang, 1976b). With this amount of fluid removed, the system would adequately take care of the sodium chloride and water from the uraemic patient. The only other things to be looked after would be urea, potassium, and phosphate. With the further development of urea adsorbents, for instance, oxystarch (Giordano *et al.*, 1975; Friedman *et al.*, 1975), urease-ammonia adsorbent

(Chang and Loa, 1970; Sparks, 1975; Gardner *et al.*, 1975; Asher *et al.*, 1975), and phosphate adsorbent, this ACAC haemoperfusion-ultrafiltration system would form a portable, miniaturised, efficient type of artificial kidney for use in chronic renal failure patients.

### 19.6 Severe Acute Drug Intoxication

Since activated charcoal is such a broad spectrum adsorbent it should not be too surprising that in its microencapsulated haemoperfusive form it performs effectively in removing many drugs. Extensive studies carried out in animals in our laboratory have demonstrated its value for use in acute intoxication due to salicylate, barbiturates, Placidyl, methaqualone, methyprylon, glutethimide, digoxins, and others (Chang, 1969, 1972a). It has also been used in patients with grade IV coma acute drug intoxication (Chang *et al.*, 1973a, 1973b; Chang, 1975a). Up to now, a total of 10 patients have been treated for the following acute drug intoxications: methyprylon (clearance. 230 ml/min), glutethimide (clearance: 150 - 200 ml/min), methaqualone (clearance: 230 ml/min), phenobarbital (clearance: 228 ml/min), secobarbital (clearance: 150 ml/min), and salicylate (clearance: 150 ml/min). The clearances obtained are many times higher than with the 'standard' haemodialysers. All 10 patients treated recovered, and some of them recovered consciousness from grade IV coma during the 2 h haemoperfusion. The following factors require consideration in such cases of acute intoxication.

(1) The adsorbing capacity and the affinity of the activated charcoal for a given drug.
(2) The most effective use of microencapsulated charcoal obtains when a large fraction of the absorbed drug is present in the circulating blood or in rapid equilibration with the circulating blood. Unlike the 'standard' haemodialysis system, the haemoperfusion system can effectively remove both free and plasma protein-bound drugs. For example, glutethimide clearance for haemoperfusion is between 150 - 230 ml/min as compared to 60 ml/min for standard haemodialysis (Chang, 1975a).
(3) For the class of drugs which are mostly bound intracellularly to tissue with only a very small fraction present in the circulating blood (e.g. valium, digoxin, and others), even though the clearance might be high so far as removal from the circulating blood is concerned, only a very small amount of the drug in total can be removed from the body.

Thus when haemoperfusion is used for the treatment of acute drug intoxication where the drug is present mostly in the circulating blood then the ACAC haemoperfusion provides an extremely effective and safe system for treatment. Haemoperfusion for acute intoxication is now being carried out by many centres using different forms of immobilised charcoal systems (Lesch *et al.*, 1976; Vale *et al.*, 1975; Winchester *et al.*, 1974; Barbour, 1976; Baltzer, 1976; Carvallo *et al.*, 1976; Hill, 1975; Hill *et al.* (see p. 123); Martin *et al.*, 1975; Chang, 1969, 1972b, 1975a; Chang *et al.*, 1973a, 1973b).

### 19.7 Liver Failure

Our centre first reported the treatment of a hepatic coma patient with haemoperfusion showing that each of the three successive applications improved the patient's consciousness (Chang, 1972b). This has stimulated further interest in the possible use of microencapsulated charcoal systems for the treatment of liver failure (Chang and Migchelsen, 1973; Chang, 1975a and b, 1976b, Gazzard *et al.*, 1974; Amano *et al.*, 1976a and b; Blume *et al.*, 1976). In the original publication (Chang, 1972b), it was suggested that the effectiveness of the haemoperfusion approach in improving the patient's consciousness may be due to the removal of some toxins resulting from liver failure. Since this first report in 1972, we have carried out treatment only on a small number of liver failure patients because it was felt that although it is effective in improving the patient's consciousness, the effectiveness in improving the survival of patients has not been absolutely demonstrated as yet (Chang, 1972b, 1975a and b, 1976b, Chang and Migchelsen, 1973). In the meantime, another centre has carried out very extensive clinical trials on the treatment of patients with liver failure using the Smith and Nephew coated charcoal system (Gazzard *et al.*, 1974). The initial, very promising improvement in survival rate reported by this group has not been repeated in their more recent studies (Weston, 1975). It has been suggested that this could possibly be due to the observation that the Smith and Nephew system may not be completely compatible with platelets and may have caused platelet aggregation. This could possibly explain the observed severe hypotension (Weston, 1975). Workers using the albumin-collodion coated charcoal haemoperfusion system did not report problems of platelet aggregation (Chang and Migchelsen, 1973; Chang, 1975b, 1972b, 1976b; Amano *et al.*, 1976a and b; Blume *et al.*, 1976; Amano *et al.*, 1976 and b). When the albumin-collodion coated system is primed with a 5 g % albumin solution, no hypotensive episode is observed in patients whose initial blood pressure may have been labile.

It should be emphasised again that the liver is an extremely complex organ and it should not be expected that such a simple system as coated charcoal haemoperfusion would be capable of replacing it. Until a more complete artificial liver support *system* is available, one should not expect prematurely that coated charcoal haemoperfusion could be a substitute for the liver and maintain the life of all patients with severe liver failure. Any clinical trial based on this assumption would not be valid. On the other hand, it has been demonstrated that the coated charcoal haemoperfusion is capable of improving the consciousness of grade IV hepatic coma patients and, in some cases, prolonging their survival (Chang and Migchelsen, 1973; Chang, 1972b, 1975a and b, 1976b; Gazzard *et al.*, 1974; Amano *et al.*, 1976a and b; Blume *et al.*, 1976). This system is no doubt removing some toxic components from the liver failure patients, and could form an important component of a complete artificial liver system.

In the construction of such a complete artificial liver system, other components would no doubt have an important role to play. For instance, microencapsulation of ion-exchange resins for the removal of ammonia as reported earlier (Chang,

1966); albumin entrapped-gel system for removal of bilirubin (Plotz *et al.*, 1974); Amberlite adsorbents (Rosenbaum, 1975); microencapsulated enzymes (Chang, 1972a); other types of immobilised enzymes and microsomes (Chang, 1977), or liver tissue haemodialysers (Nosé *et al.*, 1975) could form other components of the artificial liver support system.

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## 19.9 References

Amano, I., Iwatsuki, S., Manji, T., Saito, A., Takahira, H., Ohta, K. and Maeda, K. (1976a). Artificial liver assist. *Abstract Amer. Soc. Artif. Int. Organs*, **5**, 3.

Amano, I., Iwatsuki, S., Manji, T., Saito, A., Takahira, H., Ohta, K., and Maeda, K. (1976b). Charcoal haemoperfusion for hepatic encephalopathy. *Kidney International* (in press).

Asher, W. J., Bovee, K. C., Frankenfeld, J. W., Hamilton, R. W., Henderson, L. W., Holtzapple, P. G., and Li, N. N. (1975). Liquid membrane system directed toward chronic uraemia. *Kidney International*, **7**, S409.

Baltzer, G. (1976). Neue Wage in der Behandlung akuter Intoxikationen. *Der Internist*, **17**, 322-323.

Barbour, B. (1976). Fixed bed charcoal haemoperfusion for acute drug intoxication. *Kidney International* (in press).

Blume, U., Helmstaedt, D., Sybrecht, G., Baldamus, C., Sussman, P., Heyer, U., Schmidt, E., Schmidt, E. (1976). Hämperfusionstherapie des akuten Leberversagens. *Dtsch. Med. Wschr.*, **14**, 559.

Carvallo, A., Ramirez, B., Honig, H., Knepshield, J., Schreiner, G. E. and Gelfand, M. C. (1976). Treatment of digitalis intoxication by charcoal haemoperfusion. *Abstract Amer. Soc. Artif. Int. Organs*, **5**, 14.

Chang, T. M. S. (1964). Semipermeable microcapsules. *Science*, **146**, 524.

Chang, T. M. S. (1966). Semipermeable aqueous microcapsules (artificial cells) with emphasis on experiments in an extracorporeal shunt system. *Trans. Amer. Soc. Artif. Int. Organs*, **12**, 13.

Chang, T. M. S. (1969). Removal of endogenous and exogenous toxins by a microencapsulated adsorbent. *Can. J. Physiol. Pharm.*, **47**, 1043.

Chang, T. M. S. (1972a). *Artificial Cells*, Thomas, Springfield, Illinois, USA.

Chang, T. M. S. (1972b). Haemoperfusions over microencapsulated adsorbent in a patient with hepatic coma. *Lancet*, **2**, 1371.

Chang, T. M. S. (1972c). Recent trends in biocompatible materials and microcapsular absorbents for kidney function replacement, in R. M. Kenedi (Ed.) *Perspectives in Biomedical Engineering*, Macmillan Press, London.

Chang, T. M. S. (1974). Platelet-surface interaction: Effect of albumin coating or heparin complexing on thrombogenic surfaces. *Can. J. Physiol. Pharm.*, **52**, 275.

Chang, T. M. S. (1975a). Microencapsulated adsorbent haemoperfusion for uremia, intoxication, and hepatic failure. *Kidney International*, **7**, S387.

Chang, T. M. S. (1975b). Experience with treatment of acute liver failure patients by haemoperfusion over biocompatible microencapsulated (coated) charcoal, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 229.

Chang, T. M. S. (1976a). Microcapsule artificial kidney including updated preparative procedures and properties. *Kidney International* (in press).

Chang, T. M. S. (1976b). Haemoperfusion alone and in series with ultrafiltration or dialysis for uraemia, poisoning, and liver failure. *Kidney International* (in press).

Chang, T. M. S. (1977). *Biomedical Applications of Immobilized Enzymes and Proteins*, Plenum, New York.

Chang, T. M. S. and Loa, S. K. (1970). Urea removal by urease and ammonia adsorbent in the intestine. *Physiologist*, **13**, 70.

Chang, T. M. S. and Malave, N. (1970. The development and first clinical uses of semi-permeable microcapsules (artificial cells) as a compact artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **16**, 141.

Chang, T. M. S. and Migchelsen, M. (1973). The characterization of possible 'toxic' metabolites in patients with chronic renal failure and hepatic coma. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 314.

Chang, T. M. S., Pont, A., Johnson, L. J. and Malave, N. (1968). Response to intermittent extracorporeal perfusion through shunts containing semipermeable microcapsules. *Trans. Amer. Soc. Artif. Int. Organs*, **15**, 163.

Chang, T. M. S., Gonda, A., Dirks, J. and Malave, N. (1971). Clinical evaluation of chronic intermittent or short-term haemoperfusion in patients with chronic renal failure using semipermeable microcapsules (artificial cells) formed from membrane coated activated charcoal. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 246.

Chang, T. M. S., Gonda, A., Coffey, J., Dirks, J. and Burns, T. (1972). ACAC microcapsule artificial kidney for long-term and short-term management of patients with chronic renal failure. *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 465.

Chang, T. M. S., Coffey, J. F., Barre, P., Gonda, A., Dirks, J. H., Levy, M., and Lister, C. (1973a). Microcapsule artificial kidney: treatment of patients with acute drug intoxication. *Can. Med. Assoc. J.*, **108**, 429.

Chang, T. M. S., Coffey, J. F., Lister, C., Stark, R. and Taroy, E. (1973b). The clearance of the ACAC microcapsule artificial kidney for glutethimide, methprylon, and methaqualone: *in vitro* and in patients. *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 87.

Chang, T. M. S., Migchelsen, M., Coffey, J. F., and Stark, R. (1974). Serum middle molecule levels in uraemia during long-term intermittent haemoperfusion with the ACAC (coated charcoal) microcapsule artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs*, **20**(A), 354.

Chang, T. M. S., Chirito, E., Barre, P., Cole, C., Hewish, M. (1975). Clinical performance characteristics of a new combined system for simultaneous haemoperfusion-haemodialysis-ultrafiltration in series. *Trans. Amer. Soc. Artif. Int. Organs*, **21**, 502.

Chang, T. M. S., Chirito, E., Barre, P., Cole, C., Lister, C. and Resurreccion, E. (1976). Clinical evaluation of the clearance profiles of a portable, compact, dialysate-free system incorporating microencapsulated charcoal haemoperfusion for blood purification with ultrafiltration for fluid removal. *J. Dialysis* (in press).

Demyttenaere, M. H., Maher, J. F. and Schreiner, G. E. (1967). Haemoperfusion through a charcoal column for glutethimide poisoning. *Trans. Amer. Soc. Artif. Int. Organs*, **13**, 190.

Dunea, G. and Kolff, W. J. (1965). Clinical experiments with the Yatzidis charcoal artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs*, **11**, 178.

Dunea, G., Rizvi, Z. H., Mamdaru, B. H., Anicama, H. J. and Mahurkar, S. D. (1976). Charcoal haemoperfusion and combination dialysis-haemoperfusion in chronic renal failure. *Abstract Amer. Soc. Artif. Int. Organs*, **5**, 22.

Friedman, E. A., Laugani, G. B. and Beyer, M. M. (1975). Life prolongation in nephrectomized rats fed oxidized starch and charcoal. *Kidney International*, 7, S377.

Gardner, D. L., Emmerling, D. C., Williamson, D. W., Baytos, W. C. and Hassler, C. R. (1975). Encapsulated adsorbents for removal of nitrogenous metabolites via oral ingestion. *Kidney International*, 7, S393.

Gazzard, B. G., Weston, M. J., Murray-Lyon, I. M., Flax, H., Record, C. O., Portman, B., Langley, P. G., Dunlop, E. H., Mellon, P. J., Ward, M.D. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminating hepatic failure. *Lancet*, 1 (7870), 1301.

Giordano, C., Esposito, R. and Pluvio, M. (1975). Oxycellulose and ammonia-treated oxystarch as insoluble polyaldehydes. *Kidney International*, 7, S380.

Hagstam, K. E., Larsson, L. E. and Thysell, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital intoxication and uremia including histological findings. *Acta. Med. Scand.*, **180**, 593.

Hill, J. B. (1975). *B-D haemo-detoxification systems*. Pamphlet from Becton, Dickinson, & Co, Research Centre, North Carolina, pp. 1-4.

Lesch, P., Blume, V., Scheibe, G., Sussmann, P., Schmidt, F. W., Bartels, M., and Okonek, S. (1976). Haemoperfusion durch verkapselte Aktivkolile Zur Therapie Exogener und endogener Intoxikationen. *Klin. Wschr.*, **54**, 509.

Martin, A. M., Mitchell, D. C., Gibbins, J. K., Devapal, D., Trinder, P. (1975). Charcoal haemoperfusion in the management of severe poisoning. *Brit. Med. J.*, **1** (5948), 5.

Nosé, Y., Koshino, I., Castino, F., Yoshida, K., Carse, G., Kambic, H., Scheucher, K., Kretx, A. P. and Malchesky, P. S. (1975). Further assessment of liver tissue materials for extracorporeal hepatic assists, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 202.

Plotz, P. H., Berk, P. D., Scharschmidt, B. T., Gordon, J. K. and Vergalla, J. (1974). Removal of bilirubin by albumin-coated conjugated agarose beads. *J. Clin. Invest.*, **53**, 778.

Rosenbaum, J. L. (1975). Biocompatibility of resin haemoperfusion, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, pp. 118.

Silverstein, M. D., Ford, C. A., Lysaght, M. J. and Henderson, C. W. (1974). Treatment of severe fluid overload by ultrafiltration. *New Engl. J. Med.*, **291**, 747.

Sparks, R. E. (1975). Gastrosorbents in the therapy of uraemia: Inferences from intestinal loop dialysis. *Kidney International*, 7, S373.

Vale, J. A., Rees, A. J., Widdop, B., and Goulding, R. (1975). The use of charcoal haemoperfusion in the maintenance of severely poisoned patients. *Brit. Med. J.*, **1** (5948), 5.

Van Wagenen, R. A., Steggall, M., Lentz, D. J. and Andrade, J. D. (1975). Activated carbons for medical applications. *In vitro* microparticle characterisation and solute adsorption. *Biomat. Med. Dev. Artif. Organs*, **3**, 319.

Watson, W. J. and Chang, T. M. S. (1975). Platelet-surface interaction: Effects of Dextran 70 on platelet retention in extracorporeal surfaces. *Biomat. Med. Dev. Artif. Organs*, **3** (4), 489.

Weston, M. J. (1975). Discussion, in *Conference on Sorbents in Uraemia*, Downstate Medical Centre, Brooklyn, New York, November 3 - 4, 1975.

Winchester, J. F., Tilstone, W. J., Edwards, R. O., Gilchrist, T. and Kennedy, A. C. (1974). Haemoperfusion for enhanced drug elimination – a kinetic analysis in paracetamol poisoning. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 358.

Winchester, J. F., Apiliga, M. T., MacKay, J. M., Kennedy, A. C. (1975). Haemodialysis with charcoal haemoperfusion. *Proc. Eur. Dial. Transpl. Assoc.*, **12**, 526.

Yatzidis, D. (1964). A convenient haemoperfusion micro-apparatus over charcoal for the treatment of endogenous and exogenous intoxications. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.*, **1**, 83.

# 20

# THE ROLE OF CHARCOAL HAEMOPERFUSION IN THE MANAGEMENT OF ACUTE POISONING BY DRUGS

G. N. VOLANS, J. A. VALE, P. CROME, B. WIDDOP and R. GOULDING

**Summary**

Haemoperfusion has been carried out, in a series of 21 patients suffering from acute drug overdosage, using a commercially available column containing activated charcoal coated with acrylic hydrogel. The patients selected were in all cases those with evidence of severe intoxication and very high plasma drug levels together with complications such as bronchopneumonia. Sixteen patients survived and five died, four from cerebral damage which occurred before haemoperfusion and one from a complication of disease unrelated to the drug overdose. The treatment was free from major side effects and in the patients who survived it appeared to make a real contribution towards recovery. A survey of severely poisoned patients referred to the Poisons Unit for laboratory analysis suggests that this method of treatment may have an important role to play in reducing the mortality and morbidity of the severely poisoned patient, particularly in those cases who have taken short or medium acting barbiturates or nonbarbiturate hypnotics. It is suggested that this technique is now suitable for further clinical evaluation and that this should be carried out at specialist, regional units with adequate laboratory facilities.

## 20.1 Introduction

Charcoal haemoperfusion was recognised as a potentially useful treatment for acute poisoning as far back as 1964 (Yatzidis, 1964). Full clinical evaluation of this technique has not been possible, however, because of the adverse effects

**Table 20.1**

Complications associated with haemoperfusion using uncoated activated charcoal

| | |
|---|---|
| Marked thrombocytopaenia<br>Fibrinogen loss<br>Pyrogen reaction | Yatzidis (1964) |
| Charcoal embolism | Hagstam *et al.*, (1966) |
| Leucopaenia | Rosenbaum *et al.*, (1968) |

associated with the mixing of blood with activated charcoal (table 20.1). With the development of coated charcoals, these effects have been overcome to the extent that haemoperfusion is now a clinical reality (Andrade *et al.*, 1971; Chang *et al.*, 1973; Vale and Goulding, 1975; Vale *et al.*, 1975). It is now possible to review our experiences with charcoal haemoperfusion in patients suffering from acute poisoning due to drugs over the period of 27 months from December 1973 to March 1976. In addition, this report includes a survey of severely poisoned patients who took similar doses of drugs but who were not treated by charcoal haemoperfusion. From these two surveys an evaluation has been made of the clinical role of charcoal haemoperfusion.

## 20.2 Methods

*Patient selection*

Patients were considered for treatment by haemoperfusion only after toxicological analyses had disclosed the identity of the drug and shown this to be present in high concentrations in the plasma (table 20.2a). These findings alone were not considered a sufficient indication for haemoperfusion and treatment was not undertaken unless there were also good clinical grounds to suggest that the patient might not recover without active treatment (table 20.2b).

**Table 20.2**

Criteria for patient selection for haemoperfusion

| (a) Plasma drug levels not less than: | mg/l |
|---|---|
| Phenobarbitone and barbitone | 100 |
| Other barbiturates | 50 |
| Glutethimide | 40 |
| Methaqualone | 40 |
| Ethchlorvynol | 150 |
| Meprobamate | 100 |
| Trichloroethanol | 50 |
| Salicylates | 800* |

(*or 500 mg/l if arterial pH is < 7.34 more than 4 h after ingestion)

Plus at least one of the following clinical features.

(b) Clinical features

Severe clinical intoxication, e.g. grade IV coma, hypotension, hypothermia, hypoventilation.

Progressive deterioration or failure to improve in spite of good supportive management.

Prolonged coma with complications, e.g. pneumonia, chronic respiratory disease.

*Haemoperfusion technique*

In all cases, the haemoperfusion column contained 300 g, 5 - 10 mesh, coconut shell charcoal coated with an acrylic hydrogel to a thickness of 5 μm (2% by weight) (Haemocol, Smith and Nephew Research Ltd). Before use, the columns, which are supplied sterile and filled with saline, were primed with 2 l of sodium chloride (0.9% w/v) to ensure there was no leakage of visible charcoal particles and that no large volumes of air were trapped in the column. Heparin (1 unit/ml) was added to that part of the priming solution which was retained in the column prior to use. In use, an upward flow was produced through the column by the use of a Watson Marlow roller pump and flow rates were of the order of 200 - 300 ml/min as measured by bubble transit. A bolus of 5000 units of heparin was injected into the arterial line at the commencement of haemoperfusion and heparinisation was controlled by adjusting the rate of a constant infusion of heparin into the circuit such that a plasma heparin concentration of 1.0 - 3.0 units/ml was achieved. Perfusion was continued until clinical recovery or until plasma drug concentration had been reduced to subtoxic levels.

*Biochemical and haematological methods*

Blood was taken before, after and sometimes during perfusion for estimation of electrolytes, urea, calcium, phosphate, magnesium, alkaline phosphate, glucose, proteins, bilirubin, cholesterol, aspartate and amino-transferases, uric acid, creatinine, fatty acids, fat soluble vitamins, blood picture, coagulation profile and drug levels.

In addition samples were taken routinely during perfusion for estimation of drug levels (pre and post-column), platelet, leucocyte and heparin levels. The biochemical and haematological estimations were carried out by standard techniques; heparin concentrations were determined by a modification of the method of Pitney (1968); platelets were measured by a Coulter F electronic counter; barbiturate and glutethimide levels were measured by a specific gas liquid chromatographic technique (Flanagan and Withers, 1972); salicylate by the method of Trinder (1954).

*Evaluation of the results of haemoperfusion*

Efficacy of the technique was recorded in terms of the eventual outcome of treatment, the time taken to regain consciousness, the incidence of complications commonly associated with drug overdosage and the plasma clearance of the drugs. Its safety was evaluated on the basis of haematological and biochemical indices together with any clinical observations thought to relate to adverse effects from haemoperfusion.

## 20.3 Cases Not Treated by Haemoperfusion

Since it was not considered possible to study a true 'control series' of patients who were similarly poisoned but were not treated by haemoperfusion, an attempt has been made to evaluate the role of haemoperfusion by conducting a retro-

spective survey of all the cases of severe intoxication (as defined by the blood level criteria in table 20.2a), which were referred to the Poisons Unit during 1975. For each of these cases, the type of treatment used was recorded and the outcome was assessed in terms similar to those used for the assessment of haemoperfusion, except that it was not possible to estimate the plasma drug clearance.

## 20.4 Results For Cases Treated by Haemoperfusion

*Clinical response*

During the 27 month period a total of 21 patients were treated by haemoperfusion. Table 20.3 lists the cases according to the drugs which were taken and fuller clinical details are given in table 20.4. Deaths occurred in five patients who had taken short or medium acting barbiturates. Of these, four had suffered from at least one cardiorespiratory arrest before haemoperfusion commenced whilst the fifth was a diabetic who died 15 days after haemoperfusion from a respiratory infection thought to be secondary to problems associated with control of the diabetes. All the surviving patients recovered consciousness within 33 h of starting haemoperfusion. Those poisoned with short or medium acting barbiturates took longer to recover consciousness (and therefore required a longer period of haemoperfusion) than those who had taken phenobarbitone (table 20.5). Since most of the patients were transferred to Guy's Hospital from other hospitals, the total duration of coma since the first arrival in hospital was somewhat greater than the duration of haemoperfusion. Although the majority of the patients had developed complications associated with coma before starting haemoperfusion, none of the survivors developed any further complications of that type.

**Table 20.3**

Haemoperfusion cases: December 1973 to March 1976

| *Drug* | *Number of patients* | *Number of deaths* |
|---|---|---|
| Short-acting barbiturates | 15 | 5 |
| Long-acting barbiturates | 4 | 0 |
| Glutethimide | 1 | 0 |
| Methylsalicylate | 1 | 0 |
| Total | 21 | 5 |

*Efficacy and safety of charcoal haemoperfusion*

The clearance data are summarised in table 20.6. It can be seen that worthwhile amounts of the drug were removed in all cases, although it is notable that there was a considerable variation in the clearance achieved even between cases where the flow rate was identical.

**Table 20.4**

Clinical details and plasma drug levels in 21 patients treated by haemoperfusion

| *Patient* | *Age* | *Sex* | *Agent* | *Length of perfusion (h)* | *Plasma level (mg/l)* | | *Outcome* |
|---|---|---|---|---|---|---|---|
| | | | | | *Pre-perfusion* | *End of perfusion* | |
| 1 | 56 | F | Butobarbitone | $4\frac{1}{2}$ | 68 | 32 | Recovered |
| 2 | 23 | F | Phenobarbitone | 6 | 260 | 110 | Recovered |
| 3 | 31 | M | Phenobarbitone | 7 | 144 | 66 | Recovered |
| 4 | 31 | M | Glutethimide | 6 | 78 | 15 | Recovered |
| 5 | 56 | M | Pentobarbitone | 8 | 53 | 20 | Died† |
| 6 | 62 | M | Butobarbitone | 4 | 31 | 25 | Died† |
| 7 | 40 | M | Amylobarbitone Na | $12\frac{1}{2}$ | 91 | 15 | Recovered |
| 8 | 33 | F | 'Tuinal'* | 8 | 55 | 17 | Recovered |
| 9 | 48 | M | Methyl Salicylate | 3 | 86 | 44 | Recovered |
| 10 | 28 | F | Phenobarbitone | $5\frac{1}{2}$ | 243 | 161 | Recovered |
| 11 | 19 | F | 'Tuinal'* | 6 | 41 | 25 | Recovered |
| 12 | 34 | F | Phenobarbitone | $13\frac{1}{2}$ | 197 | 40 | Recovered |
| 13 | 56 | F | Butobarbitone | $9\frac{1}{2}$ | 86 | 36 | Recovered |
| 14 | 43 | F | 'Tuinal'* | 18 | 60 | 27 | Recovered |
| 15 | 22 | M | Butobarbitone | 19 | 43 | 7 | Died† |
| 16 | 27 | M | Butobarbitone | 17 | 72 | 20 | Recovered |
| 17 | 31 | M | 'Tuinal'* | $14\frac{1}{2}$ | 54 | 10 | Recovered |
| 18 | 27 | F | Mixed‡ | $30\frac{1}{2}$ | 55 | 12 | Recovered |
| 19 | 61 | F | Pentobarbitone | 26 | 40 | 27 | Died† |
| 20 | 21 | M | 'Tuinal'* | 24 | 60 | 13 | Recovered |
| 21 | 52 | F | 'Tuinal'* | 31 | 42 | 7 | Died ϕ |

*Amylobarbitone and quinalbarbitone
†Cerebral anoxic damage
‡Barbiturate and methaqualone
ϕDiabetic. Pneumonia.

**Table 20.5**

Duration of coma in patients treated by haemoperfusion

| *Hypnotic agent ingested* | *Time (h) to recover consciousness** |
|---|---|
| Short-acting barbiturates (9) | Mean 15.4 (range 4.5 - 33) |
| Long-acting barbiturates (4) | Mean 8.0 (range 5.5 - 13.5) |

*Patient responds at least to minimal stimuli (grade II)

**Table 20.6**

Haemoperfusion using coated charcoal. Drug clearance data (mean results and ranges)

| *Agent* | *n* | *Blood flow ml/min* | *Clearance ml/min* | *Length of perfusion (h)* | *Amount removed (mg)* |
|---|---|---|---|---|---|
| Butobarbitone | 5 | 215 ( 75 - 300) | 71 (50 - 120) | 11 (4 - 19) | 1446 ( 375 - 3172) |
| Tuinal | 6 | 191 (150 - 200) | 58 (33 - 101) | 17 (6 - 31) | 2272 ( 725 - 3986) |
| Phenobarbitone | 4 | 187 (100 - 250) | 80 (44 - 120) | 9 (5½ - 13½) | 4635 (2410 - 6925) |
| Pentobarbitone | 2 | 200 | 41 (32 - 50) | 17 (8 - 26) | 1767 (1728 - 1806) |
| Glutethimide | 1 | 200 | 125 | 6 | 1220 |
| Methyl Salicylate | 1 | 150 | 57 | 3 | 4041 |

No evidence of charcoal embolism was discerned by careful histological examination of tissues from the patients who died. Likewise there were no pyrogen reactions and, although fibrinogen levels were not measured, no obvious problems resulted from their reduction. The platelet counts fell during haemoperfusion in all except one of the patients. This fall was maximal within the first hour and the mean maximal fall was approximately 40% (Vale *et al.*, 1976). Thereafter, no significant falls in the platelet counts occurred. Small falls in the leucocyte counts were also noted but, by the end of haemoperfusion, the counts had either returned to the starting level or were higher. Heparin requirements varied from 10 to 50 units/kg body weight/h. No serious side effects were noted from this regime, although bleeding was found to occur from the sites of trauma where the A-V shunt and intravenous catheters had been inserted. Only one of the 21 cases lost sufficient blood to require transfusion and the column clotted in only one case. (In this instance the haemoperfusion was being performed at another hospital and heparin levels were not being monitored.) Although various biochemical values were investigated during perfusion there were no findings of clinical significance (Vale *et al.*, 1976).

### 20.5 Results For Cases Not Treated by Haemoperfusion

During 1975 the Poisons Unit Laboratory carried out qualitative and quantitative estimations on 768 cases of drug overdosage. The survey of these results revealed a total of 40 cases with plasma drug concentrations exceeding those listed in the criteria for haemoperfusion (table 20.2). Four of these cases were treated by haemoperfusion. Of the remainder, some were referred to us too late or were admitted to hospitals so far from Guy's Hospital that transfer of the patient was not feasible. The rest were considered for haemoperfusion but subsequently rejected on the grounds that the clinical features suggested that recovery was likely without further active therapy.

Table 20.7 records the details of the drugs taken by these patients. The cases of salicylate poisoning, ethchlorvynol poisoning and three of the cases of poisoning due to phenobarbitone (long-acting barbiturate) were treated by

**Table 20.7**

Cases not treated by haemoperfusion but in whom the plasma drug levels satisfied the criteria for haemoperfusion

| *Drug* | *Number of cases* | *Number of deaths* |
|---|---|---|
| Long-acting barbiturates | 8 | 1 |
| Short-acting barbiturates | 20 | 2 |
| Trichloroethanol | 5 | 1 |
| Ethchlorvynol | 1 | – |
| Glutethimide | 1 | – |
| Salicylate | 1 | 1 |
| Total | 36 | 5 |

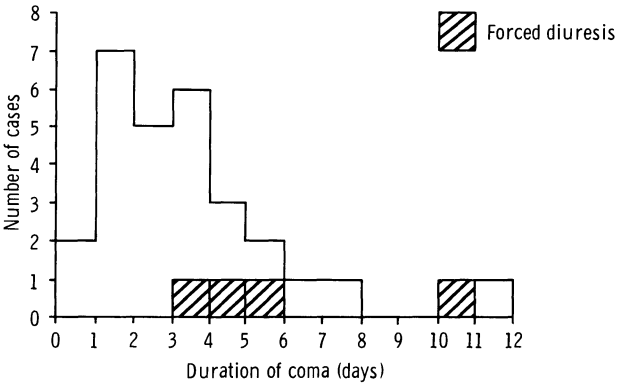


Figure 20.1 Duration of coma in 29 patients who recovered from severe drug overdosage. All had very high plasma drug levels (see text). Treatment was supportive only or supportive plus forced alkaline diuresis. (The available records for two of the cases did not give an accurate account of the duration of the coma)

forced diuresis; the remainder received full supportive therapy including assisted ventilation in most cases. Four patients died in coma whilst a fifth died from a pulmonary embolus seven days after regaining consciousness. Further complications of the coma were recorded in another nine patients; seven cases of pneumonia (one with shock lung) and two cases of permanent cerebral damage. The duration of coma in these patients is shown in figure 20.1 where it can be seen that only 9 out of 29 regained consciousness in less than 48 hours.

## 20.6 Discussion

The majority of patients acutely poisoned by drugs recover physically without any special treatment whilst most of the remainder are treated supportively along the lines first advocated by Clemmesen and Nillson (1961). These supportive measures are an essential part of the treatment of every seriously poisoned patient but, for the small group of such patients who have extremely high drug levels, it seems reasonable to suggest that active removal of the drug would be advantageous both in increasing survival and in decreasing the complications associated with coma. The survey of our laboratory records confirms the belief that very few patients are present with plasma drug levels sufficient to warrant active treatment (Goulding, 1975) and emphasises the role of the laboratory in selecting such cases.

In our experience charcoal haemoperfusion has proved to be a safe technique which is simple to operate. When compared with haemodialysis and forced diuresis, it appears the only effective technique for the removal of the short and medium-acting barbiturates as well as the nonbarbiturate hypnotics. When used for the treatment of overdosage with the long-acting barbiturates, haemo-

perfusion appears more efficient than forced diuresis and equally efficient but less expensive and less complex than haemodialysis.

The variation in drug clearance presumably reflects the difficulty in ensuring that the charcoal particle size and the coating remain identical in each production batch, notwithstanding the fact that most rigorous specifications are adhered to strictly (Fennimore *et al.*, 1975). For example, a small increase in the average particle size, although within the 5 - 10 mesh limits, will reduce the overall surface area for adsorption and hence the rate of uptake of the drug from the circulation.

Whatever the cause of this variation in clearance, the technique has proved clinically safe and useful. It is to be hoped that further research into the use of charcoal and other adsorbents will improve the efficacy of haemoperfusion and that continuing clinical experience will define more closely its applications. It would now seem reasonable for this technique to be generally available provided that it is carried out by specialist, regional units with adequate laboratory facilities.

## 20.7 Acknowledgements

We would like to record our thanks to the physicians who referred patients to us; to the surgeons at Guy's Hospital who inserted the shunts and to Mr T. Thomas and his colleagues who monitored the heparin levels. Miss M. C. Anderson undertook the search into the Poisons Unit records; the nursing care of the patients was undertaken by the staff of Starling Ward, Guy's Hospital and the biochemists of the Poisons Unit undertook the toxicological analyses.

## 20.8 References

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastagir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood perfusion: Hema/activated carbon. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 222.

Chang, T. M. S., Coffey, J. F., Barre, P., Gonda, A., Dirks, J. H., Levy, M. and Lister, C. (1973). Microcapsule artificial kidney: Treatment of patients with acute drug intoxication. *Can. Med. Assoc. J.*, **108**, 429.

Clemmesen, C. and Nillson, E. (1961). Therapeutic trends in the treatment of barbiturate poisoning: the Scandanavian method. *Clin. Pharmacol. Ther.*, **2**, 200

Fennimore, J., Watson, P. A., Munro, G. D. and Kolthammer, J. C. (1975). The design and evaluation of a convenient carbon haemoperfusion system. *Proc. Eur. Soc. Artif. Organs*, **1**, 90.

Flanagan, R. J. and Withers, G. (1972). A rapid micro-method for the screening and measurement of barbiturates and related compounds in plasma by gas-liquid chromatography. *J. Clin. Pathol.*, **25**, 899.

Goulding, R. (1975). Hamoperfusion zur behandlung von intoxikationen. *Aktnelle Nephrologie*, **4**, 88.

Hagstam, K. E., Larsson, L. E. and Thysell, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital intoxication and uraemia, including a list of pathological findings. *Acta Med. Scand.*, **180**, 593.

Pitney, W. R. (1968). In J. V. Dacie and S. M. Lewis (Eds.), *Practical Haematology,* 4th edition, Churchill, London.

Rosenbaum, J. L., Ronquillo, E. and Argyres, S. N. (1968). Column haemoperfusion and haemodialysis techniques to treat barbiturate intoxication in dogs. *J. Albert Einstein Med. Centre*, **16**, 67.

Trinder, P. (1954). Rapid determination of salicylate in biological fluids. *Biochem. J.*, **57**, 301.

Vale, J. A. and Goulding, R. (1975). The use of an acrylic hydrogel coated charcoal haemoperfusion system in the treatment of severely poisoned patients. *Proc. Eur. Soc. Artif. Organs*, **1**, 94.

Vale, J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975). Use of charcoal haemoperfusion in the management of severely poisoned patients. *Brit. Med. J.*, **1**, 5.

Vale, J. A., Thomas, T., Widdop, B. and Goulding, R. (1976). Use of charcoal haemoperfusion in poisoned patients; a review of 20 cases. *Proc. Eur. Soc. Artif. Organs*, 2, 239.

Yatzidis, H. (1964). A convenient haemoperfusion microapparatus over charcoal for the treatment of endogenous and exogenous toxins. 1. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.*, **1**, 83.

# 21

# CLINICAL APPLICATION OF HAEMOPERFUSION IN URAEMIA

J. F. WINCHESTER

**Summary**

Activated charcoal haemoperfusion offers potential clinical benefit for the treatment of end-stage chronic uraemia. Haemoperfusion has been shown to have plasma creatinine and plasma urate clearances superior to those obtained with conventional haemodialysis at comparable blood flow rates through the respective devices. Urea and phosphate removal, however, is inferior to conventional dialysis while water and electrolyte removal is negligible. Haemoperfusion therefore requires combination with dialysis or ultrafiltration apparatus in patients without high urine volumes in order to remove sufficient water and electrolytes. Molecules of 350 - 1500 daltons are removed more efficiently than by dialysis, and offer potential benefit to patients with specific complications of dialysis therapy such as peripheral neuropathy and pericarditis.

Haemostatic changes occurring with haemoperfusion are minor and present little clinical hazard to patients, although it would be desirable to reduce platelet loss to a minimum before repeated use of these devices is recommended.

Total solute removal of creatinine and urate, in a 2 h combined dialysis - ultrafiltration - haemoperfusion system is less than a longer period of conventional dialysis, but would appear to be sufficient for stable management of uraemic patients.

## 21.1 Introduction

Clinical nephrology, as a medical speciality, began in 1836 when Richard Bright described the disease 'nephritis'. In modern times this condition is responsible for end-stage renal failure in the majority of patients receiving regular dialysis therapy in Europe (Brunner *et al.*, 1975) and in USA (Bryan, 1974). The term 'dialysis' was first used in 1854 by Thomas Graham to describe the passage of urea across a parchment 'membrane' but it was not until Kolff (1944) introduced the first workable artificial kidney that the physicochemical property of dialysis was put to clinical use in the treatment of uraemic man. Since the introduction of dialysis many thousands of patients have been treated, dialysis technology has advanced and improvement in survival of patients with chronic renal failure has been achieved. Despite all the improvements in conventional dialysis, however, it has been appreciated that the anaemia of chronic renal failure is not reversed, bone disease may continue unabated, hormonal deficiencies and sexual problems are frequent and neurological manifestations of uraemia, particularly peripheral

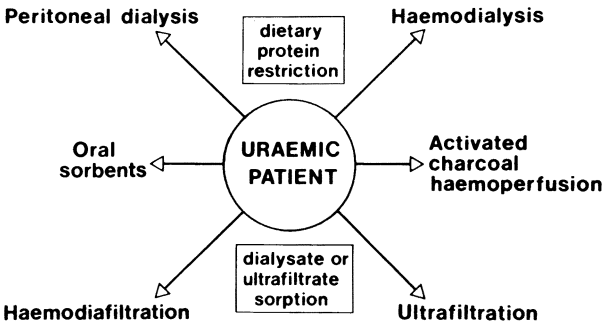


Figure 21.1 Treatment of end-stage chronic uraemia

neuropathy, may progress. These manifestations of uraemia are thought to be due to 'uraemic toxins' and have stimulated research for such toxins and for new approaches to increasing dialysis efficiency. The use of sorbents in the treatment of uraemia appears to be a particularly fruitful therapeutic approach (figure 21.1) and haemoperfusion has received a great deal of interest.

Figure 21.1 shows current techniques in the therapy of uraemia. The upper portion of the figure depicts 'conventional' therapy and the lower portion depicts current research techniques, much of which will be discussed elsewhere in this publication.

## 21.2 Haemoperfusion in Uraemia

Muirhead and Reid (1948) first described the use of cation and anion exchange resin haemoperfusion to remove urea from animals but no further reports on clinical use have been forthcoming. It was not until 1964 that Yatzidis, using uncoated charcoal, reported the use of haemoperfusion in man, to remove creatinine, uric acid, guanidine bases, indican, phenolic compounds and organic acids. Chang *et al.* (1971) confirmed that in uraemic man charcoal haemoperfusion could remove the above substances but that, in addition, it could remove amino acids (Chang and Migchelsen, 1973) and medium molecular weight substances (MMWS) (Chang and Migchelsen, 1973; Chang *et al.*, 1974). The author has also confirmed that charcoal haemoperfusion can remove creatinine, uric acid and MMWS (Winchester *et al.*, 1975b, 1976).

Creatinine and urate clearances from plasma with haemoperfusion alone are greater than with conventional dialysis (Chang *et al.*, 1971; Chang, 1974) and combination of charcoal with haemodialysis greatly augments solute clearance (Winchester *et al*, 1975b, 1976).

Table 21.1 shows reported solute removal during haemoperfusion using 100 - 300 g activated charcoal as the sorbent. It can be seen that activated charcoal haemoperfusion *in vivo* for 2 - 3 h can remove approximately 1.5 - 2.0 g creatinine, and 0.5 - 1.5 g of uric acid, with negligible urea or phosphate removal. Urea removal, however, may be significant with the petroleum based charcoals (Ota *et al.*, 1976).

**Table 21.1**

Solute removal with charcoal haemoperfusion

| *Author/ date* | *Charcoal system* | *Creatinine* | *Urate* | *PHosphate* | *Urea* |
|---|---|---|---|---|---|
| Yatzidis (1964) | Merck *in vitro* | 0.8 - 0.85g | 0.75 - 0.8g | Traces | 3 - 4g |
| Dunea (1965) | Merck *in vitro* | 4.5 g | | | |
| | in man | 1 - 3 mg% fall | 2 - 4 mg% fall | nil | nil |
| Chang (1975) | Fisher combined with dialysis | 3.0 ± 0.3g (at serum creatinine 15 mg%) | 1.4 ± 0.2g | nil | 20.7 ± 1.6g |
| | | 2.0 ± 0.2g (at serum creatinine 10 mg%) | 0.7 ± 0.1g | nil | 10.3 ± 0.8 |
| Winchester (1975b) | Sutcliffe Speakman with dialysis | 1.3g | 0.75g | nil | 14.5g |
| Winchester *et al.* (1976) | Sutcliffe Speakman with dialysis | 1.72g | 0.76g | nil | 16g |
| Winchester (1976) | Sutcliffe Speakman | 1.5g | 0.64g | nil | nil |
| Dunea (1975) | Becton Dickinson with dialysis | 3.4 ± 1.4 mg% fall<br>6.7 ± 0.5 mg% fall | 2 ± 1 mg% fall | 0.5 - 2 mg% fall | nil |
| Malchesky (1976) | Charcoal capillary *in vitro* | 1.5g | 1.6 | – | – |
| Ota (1976) | Petroleum based | 44.3 % fall | 53.2 % fall | | 37 % fall |

It has been demonstrated that in uraemia larger molecules (in the 350 - 1500 dalton range) may be removed by charcoal haemoperfusion (Chang *et al.*, 1974; 1975, Winchester *et al.*, 1975b; 1976). Leber *et al.* (1976) have shown that these MMWS prevent globin synthesis *in vitro*, and can be successfully removed from serum by haemoperfusion. Amino acids, benzoyl-L-cysteine, L-phenylalanyl – L-phenylalanine tetra-L-phenylalanine are removed by charcoal haemoperfusion *in vitro* (Chang and Migchelsen, 1974). Gazzard *et al.* (1974) have reported that in hepatic coma, although many amino acids are extracted by charcoal haemoper-

fusion, the arterial levels of phenylalanine, tyrosine and methionine only were significantly reduced.

In uraemic patients treated with haemoperfusion alone, the arterial levels of the amino acids serine and cystine were significantly reduced, whereas dialysis or combined dialysis/haemoperfusion resulted in significant falls of many other amino acids in addition to serine and cystine.

## 21.3 Haemostatic Changes

The initial problems with platelet depletion (Dunea and Kolff, 1965) and small particle embolisation (Hagstam *et al.*, 1966) associated with unwashed and poorly coated charcoal have been largely overcome with suitable polymer coatings (Chang, 1966; Andrade *et al.*, 1971; Winchester *et al.*, 1974; 1975a). In most studies of haemoperfusion in uraemic man, an average 30 - 40% fall in platelet count occurs.

In a study of combined dialysis/charcoal haemoperfusion, no significant fall in platelet count was observed in control dialysis alone, but an average 30% fall in platelet count was observed after combined haemodialysis/charcoal haemoperfusion (Winchester *et al.*, 1975b; Winchester, 1976). However no significant changes in coagulation tests or coagulation factors occurred with either haemodialysis or combined haemodialysis/haemoperfusion.

Chang *et al.* (1971) have reported an average terminal platelet count of 91.8 ± 11.8% of initial platelet count, after haemoperfusion with albumin-collodion coated charcoal for uraemia.

## 21.4 Discussion

Activated charcoal haemoperfusion possesses useful biomedical properties for removal of uraemic toxins (Yatzidis, 1964; Dunea and Kolff, 1965; Chang 1969; Chang *et al.*, 1968, 1970, 1972). Activated charcoal, however, has been used in other systems for treatment of uraemia. Taken orally it removes uraemic toxins from the gastrointestinal tract (Sparks, 1975; Friedman *et al.*, 1975; 1976); it is now used as a component of dialysate regeneration apparatus (Gordon *et al.*, 1970) and as a major component in a wearable artificial kidney (Stephen *et al.*, 1975).

Charcoal haemoperfusion using 200 - 300 g charcoal does not possess the ability to remove water, ions (particularly sodium and potassium) and urea in significant quantities. These disadvantages impose major restrictions on the use of charcoal haemoperfusion alone for treatment of uraemia, and have necessitated the use of charcoal haemoperfusion in combination with dialysis for maintenance of patients with end-stage chronic azotaemia (Chang *et al.*, 1975; Winchester *et al.*, 1976). The greatest appeal of charcoal haemoperfusion in uraemia is that middle molecular weight substances, which are thought to be responsible for continuing uraemic symptoms despite dialysis (Babb *et al.*, 1971, 1972; Bergstrom and Furst, 1976), are removed by haemoperfusion alone (Chang *et al.*, 1974) or combined dialysis/haemoperfusion (Chang *et al.*, 1975; Dunea *et al.*, 1976; Winchester *et al.*,

1976). There is evidence that specific peptide subpeaks in the middle molecule range (350 - 1500 daltons) are removed by adsorption on charcoal in a sorbent-based dialysate system (Gordon *et al.*, 1976). Recently dialysis with polyacrylonitrile membranes has been shown to remove middle molecules (Funck-Brentano *et al.*, 1975; 1976).

Yatzidis (1964) and Chang *et al.* (1975) have proposed that a 2 h haemoperfusion is sufficient repetitive maintenance time for dialysis patients, since solute clearance is greater for creatinine and urate when compared with dialysis. While this proposal is attractive for reducing dialysis time, total small solute removal is not substantially greater than conventional haemodialysis for the same duration, and is less effective than longer periods of dialysis alone (Winchester *et al.*, 1976). However, this finding should not discourage the clinical investigation of haemoperfusion in uraemia, since it is by no means certain if small molecules such as urea (Johnson *et al.*, 1975) or creatinine (Jones and Burnett, 1975) exert toxicity until maximal levels are reached. Moreover, the quantities of urea, creatinine and urate which are desirable to remove in uraemic man are unknown, although it would appear to be important to determine such quantities for individual patients (Babb *et al.*, 1975) for their maintenance in a stable, symptom-free state. There is some evidence, however, that haemoperfusion combined with dialysis for 2 h three times weekly is sufficient maintenance therapy for dialysis patients (Chang *et al.*, 1975), and combination with ultrafiltration (Amicon 0.2m$^2$ capillary ultrafiltrator) can achieve fluid loss of 2.5 l in a 2 h period (Chang *et al.*, 1976).

The other small solute, desirable to reduce in chronic renal failure, is inorganic phosphate. Unfortunately, this substance is removed only inefficiently by both dialysis and haemoperfusion. Aluminium hydroxide can be used to prevent (or reduce) gastrointestinal absorption of phosphate, but there is some concern that aluminium deposited in tissues may be responsible for dialysis dementia, a fatal complication of dialysis (Ward *et al.*, 1976) although it is by no means certain that ingestion of phosphate binding gels is responsible for the aluminium intoxication (Flendrig *et al.*, 1976).

The role of charcoal haemoperfusion in the treatment of uraemic patients has yet to be defined, but it is almost certain that charcoal sorption will require to be combined with dialysis or ultrafiltration apparatus for clinical application. Haemoperfusion appears to be more efficient than dialysis for removal of the middle molecules and offers great potential therapeutic benefit for patients with specific complications of dialysis. The demonstration of the long-term efficacy of charcoal haemoperfusion (or any other innovative procedure) will require carefully controlled clinical evaluation. Most workers try to achieve a total solute removal per day of 30 g urea, 1.5 g of creatinine and 1.0 g uric acid. These values are the average amounts of small solutes in a 1.5 l volume of urine in normal man per day. It would seem desirable to achieve this volume of fluid and solute removal with any device used in renal failure. However, there is no knowledge regarding the generation rate or removal of middle molecules in normal or uraemic man.

## 21.5 References

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood perfusion HEMA/activated carbon. *Trans. Amer. Soc. Artif. Int. Organs,* **17**, 222 - 228.

Babb, A. L., Popovich, R. P., Christopher, T. G. and Scribner, B. H. (1971). The genesis of the square metre-hour hypothesis. *Trans. Amer. Soc. Artif. Int. Organs,* **17**, 81 - 91.

Babb, A. L., Farrell, P. C., Uvelli, D. A. and Scribner, B. H. (1972). Haemodialyser evaluation by examination of solute molecular spectra. *Trans. Amer. Soc. Artif. Int. Organs,* **18**, 98.

Babb, A. L., Strand, M. J., Uvelli, D. A., Milutinovic, J. and Scribner B. H. (1975). Quantitative description of dialysis treatment: a dialysis index. *Kidney International,* **7**, S23 - 29.

Bergstrom, J. and Furst, P. (1976). Uraemic middle molecules. *Clin. Nephrol.* **5**, 143 - 152.

Bright, R. (1836). Cases and observations illustrative of renal disease accompanied by the secretion of albuminous urine. *Guy's Hospital Report,* **1**, 338 - 379.

Brunner, F. P., Giesecke, B., Gurland, H. J., Jacobs, C., Parsons, F. M., Scharer, K., Seyffart, G., Spies, G. and Wing, A. J. (1975). Combined report on intermittent dialysis and transplantation in Europe. *Proc. Eur. Dial. Transpl. Assoc.,* **12**, 3 - 64.

Bryan, F. A. (1974). Sixth Annual Progress Report 1973 - 1974: The National Dialysis Registry. National Institutes of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014.

Chang, T. M. S. (1966). Semipermeable aquaeous microcapsules ('artificial cells'): with emphasis on experiments in an extracorporeal shunt system. *Trans. Amer. Soc. Artif. Int. Organs,* **12**, 13.

Chang, T. M. S. (1969). Removal of endogenous and exogenous toxins by a microencapsulated adsorbent. *Can. J. Physiol. Pharmacol.,* **47**, 1043 - 1045.

Chang, T. M. S. (1974). A comparison of semipermeable microcapsules and standard dialysers for use in separation. *Separation and Purification Methods,* **3**, (2), 245 - 262.

Chang, T. M. S., Pont, A., Johnson, L. J. and Malave, N. (1968). Response to intermittent extracorporeal perfusion through shunts containing semipermeable microcapsules. *Trans. Amer. Soc. Artif. Int. Organs,* **14**, 162 - 168.

Chang, T. M. S., and Malave, N. (1970). The development and first clinical use of semipermeable microcapsules (artificial cells) as a compact artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **16**, 141 - 148.

Chang, T. M. S., Gonda, A., Dirks, J. H. and Malave, N. (1971). Clinical evaluation of chronic intermittent and short-term haemoperfusion in patients with chronic renal failure using semipermeable microcapsules (artificial cells) formed from membrane coated activated charcoal. *Trans. Amer. Soc. Artif. Int. Organs,* **17**, 246 - 252.

Chang. T. M. S., Gonda, A., Dirks, J. H., Coffey, J. F. and Burns, T. (1972). ACAC microcapsule artificial kidney for the long-term and short-term manage-

ment of patients with chronic renal failure. *Trans. Amer. Soc. Artif. Int. Organs,* **18**, 465 - 472.

Chang, T. M. S. and Migchelsen, M. (1973). Characterisation of possible 'toxic' metabolites in uraemia and hepatic coma based on the clearance spectrum for larger molecules by the ACAC microcapsule artificial kidney. *Trans. A ner. Soc. Artif. Int. Organs,* **19**, 314 - 319.

Chang, T. M. S., Migchelsen, M., Coffey, J. F. and Stark, A. (1974). Serum middle molecule levels in uraemia during long-term intermittent haemoperfusions with the ACAC (coated charcoal) microcapsule artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 364 - 371.

Chang, T. M. S., Chirito, E., Barre, B., Cole, C. and Hewish, M. (1975). Clinical performance-characteristics of a new combined system for simultaneous haemo-perfusion-haemodialysis-ultrafiltration in series. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 502 - 508.

Chang, T. M. S., Chirito, E., Barre, P. and Resureccion, E. (1976). The use of ACAC microencapsulated charcoal haemoperfusion in series with Amicon ultrafiltration for patients with chronic renal failure. *Abstracts, Amer. Soc. Artif. Int. Organs,* **5**, 15.

Dunea, G. and Kolff, W. J. (1965). Clinical experience with the Yatzidis charcoal artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **11**, 178 - 182.

Dunea, G., Rizvi, S. H., Mamdani, B. H., Anicama, H. J. and Mahurkar, S. D. (1976). Charcoal hemoperfusion and combination dialysis-haemoperfusion in chronic renal failure. *Abstracts Amer. Soc. Artif. Int. Organs,* **5**, 22.

Flendrig, J. A., Kruis, H. and Das, H. A. (1976). Aluminium intoxication: the cause of dialysis dementia? *Proc. Eur. Dial. Transpl. Assoc.,* **13**, 355.

Friedman, E. A. (1976). Oxidised starch and oral charcoal in uraemic patients. *Kidney International* (in press).

Friedman, E. A., Laungani, G. B. and Beyer, M. M. (1975). Life prolongation in nephrectomized rats fed oxidized starch and charcoal. *Kidney International,* **7**, 377 - 379.

Funck-Brentano, J. L., Man, N. K., Sausse, A., Cueille, G., Zingraff, J., Drueke, T., Jungers, P. and Billon, J. P. (1975). Neuropathy and 'middle' molecule toxin. *Kidney International,* **7**, 352 - 356.

Funck-Brentano, J. L., Man, N. K., Sausse, A., Zingraff, J., Cloix, J. F., Becker, A. and Cueille, G. (1976). Characterisation of a 1100 - 1300 MW uraemic neurotoxin. *Trans. Amer. Soc. Artif. Int. Organs,* **22**, 163.

Gazzard, B. G., Portmann, B. A., Weston, M. J., Langley, P. G., Murray-Lyon, I. M., Dunlop, E. H., Flax, H., Mellon, P. J., Record, C. O., Ward, M. B. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet,* **1**, 1301 - 1307.

Gordon, A., Gral, T., De Palma, J. R., Greenbaum, M. A., Marantz, L. B., MacArthur, M. J. and Maxwell, M. H. (1970). A sorbent-based low-volume dialysate system. Preliminary studies in human subjects. *Proc. Eur. Dial. Transpl. Assoc.* **7**, 63 - 68.

Gordon, A., Furst, P., Bergstrom, J., Lewin, A., Rosenfeld, M. D. J., Roberts, M. and Maxwell, M. H. (1976). Adsorption of uremic toxins. *Proc. 6th Int. Cong. Nephrol.* (in press).

Graham, T. (1854). Bakerian lecture on osmotic force. *Phil. Trans. Roy. Soc., London,* p. 177.

Hagstam, K. E., Larsson, L. E. and Thysell, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital intoxication and uraemia, including histopathologic findings. *Acta. Med. Scand.,* **180**, 593 - 603.

Johnson, W. J., Hagge, W. W., Wagoner, R. D., Dinapoli, R. P. and Rosevear, J. W. (1975). Toxicity arising from urea. *Kidney International,* 7, S288 - 293.

Jones, J. D. and Burnett, P. C. (1975). Creatinine metabolism and toxicity. *Kidney International,* 7 S294 - 298.

Kolff, W. J., and Berk, H. T. J. (1944). Artificial kidney: dialyser with great area. *Acta Med. Scand.,* **117**, 121 - 134.

Leber, H. W., Neuhauser, M., Goubeaud, G. and Schutterle, W. (1976). Removal of toxic middle molecular solutes from uraemic serum by haemoperfusion. *Abstracts, Eur. Dial. Transp. Assoc.,* p. 265.

Malchesky, P. S., Nokoff, R., Varnes, W. and Nosé, Y. (1976) The charcoal capillary hemoperfusion system. *Proc. 13th Eur. Dial. Transpl. Assoc.,* **13**, 242.

Muirhead, E. E. and Reid, A. F. (1948). A resin artificial kidney. *J. Lab. Clin. Med.,* **33**, 841 - 844.

Ota, K., Ohta, T., Kobayashi, M., Yoshida, S., Kaneko, I., Agishi, T. Sugihara, M. (1976). Petroleum based activated charcoal for direct haemoperfusion. *Proc. Eur. Dial. Transp. Assoc.,* **13**, 250.

Sparks, R. E. (1975). Gastrosorbents in the therapy of uraemia; inferences from intestinal loop dialysis. *Kidney International,* 7, S373 - 376.

Stephen, R. L., Jacobsen, S. C., Atkin-Thor, E. and Kilff, W. J. (1975). Portable/wearable artificial kidney (WAK) - initial evaluation. *Proc. Eur. Dial. Transpl. Assoc.,* **12**, 26.

Ward, M. K., Pierides, A. M., Fawcett, P., Shaw, D. A., Perry, R. H., Tomlinson, B. E. and Kerr, D. N. S. (1976). Dialysis encephalopathy syndrome. *Proc. Europ. Dial. Transpl. Assoc.,* **13**, 348.

Winchester, J. F. (1976). Working luncheon on adsorbent haemoperfusion for uraemia, acute intoxication and liver failure. *22nd Amer. Soc. Artif. Int. Organs,* San Francisco.

Winchester, J. F., Tilstone, W. J., Edwards, R. O., Gilchrist, T. and Kennedy, A. C. (1974). Haemoperfusion for enhanced drug elimination - a kinetic analysis in paracetamol poisoning. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 358 - 363.

Winchester J. F., Edwards, R. O., Tilstone, W. J. and Woodcock, B. G. (1975a). Activated charcoal haemoperfusion and experimental acetaminophen poisoning. *Toxicol. Appl. Pharmacol.* **31**, 120 - 127.

Winchester, J. F., Apiliga, M. T., Mackay, J. M. and Kennedy, A. C. (1975b). Haemodialysis with charcoal haemoperfusion. *Proc. Eur. Dial. Transpl. Assoc.*, **12**, 526 - 533.

Winchester, J. F. Apiliga, M. T. and Kennedy, A. C. (1976). Short term evaluation of charcoal haemoperfusion combined with dialysis in uraemic patients. *Kidney International* (in press).

Yatzidis, H. (1964). A convenient haemoperfusion micro-apparatus over charcoal for the treatment of endogenous and exogenous intoxications. Its use as an artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.,* **1**, 83 - 87.

# 22

# THE CLINICAL USE OF A CARBON HAEMOPERFUSION COLUMN

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**Summary**

Interim results on some clinical applications of haemoadsorption are presented. The technique is considered a simple, safe and effective method of treating severe poisoning due to glutethimide and short acting barbiturates. There are also encouraging indications for the use of haemoperfusion on a repeated basis (alternated with haemodialysis) in the control of the uraemic state.

## 22.1 Introduction

More than 30 years after its introduction we are still learning how to use the artificial kidney and how to define adverse reactions. Such reactions to formalin sterilisation with the production of auto antibodies (Fassbinder *et al.*, 1976), the migration of plasticisers (Neergard *et al.*, 1971; Ono *et al.*, 1975; Gibbins *et al.*, 1976) from A-V lines, and a reappraisal of the duration and efficiency of dialysis have been topics of study in the last two years. Perhaps we now realise the limits of the artificial organ in therapy and no longer make extravagant claims such as Murray's suggestion (Murray *et al.*, 1948) of its use in the removal of a foetus, its trials in schizophrenia (Tholen *et al.*, 1967) and the hope of its use in liver failure (Keynes, 1968; Chalmers, 1968; Pirola *et al.*, 1969).

## 22.2 Haemoperfusion Column Development

Haemoadsorption techniques are still in a very early phase of development and many changes and improvements are inevitable. Manufacturers and clinicians together must ensure that the system is free from adverse effects, that it is consistent and predictable in performance and that the indications for its use have been ascertained.

The development of the Gambro charcoal column illustrates the use of these guiding concepts.

The first generation of columns developed contained 100 - 150 g acrylic copolymer membrane coated charcoal with good surface blood compatibility and promising *in vitro* transport characteristics (Gilchrist *et al.*, (1975). Perfusions combined with dialysis in a series of glutethimide poisoning cases (Martin *et al.*, 1975) demonstrated their potential (table 22.1). Five cases were treated in grade IV coma

**Table 22.1**

Glutethimide poisoning. Combined haemodialysis and haemoperfusion (100 - 150 g acrylic copolymer coated charcoal).

| *Initial blood level* | *Blood level after 2 h (dialysis - perfusion)* |
|---|---|
| mg/l | mg/l |
| 49 | 22 |
| 41 | 25 |
| 49 (mean 55) | 21 (mean 31) |
| 75 | 48 |
| 63 | 40 |

with initial blood levels ranging from 41 to 75 mg/l. The column was run in series with a 1 $m^2$ dialyser and the post treatment blood levels ranged from 48 to 21 mg/l after 2 h therapy. Clearance of the column and dialyser together ranged from 83 to 120 ml/min.

The use of this copolymer coating was discontinued in the Gambro columns in view of the requirement for formalin sterilisation of the membrane, and the possibility of the persistence of trace quantities of dimethylformamide, the polymer solvent. The membrane material now used is cellulosic for which the following appropriate coating technique was devised (Thysell *et al.*,(1976). The charcoal is carefully washed, dried and then spray coated with cellulose acetate dissolved in acetic acid. The cellulose acetate is then converted to a cellophane membrane by hydrolysis in alkaline solution and adsorbed acetic acid converted to sodium acetate. The hydrolysis determines the biocompatibility and the permeability. Gradual optimisation of the hydrolysis process has dramatically improved both these properties.

**Table 22.2**

Fines count. (Gambro haemoperfuser column)

| *Column condition* | *Fines diameter* μm | *Fines count per ml* | |
|---|---|---|---|
| | | In | Out |
| Before autoclaving | 2 | | 207 ± 14 |
| | 5 | | 25 ± 2 |
| After wash through prior to clinical use | 2 | 85 ± 11 | 279 ± 22 |
| | 5 | 8 ± 1 | 15 ± 1 |

### 22.3 Current Column Specifications and Performance

The column specification is as follows: 300 g cellophane coated extruded charcoal charge; saline primed and autoclaved; a priming volume of 400 ml, including blood lines and a fines production rate before autoclaving well below the British Pharmacopoeial recommendations for infusing liquids namely:

$$207 \pm 14/\text{ml} > 2\,\mu \text{ diameter}$$
$$25 \pm 2/\text{ml} > 5\,\mu \text{ diameter}$$

After priming with 500 ml dextrose and 2 l of saline, studies have shown (Jonsson, 1976) a change in the fines count due to the additional fines actually entering from the infusion fluids – a situation similar to that tolerated by regular haemodialysis patients. The change in fines count is shown in table 22.2.

A series of rabbit sacrifice experiments failed to reveal evidence of fines embolisation (Thysell *et al.*, 1976). The residual blood volume noted after clinical use was 1.6 ml (a mean of 5 deteminations by haemoglobinometry).

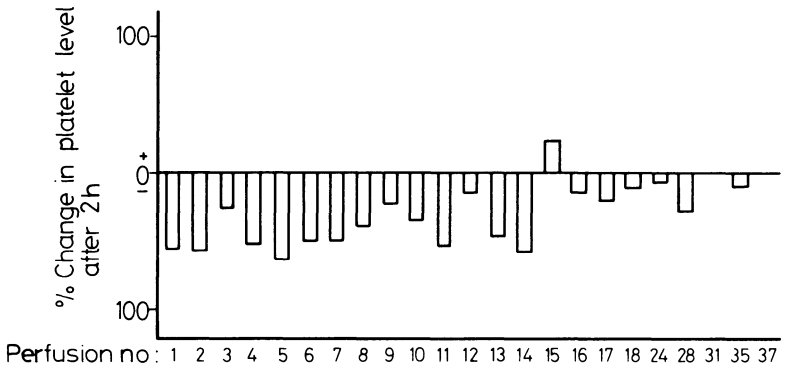


Figure 22.1 Change in platelet levels in sheep. (Early Gambro columns with cellulosic coated charcoal granules.)

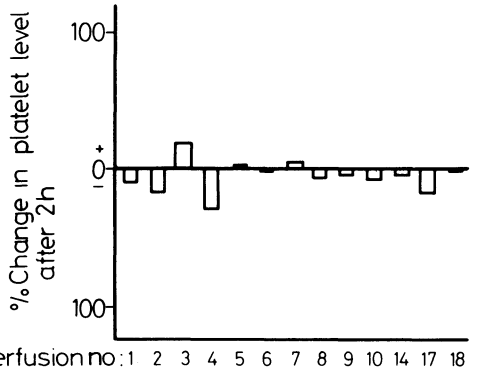


Figure 22.2 Change in platelet levels in sheep. (Gambro columns optimised for biocompatibility and permeability.)

Prior to human clinical evaluation a series of sheep perfusions on a twice weekly basis was commenced is still under study. More than 42 treatments have been carried out so far on the sheep with no evidence of adverse effects or long-term haematological abnormality.

The early cellulosic coated charcoal columns were associated with a repeated transient fall in platelet levels in the sheep as shown in figure 22.1. As a result of the hydrolysis optimisation now attained there is a marked improvement in biocompatibility and permeability. This is seen in the recent sheep perfusions where changes in platelet levels are within the error of the counting method used, and permeability is greatly enhanced (figure 22.2).

## 22.4 Clinical Evaluation

The technique was applied at this stage to patients suffering from acute amylobarbitone, pentobarbitone and paraquat poisoning.

The clearances obtained for short acting barbiturates were certainly superior to haemodialysis and peritoneal dialysis (Bloomer, 1965; table 22.3) with a removal rate of up to 375 mg/h. Paraquat removal was also accelerated by haemoperfusion as illustrated in table 22.4.

**Table 22.3**

Clearances obtained for short acting barbiturate poisoning.
(Gambro columns 300 mg cellulosic coated)

| *Patient* | *Blood flow ml/min* | *Perfusion time min* | *Clearance ml/min* | *Pre-drug levels mg/l* | *Post-drug levels mg/l* |
|---|---|---|---|---|---|
| N.D. | 240 | 90 | 38 | 36 | 21 |
| M.C. | 250 | 30 | 30 | 28 | 18 |
| M.B. | 218 | 20 | 43 | 38 | 19 |
| | 240 | 40 | 10 | | |
| | 240 | 60 | 21 | | |
| | 240 | 90 | 10 | | |

Amylobarbitone removal rate up to 375 mg/h

With these earlier columns platelet drops ranged from 0 to 66% with a mean of 35%, but the magnitude of this drop is heavily weighted by the platelet changes in the paraquat case in which coagulation occurred within a dialyser used prior to perfusion.

A case of 2 h haemoperfusion in glutethimide poisoning demonstrates the immediate fall in total and rise in free glutethimide in the first hour and the rebound after therapy (figure 22.3).

Ten 2 h haemoperfusions have also been carried out on a patient with end-stage renal failure, alternating with haemodialysis, over a 2 month period (table 22.5). With improvement in the hydrolysis technique the permeability of the later columns

**Table 22.4**

Paraquat poisoning. (Gambro columns 300 mg cellulosic coated)

A. R. age 37 years

Ingestion 01.30 hours 30.11.75.
Gastric lavage within 1 hour
Haemoperfusion commenced 05.45 hours
Treatment continuous to 9.12.75
Consisting of: haemoperfusion x 3
haemodialysis x 8
peritoneal dialysis
Intitial blood level 0.38 μg/ml
Urine +ve for 8 days. Gastric concentration 2 g/l.

| *Perfusion* | *Dialysis* |
|---|---|
| A 0.38 0.33 | A 0.06 |
| V 0.34 0.25 | V 0.02 |
| *Blood flow* | |
| 320 280 | 120 |
| 42 67 ml/min | 80 ml/min |
| *Paraquat levels* | |
| pre 0.38 | pre 0.24 |
| post 0.24 | post 0.06 |

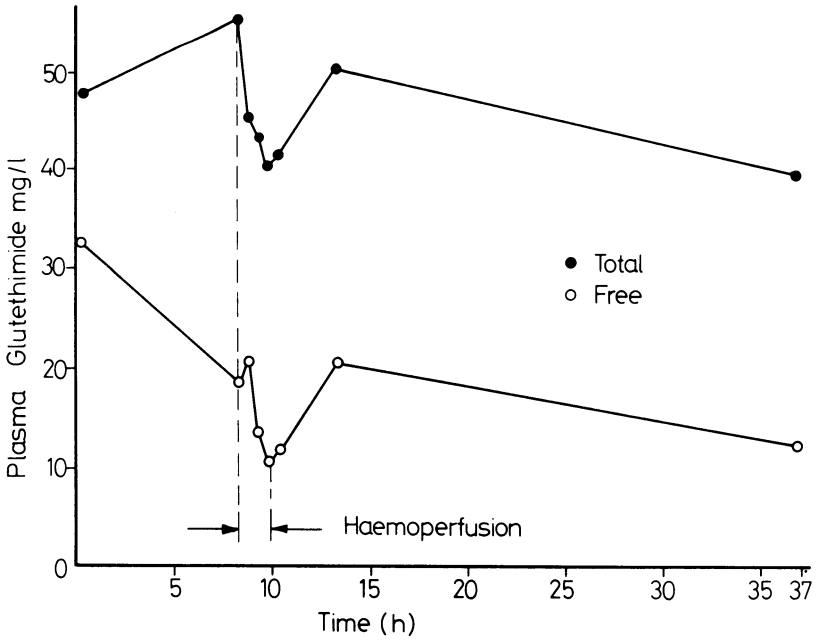


Figure 22.3 Glutethimide poisoning: 2 h haemoperfusion.

**Table 22.5**

Intermittent haemoperfusion: 2 h perfusions with blood flow 240 – 280 ml/min

| *Creatinine μmol/l* | | *Uric acid m mol/l* | |
|---|---|---|---|
| *Pre-perfusion* | *Post-perfusion* | *Pre-perfusion* | *Post-perfusion* |
| 940 | 550 | 0.53 | 0.28 |
| 960 | 790 | 0.54 | 0.43 |
| 1030 | 790 | 0.56 | 0.4 |
| 1040 | 880 | 0.51 | 0.42 |
| 950 | 510 | 0.51 | 0.27 |
| 940 | 490 | 0.56 | 0.26 |
| 840 | 420 | 0.50 | 0.23 |

**Table 22.6**

Intermittent haemoperfusion: platelet count pre and post-haemoperfusion
Perfusion 2 h, blood flow 240 – 280 ml/min

| *Platelet count* $\times 10^3$ | |
|---|---|
| *Pre-perfusion* | *Post-perfusion* |
| 240 | 205 |
| 165 | 165 |
| 145 | 130 |
| 135 | 125 |
| 160 | 180 |

was markedly increased resulting in a 50% fall in creatinine and uric acid after 2 h. There was no significant platelet abnormality during or after any of the haemoperfusions (table 22.6).

## 22.5 Conclusions

Haemoperfusion is a safe, effective and simple method of treating severe poisoning due to glutethimide and short acting barbiturates with clearances superior to haemodialysis.

A 2 h haemoperfusion in end-stage renal failure has been shown to reduce the concentrations of creatinine and uric acid by 50%. Used in the short term on a repeated basis, alternated with haemodialysis, satisfactory symptomatic and biochemical control of the uraemic state was noted.

Repeated use of the Gambro haemoperfusion column was not associated with any significant platelet abnormality or change.

## 22.6 References

Bloomer, H. A. (1965). *New Engl. J. Med.*, 272, 1309.

Chalmers, T. C.,(1968). *Med. Clin. N. Amer.*, **52**, 1475.

Fassbinder, W., Pilar, J., Shevermann, E. and Koch, K. M. (1976). *Proc. Eur. Dial. Transpl. Assoc.*, **13**, 333.

Gibbins, J. K., Martin, A. M., and Herbert, R. (1976). *Abstracts Eur. Dial. Transpl. Assoc.*, p. 348.

Gilchrist, T., Jonsson, E., Martin, A. M., Naucler, L., Cameron, A. and Courtney, J. M. (1974). Development of the Strathclyde haemoperfusion system, in R. Williams and I. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 319.

Jonsson, E., (1976). Personal communication.

Keynes, W. M. (1968). *Lancet*, 2, 1236.

Martin, A. M., Mitchell, D. C., Gibbins, J. K. Devapal, D. and Trinder, P. (1975). *Brit. Med. J.*, 1, 392.

Murray, G., Delorme, E. and Thomas, N. (1948). *J. Amer. Med. Assoc.*, **137**, 1596.

Neergard, J., Nielson, B., Faurby, V., Christensen, D. H. and Nielson, O. F. (1971). *Scand. J. Urol. Nephrol.*, **5**, 141.

Ono, K., Tatsukawa, R. and Wakimoto, T. (1975). *J. Amer. Med. Assoc.*, **234**, 948.

Pirola, R. C., Ham, J. N. and Elmslie, R. G. (1969). *Gut*, **10**, 898.

Thysell, H., Lindholm, T., Heinegard, D., Jonsson, E., Nyclen, U., Svensson, T., Bergkuist, G. and Gullberg, C. A. (1976). *Proc. Eur. Soc. Artif. Organs*, **2**, 212.

Tholen, H., Strickler, E., Feer, H., Massini, M. A. and Staub, H. (1960). *Dtsch. Med. Wschr.*, **85**, 1012.

# 23

# HAEMOPERFUSION IN THE TREATMENT OF ACUTE POISONING

J. L. ROSENBAUM

**Summary**

Amberlite XAD-4 is an uncharged, macroreticular styrene resin with a specific adsorptive attraction for lipid-soluble, organic molecules. Thirteen patients with life-threatening drug overdose were haemoperfused on 15 occasions with a 312 g Amberlite XAD-4 resin column with a blood flow rate of 300 ml/min. The mean hourly column clearance of glutethimide was 266±23 ml/min and barbiturate was 242±41 ml/min for treatment sessions extending from $2\frac{1}{2}$ to 10 h. In five of the patients, column clearances were determined separately for plasma and erythrocyte fractions of the perfused blood. In three patients with glutethimide intoxication the pre and post-perfusion blood revealed minimal differences between plasma and erythrocyte concentrations, whereas two patients with barbiturate intoxication had much higher drug concentrations in the plasma fractions of the pre-perfusion blood. All patients had a marked reduction in both plasma and erythrocyte drug concentrations in the post-perfusion blood. The data suggest utilising whole blood to accurately measure drug concentrations.

The patients demonstrated dramatic clinical improvement during haemoperfusion with no evidence of significant toxicity or reaction to the procedure. Column haemoperfusion with Amberlite XAD-4 resin is more effective than any other known method of removing barbiturates and glutethimide from the blood of overdosed patients.

## 23.1 Background

Persusion of blood through various adsorbent and ion exchange substances is more efficient and simpler than haemodialysis for the removal from the circulation of many drugs commonly involved in suicidal and accidental overdose. Haemoperfusion through a column of activated charcoal was shown to remove barbiturates from the blood of intoxicated patients (Yatzidis, 1966). Unfortunately, this was not a feasible method for treatment due to platelet adsorption by the charcoal and to fragmentation of the charcoal particles with release into the systemic circulation (Hagstam *et al.*, 1966). This has been effectively corrected by coating the charcoal particles with semipermeable membranes (Andrade *et al.*, 1975). Blood perfused at a flow rate of 300 ml/min through a 300 g charcoal column coated with cellulose nitrate and human albumin has resulted in a clearance of 150 ml/min of glutethimide in one patient and 200 ml/min of methaqualone over a 2 h interval in another patient (Chang, 1975). In another report five patients with glutethimide and one

with barbiturate overdose had their blood perfused at 200 ml/min through a 300 g column of charcoal coated with an acrylic hydrogel. The barbiturate clearance was 50 to 120 ml/min and the average glutethimide clearance was 125 ml/min with a 38 to 50% reduction in platelet concentration (Vale *et al.*, 1975).

Also, uncoated ion-exchange and uncharged adsorbent resins have been used for haemoperfusion in the treatment of drug overdose. These resins can be produced inexpensively in large quantities. Phenobarbital was shown to be removed from the blood of intoxicated dogs by haemoperfusion with a column containing 500 g of the anion-exchange resin Amberlite IRA 900 (Nealon *et al.*, 1966). The resin was mixed as a 2:1 ratio of chloride to bicarbonate. The column had no effect on the pH or electrolyte composition of the perfused blood. This system was effective in adsorbing salicylate (Rosenbaum *et al.*, 1970). Three intoxicated dogs were haemoperfused for 2 h. The column clearance of salicylates ranged from 96±85 to 190±47 ml/min with a mean reduction of serum salicylate concentration from 42.9 to 9.8 mg/100 ml. Also, there was a fall in leucocyte and platelet concentrations due to direct adsorption onto the resin bed.

### 23.2 Haemoperfusion with Amberlite

Amberlite XAD-2 is an uncharged, macroreticular, styrene-divinylbenzene copolymer with a specific adsorptive attraction for lipid-soluble molecules including barbiturates, glutethimide, ethchlorvynol, methaqualone, chloroquine and tricyclic depressants. It may be autoclaved or gas sterilised. The particles do not easily fragment. Haemoperfusion with a 650 g column of Amberlite XAD-2 was markedly effective in clearing barbiturates, glutethimide, and ethchlorvynol from intoxicated dogs and patients (Rosenbaum *et al.*, 1970; Rosenbaum *et al.*, 1971; Rosenbaum, 1975a). The intoxicated patients had an average reduction in circulating platelet concentration of 40% with no evidence of clinical toxicity. Haemoperfusion was shown to be more effective than haemodialysis by treating five patients with drug overdose using a haemodialysis and Amberlite XAD-2 haemoperfusion system in parallel (Rosenbaum *et al.*, 1970).

Amberlite XAD-4 is chemically identical to Amberlite XAD-2 but differs in physical properties with a larger surface area of 750 $m^2/g$ compared to 330 $m^2/g$. The increased adsorptive capacity for lipid soluble molecules was measured *in vitro* by circulating a solution containing 20 mg/100 ml of phenobarbital through 650 g (312 g dry weight) resin columns of Amberlite XAD-2 and XAD-4 (Rosenbaum, 1975b). At a flow rate of 200 ml/min the phenobarbital was cleared by the Amberlite XAD-4 column at a rate exceeding 190 ml/min for over 4 h; over 13 g of phenobarbital were removed in 6 h with a final clearance rate of 150 ml/min. This was distinctly superior to the performance of the Amberlite XAD-2 column. Five dogs were intoxicated with oral phenobarbital and treated for 6 h of haemoperfusion with the 650 g Amberlite XAD-4 column at a flow rate of 200 ml/min. In addition, 1 g per hour of phenobarbital was infused intravenously during the last 4 h of haemoperfusion. Drug clearance by the column ranged from 187 to 197 ml/min. An additional five dogs were intoxicated with oral methaqualone. They

were haemoperfused for 6 h with a flow rate of 200 ml/min. The drug clearances ranged from 195 to 200 ml/min.

If perfusate is passed through a modified Dacron-wool filter in the return line from the column, particulate resin particles are almost completely removed. Using a 0.8 micron filter, a saline solution was perfused through 10 DX-60 cartridges of Amberlite XAD-4 at a flow rate of 300 ml/min over 8 h. (The disposable DX-60 cartridge contains 312 g dry weight of Amberlite XAD-4. It is designed for the treatment of drug intoxication by Extracorporeal Medical Specialties, Inc, King of Prussia, Pennsylvania, USA.) The filter contained an average of only 5.9 particles/h from 5 to 25 microns, 1.1 particles/h from 25 to 50 microns, and no particles over 50 microns in size.

### 23.3 Clinical Assessment

The result of haemoperfusion with the 650 g Amberlite XAD-4 column has been reported in eight patients with severe intoxication with a variety of drugs including glutethimide, pentobarbital, secobarbital and amobarbital (Rosenbaum *et al.*, 1976a and b). Haemoperfusion extended from $2\frac{1}{2}$ to 10 hours with a blood flow rate of 300 ml/min. No clinical toxicity was noted. The average platelet concentration decreased from 229000 ± 98000/ml to 115000 ± 40000/ml during the procedure, and returned to 182000 ± 97000/ml 18 to 24 h later. Seven of eight patients demonstrated a dramatic clinical response usually with spontaneous stabilisation of the blood pressure and respiratory rate by 30 to 45 min, response to painful stimuli within 45 min, and response to verbal command by the end of haemoperfusion. Two patients with glutethimide intoxication had a modest clinical rebound after haemoperfusion, but not enough to require a repeat treatment. One patient ingested 75 g of glutethimide requiring 3 daily haemoperfusions for 9, 10, and 8 h respectively. After the first haemoperfusion, the blood concentration of glutethimide only decreased from 11.0 to 9.5 mg/100 ml in spite of removing 13.4 g of drug. There was no evidence of column saturation during the procedure. This suggests a rapid movement of glutethimide from the intracellular compartment and the gastrointestinal lumen into the extracellular compartment. On the second day the patient responded to verbal command during haemoperfusion while the blood glutethimide concentration remained over 5.0 mg/100 ml. This occurred in spite of persistent dependency on respiratory control and a vasoactive drug infusion. In contrast, three patients were in deep coma prior to haemoperfusion with relatively low serum concentrations of glutethimide. This emphasises the poor correlation of depth of coma with serum concentration of glutethimide. Also, it is possible that the more toxic metabolite, 4-hydroxyglutethimide, contributed to the comatose state (Hansen *et al.*, 1975). Occasional patients have demonstrated a delayed improvement in coma for several days following effective removal of the intoxicant drug by haemoperfusion with either the Amberlite XAD-2 or the XAD-4 column. This coma was followed by complete clinical recovery within a few days (Rosenbaum *et al.*, 1970; Rosenbaum *et al.*, 1976a and b). It appears that rapid removal of glutethimide and barbiturates by haemoperfusion allows the identification of

the more common rapid responder, in whom the coma is likely due to the drug excess itself, and the less common slow responder in whom associated ischaemia or drug toxicity to the central nervous system may be important contributing factors to the coma.

### 23.4 Plasma and Whole Blood Drug Concentrations

Haemoperfusion and haemodialysis clearance data generally are assumed to represent whole blood passing through the column or dialyser although the drug concentrations are usually calculated from plasma. However, the plasma may not necessarily reflect erythrocyte concentration of a solute being rapidly removed by an artificial device (Nolph *et al.*, 1974; Rosenbaum *et al.*, 1976a and b). Therefore, we should not freely substitute plasma concentration for blood concentration in calculating indices of solute removal from perfused blood.

One previously reported patient and four additional patients with life-threatening drug intoxication had haemoperfusion with a 650 g column of Amberlite XAD-4 resin with a blood flow rate of 300 ml/min (Rosenbaum *et al.*, 1976a and b). Patient 1 was a 32 year old man with combined amobarbital and secobarbital ingestion; patient 2 was a 66 year old woman with glutethimide intoxication; patient 3 was a 74 year old man with glutethimide intoxication; patient 4 was a 34 year old man with phenobarbital intoxication; patient 5 was a 61 year old woman with com-

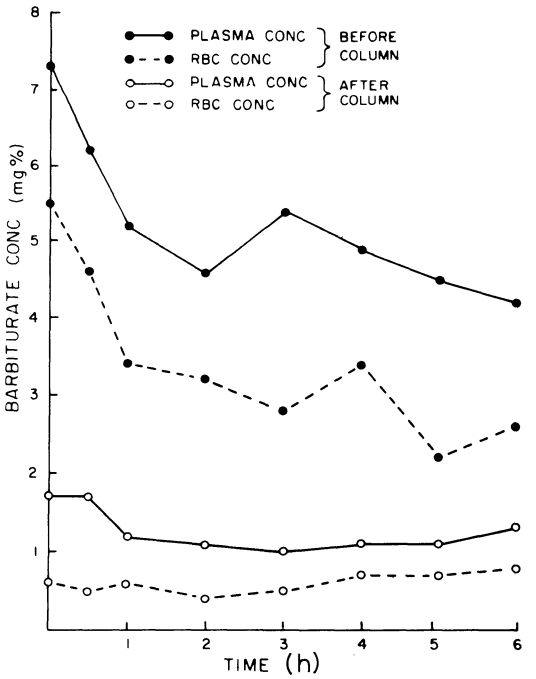


Figure 23.1 Blood barbiturate concentrations in patient 1 during 6 h of haemoperfusion with an Amberlite XAD-resin column

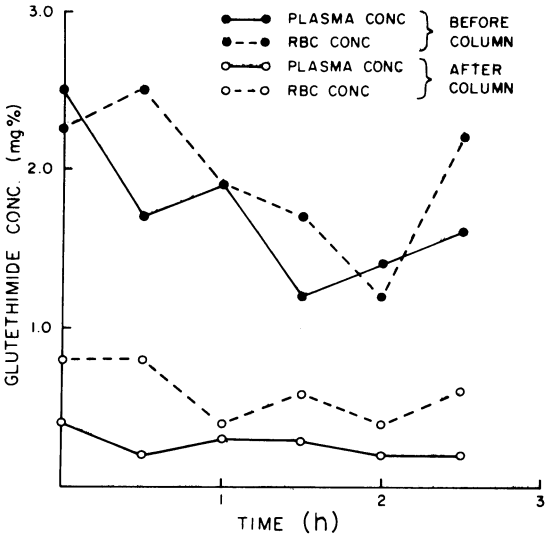


Figure 23.2 Blood glutethimide concentrations in patient 2 during 2½ h of haemoperfusion with an Amberlite XAD-4 resin column

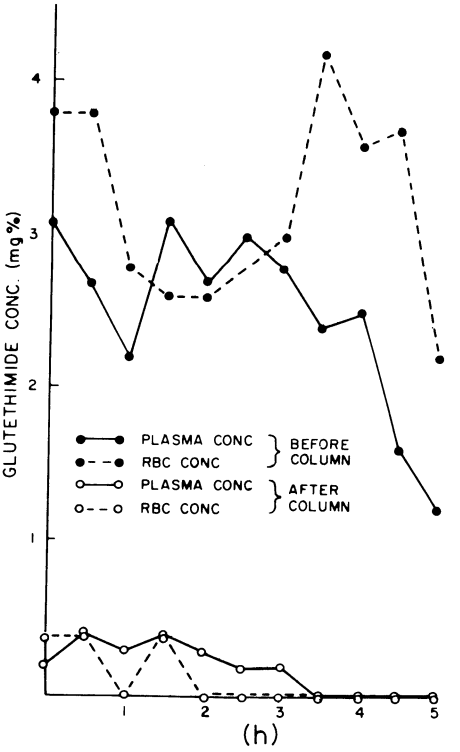


Figure 23.3 Blood glutethimide concentrations in patient 3 during 5 h of haemoperfusion with an Amberlite XAD-4 resin column

bined glutethimide and diazepam intoxication. All patients required a vasoactive drug infusion and respiratory assist at the start of haemoperfusion. The first four patients responded dramatically and were responsive to verbal command by the conclusion of haemoperfusion. The fifth patient had a modest response and reacted to painful stimuli within 1 h of haemoperfusion (associated diazepam intoxication probably had a dampening effect on the response). No toxic effects or rebound coma were noted. In all patients the plasma was immediately separated from the erythrocytes after obtaining each blood sample. The drug concentrations were determined separately for erythrocytes and plasma. The column clearance and quantitative removal rates were calculated according to previously described formulae which were further adapted for separate plasma and erythrocyte determinations (Rosenbaum and Mandanas, 1967). Patients 1 and 2 had drug concentrations determined by the colorimetric method of Baer (1965); patients 3, 4 and 5 by a gas-liquid chromatography technique with a DC-200 column.

Patients 4 and 5 had no appreciable 4-hydroxyglutethimide peak in any of the blood determinations. However, it could have been masked by the diazepam peak in patient 5. Figures 23.1 to 23.5 represent the relationships of inflow and outflow

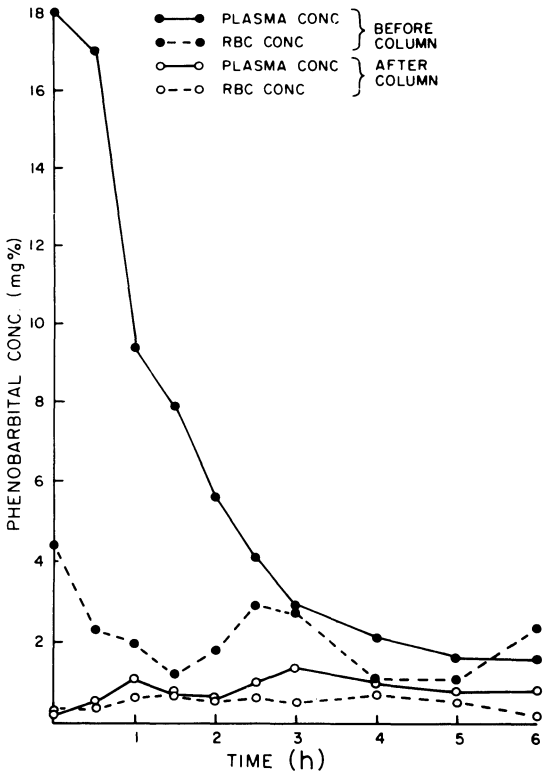


Figure 23.4 Blood phenobarbital concentrations in patient 4 during 6 h of haemoperfusion with an Amberlite XAD-4 resin column

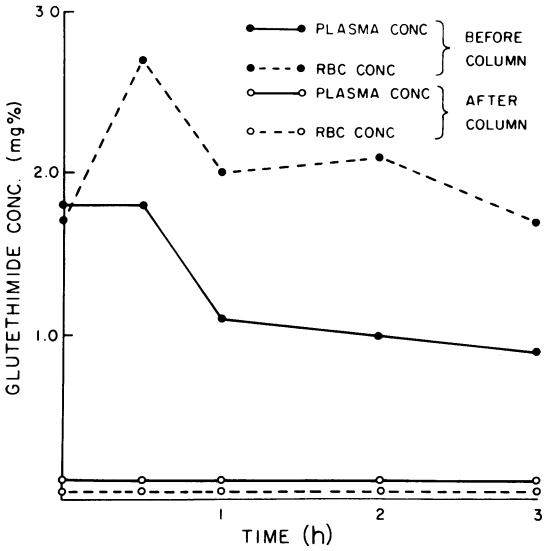


Figure 23.5 Blood glutethimide concentrations in patient 5 during 3 h of haemoperfusion with an Amberlite XAD-4 resin column

**Table 23.1**

Clearance of drug during haemoperfusion by a 650 g column of Amberlite XAD-4 resin in patients with acute intoxication. Blood flow rate through the column was 300 ml/min

| *Patient* | *Drug* | *0 h* | *1 h* | *2 h* | *3 h* | *4 h* | *5 h* | *6 h* |
|---|---|---|---|---|---|---|---|---|
| | Column clearance from plasma fraction (ml/min) | | | | | | | |
| 1 | Amobarbital-Secobarbital | 161 | 163 | 165 | 161 | 142 | 136 | 124 |
| 2 | Glutethimide | 176 | 154 | 157 | – | – | – | – |
| 3 | Glutethimide | 177 | 155 | 160 | 167 | 183 | 183 | – |
| 4 | Phenobarbital | 198 | 162 | 166 | 94 | 97 | 95 | 95 |
| 5 | Glutethimide | 192 | 198 | 201 | 195 | – | – | – |
| | Column clearance from erythrocyte fraction (ml/min) | | | | | | | |
| 1 | Amobarbital-Secobarbital | 80 | 72 | 74 | 84 | 93 | 82 | 83 |
| 2 | Glutethimide | 59 | 92 | 78 | – | – | – | – |
| 3 | Glutethimide | 99 | 120 | 102 | 120 | 117 | 117 | – |
| 4 | Phenobarbital | 92 | 112 | 76 | 96 | 34 | 62 | 101 |
| 5 | Glutethimide | 108 | 102 | 99 | 105 | – | – | – |

concentration of the intoxicant drugs during haemoperfusion. Tables 23.1 and 23.2 record the clearance and quantitative removal rates of the drugs by the column. Patient 4 had a much lower phenobarbital concentration in the erythrocytes compared to the plasma in the inflow blood at the start and during the first

**Table 23.2**

Quantity of drug removed during haemoperfusion by a 650 g column of Amberlite XAD-4 resin in patients with acute intoxication. Blood flow rate through the column was 300 ml/min

| *Patient* | *Drug* | *1 h* | *2 h* | *3 h* | *4 h* | *5 h* | *6 h* | *Total* |
|---|---|---|---|---|---|---|---|---|
| | Column adsorption from plasma fraction (mg) | | | | | | | |
| 1 | Amobarbital-Secobarbital | 588 | 483 | 488 | 470 | 446 | 394 | 2869 |
| 2 | Glutethimide | 198 | 127 | 71* | - | - | - | 396 |
| 3 | Glutethimide | 263 | 263 | 338 | 270 | 190 | - | 1324 |
| 4 | Phenobarbital | 1718 | 766 | 354 | 144 | 106 | 90 | 3178 |
| 5 | Glutethimide | 190 | 127 | 114 | - | - | - | 431 |
| | Column adsorption from erythrocyte fraction (mg) | | | | | | | |
| 1 | Amobarbital-Secobarbital | 207 | 139 | 141 | 165 | 149 | 119 | 920 |
| 2 | Glutethimide | 100 | 78 | 42* | - | - | - | 220 |
| 3 | Glutethimide | 226 | 185 | 159 | 245 | 232 | - | 1047 |
| 4 | Phenobarbital | 153 | 62 | 144 | 92 | 31 | 94 | 576 |
| 5 | Glutethimide | 143 | 124 | 116 | - | - | - | 383 |

*Haemoperfusion was discontinued after 2½ h.

2 h of haemoperfusion. Also, there was a persistent reduction in inflow erythrocyte barbiturate concentration in patient 1 throughout haemoperfusion. In contrast, the erythrocyte concentration of glutethimide was similar to or higher than the plasma concentrations in patients 2, 3 and 5. Haemoperfusion had no appreciable effect on the plasma and erythrocyte concentrations of diazepam. The 0 - 1 - 2 - 3 h blood samples revealed concentrations of diazepam of 29 - 25 - 20 - 28 mg/100 ml in pre-perfusion plasma, 25 - 21 - 20 - 29 mg/100 ml in post-perfusion plasma, 15 - 17 - 21 - 17 mg/100 ml in pre-perfusion erythrocytes and 20 - 24 - 32 - 27 mg/100 ml in post-perfusion erythrocytes. All patients had a sharp reduction in erythrocyte and plasma concentration of glutethimide and barbiturates in the outflow blood. The Amberlite XAD-4 resin column demonstrated excellent clearance of glutethimide and barbiturate, but not diazepam, from both erythrocytes and plasma. As suggested by these data, concentration of certain intoxicants may be either higher or lower in erythrocytes compared to plasma, possibly depending on protein-binding sites, solubility factors, metabolism within the red blood cell or restricted cell membrane transfer. Until more data are available with regard to plasma versus erythrocyte concentration of intoxicants, it is advisable to determine whole blood levels for precise calculations of column clearance and removal rates during haemoperfusion.

## 23.5 Conclusions

Haemoperfusion with the Amberlite XAD-4 resin column has consistently demonstrated exceptionally high clearance rates for barbiturates, glutethimide, eth-

chlorvynol and methaqualone. It has not shown any appreciable toxicity and has resulted in dramatic clinical responses. The resin does not require any protective coating thereby allowing an inexpensive preparation and a highly effective adsorptive surface. Haemoperfusion with adsorbent columns dramatically shortens the interval of drug-induced coma and assists in restoring the spontaneous vital functions of the body. Therefore, we should anticipate that haemoperfusion will result in a reduction in morbidity and mortality for patients with severe, life-threatening drug intoxication.

## 23.6 Acknowledgements

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## 23.7 References

Andrade J. D., Coleman, D. L., Kim, S. W., and Lentz, D. J. (1975). The coating of activated carbons for optimal blood compatibility, in R. Williams and I. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London, pp. 84 - 92.

Baer D. M. (1965). A simple colorimetric method for detection of barbiturates, suitable for the small clinical laboratory. *Amer. J. Clin. Pathol.,* **44**, 114 - 117.

Chang, T. M. S. (1975). Microencapsulated adsorbent haemoperfusion for uraemia, intoxication and hepatic failure. *Kidney International,* **7**, S387 - S392.

Hagstam, K., Larsson, L. and Thysell, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital and uraemia, including histopathologic findings. *Acta Med. Scand.,* **180**, 593 - 603.

Hansen, A. R., Kennedy, K. A., Ambre, J. J. and Fischer, L. J. (1975). Glutethimide poisoning. *New Engl. J. Med.,* **292**, 250 - 252.

Nealon, T. F., Sugerman, H., Shea, W. and Fleegler E. (1966). An extracorporeal device to treat barbiturate poisoning. *J. Amer. Med. Assoc.,* **197**, 158 - 160.

Nolph, K. D., Bass, O. E. and Maher, J. F. (1974). Acute effects of haemodialysis on removal of intracellular solutes. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 622 - 627.

Rosenbaum, J. L. (1975a). Biocompatibility of resin haemoperfusion, in R. Williams and I. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London, pp. 118 - 125.

Rosenbaum J. L. (1975b). Resin haemoperfusion in the treatment of acute drug intoxication. *Ind. Eng. Chem. Res. Devel.* **14**, 99 - 101.

Rosenbaum J. L. and Mandanas, R. (1967). Treatment of phenobarbital intoxication in dogs with an anion-recirculation peritoneal dialysis technique. *Trans Amer. Soc. Artif. Int. Organs,* **13**, 183 - 189.

Rosenbaum J. L., Winsten, S., Kramer, M. S., Moros, J. and Raja, R. (1970). Resin haemoperfusion in the treatment of drug intoxication. *Trans Amer. Soc. Artif. Int. Organs,* **16**, 134 - 140.

Rosenbaum J. L., Kramer, M. S., Raja R. and Boreyko, C. (1971). Resin haemoperfusion : A new treatment for acute drug intoxication. *New Engl. J. Med.,* **284**, 874 - 877.

Rosenbaum J. L., Kramer, M. S. and Raja, R. (1976a). Resin haemoperfusion for acute drug intoxication. *Arch. Int. Med.,* **136**, 263 - 266.

Rosenbaum J. L., Kramer, M. S., Raja, R., Winsten, S. and Dalal, F. (1976b). Haemoperfusion for acute drug intoxication. *Kidney International* (in press).

Vale, J. A., Rees, A. J., Widdop, B, and Goulding, R. (1975). Use of charcoal haemoperfusion in the management of severely poisoned patients. *Brit. Med. J.,* **1**, 5 - 9.

Yatzidis, H. (1966). Paper presented at The Third International Congress on Nephrology.

# 24

# AN ANIMAL MODEL FOR ADSORBENT EVALUATION

R. K. MEDD

**Summary**

The criteria for selection of an animal species for haemoadsorbent evaluation are discussed with reference to proof of efficacy and safety. The basic methods of access to the vascular system are described, together with the flow rates and pressures attainable. The problems associated with animal management are considered in relation to the comparison between haemoadsorbent, haemodialysis and negative control experiments. A number of factors involved in the design of safety evaluation studies are mentioned with a discussion of the difficulties posed in interpretation.

## 24.1 Introduction

In the experimental evaluation of haemoadsorption systems, there are two distinct prerequisites: the demonstration both of efficacy in adsorption of the substance(s) to be eliminated and their safety in use. Preliminary investigations of efficacy in adsorption may be performed *in vitro* and confirmed *in vivo* on miniaturised systems using the rat or rabbit where detection of the toxic product can be performed on minute volumes of blood. These systems equally permit the investigation of limited haematological and biochemical variables, again within severe volumetric restrictions. Whilst comprehensive histopathological investigation is practical on completion of haemoperfusion, investigations involving a recovery period or safety evaluation by repeated perfusions on the same animal are rarely practicable in rodents or lagomorphs.

## 24.2 Safety and Efficacy of Drugs and Devices

Regulatory requirements governing the testing, efficacy and use of haemoadsorption systems are in the formative stages in some countries and less advanced in others. Therefore, at present, responsibility for the use of such systems remains with the clinician and his assessment of the safety and efficacy of a particular adsorbent. Whilst the haemoadsorption devices may originate from academic research or commercial laboratories, neither can expect clinical trials to be commenced without providing experimental evidence of efficacy and safety. Apart from ethical considerations, it seems unlikely that any regulations will be less stringent in principle than those already applied to existing and potential

drugs. Such requirements include ophthalmological and neurological, as well as general clinical examinations; some form of abuse to demonstrate safety is needed (either repetitive use or disproportion between size of haemoadsorption system and blood volume); apart from full histopathology, including all major organ weights, an extensive range of haematological and biochemical investigations requires a considerable volume of blood, even in a well equipped laboratory. In considering safety, a disproportion which seems clinically practical is a human size device connected to an experimental animal of not less than 8 kg bodyweight.

## 24.3 Requirements for Modelling

In selecting any experimental animal in safety evaluation tests, the following points must be considered.

(1) Size and general physical characteristics, including handling temperament.
(2) Availability and quality (natural incidence of disease, etc).
(3) Cost.
(4) Existence of accepted normal values and ranges of variables for the species employed.

The temperament of the monkey, including interference with fistula sites, makes it of doubtful suitability. The sheep and the pig receive a toxicological prejudice based largely on the absence of background information. The pig is used increasingly in safety evaluation, but personal experience and reports of others reveal considerable difficulty in maintaining vascular patency; the sheep may well prove very useful in the future, but it has the physiological disadvantage of being a ruminant, unlike the pig which is monogastric. At present the dog, and in particular the laboratory Beagle, fulfils the experimental requirements for haemoadsorption evaluation *in vivo* with the least disadvantage; the main difficulty is the necessity for anaesthesia or sedation during perfusion, which in itself introduces another variable.

Access to the vascular system may only be effected in the following three ways: exteriorised carotid artery/jugular vein shunt, femoral artery/saphenous or cephalic vein percutaneous catheterisation, or double cannulation of the posterior vena cava via the femoral vein. The exteriorised shunt has the advantage that it can be inserted at any period prior to haemoperfusion and at termination the animal can be disconnected from the system even in the presence of abnormal blood coagulation; the shunt is well tolerated and patency can be maintained for up to six weeks without heparinisation. If the shunt is inserted at the time of perfusion (either under specific anaesthesia or by the narcosis caused by the experimental conditions), a limited amount of blood extravasation may ensue following heparinisation for haemoperfusion, but this rarely exceeds 5 ml over a period of 6h. Shunt access also permits repeated perfusion procedures. Percutaneous puncture of the femoral artery is a simple technique and may even be performed under local analgaesia in the conscious dog. The disadvantages lie in the fact that the catheter must be supervised for the entire duration of its presence to prevent

removal; because of the anatomical location of access in the proximal part of the limb, it is not easy to control haemorrhage on removal in the presence of a prolonged coagulation time; because of the anatomical absence of adequate collateral circulation, closure of the arteriopuncture surgically to leave vascular patency is obligatory instead of ligation in the presence of uncontrollable haemorrhage. Double lumen venous cannulation is a perfectly feasible technique to gain access to the vascular system, but venous-venous circulation has not yet been evaluated in comparison to arterio-venous access.

### 24.4 Simulation of the Clinical Situation

In the course of system evaluation, criteria of flow rate may be specified, especially when attempts are being made to compare different adsorbents. The exteriorised arterio-venous shunt permits blood flow rates of 200 ml/min in animals of body-weight 10 kg or more for periods of at least 3 h, providing the systolic blood pressure does not fall below 110 mm Hg.

Experimental evidence of cerebral anoxia has been produced with a flow rate of 200 ml/min after 6 h when the systolic blood pressure has intermittently fallen to 90 mm Hg. It seems feasible that with this method of access it is possible to divert most of the blood from the common carotid artery into the perfusion system. Perfusion at a flow rate of 100 ml/min is safe for at least 10 h. With the femoral arterial catheter, reduced blood pressure leads to collapse of the posterior aorta which causes pump shut-down in pressure monitored pumping systems. However, a 14 g catheter allows flow rates of up to 300 ml/min to be obtained, providing arterial pressure is adequate, and with a pump this performance is comparable to the exteriorised shunt. Without a pump, a shunt will produce flow rates in excess of 300 ml/min, but only 180 ml/min will be maintained unassisted through the femoral arterial catheter. One contributory cause to this slowed rate is the venous pressure encountered in the cephalic or saphenous line, even with a pump, especially in perfusion in excess of 3 h. This can rise as high as 60 mm Hg. Whilst in itself this is not disadvantageous in evaluation of adsorbents by perfusion, it does cause difficulties in standard haemodialysis due to consequent high ultrafiltration; if comparison is made between haemoadsorption and haemodialysis, it is important that the operating conditions are similar. It could additionally prove a disadvantage when considering the use of liquids in transmembrane adso ption systems.

Perhaps the main disadvantage of this dog model is that any prolonged period of haemoperfusion requires sedation, neuroleptanalgaesia or anaesthesia. In many instances during simulation of a clinical situation for the evaluation of haemoadsorption, sedation or narcosis is a useful and direct consequence. If this is not a sequel, sedation and neuroleptanalgaesia are often less satisfactory than judicious barbiturate anaesthesia (not overdosage), since they frequently produce marked falls in blood pressure and reduced cardiac output, cardiac arhythmias and reduced respiratory minute volume; this is not observed with barbiturate (Silberschmid *et al.*, 1976). It is appreciated that many haemoadsorbent systems efficiently

remove barbiturate and that supplements of barbiturate may be required frequently; the barbiturate amounts may be reduced by the use of synergistic drugs at levels inadequate to produce the side effects seen if they were employed as the sole sedatives.

## 24.5 Safety Evaluation

While evaluating a haemoadsorption system for safety, by using the following methods it is possible to establish a safety margin.

(1) Establishing a complete record of physical changes.
(2) Employing a unit of human size, thus establishing a margin by disproportion.
(3) Maintaining flow rates approaching those intended for man, whilst using an animal of approximately 1/7th of the bodyweight.
(4) Performing the standard toxicological investigations employing a post-perfusion period of many weeks if necessary.
(5) Employing the system repeatedly when intended for clinical use on one occasion only (Medd *et al.*, 1974).

It is indeed practical to apply the standard toxicological screen, such as might be used on a dog model undergoing an acute or chronic drug safety evaluation study. The interpretation of these results and also the suitability of this standard screen are more difficult to assess. In safety evaluation of haemoadsorption systems, there is no such state as a 'no effect' level; it is also equally important to distinguish between the effects of the technique of haemoperfusion and the effect of the adsorbent material (Medd *et al.*, 1975). Ideally, the control situation should be that of haemoperfusion using the adsorbent container without adsorbent, but in practice it is more usual to observe and record changes due to ancillary agents (such as barbiturate, hypnotic, heparin) over a similar period to perfusion; the effect of the adsorbent material is then regarded as the difference between the two. Such conclusions should be weighed with great care in clinical as well as biochemical and biomedical engineering contexts. Ultimately, haemoperfusion may be used repeatedly in medical conditions, for example, as an adjunct to or replacement for haemodialysis. Under these circumstances, it will be particularly important to investigate the effect of adsorbents on hormones, steroids, enzymes and cofactors which are assessed in chronic toxicology by the manifestation of changes clinically or pathologically detectable in the animal as a whole. In this instance, it will be necessary to detect actual circulating levels and also to evaluate immunological changes due to possible breakdown of the animal's own cells (e.g. IgM increase due to leucocyte DNA release) or to haptenes incorporating substances leached out of the adsorbent or haemoperfusion system. So complex can evaluation and interpretation become that the safety of new haemoadsorbents may be more readily evaluated in initial stages by comparison with existing accepted haemoadsorbents or haemodialysis.

**Table 24.1**

Haematological changes resulting from a high dose of quinalbarbitone without perfusion

| *Time (h)* | *PCV %* | *Hb g%* | *RBC $10^6$/ml* | *WBC $10^3$/ml* | *N* | *L* | *E* | *B* | *M* | *Platelets $10^3$/ml* |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 32 | 9.2 | 3.4 | 14.8 | 56 | 42 | 2 | 0 | 0 | 160 |
| 2 | 27 | 8.8 | 3.2 | 6.0 | 64 | 30 | 4 | 0 | 0 | 175 |
| 3 | 26 | 8.6 | 3.0 | 7.0 | 86 | 14 | 0 | 0 | 0 | 150 |
| 4 | 28 | 8.8 | 3.3 | 9.8 | 92 | 8 | 0 | 0 | 0 | 200 |
| 5 | 37 | 12.2 | 4.6 | 14.8 | 84 | 16 | 0 | 0 | 0 | 220 |
| 7 | 29 | 8.6 | 3.6 | 12.0 | 86 | 14 | 0 | 0 | 0 | 200 |
| 24 | 29 | 8.6 | 3.6 | 16.6 | 94 | 6 | 0 | 0 | 0 | 120 |

## 24.6 Evaluation of Safety and Efficacy

Evaluation of haemoadsorbents for combined efficacy and safety under simulated clinical conditions can present even more of a problem (Medd *et al.*, 1974). Firstly, it is necessary to simulate entry of the drug or metabolite into the blood stream in a manner equivalent to that seen in man; while intravenous administration may demonstrate simple clearance across the adsorbent, it rarely replicates a clinical situation in which considerable amounts have been deposited in body tissues and fluids. Metabolism may be different, as with paracetamol, where a single massive dose produces profound methaemaglobinanaemia, without apparent irrevocable delayed liver damage if the initial dose is not acutely fatal; amitriptyline fails to induce cardiac arrythmias. Such differences do not necessarily interfere with the evaluation of the adsorbent, but make clinical assessment of the likely absorbed dose difficult and experimental results are confirmed solely by laboratory results in retrospect (Medd *et al.*, 1974). It is of equal importance to establish the effect of the drug in overdosage or the metabolite on the experimental animal without haemoperfusion, if possible using a previously perfused animal as its own control. As with quinalbarbitone at 45 mg/kg intravenously, quite profound changes can occur (table 24.1) which might otherwise have adversely affected the evaluation of the adsorbent.

**Table 24.2**

Results with possible application to man. Comparison of leucocyte and platelet charges

| | *% Haematological changes* | | | |
|---|---|---|---|---|
| | *Leucocytes* | | *Platelets* | |
| | 5 h | 24 h | 5 h | 24 h |
| XAD-2 | 25 | 99 | 26 | 41 |
| Charcoal | 94 | 154 | 101 | 73 |
| Dialysis | 95 | 125 | 53 | 51 |
| Support | 97 | 116 | 97 | 77 |

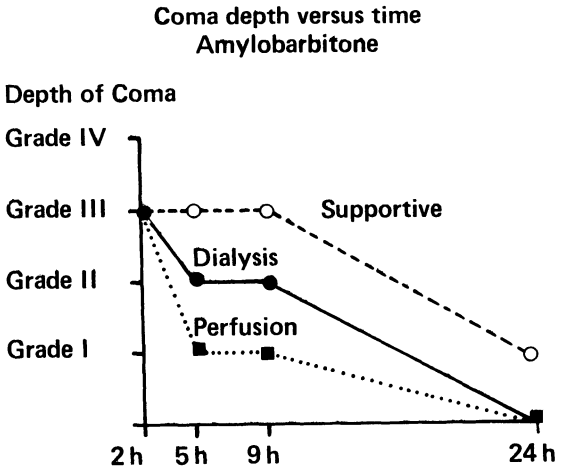


Figure 24.1 Results with possible application to man. Clinical effect as assessed by depth of coma.

**Table 24.3**

Comparison of drug clearances with different materials and methods

| | *In vivo* drug clearances (ml/min) | | |
|---|---|---|---|
| | XAD-2 | Charcoal | Dialysis |
| Amylobarbitone | 86 | 66 | 26 |
| Quinalbarbitone | 81 | 34 | 9 |
| Phenobarbitone | 89 | 60 | 27 |
| Glutethimide | 83 | 72 | 16 |
| Methaqualone | 100 | 56 | — |
| | | All at flow rate 100 ml/min | |

## 24.7 Conclusions

The purpose of an experimental model is to provide results which are applicable to man (tables 24.1 and 24.2, figure 24.1). The extrapolation of experimental data to man is never without hazard. The best that any animal results can do is to enable the clinician to receive information sufficiently reliable and complete to embark on limited and closely supervised clinical trials on patients. Inevitably, certain differences, such as the vulnerability of canine platelets compared to human platelets, will be found and some converse defects may also come to light. However, the beagle dog does permit evaluation of a haemoadsorbent *per se*, as well as in comparison to more conventional methods such as forced diuresis, haemodialysis (table 24.3), or plain supportive or therapeutic medication. In time, it is possible that better models, and desirably non-animal, will be found, but in so doing it will also be necessary to ensure the generation of adequate background data upon which to base an informed opinion.

## 24.8 References

Medd, R. K., Widdop, B., Braithwaite, R. A., Rees, A. J. and Goulding, R. (1974b). Comparison of haemoperfusion and haemodialysis in the therapy of barbiturate intoxication in dogs. *Arch. Toxicol.*, **31**, 163 - 174.

Medd, R. K., Widdop, B., Braithwaite, R. A. and Rees, A. J. (1975). Treatment of experimental drug intoxication in dogs by haemodialysis and haemoperfusion. *Proc. Eur. Soc. Artif.* Organs, **1**, 87 - 90.

Silberschmid, M., Bagger, H., Dupont, B. A. and Lyager, S. (1976). Stable Cardiac output during Nembutal anaesthesia in dogs. *Eur. Surg. Res.*, **8**, Suppl. 1, 113.

# 25

# CHRONIC URAEMIA–HAEMODIALYSIS OR HAEMOPERFUSION?

H. W. LEBER, M. NEUHÄUSER and G. GOUBEAUD

**Summary**

The plasma concentrations of several low molecular solutes accumulating in renal failure have been measured before and after a 2 h haemoperfusion through coated activated carbon or Amberlite XAD-4. Despite a marked decrease of the plasma concentration of creatinine, haemoperfusion did not influence the plasma concentrations of guanidines, aromatic amines and urea. Phenolic acids decreased only slightly during haemoperfusion.

With regard to the removal of middle molecular solutes from uraemic plasma, a decrease of the integrated absorption at 206 nm and a diminished toxicity towards the porphobilinogen synthesis of normal blood cells have been found after haemoperfusion and haemodialysis. From our results it is concluded that haemoperfusion has no advantages over haemodialysis.

## 25.1 Introduction

In 1964 it was demonstrated by Yatzidis that several solutes accumulating in renal failure, including creatinine, uric acid, guanidines, phenolic acids and indican could be eliminated by charcoal adsorption both *in vitro* and *in vivo*. The treatment of uraemic patients by haemoperfusion (Yatzidis *et al.*, 1965), however, did not attain general importance due to the insufficient removal of urea, electrolytes and water and the unsatisfactory biocompatibility of the charcoal preparations.

In recent years the biocompatibility of charcoal has been improved particularly by coating with polymers or albumin (Rosenbaum *et al.*, 1968; Chang, 1969; Andrade *et al.*, 1971). Today, haemoperfusion is well acknowledged in the treatment of poisoned or drug-overdosed patients (Chang *et al.*, 1973; Vale *et al.*, 1975; Winchester *et al.*, 1974; Rosenbaum, 1975; Leber *et al.*, 1976). Regarding the application of haemoperfusion to uraemic patients, extensive studies were undertaken by Chang *et al.* (1971, 1972, 1973, 1974, 1975).

Measuring the removal of creatinine, uric acid, guanidines and middle molecules (mol. wt. 500 - 1300 daltons) Chang claimed that a haemoperfusion through artificial cells, containing activated charcoal, for 2 h is as effective as a haemodialysis with a 1.0 $m^2$ coil dialyser for 6 h. With regard to middle molecular solutes the significance of Chang's results are limited for the following three reasons.

(1) Clearance data were evaluated with model substances which are not known to occur in uraemic patients.

(2) The separation method used for middle molecules was very rough resulting in poor selectivity.
(3) It was not investigated whether the toxicity of middle molecules from uraemic serum had decreased after haemoperfusion. It remains open, therefore, whether it was toxic material or essential blood constituents (at this molecular weight) which were removed.

These limitations and the fact that different adsorbents are now commercially available prompted us to reinvestigate whether haemoperfusion could be an alternative or a supplement to haemodialysis therapy. To answer this question we investigated especially the elimination of middle molecules from uraemic serum by haemoperfusion and haemodialysis. The removal of middle molecular solutes was determined firstly by measuring the integrated absorption at 206 nm of gelfiltrates from plasma ultrafiltrates which is the usual way of quantifying middle molecules (Goubeaud, 1976). Secondly we tried to find out whether it was toxic material or essential blood constituents that were adsorbed.

As a toxicity test the inhibition of the porphobilinogen synthesis in blood cells from healthy subjects by middle molecular fractions from uraemic serum was measured. This procedure was chosen because our earlier results had established that porphobilinogen synthesis is inhibited in blood cells from uraemic patients due to an inhibition of the corresponding enzyme, δ-aminolevulinic acid dehydrase (Leber *et al.*, 1974, 1975) and that this effect could be imitated in blood cells from subjects with normal kidney function by adding middle molecular fractions from uraemic serum to the assay system *in vitro* (Goubeaud *et al.*, 1976).

## 25.2 Methods

Six chronic uraemic patients from the intermittent haemodialysis programme of our hospital were haemoperfused once a week for 2 h followed by 3 h dialysis with the PAN-membrane (RP 6, Rhône-Poulenc) instead of the regular dialysis routine with the dialyser alone. For haemoperfusion purposes either a coated activated carbon cartridge (Haemocol, Smith and Nephew) or an Amberlite XAD-4 resin (Extracorporeal) was used. The blood flow was maintained at 250 ml/min both during haemoperfusion and haemodialysis. The methods for determining the low and middle weight molecules were published elsewhere (Goubeaud, 1976, Leber *et al.*, 1971; Leber, 1972). Details of the measurement of the enzyme δ-aminolevulinic acid dehydrase have also been described previously (Leber *et al.*, 1974). To measure the influence of haemoperfusion and haemodialysis on the toxicity of middle molecules 100 µl of gelfiltrates representing the middle molecular range (fraction 3 - 8, see figure 25.2) from uraemic serum (5 ml) were added to the enzyme assay mixture (Leber *et al.*, 1974, Goubeaud *et al.*, 1976) before and after haemodialysis and haemoperfusion. *Statistical evaluations* were carried out using the X-Test according to van der Waerden and Nievergelt (1956).

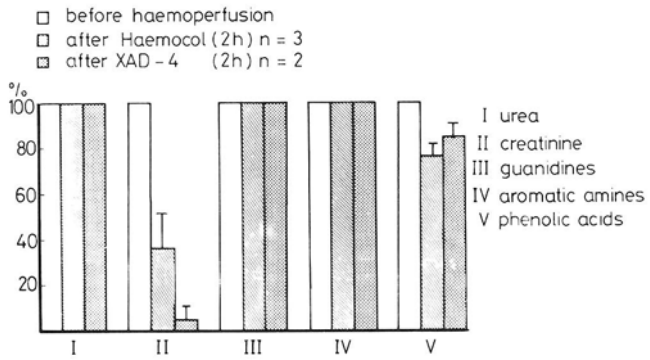


Figure 25.1 Plasma concentrations of several low molecular weight solutes occurring in renal failure before and after haemoperfusion

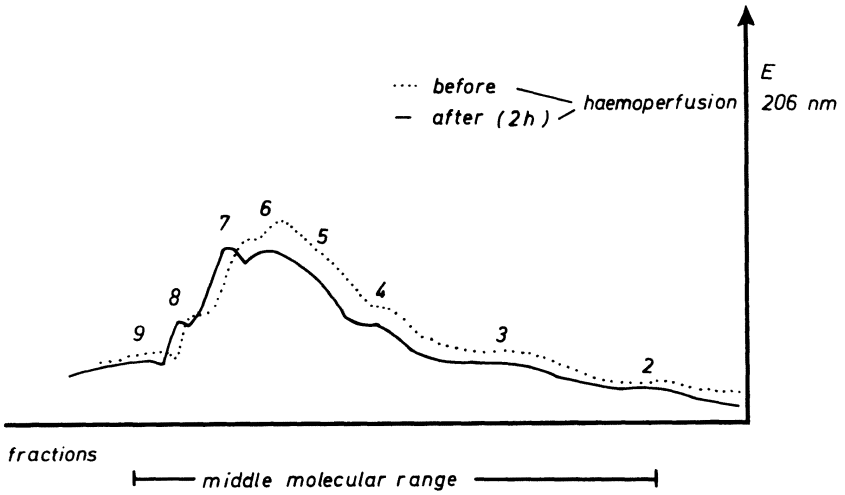


Figure 25.2 Absorption at 206 nm of gelfiltrates from uraemic plasma before and after haemoperfusion

## 25.3 Results and Discussion

Figure 25.1 depicts the plasma concentrations of some low molecular weight solutes from uraemic serum before and after haemoperfusion. It can be clearly seen that a marked adsorption of creatinine takes place both when Amberlite XAD-4 or coated charcoal is used. With regard to creatinine adsorption Amberlite proved to be more efficient than charcoal. From all the other solutes shown only phenolic acids are removed significantly by haemoperfusion, the two systems differing slightly but significantly. Interestingly the plasma concentrations of guanidines and aromatic amines were not influenced by haemoperfusion. This is in contrast to our earlier *in vitro* findings (unpublished). Considering our

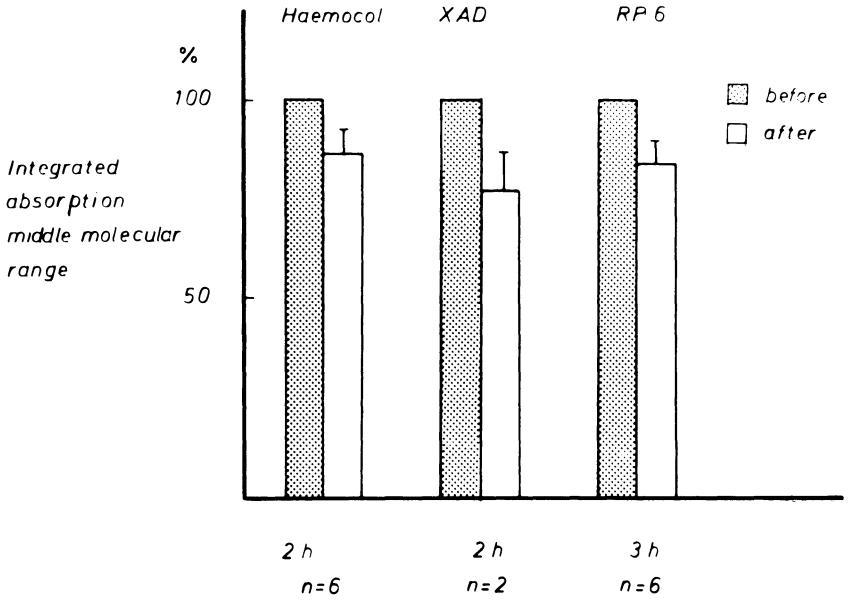


Figure 25.3 Integrated absorption (206 nm) of gelfiltrates (middle weight molecular range) from uraemic plasma before and after haemoperfusion or haemodialysis

previously published results (Leber *et al.*, 1971; Leber, 1972), which demonstrate that all the solutes listed in figure 25.1 are decreased after haemodialysis, it may be concluded that, in contrast to Chang and his coworkers' results (1971, 1972, 1973, 1974, 1975) with respect to solutes of low molecular weight accumulating in renal failure, haemodialysis is more effective than the systems of haemoperfusion investigated.

Figure 25.2 demonstrates the absorption at 206 nm of gelfiltrates from ultrafiltrates of uraemic serum before and after haemoperfusion.

From figure 25.3 it can be seen that the integrated absorption at 206 nm of the middle molecular range after haemoperfusion (2 h) is diminished to the same extent as after haemodialysis (3 h).

From figure 25.4 it can be concluded that the inhibiting effect of middle molecules from uraemic serum on the porphobilinogen synthesis in normal blood cells is significantly decreased after haemoperfusion and haemodialysis. Similar results were obtained with regard to the influence of middle molecules on globin synthesis (unpublished). The most pronounced decrease of the enzyme inhibition was found after Haemocol perfusion (figure 25.4) and the PAN-membrane dialysis, whereas XAD-4 was less efficient. Hence, it is concluded that toxic materials are removed by haemoperfusion and haemodialysis. Which essential blood constituents are eliminated as well has yet to be elucidated.

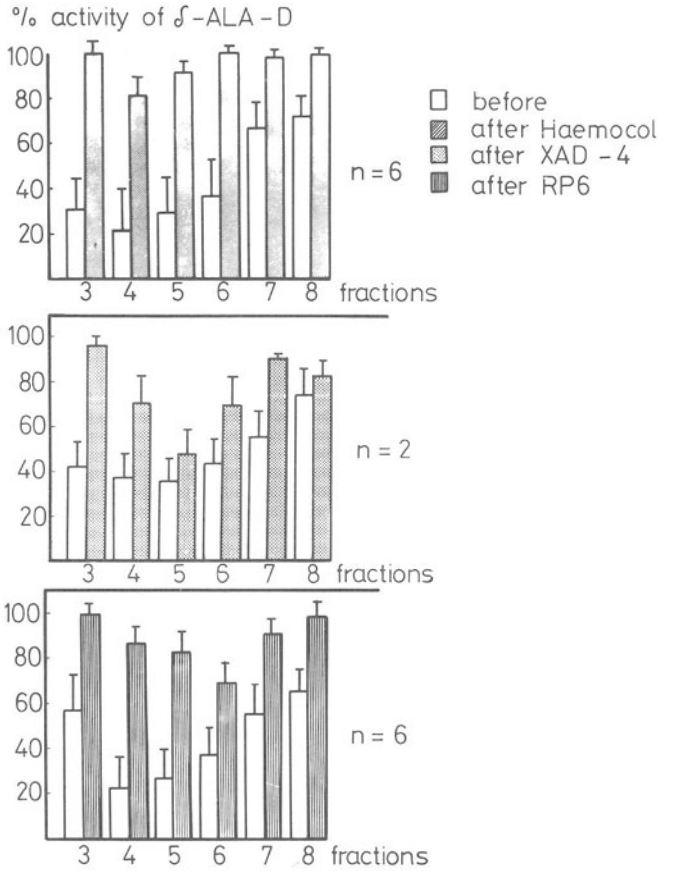


Figure 25.4 Influence on the activity of the enzyme δ-aminolevulinic acid dehydrase of several middle weight molecular fractions from uraemic serum isolated before and after haemoperfusion or haemodialysis

In our opinion uraemic patients should undergo haemoperfusion regularly only if the following two conditions are fulfilled.

(1) Availability of sorbent systems which remove 'uraemic toxins' better than haemodialysis.
(2) Demonstration that sorbent systems are better than haemodialysis in preventing uraemic complications such as anaemia, neuropathy and osteopathy.

## 25.4 Conclusions

According to our results haemoperfusion through charcoal or Amberlite XAD-4 has no significant advantage compared to the PAN-membrane dialysis with regard to the removal of known 'uraemic toxins'. Even though the integrated absorption and the toxicity of middle molecular solutes from uraemic serum are decreased in

a shorter time than with the dialyser, there remains the main disadvantage of the sorbent systems investigated so far: the insufficient removal of some low molecular weight substances which accumulate in renal failure. Therefore in our present state of knowledge haemoperfusion through charcoal or Amberlite XAD-4 *alone* cannot be regarded as an alternative to haemodialysis.

Additionally we have no indication from our results that haemoperfusion through charcoal or Amberlite XAD-4 could be beneficial for uraemic patients as a supplement to haemodialysis. This contradiction to the results of Chang's group might be due to the differences in the sorbent systems investigated and in the parameters measured.

We consider our experiments to be preliminary since, due to the limited number of haemoperfused cases, no comparison of clinical data was possible between haemoperfused and haemodialysed patients.

To answer the question concerning the benefit of haemoperfusion in uraemia definitively, further studies are necessary, including long-term clinical investigations, because too little is known about the identity and the influence of 'uraemic toxins'. Therefore, the use of haemoperfusion for the present should be limited only to applications which will assist elucidation of the problem.

## 25.5 References

Andrade, J. D., Kunitomo, K., Van Wangenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 222.

Chang, T. M. S. (1969). *Can. J. Physiol. Pharmacol.*, **47**, 1043.

Chang, T. M. S., Gonda, A., Dirks, J. H. and Malave, N. (1971). *Trans.Amer. Soc. Artif. Int. Organs*, **17**, 246.

Chang, T. M. S., Gonda, A., Dirks, J. H., Coffey, J. F. and Lee-Burns, T. (1972). *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 465.

Chang, T. M. S., Coffey, J. F., Lister, C., Tarcy, E. and Stark, A. (1973). *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 87.

Chang, T. M. S. and Migchelsen, M. (1973). *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 314.

Chang, T. M. S. and Migchelsen, M., Coffey, J. F. and Stark, A. (1974). *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 364.

Chang, T. M. S., Chirito, E., Barre, B., Cole, C. and Hewish, M. (1975). *Trans. Amer. Soc. Artif. Int. Organs*, **21**, 502.

Goubeaud, G. (1976). Habilitationsschrift, Giessen.

Goubeaud, G., Leber, H. W., Schott, H. H. and Schütterle, G. (1976). *Proc. Eur. Dial. Transpl. Assoc.*, **13**, 371.

Leber, H. W., Ampt, B. and Schütterle, G. (1971). In *Aktuelle Probleme der Dialyseverfahren und der Niereninsuffizienz*, Bindernagel, Friedberg, p. 436.

Leber, H. W. (1972). Mitteilungen der Arbeitsgem. für Klin. *Nephrologie*, **1**, 44.

Leber, H. W., Sinning, P. and Schütterle, G. (1974). *Proc. Eur. Dial. Transpl. Assoc.*, **11**, 121.

Leber, H. W., Baumgarten, C., Goubeaud, G., Matthias, R. and Schütterle, G. (1975). *Proc. Eur. Dial. Transpl. Assoc.*, **12**, 246.

Leber, H. W., Geibler, R. H., Faber, M. and Post, D. (1976). *Klin. Wschr.*, **54**, 517.

Rosenbaum, J. L. (1975). *Ind. Eng. Chem. Prod. Res. Dev.*, **14**, 99.

Rosenbaum, J L., Rouquillo, E. and Argyras, S. N. (1968). *J. Albert Einstein Med. Cent.*, **16**, 67.

Yatzidis, H. (1964). *Proc. Eur. Dial. Transpl. Assoc.*, **1**, 83.

Yatzidis, H., Oreopoulos, D., Triantaphyllidis, G., Voudidari, S., Tsaparas, N., Gavras, C. and Stavroulaki, A. (1965). *Lancet* **II**, 216.

Vale, J. E., Rees, A. J., Widdop, B. and Goulding, R. (1975). *Brit. Med. J.*, **1**, 5.

van der Waerden, B. L. and Nievergelt, E. (1956). *Tafeln sum Vergleich zweier Stichproben mittels X-Test und Zeichentest*, Springer, Berlin.

Winchester, J. F., Tilstone, W. J., Edwards, R. O., Gilchrist, T. and Kennedy, A. C. (1974). *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 358.

# SESSION C DISCUSSION

*Chang* started the discussion by presenting the results of *in vivo* animal studies with coated and uncoated charcoal. First he showed that *both* cause significant platelet depletion. However, a collodion coating superimposed with a layer of albumin had been found to reduce this depletion. Secondly, discussing results of particle release studies (using a Coulter counter, and a Travenol rotary pump at 300 ml/min flow rate, 300 mm Hg pulse pressure) he submitted that these demonstrated an increase in particle release from uncoated charcoal but no such increase from coated charcoal. Partial obstruction of the arterial line, simulating that during haemodialysis and haemoperfusion, produced a further increase in particle release from the uncoated but not from the coated charcoal. Many years of experience with coated charcoal had given *no* histopathological evidence of charcoal emboli, and he had never found that the coating membrane came off the charcoal during autoclaving. He therefore thought it possible that in Hill's comparison of coated and uncoated charcoal particle release, the coating had not been properly applied. He asked Hill for comment on these remarks together with more detail on his 'bumping' tests, and whether when immobilising charcoal on an adhesive surface it was possible for the adhesive to be drawn by surface tension over the particles in a thin film. He added that since scanning electron microscopy could not be used to show such a coating, had other tests been devised for his fixed-bed system?

*Hill* felt that while comparisons were difficult because of differences in weight of charcoal charge, particle size, distribution, etc., they had to be made. Clinical data on platelet losses were difficult to obtain and were variable, yet initially all studies, except those of Chang, had shown a 30 - 40% platelet drop during haemoperfusion.

Regarding the suggestion of accidental coating of the sorbent by adhesive, *Hill* commented that the adhesive used was chlorosulphonated polyethylene and that in the early development stages too much had been used with resultant loss of solute uptake. This indicated that the charcoal had indeed been coated! However, the system used at present showed no such large uptake loss so it was likely that the charcoal was simply stuck to the adhesive rather than being coated by it. Providing more detail on the bumping tests used, he said that in the pulsing system the pump inlet was constricted to give pressures of approximately +200 mm Hg to −200 mm Hg, 3 times per second when operating at full speed. One reason for discrepancy in results might be the tightness of packing in the column since, if the charcoal was held tightly, pulsation had little effect whereas if it was loosely held problems could occur.

On a new topic *Friedman* asked Hill if he thought that in quantifying the effect

of coating charcoal it might be desirable to classify the penetration into charcoal of all compounds on the basis of their lipid affinity. Charcoal had a strong surface affinity for lipids which therefore might enhance its attraction for hydrophobic rather than hydrophilic substances. Thus, lipid coating of the membrane or the charcoal might subsequently influence the adsorption of other compounds. On this topic *McClure* wished to know if the results of Hill's tests could be affected by the relative hydrophilicities of solute and membrane rather than simply molecular weight. *Hill* replied that there were reported instances of one substance interfering with the uptake of another but that they had been surprised by the *absence* of this effect in clinical use. Ethanol, reputed not to be adsorbed by charcoal, in fact *is adsorbed* but since the dose tended to be in g/kg rather than mg/kg the column was saturated in a very short time. Most self-poisonings were ethanol-associated and yet an ethanol-saturated column still adsorbed phenobarbital and salicylate.

*Park* wished to know what coating *method* Hill had used in his comparisons with uncoated charcoal: that of Chang, Smith and Nephew, or Strathclyde? Was the lack of observed particle release from uncoated carbon in any way due to the type of outlet filtering system? *Hill* replied that of the data he presented only those relating to the dog platelet was obtained from material coated by his group. The method used was the collodion technique described by Chang, which was carried out according to directions available to them. At that level of coating, which interfered with solute removal, particle release still occurred on pulsing. Polyurethane filters of approximately 40 $\mu$m pore size were used at the ends of the column and did not affect the results.

The subject of longer-term platelet loss was raised by *McClure* who asked Hill whether his studies had been followed up on a long-term basis (as suggested by de Jong) to establish the presence and magnitude of any secondary 'dip' in thrombocyte numbers. *Hill* said that platelet levels had been studied over some time and that the results were very similar to those given by de Jong. In the exchange of views that followed it was stated that albumin coating reduced platelet loss (*Chang*), but that collodion coating alone did not enhance this effect (*Hill*). *Fennimore* claimed that Haemocol used without albumin and only with a polymer (hydrogel) coating did appear to enhance blood compatibility. *Denti* commented on the difference in platelet behaviour as seen on scanning electron micrographs of coated and uncoated carbon granules exposed to blood for 30 min. He pointed to the need for extreme caution when drawing conclusions on blood compatibility from simple experiments. Only extensive clinical data from purpose designed experiments clarified the relative merits of different coatings and uncoated carbon.

*Martin* opened the discussion on the Wearable Artificial Kidney (WAK) and asked Walker if this device was aesthetically acceptable to patients, and what were its particular advantages. *Walker*, while stating that he was neither initiator nor advocate for the WAK, felt that its advantages were at this stage more psychological than functional. For example, the device was relatively small and thus enabled the patient to feel 'in charge' of the machine rather than the converse. *Denti* had been involved with this project and he believed that a truly wearable artificial kidney

system would evolve when the urea module problems were overcome and the overall size of the device further reduced.

*Rosenbaum* commented on Fennimore's particle release value (25 particles/ml) and its comparison to the British Standard for saline infusion solutions (1000 particles/ml). If haemoperfusion took place for 2 h, 3 times a week (a total of 6 h) approximately 120 l of blood would have passed through the column and therefore about 30 000 particles released into the blood stream. While these values were probably quite acceptable for *acute* intoxication, their comparison with the British Standard values was irrelevant for *repeated* perfusions. *Fennimore* accepted this as an exceedingly valid point and stated that the British Standard value was used purely for reference. In connection with particle release, it was essential to consider the part played by other components of the extracorporeal circuit. *Chang* referred to Andrade's observations of particle generation with plastic tubing. Chang's group had carried out particle release studies on dogs which had been perfused 3 or 4 times for 2 h each time. No particles were found on histological examination of tissue samples, and there was no microscopic evidence of particle release when 2 l of liquid were perfused through 300 g of charcoal for 2 h. *Denti* remarked that histological examination was not perhaps the best method for particle detection, and that at present all available columns appeared to have a lower particle generation rate than the tubing of a roller pump. However, caution must be exercised both in chronic systems and in test system design. For example, the use of a Millipore filter to quantify particle release could be misleading due to weight changes of the filter itself through hydration. The only practical methods were Coulter counting or the use of Nuclepore filters. These gave good results for a well constructed column releasing 3 to 10 $\mu$g of particulate matter per hour. The possibility of dissolution or migration of coating into the blood was raised by *Martin*, particularly in relation to prolonged perfusions. *Fennimore* responding, said that this had been investigated and that no evidence for such an effect had been found. *Hill* contributed comments on the toxicity of fine particles: charcoal fragments of maximum size 40 $\mu$m and in quantities of mg/kg had been injected into animals. The lethal dose for a rat was 100 mg/kg and for a dog 40 mg/kg. However, some animals injected with large amounts (20 mg/kg) of such charcoal died naturally 256 days later and showed no adverse reaction to the presence of charcoal in the tissues. The significance, however, of such results was difficult to determine and relate to the haemoperfusion situation. As yet, there was neither a technique to measure particle generation as *blood was passed through a column* nor any notion of how this could be done. *Friedman* recalled that treatment of subacute bacterial endocarditis in man was once the intravenous injection of 10 to 50 g of charcoal, and that Dr Kolff had observed that although this didn't do the patient much good, nor did it appear to do any harm. However, chronic rather than acute exposure was of greater concern since the question of acute toxicity had to an extent been settled by clinical experience. *Denti* emphasised that the lethal dose of carbon particles in rabbits was dependent on the *rate* of infusion and that fast infusion of 120 mg (±10) could be lethal. *Rosenbaum* quoted

work with dogs on recirculated peritoneal dialysis which had been injected with 50 g of carbon slurry in the peritoneum, and sacrificed one month later. Histopathological examination showed that although the carbon was completely embedded in the peritoneal tissue there was no inflammatory reaction. *Hughes* felt that the elution of plasticisers and stabilisers used in the manufacture of haemoperfusion devices must also be considered. It was essential that as well as carbon particles, toxic chemicals were taken into account when assessing the overall biocompatibility of a device.

The topic of albumin coating was raised by *Nosé* who asked Chang if he had used aldehyde during the coating procedure. The use of albumin alone did not seem to improve blood compatibility but this was remedied by exposing the coating to either heat or aldehyde. *Chang's* devices were steam autoclaved for sterilisation and cooled prior to the surface adsorption of human albumin and he thought that Nosé had most likely used a surface which would not adsorb albumin unless it was attached by cross-linkage.

*Maini* asked Chang if the ultrafilter in the combined albumin coated activated carbon device was re-usable and posed the general question: might it be advantageous to use a high porosity dialyser of lower priming volume in conjunction with closed circuit dialysate regeneration? In such a system *uncoated, unsterile* carbon and other nonbiocompatible materials could be used for potassium and phosphate removal without increasing complexity or cost. *Chang* in reply said that re-use of the ultrafilter had not been attempted. Regarding the second point, the system proposed would have to be tested and compared to others; whether it would be better and less expensive was impossible to tell at this stage.

*Martin* asked how the length of Chang's treatment time (2 h) in either drug intoxication or chronic renal failure had been decided. Had he had any haemoadsorption experience of tricyclic antidepressant poisoning or endotoxin shock in acute renal failure? *Chang*'s use of 2 h had been defined through clinical trials, although this time was sometimes extended to 3 h depending on patient recovery. Haemoperfusion had not been tried with tricyclic antidepressants because of the numerous intracellular problems.

*Volans* wished to question Martin on the use of haemoperfusion to treat overdose of tricyclic antidepressants. He had some reservations about this treatment since the amount of drug removed would be so small. However, it was possible that this small amount could be having a pharmacological effect on the patient, thereby threatening his life. Controlled investigation with haemoperfusion must therefore be very carefully considered, but until that time manufacturers should not recommend the use of perfusion for tricyclic poisoning. One or two investigators had already done this without, he thought, substantiating their claims. *Martin* had treated two such patients with haemoperfusion, one of which was now being treated for the third time without ill effect.

*Black*, returning to the question of endotoxic shock raised by Martin, mentioned that a group in Buffalo, USA had tried haemoperfusion treatment (using coated carbon) of dogs with no success, probably because of the size of molecule

involved. *Maini* stated that Wilkinson in London had investigated endotoxin removal by coated carbon unsuccessfully. However, Less and Levvy had shown that *finely ground* active carbon was successful in removing endotoxin where granular carbon was not. *Hill* did not think that the size of the carbon particles was as significant as the coating. He emphasised that when discussing substance-removal by carbon it was essential to state whether or not the carbon was coated. His group used uncoated carbon which removed amino acids far more efficiently than coated carbon.

*Winchester* questioned Volans on the topic of platelet loss in salicylate overdose. Had he noticed a greater platelet depletion in patients with salicylate poisoning? This effect had been observed both in dog experiments and in one human case. *Volans* had experienced only one such case but he would have expected to encounter such an effect.

*Martin* posed a general question to all those tempted to use haemoperfusion as a treatment for poisoning. Do you think that haemoperfusion should only be used in specialised centres where blood levels can be frequently and accurately measured? *Volans* thought haemoperfusion to be a technique which should be available regionally, perhaps on the same basis as haemodialysis. Even if (as *Martin* had pointed out) patients who might benefit from perfusion were on ventilators, they could still be transported to a haemoperfusion centre. *Winchester* commented that although haemodialysis was quite a complex procedure, haemoperfusion was not as simple as was being suggested and that it should indeed be a method of treatment reserved for specific centres. *Rosenbaum* disagreed with this and thought that haemoperfusion was a technique which could be learned by any interested toxicologist or nephrologist. He saw no reason why it should be limited to certain centres since the whole advantage of the treatment was that it could be used where the patients were, for example, in the small hospitals. Most of the patients *were* too ill to be transported and for this reason haemoperfusion should be available on a widespread basis. *Chang* asked Rosenbaum if he agreed that perhaps the clinical trial testing stage of the device should be confined to large centres and transferred to the small hospitals only when it had been proved safe and effective. *Rosenbaum* thought that haemoperfusion had already been demonstrated to be clinically useful and that as long as the device was used in a knowledgeable fashion it could be made widely available.

At this point *Kennedy* asked Martin for more details as to why he was attracted to haemoperfusion treatment in acute renal failure. Was it not a major disadvantage of haemoperfusion that potassium, water and urea removal were limited? *Martin* replied that he was not considering the use of haemoperfusion alone, but that he was distressed at the mortality encountered even with prophylactic dialysis. Although he might be fantasising, it was possible, particularly with those patients suffering from endotoxaemic shock, that some unknown substance could be removed. Nevertheless this consideration had precipitated him into trying haemoperfusion after haemodialysis (perhaps the following day) in those patients suffering from septicaemic shock who otherwise would almost certainly die. *Kennedy*

wished to point out the enormous difficulties there would be in getting a controlled trial to prove Martin's point. *Martin* thought that these difficulties already existed in acute renal failure and that it was debatable whether or not dialysis was of any major advantage.

*Fairweather* asked if there was any problem with the removal of the deacetylating alkaline material used during the coating procedure of the Gambro haemoperfusion column, and *Park* wanted to know whether any of this coating was removed during the deacetylation process which would perhaps leave a less than 2% final coating of 'cellophane'. *Jonsson* replied that there was no problem of toxicity since acetic acid was the solvent used and the acetate ion was harmless. Regarding Park's question, a small amount of cellulose acetate was removed during hydrolysis but this was only a few percent of the total coating.

*Gibbons* was concerned that Courtney had chosen the amine copolymer for a charcoal coating since it had been established (at least in *in vivo* subcutaneous implant experiments and in *in vitro* cell culture) that such a concentration of the amine group in a copolymer would cause excessive cell adhesion. It had also been found in the *in vivo* case that the fibrous capsule which formed round the subcutaneous implant was markedly different from the normal and while he recognised that the application was for *acute* haemoperfusion he thought that cell and particularly leucocyte changes ought to be studied. *Courtney* agreed that while acute versus chronic application must be kept in mind, he did not believe that a positively charged polymer indicated a bad coating. It was possible, for example, to attach heparin to the polymer which would then attract a different type of protein layer.

*Martin* raised a new topic, that of paraquat poisoning, by asking at what blood levels should treatment be attempted. *Widdop* said that this was a difficult question to answer since the level of paraquat in the blood must be lowered quickly in order to be effective. He thought that 2 μg paraquat/ml blood was the kind of value below which treatment might prove effective.

Session D

# ADSORBENTS IN THERAPEUTIC MEDICINE AND ASSOCIATED TOPICS (CONTINUED)

*Chairman*
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# 26

# THROMBOGENESIS CAUSED BY BLOOD -FOREIGN SURFACE INTERACTION

J. FEIJEN

**Summary**

Although the use of artificial organs in medicine has become well accepted, the blood compatibility of these devices needs further improvement. A survey of the blood damage caused by extracorporeal circuits is presented, followed by a discussion on mechanisms of blood-foreign material interactions. Attention is given to recent developments in the area of protein adsorption, platelet adhesion and activation of the intrinsic coagulation with special attention to the influence of flow conditions. Finally, some conclusions and future perspectives are outlined.

## 26.1 Introduction

The use of artificial organs, such as oxygenators, haemodialysers and haemoperfusion systems in medicine has become well accepted. According to their specific functions, these devices have to meet a range of different requirements such as, adequate gas transfer or removal of metabolites. However, in all cases blood will be exposed to foreign materials under different flow conditions. This will cause changes in the recirculating blood. The modified blood interacts with the living body and a dynamic system is established. Within this system, we have to investigate the nature and cause of the events which will ultimately lead to the damage of blood. Up to now, a great deal of effort has been put into the testing of materials and devices by the use of *in vitro, ex vivo* and *in vivo* tests. The results obtained by using different test systems are frequently not comparable because the experimental conditions differ and the parameters which are used to describe the interaction of blood with systems outside the body show a wide variation.

Only a few tests have become accepted as standardised test systems, e.g. the vena cava implant test for thrombogenicity (Gott *et al.*, 1969; Gott and Furuse, 1971 and Daniels *et al.*, 1975). The results of this test only give information about the behaviour of different materials placed in one specific position. On the other hand, the *in vivo* testing of complete extracorporeal circuits gives insight only in respect of the total effect of a combination of different materials under different flow conditions. In order to achieve improvements in the future, it will be necessary to study the mechanisms of interaction of blood with individual foreign surfaces under varying conditions on a molecular and cellular level. The results

can then be used for the construction of improved devices, which have now been developed mainly on an empirical basis.

Furthermore, we have to extend our knowledge about the detection of relevant changes in the blood caused by extracorporeal circuits and the impact of these changes for specific applications of these systems.

## 26.2 Blood Damage Caused by Extracorporeal Circuits

Several authors (de Leval *et al.*, 1975; Dutton *et al.*, 1974; Bartlett *et al.*, 1974; Awad *et al.*, 1974; de Jong *et al.*, 1975; Bloom *et al.*, 1974 and Lindsay *et al.*, 1973) have reported on the modifications of blood by extracorporeal circuits. Table 26.1 shows a survey of the phenomena which are frequently observed, when foreign materials are contacted with blood. Most of the alterations also play a role in thrombogenesis caused by extracorporeal circuits. Again we have to realise that two effects contribute to the observed phenomena, namely contact of

**Table 26.1**

Survey of the induced changes of blood components as a result of blood foreign surface interaction

| *Blood component* | *Induced changes* | *References* |
|---|---|---|
| (1) *Proteins* Albumin, γ-Globulin, Fibrinogen etc. | Adsorption, conformational changes, increased enzymatic degradation | Brash and Lyman (1971) Baier *et al.* (1971) Olsen *et al.* (1973) |
| Clotting factors | Activation of the intrinsic coagulation causes the activation of clotting factors resulting in the ultimate conversion of fibrinogen into fibrin. | Vroman *et al.* (1971) Feijen *et al.* (1976) Stormorken (1971) |
| (2) *Lipids* | Adsorption, conformational changes | Brash and Lyman (1971) |
| (3) *Formed elements* Platelets | Adhesion, release aggregation, platelet plug formation, deterioration of function, removal from the circulation, interference with intrinsic coagulation | Salzman (1971) |
| Erythrocytes | Adhesion, release, haemolysis, removal from circulation, deterioration of function, interference with coagulation mechanism | Bernstein (1971) Stormorken (1971) |
| Leucocytes | Adhesion, deterioration of function, removal from circulation | Kusserow *et al.* (1971) |

blood with the foreign surfaces and circulation of blood under non-natural flow conditions.

The circulation of blood under non-natural flow conditions might lead to alteration of elements in the flowing blood. These altered elements may show a different interaction with foreign surfaces as compared with the nonmodified elements. When interaction of any blood element with the foreign surface has taken place and adsorption or adhesion has occurred, the flow conditions near the surface are important for subsequent events such as desorption, denaturation, release, damage, etc. Extracorporeal circuits generally consist of a number of components, e.g. pump, heat exchanger, cardiotomy reservoir, sucker, blood filter. To improve the performance of the whole circuit with respect to blood damage, we have to know the contribution of each component. The effects of a membrane and a bubble oxygenator on dog blood were investigated by de Leval *et al.*, (1975). In both cases the use of cardiotomy suction has a marked deleterious effect on platelet number and function. Therefore, we have to realise that, for instance, improvement of membrane materials under certain circumstances might not show any effect on the performance of the whole system. At this time, no extracorporeal circuit is available which can function without causing any blood damage. However, when heparin is used as an anticoagulant, some haemodialysers and oxygenators function reasonably well for limited periods of time. One of the questions which remains to be answered is the tolerance of each type of blood damage in a specific application. For instance, in the case of short-term applications of oxygenators (cardiac surgery), the disappearance of platelets from the circulation and the deterioration of the function of the remaining ones can be tolerated (the life time of platelets is relatively short). For long-term applications (respiratory insufficiency), the risks of internal bleeding become high. The damage of erythrocytes causes a more long-term effect (their life time is much longer than that of platelets or leucocytes). The disappearance and loss of function of leucocytes might increase the risks of infection (Kusserow *et al.*, 1971). Determination of the effects of the different types of blood damage (short and long term) on the condition of the patient requires a more detailed and thorough study.

## 26.3 Mechanisms of Blood-Foreign Material Interactions

When a foreign material is contacted with blood a sequence of events takes place, which is represented in Figure 26.1. Protein adsorption occurs immediately, frequently followed by platelet adhesion and activation of the intrinsic coagulation. The last two phenomena occur more or less independently (Feijen *et al.*, 1976). It must be assumed that in the later stages of blood clotting on a foreign surface, there will be a mutual interaction between the coagulation process and platelet aggregation. When thrombin is formed, platelet release will take place, followed by the formation of platelet aggregates. On the other hand, when the platelet release reaction takes place, ADP will be released and platelets which are stimulated by ADP can initiate the intrinsic coagulation by activating factor XII.

Also, erythrocytes can adhere to the adsorbed protein layer. Under certain con-

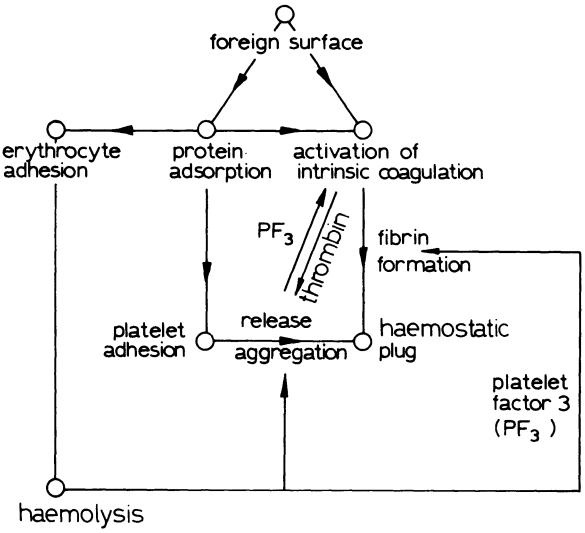


Figure 26.1 Schematic representation of events taking place when a foreign material is contacted with blood

ditions haemolysis can take place and erythrocyte ghosts induce the platelet release reaction, probably triggered by an attempted phagocytosis of the ghosts by the platelets (Stormorken, 1971). Coagulation is then promoted by a platelet factor III activity. Furthermore, leucocytes can adhere and the way in which they interfere with the other processes is not known. Platelet aggregation and fibrin deposition lead to the formation of a thrombus.

Different surface parameters (wettability, surface tension, critical surface tension, interfacial tension, surface charge ($\zeta$ potential), conductivity, domain structure) have been used for the correlation with the blood-foreign surface interaction phenomena previously mentioned. Up to now it has not been possible to use a single parameter for the prediction of blood-surface interaction behaviour (Feijen *et al.*, 1976).

We shall now discuss recent developments in the area of protein adsorption, platelet adhesion and activation of the intrinsic coagulation with special attention to the influence of flow conditions.

*Proteins*

When a foreign material is contacted with blood or plasma, protein adsorption will take place. The adsorbed protein layer modified the surface and this is important for subsequent events such as platelet adhesion. Relevant review papers are listed in table 26.1. The following features, outlined below, are important.

What is the relationship between the character of the surface, the rate of protein adsorption and the composition and thickness of the adsorbed protein layer?

At this moment we are not able to answer this question. Neither is it possible to correlate preferential protein adsorption or mono or multi-layer formation with macroscopical surface parameters. Especially, the rate of protein adsorption and possible preferential adsorption probably play an important role in initial blood-facing surface events such as the activation of the intrinsic coagulation. In general, proteins interact more strongly with hydrophobic surfaces than with hydrophilic ones (Hoffman, 1974). Brash *et al.* (1969) state that proteins are physically but irreversibly adsorbed onto hydrophobic surfaces as close-packed monolayers in the native state (end-on adsorption). Recently we have detected two plateau values in the adsorption isotherm of human serum albumin onto polystyrene, which correspond with side-on adsorption ($2.1 \times 10^{-6}$ kg/m$^2$) and end-on adsorption ($5.9 \times 10^{-6}$ kg/m$^2$) (van der Scheer *et al.*, 1976). Hydrophilic surfaces seem to adsorb less protein than hydrophobic surfaces (Brash and Lyman, 1971).

Conformational changes of protein caused by the adsorption on different surfaces may play an important role. It is well known that proteins spread and denature at blood-air interfaces (Lee *et al.*, 1971). Although Brash and Lyman (1969) state that proteins are adsorbed on hydrophobic surfaces in the native state, a very limited amount of work has been carried out to detect conformational changes. It can be imagined that, for instance, conformational changes of protein will occur when side-on adsorption is changed into end-on adsorption. The effect of small changes in the conformation of proteins caused by specific molecular surface interactions on phenomena such as platelet adhesion is presently being studied in our laboratory.

Reversibility of protein adsorption is an important factor which determines the ultimate dynamic equilibrium state of the protein coating on the surface. Exchange studies have been performed by Vroman *et al.*, (1971), Brash and Lyman (1971), Gonzalez and MacRitchie (1970), Brash *et al.*, (1974) and van der Scheer *et al.* (1976). Generally, proteins are exchanged more easily on hydrophilic surfaces than on hydrophobic ones. We found that in the case of albumin adsorption on polystyrene and silicone rubber, the exchange is greatly facilitated by the presence of albumin in the exchange solution (van der Scheer, 1976). The same behaviour has been found by Brash *et al.* (1974) when albumin is adsorbed on polyethylene, polyurethane and Cuprophane.

The influence of flow conditions on protein adsorption has been investigated by Brash and Lyman (1975) and Lyman *et al.* (1971). These authors state that the initial rate of adsorption of albumin to polyethylene is lowered under conditions of turbulent flow, while the plateau value of surface concentration corresponds to a multilayer (mono-layer under laminar flow and static conditions). A similar experiment with poly (dimethyl siloxane) shows no appreciable adsorption of albumin. Denaturation of protein in the solution occurs under both flow conditions but the rate in turbulent flow is about twice that in laminar flow. Brash *et al.* (1974) report that albumin desorption and exchange on polyethylene at low albumin solution concentrations (10 mg %) is negligible. However, under flow conditions (shear strain rate at the wall 300/s) the exchange is significant (20%);

an increase in shear rate does not lead to an increase in the amount of exchange, suggesting that the protein layers comprise two types of site, one removable by the shear forces and the other not.

*Platelets*

Adhesion on foreign surfaces has been related to protein adsorption by several authors (Falb *et al.*, 1967; Brash and Lyman, 1971; Vroman *et al.*, 1971; Salzman *et al.*, 1969; Baier *et al.*, 1971; Lee and Kim, 1974).

In general, fibrinogen and $\gamma$-globulin adsorbed on a surface promote platelet adhesion (Lyman *et al.*, 1968, 1970a and b, 1973; Packham *et al.*, 1969; Vroman, 1971; Mason *et al.*, 1973, Lee *et al.*, 1974), whereas albumin adsorption shows decreased or no platelet adhesion. The interaction of platelets and adsorbed fibrinogen or $\gamma$-globulin has been explained by Kim *et al.* (1974) and Lee *et al.* (1974) by the assumption of the formation of a complex between glycosyl-transferases located in the platelet membrane with incomplete heterosaccharides of fibrinogen and $\gamma$-globulin. Sugar nucleotides can then be transferred by these transferases from the platelet to the protein (not possible with albumin because saccharide chains are not present). This mechanism is similar to the mechanism proposed by Jamieson (1973) for the interaction of platelets with collagen.

A variety of compounds such as serotonin and ADP can be *released from the platelets* by stimuli such as thrombin. Both ADP and thrombin cause subsequent platelet aggregation. Then platelets irreversibly stick together and a haemostatic plug is formed. The reversibility of platelet adhesion and the mechanism of platelet release induced by foreign surfaces require further study.

The response of human platelets to shear stress has recently been investigated by Brown *et al.* (1975) and Goldsmith *et al.* (1975). Brown and coworkers have carried out studies with PRP (platelet rich plasma) in a Rice University viscometer at 20 - 22°C. Acid phosphatase release is used as an index of platelet destruction. A threshold shear stress of approximately 15 $N/m^2$ for extensive acid phosphatase release is observed. It seems that *turbulent flow* causes somewhat higher levels of damage at higher shear stresses, but the threshold shear stress equals that for *laminar flow.* The platelet release reaction is measured by the serotonin content of platelets. Shear stresses of 5 $N/m^2$ applied for 5 min show a significant reduction of the serotonin content, whereas 50% of the serotonin content of platelets is released within 30 s when a shear stress of 15 $N/m^2$ is applied. Platelet count studies in which the shear stress or the shear stress application times are varied indicate that partially reversible platelet aggregation occurs in response to shear stress. Platelet aggregation studies in response to ADP show that the aggregating effects of ADP are lost when platelets are exposed for 5 min to shear stresses of 10 $N/m^2$ or more. Also, the aggregation of platelets by ADP, still observed when a shear stress of 15 $N/m^2$ is applied for 45 s, disappears when the duration of this shear stress is prolonged to 60 s or more.

Goldsmith *et al.* (1975) have investigated the effects of high and variable shear stress on the platelet release reaction (thrombin induced serotonin) and aggregation.

Platelets suspended in Tyrodes-albumin solution are subjected to laminar oscillatory flow at 37°C in polyethylene tubes. Oscillatory flow for 20 min at time averaged velocity gradients at the tube wall of 2350/s (shear stress 1.5 $N/m^2$) causes an appreciably higher thrombin induced release of serotonin than in control suspensions. This difference is not apparent at wall velocity gradients below 2000/s and for periods of flow less than 5 min. No significant serotonin release as a result of oscillatory flow alone is observed. Sheared suspensions do not contain platelet aggregates and thrombin induced aggregation is similar to paired control suspensions. Klose *et al.* (1976) report that platelets in citrated plasma undergoing shear flow (shear strain rate 25 - 115/s) in a cone-in-plate viscometer form aggregates within 10 min. The pre-treatment of PRP seems to be important in this respect. The adhesion rate of platelets to different surfaces as a function of shear rate and haemotocrit has been studied by Feuerstein *et al.* (1975) using pig platelets and erythrocytes in citrated plasma or Tyrode's solution (0.35 weight percent albumin) in a rotating probe device. The experimental data can best be described by taking into account the diffusion as well as the adhesion reaction at the surface.

$$M = Co\left(-\frac{D}{k} + \frac{2}{\pi^{1/2}}\sqrt{Dt}\right)$$

$M$ is the surface platelet concentration, $Co$ is the bulk platelet concentration and $t$ is time.

The platelet diffusion coefficient $D$ ($\sim 10^{-7}$ $cm^2/s$) shows a 5 - 10 fold increase when the haematocrit is increased from 15 to 45%. A straightforward shear rate dependence of $D$ is not observed. The surface reaction constant $k$ (2.5 - 13.2 $\times 10^{-5}$ cm/s) shows an increase with haematocrit increase up to 35% followed by a levelling off at higher haematocrit values. No shear rate dependence of $k$ is observed. Experiments carried out with different surfaces (glass, polyurethane and polystyrene) at a shear rate of 19.1/s and at different haematocrit values indicate that adhesion levels after 5 or 10 min are independent of the surface type but dependent on haematocrit values. The authors suggest that equilibrium adsorption is reached on these surfaces and that the presence of more red cells leads to an increased interaction energy, which increases the total surface area available for platelet adhesion. On the other hand, Butruille *et al.* (1975) carrying out experiments with a rotating disc covered with a polyurethane strip (type A, no. 705, SRI) in heparinised canine blood, found that the diffusion constant of platelets is shear sensitive in certain shear rate areas and that at high shear rates, the shear rate interferes with the adhesion reaction. The adhesion patterns on the disc are isotropic when blood is sheared at low rates, and anisotropic when formed from blood sheared at higher rates. The release of an adhesion-potentiating substance from platelets already adhering to the surface affects platelets which approach the surface. Platelet clusters are formed, because the probability of platelets reacting with the surface is increased by their proximity to an already adhered platelet, which may be regarded as the source of an adhesion-potentiat-

ing substance. The diffusion controlled platelet adhesion combined to the clustering process suggests that platelets that do not adhere are transformed at least temporarily into a passive state with respect to the surface. Friedman *et al.* (1971) have investigated different materials in an *ex vivo* flow chamber using heparinised canine blood. It has been concluded that the initial platelet adhesion (first measurements after about 5 min exposure time) is a diffusion limited process.

In general we can conclude that in studying platelet-foreign surface interaction as a function of shear rate and haematocrit the following features are important.

(1) Initial platelet adhesion sometimes depends on the foreign surface (Lyman *et al.*, 1970a and b), but in other experiments it is diffusion controlled (Friedman *et al.*, 1971). (A possible explanation is given by Turitto and Leonard (1972) who assumed a foreign surface-dependent protein adsorption followed by platelet adhesion, which depends on the surface concentration of reacted protein and the concentration of platelets adjacent to the surface).
(2) Platelets may be aggregated under the influence of shear; thus a different adhesion behaviour under different flow circumstances might be found.
(3) When citrated plasma is used, the effect of removal of $Ca^{2+}$ may play an important role, whereas the influence of the administration of heparin to blood has also to be taken into account.
(4) When platelets adhere to a foreign surface, the surface as well as flow conditions influence release of platelet aggregating substances, which affect the behaviour of other platelets approaching the surface.

*Activation of the intrinsic coagulation*

The mechanism of the activation of the intrinsic coagulation in the presence of foreign surfaces is still not well understood. Vroman (1967) has investigated the influence of the presence of fibrinogen on the activation of factor XII. When experiments with plasma and fibrinogen deficient plasma are carried out no difference is observed in the activation of factor XII. At the moment we are investigating the effect of preadsorption of proteins onto different surfaces on the activation of factor XII. It is generally agreed that adsorption of factor XII is necessary for the activation. The subsequent reaction between factor XIIa and factor XI seems also to take place on the surface (Haanen, 1969). When factor XII is adsorbed, the molecule is possibly uncoiled thereby showing its enzymatically active centre, necessary for the activation of factor XI (Donaldson *et al.*, 1965). Factor XII is rapidly adsorbed and activated onto negatively charged surfaces such as glass. When stimulated by ADP, platelets can also activate factor XII (Walsh, 1972).

The possible activation of factor XII in solution by shear stress has to be investigated. This can be imagined either directly by causing conformational changes or indirectly by causing the release of ADP from platelets. The *influence of flow conditions* on the adsorption of factor XII and the desorption of complex XIIa-XI needs further investigation.

## 26.4 Conclusions and Future Perspectives

In order to make progress in improving the blood compatibility of different devices, we have to study in detail the contributions of foreign materials, flow conditions and their interaction to blood compatibility. There are several ways to improve biomaterials. Andrade *et al.* (1973) are looking for ideal hydrogen grafts, which prevent protein adsorption, activation of factor XII and platelet adhesion. The structure of water plays a dominant role in this approach. Heparinisation of materials has been carried out by several authors (e.g. Leiniger *et al.*, 1972; Lagergren *et al.*, 1974). Most authors try to prevent the leaching of heparin from the surface. Heparin covalently bound to surfaces cannot leach off the surface. However, these surfaces are inferior as compared to the ionically heparin bound surfaces. It is possible that we will have to make heparinised surfaces which also leach a controlled amount of heparin (Tanzawa *et al.*, 1973). In this way both activation of factor XII and platelet adhesion is prevented. In our laboratory, heparin analogues with a substantially higher molecular weight than heparin are bound to surfaces in order to investigate the necessity for controlled leaching of anticoagulants from surfaces.

While materials such as certain polyurethanes which selectively adsorb albumin (Lyman *et al.*, 1975) may prevent platelet adsorption, the activation of the intrinsic coagulation still needs further study. Silica-free silicone rubber seems to have great potential for application in membrane oxygenators (Kolobow *et al.*, 1974).

It is also worthwhile to investigate further the use of extra-corporeal circuits in combination with drugs which prevent platelet adhesion, aggregation and release. Fundamental research to relate selective protein adsorption with surface parameters has to be carried out.

## 26.5 Acknowledgement

The author thanks Dr Tom Beugeling for his invaluable advice and stimulating discussions. The members of the Biomaterials Group are gratefully acknowledged for their contribution to this paper. Finally I thank Mrs Hammink-Siers for typing the manuscript.

## 26.6 References

Andrade, J. D., Lee, H. B., Jhon, M. S., Kim, S. W., and Hibbs, J. B. (1973). Water as a biomaterial. *Trans. Amer. Soc. Artif. Int. Organs,* **19**, 1.

Awad, J. A., Fortin, B., Bernier, J. P. and Leclerc, R. (1974). Red blood cell survival after perfusion with a membrane oxygenator. *Amer. J. Surg.*, **127**, 535.

Baier, R. E., Loeb, G. I. and Wallace, G. T. (1971). Role of an artificial boundary in modifying blood proteins. *Fed. Proc.*, **30**, 1523.

Bartlett, R. H., Gazzaniga, A. B., Fong, S. W. and Burns, N. E. (1974). Prolonged extracorporeal cardiopulmonary support in man. *J. Thorac. Cardiovasc. Surg.*, **68**, 918.

Bernstein, E. F. (1971). Certain aspects of blood interfacial phenomena, red blood cells. *Fed. Proc.*, **30**, 1510.

Bloom, S., Sapol, W., Wonders, T., Berger, S. and Salzman, E. (1974). Platelet destruction during 24 hours membrane lung perfusion. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 299.

Brash, J. L. and Lyman, D. J. (1969). Adsorption of plasma protein in solution to uncharged, hydrophobic polymer surfaces. *J. Biomed. Mater. Res.,* **3**, 175.

Brash, J. L., and Lyman, D. J. (1971). Adsorption of proteins and lipids to non-biological surfaces, in M. L. Hair (Ed.), *Chemistry of Biosurfaces,* Dekker, New York, chapter 5.

Brash, J. L., Uniyal, S. and Samak, Q. (1974). Exchange of albumin adsorbed on polymer surfaces. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 69.

Brown, C. H. III, Lemuth, R. F., Hellums, J. D., Leverett, L. B. and Alfrey, C. P. (1975). Response of human platelets to shear stress. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 35.

Butruille, Y. A., Leonard, E. F. and Litwak, R. S. (1975). Platelet-platelet interactions and nonadhesive encounters on biomaterials. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 609.

Daniels, A. U., Mortensen, J. D. and Allen, S. D. (1975). Protocol vena cava implant test for thrombogenicity, Revision 1 TR 149 - 006, Utah Biomedical Test Laboratory. University of Utah Research Institute, 520 Wakana way, Salt Lake City, Utah 84108, 15 August 1975.

Donaldson, W. H., Ratnof, O. D., (1965). Hageman factor: Alterations in physical properties during activation. *Science,* **150**, 754.

Dutton, R. C., Edmunds, L. H., Hutchinson, J. C. and Roe, B. B. (1974). Platelet aggregate emboli produced in patients during cardiopulmonary bypass with membrane and bubble oxygenators and blood filters. *J. Thorac. Cardiovasc. Surg.,* **67**, 258.

Falb, R. D., Pakahashi, M. T., Grode, G. A. and Leiniger, R. I. (1967). Studies on the stability and protein adsorption characteristics of heparinised polymer surfaces by radioisotope labelling techniques. *J. Biomed. Mater. Res.,* **1**, 239 - 251.

Feijen, J., Beugeling, T., Bantjes, A. and Smit Sibinga, C. Th. (1976). Biomaterials and interfacial phenomena, in D. N. Ghista, E. van Vollenhoven and W. J. Yang (Eds.) *Cardio-Circulatory Analysis of Aspects of Diagnosis, Assist, Therapeutic and Prosthetic Devices,* vol. IV, *Prostheses, Assist, Artificial Organs and Biomaterials,* Delft University Press, Delft, chapter 22.

Feuerstein, I. A., Brophy, J. M. and Brash, J. L. (1975). Platelet transport and adhesion to reconstituted collagen and artificial surfaces. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 427.

Friedman, L. I. and Leonard, E. F. (1971). Platelet-adhesion to artificial surfaces: consequences of flow, exposure time, blood condition and surface nature. *Fed. Proc.,* **30**, 1641.

Goldsmith, H. L., Yu, S. S. K. and Marlow, J. (1975). Fluid mechanical stress and the platelet, *Thrombos. Diathes. Haemorrh.,* **34**, 32.

Gonzalez, G. and MacRitchie, F. (1970). Equilibrium adsorption of proteins, *J. Colloid. Interface Sci.,* **32**, 55.

Gott, V. L., Ramos, M. D., Najjar, F. B., Allen, J. L. and Becker, K. E. (1969). The *in vivo* screening of potential thromboresistant materials, in R. J. Hegyeli, (Ed.), *Proceedings of the Artificial Heart Program Conference,*

National Institutes of Health, Bethesda, Maryland, June 9 - 13, 1969, U. S. Government Printing Office, Washington, D. C. p. 181.

Gott, V. L. and Furuse, A. (1971). Antithrombogenic surfaces, classification and *in vivo* evaluation, in *Federation Proceedings, Conference on Mechanical Surface and Gas Layer Effects on Moving Blood,* San Diego, California, January 1971, Federation of American Societies for Experimental Biology, Maryland, pp. 1679 - 1687.

Haanen, C. (1969). The nature of the factor IX activating principle, in H. C. Hemker, E. A. Loelinger and J. J. Veltkamp (Eds.), *Human Blood Coagulation,* Leiden University Press, Leiden, pp. 58 - 72.

Hoffman, A. S. (1974). Principles governing biomolecule interactions at foreign surfaces. *J. Biomed. Mater. Res. Symposium,* **5**, 77.

Jamieson, G. A. (1973). Role of glycoproteins in platelet function, in E. Gerlach, K. Moser, E. Deutsch and W. Wilmanns (Eds.), *Erythrocytes, Thrombocytes, Leucocytes,* Georg Thieme, Stuttgart, p. 209.

de Jong, J. C. F., Smit Sibinga, C. Th. and Wildevuur, Ch. R. H. (1975). Thrombocytopenia in extracorporeal circulation (ECC). II: Bubble oxygenator versus artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 40.

Kim, S. W., Lee, R. G., Oster, H., Coleman, D., Andrade, J. D., Lentz, D. J. and Olsen, D. (1974). Platelet adhesion to polymer surfaces, *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 449.

Klose, J. J., Rieger, H., Heinich, L. and Schmid-Schonbein, H. (1976). A new method for the study of platelet rheology in artificial flow. *Thrombos. Research,* in press.

Kolobow, T., Stool, E. W., Weathersby, P. K., Pierce, J., Hayano, F. and Suaudeau, J. (1974). Superior blood compatibility of silicone rubber free of silica filler in the membrane lung, *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 269.

Kusserow, B., Lanow, R. and Nichols, J. (1971). Perfusion and surface-induced injury in leucocytes, *Fed. Proc.,* **30**, 1516.

Lagergren, H., Olsson, P., Swedenborg, J. (1974). Inhibited platelet adhesion. A non-thrombogenic characteristic of a heparin-coated surface, *Surgery,* **75**, 643.

Lee, W. H. and Hairston, P. (1971). Structural effects on blood proteins at the gas-blood interface, *Fed. Proc.,* **30**, 1615.

Lee, R. G., and Kim, S. W. (1974). The role of carbohydrate in platelet adhesion to foreign surfaces, *J. Biomed. Mater. Res.,* **8**, 393.

Leininger, R. I., Crowley, J. P., Falb, R. D. and Grode, G. A. (1972). Three years experience *in vivo* and *in vitro* with surfaces and devices treated by the heparin complex method, *Trans. Amer. Soc. Artif. Int. Organs.* **18**, 312.

de Leval, M. R., Hill, J. D., Mielke, C. H., Macur, M. F. and Gerbode, F. (1975). Blood platelets and extracorporeal circulation, kinetic studies on dogs on cardiopulmonary bypass, *J. Thorac. Cardiovasc. Surg.,* **69**, 144.

Lindsay, R. M., Prentice, C. R. M., Burton, J. A., Ferguson, D. and Kennedy, A. C. (1973). The role of the platelet-dialysis membrane inter-action in thrombus formation and blood loss during hemodialysis, *Trans. Amer. Soc. Artif. Int. Organs,* **19**, 487.

Lyman, D. J., Brash, J. L., Chaikin, S. W., Klein, K. G. and Carini, M. (1968). The effect of chemical structure and surface properties of synthetic polymers on

the coagulation of blood. II: Protein and platelet interaction with polymer surfaces, *Trans. Amer. Soc. Artif. Int. Organs,* **14**, 250.

Lyman, D. J., Klein, K. G., Brash, J. L. and Fritzinger, B. K. (1970a). Interaction of platelets with polymer surfaces, *Thrombosis Diathes. Haemorrh.,* **23**, 120.

Lyman, D. J., Klein, K. G., Brash, J. L., Fritzinger, B. K., Andrade, J. D. and Bonomo, F. S. (1970b). Platelet interaction with protein-coated surfaces: An approach to thromboresistant surfaces, *Thrombos. Diathes. Haemorrh., Suppl.*, **42**, 109.

Lyman, D. J., Metcalf, L. C., Albo, D., Richards, K. F. and Lamb, J. (1974). The effect of chemical structure and surface properties of synthetic polymers on the coagulation of blood. III: *In vivo* adsorption of proteins on polymer surfaces, *Trans. Amer. Soc. Artif. Organs,* **20**, 474.

Lyman, D. J., Knutson, K., McNeill, B. and Shibatani, K. (1975). The effects of chemical structure and surface properties of synthetic polymers on the coagulation of blood. IV: The relation between polymer morphology and protein adsorption, *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 49.

Mason, R. G., Shermer, R. W., Zucker, W. H. and Brinkhous, K. M. (1973). Platelet and plasma protein reactions with foreign surfaces, in E. Gerlach, K. Moser, E. Deutsch and W. Wilmanns (Eds.), *Erythrocytes, Thrombocytes, Leucocytes,* Georg Thieme, Stuttgart, p. 263.

Olsen, D. A. and Kletschka, H. D. (1973). Biophysical interactions of blood proteins with polymeric and artificial surfaces, in J. F. Danielli, M. D. Rosenberg and D. A. Cadenhead (Eds.), *Progress in Surface and Membrane Science,* Academic Press, New York, p. 331.

Packham, M. A., Evans, G., Glynn, M. F. and Mustard, J. F. (1969). The effect of of plasma proteins on the interaction of platelets with glass surfaces, *J. Lab. Clin. Med.,* **73**, 686.

Salzman, E. W. (1971). Role of platelets in blood-surface interaction, *Fed. Proc.,* **30**, 1503.

Salzman, E. W., Merrill, E. W., Binder, A., Wolf, C. F. W., Ashford, T. P. and Austen, W. G. (1969). Protein-platelet interaction on heparinized surfaces, *J. Biomed. Mater. Res.,* **3**, 69.

Scheer, A. van der, Feijen, J., Wubben, F. J., Krugers Dagneaux, D. G. L. C. and Smolders, C. A. (1976). The adsorption of human serum albumin at polymer-water interfaces, the influence of labelling on the adsorption behaviour and the reversibility of the adsorption, *J. Colloid. Interface Sci.* (in press).

Stormorken, H. (1971). Platelets, thrombosis and haemolysis, *Fed. Proc.,* **30**, 1551.

Tanzawa, H., Mori, Y., Harumiya, N., Miyama, H., Hori, M., Ohshima, N. and Idezuki, Y. (1973). Preparation and evaluation of a new athrombogenic heparinized hydrophilic polymer for use in cardiovascular system, *Trans. Amer. Soc. Artif. Int. Organs,* **19**, 188.

Turitto, V. T. and Leonard, E. F. (1972). Platelet adhesion to a spinning surface, *Trans. Amer. Soc. Artif. Int. Organs.* **18**, 348.

Vroman, L., (1967). Surface activity in blood coagulation, in W. M. Seegers (Ed.), *Blood Clotting Enzymology,* Academic Press, New York, pp. 279.

Vroman, L. (1971). Identity and significance to clotting and platelet adhesion, of proteins adsorbed out of plasma onto artificial kidney membrane materials. Progress Report PB - 204601, NIAMD, National Technical Information Service, Springfield, Virginia.

Vroman, L., Adams, A. L. and Klings, M. (1971). Interactions among human blood proteins at interfaces, *Fed. Proc.*, **30**, 1494.

Walsh, P. N. (1972). The role of platelets in the contact phase of blood coagulation, *Brit. J. Haemat.*, **22**, 237.

# 27

# PLATELET-FOREIGN SURFACE INTERACTIONS WITH OXYGENATOR MEMBRANES

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**Summary**

The interaction of platelets and oxygenator membranes is of clinical importance. The membranes have an affinity for platelet retention which may, *per se*, cause a haemostatic defect while the release reaction may cause the formation of harmful microemboli of aggregated platelets together with possible vasoactive phenomena. With regard to the release reaction a plasma factor, possibly associated with complement activation, develops following contact of plasma or whole blood with membranes used in oxygenators and dialysis. In the development of biocompatible membranes for such devices platelet-foreign surface interactions, as described, below, require to be considered.

## 27.1 Introduction

The deleterious results of the interaction of blood with artificial surfaces are recognised increasingly as an important problem in medicine. The expanding use of prosthetic heart valves, prosthetic blood vessels, vascular cannulae and catheters, arterio-venous shunts and the extracorporeal circulations of the artificial kidney and lung has clearly indicated that biocompatible materials need to be developed. To allow such development, extensive efforts are required to correlate the properties of materials with changes in blood components and to this end many laboratories are engaged in the examination of protein deposition, loss of formed blood elements, activation of contact coagulation factors, etc, onto or by different foreign surfaces. A principal interest of these laboratories is that of the platelet-foreign surface interaction which appears to be of considerable importance in prolonged perfusion by extracorporeal oxygenators. Platelet concentration decreases to almost zero following 12 h of circulation using a bubble oxygenator and uncontrollable bleeding inevitably occurs (Fong *et al.*, 1974). Thrombocytopenia also occurs with non-gas interface systems but the degree is less than that encountered with the bubble oxygenator and prolonged perfusion is possible in man (Bartlett *et al.*, 1974; Hill *et al.*, 1974; Zapol *et al.*, 1975). Nevertheless, compromised haemostasis may still be a problem to which thrombocytopenia and impaired platelet function will contribute. In addition it is possible that platelets

may release biologically active substances into the circulation with damaging effects on other organs (Hollenberg *et al.*, 1963) or may aggregate *in vivo* leading to patient embolisation of, for example, retinal vessels (Williams, 1971). Because of these potential hazards we have carried out both animal *ex vivo* and laboratory *in vitro* studies of the interrelationship between platelets and foreign surfaces and were able to make some observations which may be of value to the further develoopement of biocompatible membranes for oxygenation.

## 27.2 Experimental Design, Methodology and Results

*Study one*

*To study the effect of an anti-platelet agent on platelets and pulmonary responses to the onset of membrane oxygenator perfusion.*

A method of initiating membrane oxygenator perfusion in sheep was established and was designed to isolate the effects of blood-oxygenator interaction from other factors (e.g. haemodilution by priming, pump-induced trauma to platelets, heparinisation, etc) which might affect platelet loss. With this model the early platelet loss during perfusion was investigated and a pulmonary vascular response related to this platelet loss was identified.

*Methods* Female Suffolk sheep weighing 30 kg were cannulated for veno-venous perfusion under local anaesthesia using ketamine hydrochloride (22 mg/kg) and 2% Xylocaine. A polyethylene catheter (PE 190) was inserted into the left carotid artery and advanced to the aortic arch for arterial pressure recordings. A number 7 French, Swan-Ganz, thermodilution catheter, especially modified to have the proximal injection site 12.5 cm from the thermistor tip, was inserted into the left jugular vein and advanced into the pulmonary artery and secured to permit pulmonary artery and pulmonary wedge pressure recording. Cephalad to the Swan-Ganz insertion site a 4.75 mm (0.187 in) ID, wire reinforced, polyurethane cannula (Johnson, Peabody, Massachusetts) was inserted centrally into the left jugular vein to be used as a venous return cannula. A 5.55 mm (0.219 in) ID cannula of similar material was inserted cephalad for venous drainage of the left side of the head. A number 28 Argyle thoracic catheter was inserted centrally via the right jugular vein, to the level of the diaphragm for inferior vena caval drainage. The cephalic end of the right jugular vein was cannulated, as on the left side, for venous drainage of the head. The proximal and distal neck cannulae were temporarily connected as venous shunts until the animal was ready to be connected to the perfusion apparatus. Prior to cannulation 1.25 mg/kg of heparin were administered systemically and the dose repeated 30 min later. Following cannulation the animal was returned to its cage and allowed to recover until it was alert and standing (1 to 2 h on average).

The perfusion circuit, described in detail elsewhere (Duffin *et al.*, 1976; Birek *et al.*, 1976), is depicted in figure 27.1. Venous blood is gravity drained to a 100 ml volume, distensible, silicone rubber reservoir which activates a servocon-

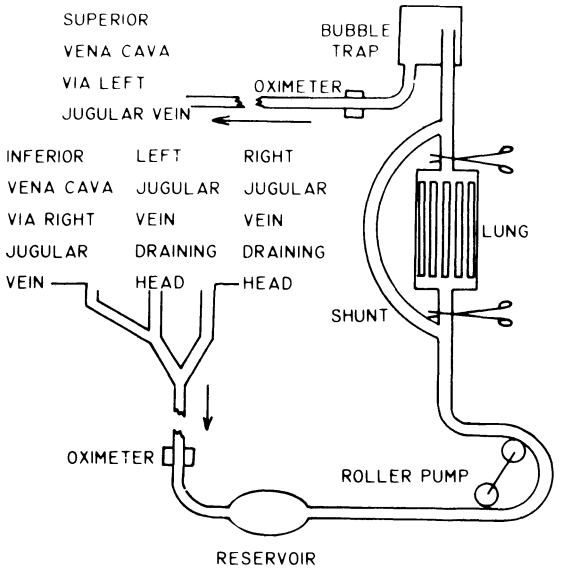


Figure 27.1 Schematic diagram of perfusion circuit. During control perfusion the oxygenator is excluded and flow is through the shunt

trolled Sarns roller pump. This, in turn, pumps blood from the reservoir through the membrane lung and back to the animal. All surfaces in the circuit are silicone rubber with the exception of the polycarbonate tubing connectors (Cobe Laboratories, Denver, Colorado). The perfusion circuit, excluding the membrane oxygenator, has a priming volume of 900 ml and a surface area of 0.18 $m^2$. The membrane oxygenator utilised is a spiral coil membrane lung (Sci-Med Incorporated, Minneapolis, Minnesota) with a priming volume of 300 ml.

The circuit was primed with Ringer's Lactate solution, 50 mg of heparin and 0.75 g of sodium bicarbonate being added to each litre. After priming, the inflow and return lines were attached to each other and the priming solution heated to 39°C by an external heat source and circulated through the membrane oxygenator to eliminate trapped air bubbles for 1 h. After this time the membrane oxygenator was excluded from the circuit with clamps while perfusion continued through a bypass silicone rubber tubing shunt. The sheep was then attached to the circuit as shown in figure 27.1.

Perfusion commenced at a rate of 200 ml per min with the oxygenator excluded. After 15 min, perfusion was increased to 1 l/min and maintained at this rate for 90 min. The flow was then reduced again to 200 ml/min for 15 min. This 2 h perfusion, with the membrane excluded, is referred to as the 'control perfusion' and permitted the animal to adjust to the haemodilution from the priming solution, and to any effects of the perfusion itself, before the oxygenator was inserted. During this time the heparin administration was regulated to maintain an activated clotting time of approximately 400 seconds (measured by Hemochron, Interna-

tional Technedyne Corp., Edison, New Jersey); this required a continuous infusion of heparin of between 0.5 and 1 mg/kg/h. With the flow at 200 ml/min, the membrane oxygenator was abruptly introduced into the circuit by unclamping the oxygenator and clamping the bypass shunt. Oxygen flow through the gas phase of the oxygenator was then begun at 1 l/min. Perfusion was maintained at 200 ml/min for 16 min and then abruptly increased to 1 l/min for an additional 4 h.

The arterial and Swan-Ganz catheters were attached to Statham transducers (P23D 1a) connected to a Hewlett-Packard multichannel pen recorder for continuous recording of the arterial and pulmonary pressures. Cardiac output was determined by the thermodilution method, using an Edwards model 9510 cardiac output computer. Pulmonary vascular resistance was calculated as the difference between mean pulmonary artery and wedge pressures (mm Hg) divided by the cardiac output (l/min).

Haematocrit was measured by the microcentrifuge method and platelet counts by the Coulter counter (Model $F_N$ – Coulter Electronics Inc, Florida). These measurements were determined in duplicate at the time of cannulation, after the control perfusion and at intervals after the introduction of the oxygenator into the perfusion circuit (2,4,8,16,32,60,120 and 240 minutes). The values at the end of the 2 h control period were considered to be the baseline values. All other platelet counts were corrected for changes in haematocrit due to haemodilution and blood sampling and were expressed as a percentage of the baseline counts.

Using this experimental protocol, two groups of animals were studied. Group I animals, referred to as the control group, consisted of three sheep which underwent perfusion as outlined above. Group II animals, consisting of four sheep, received 1 to 1.5 g of sulphinpyrazone (Geigy) injected over 1 h directly into the perfusion circuit during the period of 'control perfusion'. In all other respects, both groups of animals were treated identically.

*Results: haemodynamic* All animals remained haemodynamically stable during the 2 h control perfusion. However, 8 to 10 min after introduction of the oxygenator into the circuit, group I (non-sulphinpyrazone) animals developed a sudden marked increase in pulmonary artery pressure. The pulmonary vascular resistance increased at least two-fold in all of these animals, and gradually returned to normal over the next 60 to 90 min. When the pulmonary artery pressure began to rise, these animals became noticeably less alert and less active.

In contrast, the sulphinpyrazone-treated animals showed no change in pulmonary artery pressure or pulmonary vascular resistance following the introduction of the membrane oxygenator or at any time during the ensuing 4 h perfusion. No apparent clinical change occurred in these animals and they remained active and alert.

Figure 27.2 illustrates the pulmonary artery pressure in both groups of animals during the first hour of oxygenator perfusion. The pulmonary vascular resistance for both groups is illustrated in figure 27.3.

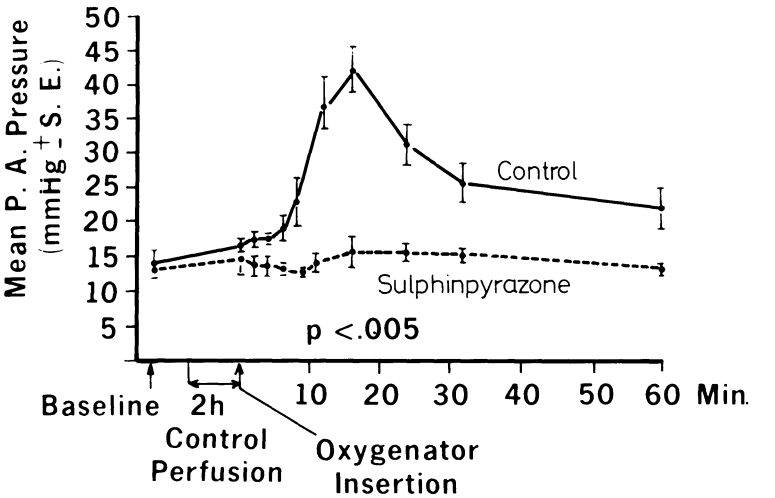


Figure 27.2 Mean pulmonary artery pressures in group I (control) and group II (sulphinpyrazone-treated) animals over periods of control and test perfusions

*Results: platelet counts* When corrected for changes in haematocrit there was no significant change in the platelet count from the time of cannulation to the end of the 2 h control perfusion in either group of animals. Following insertion of the oxygenator, group I animals had an early and significant fall in the platelet count during the initial 16 min of perfusion. A further, more gradual decline subsequently occurred with 46% of the baseline platelet count remaining by the end of the 4 h of oxygenator perfusion.

Sulphinpyrazone animals (group II) had an early, slight fall in the platelet count with a subsequent rebound. At the end of the 4 h perfusion, 86% of the baseline platelet count remained in these animals, a significant improvement on the

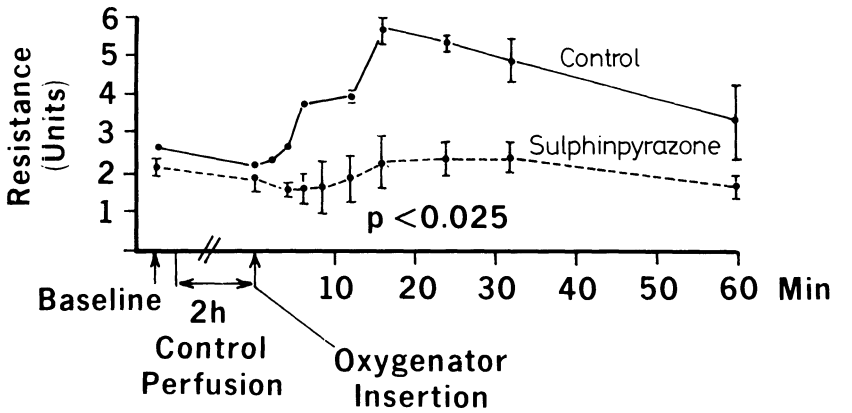


Figure 27.3 Pulmonary vascular resistances in group I (control) and group II (sulphinpyrazone-treated) animals during perfusion periods

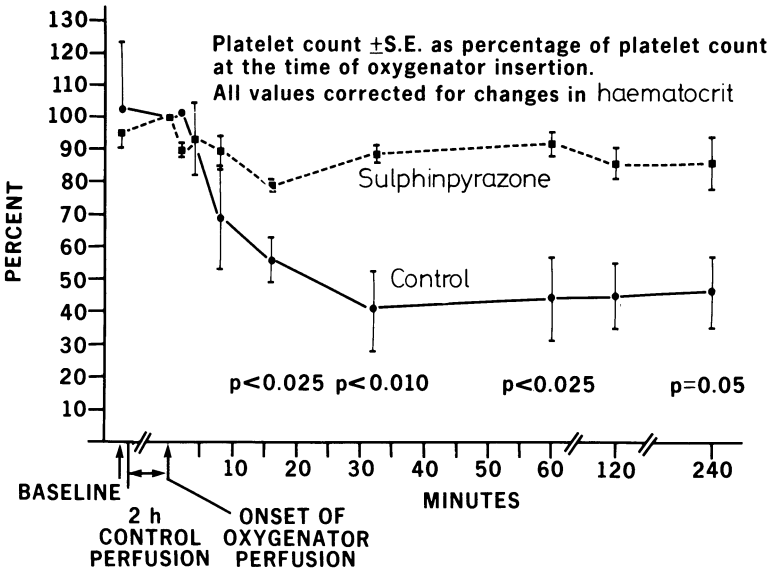


Figure 27.4 Platelet counts in group I (control) and group II (sulphinpyrazone-treated) animals during perfusion periods

group I results. Figure 27.4 shows the effect of oxygenator perfusion on the platelet count for each group and indicates the significance of differences at varying times.

In one of the sulphinpyrazone-treated animals, oxygenator perfusion was extended to 12 h, at which the baseline platelet count increased by 6%. In a second sulphinpyrazone-treated animal, perfusion was extended to 24 h, resulting in preservation of 78% of the baseline platelet count. All remaining perfusions in group I and group II animals were terminated after 4 h.
(Full details and discussion of these experiments, which were carried out in the Department of Surgery, University of Toronto, are given by Birek *et al.*, 1976).

*Study two*

*To examine the platelet-retaining properties of various oxygenator membranes.*

An *in vitro* method has been developed to examine the platelet-retaining properties of various membranes used in extracorporeal devices. It has been extensively used in studies of artificial kidney membranes where a direct correlation between the *in vitro* platelet retention and both the *in vivo* degree of thrombus formation and the post dialysis fall in platelet count has been observed (Lindsay *et al.*, 1973a). The design of the method lends itself to studies both of pharmacological agents with an anti-platelet effect and of different surfaces (Lindsay *et al.*, 1973b). The method was used to study the platelet-retaining properties of certain membranes used in membrane oxygenators.

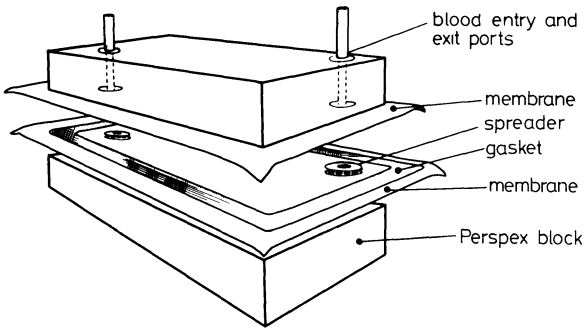


Figure 27.5 Exploded diagram of membrane-test cell system for measurement of platelet retention

*Methods* The membrane test cell system is fully described elsewhere (Lindsay *et al.*, 1973b; Leonard *et al.*, 1974). It consists of two Perspex blocks so constructed as to allow the introduction of blood between two sheets of membranes by means of unplasticised poly(vinyl chloride) entry ports and plastic (Kematal, ICI, UK) spreaders. The two spreaders and a plastic (low density polyethylene) gasket maintain a blood compartment height of 0.07 cm. The compartment, which is 12.8 cm long and 10.5 cm wide, has a volume of 9 ml. An exploded view of the cell is shown in figure 27.5. To measure platelet retention, membrane sheets cut larger than the test cell are washed twice in distilled water and then immersed in Owren's buffer (pH 7.35, 300 mOsmol/kg) for 5 min. They are then placed in a test cell, the two blocks of which are clamped together. The cell is then primed with 9 ml Owren's buffer and emptied after 10 min. For each test cell experiment 9 ml venous blood from a healthy volunteer are taken into a plastic syringe and mixed with 1 ml of 3.8% sodium citrate in a plastic tube; 1 ml of this blood is taken for platelet counting, the rest being introduced into the test cell by plastic syringe in a standard fashion. The blood ports are then clamped and the test cell rocked by hand, to an angle of 30° at 5 s intervals. The rocking is carried out using a simple seesaw device which also allows two test cells to be used simultaneously. After 10 min the test cell is emptied by opening the ports and withdrawing the blood into a plastic syringe. The withdrawn blood is gently mixed in a plastic tube and a 1 ml sample taken for a second platelet count. The samples for platelet counts have ethylenediamine tetraacetic acid (EDTA) added to them (1 mg/ml final concentration) and are counted electronically using a Coulter ZBI counter (Coulter Electronics Inc., Florida). Such electronic counts have been shown to have a good correlation with microscopic counts even on post-foreign surface contact samples (Lindsay *et al.*, 1975). By comparing the pre- and post-test cell platelet count, the number of platelets retained within the test cell is calculated. The drop in platelet count expressed as a percentage of the initial count gives a value termed 'platelet retention'. Using twin test cells of identical construction and the rocking device, paired estimations for platelet retention can

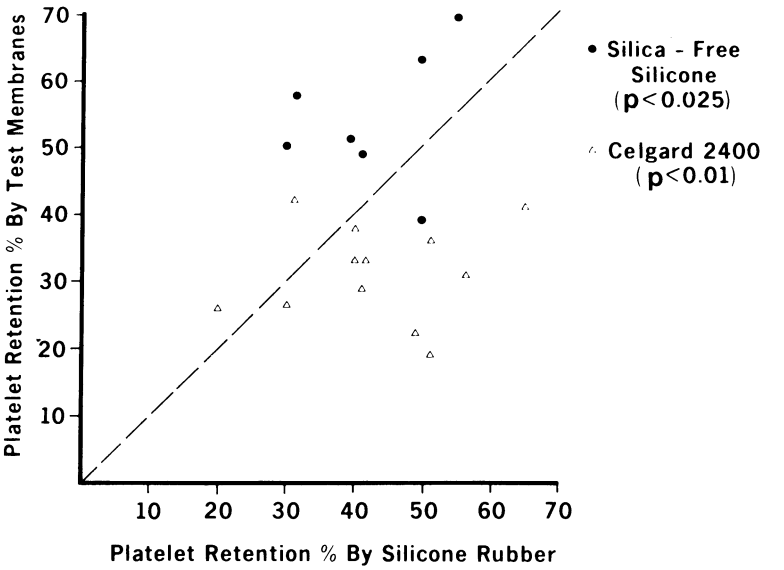


Figure 27.6 Paired platelet retention (%) results for reference membrane (silicone rubber) versus test membranes (silica-free silicone rubber and Celgard 2400)

be made. If both test cells contain identical membranes an excellent correlation is obtained between the two sets of results (Lindsay *et al.*, 1973b). Thus, a test membrane may be inserted into one test cell and compared directly with a standard reference membrane by examination of the paired platelet retention results.

Using this technique, a 0.23 mm (0.009 in) thick silicone rubber membrane (Baxter-Travenol, Chicago) was chosen as the reference material and test membranes were Celgard 2400 (a microporous polypropylene film; Celanese Plastics Co, Greer, South Carolina) and a silica-free silicone rubber membrane (Sci-Med Incorporated, Minneapolis, Minnesota).

*Results* Twelve paired studies were made between Celgard and reference silicone and the respective mean platelet retention values were 31 ± 7.2% and 43 ± 12% (M ± SD). A paired '*t*' test showed that significantly fewer platelets adhered to the Celgard than to the silicone ($p < 0.01$). Seven comparisons were made between the silica-free silicone material and the reference silicone; the former was shown to have a significantly greater platelet retention than the latter ($p < 0.025$). The individual plots for these experiments are shown in figure 27.6

*Study three*

*To determine whether contact between blood and oxygenator membranes causes serotonin release from platelets.*

We have noted that a reduction of intraplatelet serotonin concentration occurs during the course of haemodialysis in studies of platelets from uraemic patients

receiving regular dialysis therapy; this may be, at least in part, secondary to serum complement activation and not merely due to the mechanical stresses of blood flowing over nonbiological surfaces. Whatever the mechanism it is obvious that the release of serotonin and other substances from platelets may be of importance both vasoactively and in causing secondary aggregation of circulating platelets which, in turn, may lead to patient microembolisation and end organ damage. Because of this, we have studied the effect of incubation of whole blood, platelet rich plasma (PRP) and platelet poor plasma (PPP) with silicone rubber membrane, as used in an oxygenator, on the endogenous intraplatelet serotonin content and on the release of exogenous $^{14}C$ labelled serotonin. Finally we examined the effect of such release on healthy platelets.

*Methods and results* Initial experiments were carried out to examine the effect of incubation of whole blood or PPP with a silicone rubber membrane on the intraplatelet endogenous serotonin concentration and upon plasma total haemolytic complement ($CH_{50}$) levels. 20 ml whole blood were taken on five occasions from four healthy volunteers, who had not taken any drug known to have an antiplatelet action, and were anticoagulated with heparin (MTC Pharmaceuticals Canada Ltd, final concentration 4 units/ml). 10 ml of this blood were placed into a plastic, shallow container in contact with 265 $cm^2$ of a 0.038 mm (0.0015 in) thick silicone rubber membrane (Baxter-Travenol, Chicago) which had been previously washed first in distilled water and then soaked for 10 min in isotonic phosphate buffer (ph 7.4, Fisher Scientific). The containers and their contents were allowed to stand still on the bench. The remaining 10 ml were centrifuged at 273G for 13 min and the supernatant PRP obtained. A sample from this gave a pre-contact intraplatelet serotonin concentration. The remaining PRP was centrifuged at 400G for 20 min and PPP obtained. 1 ml of this was used for estimation of $CH_{50}$. After 1 h at room temperature the blood samples were removed from the plastic containers and PRP and PPP harvested as above. Post-membrane contact intraplatelet serotonin and $CH_{50}$ values were then measured. On two occasions only was a time control also run; on these occasions a further 10 ml of whole blood were taken and placed into a plastic container, but not in contact with membrane, and left to stand for 1 h. Serotonin was measured fluorimetrically by a modification of the method of Drummond and Gordon (1974) on known volumes of lysed platelets. To estimate the intraplatelet concentration, the lysed platelet result is corrected for platelet number and platelet volume. The latter were obtained electronically using a Coulter ZBI counter coupled to a Coulter Channelyser (Coulter Electronics Inc, Florida). $CH_{50}$ was measured by the method of Kabat and Mayers (1971). The results, which are listed in table 27.1, show that on 4 out of 5 occasions there was a fall in intraplatelet endogenous serotonin concentration (this fall was significant by paired '*t*' test; $p < 0.05$; $n = 5$) and in $CH_{50}$ ($p < 0.05$; $n = 5$) after contact with the membrane. Falls of similar magnitude did not occur with the two-time controlled studies. On the one occasion that the intraplatelet serotonin level did not fall there was no evidence of complement activation.

Table 27.1

Effect of whole blood-silicone membrane contact on complement activation and intraplatelet 5HT.

| *Experiment* | *$10^{-6}$ pmol/$\mu^3$ Intraplatelet 5HT* | | *$CH_{50}$ (units)* | |
|---|---|---|---|---|
| | *Control* | *Post-contact* | *Control* | *Post-contact* |
| 1 | 1.04 | 0.69 | 122 | 66 |
| 2 | 1.34 | 0.54 | 96 | 85 |
| 3 | 1.34 | 0.32 | 96 | 58 |
| 4* | 0.63 | 0.81 | 63 | 67 |
| 5 | 1.15 | 0.83 | 138 | 101 |
| | ($p < 0.05$) | | ($p < 0.05$) | |

(*No fall in either 5HT or $CH_{50}$)

Furthermore, on consideration of all pre- and post-contact results a significant linear relationship between $CH_{50}$ and serotonin values was evident ($r = 0.67$, $p < 0.05$) as shown in figure 27.7. On five occasions 40 ml heparinised whole blood were obtained as above and centrifuged to harvest PPP and PRP. The previous experiment was then repeated but this time PPP was placed in the plastic containers, one of which contained the membrane, so that all experiments were time controlled. After 1 h of contact the PPP was removed and 5 ml added to 5 ml PRP, mixed and allowed to stand at room temperature for a further 1 h. The platelet containing plasma was then removed from the containers and intraplatelet serotonin levels measured. As shown in table 27.2, on four occasions a fall in serotonin was obtained, and by paired '*t*' test the fall was significant ($p < 0.05$; $n = 5$) on comparison of the post-membrane contacted plasma values with the time control results.

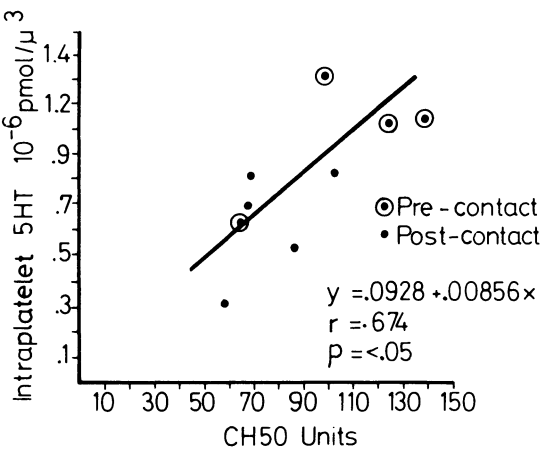


Figure 27.7 Relationship between intraplatelet serotonin (5HT) and $CH_{50}$ levels pre and post-contact with silicone rubber.

**Table 27.2**

Effect of PPP-silicone membrane contact on intraplatelet 5HT after subsequent PRP incubation

| *Experiment* | *Intraplatelet 5 HT* $10^{-6}$ *pmol/μ*$^3$ | |
|---|---|---|
| | *Control* | *Post-contact* |
| 1 | 0.92 | 0.69 |
| 2 | 1.44 | 1.10 |
| 3 | 1.29 | 1.20 |
| 4 | 0.63 | 0.67 |
| 5 | 0.94 | 0.57 |
| | ($p < 0.05$) | |

In an attempt to improve the sensitivity of these studies a further five experiments were carried out using platelets containing $^{14}C$ labelled serotonin. For each experiment 50 ml whole blood were taken from healthy volunteers into plastic tubes; on two occasions the blood was anticoagulated with heparin to give a final concentration of 4 units/ml and on the other occasion 3.8% sodium citrate was used (9 parts blood: 1 part sodium citrate). The anticoagulated blood was centrifuged at 273G for 13 min and then at 400G for 20 min to harvest PRP and PPP. The PRP platelet count was adjusted to 200 000/mm$^3$ for each donor using his PPP. The PRP was then incubated with $^{14}C$ labelled serotonin (55 mCi/mmole; Amersham-Searle, Oakville, Ontario) according to the method of David and Herion (1972) and a sample checked to ensure that over 90% of radioactivity had been taken up by the platelets. The labelled PRP was then added to a plastic tube containing 0.038 mm (0.0015 in) thick silicone rubber membrane (Baxter-Travenol Inc, Chicago), using 26.5 cm$^2$ of membrane per 1 ml PRP (approximate volume used per experiment 10 ml). The membrane had been previously washed with distilled water and then soaked in phosphate buffer (pH 7.4, Fisher Scientific). An equal volume of labelled PRP was added to an identical but empty plastic tube as a control. After 1 h standing still at room temperature the tubes were gently inverted five times and then 1 ml PRP taken from each. 100 μl of 0.4% EDTA and 50 μl of 0.9% normal saline were added to the PRP which was then centrifuged at 12 500G at 0°C for 10 min. 200 μl of supernatant were removed and air dried on glass fibre filter paper which in turn was placed in a counting vial containing 20 ml of PCS solubiliser scintillation fluid (Amersham-Searle, Oakville, Ontario) and counted for $^{14}C$ activity. Each sample was counted in triplicate and the mean value compared with the amount of $^{14}C$ activity in a PPP sample obtained pre-incubation (and prepared in an identical fashion to the above) and each result was expressed as a percentage of this latter value. The results obtained from the post-membrane contacted samples were 134% (sodium citrate anticoagulation), 183%, 115%, 159% and 150% (all heparin) as compared with a range of 96% to 105% from the time controlled nonmembrane contacted samples. These results indicated that

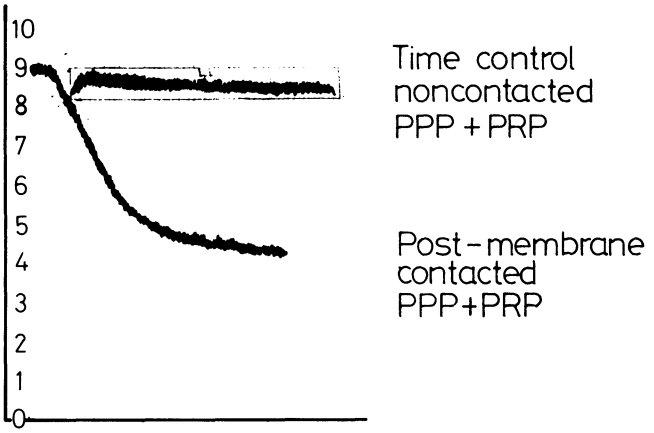


Figure 27.8 The effect of contact between platelet poor plasma (PPP) and platelet rich plasma (PRP) on platelet aggregation; 0.1 ml PPP (post-membrane contact and fibre control) added to 0.5 ml PRP

contact with the silicone rubber membrane had caused the platelets to release $^{14}C$-labelled serotonin.

On two further occasions PRP was taken from the plastic tubes (i.e. after 1 h at room temperature) and centrifuged at 400G for 20 min and the PPP removed. 0.1 ml of this PPP was then added to 0.5 ml PRP (200 000 unlabelled platelets/$mm^3$) obtained from the same donor, and placed in an aggregometer (model PAP-2A, Bio/Data Corp, Willow Grove, Pa.). On both occasions the PPP obtained from the tube containing the membrane caused platelet aggregation while that obtained from the control tube did not. The aggregation curves obtained from one of these experiments is shown in figure 27.8.
(Studies 2 and 3 were carried out in the Renal Laboratory, Victoria Hospital, London.)

### 27.3 Discussion

Haemostatic and thromboembolic complications following cardiopulmonary bypass may result from alterations in platelet function (Clowes, 1960; Schmidt *et al.*, 1961, Heiden *et al.*, 1975). In addition, microembolisation of platelet aggregates formed during the bypass has been implicated in the pathogenesis of cerebral, pulmonary and other postoperative complications (Becker *et al.*, 1974; Dutton *et al.*, 1974, Swank *et al.*, 1963; Carlson *et al.*, 1973 and Williams, 1971). Therefore, studies of platelet-foreign surface interactions are of potential clinical importance. The experiments described are preliminary in nature but nevertheless indicate the importance of the platelet-oxygenator membrane interaction and give some insight into the mechanism of platelet aggregate formation.

The first study was of an animal model designed to separate the effects on platelets of haemodilution, mechanical pumping, flow circuit fluid mechanics,

and heparin administration from the effects due to exposure to a membrane oxygenator. The study showed that, concomitant with the introduction of the membrane into the circuit, platelet numbers fell and pulmonary vascular resistance (and hence pulmonary artery pressure) rose. This effect was abolished when the animals were pre-treated with sulphinpyrazone, an agent which will inhibit the platelet release reaction (Packham and Mustard, 1968; Packham *et al.*, 1967), will prolong platelet survival and reduce platelet turnover (Smythe *et al.*, 1965). The study strongly suggests, therefore, that the platelet-membrane interaction caused the increase in pulmonary vascular resistance by either the formation of platelet aggregates, which would be trapped in the pulmonary capillaries, or the release of vasoactive substances from the platelets or, indeed, both factors may have been operative.

With this knowledge, studies 2 and 3 were carried out and these demonstrated, first of all, that the standard silicone rubber oxygenator membrane has considerable affinity for platelets judged by a mean platelet retention of 43% (± 12: ± S.D.) using the membrane test cell system of Lindsay *et al.* (1973b). Such platelet retention may be of importance clinically by depleting the patient of platelets and, thus, causing a haemostatic problem. In addition, platelet retention is likely to be followed by the release of serotonin, and other substances, which may lead to secondary aggregation of platelets circulating in the local microenvironment and thus may play a role in the formation of microemboli. In states of lower flow, such as that found in the extracorporeal circuits of the artificial kidney, platelet retention by dialysis membranes may be followed by thrombus formation in spite of adequate heparinisation (Lindsay *et al.*, 1972a and b). This is undesirable because of the problems of masking of transport surfaces, embolisation to the patient and blood loss. Indeed, a correlation has been found between the *in vitro* platelet retention by dialysis membranes and the *in vivo* degree of thrombus formation upon those membranes (Lindsay *et al.*, 1973a). The higher blood flows of the cardio-pulmonary bypass probably prevent thrombus formation upon oxygenator membrane surfaces but the platelet-adhesion-aggregation reaction is likely to proceed with its possible sequelae. In view of this, such platelet retention studies may be of value in biocompatibility testing and the present data demonstrate that Celgard has significantly less affinity for platelets than silicone rubber while a silica-free-silicone membrane had greater platelet retention (figure 27.6).

These subsequent *in vitro* studies also demonstrate that mere contact of whole blood or platelet rich plasma with a silicone rubber membrane will lead to the release of serotonin (and, most likely, of other substances) which in turn will cause secondary platelet aggregation (figure 27.8) and which may also play a vasoactive role. As yet, the mechanism for this release reaction is not clear but activation of the plasma complement system appears to be associated. The fact that plasma, previously contacted with the membranes, can induce the release of serotonin (as judged by a reduction in intraplatelet endogenous serotonin concentration) from platelets which were not in contact with that surface is extremely interesting. The significant linear relationship between platelet intracellular sero-

tonin and $CH_{50}$ does suggest a cause and effect situation although further studies are required to prove this and these are underway. Plasma complement activation has been found, incidentally, in patients undergoing haemodialysis and is associated with pulmonary margination of leucocytes (Fehr *et al.*, 1975) and with a reduction in intraplatelet serotonin levels.

## 27.4 Acknowledgements

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## 27.5 References

Bartlett, R. H., Gazzaniga, A. B., Fong, S. W. and Burns, N. E. (1974). *J. Thorac. Cardiovasc Surg.*, **68**, 918.

Becker, E. M., Smith, M. R. and Dorell, A. R. C. (1974). *Ann. Surg.*, **179**, 52.

Birek, A., Duffin, J., Glynn, M. F. X. and Cooper, J. D. (1976). *Trans. Amer. Soc. Artif, Int. Organs,* **22**, 94.

Carlson, R. G., Lande, A. J., Ivey, L. A., Subramanian, V. A., Bloch, J. R., Rogos, B., Landis, B., Baxter, J., Patterson, R. and Lillehei, C. (1973). *Surgery*, **72**, 913.

Clowes, G. H. A. (1960). *Physiol. Rev.*, **40**, 826.

David, J. L. and Herion, F. (1972). In Mannucci and Gorini (Eds.), *Platelet Function and Thrombosis, A Review of Methods. Advances in Experimental Medicine and Biology*, vol. 34, p. 335.

Drummond, A. H. and Gordon, J. L. (1974). *Thrombus. Diathes. Haemorrh.*, **31**, 366.

Duffin, J., Martin, B. and Cooper, J. D. (1976). *Can. Anaes. Soc. J.* (in press).

Dutton, R. C., Edmunds, L. H., Hutchinson, J. C. and Roe, B. B. (1974). *J. Thorac. Cardiovasc. Surg.*, **67**, 259.

Fehr, J., Graddock, P. R. and Jacob, H. S. (1975). *Amer. Soc. Hemat. Abstracts*, no. 162, 100.

Fong, S. W., Burns, N. E., Williams, G., Woldanski, C., Gazzaniga, A. B. and Bartlett, R. H. (1974). *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 239.

Heiden, D., Mielke, C. H., Rodvien, R. and Hill, J. D. (1975). *J. Thorac. Cardiovasc. Surg.*, **70**. 644.

Hill, J. D., Ratliff, J. L., Fallat, R. J., Tucker, H. J., Lamy, M., Dietrich, H. P. and Gerbode, F. (1974). *J. Thorac. Cardiovasc. Surg.*, **68**, 905.

Hollenberg, M., Pruett, R. and Thal, A. (1963). *J. Thorac. Cardiovasc. Surg.*, **45**, 402.

Kabat, E. A. and Mayers, M. M. (1971). In *Experimental Immunochemistry*, Thomas, Springfield, Illinois, p. 133.

Leonard, E. F., Colton, C. K., Craig, L. D., Gessler, R. M., Klein, E., Lontz, J. F., Lyman, D. L., Mason, R. G. and Nosell, H. L. (1974). Department of Health, Education and Welfare, publication no. (NIH) 74-605.

Lindsay, R. M., Prentice, C. R. N., Davidson, J. F., Burton, J. A. and McNicol, G. P. (1972a). *Brit. Med. J.*, **4**, 454.

Lindsay, R. M., Ferguson, D., Prentice, C. R. M., Burton, J. A. and McNicol, G. P. (1972b). *Lancet*, **2**, 1287.

Lindsay, R. M., Prentice, C. R. M., Burton, J. A., Ferguson, D. and Kennedy, A. C. (1973a). *Trans. Amer. Soc. Artif. Int. Organs*, **19**, 487.

Lindsay, R. M., Prentice, C. R. M., Ferguson, D., Muir, W. M. and McNicol, G. P. (1973b). *Brit. J. Haemat.*, **24**, 377.

Lindsay, R. M., Koens, F. and Linton, A. L. (1975). *J. Lab. Clin. Med.*, **86**, 5.

Packham, M. A., Warrior, E. S., Glynn, M. F., Senyi, A. S. and Mustard, J. F. (1967). *J. Exp. Med.*, **126**, 171.

Packham, M. A. and Mustard, J. F. (1968). *Proc. Soc. Exp. Biol. Med.*, **130**, 72.

Schmidt, P. J., Peden, J. C., Brecher, G., Baranovsky, I. (1961). *New Engl. J. Med.*, **265**, 1181.

Smythe, H. A., Ogryzlo, M. A., Murphy, E. A. and Mustard, J. F. (1965). *Can. Med. Assoc. J.*, **92**, 818.

Swank, R. L., Hirsch, H., Breuer, M. and Hissen, W. (1963). *Surg. Gynecol. Obstet.*, **117**, 547.

Williams, I. M., (1971). *Lancet*, **2**, 688.

Zapol, W. M., Quist, J., Pontoppidan, H., Liland, A., McEnany, T. and Laver, M. B. (1975). *J. Thorac. Cardiovasc. Surg.*, **69**, 439.

# 28

# INTERACTION OF PLATELETS WITH SURFACES: A FACTORIAL STUDY OF ADHESION AND ASSOCIATED RELEASE OF SEROTONIN

J. L. BRASH and S. J. WHICHER

**Summary**

Adhesion of platelets to several polymer surfaces and the release of serotonin from adherent platelets have been investigated. The platelets were washed, labelled with $^{51}Cr$ and $^{14}C$-serotonin and suspended in media containing different concentrations of red cells, albumin and fibrinogen. The apparatus was a couette flow device operated under well-characterised flow and transport conditions. Adhesion and release were measured by counting radioactivity of both isotopes on the surface and comparing to aliquots of suspension of known platelet concentration. A factorial experimental design was used to obtain estimates of the effects of haematocrit, platelet count, fibrinogen concentration and albumin concentration on adhesion and release. This approach also allowed estimates of the effects of the different surfaces, independent of any of these 'environmental' factors. The surfaces studied formed a series of segmented polyether urethanes and glass. Adhesion was found to be strongly dependent on the surface properties and decreased with increasing hydrophilicity of the polyurethanes. Release was somewhat less dependent on surface properties but increased with increasing hydrophilicity. The most important environmental factors for adhesion were haematocrit and fibrinogen concentration, both of which caused an increase. For release, haematocrit and fibrinogen were also important. However, release was found to decrease as the levels of these factors increased. Thus, the effects of surface properties and environmental factors were found to be opposite for the adhesion and release response.

## 28.1 Introduction

The interaction of platelets with surfaces in contact with blood is recognised as being important in the process of thrombogenesis. That the interaction begins with an adhesion event is well established and a considerable effort has been expended in studying the details of platelet adhesion to both subendothelial and artificial surfaces (Baumgartner *et al.*, 1976; Cazenave *et al.*, 1975; Brash *et al.*, 1976; Friedman *et al.*, 1970). However, little is known of the sequence of events following adhesion and leading up to the elaboration of a thrombus. Even less is known of the determinants which cause thrombus generation to

follow platelet adhesion in one situation and not in another, although it is true that some platelet adhesion is observed on all surfaces, even those that are thromboresistant. It might reasonably be expected that in situations where thrombus is formed, platelets that adhere undergo the release reaction, thus generating locally high concentrations of ADP and causing surface-localised platelet aggregation to occur. Accordingly, the experiments reported here are designed to determine the extent to which platelets that adhere to various surfaces subsequently or simultaneously undergo release, and thus hopefully to establish an additional criterion of thrombogenic tendency.

The surfaces used in this study were chosen from a group of segmented polyether urethanes. These materials are chemically similar but their compositional differences give rise to varying hydrophilicity. Segmented polyurethanes have been shown to be useful as cardiovascular prosthetic materials with their wide range of mechanical behaviour and their good, but little understood, blood compatibility (Brash *et al.*, 1973). Glass was also studied as a reference material, in order to allow placement of this work into the context of available literature on platelet-surface interaction.

The measurement of adhesion and release is based on double labelling of the platelets. $^{51}$Cr-sodium chromate, which labels the plasma membrane and cytoplasm (Steiner *et al.*, 1970), has been used as a measure of adhesion. $^{14}$C-serotonin has been used to label the amine storage granules, and the loss of $^{14}$C from adherent platelets then serves as a measure of the release reaction accompanying adhesion.

## 28.2 Experimental Methods and Design

*Platelet preparations*

Suspensions of washed platelets were prepared essentially as described by Mustard *et al.*, (1972). Pig blood was collected from the carotid artery into acid citrate dextrose (ACD) anticoagulant. The platelets were then separated by centrifugation, washed three times and suspended in Tyrode solution containing albumin (0.35 g/100 ml) and apyrase. The platelets were labelled in the first wash by incubating with $^{51}$Cr-$Na_2CrO_4$ and $^{14}$C-serotonin for 45 min at 37 °C and were suspended finally at the desired platelet count in Tyrode solution with appropriate protein and red cell content (see Experimental design p. ■■■) and with apyrase.

Albumin was bovine fraction V from ICN (Montreal). Fibrinogen was human Grade L from Kabi (Stockholm). Red cells were obtained from the same pig blood that provided the platelets. They were washed three times in a Tyrode-ACD medium as described previously (Feuerstein *et al.*, 1975).

*Surfaces*

Glass tubing was cleaned by treating sequentially with HCl, NaOH and distilled water.

Segmented polyurethanes were synthesised as described elsewhere (Brash *et al.*, 1973). Specimens for platelet studies were prepared by coating on Delrin tubing from solutions of the polymers in dimethylformamide or dimethylsulphoxide. These polyurethanes are based on methylene bis (4-phenyl isocyanate), ethylene diamine and either polyoxypropylene (PPG) or polyoxyethylene glycol (PEG). Three different materials were used in the present study. These are based respectively on PPG of molecular weight 1200, PEG 600 and PEG 1540. The resulting polymers are henceforward referred to as PPG 1200, etc. They are chemically similar but have different mechanical properties and varying water absorption when equilibrated in aqueous media. Water contents, determined gravimetrically, were found to be: PPG 1200, 3.2%; PEG 600, 16.9%; PEG 1540, 66.8%. Before carrying out adhesion measurements, the coated specimens were equilibrated in Tyrode solution for 24 h.

*Measurement of adhesion and release*

The surfaces were exposed to platelet suspensions in a couette flow device as described in detail previously (Feuerstein *et al.*, 1975). Flow and mass transport conditions are well defined in this system. The device consists basically of a cylindrical probe the surface of which is coated with the test material. The probe rotates concentrically in a test tube containing the platelet suspension. In the present work, all runs were conducted for 8 min at a rotational speed of 100 rev/min (wall shear rate 19.1/s) and a temperature of 37°C.

After exposure to the platelet suspension the probes are withdrawn and shaken to remove excess fluid, then rinsed by rotating for 2 min in Tyrode solution.

*Radioactivity counting*

To remove platelets from the surface for counting, the rinsed specimens are placed for 2 h in counting vials each containing 10 ml of a scintillation cocktail. The solid specimens are then removed and the liquid extract counted for both $^{51}Cr$ and $^{14}C$ in a Phillips 4501/01 automatic scintillation analyser.

Adhesion and release are determined by comparing the radioactivity of the surface with that of an aliquot of suspension of known platelet concentration. In the absence of demonstrable platelet lysis, the surface count of $^{51}Cr$ can be used to compute the number of platelets adhering per unit area when compared to the suspension count. The surface count of $^{14}C$, when compared to the $^{51}Cr$ surface count and to the solution $^{14}C$ count, will show a proportionate decrease if release of serotonin has accompanied adhesion. The amount of the decrease can be converted into quantitative release parameter.

Quench corrections are applied via an external standard channels ratio method. This involves the addition of known amounts of radioactivity to a typical unlabelled experimental solution. By comparing the measured to the known radioactivity an estimate of quenching can be obtained. Corrected counting rates are used to compute adhesion number and percent release as follows:

$$\text{Adhesion number} = \text{Number of platelets per } 1000\ \mu m^2$$

$$= \frac{(\text{platelet count per } \mu l) \times Cr \times 1000}{\text{surface area } (\mu m^2)}$$

$$\text{Percent release} = \text{percent } {}^{14}C \text{ radioactivity released from adherent platelets}$$

$$= (A\text{-}B)/B$$

$$\text{where A} = \frac{{}^{51}Cr \text{ cpm on surface}}{{}^{51}Cr \text{ cpm per } \mu l \text{ of suspension}}$$

$$B = \frac{{}^{14}C \text{ cpm on surface}}{{}^{14}C \text{ cpm per } \mu l \text{ suspension}}$$

*Experimental design*

As well as the surface properties themselves, various 'environmental' factors have been shown to be important for platelet adhesion. Among these are the protein composition of the medium (Packham *et al.*, 1969; Brash *et al.*, 1976) and haematocrit (Feuerstein *et al.*, 1975). On the other hand, little is known of the effect of these factors on release from adherent platelets. Since it seemed they might be important and since the methods used in this work allow closely controlled variation of these factors, we have attempted to elucidate their effects on both adhesion and release. This has been done using a $2^k$ factorial experimental design as described by Box and Hunter (1961). With this approach the individual effects of $k$ factors can be estimated as well as the interaction (i.e. synergistic) effects for any combination of the factors. The estimates obtained are entirely uncorrelated one with another.

In the present work four factors have been chosen for study, namely, albumin concentration, fibrinogen concentration, platelet count and haematocrit. In the two level factorial design the values of each factor are set at one of two levels, low and high. All possible combinations of high and low levels of the factors are considered, thereby generating a total of $2^k$ experiments for $k$ factors. The levels of the factors are scaled so that their design values are + 1 (high), − 1 (low) or 0 (centre of the range). Table 28.1 shows the design matrix for a $2^4$ design relevant to the present work, and indicates the scaling factors required to convert from scaled to conventional units.

By suitable analysis of the results (Box *et al.*, 1961), numerical estimates of the effects of changing the value of each factor from its low to its high level are obtained.

## 28.3 Results

Table 28.2 shows adhesion number and percent release data for the four surfaces studied. Two sets of data were obtained for each set of conditions, and the results are shown as means. The data for PEG 1540 are incomplete so the

**Table 28.1**

Experimental design-suspension concentration levels ($x$) of environmental factors. (1) The values used to calculate the two factor interactions are obtained by multiplying together the two appropriate columns. (2) Two runs were made for each set of conditions. (3) The experiments were conducted in random order, two on a given day

| *Experiment* | *Factor 1 Albumin concentration ($x_1$)* | *Factor 2 Fibrinogen concentration ($x_2$)* | *Factor 3 platelet count ($x_3$)* | *Factor 4 Haematocrit ($x_4$)* |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 |
| 2 | −1 | 1 | 1 | 1 |
| 3 | 1 | −1 | 1 | 1 |
| 4 | −1 | −1 | 1 | 1 |
| 5 | 1 | 1 | −1 | 1 |
| 6 | −1 | 1 | −1 | 1 |
| 7 | 1 | −1 | −1 | 1 |
| 8 | −1 | −1 | −1 | 1 |
| 9 | 1 | 1 | 1 | −1 |
| 10 | −1 | 1 | 1 | −1 |
| 11 | 1 | −1 | 1 | −1 |
| 12 | −1 | −1 | 1 | −1 |
| 13 | 1 | 1 | −1 | −1 |
| 14 | −1 | 1 | −1 | −1 |
| 15 | 1 | −1 | −1 | −1 |
| 16 | −1 | −1 | −1 | −1 |
| 17 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 |

Scaling factors: to obtain suspension concentrations in conventional units:

$$C_1\,(\text{mg\%}) = 17.5\,(1 + x_1); C_2\,(\text{mg\%}) = 15.0\,(1 + x_2),$$

$$C_3\,(\text{pl}/\mu\text{l}) = 10^5(5 + 2x_3); C_4\,(\text{vol\%}) = 17.5\,(1 + x_4).$$

estimates of effects for this surface are somewhat correlated with each other. The experimental conditions in column 1 correspond to the rows of the design matrix (table 28.1). Also shown are the mean values averaged over all the experimental conditions. These values represent estimates of the relative effects of the different surfaces, uncorrelated with any of the environmental factors. It can be seen that adhesion decreases in the series 1200 PPG, 600 PEG, and 1540 PEG and this is the order of increasing hydrophilicity. Percent release for platelets that have adhered shows the opposite trend and increases with increasing hydrophilicity of the polyurethanes. The differences in percent release are not highly significant, however, whereas those for adhesion are significant at the 5% level ($t$-statistic).

Table 28.3 shows the main effects on adhesion and release of the four suspension factors. These are computed as described by Box and Hunter (1961) by summing for the appropriate column of the design matrix the products

**Table 28.2**

Measured values of adhesion* and release reaction† for platelet interaction with various surfaces

| *Experimental conditions*‡ | *Glass* | | *PPG 1200* | | *PEG 600* | | *PEG 1540* | |
|---|---|---|---|---|---|---|---|---|
| | *Adhesion* | *Release* | *Adhesion* | *Release* | *Adhesion* | *Release* | *Adhesion* | *Release* |
| 1 | 33.64 | 22.5 | 95.57 | 19.9 | 34.94 | 38.3 | 1.43 | 21.6 |
| 2 | 29.54 | 7.5 | 35.20 | 0 | 21.34 | 12.8 | 0.86 | 18.7 |
| 3 | 2.68 | 47.2 | 2.57 | 48.0 | 1.82 | 34.0 | 0.75 | 58.3 |
| 4 | 24.06 | 44.4 | 36.35 | 55.1 | 3.72 | 70.8 | 0.89 | 76.6 |
| 5 | 11.03 | 34.6 | 33.70 | 44.6 | 14.71 | 45.0 | 0.90 | 44.6 |
| 6 | 9.91 | 20.7 | 33.48 | 14.5 | 5.59 | 56.2 | 1.33 | 55.7 |
| 7 | 7.15 | 33.0 | 2.21 | 55.8 | 2.49 | 49.4 | | |
| 8 | 6.08 | 17.6 | 19.48 | 42.7 | 6.81 | 52.9 | 0.74 | 65.9 |
| 9 | 3.08 | 0.7 | 2.16 | 11.3 | 5.66 | 34.2 | 1.31 | 36.4 |
| 10 | 2.75 | 42.4 | 11.73 | 56.1 | 13.01 | 57.5 | | |
| 11 | 3.23 | 19.2 | 2.84 | 28.6 | 1.99 | 21.4 | | |
| 12 | 1.71 | 0 | 1.75 | 10.1 | 1.92 | 3.6 | 0.79 | 28.9 |
| 13 | 1.14 | 0 | 1.75 | 22.5 | 1.26 | 33.7 | | |
| 14 | 2.45 | 33.0 | 12.02 | 37.2 | 16.11 | 27.4 | 0.95 | 46.0 |
| 15 | 0.77 | 28.0 | 1.04 | 52.3 | 1.14 | 46.1 | 0.69 | 73.4 |
| 16 | 3.54 | 34.2 | 9.71 | 76.9 | 7.33 | 82.4 | | |
| 17 (+ 18) | 27.84 | 42.0 | 36.3 | 38.6 | 8.80 | 31.5 | 1.42 | 54.6 |
| Mean | 11.02 | 26.0 | 20.98 | 36.3 | 8.75 | 40.7 | 1.01 | 48.8 |
| 95% confidence interval | 15.0<br>7.04 | 31.8<br>20.3 | 29.23<br>12.73 | 43.7<br>28.8 | 11.80<br>5.69 | 47.5<br>33.9 | 1.16<br>0.87 | 56.3<br>41.4 |

*Platelets per 1000 $\mu m^2$

†Percent serotonin released from adhering platelets.

‡See table 28.1

**Table 28.3**

Main effects of environmental factors on adhesion and release reaction of platelets interacting with various surfaces.

| Surface | Grand average‡ | | Effects β* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Albumin | | Fibrinogen | | Platelet count | | Haematocrit | |
| | Adhesion | Release | Adhesion | Release | Adhesion | Release | Adhesion | Release | Adhesion | Release |
| Glass | 11.02 | 26.0 | −2.16 | 2.2 | 5.54 | −18.0† | 7.32 | −15.6† | 13.18 | −15.6† |
| PPG 1200 | 20.98 | 36.3 | −2.30 | 2.0 | 18.76 | −45.8† | 9.42 | −27.4† | 27.0† | −38.4† |
| PEG 600 | 8.75 | 40.7 | −1.48 | −2.2 | 10.68† | −47.0† | 3.62 | −28.8† | 5.38 | −14.0 |
| PEG 1540 | 1.01 | 48.8 | −0.02 | −14.0† | 0.36 | −36.8† | 0.20 | −31.4† | −0.04 | 2.2 |

*β is amount by which the adhesion number (platelets/1000 $\mu m^2$) or % release changes when the factor changes from its low to its high value (see text)

†Effects significantly different from zero at 95% confidence level.

‡See table 28.2.

**Table 28.4**

Two factor interaction effects of environmental factors on adhesion and release reaction of platelets interacting with various surfaces.
A: albumin concentration, F: fibrinogen concentration, P: platelet concentration, H: haematocrit.

| | Effects β | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A-F | | A-P | | A-M | | F-P | | F-H | | P-H | |
| | Adhesion | Release | Adhesion | Release | Adhesion | Release | Adhesion | Release | Adhesion | Release | Adhesion | Release |
| Glass | 3.22 | −15.6* | −1.70 | 3.8 | −1.60 | 16.6* | 3.80 | −8.6 | 5.70 | −16.6* | 6.62 | −6.2 |
| PPG 1200 | 12.36 | −18.0 | 6.70 | −11.2 | 4.56 | 9.0 | 3.64 | −2.2 | 15.68 | −33.4* | 10.92 | −8.8 |
| PEG 600 | 1.6 | 0.4 | 2.58 | −5.6 | 5.6 | −19.8 | 5.70 | −11.8 | 4.76 | −24.6* | 4.44 | −13.4 |
| PEG 1540 | 1.18 | 1.0 | 1.32 | −9.2 | −1.1 | −22.8* | −1.14 | −16.8 | 1.10 | −9.2 | 0.98 | 16.2 |

*Effects significantly different from zero at 95% confidence level.

(observation × corresponding matrix element) and dividing the sum by N/2 where $N = 2^4$. These estimates, $\beta$, represent the amount by which the adhesion number (platelets/1000 $\mu m^2$) or percent release changes when the environmental factor changes from its low to its high value for any set of conditions. For example, the effect of changing from zero to 35% haematocrit with a glass surface is to increase adhesion by 13.18 platelets/1000 $\mu m^2$ and to decrease percent release by 15.6. Confidence intervals for the estimates of the effects are not shown in table 28.3 but in cases where the effect is significantly different from zero the value is marked with a double asterisk. Thus it can be seen that albumin concentration has little effect on adhesion or release, fibrinogen concentration has a significant negative effect on release, but affects adhesion only on PEG 600. Platelet count does not significantly affect adhesion but has a negative effect on release while haematocrit has a positive effect on adhesion to PPG 1200 and PEG 1540 and a negative effect on release for PPG 1200 and glass. It is noteworthy that the factors studied have generally negative effects on release and positive effects on adhesion.

In table 28.4 are presented two-factor interaction effects derived from the factorial design and analysis. As can be seen the only effects significantly different from zero are effects on release. These are principally for factor pairs involving haematocrit, namely albumin-haematocrit and fibrinogen-haematocrit.

## 28..4 Discussion

The present study shows that substantial release of serotonin occurs from platelets that adhere to glass and polyurethanes. The mean percent release values (table 28.2) vary between 26 and about 50 although only glass versus PEG 600 and glass versus PEG 1540 are significantly different at the 5% probability level.

Adhesion is significantly different for all pairs of surfaces except glass versus PPG 1200 and glass versus PEG 600. Using the same apparatus it was previously concluded that no difference in saturation levels of adhesion existed among various surfaces (Brash *et al.*, 1976). In these earlier studies the range of conditions was not as great and adhesion data were not averaged over all conditions as in the present work. The PEG 1540 surface, which has not been studied previously, is particularly notable for its low value of adhesion.

It is of interest to note that within the polyurethane series the percent release increases with hydrophilicity whereas the adhesion number decreases. No convincing explanation can be offered for these opposing trends but the observations suggest that platelet surface density in a given test system should not be taken as anything but a crude indicator of overall platelet response, nor should percent release alone be used as the criterion of platelet-surface reactivity. A better index might be the product of adhesion number and percent release. On this basis the surfaces in the present study would rank in the same order as on the basis of adhesion number alone, except that PEG 600 would appear as a more reactive surface than glass, the opposite to what would be concluded from adhesion number.

The work of Packham *et al.* (1969) has some relevance to the present results. These authors measured release of serotonin into the suspending fluid from platelets that were exposed to glass, siliconised glass, fibrinogen, albumin, and γ-globulin-coated glass. Some release was observed with all surfaces studied but was most marked with the γ-globulin-coated glass. Inhibitory effects of added gelatin and albumin were also noted, the latter in agreement with the present results.

Lindsay *et al.* (1976) have also reported that contact of platelets with silicone rubber surfaces such as are used in membrane oxygenators, causes release of serotonin. Such release can be detected as a significant drop in intra-platelet serotonin in the platelet suspension implying that nonadhesive as well as adhesive encounters cause serotonin release. These authors also reported a simultaneous activation of the plasma complement system and suggested an association of this with the platelet release reaction.

Recently, Baumgartner *et al.* (1976) reported on the disappearance of organelles (determined microscopically) from platelets that had adhered from flowing whole blood to various surfaces. They found that platelets adherent to collagen fibres or subendothelium lost virtually all of their amine storage granules whereas the loss on an epoxide resin or elastin surface was only about 10%. These are respectively larger and smaller extents of release than observed for any of the surfaces studied here, but support the conclusion that adhesion is in general accompanied by at least some release of granule constituents. These authors concluded that release is a more surface-specific effect than adhesion and that the two phenomena are at least partly independent. In the present work, surface specificity is more apparent for adhesion than release.

The effects of protein, red cell and platelet content of the medium on adhesion and release (tables 28.3 and 28.4) are deserving of further comment. For adhesion the most important environmental factors are haematocrit and fibrinogen concentration. Red cell effects on adhesion have been noted previously (Feuerstein *et al.*, 1975; Brash *et al.*, 1976) and there is widespread recognition of the effects of red cells in augmenting platelet transport (Goldsmith, 1971; Turitto *et al.*, 1972). Substantial haematocrit-platelet count interaction effects (table 28.4) support this concept. Fibrinogen may exert its effect on adhesion by adsorption to the platelet membrane and/or to the test surface. An enhancement of adhesion on a pre-adsorbed fibrinogen layer has been observed by Packham *et al.* (1969). Also Mason *et al.* (1971) have noted the increase of platelet adhesion to glass with increase in fibrinogen concentration.

In contrast to their positive effects on adhesion, the various environmental factors exert mainly negative effects on release of serotonin. Fibrinogen has a particularly strong inhibitory effect, but again it is not possible to deduce whether this is exerted through adsorption to the contact surface or to the plasma membrane. Release is also inhibited by red cells and by platelet concentration itself, and mechanistic bases for these effects are even more difficult to envisage. Significant two factor interactions are fewer than main

effects and again, for the most part, are negative, the most important of them being the fibrinogen-red cell interaction. Finally, it should be mentioned that aggregates on the surface or in the suspension were not detected, and any release of ADP implied by the results presented here must have been insufficient to cause detectable aggregation. Longer-term experiments or experiments in which coagulation can interact with platelet reactions may be required to connect the effects reported here with ultimate thrombus formation.

## 28.5 Acknowledgements

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## 28.6 References

Baumgartner, H. R., Muggli, R., Tschopp, T. B. and Turitto, V. T. (1976). Platelet adhesion, release and aggregation in flowing blood: effects of surface properties and platelet function. *Thrombos. Diathes. Haemorrh.*, **35**, 124.

Box, G. E. P. and Hunter, J. S. (1961). The $2^{k-p}$ fractional factorial designs. Part I. *Technometrics*, **3**, 310.

Brash, J. L., Fritzinger, B. K. and Bruck, S. D. (1973). Development of block copolyether-urethane intra-aortic balloons and other medical devices. *J. Biomed. Mater. Res.*, 7, 313.

Brash, J. L., Brophy, J. M. and Feuerstein, I. A. (1976). Adhesion of platelets to artificial surfaces: effect of red cells. *J. Biomed. Mater. Res.*, **10**, 429.

Cazenave, J. P., Packham, M. A., Guccione, M. A. and Mustard, J. F. (1975). Inhibition of platelet adherence to damaged surface of rabbit aorta. *J. Lab. Clin. Med.*, **86**, 551.

Feuerstein, I. A., Brophy, J. M. and Brash, J. L. (1975). Platelet transport and adhesion to reconstituted collagen and artificial surfaces. *Trans. Amer. Soc. Artif. Int. Organs*, **21**, 427.

Friedman, L. I., Liem, H., Grabowski, E. F., Leonard, E. F. and McCord, C. W. (1970). Inconsequentiality of surface properties for initial platelet adhesion. *Trans. Amer. Soc. Artif. Int. Organs*, **16**, 63.

Goldsmith, H. L. (1971). Red cell motions and wall interactions in tube flow. *Fed. Proc.*, **26**, 1578.

Mason, R. G., Read, M. S. and Brinkhous, K. M. (1971). Effect of fibrinogen concentration on platelet adhesion to glass. *Proc. Soc. Exp. Biol. Med.*, **137**, 680.

Mustard, J. F., Perry, D. W., Ardlie, N. G. and Packham, M. A. (1972). Preparation of suspensions of washed platelets from humans. *Brit. J. Haemat.*, **22**, 193.

Packham, M. A., Evans, G., Glynn, M. F. and Mustard, J. F. (1969). The effect of plasma proteins on the interaction of platelets with glass surfaces. *J. Lab. Clin. Med.*, **73**, 686.

Steiner, M. and Baldini, M. (1970). Subcellular distribution of $^{51}Cr$ and characterisation of its binding sites in human platelets. *Blood*, **35**, 727.

Turitto, V. T., Benis, A. M. and Leonard, E. F. (1972). Platelet diffusion in flowing blood. *Ind. Eng. Chem. Fundam.*, **11**, **216**.

# 29

# PLATELET-PROTEIN INTERACTIONS: ROLE OF PROTEINS IN PLATELET AGGREGATION AND ADHESION

S. W. KIM

**Summary**

The phenomena of platelet aggregation and adhesions, which are important in haemostasis and thrombosis, have been studied by many investigators without much success. It is hypothesised that platelet aggregation is mediated by an excess binding between platelets and glycoproteins and platelet adhesion to surfaces occurs through the formation of complex bridges between platelets and adsorbed glycoproteins. We have studied the interactions of oligosaccharide groups in fibrinogen and gamma globulins with platelets. The results provide evidence for the involvement of platelet-protein reactions in platelet aggregation and adhesion to surfaces.

## 29.1 Introduction

Normal blood circulation within the body has an effective infinite clotting time. Platelets circulate in the blood as round disc-shaped cells that adhere neither to each other nor to normal endothelium. However, platelets aggregate in normal haemostasis and unusual platelet functions account for the bleeding symptoms in a wide variety of clinical conditions. Platelets also adhere to foreign surfaces upon contacting blood and this causes thrombus formation. It has been known that platelet functions for haemostasis and thrombosis involve multifactors and a complicated biological process. Many investigators have studied the related problems in this area based on various aspects of platelet physiology and biochemistry (Weiss, 1972).

The present communication summarises our early results and recent data on platelet-protein interactions which are applicable for describing a possible mechanism of platelet aggregation and adhesion. More emphasis will be given on the mechanism of platelet adhesion to polymer surfaces.

## 29.2 Basic Concepts

The concept of a plasmatic atmosphere of proteins necessary for optimum platelet function was formulated by Roskam (1923) more than 50 years ago. It has been postulated that several plasma procoagulants, most notably fibrinogen

and Hageman factor, play an important role in the platelet aggregation. Numerous reports have dealt with the identification of clotting factors that presumably, through adsorption to the platelet surface, create the right plasmatic atmosphere and allow platelets to fulfil the essential role of releasing clotting accelerator substances. However, an exact definition of the plasmatic atmosphere as it applies to protein requirements for platelet aggregation and adhesion has not been formulated. Recently Bang *et al.* (1972) showed that three proteins, fibrinogen, gamma globulins and Hageman factor, are capable of restoring aggregation capability of washed human platelets. Packham *et al.* (1969) demonstrated that an albumin precoated surface showed little platelet adhesion, but that fibrinogen or gamma globulin coated surfaces had more adhering platelets. Mason *et al.* (1971) indicated that fibrinogen is required for platelet adhesion to glass and that the fibrinogen must be adsorbed to glass first so that the platelets adhere to the adsorbed fibrinogen films (Zucker and Vroman, 1969). This is consistent with electron microscopy studies (Mason *et al.*, 1971) in which platelets were found adhering to an adsorbed protein layer. Pre-adsorbed gamma globulin has also been shown to induce platelet adhesion and release (Packham *et al.*, 1969).

It is very interesting to note that fibrinogen and gamma globulin (predominantly IgG immunoglobin) contain oligosaccharide chains of the same structural pattern with the sequence:

N-acetylneuraminic acid (or fucose) → galactose →

N-acetylglucosamine → mannose → N-acetylglucosamine →

amino acid asparagine.

Albumin does not contain such oligosaccharide chains (Sprio, 1970). Fibrinogen probably contains six oligosaccharide chains occurring in three pairs on the molecule (Mester, 1968). IgG has two or three carbohydrate chains. Each chain is highly branched with one N-acetylneuraminic acid, three N-acetylglucosamine and two fucose residues as the terminal units (Clamp and Putnam, 1964). Because of the microheterogeneity of glycoprotein it is suggested that fibrinogen and gamma globulin would contain incomplete oligosaccharide chains showing terminal galactose, N-acetylglucosamine and even mannose residues as well as the complete unit with terminal N-acetylneuraminic acid. The significance of this statement will become apparent.

A number of platelet membrane-associated enzymes have been isolated. Of particular interest is a glycoprotein called galactosyl transferase (Bosmann, 1971) and a glycoprotein: N-acetylneuraminic acid transferase (Bosmann, 1972). Both are located in the platelet plasma membrane and require divalent cations for activity. Glycoprotein: galactosyl transferase catalyses the reaction:

UDP-galactose + acceptor → acceptor-galactose + UDP.

The highest activity occurs when the acceptor has N-acetylglucosamine as the terminal residue. The glycoprotein: N-acetylneuraminic acid transferase catalyses:

CMP-N-acetylneuraminic acid + acceptor → acceptor-N-acetylneuraminic acid + CMP.

Both of these enzymes have the capability of complexing, along with the proper sugar nucleotide donor and divalent cation, with a glycoprotein containing incomplete oligosaccharide chains. Bosmann (1972) also demonstrated this phenomenon by proving that glycoprotein: N-acetylneuraminic acid transferase demonstrated activity with prothrombin as the glycoprotein acceptor, particularly when further N-acetylneuraminic acid residues were removed by neuraminidase.

Recently, we found that the interaction of washed platelets with galactose oxidase-treated fibrinogen is about five times less than with native fibrinogen. Similar trends were obtained with the use of gamma globulin. The aggregating capability of platelets with galactose oxidase-treated fibrinogen was much lower than with native fibrinogen (Kim *et al.*, 1976a). This experiment shows indirect evidence for the involvement of the oligosaccharide group in the platelet aggregation. Glycoproteins can be bridges between platelet and platelet but the excess binding of glycoproteins to the platelet surface may cause a drastic change of cell nature resulting in platelet release reaction. These experiments are being continued by our group towards a detailed understanding of platelet aggregation.

Several investigators (Baier and Dutton, 1969; Varco 1971; Williams *et al.*, 1973) have found that foreign surfaces adsorb plasma proteins when exposed to blood. Since platelet adhesion to the surface is the first observable event occurring in clotting on foreign surfaces and since platelets are known to participate in haemostatic and coagulation events, the indication is that platelet adhesion plays a major role in the *in vivo* initiation of thrombus formation on foreign surfaces. Our studies indicated that proteins were readily adsorbed from their aqueous solutions or from human plasma onto a variety of hydrophobic polymers. Adsorption of albumin, gamma globulin and fibrinogen from single solutions onto several hydrophobic polymers was studied (Brash and Lyman, 1968; Lee and Kim, 1974a) using internal reflection infrared spectroscopy. The adsorption isotherm appeared to be of Langmuir-type. The calculated rate and amount of protein adsorbed were dependent upon the nature of polymer substrate and flow rate of the solution (Kim *et al.*, 1975). Competitive adsorption of the plasma proteins onto three hydrophobic polymer surfaces has been investigated (Lee *et al.*, 1974) by using $^{125}$I radiolabelled proteins to determine the rate and adsorbed concentration of each protein from a mixture of albumin, gamma globulin and fibrinogen in PBS solution. Fresh plasma was also used for adsorption studies in a similar manner (Kim *et al.*, 1976b). The pattern of competitive adsorption from solution was similar to that from human plasma.

We proposed (Lee and Kim, 1974b, Kim *et al.*, 1974) that platelet adhesion to polymer surfaces is mediated by formation of enzyme-substrate complex bridges between platelet glycosyl transferases and incomplete heterosaccharides present in surface adsorbed glycoproteins. Therefore, the adsorbed protein layer

functions as the acceptor to determine platelet adhesion. Hydrophobic polymers adsorb proteins readily. However, the surface composition of each protein and the adsorption rates are dependent upon the the type of polymer surface. With hydrophilic gels such as poly(hydroxyethyl methacrylate), preliminary experiments indicate that little protein is adsorbed if that protein is desorbed depending on the water percentage in the polymer gel.

When blood contacts a polymer surface, plasma proteins adsorb on the surface until an equilibrium plateau concentration is reached. A glycosyl transferase on a platelet interacts with a nucleotide diphosphate monosaccharide and a divalent cation with a glycoprotein acceptor adsorbed to the foreign surface. The stability of the complex would determine the strength and duration of the adhesion. Platelets have been found (Weiss, 1972) to contain a variety of glycosidases. It is conceivable that the platelet glycosidases could cleave terminal N-acetylneuraminic acid residues, thereby exposing new acceptors on the glycoproteins for continued complex formation and adhesions.

### 29.3 Platelet-Protein Interaction Mechanism

Our observations are consistent with the following proposed mechanism.

(1) The interactions of washed bovine platelets with fibrinogen or gamma globulins are about five times greater than with galactose oxidase-treated fibrinogen or gamma globulins. Albumin binding to platelets is negligible as shown by Salzman (1972). Platelet aggregation caused by galactose oxidase-treated fibrinogen is minimal.
(2) The first *in vitro* series of experiments assayed two glycosyl transferases using adsorbed plasma proteins on Silastic rubber tubing (polydimethylsiloxane, Dow Corning, medical grade) as acceptors. Sugar nucleotides could be transferred onto adsorbed gamma globulin and fibrinogen but not onto adsorbed albumin.
(3) The second group of experiments quantified platelets adhering to tubing coated with protein and subjected to treatments to remove different carbohydrate residues.
(4) Using whole unheparinised bovine blood, more platelets adhered to fibrinogen or gamma globulin coatings which had been either desialised or had both sialic acid and galactose removed than to the same coatings when untreated.
(5) In the plasma adsorption experiments, fibrinogen is at all times the major constituent adsorbing to hydrophobic polymers. Hydrophobic polymers include polyether-urethane-urea based on polypropylene glycol, methylene-bis-4-phenylisocyanate, and ethylene diamine (PEUU), Silastic rubber (SR), and fluorinated ethylene/propylene copolymer (FEP, Dupont's Teflon). However, most of the protein adsorbed early on PEUU, a relatively nonthrombogenic polymer, is albumin. Albumin is only a minor contributor to the early FEP, a relatively thrombogenic polymer, and adsorbs intermediately on SR having intermediate thrombogenicity. By comparison with the weight percentage of the proteins in the bulk solution (albumin 55%, gamma globulin 28%, and fibrinogen 17%) it

is apparent that in no case are the adsorbed percentages similar to those in the solution. This means that the surface selects between the proteins and does not adsorb them only in relation to their numbers. Biomer (Ethicon Co.), which is noteworthy for its relative haemocompatibility when utilised in total heart implants, adsorbs very large amounts of albumin upon contacting human plasma (Kim *et al.*, 1976b).

(6) Hydrogels such as poly(hydroxyethyl methacrylate), which have been known as good blood compatible materials, due to relatively high water content, showed lower amounts of adsorbed proteins with longer plateau times. Therefore, platelets would show little tendency to adhere to such proteinated surfaces due to the presence of few acceptor sites. This is verified by the fact that platelet studies on hydrogel-coated carbon show very little platelet adhesion (Andrade, 1971).

Barber and Jamieson (1971) and Bosmann (1972) suggested that the carbohydrate side chains of collagen may be involved in the adherence of platelets to collagen. Though the lack of evidence for the hypotheses has been reported by other investigators (Muggli and Baumgartner, 1973; Cazenave *et al.*, 1974), this can be accepted considering that any surface, including collagen, adsorbs proteins to form a proteinous layer upon contacting platelet rich plasma or blood. The protein layer may determine the extent of platelet adhesion as previously described.

A question might arise from the above mentioned mechanisms as to why platelets fail to adhere to each other though blood normally contains platelets, proteins and $Ca^{2+}$. The following schemes can provide the answer:

$$\text{platelets} + \text{glycoproteins} \rightleftharpoons \text{platelets-glycoproteins} \quad \text{(a)}$$

This is a reaction scheme between platelets and plasma glycoproteins (mainly fibrinogen and gamma globulins). The equilibrium is toward the direction of the left side. This can be considered for the normal platelets or physiological condition. However,

$$\text{platelets} + \text{glycoproteins} \rightleftharpoons \text{platelets-glycoproteins} \quad \text{(b)}$$

For this reaction, the equilibrium in (a) is modified by excess binding of glycoprotein with platelets due to the favourable forward reaction. The changes of platelet cell surfaces either by drugs or abnormal physiological conditions may cause the shift of equilibrium resulting in platelet aggregation. Excess glycoproteins on cells may become bridges and/or induce platelet release reaction causing aggregation.

The last scheme is platelet adhesion to surfaces:

$$\text{platelet} + \text{adsorbed glycoproteins} \longrightarrow \text{platelet-adsorbed glycoproteins} \quad \text{(c)}$$

This is assumed to be an irreversible process and platelets adhere to foreign surfaces through the layer of adsorbed proteins as discussed previously.

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## 29.5 References

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood perfusion: HEMA/ activated carbon *Trans. Amer. Soc. Artif. Int. Organs,* **17**, 222.

Baier, R. E. and Dutton, R. C. (1969). Initial events in interactions of blood with a foreign surface. *J. Biomed. Mater. Res.*, **3**, 191.

Bang, N. U., Heidenreich, R. O. and Trygstad, G. W. (1972). Plasma protein requirements for human platelet aggregation. *Ann. New York Acad. Sci.*, **201**, 280.

Barber, A. J. and Jamieson, G. A. (1971). Platelet collagen adhesion characterisation of collagen glucosyltransferase of plasma membranes of human blood platelets. *Biochim. Biophys. Acta*, **252**, 533.

Bosmann, H. B. (1971). Platelet adhesiveness and aggregation: the collagen: glucosyl polypeptide: N-acetyl galactosaminyl and glucoprotein: galactosyl transferases of human platelets. *Biochem. Biophys. Res. Comm.*, **43**, 118.

Bosmann, H. B. (1972). Platelet adhesiveness and aggregation: surface sialic acid, glycoprotein: N-acetylneuraminic acid transferase and neuraminidase of human blood platelets. *Biochim. Biophys. Acta*, **279**, 456.

Brash, J. L. and Lyman, D. J. (1968). Adsorption of plasma proteins in solution to uncharged hydrophobic polymer surfaces. *J. Biomed. Mater. Res.*, **3**, 175.

Cazenave, J. P., Guccione, M. A., Mustard, J. F. and Packham, M. A. (1974). Lack of effect of UDP, UDPG and glucosamine on platelet reactions with collagen. *Thrombos. Diathes. Haemorrh.*, **31**, 521.

Clamp, J. R. and Putnam, F. W. (1964). The carbohydrate prosthetic group of human $\gamma$-globulin. *J. Biol. Chem.*, **239**, 3233.

Kim, S. W., Lee R. G., Coleman, D., Oster, H., Andrade, J. D. and Olsen, D. B. (1974). Platelet adhesion to polymer surfaces. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 449.

Kim, S. W., Lee, E. S. and Lee, R. G. (1975). *Adsorption of Blood Proteins onto Polymer Surfaces. Advances in Chemistry Series*, American Chemical Society, Vol. 145, 218.

Kim, S. W., Lee, E. S. and Lee, R. G. (1976a): Effect of oligosaccharide terminal groups of glycoprotein on platelet aggregation. Personal communication.

Kim, S. W., Wisniewski, S., Lee, E. S. and Winn, M. L. (1976b). Role of protein and fatty acid adsorption on platelet adhesion and aggregation at blood polymer interface. *J. Biomed. Mater. Res.* (in press).

Lee, R. G. and Kim, S. W. (1974a). Adsorption of proteins onto polymer surfaces: adsorption isotherm and kinetics. *J. Biomed. Mater. Res.*, **8**. 251.

Lee, R. G. and Kim, S. W. (1974b). The role of carbohydrate in the platelet adhesion to foreign surfaces. *J. Biomed. Mater. Res.*, **8**, 393.

Lee, R. G., Adamson, C. and Kim, S. W. (1974). The competitive adsorption of plasma proteins onto polymer surfaces. *Thrombos. Research*, **4**, 485.

Mason, R. G., Read, M. S. and Brinkhaus, K. M. (1971). Effect of fibrinogen concentration on platelet adhesion to glass. *Proc. Soc. Exp. Biol. Med.*, **137**, 680.

Mester, L. (1968). In K. Laki (Ed.), *Fibrinogen,* Marcel Dekker, New York, p. 165.

Muggli, R. and Baumgartner, H. R. (1973). Collagen induced platelet aggregation: requirement for tropocollagen multimers. *Thrombos. Research,* **3**, 715.

Packham, M. A., Evans, G., Glynn, M. F. and Mustard, J. F. (1969). The effect of plasma proteins on the interaction of platelets with glass surfaces. *J. Lab. Clin. Med.*, **73**, 686.

Roskam, J. (1923). Contribution à l'étude de la physiologie normale et pathologique du globulin. *Arch. Intern. Physiol.*, **20**, 241.

Salzman, E. W. (1972). In M. L. Hiar (Ed.), *Chemistry of Biological Surfaces,*, vol. 2, Marcel Dekker, New York.

Spiro, R. G. (1970). Glycoproteins. *Ann. Rev. Biochem.*, **39**, 599.

Varco, R. L. (Ed.) (1971). *Conference on Moving Blood.* Fed. Amer. Soc. Exp. Biology.

Weiss, H. J. (Ed.) (1972). *Platelets and Their Role in Haemostasis,* New York Academy of Science, vol. 201.

Williams, D. F., Roaf, R., Maisels, D. O., Temple, L. F. and Wright, F. T. M. (1973). *Implants in Surgery,* Saunders, London.

Zucker, M. B. and Vroman L. (1969). Platelet adhesion induced by fibrinogen adsorbed onto glass. *Proc. Soc. Exp. Biol. Med.*, **131**, 318.

# 30

# HAEMOSTATIC CHANGES INDUCED BY ADSORBENT HAEMOPERFUSION

J. F. WINCHESTER

**Summary**

This paper reviews the haemostatic changes occurring with haemoperfusion in man and animals. Suitably coated activated charcoal produces least change in haemostatic elements while uncoated activated charcoal or ion-exchange resins may produce profound changes in the formed elements of blood during haemoperfusion. Considerable differences exist between animals and man, and between individuals in terms of platelet response to haemoperfusion and make direct extrapolation of compatibility testing from animals to man difficult and possibly inappropriate. It is suggested that *in vitro* evaluation of compatibility of haemoperfusion materials for human blood be assessed prior to application in man. In common with most extracorporeal devices comprising nonbiological materials, the 'ideal' nonthrombogenic surface properties of haemoperfusion materials have not been defined. However, innovations in polymer technology, encapsulation techniques and, possibly, new antiplatelet agents are developing trends for improving the haemocompatibility of haemoperfusion devices for repeated clinical use.

## 30.1 Introduction

Haemoperfusion is a technique which involves passage of anticoagulated blood through a device containing granules of sorptive agents (activated charcoal), or ion-exchange polystyrene-based resins, to remove unwanted substances. Since the technique involves intimate contact of blood with a large area of artificial surface, haemoperfusion is invariably accompanied by changes in the formed elements of the blood, with or without accompanying changes in the protein constituents of whole blood. The least biocompatible surfaces produce the most profound changes in haematology, and improvement in the artificial surface in contact with blood ameliorates any haematological effects. While the above statements hold for most artificial surfaces in contact with blood (haemodialysis membranes, prosthetic heart valves, and other vascular prostheses) in general it may be stated that to date an ideal haemoperfusion system has not been developed which completely abolishes the changes in haemostatic elements observed with clinical and laboratory use. Over the last 10 years there has been a growing interest in sorbent haemoperfusion devices for clinical use, and many human lives have been salvaged when such devices have been applied, especially in treating severely poisoned patients. The purpose of this communication is to

**Table 30.1**

Comparison of types of uncoated charcoal used in haemoperfusion

| *Author and date* | *Type of charcoal* | *Test animal* | *Condition being treated* | *Protein change* | *Platelet drop %* | *Bleeding problems* |
|---|---|---|---|---|---|---|
| Yatzidis (1964) | Merck | Dog | Normal | fibrinogen (60%) plasma proteins 5% | 30 - 50· | No |
| Hagstam *et al.* (1966) | Merck | Rabbit | Normal/ poisoned/ uraemic | No figures | No figures | Yes (6/40) |
| Dutton *et al.* (1969) | National Carbon | Dog/ cat | Normal | No figures | 50 - 80 | No mention |
| Barakat and MacPhee (1970) | Sutcliffe -Speak - man | Dog | Uraemic/ hepatic | No figures | 40 ± ·26.1 | No mention |
| Andrade *et al.* (1971) | Columbia | Sheep | Uraemic | No figures | 50 - 70 | No mention |
| Chang *et al.* (1974) | Fisher | Dog | Normal | No figures | 50 | No mention |
| Maini and Winches- ter (1975) | Norit- Clydesdale | Dog | Poisoned | No figures | 40 | No |
| Thysell *et al.* (1976) | Norit- Clydesdale | Rabbit | Normal | No figures | 30 ± 7 | No mention |
| Dunea *et al.* (1976) | Becton, Dickinson | Man | Uraemic | No figures | 53 ± 21 dextran 26 ± 17 albumin | No but bleeding time in- creased |

review the haemostatic changes occurring with haemoperfusion in animals and man and to suggest procedures for testing haemocompatibility of haemoperfusion devices and studying the mechanisms underlying the coagulation changes. Likely future trends in the development of biocompatible devices will also be outlined.

## 30.2 Haemostatic Changes With Sorbent Haemoperfusion

*Uncoated charcoal*

Different types of uncoated (nonpolymer coated) charcoal have been used in haemoperfusion devices. Most have been tested in animals and found to produce such large platelet depletion militating against their use in man (table 30.1). More recently, however, uncoated charcoal has been used in man (Dunea *et al.*, 1975) in the treatment of uraemia without profound platelet depletion. In general it is coated charcoal which produces least platelet depletion and this form has been used extensively in man.

It would seem that prior treatment of uncoated charcoal with albumin will reduce the magnitude of platelet loss during haemoperfusion, but most workers use coatings which will reduce attrition of charcoal and subsequent small particle release. (Van Wagenen *et al.*, 1975; Courtney *et al.*, 1975) as well as reducing platelet loss on charcoal.

*Coated charcoal*

Much more experience has been gained with the use of coated charcoal in animals and man (table 30.2). Most workers have examined only the effect of haemoperfusion on platelet counts but a small number of authors have also investigated the changes occurring in the coagulation mechanism (Abouna *et al.*, 1975; Gazzard *et al.*, 1974; Winchester *et al.*, 1976).

In the United Kingdom most human experience has been gained using an acrylic hydrogel coated charcoal originally developed for severe drug poisoning (Fennimore *et al.*, 1975) and used for clinical poisoning (Vale *et al.*, 1975), but also for the treatment of fulminant hepatic failure (Gazzard *et al.*, 1974) and for the treatment of uraemia (Winchester *et al.*, 1975b, 1976). In addition a system based on acrylic copolymer coated charcoal, designed by the University of Strathclyde, for direct toxin removal from blood (Courtney *et al.*, 1973) has been used clinically for the treatment of drug overdose, chronic uraemia, and hepatic failure (Martin *et al.*, 1975; and Gilchrist *et al.*, 1975). In North America most of the clinical experience has been gained with the use of the specially designed column of Chang *et al.* (1968) which utilises albumin-collodion coated charcoal (microcapsules). Chang *et al.* (1974, 1975) claim that negligible platelet depletion occurs in uraemic man during haemoperfusion with this device. Again it would seem that an albumin coating confers a more superior haemocompatibility than collodion alone.

*Resins*

Most resins used either clinically or in animals have been of neutral type, but ion-exchange resins have also been investigated with a view to poison removal (table 30.3). In all cases reported to date a polymer coating has not been used to improve the biocompatibility.

**Table 30.2**

Comparison of types of coated charcoal used in haemoperfusion

| *Author and date* | *Charcoal* | *Coating* | *Test animal* | *Condition being treated* | *Protein change* | *Platelet drop %* | *Bleeding problems* |
|---|---|---|---|---|---|---|---|
| Chang (1969) | Fisher | Cellulose nitrate | Dog | Normal (N) | No figures | 44 ± 7 | No |
| | Fisher | Albumin cellulose | Dog | N | No figures | 10 ± 10 | No |
| | Fisher | Heparin cellulose | Dog | N | No figures | 0 | No |
| Yatzidis (1971) | Merck | Cellulose acetate | Man | Poisoned | Fibrinogen 40 - 80% fall | Falls | No mention |
| Gazzard *et al.* (1974) | Sutcliffe-Speakman | Acrylic hydrogel | Man | Hepatic | II, V, IX, X not removed | 30% in 35<br>30 - 60% in 7 | No |
| Chang *et al.* (1974) | Fisher | Albumin collodion | Man | Uraemic | No figures | negligible | No |
| Winchester *et al.* (1974, 1975a) | Norit-Clydesdale | Acrylic | Dog | Poisoned | No figures | Falls | No |
| Abouna *et al.* (1975) | Norit-Clydesdale | Acrylic | Dog | Hepatic | II, VII, IX, X fall | 50 | No |
| Martin *et al.* (1975) | Norit-Clydesdale | Acrylic | Man | Poisoned/ Uraemic/ Hepatic | | Up to 25% | No |
| Thysell *et al.* (1976) | Norit-Clydesdale | Cellophane | Rabbit | N | No figures | 27 ± 7 | No |
| Amano *et al.* (1976) | Petroleum | Collodion | Man | Hepatic | No figures | 15 | ? |
| Winchester *et al.* (1976) | Sutcliffe-Speakman | Acrylic hydrogel | Man | Uraemic | No change in coagulation factors I - XII | 30 | No |

**Table 30.3**

Ion-exchange resins used in poison removal

| *Author and date* | *Resin* | *Test animal* | *Condition being treated* | *Protein change* | *Platelet drop %* | *Bleeding problems* |
|---|---|---|---|---|---|---|
| Rosenbaum *et al.* (1970) | XAD-2 | Man | Poisoned | No figures | 37.8 ± 23.6 | No mention |
| Rosenbaum *et al.* (1971) | XAD-2 | Man | Poisoned | No figures | 46.2 ± 10.5 | No mention |
| Rosenbaum *et al.* (1976) | XAD-4 | Man | Poisoned | No figures | 53.8 ± 13.9 | No mention |
| Maini and Winchester (1975) | Zerolit 225 | Dog | Poisoned | No figures | 50 | No |
| Weston *et al.* (1974) | XAD-2 | Dog | Hepatic | No figures | 79.4 ± 3.4 | No |

While resins appear to be more efficient than charcoal in terms of poison removal per gram of sorbent, platelet depletion would seem to be greater than coated charcoal and is similar to uncoated charcoal in terms of platelet loss.

## 30.3 Test-Column System

The above remarks on charcoal and resins give no indication of the underlying mechanisms involved in platelet-artificial surface interaction during haemoperfusion. Moreover, animal studies are not strictly comparable with the human situation, since it is known that species variation occurs in response of platelets to anticoagulants and foreign surfaces (Fidlar and Jacques, 1948; Eika and Godal, 1971).

We have developed a test system which examines the passage of fresh human blood (anticoagulated with heparin or sodium citrate) *in vitro* through coated or uncoated charcoal for 2 h. The polymer coating utilised was acrylonitrile/dimethylaminoethyl methacrylate copolymer (AN/DMAEMA) originally developed as a haemodialysis membrane (Muir *et al.*, 1971). We have shown (Winchester *et al.*, 1975c) that in heparinised blood, platelet depletion was 90% with uncoated charcoal haemoperfusion but was 15% with AN/DMAEMA coated charcoal haemoperfusion. Citrated blood resulted in equal platelet depletion (20%) with either coated or uncoated charcoal haemoperfusion. Coagulation factors II, V, IX, X and XII were reduced with either uncoated or coated charcoal haemoperfusion, but associated changes in coagulation tests (KCCT, PTT, etc) only occurred with uncoated charcoal haemoperfusion. Platelet rich plasma (heparinised) haemoperfusion resulted in small changes in platelet counts after passage through coated or uncoated charcoal.

The results of this study suggest that haemoperfusion of heparinised human blood over charcoal will result in less platelet depletion and less changes in coagulation factors if the charcoal is coated with copolymer. Moreover red cell free perfusion produces little platelet loss and suggests that red cell adenosine diphosphate may play a crucial role in producing platelet aggregation/adhesion to the artificial surfaces in intimate contact with blood during haemoperfusion. The results are somewhat similar to platelet changes occurring with the passage of blood through glass beads (Hellem, 1960), where adenosine diphosphate (ADP) has been shown to be important in producing platelet adhesion. We have also noted that haemoperfusion of whole blood produces a small but significant rise in plasma haemoglobin and this lends weight to the hypothesis that red cell ADP is important in causing platelet adhesion to charcoal.

## 30.4 Discussion

The passage of blood through any extracorporeal device will result in changes in the haemostatic elements and considerable research is being undertaken to elucidate the mechanisms involved in platelet-artificial surface interaction and to improve the artificial surfaces (Bruck, 1974; Nyilas *et al.*, 1975). The major problem with application of haemoperfusion in animals and man is that there is large individual variation in response of platelets to haemoperfusion, in normal or in diseased animals or man. It is therefore not possible to directly extrapolate results from animals to man and probably would be more useful to undertake *in vitro* laboratory testing of haemoperfusion materials with human blood before clinical or animal evaluation. Moreover it would be worthwhile examining in detail *in vitro* the difference between uraemic blood or blood from patients with hepatic failure and comparing the results with normal. There is some indication that the platelet response to haemoperfusion in patients with fulminant hepatic failure is associated with the formation of platelet aggregates measured by increase in screen filtration pressure (SFP) (Weston and Williams, 1976). This rise in SFP has not been observed in patients treated with similar haemoperfusion devices in uraemia (Winchester, unpublished observations) and could be accounted for by the known platelet defect in uraemia which can be corrected by dialysis (Stewart and Castaldi, 1967).

Uncoated charcoal in haemoperfusion apparatus usually produces profound platelet loss, but the prior addition of albumin protects against the same degree of platelet depletion as reported by Dunea *et al.* (1976). In general, however, it is unlikely that uncoated charcoal will find a place for repeated clinical use since there is the attendant risk of particle embolisation (Hagstam *et al.*, 1966) although this can be diminished to some extent by using a fixed bed charcoal device (Hill, 1976).

Albumin coating is known to prevent platelet adhesion to artificial surfaces *in vitro* (Lyman *et al.*, 1970; Chang, 1969; Salzman *et al.*, 1969; Packham *et al.*,

1969) and has been shown to be useful in reducing platelet losses during haemoperfusion *in vivo* (Chang, 1969, 1974). The use of albumin would appear to be justified at present but long-term use of albumin may be associated with anaphylactic reactions and hepatitis, for example, although these complications are rare.

The likely future trends in improving haemocompatibility of charcoal will be primarily directed at polymer research - from this aspect polymers which are suitable for heparin bonding, such as those which can be modified to contain quaternary amine groups (Eriksson *et al.*, 1967; Lindsay *et al.*, 1976), have been shown to reduce platelet adhesion *in vitro*. For a review of biocompatible surfaces for microencapsulation of sorbents see Chang (1973). Recently immobilisation of prostaglandins on artificial surfaces has been shown to reduce platelet adhesion (Grode *et al.*, 1974). Preparation of biomaterials by removal of gas nuclei has been associated with reduced platelet adhesion (Ward *et al.*, 1974).

Heparin sulphate in solution, while probably the most suitable anticoagulant for clinical use, is known to produce platelet adhesion and aggregation to ADP (Eika, 1972; Thomson *et al.*, 1973) and not to prevent haemostatic changes occurring with haemoperfusion (Abouna *et al.*, 1975; Gazzard *et al.*, 1974). Since heparin does not prevent haemostatic changes in various extracorporeal circuits other workers have suggested that citrate should be used as the anticoagulant (despite the inherent difficulties in replenishing and maintaining normal calcium levels in effluent blood) in extracorporeal circuits (Berk, 1976; Neal, 1976).

Another method of improving biocompatibility involves total encapsulation of carbon, either embedded in hydroxyethyl cellulose fibres (Davis *et al.*, 1974) or contained between plates of haemodialysis membranes (Castino *et al.*, 1976) or packed inside hollow fibres of Cuprophan membrane (Malchesky *et al.*, 1976).

The types of charcoal used in haemoperfusion devices, aside from differing adsorptive properties, differ also in haemocompatibility (Walker *et al.*, 1976; Denti *et al.*, 1976) and improvement in haemocompatibility may also be achieved by choosing a carbon with a smooth, nonangular surface. The most recent addition to carbon technology has been the introduction of a spherical, petroleum-based charcoal (Amano *et al.*, 1976) which theoretically should produce improved haemocompatibility when compared to conventional carbons.

There is also a developing interest in the use of antiplatelet agents (such as sulphinpyrazone, aspirin) for preventing platelet loss in extracorporeal circuits (Lindsay *et al.*, 1972) and if repeated use of haemoperfusion is proposed then such therapy may be useful. Other pharmacological substances have been shown to reduce platelet adhesion to surfaces (Cazenave *et al.*, 1974).

There is no doubt that haemoperfusion over activated charcoal is a developing and potentially useful technique for clinical use. It would seem likely that repeated clinical use will be necessary in fulminant hepatic failure and uraemia. Haemocompatibility is of the utmost importance if repeated clinical use is contemplated.

## 30.5 References

Abouna, G. M., Gilchrist, T., Pettit, J. E., Boyd, N. D., Todd, J. K., Courtney, J. M. and Maini, R. (1975). Haemoperfusion with activated charcoal in treatment of experimental acute hepatic failure, in R. Williams and I. M. Murray-Lyon (Eds.)., *Artificial Liver Support,* Pitman Medical, London, p. 180.

Amano, I., Iwatsuki, S., Saito, A., Manji, T., Takahira, H., Ohta, K. and Maeda, K. (1976). Microencapsulated beads-type petroleum charcoal in the artificial liver assist. *Abstracts Amer. Soc. Artif. Int. Organs,* **5**, 3.

Andrade, J. D., Kunitomo, K., Van Wagenen, R., Kastigir, B., Gough, D. and Kolff, W. J. (1971). Coated adsorbents for direct blood perfusion: HEMA/activated carbon. *Trans. Amer. Soc. Artif. Int. Organs.*, **17**, 222 - 228.

Barakat, T. and MacPhee, I. W. (1970). Experiments with an extracorporeal carbon column as a simplified artificial kidney. *Brit. J. Surg.*, **57**, 580 - 583.

Berk, P. D. (1976). Haemoperfusion in hepatic failure. *Kidney International* (in press).

Bruck, S. D. (1974). *Blood Compatible Synthetic Polymers,* Thomas, Springfield, Illinois.

Castino, F., Scheucher, K., Malchesky, P. S., Koshino, I. and Nose, Y. (1976). Microemboli free blood detoxification utilising plasma filtration. *Trans. Amer. Soc. Artif. Int. Organs,* **11**, 637.

Cazenave, J. P., Packham, M. A., Guccione, M. A. and Mustard, J. F. (1974). Inhibition of platelet adherence to a collagen-coated surface by nonsteroidal anti-inflammatory drugs, pyrimido-pyrimidine and tricyclic compounds and lidocaine. *J. Lab. Clin. Med.*, **83**, 797 - 806.

Chang, T. M. S. (1969). Removal of endogenous and exogenous toxins by a microencapsulated adsorbent. *Can. J. Physiol. Pharmacol.*, **47**, 1043 - 1045.

Chang, T. M. S. (1973). Recent trends in biocompatible materials, and microcapsular adsorbents for kidney function replacement, in R. M. Kenedi (Ed.), *Perspectives in Biomedical Engineering,* Macmillan, London and Basingstoke, pp. 39 - 43.

Chang, T. M. S., Pont, A., Johnson, L. J. and Malave, N. (1968). Response to intermittent extracorporeal perfusion through shunts containing semipermeable microcapsules. *Trans. Amer. Soc. Artif. Int. Organs,* **14**, 163 - 168.

Chang, T. M. S., Migchelsen, M., Coffey, J. F. and Stark, R. (1974). Serum middle molecule levels in uraemia during long-term intermittent haemoperfusion with the ACAC (coated charcoal) microcapsule artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 364 - 371.

Chang T. M. S., Chirito, E., Barre, B., Cole, C. and Hewish, M. (1975). Clinical performance-characteristics of a new combined system for simultaneous haemoperfusion-haemodialysis-ultrafiltration in series. *Trans. Amer. Soc. Artif. Int. Organs,* **21**, 502 - 508.

Courtney, J. M., Gilchrist, T., Edwards, R. O. and Walker, J. M. (1973). A new coated adsorbent for blood perfusion. *Proc. 10th Intern., Conf. Med. Biol. Eng.* **10**, 33.

Courtney, J. M., Gilchrist, T., Hood, R. G. and Townsend, W. B. (1975). A novel method for preparing coated carbon granules. *Proc. 2nd Eur. Soc. Artif. Organs*, **2**, 210.

Davis, T. A., Cowsar, D. R., Harrison, S. D. and Tanquary, A. C. (1974). *Trans. Amer. Soc. Artif. Int. Organs,* A **20**, 353.

Denti, E., Walker, J. M., Tessore, V., Luboz, M. P. and Malinverni, A. (1976). Haemocompatibility problems in blood detoxification by activated carbon. *Proc. 2nd Eur. Soc. Artif. Organs,* **2**, 215.

Dunea, G., Rizvi, Z. A., Anicama, H. J., Mamdani, B. H. and Mahurkar, S. D. (1975). Charcoal haemoperfusion – *in vivo* and *in vitro* studies. *Kidney International,* **8**, 424.

Dunea, G., Rizvi, Z. H., Mamdani, B. H., Anicama, H. J. and Mahurkar, S. D. (1976). Charcoal haemoperfusion and combination dialysis – haemoperfusion in chronic renal failure. *Abstracts Amer. Soc. Artif. Int. Organs,* **5**, 22.

Dutton, R. C., Dedrick, R. L. and Bull, B. S. (1969). A simple technique for the experimental production of acute platelet deficiency. *Thrombos. Diathes. Hemorrh.,* **21**, 367 - 372.

Eika, C. (1972). On the mechanism of platelet aggregation indiced by heparin, protamine and polybrene. *Scand. J. Haemat.,* **9**, 248 - 257.

Eika, C. and Godal, H. C. (1971). Thrombocytopenia in rabbits induced by heparin, protamine and polybrene. *Scand. J. Haemat.,* **8**, 481 - 486.

Eriksson, J. C., Gillbert, G. and Lagergren, J. (1967). A new method for preparing nonthrombogenic plastic surfaces. *J. Biomed. Mater. Res.* **1**, 301.

Fennimore, J., Watson, P. A., Munro, G. D. and Kolthammer, J. C. (1975). The design and evaluation of a convenient carbon haemoperfusion device. *Proc. Eur. Soc. Artif. Organs,* **1**, 90 - 94.

Fidlar, E. and Jacques, L. B. (1948). The effect of commercial heparin on the platelet count. *J. Lab. Clin. Med.,* **33**, 1410 - 1423.

Gazzard, B. G., Portmann, B. A., Weston, M. J., Langley, P. G., Murray-Lyon, I. M., Dunlop, E. H., Flax, H., Mellon, P. J., Record, C. O., Ward, M. B. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet,* **1**, 1301 - 1307.

Gilchrist, T., Jonsson, E., Martin, A. M., Naucler, L., Cameron, A. and Courtney, J. M. (1975). Development of Strathclyde haemoperfusion system, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London, p. 319.

Grode, G. A., Pitman, J., Crowley, J. P., Leininger, R. I. and Falb, R. D. (1974). Surface immobilised prostaglandin as a platelet protective agent. *Trans. Amer. Soc. Artif. Int. Organs,* A **20**, 38 - 41.

Hagstam, K. E., Larsson, L. E. and Thysell, H. (1966). Experimental studies on charcoal haemoperfusion in phenobarbital intoxication and uraemia, including histopathologic findings. *Acta Med. Scand.,* **180**, 593 - 603.

Hellem, A. J. (1960). The adhesiveness of human blood platelets *in vitro*. *Scand. J. Clin. Lab. Invest.,* **12**, suppl. 51, 1 - 117.

Hill, J. B. (1976). The rationale for fixed bed charcoal in haemoperfusion. *Kidney International* (in press).

Lindsay, R. M., Prentice, C. R. M., Ferguson, D., Burton, J. A. and McNicol, G. P. (1972). Reduction of thrombus formation on dialyser membranes by aspirin and RA 233. *Lancet,* **2**, 1287 - 1290.

Lindsay, R. M., Rourke, J., Reid, B., Friesen, M., Linton, A. L., Courtney, J. and Gilchrist, T. (1976). Platelets, foreign surfaces and heparin. *Trans. Amer. Soc. Artif. Int. Organs,* **22**, 292.

Lyman, D. J., Klein, K. G., Brash, J. L. and Fritzinger, B. K. (1970). The interaction of platelets with polymer surfaces 1. Uncharged hydrophobic polymer surfaces. *Thrombos. Diathes. Hemorrh.,* **23**, 120.

Maini, R. and Winchester, J. F. (1975). Removal of paraquat from blood by haemoperfusion over sorbent materials. *Brit. Med. J.,* **3**, 281 - 282.

Malchesky, P. S., Nokoff, R. and Nose, Y. (1976). The charcoal capillary haemoperfusion system. *Abstracts Amer. Soc. Artif. Int. Organs,* Vol. 5 (22nd annual meeting), p. 52.

Martin, A. M., Mitchell, D. C., Gibbins, J. K., Devapal, D. and Trinder, P. (1975). Charcoal haemoperfusion in the management of severe poisoning. *Brit. Med. J.,* **1**, 392.

Muir, W. M., Courtney, J. M., Gray, R. A. and Ritchie, P. D. (1971). Perm-selective dialysis membranes. 1. Films based on dimethylamino-ethyl methacrylate copolymers. *J. Biomed. Mater. Res.,* **5**, 415 - 445.

Neal, M. (1976). Haemodialysis using citrate for regional anticoagulation. American Association Nephrology Nurses and Technicians.

Nyilas, E., Chiu, T. H., Morton, W. A., Lederman, G. A., Herzlinger, G. A. and Cumming, R. D. (1975). Physicochemical studies on artificial surface/protein/platelet interaction. *Thrombos. Diathes. Hemorrh.,* **34**, 555.

Packham, M. A., Evans, G., Glynn, M. F. and Mustard, J. F. (1969). Effect of plasma proteins on interaction of platelets with glass surfaces. *J. Lab. Clin. Med.,* **73**, 686.

Rosenbaum, J. L., Winsten, S., Kramer, M. S., Moros, J. and Raja, R. (1970). Resin haemoperfusion in the treatment of drug intoxication. *Trans. Amer. Soc. Artif. Int. Organs,* **16**, 134 - 140.

Rosenbaum, J. L., Kramer, M. S., Raja, R. and Boreyko, C. (1971). Resin haemoperfusion: a new treatment for acute drug intoxication. *New Engl. J. Med.,* **284**, 874 - 877.

Rosenbaum, J. L., Kramer, M. S. and Raja, R. (1976). Resin haemoperfusion for acute drug intoxication. *Arch. Intern. Med.,* **136**, 263 - 266.

Salzman, E. W., Merrill, E. W., Binder, A., Wolf, C. F. W., Ashford, T. P. and Austen, W. G. (1969). Protein-platelet interaction on heparinized surfaces. *J. Biomed. Mater. Res.,* **3**, 69.

Stewart, J. H. and Castaldi, P. A. (1967). Uraemic bleeding: a reversible platelet defect corrected by dialysis. *Quart. J. Med.,* **36**, 409.

Thomson, C., Forbes, C. D. and Prentice, C. R. M. (1973). The potentiation of platelet aggregation by heparin *in vitro* and *in vivo. Clin. Sci. Mol. Med.,* **45**, 485 - 494.

Thysell, H., Lindholm, T., Heinegard, H., Henrikson, H., Jonsson, E., Tylen, U., Svensson, T., Bergkvist, G. and Gulberg, C. A. (1976). A haemoperfusion column using cellophane coated charcoal. *Proc. Eur. Soc. Artif. Organs,* **2** 2, 212.

Vale, J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975). Use of charcoal haemoperfusion in the management of severely poisoned patients. *Brit. Med. J.,* **1**, 5 - 9.

Van Wagenen, R. A., Steggale, M., Lentz, D. J. and Andrade, J. D. (1975). Activated carbons for medical applications. *In vitro* microparticle characterisation and solute adsorption. *Biomat. Med. Dev. Artif. Organs,* **3**, 319 - 364.

Walker, J. M., Denti, E., Van Wagenen, R. and Andrade, J. D. (1976). Evaluation and selection of activated carbon for haemoperfusion. *Kidney International* (in press).

Ward, C. A., Ruegsegger, B., Stanga, D. and Zingg, W. (1974). Reduction in platelet adhesion to biomaterials by removal of gas nuclei. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 77 - 84.

Weston, M. J., Gazzard, B. G., Buxton, B. H., Winch, J., Machado, A. L., Flax, H. and Williams, R. (1974). Effects of haemoperfusion through charcoal or XAD - 2 resin on an animal model of fulminant liver failure. *Gut,* **15**, 482 - 486.

Weston, M. J. and Williams, R. (1976). Experience with haemoperfusion in fulminant hepatic failure. *Proc. 1st Int. Symp. Gastrointestinal Emerg.* (in press).

Winchester, J. F., Tilstone, W. J., Edwards, R. O., Gilchrist, T. and Kennedy, A. C. (1974). Haemoperfusion for enhanced drug elimination - a kinetic analysis in paracetamol poisoning. *Trans. Amer. Soc. Artif. Int. Organs,* **20**, 358 - 363.

Winchester, J. F., Edwards, R. O., Tilstone, W. J. and Woodcock, B. G. (1975a). Activated charcoal haemoperfusion and experimental acetaminophen poisoning. *Toxicol. Appl. Pharmacol.,* **31**, 120 - 127.

Winchester, J. F., Apiliga, M. T., Mackay, J. M. and Kennedy, A. C. (1975b). Haemodialysis with charcoal haemoperfusion. *Proc. Eur. Dial. Transpl. Assoc.,* **12**, 526 - 533.

Winchester, J. F., Mackay, J. M., Forbes, C. D., Blakely, E., Prentice, C. R. M., Kennedy, A. C., Harvey, B. and Gilchrist, T. (1975c). Haemostatic changes induced *in vitro* by activated charcoal haemoperfusion. *Thrombos. Diathes. Hemorrh.,* **34**, 587.

Winchester, J. F., Apiliga, M. T. and Kennedy, A. C. (1976). Short-term evaluation of charcoal haemoperfusion combined with dialysis in uraemic patients. *Kidney International* (in press).

Yatzidis, H. (1964). A convenient haemoperfusion micro-apparatus over charcoal for the treatment of endogenous and exogenous intoxications. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.,* **1**, 83 - 87.

Yatzidis, H. (1971). The use of ion-exchange resins and charcoal in acute barbiturate poisoning, in H. Matthew (Ed.), *Acute Barbiturate Poisoning,* Excerpta Medica, Amsterdam, pp. 223 - 232.

# 31

# SELECTIVE REMOVAL OF PHOSPHATE IONS FROM THE BLOOD OF URAEMIC PATIENTS

S. SIDEMAN, E. HOFFER, O. S. BETTER and S. LUPOVITCH

**Summary**

Haemoperfusion over a packed bed alumina column is suggested for the removal of excess phosphate ions from the blood of uraemic patients. The alumina particles are coated with blood compatible phosphate-permeable polymers. Poly-HEMA and collodion-based coatings have been tested in preliminary *in vitro* simulated haemoperfusion experiments with fresh human blood. Collodion based coatings exhibit good blood compatibility. Additional work is required to define the limitations and refine the proposed system.

## 31.1 Introduction

The high concentration of phosphate ions in the blood of uraemic patients, sometimes reaching levels of up to 20 mg% as compared with approximately 4 mg% in healthy humans, is due to the inability of the impaired kidneys to excrete the phosphates into the urine. The high concentration of phosphate ions stimulates the function of the parathyroid gland. As a consequence, calcium ions are released from the bones into the blood stream. Also, due to the high concentration of calcium and phosphate ions, calcium phosphates may deposit on soft tissues like the cornea or the heart, and some uraemic patients on maintenance dialysis developed intractable heart failure and atrioventricular block (Arora *et al.*, 1975). These patients had persistently high calcium/phosphorus products, including one patient who took oral phosphate binders. At autopsy, all had metastatic calcification.

Haemodialysis membranes remove phosphate ions only partially. Lowering of the phosphate level in blood can be achieved by oral administration of aluminium hydroxide Al $(OH)_3$ or aluminium carbonate gels. However, some patients reject this medication because of constipation and nausea. Cases of intestinal perforation due to obstructions caused by this medication have been reported (Townsend *et al.*, 1973; Chesney and Haughton, 1974), and dialysis dementia and encephalopathy syndrome were recently observed in uraemic patients with long time administration of $Al(OH)_3$ gels (Berlyne *et al.*, 1976; Alfrey *et al.*, 1975; Ward *et al.*, 1976; Flending *et al.*, 1976). At autopsy, aluminium was found in the brain of these patients. Though the link between oral administration of $Al(OH)_3$ and the occurrence of aluminium in the blood has not been thoroughly investigated as yet,

these findings serve as a warning against the carefree, long-term, introduction of $Al(OH)_3$ into the body.

The objective of the present programme is to develop a haemoperfusion device capable of removing excess phosphate ions from the blood of uraemic patients.

Carbon haemoperfusion was suggested by Yatzidis (1964), as a means of removing creatinine, uric acid, indican, phenolic compounds, guanidines and barbiturates from blood. To minimise blood damage and eliminate the carry-over of fine particles into the blood during haemoperfusion, Chang (1972) suggested the application of coated (microencapsulated) carbon granules. An evaluation of the various coatings was presented by Andrade *et al.* (1975). A detailed review on the biocompatibility of various coatings and on the application of coated activated carbon to remove various metabolites, toxins and overdoses of various medications has been presented by Chang (1976) and will not be repeated here. A coated activated carbon column has been commercially developed by Smith and Nephew, England. Unfortunately, activated carbon columns are ineffective in removing phosphate.

In order to find a suitable adsorbent for phosphate ions, use was made of the vast information accumulated by the environmentalists. Since phosphate ions arising from detergents or fertilisers are common pollutants, their removal from water has been thoroughly investigated and aluminium oxide, $Al_2O_3$, was suggested as an efficient adsorbent (Neufeld and Thodos, 1969). It has strong affinity towards phosphate ions even at high concentrations of chlorides, and is therefore a promising adsorbent for phosphate removal from blood (Sideman and Better, 1973). Preliminary tests, including haemoperfusion on dogs, were satisfactorily conducted in these laboratories with alumina particles coated with blood-compatible and phosphate-permeable polymers (Hoffer *et al.*, 1975). Denti *et al.* (1975) have also considered aluminium oxide as a potential candidate for haemoperfusion. Phosphate adsorption and *in vitro* blood compatibility tests were reported (Denti *et al.*, 1975) but the results were not conclusive.

The present programme includes a search for an efficient adsorbent, a search for a suitable biocompatible polymeric coating which has a high permeability for phosphate ions, development of suitable reproducible coating techniques, *in vitro* and *in vivo* biocompatibility tests, analysis of the engineering aspects associated with the design and development of a packed bed cartridge and, finally, clinical tests.

The detailed report of these various studies will be presented in due course, but for the present our attention is focused on the removal efficiency of the coated and uncoated alumina particles and on their biocompatibility with human blood.

## 31.2 Experimental

*The adsorbing material*

The choice of the proper adsorbent was dictated, *inter alia*, by the availability of the adsorbent in the form of relatively large particles – granules or beads. This is

necessary for minimising the pressure drop in the cartridge. Aluminium oxide can be obtained in the desired granular size which can be effectively coated. Hydrated zirconium oxide was eliminated because of its low particle size and high cost.

*Solutions*

A model solution was used in the screening of the various $Al_2O_3$ particles and coatings. It consisted of 15 mg% dissolved in saline (0.9% NaCl) and buffered to pH 7.4 by tris buffer. An anticoagulant, acid citrate dextrose (ACD), was used in the runs aimed at evaluating the biocompatibility of the various coatings. The ACD consisted of 0.8% disodium citrate, 2.2% trisodium citrate and 2.45% dextrose. In these tests the blood contained 15% ACD. An anticoagulant, sodium oxalate, was used in the phosphate adsorption tests. Its concentration in the blood was 0.01%.

*Coating materials and techniques*

A number of coating materials have been used. These include hydroxyethyl methacrylate (HEMA), collodion, modified collodion and (human) albumin - collodion.

For the Poly-HEMA coating the *in situ* polymerisation procedure, utilising a redox initiator system (Andrade and Kolff, 1973), was modified to allow for the much higher specific density of the alumina granules. Collodion coating was set on the particles by immersion in an organic solution. The modified collodion coating was obtained by reacting the collodion coated particles with a dichlorotriazin reagent. Albumin was chemically bound to the collodion surface via the dichlorotriazin reagent. All the coated particles were stored in water.

*Fine particles release*

Ten litres of distilled water were passed at the rate of 100 ml/min through a 100 g bed of particles, and filtered through a 0.22 $\mu$m Millipore filter.

*Stirred batch experiments*

Phosphate adsorption of coated and uncoated alumina 14 - 28 mesh particles was tested in a 500 ml Erlenmyer flask. The flask was kept at 37°C, and mixing was produced by gentle shaking. 5 g $Al_2O_3$ were placed in 100 ml model solution. Samples were taken at various time intervals, up to 2 h.

*Experimental cartridge for blood tests*

A two facet conical Perspex column, 5 cm height and (maximum) width of 3 cm. was used throughout (Sideman *et al.*, page 419 of these proceedings). The inlet and outlet entry leads were tapered Perspex cones with 1.5 cm diameter connections. The priming volume of the empty system was 28 ml. A 50 mesh stainless steel grid was placed at the bottom and the top of the column. The whole column was 'siliconised' with Dow Medical fluid no. 360. Fresh blood was obtained from the Maccabi Haematological Laboratory.

*Phosphate adsorption test*

The column contained 10 g particles and the total circulated blood volume was 100 ml. Prior to each run, the particles were washed by circulating 1 l of saline which contained 0.01% sodium oxalate. The saline solution was replaced by blood. Samples were taken from the blood reservoir. The flow rate of blood was 25 ml/min.

*Blood compatibility tests*

The column contained 25 g particles and the total circulated blood volume was 50 ml. Prior to each run the particles were washed by circulating 1 l saline which contained 15% ACD, for 12 h. The saline was replaced by blood and discarded thereafter. The flow rate of blood was 25 ml/min. Samples were taken, at various time intervals, from the column exit. Platelet, erythrocyte and leucocyte counts were taken during the various runs. The platelets and leucocytes were counted in a microscope. The erythrocytes were counted in a Coulter counter model Z - B.

*Blood chemistry*

The levels of uric acid, urea and creatinine at the beginning and the end of each run were checked. Also, the concentration of inorganic ions and the total protein content of blood were determined.

*Surface morphology*

The particles were examined with a JSM-U3 scanning electron microscope. Pictures of the particles to show surface morphology were taken before and after the blood runs. The latter were taken after fixation with glutaraldehyde. The samples were coated with gold-palladium before scanning.

## 31.3 Results and Discussion

The results for the phosphate adsorption tests of 14 - 28 mesh alumina granules, coated and uncoated in a stirred (mixed) batch, are presented in table 31.1.

**Table 31.1**

Phosphate adsorption by coated and uncoated alumina (mg % in model solution). H1 : 20% HEMA, H2 : 10% HEMA (in coating solution)

| *Time* | *Uncoated* | *Collodion 0.5%* | *Poly - HEMA* | |
|---|---|---|---|---|
| | | | H1 | H2 |
| 0 | 14 | 13.7 | 12.5 | 14.6 |
| 30 | 4.3 | 7.3 | 4.8 | 4.9 |
| 60 | 2.3 | 5.1 | 2.6 | 2.7 |
| 90 | 1.2 | 4.1 | 1.5 | 1.5 |
| 120 | 1.0 | 2.7 | 1.3 | 1.0 |

As seen in table 31.1, the poly-HEMA coating seems to present practically no resistance to the phosphate transfer, whereas the collodion coating reduces the phosphate transfer rate. These results bring out two interesting points. The first is that, though a poly-HEMA membrane is not permeable to phosphate (Moise *et al.*, 1976). these poly-HEMA coatings may still be permeable to phosphate. The second relates to the collodion permeability, which strongly depends on the conditions under which the coating is made, i.e. temperature, humidity and drying time. Also, collodion can easily be made blood compatible. The choice of the coating should therefore reflect a proper balance between the desired permeability and blood compatibility.

Fine particles, 2.1 mg, were collected on a 0.22 $\mu$m Millipore filter when uncoated alumina was thoroughly washed by ultrasonication. No particles were detected when the coated granules were subjected to the same test.

A comparison of phosphate adsorption by the uncoated and coated alumina granules is presented in table 31.2.

As seen in table 31.2, there is quite a large difference between phosphate adsorption from blood and a model solution. Evidently, some of the various proteins in the blood compete for the free alumina sites, thus reducing phosphate adsorption. The effect of the coating is also evident: 72% of the phosphate in blood was adsorbed on the 10 g uncoated alumina bed, while only 38% was adsorbed on the collodion coated bed of the same weight. It is, however, to be noted that very little physical settling of proteins occurred on either the coated or uncoated alumina particles shown in figure 31.1. This is in a marked contrast to the heavy settling of proteins on uncoated ion-exchange beads (Sideman *et al.*, page 419 of these proceedings).

The blood compatibility of the coated alumina particles was tested in the *in vitro* closed cycle system with fresh human blood. The results, summarised in table 31.3, show that the modified collodion and albumin treated collodion coating reduce the platelet count by less than 10%.

The improvement is similar to the one obtained (Chang, 1976; Chang *et al.*, 1971) by physical adsorption of albumin on collodion-coated activated charcoal.

**Table 31.2**

Comparison of phosphate adsorption from model solution and blood in a closed packed bed system (mg% in model solution and blood)

| *Time* | *Uncoated* | | *Collodion coated* | |
|---|---|---|---|---|
| | *Solution* | *Blood* | *Solution* | *Blood* |
| 0 | 10.4 | 8.93 | 10.0 | 6.34 |
| 30 | 1.57 | 4.82 | 3.20 | |
| 60 | 0.98 | | 1.67 | 5.47 |
| 90 | | 2.62 | 0.96 | 4.27 |
| 120 | 0 | 2.48 | 0.96 | 3.94 |

**Table 31.3**

Blood compatibility of various particles

| *Particles* | *Time* | *Erythrocytes* | | *Leucocytes* | | *Platelets* | |
|---|---|---|---|---|---|---|---|
| | | $\times 10^6/\mu l$ | % | $\times 10^5/\mu l$ | % | $\times 10^5/\mu l$ | % |
| Uncoated alumina | 0 | 7.04 | 100 | 13 | 100 | 1.5 | 100 |
| | 30 | | | 10.5 | 81 | 1.66 | 111 |
| | 60 | | | 9.5 | 73 | 1.48 | 98 |
| | 90 | | | 9.5 | 73 | 1.39 | 93 |
| | 120 | 6.42 | 91 | 9.4 | 72 | 0.97 | 64 |
| Collodion | 0 | 7.04 | 100 | 12.3 | 100 | 1.07 | 100 |
| | 30 | | | 9.5 | 77 | 0.66 | 61 |
| | 60 | | | 9.1 | 74 | 0.58 | 53 |
| | 90 | | | 7.9 | 64 | 0.48 | 44 |
| | 120 | 6.64 | 94 | 7.6 | 62 | 0.43 | 40 |
| Modified collodion | 0 | 3.44 | 100 | 4.9 | 100 | 1.40 | 100 |
| | 30 | 2.61 | 76 | 4.5 | 92 | 1.36 | 97 |
| | 60 | 2.70 | 78 | 4.2 | 86 | 1.33 | 95 |
| | 90 | 2.61 | 76 | 4.2 | 86 | 1.32 | 94 |
| Albumin collodion | 0 | 3.44 | 100 | 4.9 | 100 | 1.40 | 100 |
| | 30 | 2.91 | 85 | 4.7 | 96 | 1.32 | 94 |
| | 60 | 2.57 | 75 | 4.7 | 96 | 1.30 | 93 |
| | 90 | 2.89 | 84 | 4.2 | 86 | 1.30 | 93 |
| Poly-HEMA | 0 | 5.21 | 100 | 8.9 | 100 | 0.90 | 100 |
| | 30 | 4.22 | 81 | 5.7 | 64 | 0.63 | 70 |
| | 60 | 4.51 | 87 | 4.3 | 48 | 0.7 | 77 |
| | 90 | 5.01 | 96 | 1.9 | 21 | 0.63 | 70 |

The results shown by the modified collodion coating are consistent with the results obtained by modifying various polymeric surfaces with Chicago acid and Evan's blue (Nishizawa *et al.*, 1973). It seems that the attachment of negatively charged (sulphonic) groups to the surface is responsible for the improved nonthrombogeneity.

The decrease in platelet level obtained here is much smaller than that obtained in haemodialysis (Lindsay *et al.*, 1972) and much smaller than that obtained in dog (Gazzard *et al.*, 1974; Brookings and Ramsey, 1975) and human (Vale *et al.*, 1975) haemoperfusion with coated activated charcoal.

The leucocyte count fall with the modified collodion coatings is also surprisingly small. Only some 15% of the leucocytes were lost, as compared with accepted values of 40 to 60% reported in the literature (Brookings and Ramsey, 1975). Though the normal recovery rate of leucocytes is relatively fast, returning to the original level 1 h after the end of the haemoperfusion (Nishizawa *et al.*, 1973), a leucocyte-safe system is clearly advantageous.

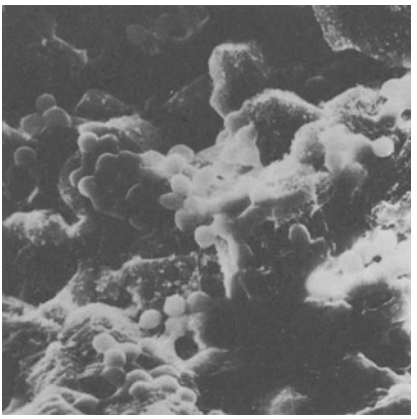

Figure 31.1 Scanning electron microscope photographs (× 1000) of (a) uncoated and (b) poly-HEMA 2% coated alumina particles after equilibration with blood and fixation with glutaraldehyde (same batch as in table 31.3)

It is perhaps interesting to note that the size distribution of the leucocytes in blood leaving the column was identical with that entering the column. Evidently, the leucocyte loss is not due to physical filtration by the particle bed but rather to physical adsorption on the surface. The different results between the $Al_2O_3$ bed and the charcoal or resin beds seem to indicate the much greater activity of the two latter, hence the greater loss of the blood components in these beds. Comparison of figure 31.1a and b with figure 44.3a and b of Sideman (p. 419, these proceedings) shows significant protein layer on the uncoated resin beads which entrap the blood cells. Here, however, the blood cells are quite loosely attached, even to the surface of the uncoated alumina.

**Table 31.4**

The concentration of various blood components at the beginning and the end of a closed circuit experiment with blood

| *Solute* | *Uncoated* | | *Collodion* | |
|---|---|---|---|---|
| | $t = 0$ | $t = 120$ min | $t = 0$ | $t = 120$ min |
| K mEq/l | 11.1 | 8 | 10.9 | 8.1 |
| Na mEq/l | 169 | 152 | 169 | 154 |
| $CO_2$ mEq/l | 9 | 7 | 7 | 6 |
| Ca mg% | 8.6 | 3.4 | 8.9 | 4.1 |
| Urea mg% | 20 | 18 | 20 | 18 |
| Uric acid mg% | 4.5 | 4.5 | 4.5 | 4.5 |
| Creatinine mg% | 0.7 | 0.7 | 0.7 | 0.7 |
| Glucose mg% | 294 | 288 | 300 | 288 |
| Total protein g% | 6.4 | 5.5 | 6 | 5.2 |
| Albumin g% | 4.7 | 4.4 | 4.5 | 4.0 |
| Globulin g% | 1.7 | 1.1 | 1.5 | 1.2 |

Blood chemistry tests are summarised in table 31.4. The results demonstrate the specific adsorption of the phosphate ions. The noted decrease of Ca is most probably due to the use of oxalate as an anticoagulant in these tests, as Ca-oxalate may have precipitated on the alumina bed during the tests.

Obviously, a noninteracting anticoagulant must be found before the application of this technique can be clinically realised.

## 31.4 Concluding Remarks

The work reported herein represents preliminary feasibility tests for utilising coated alumina particles to remove phosphate ion from the blood of uraemic patients. Additional work is required to refine and define the exact system. The application of the haemoperfusion device suggested herein in conjunction with a standard artificial kidney will allow a controlled and safe removal of excess phosphate ions. Furthermore, it may allow relaxation of the strict low-phosphate diet presently prescribed to uraemic patients.

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## 31.6 References

Alfrey, A. C., Kaehny, W. D., Nunnelley, L. L. and Smythe, W. R. (1975). The dialysis encephalopathy syndrome (DE). Possible aluminium intoxication. *Kidney International*, **8**, 422.

Andrade, J. D. and Kolff, K. J. (1973). Development of more efficient, less costly artificial kidneys: Adsorbent haemoperfusion. Annual Report to NIH, August 1973.

Andrade, J. D., Coleman, D. L., Kim, S. W. and Lentz, D. J. (1975). The coating of activated carbons for optimal blood compatibility, in R. Williams, and I. M. Murray-Lyon, (Eds.), *Artificial Liver Support*, Pitman Medical, London.

Arora, K. K., Lacy, J. P., Schacht, R. A., Martin, D. G. and Gutch, C. G. (1975). Calcific cardiomyopathy in advanced renal failure. *Arch. Intern. Med.*, **135**, 603.

Berlyne, G. M., (1976). Dialysis dementia: aluminium again. *Lancet*, **2**, 349.

Brookings, C. H. and Ramsey, J. D. (1975). Salicylate removal by charcoal haemoperfusion in experimental intoxication in dogs: Assessment of efficacy and safety. *Arch. Toxicol.* , **34**(3), 243.

Chang, T. M. S., Gonda, A., Dirks, J. and Malave, N. (1971). Clinical evaluation of chronic intermittent or short-term haemoperfusion in patients with chronic renal failure using semipermeable microcapsules (artificial cells) formed from membrane-coated activated charcoal. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 246.

Chang, T. M. S., (1972). *Artificial Cells,* Thomas, Springfield, Illinois.

Chesney, R. W. and Haughton, P. B. (1974). Tetany following phosphate anaemias in chronic renal disease. *Amer. J. Dis. Child.*, **127**, 584.

Denti, E., Luboz, M. P., Tessore, V. A., Castino, F., Gaglia, P. F. (1975). Adsorbents in haemoperfusion. *Kidney International*, 7, S401.

Flendrig, J. A., Kruis, H. and Das, H. A. (1976). Aluminium intoxication: the cause of dialysis dementia. *Proc. Eur. Dial. Transpl. Assoc.*, **13**, 355.

Gazzard, B. G., Langley, P. G., Weston, M. J., Dunlop, E. H. and Williams, R. (1974). Polymer coating of activated charcoal and its effects on biocompatibility and paracetamol binding. *Clin. Sci. Mol. Med.*, **47**, 97 - 104.

Hoffer, E., Rousseau, I., Sideman, S., Better, O. and Raziel, A. (1975). Selective removal of waste metabolites from blood serum. *Abstracts, 24th International Symposium on Macromolecules*, Jerusalem, July 1975.

Lindsay, R. M., Prentice, C. R. M., Davidson, J. F., Burton, J. A. and McNicol, G. P. (1972). Haemostatic changes during dialysis associated with thrombus formation on dialysis membranes. *Brit. Med. J.*, **11**, 454 - 458.

Moise, O., Sideman, S., Hoffer, E., Rousseau, I. and Better, O. S. (1976). Membrane permeability for inorganic phosphate ions. Technion Research and Development Foundation Research Report August 1976.

Neufeld, R. D. and Thodos, G. (1969). Removal of orthophosphates from aqueous solutions with activated alumina. *Environ, Sci. and Technol.*, **3**, 661.

Nishizawa, E. E., Wynalda, D. J. and Lednicer, D. (1973). Nonthrombogenic surface with Evan's blue, in P. Didisheim *et al.* (Eds.), *Platelets, Thrombosis and Inhibitors*, Proc. Honolulu-US-Japan Meeting, Schattauer Verlag, p. 135.

Sideman, S. and Better, O. (1973). Selective removal of waste metabolites from blood serum. Research proposal 070 147/P, Technion Research and Development Foundation, December 1973.

Townsend, C. M., Remmers, A. R., Srales, H. E. and Fish, J. C. (1973). Intestinal obstruction from medication in patients with renal failure. *New Engl. J. Med.*, **288**, 1058.

Vale, J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975). Use of charcoal haemoperfusion in the management of severely poisoned patients. *Brit. Med. J.*, **1**, 5.

Ward, M. K., Pierides, A. M., Fawcett, P., Shaw, D. A., Perry, R. H., Tomlinson, B. E. and Kerr, D. N. S. (1976). Dialysis encephalopathy syndrome. *Proc. Eur. Dial. Transpl. Assoc.*, **13**, 348.

Yatzidis, H. (1964). A convenient haemoperfusion micro-apparatus over charcoal for the treatment of endogenous and exogenous intoxications. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.*, **1**, 83.

# 32

# ADSORPTION OF BARBITURATES FROM BUFFERS, BLOOD AND INTESTINAL FLUIDS

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**Summary**

The possibility of removing drugs directly from the intestinal tract by ingestion of sorbents is discussed. This would sequester the reservoir of the drug and, in conjunction with direct blood perfusion over sorbents, provide a two-pronged method of rapid drug removal in overdose cases.

Isotherms were measured for adsorption on activated carbon and exchange on Dowex 1 of phenobarbital, pentobarbital and secobarbital in neutral solution, acid solution, stomach fluid (pig), small intestinal fluid (pig), blood (human) and plasma (human). Severe decreases in adsorption capacity were found in the intestinal fluids. Microencapsulation in selective polymers is suggested as a method of overcoming this competitive adsorption.

## 32.1 Introduction

An increasingly frequent cause of hospital admission is drug overdose. It would be of great help in the treatment of these patients to have available a rapid method of removing the various drugs from the intestine and from the blood.

There are several dangers and limitations to the use of several techniques for rapid detoxification such as haemodialysis, exchange transfusion, peritoneal dialysis and forced diuresis. The difficulties of the last three are obvious. Haemodialysis has received considerable attention in cases of massive overdoses (Maher and Shreiner, 1968). However, in barbiturate detoxification, for example, haemodialysis is only effective in the removal of barbital (non protein-bound) and phenobarbital (15% protein-bound). For other barbiturates, the removal is slow or insignificant. A better technique is needed.

The goal of this project is a two-pronged therapeutic approach to drug removal: blood perfusion over sorbents to lower plasma levels and ingestion of sorbents to tie up the drug reservoir in the intestine. Over the last 30 years there have been occasional studies aimed at the oral use of sorbents, notably activated carbon and ion-exchange resins, in the treatment of poisonings through the direct removal of the poison by sorption on the solid.

In screening studies of a large number of sorbents, Edwards and McCredie (1967) found that phenobarbital, secobarbital and barbital were very well removed

from solution by an anion-exchange resin, cholestyramine ('Cuemid': Merck, Sharp and Dohme). For example, only 0.2 g of cholestyramine removed 90% of the phenobarbital from 6 ml of a 25 mM solution. This corresponds to a sorption capacity of 155 mg drug/g resin. Under the same conditions activated carbon removed 61 - 69% of phenobarbital, secobarbital and pentobarbital. However, if bicarbonate was added to the solution, carbon removed 84 - 92% of these drugs. Cholestyramine bound nearly as well with or without the presence of bicarbonate. No isotherms were reported and these results were measured at concentrations frequently much higher than lethal blood levels.

Such data permit calculation of some adsorption isotherm points which give capacity as a function of solution concentration. The important characteristic is the increasing sorption capacity with increasing concentration. Hence, the larger the overdose the better a sorbent works.

There have been several *in vivo* tests of the removal of overdoses of barbiturates using orally administered cholestyramine or activated carbon. Edwards (1965) gave rats the minimum lethal dose of secobarbital (25 mg/200 g rat) in 4 ml of solution by gavage tube, followed by 0.4 g of cholestyramine. Of the 30 controls, 14 died in 5 - 30 min, with plasma barbiturate concentrations above 3 mg%. The plasma levels in the treated animals rose to a maximum of 1.3 mg% and none died in the 30 min test period. Activated carbon should have the same effect.

Chin *et al.* (1970) carried otu similar tests in dogs given 30 mg/kg pentobarbital or 80 mg/kg barbital sodium, followed by orally administered activated carbon equal to 5 times the drug amount. The highest blood levels in the treated animals (first hour) were 6-fold lower than the concentration in the untreated animals. In a mortality test dogs were given lethal doses of 75 mg/kg pentobarbital or 400 mg/kg of barbital sodium, followed by 5 times as much carbon within 30 min. In the controls, 9/11 and 4/6 respectively, died within 45 min. Plasma concentration of pentobarbital at death was above 4 mg%. The plasma concentration of the treated animals peaked at 3.3 mg% 45 min after drug administration. Of the treated animals 3/12 died from pentobarbital and 1/6 died from sodium barbital, a significant decrease in mortality.

Such tests have not been carried out with human subjects, but there have been similar tests on the removal of large doses of aspirin from patients by ingested activated carbon (Levy and Tsuchiya, 1972; Decker *et al.*, 1969). Since the amount of aspirin sorbed on carbon is in the same general range as that for some of the barbiturates, the results of the aspirin study with humans may be instructive. Decker *et al.* gave volunteers 3 g of aspirin and 30 g of activated carbon in aqueous slurry within 30 min. Blood samples taken after 4, 8, 12 and 24 h showed that activated carbon treatment kept the plasma concentration of aspirin at about half the concentration in the untreated controls. Maximum concentration in the controls at 4 h was 23 mg% versus 12 mg% for treated patients. Similar results were also observed in dogs by Phansalkar and Holt (1968).

Levy gave volunteers 1.0 g aspirin followed with up to 20 g of activated carbon given at various intervals. When 10 g of activated carbon were given, the urinary

excretion of aspirin, indicative of the amount absorbed, decreased by nearly 40%. The efficacy of the treatment decreased with time, but it was still of some value up to 3 h after ingestion of the aspirin. An important observation was that if both the dose of aspirin and the dose of carbon were increased by the same factor, the effectiveness of carbon in preventing absorption of aspirin increased linearly. For 1 g aspirin and 10 g carbon about 50% of the aspirin was absorbed. However, for 5 g aspirin and 50 g carbon, less than 20% of the aspirin was absorbed. This indicates the usefulness of activated carbon in cases of massive overdose. Similar effects should be seen with barbiturates.

Activated carbon can compete favourably for removal of protein-bound barbiturates. This is indicated by the work of Decker *et al.* (1971) on the dialysis of various drugs from human plasma, with activated carbon placed in the dialysate to augment removal. In dialysis without carbon, the concentration in the plasma was asymptotic to a finite level for a protein-bound drug. However, when activated carbon was placed in the dialysate, the equilibrium concentration in the plasma appeared to be zero, indicating that the drugs were bound more tightly to carbon than to the plasma proteins. In the case of phenobarbital, the presence of carbon in the dialysate increased the efficiency of removal by a factor of 5. For secobarbital, the increase was 2.4-fold.

This means that treatment with activated carbon not only could remove residual drug from the stomach, or intestinal tract, but also might remove the drug from blood by causing its permeation back from the plasma into the intestinal tract where it would be bound to the carbon.

Another means of removing barbiturates is by direct perfusion of the blood through a cartridge filled with particles of resin or activated carbon. An advantage is the possibility of using much larger amounts of carbon than could be ingested. This technique, using cartridges filled with 200 - 300 g activated carbon, has been employed on humans by Chang (1972), Yatzidis (1964, 1971), Vale *et al.* (1975) and Koffler *et al.* (1975). The method is useful even for the protein-bound drugs.

Both the use of ingestible binders and the use of a perfusion cartridge can be made much more effective. A problem with both is that activated carbon preferentially adsorbs lipids. Hence, in a milieu containing many lipids, such as the intestinal contents, many of the surface sorption sites are taken up by lipids, causing a large decrease in capacity for the drug. For example, in the case of *in vitro* barbiturate sorption on activated carbon, the addition of 0.05 M sodium taurocholate to the mixture decreases the carbon capacity for the drug by nearly a factor of two over its capacity in bicarbonate solution. A more severe example, from another field, is the attempt to adsorb creatinine from intestinal fluid of the pig. In this case, the creatinine adsorption capacity of carbon at 2 h is decreased nearly 20-fold from capacity in buffer solution.

This difficulty might be overcome if the sorbent is microencapsulated in polymeric membranes with selective permeabilities, permitting the desired drug to enter and be adsorbed, but preventing the entrance of the interfering molecules to the carbon.

Data presently in the literature do not give sufficient knowledge of capacity as a function of concentration to permit accurate design of perfusion or ingestion systems for drug detoxification which will lower plasma drug concentrations to a nonlethal level.

Hence, our first measurements have been the adsorption isotherms in buffer at neutral and acidic pH to determine the maximum amount of drug which could be removed from solution. Our most accurate measurements were made using phenobarbital in buffered solutions, but data were also obtained in pure aqueous solutions of secobarbital and pentobarbital. The sorbents employed were activated carbons and ion-exchange resins.

Since the extent of competitive adsorption which occurs is central to the study, isotherms were also measured in blood and in stomach fluid and small gut fluid from the pig (where we have shown that competitive adsorption of creatinine is similar to that obtained *in vivo* in the rat intestine).

## 32.2 Experimental Procedures

### *Sorbents*

The sorbents studied were Merck medicinal activated carbon (a fine powder), Union Carbide NXAC activated carbon (80 - 100 mesh) and Dowex-1 anion-exchange resin.

### *Fluids*

The buffers employed for adsorption of phenobarbital at constant pH were made according to standard procedures. Aqueous solutions of secobarbital and pentobarbital were adjusted to various initial pH values before contacting with sorbents. Stomach and intestinal fluids studied were obtained from the slaughterhouse and centrifuged. The supernatants were used for these studies. To assure a homogenous supply, a large volume of the fluids was treated, subdivided into small vials and frozen. It has been shown by Goldenhersh (1973) that adsorption characteristics are not changed by freezing and storage. The source of human whole blood and plasma was out-dated stock from the Red Cross.

### *Contacting*

In measuring an adsorption isotherm, known weights of the sorbents were equilibrated for 2.5 h at 38°C in a water shaker bath with solutions or biological fluids containing pentobarbital, phenobarbital or secobarbital. Concentrations were as high as 1 g/100 ml, covering the range which might occur in the stomach, intestine and blood after ingestion of a lethal dose. The 2.5 h contact time was ample, since kinetic studies showed that the largest sorbent particles were nearly at equilibrium within 1 h. After contact, the suspensions were centrifuged and the supernatant filtered before analysis.

*Analysis*

The supernatant solutions were analysed for barbiturates according to the procedure of Goldbaum (1952) which is based on the observation that the substituted barbiturates have an adsorption band in strong alkali characteristic of one resonance form, and another band at pH 10 characteristic of another resonance form. The differences in the optical densities is a highly specific identification of the various barbiturates. The difference is a maximum at 260 $\mu$m, which was used in these determinations. When pure solutions are being analysed, the differences are not required, since the optical density at constant pH follows Beer's law.

*Capacity calculations*

The capacity of the solid for the barbiturate was calculated according to the following equation:

$$X_a = \frac{V(C_f - C_o)}{100\, m}$$

where

$X_a$ = capacity (mg barbiturate/g sorbent)

$V$ = volume of solution (ml)

$C_f$ = final concentration (mg/100 ml)

$C_o$ = initial concentration (mg/100 ml)

$m$ = sorbent weight (g)

Methods were also developed, using radiolabelled phenobarbital, to increase the speed with which the isotherms could be measured and to decrease errors due to manipulation. Measurements made using the labelled drug in all the fluids employed agreed well with the accepted chemical method of measuring the barbiturate (correlation coefficient = 0.88).

## 32.3 Results

The logarithm of the capacity was plotted versus the logarithm of the equilibrium solution concentration to obtain a straight line representative of isotherms following the Freundlich equation:

$$X_a = k\, C^{1/n}$$

*Adsorption from aqueous solutions*

Isotherms for adsorption of pentobarbital, secobarbital and phenobarbital from aqueous solutions onto NXAC activated carbon are shown in figure 32.1. The solutions of secobarbital and pentobarbital were adjusted to pH 7.0 before adsorption, and the solution of phenobarbital was carried out in pH 7.45 buffer.
Figure 32.2 contains similar data for adsorption onto Merck carbon. Also shown

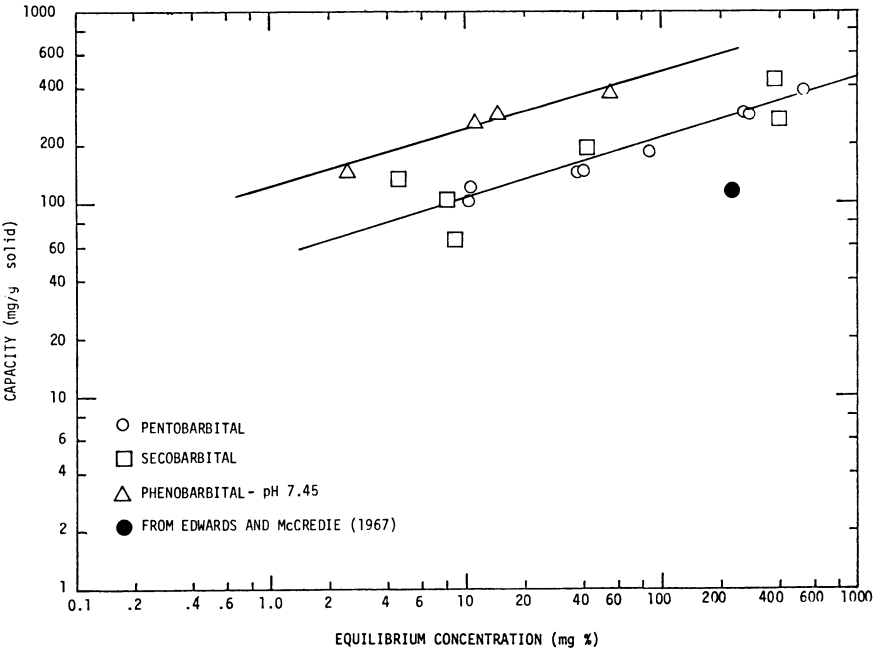


Figure 32.1 Adsorption of barbiturates on NXAC activated carbon from neutral solution.

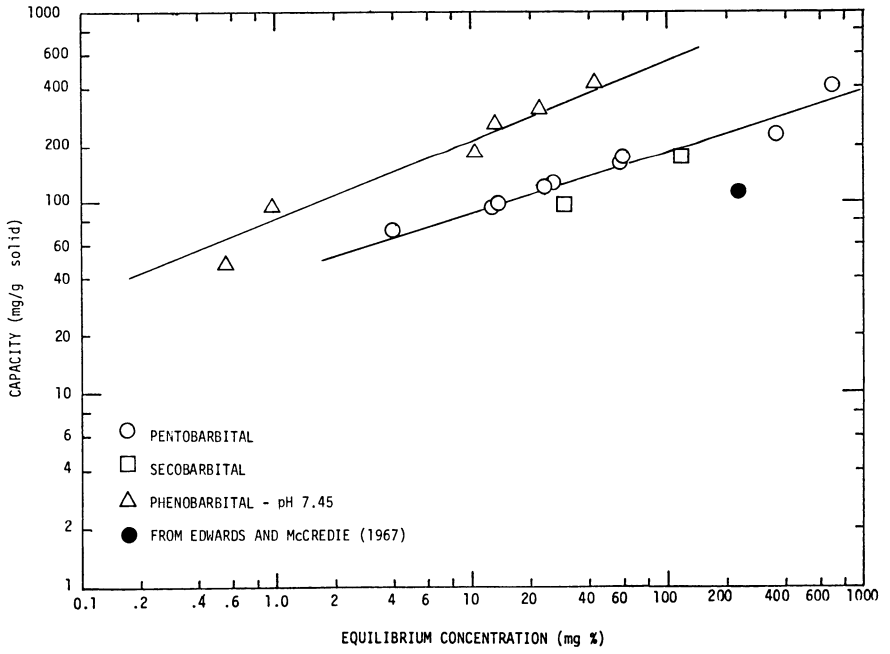


Figure 32.2 Adsorption of barbiturates on Merck activated carbon from neutral solution.

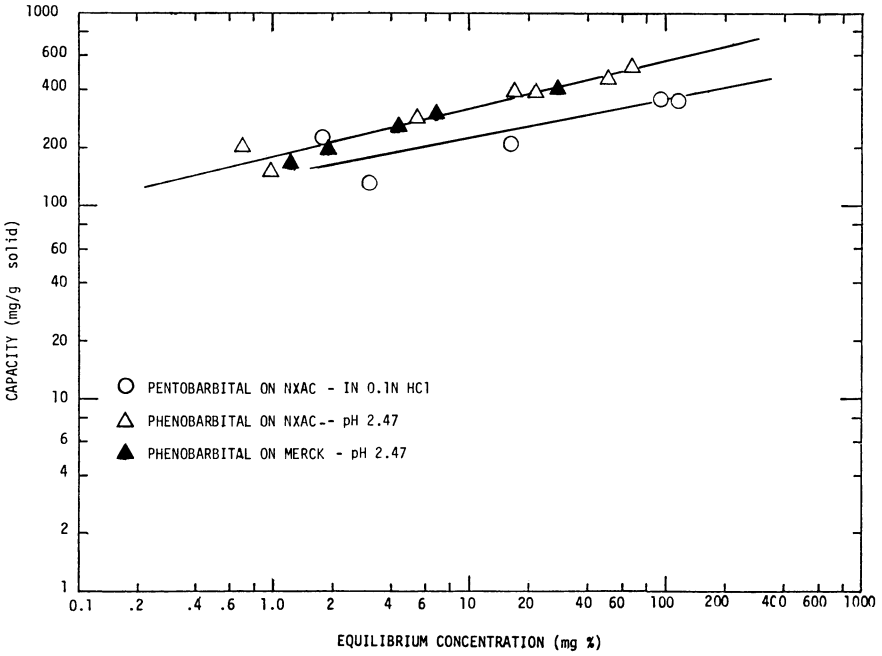


Figure 32.3 Adsorption of barbiturates on activated carbon from acid solution.

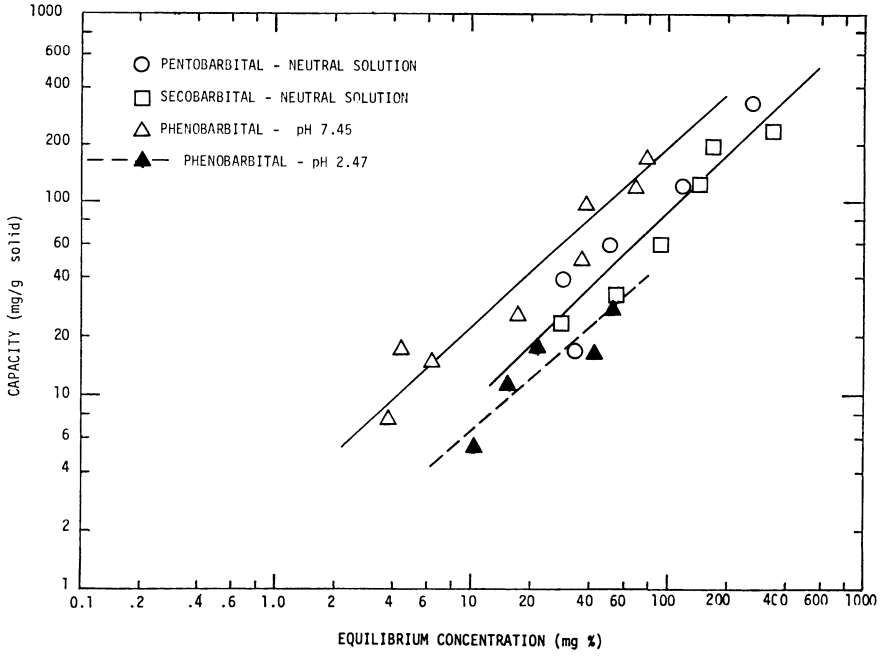


Figure 32.4 Removal of barbiturates by Dowex-1 from neutral and acid solutions.

on the plots is one point obtained by Edwards and McCredie (1967) on activated carbon (stated as 'finely particulate coconut charcoal, Fisher or Merck'). The capacity at neutral pH appears high. Approximately 10 g of carbon would be required to remove 1 g of these barbiturates at an equilibrium concentration of 10 mg%.

If the carbon were ingested for detoxification, its first function would be to remove barbiturates from the stomach. The capacity of the NXAC activated carbon for pentobarbital in 0.1 N HCl solution and for phenobarbital in pH 2.47 buffer, shown in figure 32.3, is approximately double the capacity in neutral solution. Removal of 1 g of pentobarbital at an equilibrium concentration of 10 mg% would require only 3 - 4 g of carbon. This higher capacity at low pH is probably due to the lower degree of ionisation of the pentobarbital. Carbon competes more favourably with water for less ionised molecules. Carbon appears to have high potential for binding barbiturates in the stomach.

The capacity of Dowex-1 for the barbiturates in neutral and acid solution is shown in figure 32.4. It is apparent that the resin is not as effective as the activated carbon except at very high concentrations at neutral pH.

*Adsorption from stomach fluid*

If there were no competing molecules in stomach fluid, it would be expected that the capacity would be as high as in acid buffer. However, there are many other molecules present in stomach fluid to compete for the carbon surface, and the capacity of the activated carbons is much less, as shown by the data in figure 32.5. The effect of competition is striking, causing the capacity of NXAC carbon for phenobarbital to drop by 30-fold, down to 9 mg/g at 10 mg%, and the capacity of Merck carbon to drop by a factor of 6, down to 50 mg/g. It is little wonder that activated carbon has been less effective in *in vivo* detoxification than would be predicted from the *in vitro* measurements. One of the major goals of this project is to eliminate much of the effect of this adsorption competition by encapsulating the carbon in polymeric membranes which will drastically slow the diffusion of the competing species to the carbon surface, while permitting the drug to reach the surface and be adsorbed. If this can be accomplished, then rapid detoxification by ingested adsorbents will be of practical therapeutic value.

*Adsorption from small gut fluid*

The enormous effect of competing molecules is shown by the data in figure 32.6 for adsorption in small gut fluid of the pig. The capacity of Merck carbon for phenobarbital is decreased by a factor of 30 at 10 mg%, and the capacity of NXAC cannot be read on the curve at this concentration. Cholestyramine has a higher capacity than either of the carbons, but it is too low to be practical.

*Adsorption in human blood and plasma*

Adsorption of phenobarbital from human blood and plasma was studied to determine whether competitive adsorption also occurs in these biological fluids. These data, shown in figure 32.7 will aid in design of cartridges packed with sorbents for

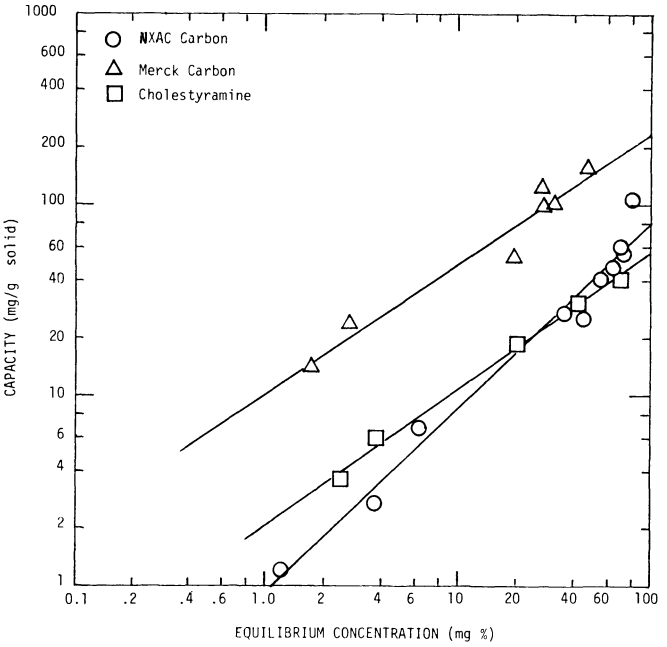


Figure 32.5 Adsorption of phenobarbital from stomach fluid (pig).

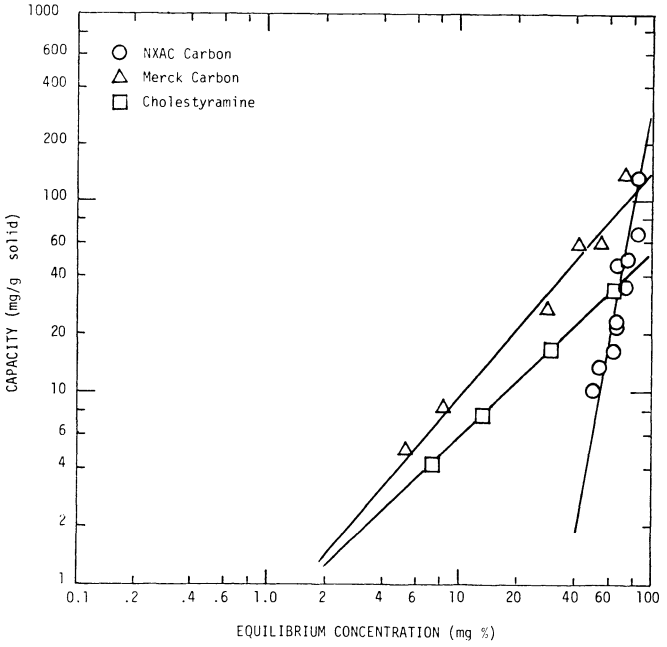


Figure 32.6 Adsorption of phenobarbital from small gut fluid (pig).

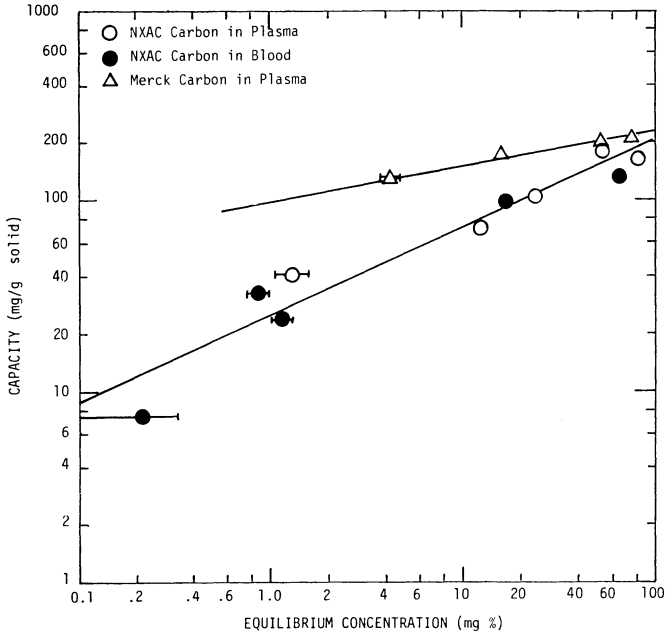


Figure 32.7 Adsorption isotherms for phenobarbital in human blood and plasma

removal of drugs directly from blood by perfusion. Data for Merck carbon in blood could not be obtained because of the difficulty in separating this finely divided adsorbent from the blood. However, the NXAC measurements show that the adsorption capacity is similar in blood and plasma, indicating that the distribution coefficient for phenobarbital between plasma and red blood cells is approximately 1.0. Hence, the data for Merck carbon in plasma should be representative of its capacity in whole human blood.

Capacity of Merck carbon has dropped by a factor of 2 compared to its capacity in neutral buffer, and NXAC has a capacity which has dropped by a factor of over 4. Hence, overcoming the competitive adsorption by microencapsulation in selectively permeable polymeric membranes should be of value also in blood perfusion, where it will permit the cartridge to be smaller, meaning that the blood will contact a small area of foreign surface.

## 32.4 Conclusions

(1) Activated carbons have a high equilibrium adsorption capacity for phenobarbital, pentobarbital and secobarbital in neutral solution. Capacities are 100 - 200 mg drug/g carbon at 10 mg%.

(2) Dowex-1 anion-exchange resin has a much lower capacity for the drug in neutral solution.

(3) In acid solution, the capacity of the activated carbons for the barbiturates is approximately twice that in neutral solution, while the capacity of the anion-exchange resin decreases.

(4) The adsorption of the barbiturates from stomach and small intestinal fluids is severely inhibited, apparently by the presence of preferentially adsorbed species, possibly lipids. Capacities decreased as much as 50-fold compared to capacities in neutral solution.

(5) Competitive adsorption also occurs in blood and plasma, with capacities decreasing up to 4-fold, compared to capacities in neutral solution.

## 32.5 Acknowledgement

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## 32.7 References

Chang, T. M. S. (1972). *Artificial Cells*, Thomas, Springfield, Illinois.

Chin, L., Picchioni, A. L. and Duplisse, B. R. (1970). The action of activated charcoal on poisons in the digestive tract. *Toxicol. Appl. Pharmacol.*, **16**, 786.

Decker, W. J., Shpall, R. A., Corby, D. G., Combs, H. F. and Paynce, C. E. (1969). Inhibition of aspirin absorption by activated charcoal and apomorphine. *Clin. Pharmacol.*, **10**, 710.

Decker, W. J., Combs, H. F., Treuting, J. J. and Banez, R. J. (1971). Dialysis of drugs against activated carbon. *Toxicol. Appl. Pharmacol.*, **18**, 573.

Edwards, K. D. G. (1965). The use of oral ion exchange resins in drug poisoning: General hypothesis and some studies on cholestyramine versus barbiturates. *Med. J. Australia*, **2**, 925.

Edwards, K. D. G. and McCredie, M. (1967). Studies on the binding properties of acidic, basic and neutral drugs to anion and cation-exchange resins and charcoal *in vitro*. *Med. J. Australia*, **1**, 534.

Goldenhersh, K. K. (1973). Adsorption of uraemic waste metabolites by activated carbon in the gastrointestinal tract. M.Sc. Thesis, Department of Chemical Engineering, Washington University, St. Louis, Missouri.

Goldbaum, L. R. (1952). Determination of barbiturates, *Anal. Chem.*, **24**, 1604.

Koffler, A., Hill, J. and Barbour, B. H. (1975). Report in *B-D Haemodetoxification System*, Becton, Dickinson and Company Research Centre, Research Triangle Park, N. Carolina, USA.

Levy, G. and Tsuchiya, T. (1972). Effect of activated charcoal on aspirin absorption in Man, part 1, *Clin. Pharmacol. Therap.*, **13**, 317.

Maher, J. F. and Schreiner, G. E. (1968). The dialysis of poisons and drugs. *Trans. Amer. Soc. Artif. Int. Organs*, **14**, 440.

Phansalkar, S. V. and Holt, L. E., Jr. (1968). Observations on the immediate treatment of poisoning. *J. Pediat.*, **72**, 683.

Vale, J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975). Use of haemoperfusion in the management of severely poisoned patients. *Brit. J. Med.*, **1**, 5.

Yatzidis, H. (1964). A convenient haemoperfusion microapparatus over charcoal for the treatment of endogenous and exogenous intoxications. Its use as an effective artificial kidney. *Proc. Eur. Dialysis and Transplant Assoc.*, **1**, 83.

Yatzidis, H. (1971). The use of ion exchange resins and charcoal in acute barbiturate poisoning, in H. Matthew (Ed.), *Acute Barbiturate Poisoning*, Excerpta Medica, Amsterdam.

# 33

# DIALYSATE REGENERATION

E. DENTI and M. BIAGINI

**Summary**

Different types of adsorbent are available today. Among them activated carbon is most studied for use in medicine. There is increasing evidence that activated carbon is able to remove some known as well as unknown uraemic toxins. The requirements for the selection of the activated carbon for use in dialysate regeneration are briefly discussed.

Dialysate regeneration is the first necessary step to reduce and simplify haemodialysis. A limited clinical experience seems to confirm that a substantial reduction of dialysate based on regeneration of activated carbon is sufficiently safe for the kidney patient. However, more research work is required to further reduce the size of dialysis machines, to achieve true portability and safe and easy use.

## 33.1 Introduction

In a broad sense an adsorbent is any material able to adsorb a substance from a solution. However, a classification of adsorbents based on the adsorption mechanism is more accurate and useful.

Table 33.1 shows a tentative classification of 'adsorbents' available to solve a variety of purification problems. It is very difficult to date exactly the first use of an adsorbent in medicine but it is almost certain that the oral use of activated carbon in poisoning was empirically known centuries ago. Today the use of adsorbents in medicine is directed to the treatment of poisoning, uraemia, and liver failure.

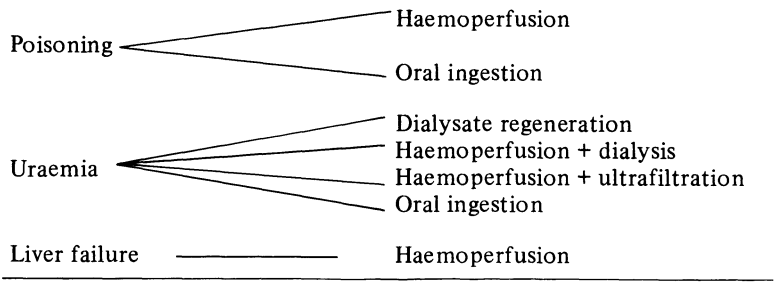
**Table 33.1**

Classification of adsorbents

| | |
|---|---|
| Aspecific | Activated carbons, clays, etc |
| | Macroreticular resins |
| Partially specific | Organic ion exchangers |
| | Inorganic ion exchangers |
| Specific | Binders (e.g. $Al_2O_3$) |
| | Oxystarch, oxycellulose |
| | Affinity chromatography Supports |

**Table 33.2**

Clinical applications of adsorbents




The method of use of the adsorbent is dependent on the specific application and the particular therapeutic problem considered. Table 33.2 shows schematically the approaches utilised for different pathologies. In the present work the use of adsorbents for dialysate regeneration is briefly discussed.

## 33.2 Dialysate Regeneration by Adsorbents

During a regular dialysis treatment the dialysate flow rate into the dialyser is 500 ml/min. The amount of dialysate used for a single treatment is dependent on the efficiency of the dialyser, the patient requirements, and on the dialysis schedule adopted. Using a low efficiency device like a Kiil dialyser the weekly quantity of dialysate is about 1200 l; with high efficiency dialysers and shorter schedules at least 600 l of dialysate are used.

Obviously a drastic reduction in the dialysate volume obtained by a regeneration system is a very attractive measure not only to reduce the cost of haemodialysis but also for size reduction (portability, wearability), simplification in the dialysate preparation and the possibility of using tap water. In reviewing the different approaches applied to the regeneration problem it is useful to discuss the principal requirements of a purification system. Our knowledge of the identity, number, role and relative quantity of uraemic toxins is still very incomplete. For this reason a detailed and quantitative approach to the removal requirements is only partially possible. Nevertheless some well known molecules may act as an index of dialysis adequacy. Kolff *et al.* (1974) have indicated the amount of ions and molecules removed from blood by 1500 ml ultrafiltration and the quantity of urine constituents which must be removed by dialysis in the case of a daily treatment.

Table 33.3 reports the approximate amount of some blood constituents removed by a single dialysis treatment during a three times a week dialysis schedule. The amount indicated has to be considered only a rough estimate not compensated for such variables as patient generation rate and dietary intake.

Moreover, the real requirement in terms of removal of uraemic toxins is also dependent on the individual patient tolerance.

**Table 33.3**

Quantities of some substances removed during a single haemodialysis treatment

| | |
|---|---|
| Urea | −30 g |
| Creatinine | −4 to 5 g |
| Uric acid | −0.4 to 2 g |
| Methyl guanidines | −0.05 g |
| Potassium | −5 g |
| Magnesium | −0.013 g |
| Sulphate | −3 g |
| Phosphates | −3 to 6 g |
| Water | −1.5 to 2 l |

A more careful consideration of the molecules and ions listed in table 33.3 shows that it is necessary to remove organic molecules, anions, cations and water. On the other hand, it is well known that the list of organic molecules removed by haemodialysis is potentially a long one. In the absence of kidney excretion an over-accumulation of any organic molecules may act by direct or by synergistic effect impairing homeostasis.

Adsorbents for organic molecules are essentially broad spectrum (activated carbons, macroreticular resins) or specific (affinity chromatography supports). The criteria of selection of the activated carbon for medical use have been discussed by Walker *et al.* (1976). Using the activated carbon for the adsorption of organic molecules from the dialysate, the requirements and the selection tests are in some cases less strict than when evaluating the activated carbons for haemoperfusion. The main selection criteria are the adsorption capacity and the medical grade quality, the attrition resistance in respect of microparticle generation being less important.

The macroreticular resins have also a good adsorption capacity for many organic molecules as pointed out by Rosenbaum *et al.* (1970) and Rosenbaum (1975). Amberlite XAD-2 and XAD-4 resins are highly effective for lipid-soluble organic molecules. Willson (1975), studied the removal of protein bound anions by Dowex-1 and Amberlite XAD-2. Both resins appear to be more active in saline than in plasma and are probably suitable for a dialysate purification circuit. The field of application of the inorganic ion exchanger in medicine was pioneered by Gordon *et al.* (1968). Their work on the use of zirconium phosphate as a selective adsorbent for ammonia made possible the realisation of the sorbent-based recirculating dialysate system marketed by the CCI Company under the trade name of Redy system.

The medical use of specific binders was studied by Rutherford *et al.* (1973) for the removal of phosphates by means of aluminium oxide. Giordano *et al.* (1972)

developed a specific urea binder, the oxystarch, active in the gastrointestinal tract and, more recently (Giordano *et al.*, 1976), an insoluble urea and ammonia binder, the oxycellulose. The use of such adsorbents for dialysis regeneration was studied by Petrella *et al.* (1974).

### 33.3 Dialysate Regeneration by Chemical or Physical Treatment of the Dialysate

A completely different approach was patented by Rhône-Poulenc (1973).

Dialysate regeneration is achieved in a two step process: oxidation of the dialysate by means of sodium hypochlorite and adsorption of the oxidised organic molecules by an activated carbon cartridge. The above mentioned French company in a patent by Bizot and Sausse (1973) claims that the same result is achieved by a controlled electrolysis of the dialysate followed by purification on activated carbon. In this case also, the organic molecules undergo an oxidation process by the effect of the sodium hypochlorite locally produced by the dialysate electrolysis.

The activated carbon adsorption of organic molecules is a temperature dependent process. For example, urea adsorption (Giordano *et al.*, 1976) is greatly improved at 0°C. The use of a device based on low temperature adsorption followed by regeneration by warming the activated carbon cartridge is at the present time in development at Naples University.

### 33.4 Clinical Experience

The organic toxins spectrum involved in uraemia has not been completely identified. For this reason the efficiency of a dialysate regeneration system must be evaluated on the basis of prolonged clinical trials. The good results of long-term clinical use of the Redy system suggest that the uraemic toxins are easily removed by the activated carbon contained in the cartridge (Lewin *et al.*, 1972). Until now the Redy system is the only one complete dialysate regeneration system able to work with a very small volume of dialysate (about 5 l). However, relatively complex equipment is necessary to infuse the ions ($Ca^{2+}$, $Mg^{2+}$) adsorbed from the regeneration cartridge.

Maeda *et al* (1973) have utilised a regeneration of 30 l of dialysate over a cartridge containing 500 g of activated carbon and 200 g of alumina and more recently (1974) have reduced the dialysate to 10 l.

A limited set of clinical data is available to date. The wearable artificial kidney under development at the University of Utah (Walker *et al.*, 1976, see p. 139 of these proceedings) has been also submitted to limited clinical trials (Stephen *et al.*, 1975).

Following extensive *in vitro* experiments a limited clinical experiment was performed by us to compare patients' well-being and biochemistry following a period of treatment with a low volume activated carbon regenerated dialysate. A dialysis monitor produced by Sorin Co, 13040 Saluggia, Italy was utilised. This device contains a disposable 300 g activated carbon cartridge as indicated in figure 33.1.

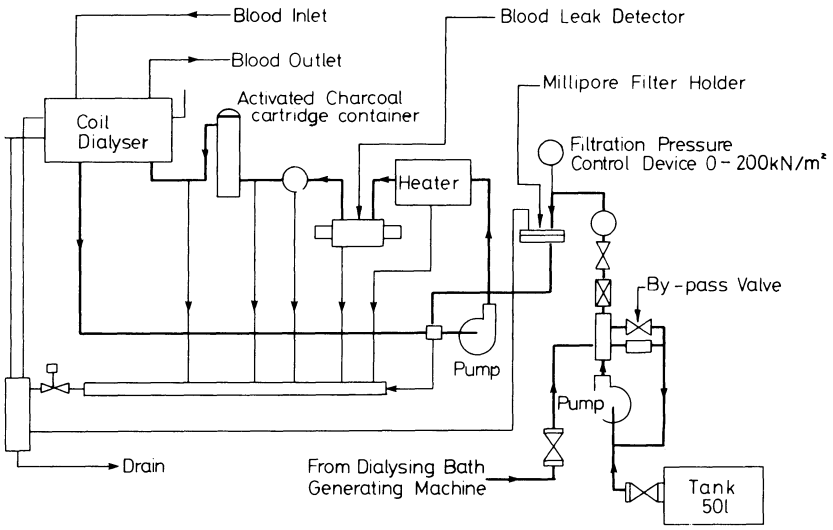



Figure 33.1 Circuit of monitor with dialysis bath regeneration

A variable flow of fresh dialysate (0 - 1000 ml/min is pumped into the canister through a 0.22 Millipore filter in order to prevent bacterial growth. Two operational procedures were adopted: recirculating single pass, dialysate flow rate 500 ml/min, without adsorbing cartridge, total dialysate volume 190 litres; dialysate partially recycled, flow rate of fresh dialysate 110 ml/min, 300 g of activated carbon in the cartridge, total dialysate volume 50l.

Three patients well stabilised and previously treated with a standard dialysis technique were treated for 3 months with the first procedure and then for a period of respectively 16, 9 and 13 months with the second procedure. The patients were on a protein-free, sodium-restricted (30 mEq/day) diet.

The results of this experiment are summarised in table 33.4. Moreover, during 520 dialyses performed with the second procedure pyrogenic reactions never occurred. The nerve conduction velocity (ulnar) measured at the end of the clinical tests varied from 41 to 44 m/s showing normal values. No patient showed radiographic symptoms of uraemic osteodystrophy. The arterial pressure and ECG were normal. All three patients were fully rehabilitated according to the EDTA criteria (Cameron *et al.*, 1970). The predialysis urea concentration was significantly higher during the use of low volume regenerated dialysate (second procedure) compared with the mean values measured during the standard treatment. Nevertheless, all three patients have been enjoying relatively good health.

## 33.5 Conclusions

There is increasing evidence that it is possible to reduce the dialysate volume through a regeneration treatment. The reduction of dialysate from 50 to 20 l (depending on the dialysis frequency) is probably immediately possible using simple

**Table 33.4**

Clinical test: standard dialysis versus reduced volume regenerated dialysate. Dialysis schedules – 1: standard treatment with SP 25 (Dasco) coil dialyser twice a week for 6 h (190 l dialysate), 2: experimental treatment with SP 75 coil dialyser, 50 l dialysate regenerated over a 300 g activated carbon cartridge twice a week for 6 h

| *Patient* | | | *Blood chemistry (mean values)* | | | | | | *Well-being subjective evaluation from + to +++++* | *Type of treatment* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Name* | *Sex* | *Age* | *Urea mg%* | *Creatinine mg%* | *Urate mg%* | $K^+$ *mEq/l* | $PO_4^{3-}$ *mEq/l* | *HCT %* | | |
| F.G. | M | 50 | 146 | 10.1 | – | – | 4.5 | 24 | ++++ | 1: standard without regeneration |
| | | | 189 | 11.5 | 6.0 | 5.2 | 5.6 | 27 | ++++ | 2: with regeneration for 16 months |
| S.R. | M | 27 | 159 | 7.9 | 7.5 | 4.1 | 6.1 | 24 | ++++ | 1 |
| | | | 174 | 7.0 | 6.7 | 4.2 | 6.3 | 26 | +++++ | 2 for 9 months |
| P.T. | F | 44 | 162 | 8.9 | – | 5.5 | 3.8 | 23 | ++++ | 1 |
| | | | 228 | 10.5 | 7.0 | 4.8 | 7.6 | 22 | ++++ | 2 for 13 months |

and cheap regeneration on a medical grade activated carbon cartridge of convenient size. Careful control of blood potassium and phosphate levels is mandatory to eventually correct the situation by the use of oral adsorbents. A further reduction of the dialysate will be useful in making a fully portable or wearable artificial kidney.

The spectrum of adsorbents available is large enough for the adsorption of the organic uraemic toxins and for the uptake of potassium and phosphates. The major problem to solve is the urea removal. The only procedure fully developed is the hydrolysis of urea to ammonia and the selective fixation of the ammonia on a special grade zirconium phosphate (Gordon *et al.*, 1966) as adopted in the Redy system. Nevertheless, the Redy system is too heavy and complex to be considered a wholly portable unit. We think that more effort is needed to develop a small sized urea removal system. Furthermore it will be necessary to find a way to easily and safely regulate the electrolyte balance even with 1 - 2 l of dialysate. This is not a simple goal but we believe that a substantial reduction of the size of the dialysis apparatus, following and developing further the way pioneered by Kolff, will be beneficial both for the patient and for dialysis units.

## 33.6 References

Bizot, J. and Sausse, A. (1973). Societé des Usines Chimiques Rhône-Poulenc, French Patent No. 71.44 868.

Cameron, J. S., Ellis, F. G., Ogg, C. S., Bewick, M., Boulton-Jones, J. M., Robinson, R. O. and Harrison, J. (1970). A comparison of mortality and rehabilitation in regular dialysis and transplantation. *Proc. Eur. Dial. Transpl. Assoc.*, 7, 25.

Giordano, C., Esposito, R., Randazzo, G. and Pluvio, M. (1972). Oxystarch as a gastrointestinal sorbent in uremia. *Uremia*, Thieme Verlag, Stuttgart.

Giordano, C., Esposito, R. and Pluvio, M. (1975). Oxycellulose and ammonia treated oxystarch as insoluble polyaldehydes in uraemia. *Kidney International,* 7, S380.

Giordano, C. (1976). *Conference on Sorbents in Uraemia,* Downstate Medical Centre, Brooklyn, New York, November 3-4, 1975. *Kidney International* (in press).

Gordon, A., Popovtzer, M., Greenbaum, M., Morantz, L., McArthur, M., De Palma, J. R. and Maxwell, M. H. (1968). Zirconium phosphate – a potentially useful adsorbent in the treatment of chronic uraemia. *Proc. Eur. Dial. Transpl. Assoc.,* **5**, 86 (Excerpta Medica Foundation edition, Amsterdam 1969).

Kolff, W. J., Andrade, J. D. and Coleman, D. L. (1974). Wearable portable artificial kidney – a feasibility study. Annual Report, Contract No. 4 - 2200, National Institute of Health, Bethesda, Maryland.

Lewin, A. J., Greenbaum, M. A., Gordon, A. and Maxwell, M. H. (1972). Current status of the clinical application of the Redy dialysate delivery system. *Proc. Dial. Transpl. Forum*, **2**, 53.

Maeda, K., Kawaguchi, S., Manji, T., Kobayashi, K., Ohta, K., Saito, A., Shimoji, T., Yui, T. and Hori, M. (1973). Portable artificial kidney system with adsorbents. *Proc. Eur. Dial. Transpl. Assoc.*, **10**, 298.

Maeda, K., Kawaguchi, S., Shimizu, K., Manji, T., Kobayashi, K., Ohta, K., Saito, A., Amano, I., Yoshiyama, N., Nakagawa, S. and Koshikawa, S. (1974). Ten-litre dialysate supply system with adsorbents. *Proc. Eur. Dial. Transpl. Assoc.*, **11**, 180.

Petrella, E., Orlandini, G. C. and Bigi, L. (1974). Regeneration of dialysis fluid. *Proc. Eur. Dial. Transpl. Assoc.*, **11**, 173.

Rhône-Poulenc, Societé des Usines Chimiques, (1973) French Patent No. 71.44867.

Rosenbaum, J. L., Winsten, S., Kramer, M. S., Moros, J. and Raja, R. (1970). Resin haemoperfusion in the treatment of drug intoxications. *Trans. Amer. Soc. Artif. Int. Organs*, **16**, 134.

Rosenbaum, J. L. (1975). Resin haemoperfusion in the treatment of acute drug intoxication. *Ind. Eng. Chem. Prod. Res. Dev.*, **14**, 99.

Rutherford, E., Mercado, A., Hruska, E., Harter, H., Mason, N., Sparks, R., Klahr, S., Slatopolsky, E. (1973). An evaluation of a new and effective phosphorus binding agent. *Trans. Amer. Soc. Artif. Int. Organs.*, **19**, 446.

Stephen, R. L., Jacobsen, S. C., Aktin-Thor, E. and Kolff, W. J. (1975). Portable/wearable artificial kidney (WAK) – initial evaluation. *12th Eur. Dial. Transpl. Assoc. Congress*, Copenhagen, August 1975, p. 26.

Walker, J. M., Denti, E., Van Wagenen, R., Andrade, J. D. (1976). Evaluation and selection of activated carbon for haemoperfusion. *Conference on Sorbents in Uraemia*, Downstate Medical Centre, Brooklyn, New York, November 3 - 4, 1975. *Kidney International* (in press).

Willson, R. A. (1975). Resin as adsorbents, including experimental studies in dogs with obstructive jaundice, in R. Williams and I. M. Murray-Lyon (Eds.) *Artificial Liver Support*, Pitman Medical, London, p. 109.

# 34

# AN *IN VITRO* STUDY OF THE COMPARATIVE PERFORMANCE OF SINGLE AND DOUBLE NEEDLE DIALYSIS SYSTEMS

M. M. BLACK, G. HARSTON and C. SEGASBY

**Summary**

A description is given of a pilot study of some of the parameters which can affect the performance of single and double needle dialysis systems.

The experimental approach employs a steady flow simulated circulation system in conjunction with a commercial dialysis unit. The *in vitro* test programme is designed to provide a clearer indication of those parameters which might be most significant in describing the fluid mechanics of a dialysis system.

Analysis of the results so far would suggest that a more detailed investigation of some parameters, based on clearance rates through the dialyser, should be undertaken.

## 34.1 Introduction

Single needle dialysis, as developed by Kopp, has rapidly become an accepted method of treatment for patients with chronic renal failure. Many studies have also been undertaken based on clinical tests, which present evidence of the comparable performance of single needle dialysis versus double needle dialysis. From a review of past literature it would appear that, in these tests, very little research has been undertaken on the fluid mechanics of single needle dialysis.

Previous clinical studies suggest that the performance of single needle dialysis is comparable to that of double needle dialysis. After a study based on clinical tests, Hilderson *et al.* (1974) concluded that the single needle system 'has been found to be equally as efficient as the double needle system on virtually all counts'. As will be seen later, the results of this present pilot study do not appear to give support to this statement.

Kirkham *et al.* (1974) state that it is important for users of single needle dialysis to optimise pressure and time settings in order to optimise the performance of single needle dialysis. Previous studies of single needle dialysis have not examined the fluid mechanics in terms of needle orientation and variation in arterial flows. Since the total system is dependent for its successful operation on an understanding of the fluid mechanics involved this could be a serious omission

from previous investigations. This present study is designed to investigate these fluid mechanics aspects of single needle dialysis and compare the results with similar tests carried out using the double needle approach.

## 34.2 Experimental Methods

*Simulated arterial circulation*

As shown in figure 34.1, the simulated arterial circulation comprises three tanks, two of which form a constant head system to maintain steady flow through the test section and a third as a reservoir to enable the analogue blood fluid to be pumped continually around the system. The flow rate is controlled by the valve (8).

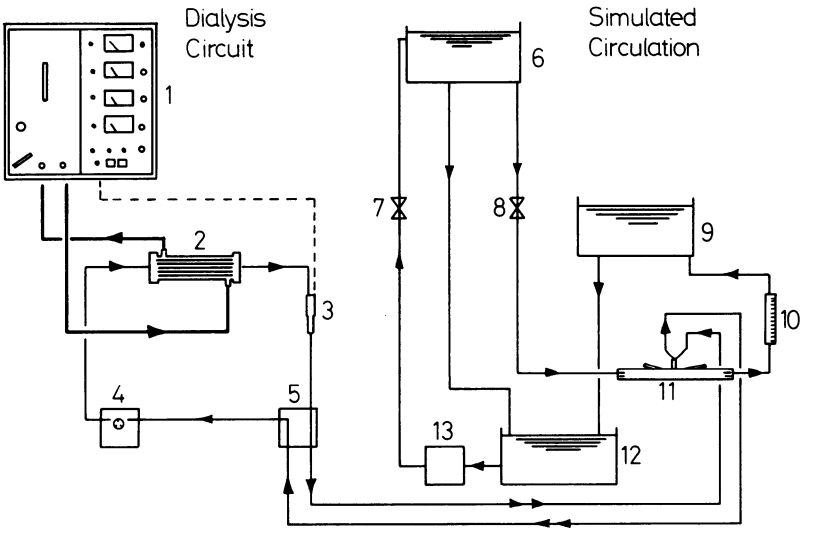



Figure 34.1 Layout of experimental test equipment. 1: Nycotron Adpac 563, 2: Cordis Dow model 4 hollow fibre dialyser, 3: bubble trap, 4: Watson Marlow Hiflow Peristaltic Pump, 5: Gambro SN1B Single Needle monitor, 6: tank 1, 7: valve, 8: valve, 9: tank 2, 10: flowmeter, 11: simulated artery, 12: tank 3, 13 pump.

The simulated artery (11) is constructed from acrylic tubing of 5 mm internal diameter into which are set three tapping points labelled A, B and C for the insertion of the needle. Positions A, B and C are at 15°, 90°, and 165° respectively, to the horizontal (figure 34.2). A simulated artery with three sets of double tapping points, arranged in a similar way, is used for the double needle study.

This configuration, with the exception of the 90° position, is analogous to needle positions with and against arterial circulation as found in the *in vivo* situation. Although it is unlikely that a 90° needle position would be used *in vivo*, it was felt desirable to include this position as a mean between the two extremes.

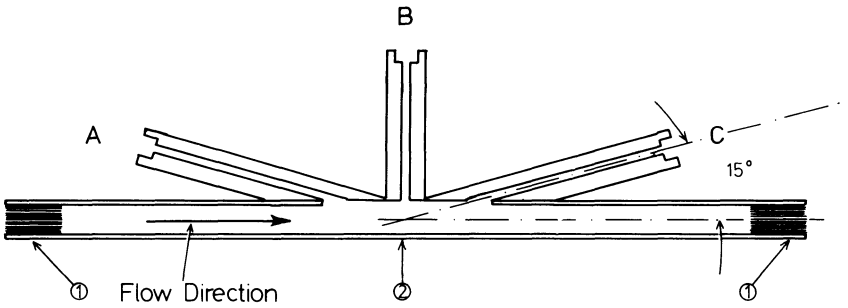



Figure 34.2 Detail of simulated arterial test section. A : 15° needle position, B: 90° needle position, C: 165° needle position, 1: flow straighteners, 2: 5 mm I.D. acrylic tubing.

*Blood analogue and simulated arterial flow rates*

We are aware that the ideal blood analogue should contain constituents of small, medium and large molecular size in order to provide an ideal simulation of the *in vivo* situation. However, for simplicity of the experimental technique in this pilot study, the blood analogue consists of 10 l of 0.9% sodium chloride solution, which is only of small molecular size.

In 1972 both Kopp and Goldberg (Kopp *et al.*, 1972; Goldberg *et al.*, 1972) found that the flow rates through well-developed subcutaneous fistulae were usually in excess of 300 ml/min. By choosing flow rates of 300, 400, and 500 ml/min the simulated arterial flow rate should represent the possible range of flows met with in the clinical situation.

*Dialysis circuit*

A commercial Nycotron Adpac 563 dialysis machine is used to pump fresh dialysing fluid, maintained at 38°C, at a rate of 600 ml/min, to a Cordis-Dow model 4 artificial kidney. The machine also monitors dialysate pressure, blood pressure and dialysing fluid concentration. Thus all these parameters could be set to the same value for each test. It should be noted that the implication of this matching amongst tests of these parameters is that the ultrafiltration rate in the dialyser should be the same in every case.

*Extracorporeal blood analogue circuit*

The fistula outflow and return tubing sets used to connect the needles to the dialyser are those used in the normal clinical situation. A Watson Marlow Hiflo persistaltic blood pump is used to maintain the required blood analogue flow.

The single needle is attached to a Y-junction tube, the branches of which go to a Gambro SN1B single needle monitor which alternately clamps off each line at various time or pressure intervals. In the present test programme this monitor was

used in the time operation mode set for five second intervals. As a result of this control system the actual flow through the circuit is of a pulsatile nature. In the double needle configuration there are individual inlet and outlet needles and lines. The flow is again controlled by a Hiflow blood pump. However, in this twin line situation, the flow in the circuit is maintained at an approximately steady value.

*Test procedure*

A dialysis was performed at each of the three simulated arterial flow rates, for each needle position, using both single and double needle. By appropriate setting of the blood pump, the mean flow through the dialyser was maintained at 100 ml/min for both single and double needle tests. Samples were withdrawn from the simulated arterial circulation over a period of 1 h. During the first half-hour the samples were taken at 5 min intervals and for the latter half at 10 min intervals. The test samples were analysed for sodium content using an I.L. 343 automatic flame photometer.

In order to analyse the results, as described in section 34.3, it is also necessary to have a value of the sodium level in the dialysing fluid prior to dialysis.

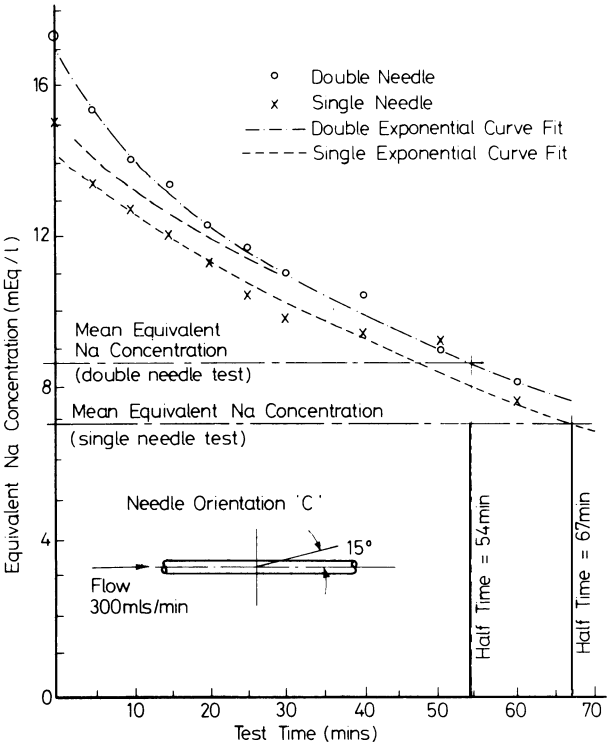



Figure 34.3 Typical exponential curve fits for single and double needle dialysis tests

## 34.3 Analysis of Test Results

From the above tests, values of sodium concentration in the simulated arterial circulation at various intervals during the course of dialysis were obtained.

Figure 34.3 shows a typical set of results where sodium concentration in the simulated arterial circulation is plotted against time of dialysis. It should be noted that plotted concentration levels are obtained by subtracting the original dialysis sodium concentration from the measured sodium concentration in the simulated arterial fluid. This procedure, which yields a graph in which the horizontal axis represents an equivalent zero sodium concentration level, simplifies the theoretical exponential curve fitting which is carried out on the test results.

In order to make comparisons of the various test results it was necessary to select a single parameter which could represent the performance of the system. For this purpose a 'half-time' for the simulated arterial sodium concentration level was used. This half-time represented the time in minutes required for the sodium concentration level in the simulated arterial circulation to reach a mean of the original dialysate and undialysed arterial fluid concentrations.

Rather than select these half-times from visual inspection of the test curves, the experimental results were analysed by computer to obtain the best single or double exponential curve fit. Having obtained and plotted the best curve, the half-time was then measured from this plot. The half-time values so obtained are shown in table 34.1. In addition, the mean values of half-times for different simulated arterial flow, neglecting needle position, and for different needle positions, neglecting simulated arterial flow values, are shown in table 34.2(a) and (b) respectively.

## 34.4 Conclusions

From table 34.1 it can be seen that half-times are consistently less for double needle dialysis, although for both single and double needle tests, the analogue blood flow per unit time through the dialyser was the same. One possible explanation for this behaviour could be the pulsatile nature of this flow in the case of the

**Table 34.1**

Half-times (in min) for different simulated arterial flow rates and needle configurations. Form of exponential fit given by s for single, d for double

| | *Single needle* | | | *Double needle* | | |
|---|---|---|---|---|---|---|
| *Flow ml/min* \ *Needle Configuration* | *A* | *B* | *C* | *A* | *B* | *C* |
| 500 | $69_s$ | $51_s$ | $62_s$ | $54_s$ | $53_d$ | $73_s$ |
| 400 | $92_s$ | $66_s$ | $82_d$ | $47_d$ | $44_d$ | $55_d$ |
| 300 | $83_s$ | $75_s$ | $67_s$ | $40_d$ | $45_d$ | $54_d$ |

single needle system since it is likely that the membrane diffusion performance is affected by the variation in flow velocity of the fluid on the blood side. Such fluctuation in flow rate inevitably leads to a variation in the concentration gradient across the membrane which, in turn, could reduce dialysis efficiency. This particular phenomenon certainly merits further investigation, which could be achieved by a more detailed study of the clearance rates across the dialyser using radioactively labelled molecules. In this way the clearance rates can be assessed accurately using appropriately placed scintillation counters.

The computer programmes used for the exponential curve fitting indicated in each case the confidence limits of the fit. In the case of the double exponential programme the results were analysed such that if they were more appropriate to a single exponential curve the programme converged accordingly. From table 34.1 it can be seen that, in general, single needle results were best fitted by a single exponential curve and the double needle by a double exponential curve. This result is also worthy of further study, as at this stage it is not clear what physical characteristics of these systems would produce this effect.

Table 34.2(a) and (b) shows the effects of needle configuration, and flow rate in the simulated arterial system. In the case of flow rate it is of interest to note that on the basis of half-times, the single needle approach performs best at the

**Table 34.2**

Mean half-times (in min) for

different simulated arterial flow rates neglecting the effect of needle position,

(a)

| *Flow ml/min* | *Single needle* | *Double needle* |
|---|---|---|
| 500 | 61 | 60 |
| 400 | 80 | 49 |
| 300 | 75 | 46 |

different needle positions neglecting the effect of simulated arterial flow rate

(b)

| *Needle position* | *Single needle* | *Double needle* |
|---|---|---|
| A | 81 | 47 |
| B | 64 | 47 |
| C | 72 | 61 |

highest arterial flow of 500 ml/min, whereas in the case of double needle dialysis, the best performance occurs at the lowest arterial flow of 300 ml/min. This result implies that the single needle approach is more likely to be successful clinically in patients with a high flow rate through the fistula.

Unfortunately, it is apparently more common to use the single needle system for patients with poor fistulae and blood flows, where it is often difficult to gain access to the circulation even with a single needle. Since the results presented are only those of a pilot study it is unreasonable to make any definitive recommendations on the use of single needle dialysis at this stage. However, the authors are planning a much fuller investigation of these problems using radioactive tracer techniques and then, hopefully, will be more able to present such recommendations.

The effect of needle position is illustrated by the results given in table 34.2(b). From these results, neglecting the mid-position B, it would appear that the single needle configuration is most efficient when the needle is inserted in the opposite direction to that of the arterial flow. In the case of double needle configuration, the best performance is achieved when the needles are inserted in the same direction as the arterial flow. The half-times for the mid-position would suggest that both systems give their best performance for this configuration. However, it is unlikely that needles could be inserted in this way in the clinical situation. Furthermore, it would again be wrong to make any strong recommendations on needle position based on the limited number of tests undertaken in this pilot study.

In conclusion, the authors believed that this present study has highlighted what might be omissions in previous research on the performance of renal dialysis systems. The results obtained so far would suggest that both arterial flow and needle configuration may play a more significant role in the overall performance of the system than has previously been considered. Most of the literature dealing with single needle dialysis suggests firstly that its performance is equivalent to that of the double needle system and, secondly, that it is less traumatic for the patient because only one needle insertion is required per treatment.

The present investigation does not support the first of these statements. In addition, the advantage of the single needle approach in terms of less trauma has led to the use of single needle dialysis in patients with less patent subcutaneous fistulae. Such patients are also likely to exhibit low levels of arterial flow and, as already noted, the results of this present investigation would indicate that patients with low blood flows are least suited for single needle dialysis.

## 34.5 Acknowledgements

The authors would like to express their thanks to Dr M. M. Platts for her advice on clinical aspects of dialysis and to Dr R. C. Smart for his assistance on the computer analysis of the results.

This work forms part of a continuing research programme being undertaken by the Sheffield University and Area Health Authority (Teaching) Department of Medical Physics.

### 34.6 References

Goldberg, E., Hill, W., Kanter, A. and Ivanovich, P. (1972). Small vessel prosthesis: An external arteriovenous fistula with funnel cannula. *Proc. Int. Med. Chicago*, **29**, 82 - 83.

Hilderson, J., Van Waelegha, J. P., Van Egmond, J., Van Haelst, J. P., Schelsraete, K. and Ringoir, S. (1974). Single needle versus double needle dialysis. *Dial. Transpl.*, 3, 6.

Kirkham, R. L., Schatten, K., and Ricks, D. (1974). Single needle versus double needle dialysis. *Dial. Transpl.*, 3, 2.

Kopp, K. F., Gutch, C. and Kolff. W. J. (1972). Single needle dialysis. *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 75.

# 35

# AN IMPROVED OXYSTARCH IN THE TREATMENT OF RENAL FAILURE

C. GIORDANO, R. ESPOSITO and M. PLUVIO

**Summary**

Gastrointestinal polyaldehyde sorbents such as oxystarch and oxycellulose provide a useful ancillary medication for uraemia. Comments, supported by *in vivo* results, are offered on improving polyaldehyde reactivity in the stomach by means of water pretreatment of the sorbent.

## 35.1 Introduction

The possibility of reducing nitrogen in uraemic patients through the administration of polyaldehydes (oxystarch or oxycellulose) has been previously documented (Giordano *et al.*, 1972; Friedman *et al.* 1974; Man *et al.* 1973). In the light of some recent experimental data obtained by us, we considered how to improve the reactivity of orally administered polyaldehydes with the ultimate aim of increasing nitrogen excretion.

We have observed that the binding capacity of oxystarch is considerably increased at an acid pH. This is due to the fact that the $H^+$ ion, and therefore the acid environment, catalyses the reaction between the aldehyde and the amino or amido groups.

Additionally the adsorbing capacity of polyaldehydes (both that of oxystarch and oxycellulose), is greatly increased when these materials are pretreated with water for at least 24 h. Such enhanced action, we believe, is due to an increase in the contact surface of the polyaldehyde due to its swelling with water. For example, figures 35.1 and 35.2 show that the reactivity of the polyaldehydes in solution is almost doubled after 24 h.

Moreover, oxystarch pretreated in water solution for 24 h reaches its maximal reactivity in 1 - 3 h instead of in 22 - 24 h. This observation emphasises the possibility of better utilisation of the acidic environment of the uraemic stomach for adsorption with water-treated oxystarch, so that the sorbent is at the optimum of its reactive capacity.

## 35.2 Materials and Methods

Seven patients with various degrees of renal failure have been studied. All were on constant nitrogen intake represented by 0.3 - 0.5 g protein/kg bodyweight. Creatinine and blood urea nitrogen (BUN) levels before treatment are shown in

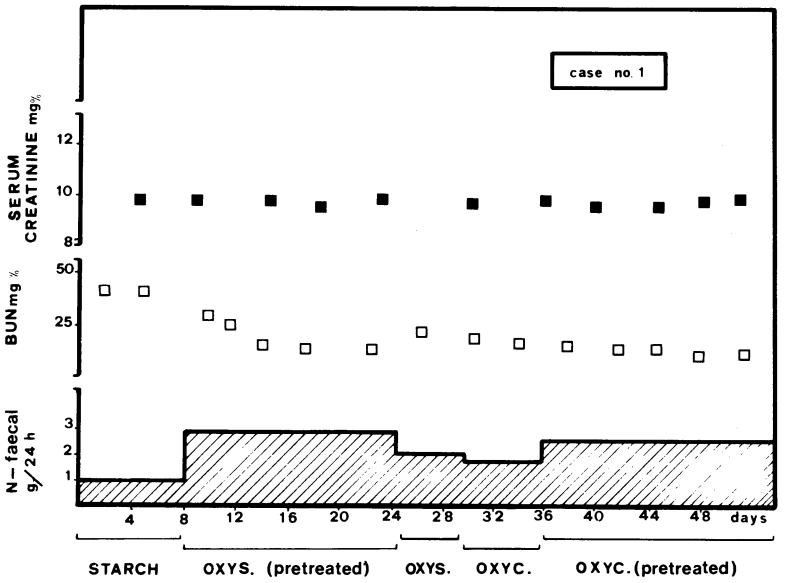



Figure 35.1 Change in reactivity of polyaldehydes with water pretreatment

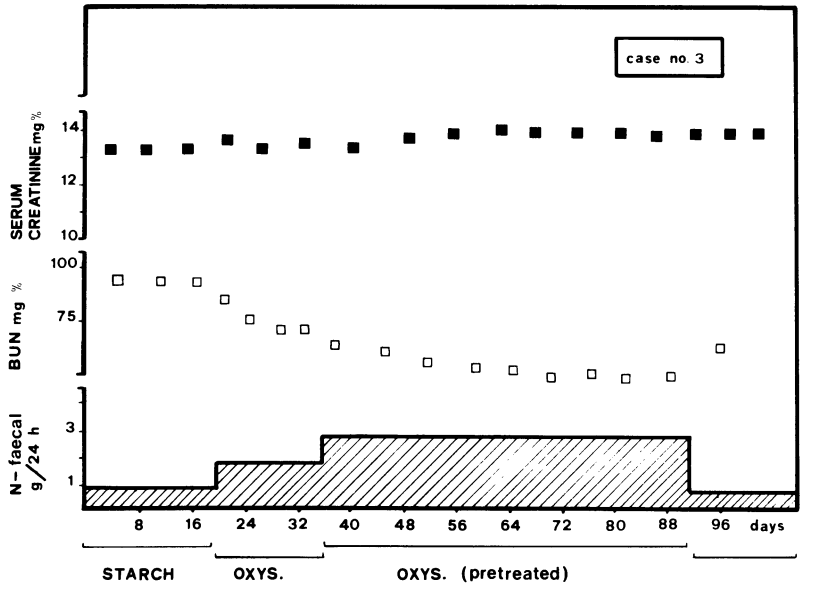



Figure 35.2 Change in reactivity of polyaldehydes with water pretreatment

table 35.1. Creatinine ranged between 7.3 and 16.2 mg% and BUN between 0.39 and 1.02 mg%. Oxystarch, and in one case oxycellulose, was left in tap water for one day prior to administration and was given by mouth in the daily amount of 25 g (dry weight) subdivided into five doses. The water pretreatment was followed by mixing the polyaldehydes with fruit jelly prior to administration, thus achieving a *moist* state for the oxystarch, and a more *palatable* form for its ingestion. The treatment period ranged from a minimum of 52 to a maximum of 80 days and, with the exception of one case, the administration sequence consisted of: starch - oxystarch - pretreated oxystarch. In the one case oxycellulose and pretreated oxycellulose were administered in place of oxystarch. Faecal nitrogen excretion, urinary nitrogen, BUN, creatinine, haemochrome and aminoacidgram were determined.

**Table 35.1**

Creatinine and blood urea nitrogen (BUN) levels of seven patients before treatment with polyaldehyde

| *Case* | *Creatinine (mg%)* | *BUN (mg%)* |
|---|---|---|
| 1 | 9.8 | 0.42 |
| 2 | 8.8 | 0.66 |
| 3 | 13.4 | 0.82 |
| 4 | 7.3 | 0.40 |
| 5 | 9.5 | 0.39 |
| 6 | 14.5 | 0.96 |
| 7 | 16.2 | 1.02 |

The sorbent urea clearance was obtained in the following way. Assuming that patients are on constant nitrogen intake and that the excess in the excretion of faecal nitrogen during oxystarch administration is the result of either urea or ammonia binding, any significant increase in faecal nitrogen during the oxystarch period in comparison to the off-sorbent (control-starch) period will represent the sorbent-induced urea clearance. Thus the procedure consists of determining the patient's urea clearance during sorbent ($T_u$) and off-sorbent ($U_c$) treatment. Then the sorbent induced urea clearance $S_c$ is given by

$$S_c = T_u - U_c$$

## 35.3 Results

Table 35.2 shows the elimination data of the average faecal nitrogen during the three periods of treatment with respectively starch (a), oxystarch (b) and pretreated oxystarch (c) in the seven cases studied. On the right of table 35.2 the variations of nitrogen excretion during the three treatment periods mentioned are reported and compared. It can be seen that the average increment during the oxystarch period is 1.15 g higher than under the starch period. Furthermore, during

**Table 35.2**

Faecal nitrogen levels of seven patients during treatment with (a) starch, (b) oxystarch and (c) water pretreated oxystarch respectively

| *N-faecal* | *g/24 h* | | | *Change in excretion* | | |
|---|---|---|---|---|---|---|
| *Case* | *a Before* | *b Oxystarch* | *c Pretreated oxystarch* | *b - a* | *c - a* | *c - b* |
| 1 | 0.85 | 1.70 | 2.05 | 0.85 | 1.20 | 0.35 |
| 2 | 1.00 | 2.55 | 3.05 | 1.55 | 2.05 | 0.50 |
| 3 | 0.75 | 1.90 | 2.40 | 1.15 | 1.65 | 0.50 |
| 4 | 0.85 | 1.25 | 2.05 | 0.40 | 1.20 | 0.80 |
| 5 | 0.90 | 2.50 | 2.70 | 1.60 | 1.80 | 0.20 |
| 6 | 0.75 | 2.00 | 2.40 | 1.25 | 1.65 | 0.40 |
| 7 | 0.80 | 2.05 | 3.10 | 1.25 | 1.25 | 1.25 |
| | | | mean ± S.E. | 1.15 ± .16 | 1.69 ± .15 | 0.54 ± .11 |

**Table 35.3**

Values presented in Table 35.2 expressed as % change in nitrogen excretion. a: before polyaldehyde administration, b: on oxystarch, c: on pretreated oxystarch

| *Case* | $\frac{b-a}{a} \times 100$ | $\frac{c-a}{a} \times 100$ | $\frac{c-b}{b} \times 100$ |
|---|---|---|---|
| 1 | 100.0 | 141.2 | 20.6 |
| 2 | 155.0 | 205.0 | 19.6 |
| 3 | 153.3 | 220.0 | 26.3 |
| 4 | 47.1 | 141.2 | 64.0 |
| 5 | 177.8 | 200.0 | 8.0 |
| 6 | 166.7 | 220.0 | 20.0 |
| 7 | 156.3 | 156.3 | 61.0 |
| mean ± S.E. | 136 ± 18% | 183 ± 14% | 32 ± 8% |
| t student | 7.76 | 13.5 | 3.8 |
| probability | $p > .0005$ | $p > .0005$ | $.005 < p < .01$ |

the pretreated oxystarch administration (c), the average excretion increase is 1.69 g compared to the starch period (a) and 0.54 g with respect to the oxystarch period (b).

Table 35.3 presents the values obtained in percentages of excretion increase. Thus there is a 136% average increase of faecal nitrogen with oxystarch and a 183% average increase for pretreated oxystarch. The average difference of faecal increment between periods (b) and (c) is 32.8%.

**Table 35.4**

Variation of % change in blood urea nitrogen (BUN) following treatment with a: starch, b: oxystarch and c: water pretreated oxystarch

| *Case* | $\frac{b-a}{a} \times 100$ | $\frac{c-a}{a} \times 100$ | $\frac{c-b}{b} \times 100$ |
|---|---|---|---|
| 1 | −29 | −50 | −30 |
| 2 | 33 | 47 | 20 |
| 3 | 26 | 32 | 8 |
| 4 | 25 | 50 | 33 |
| 5 | 21 | 51 | 39 |
| 6 | 42 | 57 | 27 |
| 7 | 32 | 45 | 19 |
| mean ± S.E. | −30 ± 3 | −47 ± 3 | −25 ± 4 |
| t student | 10.00 | 15.67 | 6.25 |
| probability | $p > .005$ | $p > .005$ | $p > .005$ |

**Table 35.5**

Efficacy of oxystarch

| *Case* | *off sorbent (starch)* | *on sorbent (oxystarch)* | *Urea clearance ml/min Increment* |
|---|---|---|---|
| 1 | 1.4 | 6.8 | 5.4 |
| 3 | 1.5 | 6.0 | 4.5 |

Table 35.4 gives the variation of BUN during the three treatment periods. The average BUN decrement under oxystarch administration was 30 mg%. When pretreated oxystarch was used, the average BUN decrement increased to 47 mg%.

Two representative cases of the urea nitrogen retention and excretion on starch and oxystarch are represented in figures 35.1 and 35.2. These patients were on constant nitrogen intake.

Estimating the sorbent urea clearance as before, an indication of the efficacy of oxystarch is given in table 35.5.

## 35.4 Discussion

In this preliminary study, results *in vivo* have broadly confirmed the theoretical premises and the *in vitro* experimental data.

Thus the conditions favourable to polyaldehyde reactivity, high urea and ammonia concentration and strongly acid pH, which exist in the stomach of uraemic patients can now be more effectively exploited. This is because the polyaldehydes, water pretreated, reach the stomach in the optimal spatial configuration. Even without such pretreatment a certain reactivity with urea, ammonia and polyaldehydes occurred, as shown by the results of faecal nitrogen

elimination *in vivo* which were uniformly superior to those obtained from the *in vitro* reactivity data at pH 7.4.

Owing to polyaldehyde pretreatment in water suspension for at least 24 h, we are now in a better position to utilise such optimal reactive properties in an acid environment. At the same time, using the polyaldehydes in a water suspension facilitates their long-term maintenance. Our experience indicates that polyaldehydes last better in a wet environment. Water removal, through drying under high vacuum or through evaporation 'on the shelf', stimulates the formation of toxic products (carbonyl radicals?) which reveal themselves macroscopically by showing a yellowish colour. This therefore implies a need to store the product in closed containers and at low temperatures. In addition, it is obviously possible to store the polyaldehydes in fruit juices or jellies, at the same time making them more palatable.

The length of time the oxystarch remains within the stomach may be prolonged by the use of drugs, such as atropine, which slow down gastric peristalsis.

Figures 35.1 and 35.2 indicate that urea clearance was minimal when patients were taking starch, it increased with oxystarch, and became even greater when pretreated oxystarch was administered. This is the real definition of the problem. An improved gastrointestinal sorbent can thus accomplish a vicarious function for a uraemic patient, comparable to an amount of renal tissue capable of 4 - 5 ml/min of urea clearance. If these results prove repeatable we definitely will have a useful subsidiary medication for uraemia.

## 35.5 References

Friedman, E. A., Fastook, J., Rattazzi, T., Beyer M. M. and Josephson A. S. (1974). Efficacy of oxidised starch as an intestinal adsorbent in uremia. *Proc. 7th Ann. Contractors Conf. NIAMDD*, Bethesda, Maryland, p. 98.

Giordano, C., Esposito, R., Randazzo, G. and Pluvio M. (1972). Oxystarch as a gastrointestinal sorbent in uremia, in Kluthe R. (Ed.), *Uremia*, Thieme, Stuttgart, pp. 132 - 138.

Man, N. K., Drueke, T., Paris J., Elizalde Monteverde, C., Rondon Nucete M., Zingraff, J. and Jungers, P. (1973). Increased nitrogen removal from the intestinal tract of uraemic patients. *Proc. Eur Dial. Transpl. Assoc.*, **10**, 143 - 151.

# 36

# OXIDISED STARCH PLUS ACTIVATED CHARCOAL IN THE NEPHRECTOMISED RAT AND URAEMIC MAN

E. A. FRIEDMAN, M. J. SALTZMAN and M. M. BEYER

*Editorial note* Significant parts of this paper have already been published. Material, however, is presented here in the context of more advanced considerations than in any previous publication.

**Summary**

Impetus for study of intestinal sorbents in the management of renal failure stems from the initial trials of Yatzidis and Giordano, who administered oral doses of activated charcoal (Yatzidis, 1964) and oxidised starch (oxystarch) (Giordano *et al.*, 1971, 1972), respectively, to uraemic patients as adjunctive therapy. Evidence of *in vitro* binding of nitrogen-containing compounds has been provided for oxystarch by Meriwether and Kramer (1976) and for charcoal by Yatzidis (1964), offering encouragement in the quest for extraction of clinically important quantities of nitrogenous wastes by ingested sorbents. Summarised herein are the results of administering a combination of oxidised starch and charcoal to two rat models of renal failure as well as a limited trial in uraemic patients.

## 36.1 Methods

Gavage fed oxidised starch and charcoal will prolong life in acutely bilaterally nephrectomised rats (Saltzman *et al.*, 1976). The mechanism of delayed death in sorbent-fed (otherwise starved) rats was explored to distinguish the lethal effect of hyperkalaemia from death due to 'uraemic toxins'. Adult male Fischer rats underwent bilaterial nephrectomy (binephrectomy) and were gavage fed oxystarch (1 g Miles Sumstar 190) and activated charcoal (1g N.F.) suspended in 10 ml tap water beginning on the first postoperative day. Groups of rats were treated as outlined below.

Group I consisted of 18 controls which were starved, and received only 10 ml water at the time of gavage feeding.
Group II, numbering 30 rats, was fed 1g charcoal in 10 ml water.
Group III, containing 29 rats, was fed 1g oxystarch in 10 ml water.
Group IV, containing 27 rats, was fed oxystarch plus charcoal in 10 ml water.

*Effect of sodium polystyrene sulphonate (Kayexalate) and sorbitol on survival*
Additional groups of binephrectomised rats were treated with drugs known to retard potassium intoxication in patients suffering acute renal failure as follows:

Group V consisted of 15 rats treated with 0.25 g of Kayexalate and 0.75 ml 70% sorbitol in 10 ml water.
Group VI contained 20 rats treated with 0.25 g of Kayexalate, 0.75 ml 70% sorbitol, 1 g oxystarch and 1 g charcoal in 10 ml water.
Rats were made potassium deficient as described by Saltzman *et al.*, (1976) by administering a potassium-free diet for seven weeks followed by binephrectomy and gavage fed sorbents outlined below.
Group VII served as a control of potassium depletion, contained 10 starved rats given 10 ml water at the time of gavage feeding.
Group VIII contained 9 rats fed oxystarch 1 g in 10 ml water.
Group IX contained 7 rats fed charcoal (1 g) in 10 ml water.
Group X contained 7 rats fed oxystarch (1 g) and charcoal (1 g) in 10 ml water.

*Glycerol-induced acute tubular necrosis*
Acute, potentially reversible tubular necrosis was produced in adult male Fischer rats by subcutaneous injection of 50% glycerol in 0.9% saline solution

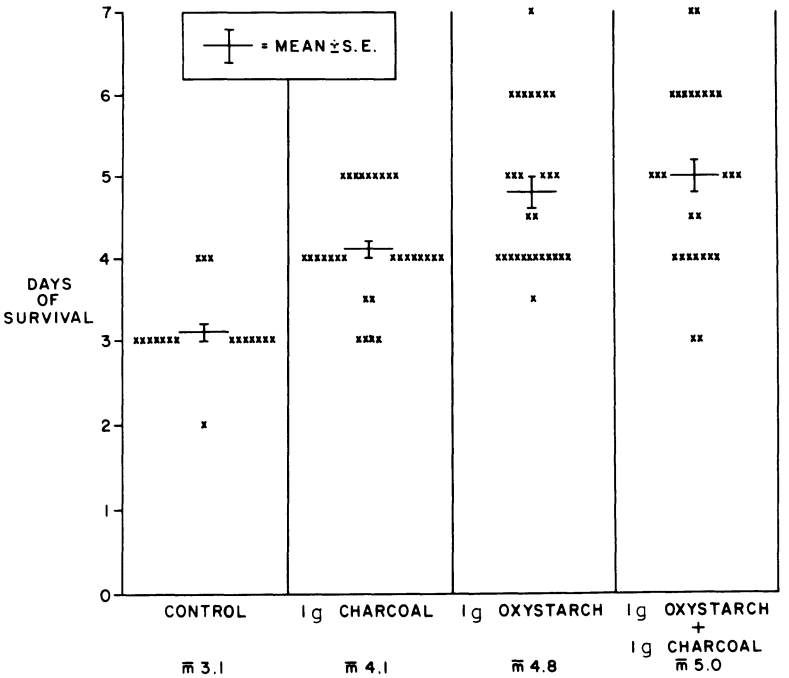



Figure 36.1 Treatment with oxystarch, charcoal, or oxystarch and charcoal improved survival for 1 to 2 days in bilaterally nephrectomised rats. (Reprinted courtesy of *Kidney International* )

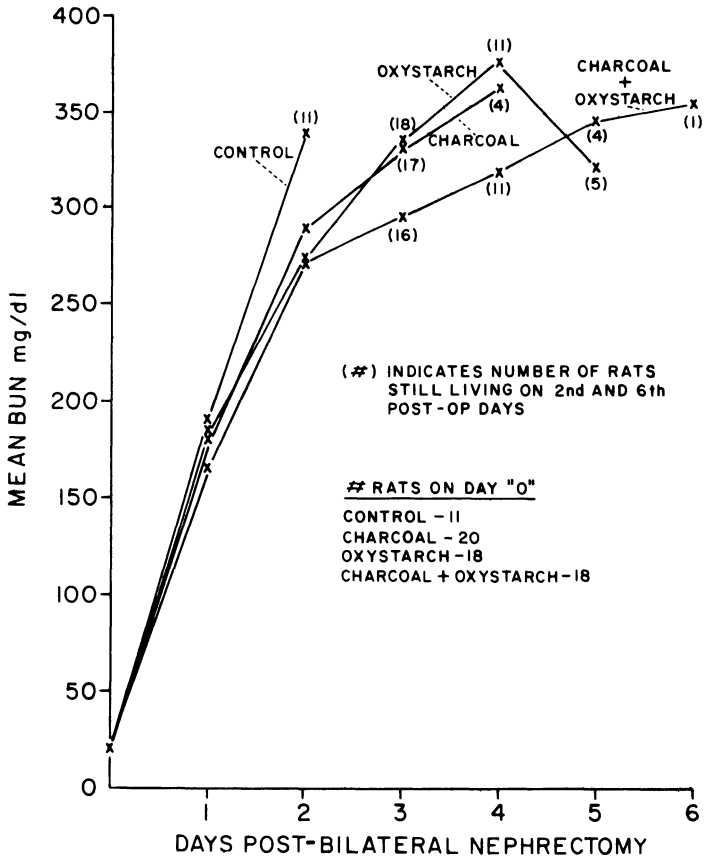



Figure 36.2 From the second post-bilateral nephrectomy day BUN elevation was lower in treated than in control rats. (Reprinted courtesy of *Kidney International)*

in a dose of 15 mg/kg body weight. Based on preliminary experiments this dose was known to cause 70% mortality. Groups of 10 control and 10 age matched treated rats were injected with glycerol and allowed water and standard feed *ad libitum*. Control rats were gavage fed water 10 ml daily while treated rats were given 1 g oxystarch (Miles Sumstar 190) in 10 ml water daily.

*Oxystarch and activated charcoal in human uraemia*

As reported elsewhere (Friedman *et al.*, 1974 and 1976) four stable uraemic urine producing patients were housed in a clinical research centre and treated with oxystarch (Miles Sumstar 190) or oxystarch plus charcoal according to the protocol detailed below.

| | |
|---|---|
| Weeks 1, 2, 3 | Adjustment to Clinical Research Centre, equilibration on 40 g protein diet, learning of collection techniques |
| Week 4 | Control balance |

| | | |
|---|---|---|
| Weeks 5, 6 | Oxystarch 8.75 g q.i.d. (four times daily) | |
| Week 7 | Control balance | |
| Weeks 8, 9 | Oxystarch 8.75 g q.i.d., plus charcoal 8.75 g q.i.d. | |
| Week 10 | Control balance | |
| Weeks 11. 12 | Oxystarch 8.75 g<br>Charcoal 8.75 g<br>Colace R 50 mg | each q.i.d. (four times daily) |
| Week 13 | Control balance | |

Pooled 7 day stool and urine collections and weekly blood samples combine to give potassium and nitrogen balance data on an invariant diet. The sampling and methods of quantification have been detailed previously (Friedman *et al.*, 1974).

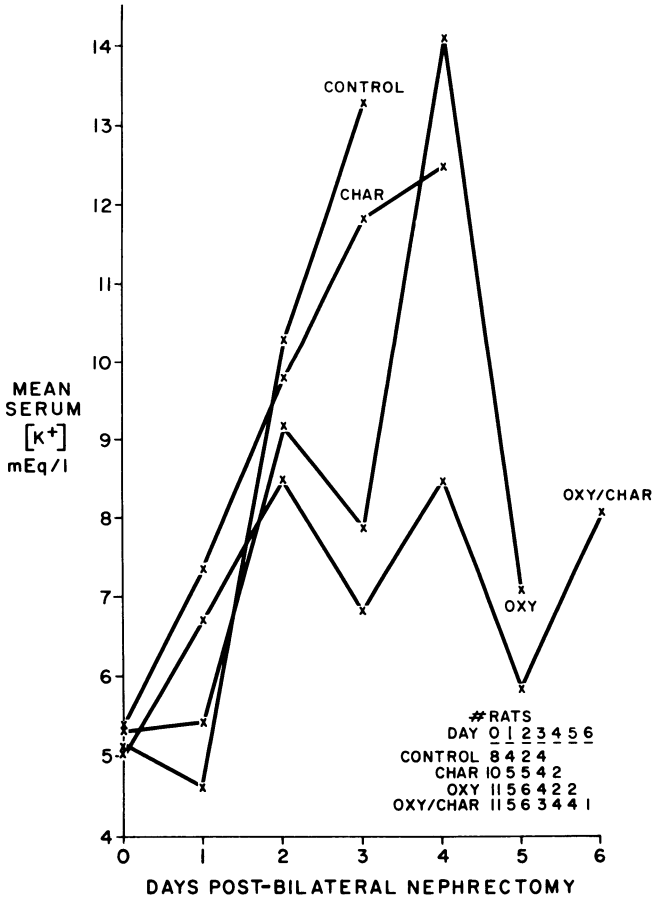



Figure 36.3 Treatment with oxystarch and charcoal retarded development of hyperkalaemia in nephrectomised rats. (Reprinted courtesy of *Kidney International)*

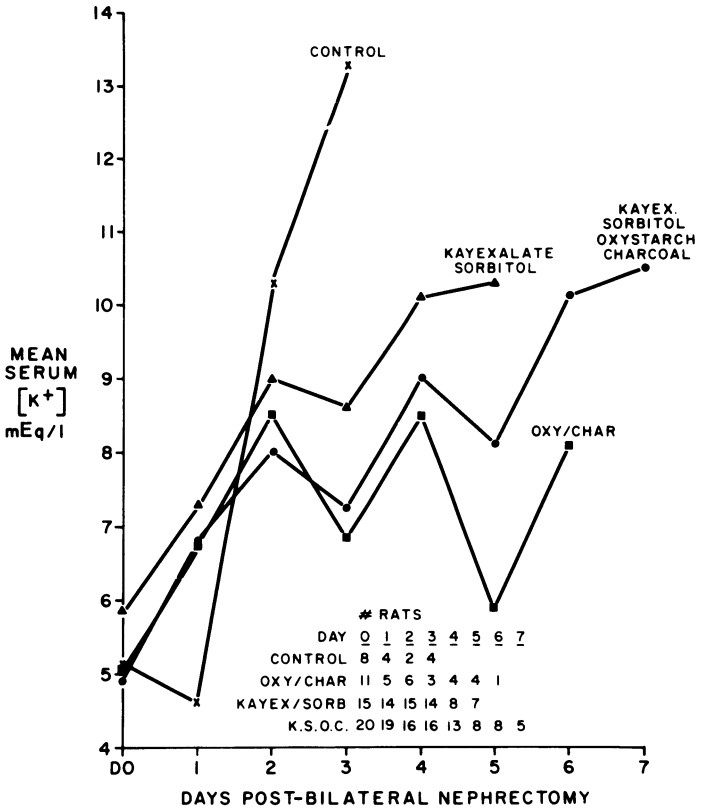



Figure 36.4 Fatal hyperkalaemia developed by the third post-nephrectomy day in controls, but was retarded in sorbent and Kayexalate treated rats. (Reprinted courtesy of *Kidney International)*

## 36.2 Results

*Binephrectomised rats*

As shown in figure 36.1, rats fed oxystarch along ($p = < 0.01$) or oxystarch plus charcoal ($p = < 0.001$) had significantly prolonged survival as compared with untreated binephrectomised controls. Feeding of both sorbents in combination was associated with the lowest blood urea nitrogen (BUN) (figure 36.2) and mean serum potassium (figure 36.3) of any treatment regimen.

Administering sodium polystyrene sulphonate (Kayexalate) and sorbitol (groups V and VI) to binephrectomised rats increased survival to a mean of 5.0 days which was equal to that obtained with oxystarch plus charcoal. Long surviving rats had retardation of post-nephrectomy hyperkalaemia (figure 36.4).

Rats subjected to a potassium depleting diet prior to nephrectomy had significantly ($p = < 0.001$) prolonged life (figure 36.5) which was not further extended by treatment with sorbents. All groups of potassium depleted rats

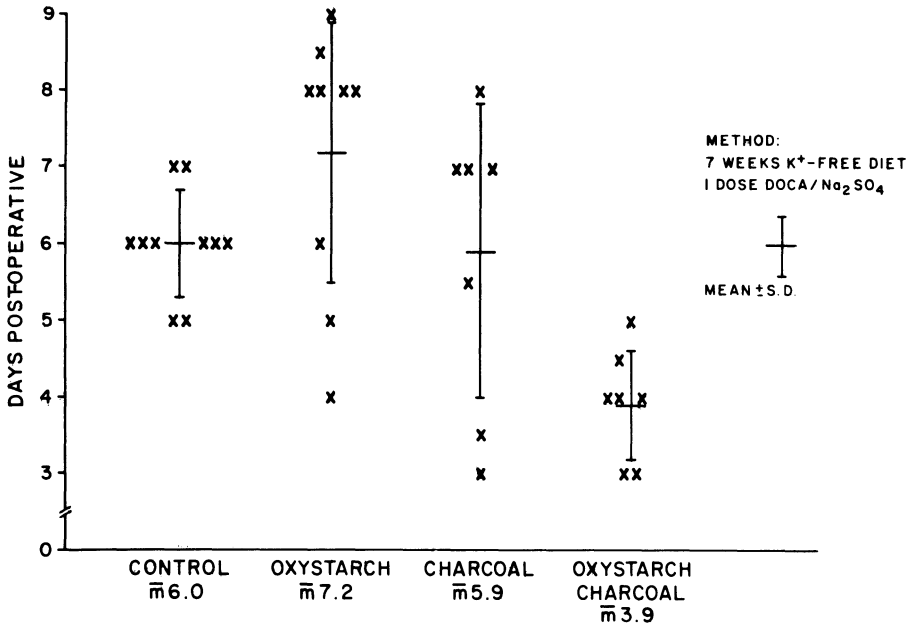



Figure 36.5 Oxystarch treatment increased survival of potassium depleted rats to a mean of 7.2 days. (Reprinted courtesy of *Kidney International)*

evinced similar degrees of hypokalaemia at the time of binephrectomy and lesser levels of hyperkalaemia agonally as compared with nondepleted controls (figure 36.6).

*Glycerol-induced renal failure*

Oxystarch proved inefficacious in improving mortality in glycerol-induced acute tubular necrosis. By the 12th day post-glycerol injection, there were three surviving controls while only one oxystarch treated rat was alive. Death rates and levels of azotaemia in the treated and control groups were similar on each day showing no significant differences throughout the study.

*Clinical trial of sorbents in uraemia*

Three of four patients tolerated sorbents well, while one patient withdrew from the study after week 10 because of discomfort produced by the soft bulky stools associated with oxystarch ingestion, a previously described side effect (Friedman *et al.*, 1974). Another patient became symptomatic of uraemia during the week 10 control period, and was placed on maintenance haemodialysis. Two patients completed all 12 weeks of the protocol, including the final treatment period in which Colace, as a wetting agent, was added to the sorbent regimen.

Mean BUN decreased 16% during each oxystarch treatment period, with no addition of fall attributable to either charcoal or Colace ingestion (figure 36.7).

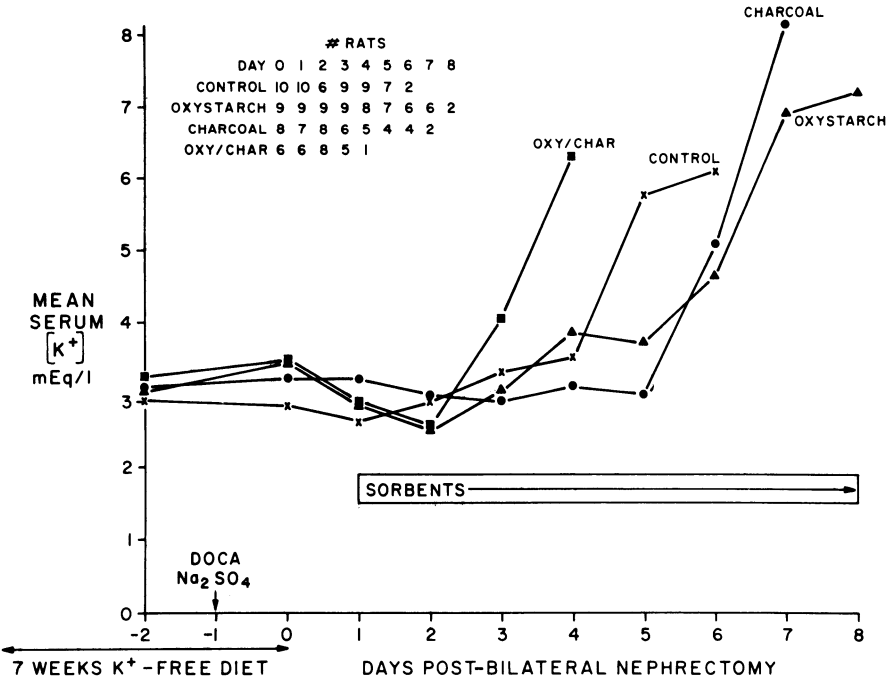



Figure 36.6 Prior potassium depletion mitigated the post-bilateral nephrectomy hyperkalaemia in all treatment groups. (Reprinted courtesy of *Kidney International)*

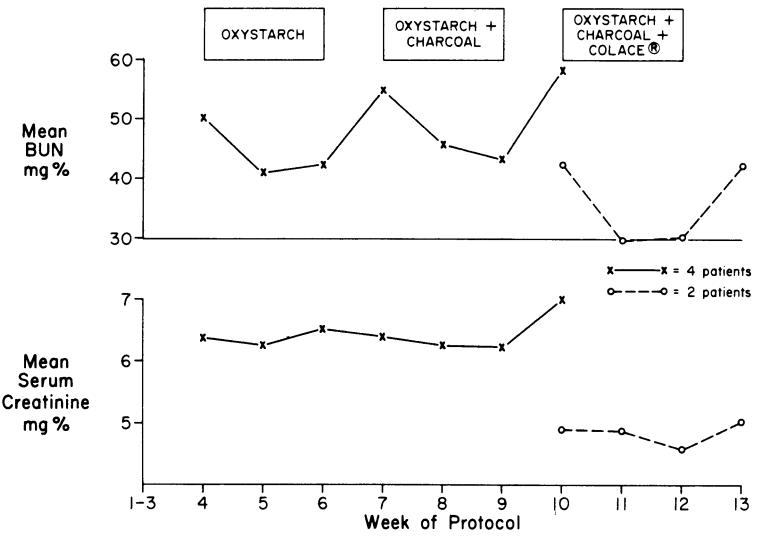



Figure 36.7 Each trial of sorbents reduced BUN level significantly, but did not affect serum creatinine level. (Reprinted courtesy of *Kidney International* )

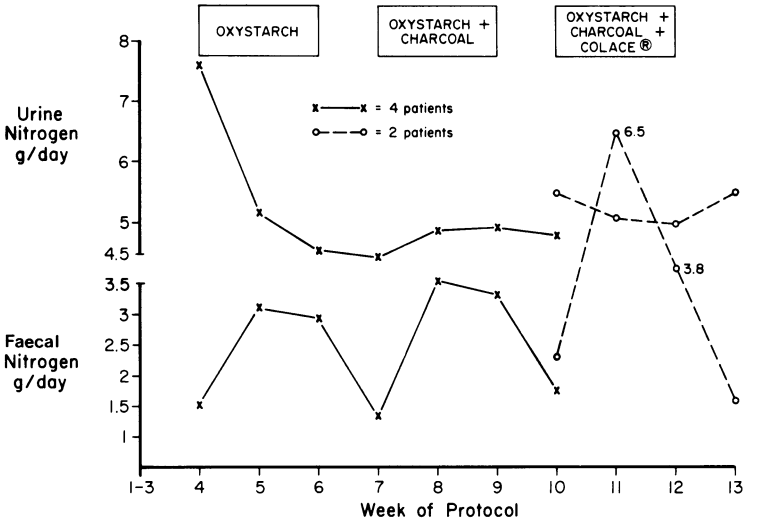



Figure 36.8 A marked increase in faecal nitrogen excretion resulted from administration of oxystarch and oxystarch plus charcoal. (Reprinted courtesy of *Kidney International)*

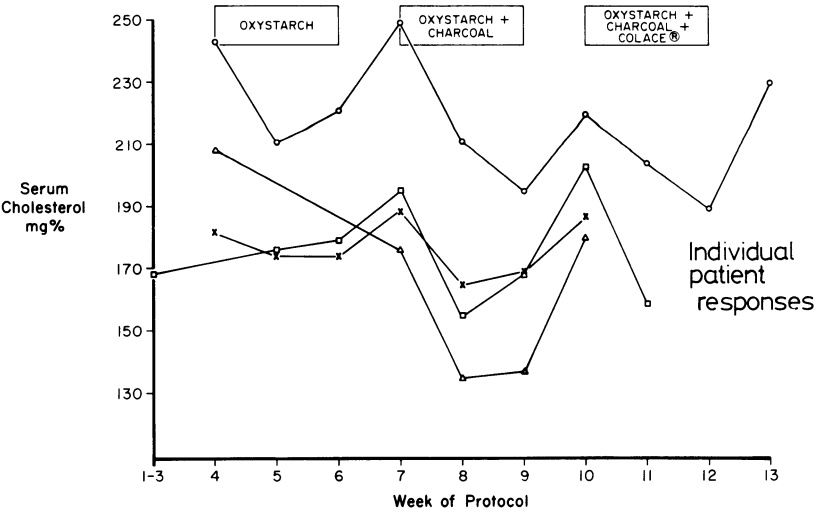



Figure 36.9 Mean serum cholesterol level fell significantly in each patient treated with oxystarch and charcoal and to a lesser extent when oxystarch alone was ingested. (Reprinted courtesy of *Kidney International)*

A significant increase in faecal potassium ($p < 0.01$) was recorded during oxystarch treatment, increasing from a control mean of 21.8 mEq/day to 43.6 mEq/day.

Net nitrogen balance (figure 36.8) was positive during control periods, but reduced to zero or slightly negative during the two trials of oxystarch and

charcoal, reflecting the expected increase in faecal nitrogen excretion during treatment.

There was no change in serum creatinine concentration (figure 36.8) during the two weeks of charcoal ingestion, although charcoal effectively adsorbs creatinine *in vitro*. A substantial fall in serum cholesterol concentration was observed in all four patients during each period of charcoal ingestion(figure 36.9).

## 35.3 Discussion

While nephrectomised rats treated with oxidised starch and charcoal evince lower BUN levels compared to untreated controls, their longer survival seems mainly attributable to a sorbent-induced retardation of development of lethal hyperkalaemia. When oxystarch and charcoal were added to the clinically efficacious regimen of Kayexalate and sorbitol, no additional increase in postnephrectomy survival resulted; thus, no specific additive beneficial consequence of sorbent binding of nitrogenous wastes was identified in the model studied. In those rats in which total body potassium depletion eliminated early death due to hyperkalaemia as a variable, no improvement in survival with sorbent treatment was demonstrable.

Sumstar 190 oxystarch has been shown to be as efficacious as Giordano's oxystarch in increasing human faecal nitrogen and potassium content. Despite this finding in urine producing patients (Giordano *et al.*, 1972; Friedman *et al.*, 1974), oxystarch treatment did not improve mortality of rats with glycerol-induced acute tubular necrosis.

No sweeping conclusion as to the ultimate clinical applicability of oxystarch and charcoal in extracting nitrogenous wastes in uraemia is warranted from the preliminary human trial conducted herein. The universally observed decrease in serum cholesterol concentration strongly suggests that lipid-like substances in the intestine compete with nitrogenous waste toxins for surface sites on activated charcoal. It will be necessary to render charcoal more selective as a nitrogen binder by microencapsulation to prevent lipid saturation of binding sites in future studies. The heretofore unrecognised property of activated charcoal as a cholesterolopenic agent warrants quantification in the trials now in progress.

## 36.4 References

Friedman, E. A., Fastook, J., Beyer, M. M., Rattazzi, T., and Josephson, A. S. (1974). Potassium and nitrogen binding in the human gut by ingested oxidized starch. *Trans. Amer. Soc. Artif. Int. Organs*, **20**, 161 - 167.

Friedman, E. A., Saltzman, M. J., Beyer, M. M. and Josephson, A. S. (1976). Combined oxystarch charcoal trial in uraemia; sorbent induced reduction in serum cholesterol. *Kidney International Supplement: Sorbents in Uraemia and Hepatic Failure* (in press).

Giordano, C., Esposito, R. and Randazzo, G. (1971). Oxystarch as a gastrointestinal sorbent in uraemia. *Workshop on Gastrointestinal Sorbents in Uraemia*,Department of Health, Education and Welfare publication no. (NIH) 72 - 78, p. 121.

Giordano, C., Esposito, R., Randazzo, G. and Pluvio, M. (1972). Trapping of urea and ammonia in the gut of uremic patients, in J. Hamburger, J. Crosnier, M. H. Maxwell (Eds.), *Advances in Nephrology from the Necker Hospital,* vol. 2, Year Book Medical Publishers, Chicago, pp. 251 - 262.

Meriwether, L. S. and Kramer, H. M. (1976). *In vitro* reactivity of oxystarch and oxycellulose. *Kidney International Supplement: Sorbents in Uraemia and Hepatic Failure* (in press).

Saltzman, M. J., Beyer, M. M. and Friedman B. A. (1976). Mechanism of life prolongation in nephrectomised rats treated with oxidised starch and charcoal. *Kidney International Supplement: Sorbents in Uremia and Hepatic Failure* (In press).

Yatzidis, H. (1964). A convenient haemoperfusion micro-apparatus over charcoal for the treatment of endogenous and exogenous intoxications. I. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.* **1**, 83 - 87.

# SESSION D DISCUSSION

In response to a question from *Nosé*, *Feijen* indicated that his tests used to study coagulation times of bovine plasma were *in vitro* and because of this there was no need for sterilisation.

*Nosé* then asked Brash how he established the hydrophilicity of the segmented polyurethane. *Brash* explained that the polyurethane discs were simply equilibrated overnight in water, the material being weighed before and after, and that the three polyurethanes had weight percent water pick-ups of 3, 17 and 65. The polymers belonged to the same family but had slightly different formulations which gave variable hydrophilicity. The hydrophobic polymer was based on polyoxypropylene and the two hydrophilic ones on polyoxyethylene. Of the latter two the least hydrophilic had a molecular weight of 600 and the other a molecular weight of 1540. It seemed to *Nosé* that in view of the data given not only the hydrophilicity of the two polymers but certain other basic properties would also be different. *Brash* disagreed since all the polymers were polyurethanes and each had a similar chemistry. He suggested that perhaps it would be more meaningful to say that although they were not precisely the same they were very similar. *Feijen* commented that both Lyman and he had emphasised the influence of the domain structure and thought that Brash might well be dealing with such differences in structure when comparing polyurethanes. He agreed that basically they were the same group of materials but, like Nosé, he felt there might be differences additional to hydrophilicity.

*Martin* remarked that it has often been suggested that a mono layer of protein was formed on a surface very soon after blood contact. He wondered therefore how the *platelet* or cell, or whatever might be damaged, actually touched the foreign surface. Blood flow had also been cited as an important factor. How important was the type and rate of flow in producing platelet changes? *Brash* suggested that the platelet/surface contact was effected by a diffusion process which might be augmented by the movement of red cells. As to the question of bare surface contact most people believed that the protein layer was the primary event and that *the platelet did not in fact contact bare surface*. He agreed with this but emphasised that the platelet was *not incapable* of adhering to a bare suface, a fact his group had confirmed by experiments with platelet suspensions in entirely protein-free systems. In fact it was found that these platelets adhered more quickly and perhaps more avidly than those in the presence of protein. Turning to the question of flow, he said that in a fast flow situation the main problem was platelet thrombosis whereas in slow flow or stagnant situations coagulation phenomena were more important. *Kim*

postulated two possible mechanisms for platelet adhesion: a *biological reaction* between the platelet and adsorbed glycoproteins and, the most likely mechanism in a protein-free system, *physical adhesion.* He did not know which of these would be the more favoured or important.

*Bjørnson* referred to Feijen's comment on the coagulation time which had been found to vary with different albumin coated surfaces. What did he consider was the mechanism behind this variation? *Feijen* thought that two factors could be involved. Firstly, the difference in the reversibility in protein adsorption when plasma contacted the surface and, secondly, that albumin itself was bound in different ways to different substances. The fact that there was an increase in coagulation time with albumin coated, hydrophobic, hydrophilic and glass surfaces, as compared to those coated with gamma globulin and fibrinogen, was not understood. He added that the mechanism for platelet adhesion in relation to protein adsorption had been only partially solved.

*Gibbons*, addressing Brash, commented that Robertson and other workers, using column techniques, had shown that in fact many haematocrit effects were either diffusion limited or diffusion independent because of volume and pathway effects. Did he feel that effective change in haematocrit was due to these factors or to some release or biochemical effect? *Brash* responded that there were two possible explanations: one that it was a physical effect and the other a biochemical effect involving ADP. He thought that all the evidence pointed to it being an effect physical in source. Control experiments had been performed which showed that pre-exposure of a surface to red cells in a platelet-free medium did not affect platelet adhesion. He believed that change in haematocrit was therefore due to a transport augmentation rather than a biochemically related or ADP effect. However, the relationship between serotonin release from adhering platelets and red cells was not really known, but it had been observed that an increase in red cell number tended to decrease the amount of serotonin released. *Gibbons* then turned to the use of polyurethanes in Brash's work by asking if heat treatment had been involved in their attachment to surfaces, since this was known to change domain structure. *Brash* explained that the polyurethanes were coated from a solution of DMF on to Delrin substrate tubing and then mounted on probes. They were then dried overnight at a temperature of 50 - 60°C with no additional heat treatment.

*Winchester* raised the topic of heparin effect on platelet reactions, commenting that heparin had recently been reported as causing immune thrombocytopaenia in several patients. He asked Lindsay if heparin had been used in his system and if he thought that the possible complement activation was important? *Lindsay* was convinced that heparin in quantities used clinically actually enhanced platelet retention by a foreign surface. He added, however, that there were certain surfaces to which heparin could be bound without leaching into the microenvironment; this thoroughly improved their antithrombogenicity. Acrylonitrile polymers had been used to demonstrate this. Nevertheless, it was possible that injected heparin could cause platelet adhesion and ADP and collagen-induced aggregation.

If this was so, and antigenic platelet fragments were released and exposed to the immune mechanism, antibodies could be formed in a long-term situation. Lindsay then went on to ask Kim if heparin had any effect on the glycoprotein-galactosyl transferase system which might cause this enhanced platelet retention. *Kim* said that this had not specifically been studied by his group but that while using heparin (as opposed to sodium citrate) as an anticoagulant, a more reasonable reaction for the galactosyl transferase system and increased platelet adhesion to the polymer had been observed.

*Maini* asked Lindsay if the sulphinpyrazone-protected platelets retained any haemostatic function or were they just 'duds'? *Lindsay* acknowledged that this was a difficult question and thought that they would retain some, but reduced, haemostatic function. He then went on to ask Brash that if adhesion was not followed by enhanced release reaction should scanning electron micrographs (SEM) not show a random scatter of single platelets rather than aggregates on the surface? Could this be an acceptable method for studying the effect of pharmacological agents on adhesion? *Brash* had not performed any such tests but said that possibly the next stage in his work would be to study the effect of various platelet suppressive agents such as aspirin. His group had done little SEM work but single platelets rather than aggregations had been seen predominantly. He agreed that there was likely to be some connection between release reaction and possible aggregation. Release occurred from all platelets adhering to a surface in amounts dependent on the hydrophilicity of the surface and the constitution of the suspension medium.

*Rosenbaum* had attempted to use retention enema in barbiturate phenol-barbitone intoxicated dogs; this was based on the use of Kayexalate for treatment of hyperkalaemia in humans. A Union Carbide carbon 200 mesh was made into the retention enema slurry. This, however, had no effect in reducing the serum concentration of the barbiturate. Regarding data on the effect on the colon of the phenobarbital adsorption, he asked Sparks whether encapsulating the carbon might improve matters. *Sparks* had not specifically tried to obtain samples from the colon of pigs used in these studies. On the subject of encapsulation affecting successful adsorption, he was concerned that the barbiturates were metabolised and perhaps already existing in the blood stream and not present in large concentrations in the colon. He wondered if perhaps other participants could inform the conference if any excretion of barbiturates occurred via faecal matter. He personally guessed that it would *not* occur.

*Kim* remarked that in Sparks' phenobarbitol adsorption studies the pH and ionisation constant of the aqueous solution were very important. Similarly, in the blood and plasma studies, the binding constant of phenobarbitone with protein was equally significant. He asked Sparks whether he had any experience of measuring free or bound phenobarbital. *Sparks* replied that in their own studies they had simply added the barbiturate directly to the blood onto the plasma; as the albumin was not removed it could be assumed that normal

binding would obtain in that particular case. He referred to Decker and co-workers (Sparks, p. 300) who had studied protein bound barbiturates and their dialysis from plasma *in vitro*. They had found that without carbon in the dialysate, the concentration of the barbiturate in the plasma decreased asymptotically to a finite level below which it could not be reduced even after very protracted dialysis. If they simply put carbon into the receiving side (i.e. the dialysate fluid side), they found that the concentration of the barbiturate in plasma decreased asymptotically to zero. From this it could be inferred that bound barbiturates could be removed. Sparks remarked that of course it was a long step from these *in vitro* studies to doing the work *in vivo*. He then commented on some of Denti's values which gave some patients' blood urea as 228 mg% for approximately 13 or 16 weeks. These levels seemed quite high and he asked Denti to comment on how far blood urea levels should be permitted to rise. For example, would it be permissible to increase the level to 500 mg%? *Denti* acknowledged the difficulty of the question and said that he did not really know the answer. Giovanetti in Pisa had tried to find the acceptable toxic level (i.e. that which produced no apparent ill effects ) in dogs and had arrived at 600 mg% for 3 months, although platelet adhesiveness changed markedly in such cases. *Denti* estimated that in the light of relevant Japanese experience a level of 300 mg% could be allowed with careful supervision.

*Leber* asked Giordano if there was any influence on the absorption of drugs from the intestinal tract when administering oxystarch to patients. *Giordano* did not know but thought it might be worthwhile investigating since the reactivity towards amino groups was small but reproducible. *Friedman* interjected that he had data showing no change in digoxin serum level (in two patients) or in requirement for antihypertensive drugs (in four patients).

*Giordano* commenting on blood urea levels pointed out that the Italian data for such levels had comprised measurement of blood urea nitrogen (BUN) and not urea. Care must also be taken to distinguish between acute and chronic uraemic patients. Russian literature contained reported cases with levels as high as 500 mg of BUN in acute renal failure and he was convinced that many of the participants must have had experience of patients with levels of 300 mg BUN or more under such conditions. He agreed, however, with Sparks that it was very seldom that comparable levels were found in patients with chronic uraemia.

*Chang* asked Giordano how much urea was removed by 25 g of oxystarch. *Giordano* first pointed out that in his calculation he had used units of dry weight and the amount of moisture present in the oxystarch was 42 - 45%, i.e. there would be 43 - 45 g of oxystarch in the form of a *moist* compound. This amount of oxystarch was able to remove 3 to 5.5 g urea, depending on whether the oxystarch was pretreated or not.

*Maini* asked Friedman if he had thought of using lipophilic sorbents such as ground Amberlite XAD-4 resins to remove lipids from the gut. He referred to Rosenbaum's work which had already shown these sorbents' high affinity for glutethimide as compared to charcoal. *Friedman* accepted this as an

interesting and valid suggestion but at the moment his work on charcoal took precedence.

*Gelfand* expressed his interest in Friedman's data concerning the fall in cholesterol and triglycerides especially in applications to moderate the appearance of arteriosclerosis in dialysate patients and normals. After hearing Friedman's early data in November his group had started to examine the triglyceride and cholesterol levels of haemoperfused patients and had found no overall change. He suggested therefore that Friedman's results could indicate failure to reabsorb bile salts, while the failure to reabsorb cholesterol and triglycerides from the gastro-intestinal tract was a purely secondary phenomenon.

*Friedman* accepted that Gelfand's suggestion could be the principal mechanism of action of cholestyramine and he could only agree that further investigation was worthwhile. *Giordano* added a comment expressing his confidence in Friedman's results and stating that the physical absorption of many lipids was a reproducible phenomenon. However, he criticised the data presented by Friedman on two counts. Firstly, unless the patient had been on the same diet for a long time the control data presented should have been gathered over a longer period. Secondly, the diet should have been prolonged for more than three weeks because the cholesterol level could have been lowered simply by a change of diet.

**Session E**

# ARTIFICIAL LIVER SUPPORT

*Chairman*
Professor R. M. Kenedi, B.Sc., Ph.D., A.R.C.S.T., C. Eng., F.I. Mech. E., M.R.Ae.S., F.R.S.E.

*Associate*
Dr. J. B. Hill, M.D., Ph.D.

# 37

# ANIMAL MODELS OF HEPATIC FAILURE FOR EVALUATION OF ARTIFICIAL LIVER SUPPORT TECHNIQUES

G. M. ABOUNA, A. Z. BARABAS, F. ALEXANDER, N. BOYD, J. K. TODD, D. W. KINNIBURGH and T. GILCHRIST

**Summary**

Three techniques of acute hepatic failure were studied and evaluated in 28 dogs. In the first, hepatic necrosis was produced by end-to-side portacaval anastomosis followed by occlusion of the common hepatic and gastroduodenal arteries for 1 h. In the second, necrosis was produced by side-to-side portacaval anastomosis followed by occlusion of the common hepatic and gastroduodenal artery for 1 h and of the portal vein for 2 h. In the third, hepatic necrosis was produced by subcutaneous injection of yellow phosphorus over a period of 3 days to a total dose of 2.5 mg/kg. Temporary arterial occlusion was carried out 24 h after portacaval anastomosis, by a 'barrel and plunger' occluding device attached to fluid-filled polyethylene tubing which was brought out subcutaneously to the exterior. All animals were studied with regard to clinical course and survival and by frequent monitoring of serum bilirubin, SGOT, LDH, alkaline phosphatase, coagulation factor analysis, free fatty acids, amino acids and octopamines. Histological examination of the liver and other organs was carried out at autopsy and at sacrifice. Hepatic coma and death occurred in 78% of the animals in group 1, 48.4% in group 2 and 100% in group 3. The mean survival times in the three groups was 27.5 h, 32.7 h and 3.7 days respectively. Clinical, biochemical and histological examination confirmed the presence of extensive but potentially reversible acute liver necrosis. It is concluded that the technique employed in group 1 is probably the most suitable model of hepatic failure since fatal coma was consistently produced, the lesion was potentially reversible and animal survival was long enough for institution and evaluation of therapy. The technique used in group 3, which is the simplest, is also a very good model but has the disadvantages of possible involvement of other organs such as the kidney, and of the necessity to take precautionary measures to protect personnel.

## 37.1 Introduction

One of the main factors which has hampered progress in our understanding of the pathogenesis of hepatic coma and in the development and evaluation of artificial liver support devices, has been the lack of a suitable and reproducible animal model of liver failure. Also, since the clinical manifestations of hepatic failure

comprise a very complex syndrome of variable pathogenesis, course and outcome, the evaluation of artificial liver support techniques in humans has failed to give consistent and reliable information. This has been evident from the many conflicting reports published during the past two decades about the clinical effectiveness of medical treatment, and of extracorporeal procedures for the treatment of hepatic failure in man, recently reviewed by Hume *et al.* (1971) and by Abouna *et al.* (1973). In order to overcome these difficulties, a suitable animal model of acute hepatic failure in a large animal such as the dog becomes mandatory if we are to maintain progress in this very difficult area of artificial organ support.

The first model of hepatic failure was that of the anhepatic animal originally described by Mann and Magath (1922) in which total hepatectomy was carried out by a two-stage procedure. This technique was recently simplified by subsequent workers, including ourselves (Serrou *et al.*, 1972) to a one-stage procedure where total hepatectomy is carried out without interruption of the inferior vena cava. However, this model, although ideal for metabolic and haemodynamic study, is not suitable for evaluating artificial liver support techniques since it is irreversible and the animal does not usually survive longer than 12 to 14 h. Likewise, total and permanent devascularisation of the liver in a two-stage procedure, as originally described by Rappaport *et al.* (1953), by Giges *et al.* (1953) and more recently by ourselves (Abouna *et al.*, 1975), is also unsatisfactory for it suffers similar drawbacks. Drug-induced hepatic necrosis by carbontetrachloride (Terblanche *et al.*, 1975), by anaesthetic agents (Harrison *et al.*, 1973) and by dimethylnitrosamine (Kuster *et al.*, 1972) have not been very successful and the hepatic failure produced is often unpredictable and of variable course.

We report on our recent experience with three models of acute hepatic necrosis in the dog all of which result in a syndrome of hepatic failure and hepatic coma and which we believe might prove suitable for evaluating artificial hepatic support techniques.

## 37.2 Materials and Methods

Twenty-eight mongrel dogs weighing 15 to 25 kg were used for this study. Before induction of hepatic failure, a catheter was inserted in the jugular vein for repeated blood sampling. Blood was drawn for baseline values of haemoglobin, WBC and platelets; bilirubin, alkaline phosphatase, SGOT, LDH and serum amylase; serum electrolytes, creatinine, BUN and blood sugar; prothrombin time and coagulation factor analysis, free fatty acids, serum amino acids and serum octopamines. All animals were given penicillin, and were maintained by intravenous fluid therapy with 1.5 to 2 l of 5% dextrose in lactated Ringer's solution every 24 h until death or until full recovery. The animals were divided into three groups.

*Group 1: end-to-side portacaval shunt and temporary occlusion of the common hepatic and gastroduodenal arteries*

Each of nine animals was anaesthetised with halothane and oxygen. Through a midline abdominal incision, the liver was exposed, all its ligamentous attachments to

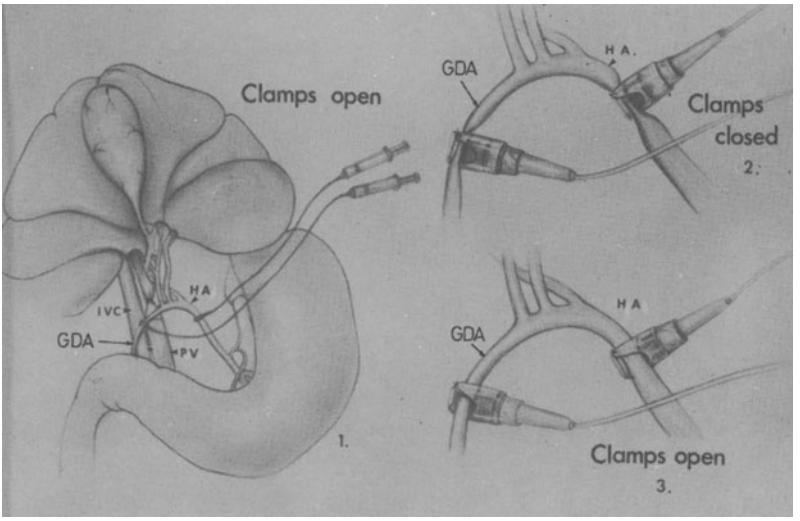



Figure 37.1 Technique of inducing acute liver necrosis (group 1), after an end-to-side portacaval shunt. Clamping devices are placed around the common hepatic and gastroduodenal arteries. On the following day, these arteries are temporarily occluded for one hour. PV portal vein, IVC inferior vena cava, HA hepatic artery, GDA gastroduodenal artery

the diaphragm and to the abdominal viscera including the gastrohepatic omentum were divided thus interrupting all collateral blood vessels, and the liver was left attached only by its vascular pedicles. A side-to-side portacaval anastomosis measuring 2 cm in length was carried out without completely occluding the portal flow as previously described by Starzl *et al.* (1959). The hepatic end of the portal vein was then ligated, thus converting this to an end-to-side anastomosis. The common hepatic artery and the gastroduodenal artery were freely mobilised and their surrounding nerve sheaths were divided. A clamping device in the form of 'barrel and plunger', constructed from a 2 ml plastic syringe, was applied around each of these two arteries in the open position (figure 37.1). The clamping device was then attached to polyethylene tubing which was brought out subcutaneously outside the abdominal cavity. When fluid is injected through this tubing, the plunger is advanced forward and thus the artery is occluded and when the fluid is withdrawn, the plunger is pulled back and the arterial occlusion is released. The abdominal incision was closed and when the animal recovered from anaesthesia, it was placed in a metabolic cage. Twenty-four hours after surgery, blood samples were drawn for the same haematological and biochemical estimations. Fluid was then injected through the tubing and both the common hepatic artery and the gastroduodenal artery were occluded. After a period of 60 min, the fluid was withdrawn and the arterial occlusion released.

The behaviour and the clinical course of the animals were continuously observed until death and blood samples were drawn at 6, 12 and 24 h after release of the

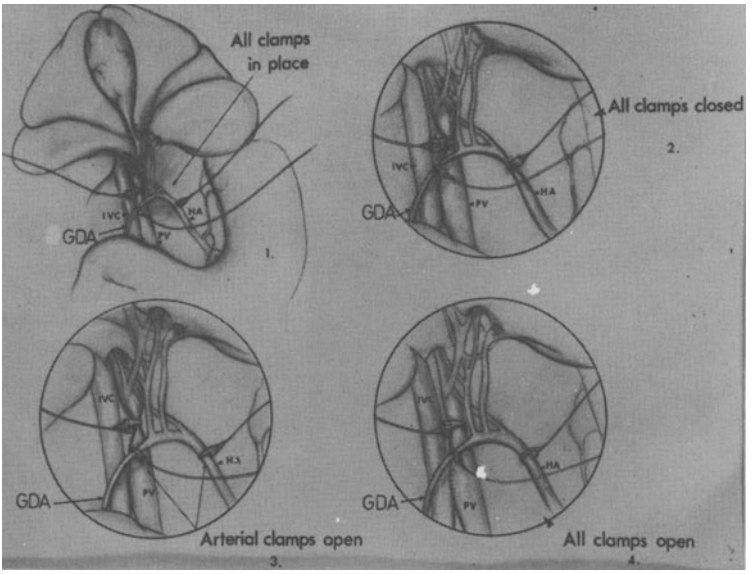



Figure 37.2 Technique of inducing acute liver necrosis (group 2). After a side-to-side portocaval shunt, clamping devices are placed around the portal vein, common hepatic and gastroduodenal arteries. On the following day, the arteries are temporarily clamped for 1 h and the distal portal vein for 2 h. PV portal vein, IVC inferior vena cava, HA hepatic artery, GDA gastroduodenal arteries

arterial clamps and once daily if the animal recovered. Autopsy examination was carried out on all animals at death and the liver and pancreas were examined histologically. Surviving animals were sacrificed at 12 to 14 days and their livers examined for evidence of regeneration.

*Group 2: side-to-side portacaval shunt and temporary occlusion of portal vein, common hepatic and gastroduodenal arteries*

Nine animals were anaesthetised and subjected to a similar surgical procedure as in group 1 except that a side-to-side portacaval anastomosis was performed and a clamping device was also applied to the portal vein distal to the portacaval anastomosis in addition to the clamping device applied around both the hepatic and the gastroduodenal arteries. The clamping device around the portal vein was constructed in a similar way from a 10 ml plastic syringe. Twenty-four hours later the portal vein, the common hepatic artery and the gastroduodenal artery were occluded for 60 min and the portal vein for 120 min (figure 37.2). Again all animals were observed clinically in metabolic cages until death or until recovery and the same post-operative care, biochemical and haematological blood tests and autopsy examinations were carried out. Surviving animals were sacrificed at 14 days.

*Group 3: phosphorus injection subcutaneously*

In 10 animals, acute hepatic necrosis was produced by deep subcutaneous injection of yellow phosphorus in oil in the following dose schedule: 1 mg/kg on day 0 and on day 1 and 0.5 mg/kg on day 2. A total dose of 2.5 mg/kg was thus given to each animal over a period of 3 days. All necessary precautions were taken to protect personnel and to prevent oxidation of phosphorus during its preparation and injection. Again, all animals were clinically observed in metabolic cages until death and daily blood samples were taken for similar haematological and biochemical estimations as in groups 1 and 2 and fluid therapy was maintained as before at 2 1/24 h. Autopsy examination was carried out at death and sections of the liver, kidney, pancreas and heart were examined histologically.

In these experiments, the haematological and the coagulation analyses were carried out by standard techniques while the routine biochemical estimations were measured by the Technicon SMA-6 and SMA-12 autoanalyser. Serum-free fatty acids were measured by modification of the method of Kelley (1965) and plasma octopamine was measured by a modification of the method of Malinoff *et al.* (1969).

## 37.3 Results

*Survival and clinical course of animals*

Table 37.1 gives details of the survival results of the animals in each of the 3 groups. The mortality from hepatic failure was nearly 78% in group 1 and 100% in group 3 but only 44% in group 2. All deaths were due to hepatic coma. The mean period of survival measured from the time of clamping of the arterial supply to the liver in groups 1 and 2 was 27.5 h and 32.7 h respectively. The mean survival time in group 3 measured from the first day of phosphorus injection was 3.7 days. Two animals in group 1 and 5 in group 2 survived hepatic coma and made eventual recovery.

**Table 37.1**

Incidence of hepatic coma, mortality and mean survival after induction of hepatic failure in the three groups

| *Group* | *Number of animal* | *Number recovering* | *Death from coma* | *Mean survival* | *Range of survival* |
|---|---|---|---|---|---|
| 1 | 9 | 2 | 77.7 | 27.5 h ± 12.0 | 16 - 40 h |
| 2 | 9 | 5 | 44.4 | 32.7 h ± 19.2 | 18 - 60 h |
| 3 | 10 | 0 | 100.0 | 3.70 days ± 0.82 | 2 - 5 days |

The clinical course of the animals of group 1 and group 2 was similar. Following portacaval anastomosis, the animals awoke from anaesthesia almost immediately and behaved quite normally. Shortly after clamping of the vascular supply 24 h later, most animals became restless but extremely weak and often vomited. Upon release of the clamps and following infusion of dextrose solution, the condition and the behaviour of the animal improved for a few hours but gradually it preferred to lie down, refused to drink or eat and within 12 h, obvious signs of hepatic failure became manifest. Somnolence, hyperventilation, acidosis, convulsions, bleeding from the wound and finally coma and death supervened. However, haemodynamic status, renal function and urinary output were maintained until death by the intravenous therapy. In the animals that survived, a similar clinical picture was observed, but within 36 to 48 h after vascular occlusion the animal began to improve clinically and within the next 2 to 3 days it was able to take food and drink. In animals of group 3, there was no change in the clinical status until after the second injection of phosphorus, when anorexia and lack of activity began to be noted. After the third injection, all animals became lethargic, often vomiting blood stained fluid and within the next 24 to 36 h, hepatic coma supervened and death followed very rapidly after onset of coma.

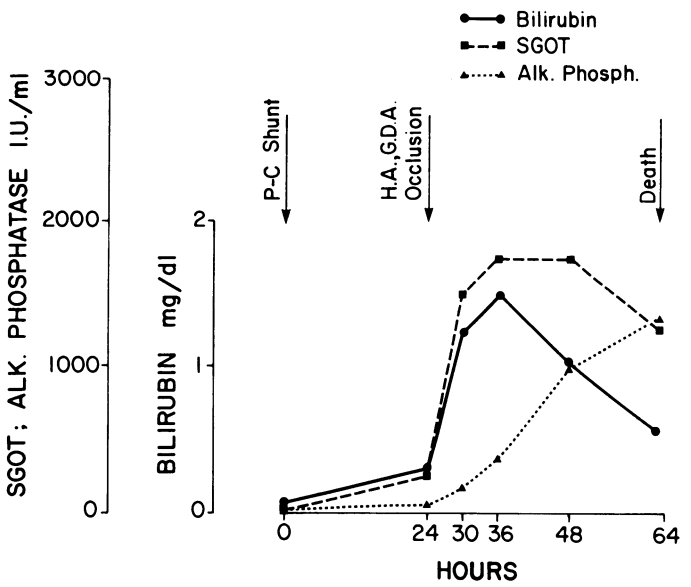



Figure 37.3 Biochemical course of one animal from group 1. Note the rise in the bilirubin, SGOT and alkaline phosphatase within 6 h after temporarily clamping of hepatic and gastroduodenal arteries. The SGOT and bilirubin decreased just before death of animal, 40 h after arterial clamping.

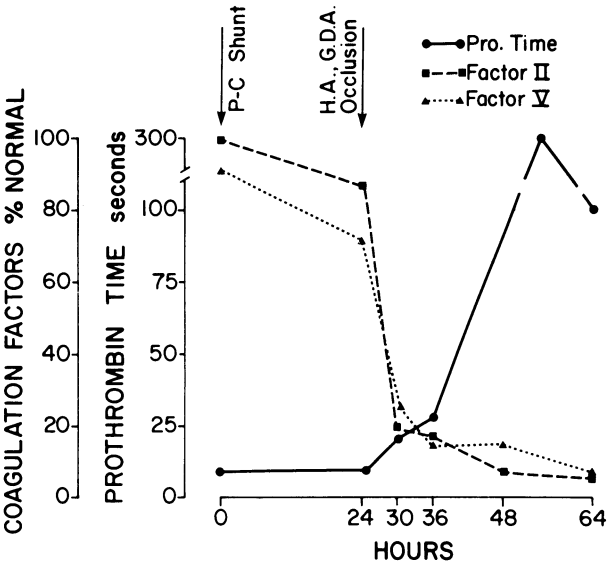



Figure 37.4 Prothrombin time and factor II and factor V levels in an animal of group 2. Note very sharp fall in the levels of clotting factors and very marked prolongation in the protime within 12 h of temporary arterial clamping

*Biochemical and haematological changes*

The biochemical and haematological pattern in animals of groups 1 and 2 was very similar. Serum bilirubin increased within 6 h after vascular occlusion and continued to rise to a level of about 1.2 to 2.6 mg/100 ml. The levels of SGOT, LDH and alkaline phosphatase increased very markedly within the first 12 h after vascular occlusion. While the serum bilirubin, the SGOT and LDH began to fall 24 h later, and just before the animal's death, alkaline phosphatase continued to increase until the animal's death (figure 37.3). The prothrombin time and the coagulation factors changed a little following portacaval shunt, but deteriorated markedly within 6 h after occlusion of the arterial supply. Very often, coagulation factors II, V, VII and IX fell to below 5% of normal at death of the animal while the prothrombin time became prolonged to more than 20 times the normal control (figure 37.4).

In the group 3 animals the rise in the serum bilirubin was much greater and began after the second injection of phosphorus. Likewise, the levels of SGOT and LDH and alkaline phosphatase continued to rise until the death of the animals (figure 37.5). The changes in the coagulation factors and the prothrombin time were similar to those of animals of groups 2 and 3. In animals which survived and made eventual recovery similar rises occurred in the serum bilirubin and the enzymes but within 24 to 36 h the bilirubin and the SGOT continued to drop, reaching normal values within 5 to 6 days although the alkaline phosphatase remained elevated until sacrifice (figure 37.6).

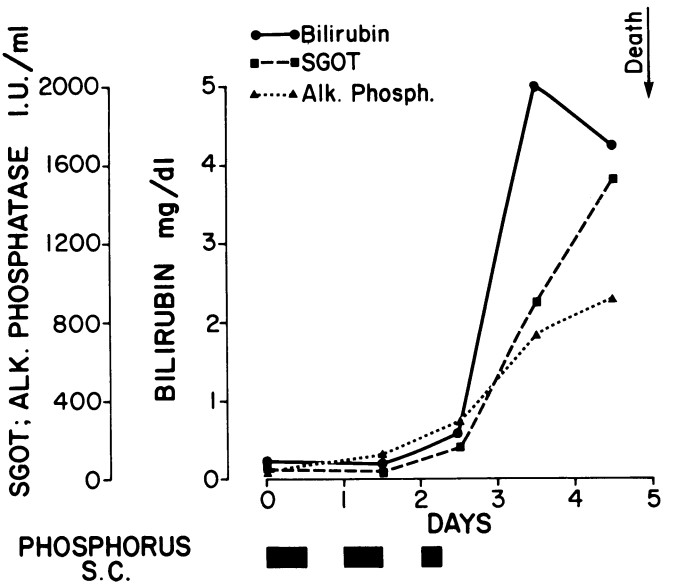



Figure 37.5 Biochemical course of a dog from group 3. Note the marked rise in bilirubin, SGOT and alkaline phosphatase following the last phosphorus injection which continue until death of animal at 5 days

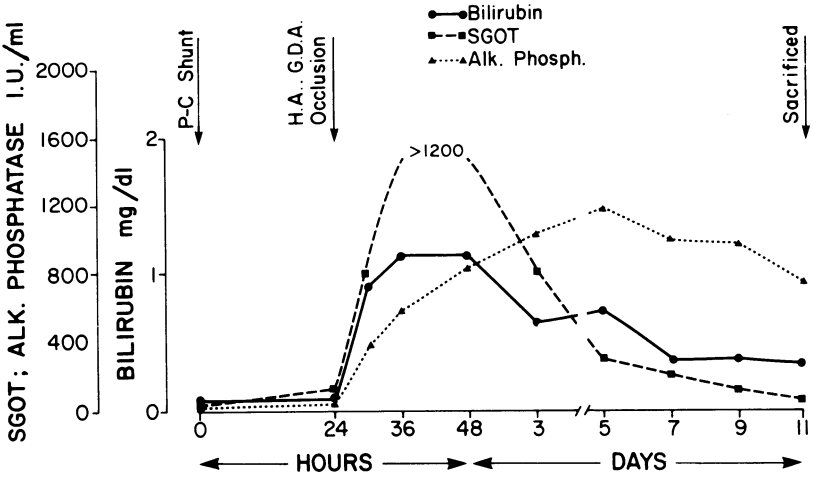



Figure 37.6 Biochemical course of one of the two animals of group 2 which recovered from hepatic coma. Note the very marked increase in the levels of serum bilirubin, SGOT and alkaline phosphatase within 12 h after temporary arterial clamping. As the animal recovered, bilirubin and SGOT fell to normal, but alkaline phosphatase remained elevated until the animal was sacrificed at 11 days.

Hypoglycaemia was a very marked feature of all animals dying in hepatic coma, particularly in group 1 and 3 animals, and sometimes reaching a value of 15 mg/100 ml. The level of free fatty acids increased by 5 to 6 fold (from 0.3 μEq/ml to 1.86 μEq/ml) during hepatic coma in the animals of all the three groups. However, while octopamine levels increased markedly from undetectable levels to a mean value of 2.2 ng/ml in group 1 and 2, the rise was extremely small, the mean of 0.43 ng/ml, in group 3. Also, while serum amylase remained normal throughout in all animals in groups 1 and 2, it increased markedly in group 3, often exceeding 500 Street-Close units (normal 35 units).

There was very little change in renal function until death. In groups 1 and 2, for example, the serum creatinine increased from a mean of 1.16 to 1.38 mg/100 ml while in group 3 animals it increased from a mean value of 0.9 to 1.28 mg/100 ml. In neither case was this rise statistically significant.

*Pathological and histological changes*

The pathological changes in the livers of groups 1 and 2 animals were similar. These showed severe centrizonal hepatic necrosis, sparing only peripheral cells around the portal area. Necrosis and atrophy of liver cells was accompanied by nuclear loss or shrinkage and reticulin stains demonstrated collapse of the hepatic architecture. No pathological changes were seen in the other organs examined. In the animals which recovered from coma, patchy lesions with active hepatocyte regeneration was found on sacrifice and the liver had shrunk to 30-40% of its original size. The livers of group 3 animals were also necrotic at autopsy. Extensive cellular changes were observed histologically throughout the liver substance with minor sparing of peripheral hepatocytes. These changes consisted of swelling and granularity of the cytoplasm giving the cells a feathery appearance with fat vaculation. Nuclei showed irregularity of the membrane, chromatin clumping and loss of nucleoli or nuclear fragmentation. Sections of the kidney showed varying degrees of degenerative changes largely in the proximal convoluted tubule and the parietal epithelium of Bowman's capsule, and in some tubules degenerate epithelial casts were seen in the tubular lumina. Generally these changes were mild, being very marked in only two of the ten dogs examined. Sections of pancreas and heart showed no detectable pathology.

## 37.4 Discussion

Owing to the very complex nature of fulminant hepatic coma in man and the marked species variation in the response to hepatic injury, the development of an ideal animal model of hepatic failure has proved difficult. Also, the fact that practical methods of resuscitation and artificial support of such an animal will eventually require repeated extracorporeal haemoperfusion techniques, the size and the ease of handling of such an animal become important. In addition, an ideal animal model will have to satisfy a number of other important criteria. These include: that the liver injury produced be extensive, uniform and very rapid; that this be reproducible; that it be potentially reversible; that it results in hepatic coma

with a very high mortality rate and that the animal survives in a fairly stable haemodynamic status long enough for the institution and evaluation of therapy.

As was pointed out earlier, the anhepatic models of Mann and Magath (1922) do not meet these criteria, mainly because of the irreversibility of the hepatic damage and the short period of survival. The permanently devascularised liver model of Rappaport *et al.* (1953) is also unsuitable because of the wide variation in the collateral blood supply and consequently in the length of survival of the animals.

The animal model described by Misra *et al.* (1972), where the hepatic artery and/or the portal vein were occluded by balloons after portacaval shunting, is similar to our models of groups 1 and 2, except that these workers ligated the gastroduodenal artery. We believe that ligation of this major vessel can have serious consequences on the viability of the duodenum and the pancreas as has been pointed out by Starzl *et al.* (1960) and, indeed, this may have accounted for the very short survival (mean 13 h) of the animals reported by Misra and his colleagues. We believe that the techniques described in this paper meet many of the criteria of a suitable model of hepatic failure particularly in groups 1 and 3. It was clear from the clinical course of the animals in group 1, and from the survival results, that this particular model is probably the more suitable since it consistently led to hepatic coma, not unlike that seen in man, and this resulted in a very high mortality of 78%. The mean survival time of the animals in this group exceeded 24 h and this in our opinion is sufficient for the institution and the evaluation of artifical support therapy. Also, the liver damage produced as determined biochemically and histologically, was extensive and uniform and was potentially reversible. When portal flow was added to the ischaemic liver as carried out in group 2, spontaneous recovery rate was increased and death following hepatic coma was markedly reduced and, consequently, both consistency and reproducibility were lost. For these reasons we believe that this latter model fails to meet the criteria of an acceptable hepatic failure model.

The third model of phosphorus-induced hepatic necrosis has many advantages but raises a number of questions. The extreme simplicity of technique, consistency, very high mortality rate and a mean survival time of 3.7 days make this a most convenient model of hepatic failure. However, in this model, hepatic coma was less predictable, came on rather suddenly and was of very short duration. Also, while hepatic necrosis was extensive and reproducible, it was not possible to exclude involvement of other organs such as the kidney. Indeed, histological examination of the kidney in these animals did show tubular epithelial degeneration in the proximal convoluted tubules. The significance of this finding could not be fully determined in view of the fact that renal function as measured by the serum creatinine and urinary output did not change significantly and certainly this change was very comparable to that seen in animals of groups 1 and 2. It is also possible that the renal tubular damage as well as the hepatic necrosis are reversible. Experience in phosphorus poisoning in animals as reported by Lawrence *et al.* (1929) and similar experience in man as reported by Rubitsky *et al.* (1949), Fletcher *et al.*

(1963) and Marin *et al.* (1971) indicate possible involvement of other organs but confirm the potentially reversible nature of the lesions produced. In our experiments, apart from the kidney, neither the heart nor the pancreas was involved histologically, although the rise in serum amylase could probably be attributed to an early toxic pancreatitis. While it has not been possible to determine the extent of involvement of extrahepatic organs, it is known that the extent of the pathological changes produced as well as the survival of the animal depend on the dose of phosphorus used and its rate of administration. The dose schedule which we employed was based on the experience of Burnell *et al.* (personal communication). These workers had observed that a dose schedule of 2.5 mg/kg given over 3 days gave the most consistent results in terms of mortality from hepatic failure. Also it is claimed by this group that the toxic effects of phosphorus given at this dose schedule are confined exclusively to liver. However, our findings of histological changes in the renal tubules do not support these claims and we are presently investigating the nature and the consequences of these renal lesions. Another disadvantage of the use of phosphorus is, of course, the rigorous precautions that have to be taken in handling the substance and in the disposal of the animals in order to protect laboratory personnel.

## 37.5 Conclusion

In conclusion, we believe that a satisfactory model of acute hepatic failure for evaluation of artificial hepatic support techniques can be readily produced by temporary ischaemia of the liver induced by end-to-side portacaval anastomosis followed, 24 h later, by occlusion of the common hepatic and gastroduodenal arteries for a period of 1 h. Phosphorus-induced hepatic necrosis, which is a much simpler technique, also produces a very good model, but we believe that this requires further careful study. We are currently using these techniques to assess the therapeutic value of haemoperfusion with activated carbon and Amberlite resin XAD-4 on the survival of animals from acute hepatic failure.

## 37.6 Acknowledgements

The work presented was supported by the Medical Research Council of Canada, Grant number MA 5601.

## 37.7 References

Abouna, G. M., Fisher, L. McA., Porter, K. A. and Andres, G. A. (1973). *Surg. Gynecol. Obstet.*, **137**, 741.

Abouna, G. M., Gilchrist, T., Pettit, J. E., Boyd, N. D., Todd, J. K., Courtney, J. M. and Maini, R. (1975). In R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 180.

Burnell, J. M., (1975, 1976) Personal communication.

Fletcher, G. F. and Galambos, J. T. (1963). *Arch. Int. Med.*, **112**, 846.

Giges, B., Dein, H. L., Sborov, V. M., Seligson, D. and Howard, J. M. (1953). *Surg. Gynecol. Obstet.*, **97**, 763.

Harrison, G. G. and Smith, J. F. (1973). *Anaesthesiology*, **39**, 619.
Hume, D. M., Mendex, G., Gayle, W. E., Smith, D. H., Abouna, G. M. and Lee, H. M. (1971). *Transpl. Proc.*, **3**, 1525.
Kelley, T. F. (1965). *Ann. Chem.*, **37**, 1078.
Kuster, G. G. and Woods, J. E. (1972). *Ann. Surg.*, **176**, 732.
Lawrence, J. S. and Huffman, M. M. (1929). *Arch. Path.*, 7, 804.
Malinoff, P. B., Landsburg L. and Axelrod, J. (1969). *J. Pharmacol. Exp. Therap.*, **170**, 253.
Mann, F. C. and Magath, T. S. (1922). *Arch. Int. Med.*, **30**, 73.
Marin, G. A., Montoya, A., Sierra, J. L., Siefra, J. L. and Senior, J. R. (1971). *New Engl. J. Med.*, **284**, 125.
Misra, M. K., P'eng, F. K., Sayhoun, A., Koshil, A., Derry, C. D., Caridis, T. and Slapak, M. (1972). *Surgery*, **72**, 634.
Rappaport, A. M., McDonald, M. H. and Boroway, Z. J. (1953). *Surg. Gynecol. and Obstet.*, **97**, 748.
Rubitsky, H. J. and Myerson, R. M. (1949). *Arch. Int. Med.*, **83**, 164.
Serrou, B., Coburg, A. J., Abouna, G. M. and Aldrete, T. (1972). *International Surgery*, **55**, 235.
Starzl, T. E., Bernhard, V. M., Cortes, N. and Benevenuto, R. (1959). *Surgery*, **46**, 880.
Starzl, T. E., Kaupp, H. A., Brock, D. R., Lazarus, R. E. and Johnson, R. U. (1960). *Surg. Gynecol. Obstet.*, **111**, 733.
Terblanche, J., Hickman, R., Miller, D. J. and Saunders, S. J. (1975). In R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 163.

# 38

# RESIN AND CHARCOAL HAEMOPERFUSION IN THE TREATMENT OF HEPATIC COMA

G. M. ABOUNA, A. Z. BARABAS, N. BOYD, J. K. TODD, F. ALEXANDER, D. W. KINNIBURGH, T. GILCHRIST and E. JONSSON

**Summary**

Some of the clinical and biochemical effects of haemoperfusion with activated carbon and Amberlite resin XAD-4 were evaluated in a potentially reversible animal model of acute hepatic coma. While the rate of ultimate recovery from hepatic failure did not differ between the treated and the control groups, there was definite though temporary clinical improvement in those animals treated by perfusion. Charcoal appeared clinically more effective as well as less hazardous than resin. In both resin and charcoal perfusion clinical improvement was frequently associated with removal of plasma-free fatty acids and octopamine but not with the amino acids generally considered to be etiological factors in hepatic coma. Resin perfusion caused greater removal of plasma proteins, bilirubin and other protein bound substances, as well as greater destruction of leucocytes and platelets. It is concluded from this preliminary study that a single haemoperfusion with either adsorbent did not alter the high mortality from hepatic coma, produced inconsistent clinical benefits and variable biochemical changes, and, by itself, such a technique may only have a limited role in the treatment of deep hepatic coma in man. A more vigorous and repeated perfusion might improve the clinical results but further investigation and development are required to reduce some of the serious hazards of this form of treatment.

## 38.1 Introduction

There has been much interest in recent years in the development and use of haemoperfusion techniques through adsorbent compounds for the treatment of hepatic failure. Activated charcoal has already been used in the treatment of patients in hepatic coma, notably by Gazzard *et al.* (1974), with encouraging but inconclusive results. Haemoperfusion through Amberlite resin, which has been successfully used in the treatment of blood intoxication by Rosenbaum (1975), has not been widely used in the treatment of hepatic failure. Haemoperfusion through these compounds is still hampered by a number of difficulties and hazards and their precise role in the therapy of hepatic coma remains undetermined.

After having developed a reproducible and potentially reversible model of hepatic failure in the dog, we proceeded to carry out an evaluation of the possible clinical and biochemical benefits as well as any serious complications of haemoperfusion with both activated charcoal and Amberlite resin XAD-4 during the treatment of experimental hepatic coma. A brief report of our preliminary findings is presented here.

## 38.2 Materials and Methods

The animal model which was used in this study has been described elsewhere on p. 351 of these proceedings by Abouna *et al.* Briefly, acute hepatic necrosis is produced in dogs by an end-to-side portacaval shunt which is followed, 24 h later, by temporary occlusion of the common hepatic and gastroduodenal arteries for a period of 60 min. The animals were divided into the following three groups 5 to 6 h after induction of hepatic necrosis.

Group 1: Treatment by haemoperfusion using activated charcoal for a period of 2 h.
Group 2: Treatment by haemoperfusion through Amberlite resin XAD-4 for the same period.
Group 3: No haemoperfusion (control group).

Haemoperfusion was carried out through an arterio-venous shunt which had been previously constructed using the carotid artery and the jugular vein and employed a technique which had been previously described by Abouna *et al.* (1975). There were six animals in group 1, six in group 2 and nine in group 3. All animals were given 1.5 to 2 l of 5% dextrose in Ringer's lactate solution per 24 h and they received long-acting Procaine Penicillin I.M. Before surgery, before and after perfusion, and daily thereafter, blood samples were taken for measurement of full blood count, platelet count, prothrombin time, coagulation factors I, II, V, VII and IX, serum electrolytes, BUN and creatinine, total serum protein, albumin, bilirubin, calcium, cholesterol, liver enzymes, plasma free fatty acids, octopamines and amino acids. The clinical condition of each animal was recorded at intervals and particularly before and after haemoperfusion. Autopsy was carried out on all animals dying in hepatic failure and 12 to 14 days after haemoperfusion.

The activated carbon was prepared at Gambro's Laboratories by a technique previously described by Thysell *et al.* (1976), which involves the coating of extruded and activated carbon with cellulosic polymer using acetic acid as the solvent. The carbon was then washed, packed and sterilised in columns containing 120 g of charcoal. The Amberlite XAD-4 resin was obtained from Rohm and Haas Chemical Company, Philadelphia. It was packed in columns containing 100 g and sterilised with ethylene oxide. Before haemoperfusion, the columns were washed with 1 to 2 l of heparinised Ringer's lactate solution containing 5% dextrose. Regional heparinisation was used in all experiments.

**Table 38.1**

Effect of haemoperfusion on the clinical status of animals. Note superiority of charcoal to Amberlite resin

| *Adsorbent* | *Improved* | *Unchanged* | *Deteriorated* |
|---|---|---|---|
| Charcoal | 4 | 1 | 1 |
| Resin | 2 | 1 | 3 |
| Total | 6 | 2 | 4 |

**Table 38.2**

Survival of animals treated by haemoperfusion. Note there is no difference in the recovery rate among the three groups but survival following charcoal perfusion was significantly longer than that following resin perfusion

| *Group* | *Number of animals* | *Number recovered* | *Death from coma (%)* | *Mean survival* (h) | *Range of survival* (h) |
|---|---|---|---|---|---|
| 1 (control) | 9 | 2 | 77.7 | 27.5 ± 12.0 | 16 - 40 |
| 2 (charcoal) | 6 | 2 | 66.6 | 46.25 ± 10.6 | 20 - 72 |
| 3 (resin) | 6 | 2 | 66.6 | 18.65 ± 4.2 | 7 - 26 |

Difference between group 2 and group 1 is short of significance ($p < 0.1 > 0.05$)
Difference between group 2 and group 3 is significant ($p < 0.05$)

## 38.3 Results

*Clinical effects and animal survival*

In most animals signs of hepatic failure appeared shortly after hepatic arterial occlusion and in some animals frank hepatic coma became manifest at the time of haemoperfusion. The clinical effects of haemoperfusion on these animals were variable. Definite clinical improvement was observed in four animals treated by charcoal haemoperfusion but only in two animals treated by haemoperfusion with resin. Whereas only one animal deteriorated following charcoal perfusion, three animals did so after resin perfusion (table 38.1). Occasionally, haemoperfusion was observed to produce dramatic but temporary improvement in that the animal was able to stand up, move around apparently quite happily, and even take a drink. Two animals from each of the three groups were able to make complete recovery and survive until sacrificed later. The remaining animals in the three different groups eventually died in coma after surviving for variable periods of time. The

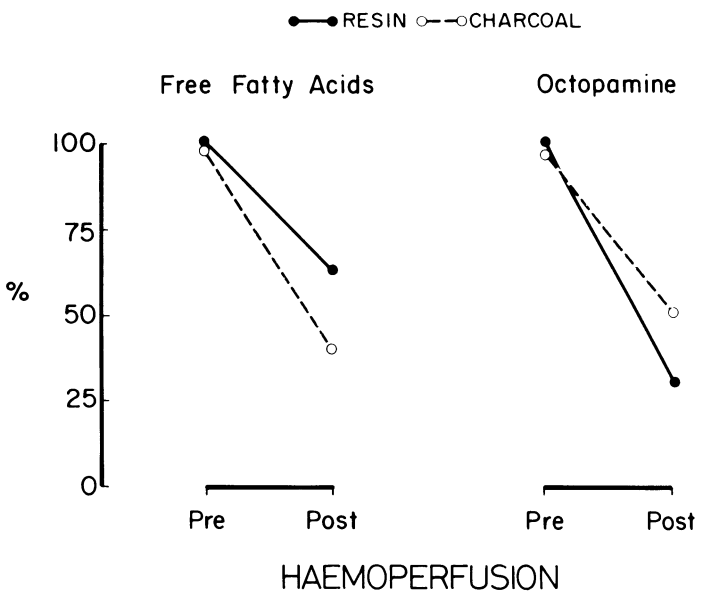



Figure 38.1 Changes in the concentration of plasma free fatty acids and octopamine following haemoperfusion. Removal of these compounds was similar after charcoal and resin haemoperfusion.

mean survival time for the animals treated with charcoal perfusion was 46.25 ± 10 h which was statistically significant ($p < 0.05$) when compared to the mean survival time of 18.65 ± 4 h in the animals treated by resin haemoperfusion, but it was not significant when compared to the mean survival time of the animals in the control group (table 38.2).

*Changes in octopamine, free fatty acids and amino acids*

Following both charcoal and resin haemoperfusion, there was considerable reduction in the concentration of plasma free fatty acids and octopamine, but there was no statistical difference between the two groups (figure 38.1). Also, in neither group was the pre-perfusion concentration of these substances very markedly elevated compared to the normal. With the onset of hepatic failure many of the eighteen amino acids examined showed marked elevation. However, following treatment with either charcoal or resin haemoperfusion, the change in the concentration of the individual amino acids was extremely variable. Resin haemoperfusion appeared very effective in removing the amino acids: glutamic acid, leucine, isoleucine, β-alanine, ornithine, tryptophan, while charcoal was effective in removing glutamic acid, isoleucine and ornithine. Neither adsorbent appeared to remove appreciable quantities of methionine, phenylalanine or tyrosine. (table 38.3).

**Table 38.3**

Changes in serum amino acids following perfusion. Note greater but variable adsorption of some amino acids after resin perfusion but little adsorption of the three amino acids methionine, tyrosine and phenylalanine

| *Amino acid* | *Resin perfusion % removed* | *Charcoal perfusion % removed* |
|---|---|---|
| Glutamic acid | 34 | 33 |
| Leucine | 38 | no change |
| Isoleucine | 39 | 63 |
| β-alanine | 38 | no change |
| Ornithine | 27 | 31 |
| Tryptophan | 43 | no change |
| Methionine | 6 | 12 |
| Phenylalanine | 5.3 | 5.7 |
| Tyrosine | no change | no change |

**Table 38.4**

Changes in several biochemical parameters during haemoperfusion. Note greater removal of serum protein, albumin, calcium and bilirubin with resin than with charcoal

| *Biochemical parameter* | *Resin haemoperfusion* | | | *Charcoal haemoperfusion* | | |
|---|---|---|---|---|---|---|
| | *Pre* | *Post* | *% Removed* | *Pre* | *Post* | *% Removed* |
| Total protein G% | 4.57 | 3.67 | 21% | 4.82 | 4.13 | 12% |
| Albumin G% | 3.43 | 2.73 | 20% | 2.98 | 2.73 | 8% |
| Calcium mg% | 9.17 | 8.4 | 13% | 8.58 | 8.53 | 0 |
| Bilirubin mg% | 0.99 | 0.7 | 29% | 0.52 | 0.42 | 19% |
| Cholesterol mg% | 124 | 100 | 19.3% | 105 | 94 | 7.8% |
| BUN mg% | 12 | 11.7 | 2.5% | 11.2 | 10.4 | 7.1% |
| Creatinine mg% | 0.88 | 0.98 | +11.2% | 0.82 | 0.87 | +6.1% |

*Changes in other biochemical parameters*

There was an appreciable decrease in the serum concentration of several of the standard biochemical parameters following haemoperfusion. Resin haemoperfusion appeared more effective in removing bilirubin and in eluting serum proteins and serum calcium. In these experiments there was no appreciable change in the concentration of serum creatinine or BUN (table 38.4).

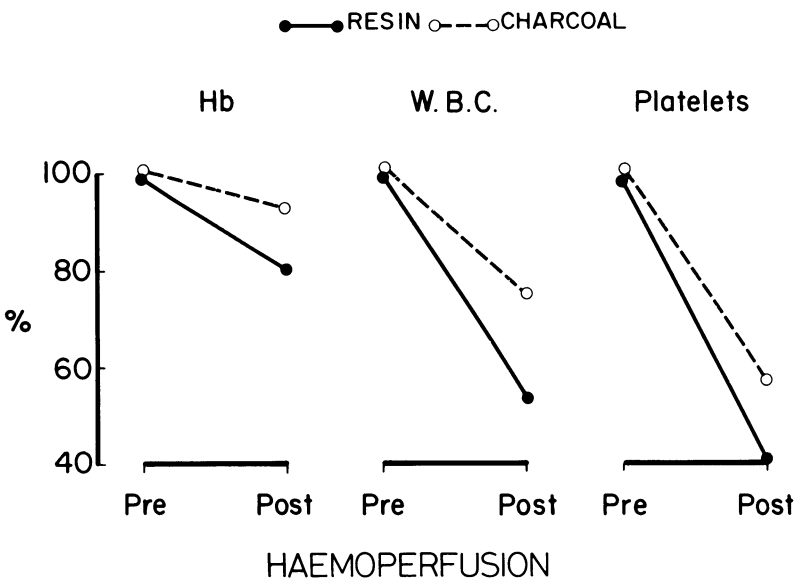



Figure 38.2 Damage to the cellular and formed elements of blood during perfusion. Note greater destruction of red cells, leucocytes and platelets following resin perfusion

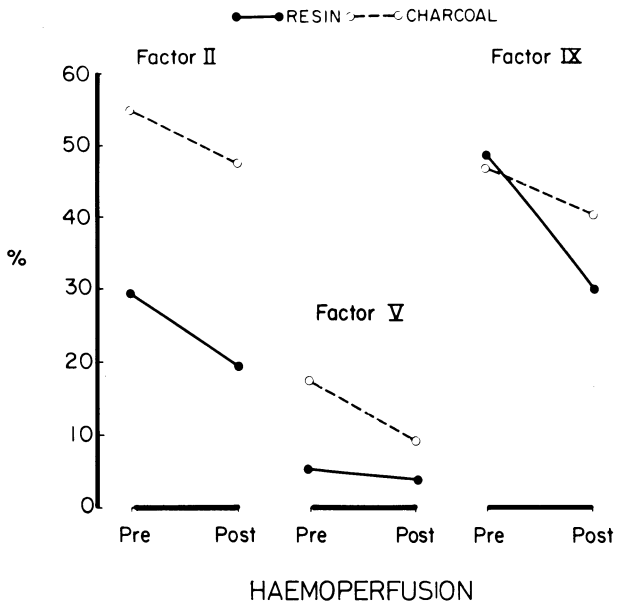



Figure 38.3 Elution of some of the already depleted coagulation factors by the perfusion columns

*Effect on blood count and coagulation factors*

Following haemoperfusion there was considerable decrease in the leucocyte and platelet counts. These changes were more marked after perfusion with resin than with charcoal (figure 38.2). There was also an appreciable decrease in the concentration of various coagulation factors with the exception of plasma fibrinogen which tended to rise or remain unchanged after perfusion (figure 38.3).

## 38.4 Discussion

The effectiveness of activated charcoal in removing water-soluble toxic substances, particularly those in the middle molecular range and the avidity of Amberlite resin in eluting lipid soluble compounds have been reported by several investigators (Yatzidis *et al.*, 1965; Dunea *et al.*, 1970; Chang *et al.*, 1970; Rosenbaum, 1975). However, the precise value of these compounds in removing the 'toxic metabolites', which are thought to be responsible for hepatic coma, remains uncertain.

The experimental model used in this study has been shown to be reproducible and potentially reversible and therefore quite suitable for the evaluation of these compounds in the treatment of experimental hepatic coma. It was observed that haemoperfusion with these adsorbent compounds did produce considerable improvement in the clinical and neurological status of some of the animals. The results indicate that charcoal haemoperfusion is probably superior to the Amberlite resin XAD-4. For, although there was no difference in the number of animals that made ultimate recovery from hepatic failure, the length of survival in those animals treated by charcoal was significantly greater than in those treated by resin. In addition, there were other important differences between the two compounds. The effect of haemoperfusion on the formed elements of the blood and particularly the platelets was far more serious during haemoperfusion with Amberlite resin than with activated charcoal. Also, the perfusion resistance was often extremely high during treatment with the resin where the pressure drop across the column sometimes exceeded 400 mm of mercury. These observations confirm some of the findings reported by Weston *et al.* (1975).

The biochemical changes observed during perfusion with both charcoal and Amberlite resin were, on the whole, very variable. While there was a consistent drop in the concentration of free fatty acids and octopamine, the changes in the concentration of amino acids were variable and quite inconsistent. Also, while most of the amino acids became elevated following induction of hepatic failure, it was not possible to show any marked adsorption of those amino acids that are generally thought to be responsible for the neurological manifestations of hepatic coma, particularly methionine, phenylalanine, tryptophan and tyrosine (Record *et al.*, 1975; Zieve, 1975). It was somewhat surprising that greater adsorption was observed in other amino acids such as leucine, isoleucine and ornithine. It was also interesting to note that these amino acids were more readily adsorbed by Amberlite resin than by activated carbon.

Changes in other biochemical parameters were also inconsistent and non-informative (table 38.4). In general, there was elution of several biochemical compounds. Again, resin appeared to be more effective in removing serum proteins and protein bound substances such as calcium, bilirubin and cholesterol. When an attempt is made to correlate the clinical improvement in the animals with the amount of the various biochemical parameters adsorbed (taking only those parameters in which the adsorption was greater than 30%), there appears to be a fairly good correlation between the clinical result and the change in octopamine and free fatty acids but not in amino acids (table 38.5).

**Table 38.5**

Relationship between clinical improvement and reduction in various biochemical parameters. Note that reduction in octopamine and free fatty acids greater than 30% was a frequent finding in the animals that showed clinical improvement. Such a relationship was not observed with regard to amino acids.

| | | *Number in which reduction in biochemical parameter* > 30% | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | *Amino acids* | | | |
| *Number of animals improved* | *Number surviving long-term* | *Octopamine* | *FFA* | *Methionine* | *Tyrosine* | *Phenylalanine* | *Tryptophan* |
| 6 | 3 | 3/4 | 4/6 | 3/6 | 1/6 | 1/6 | 0/6 |

## 38.5 Conclusions

From these findings and observations several tentative conclusions can be made.

(1) Haemoperfusion with activated carbon and Amberlite resin XAD-4 does sometimes produce a marked clinical improvement in animals with experimental hepatic failure.
(2) Such improvement is temporary and one haemoperfusion does not seem to alter the mortality rate from hepatic failure.
(3) Haemoperfusion with activated charcoal is superior to resin perfusion, both in the clinical results and in the seriousness of complications.
(4) The biochemical changes associated with charcoal and with resin haemoperfusion do not appear either specific or consistent. Although marked adsorption of free fatty acids and octopamine was seen following these perfusions, such changes were not observed in those amino acids or other compounds which are generally thought to be responsible for the neurological manifestations of hepatic coma.
(5) Haemoperfusion with either charcoal or Amberlite resin can only have a very limited role in the treatment of hepatic coma and in our opinion these techniques by themselves can do little to alter the many potentially lethal systemic and metabolic manifestations of the syndrome of hepatic failure which are often the cause of death in this condition.

(6) It is plausible that with more vigorous and repeated haemoperfusion using adsorbent compounds which are less damaging to platelets and other blood constituents, better results might be obtained. We are currently pursuing these objectives.

## 38.6 Acknowledgements

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## 38.7 References

Abouna, G. M., Gilchrist, T., Pettit, J. E., Boyd, N. D., Todd, J. K., Courtney, J. M. and Maini, R. (1975). Haemoperfusion with activated charcoal in treatment of experimental hepatic failure, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support,* Pitman Medical, London, p. 180.

Chang, J. M. and Malave, N. (1970). The development of first clinical use of semipermeable microcapsules (artificial cells) as a compact artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs,* **16**, 141.

Dunea, G. and Kolff, W. J. (1970). Clinical experience with the Yatzidis charcoal artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs*, **11**, 178.

Gazzard, B. G., Weston, M. J., Murray-Lyon, I. M., Flax, H., Record, C. O., Portman, B., Langley, P. G., Dunlop, E. H., Mellon, P. J., Ward, M. B. and Williams, R. (1974). *Lancet*, **1**, 1301.

Record, C. O., Chase, R. A., Curzam, G. and Murray-Lyon, I. M. (1975). Amino acid changes in hepatic encephalopathy, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 27.

Rosenbaum, J. L. (1975). Biocompatibility of resin haemoperfusion, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 118.

Thysell, H., Lindholm, T., Heinegard, D., Jonsson, E., Nylen, U., Svensson, T., Bergkvist, G. and Gullberg, C. A. (1976). Haemoperfusion column using cellophane-coated charcoal. *Eur. Soc. Artif. Organs*, Annual Meeting, Berlin, November 1975.

Weston, M. J., Gazzard, B. G., Flax, H. and Williams, R. (1975). Charcoal and resin haemoperfusion in dogs with acute hepatic failure, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 173.

Yatzidis, H., Voudiciari, S., Oreopoulos, D., Triantaphyllidis G., Voudidari, S., Tsaparas, N., Gavras, C. and Stavraulaki, A. (1965). Treatment of severe barbiturate poisoning. *Lancet*, **2**, 216.

Zieve, L. (1975). Metabolic abnormalities in hepatic coma and potential toxins to be removed, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 11.

# 39

# HEPATIC ASSIST 1: USE OF LIVER TISSUE IN AN EXTRACORPOREAL DEVICE

Y. NOSÉ, P. S. MALCHESKY, I. KOSHINO, F. CASTINO and K. SCHEUCHER

**Summary**

Hepatic failure and the eventual hepatic coma form a complex pathophysiological state, the aetiology of which remains in most cases undefined and for which treatment has yet to be established. The functions of the liver may be grouped for discussion into those of detoxification and metabolism. Since the function of detoxification is most notable in the early stages of hepatic failure, most attention has been directed to this method of treatment. The rationale for this approach stems from the ability of the liver to regenerate itself and therefore eventually provide total support. However, methods of detoxification alone are limiting and are not sufficient where extended periods of support are necessary, therefore, the need to provide active metabolic activity. For this purpose, liver tissue was chosen. Studies on the procurement and preparation of liver tissue in the form of slices were carried out and the preparation evaluated both in incubation chambers and in specially constructed perfusion devices. Studies have indicated the active function of these preparations for various metabolic processes.

## 39.1 Introduction

The primary causes of hepatic coma and hepatic failure have remained in most cases undefined. Therefore, the best mode of treatment has yet to be established. Two main classifications of the types of treatment employed in hepatic failure are conventional and nonconventional. Nonconventional treatments can be further classified as being nonbiological or biological. Conventional treatments are those directed to offer general support to the patient or are designed for symptomatic treatment. Bowel sterilisation, which or without lavage, lactulose administration, colon bypass, or resection, and the employment of various drugs have been tried, but these are not sufficient (Schenker *et al.*, 1974; Fischer, 1974).

Nonconventional nonbiological treatments have included haemodialysis (Kiley *et al.*, 1956) and sorbents (Schechter *et al.*, 1958) for the purpose of removing substances implicated or in excess in hepatic failure. Naturally, because of the success of dialysis in the detoxification of blood in renal failure, it was considered for hepatic support. However, with knowledge of the dialysis membrane and its

limitation of solute transport it could not be expected to be effective, particularly in the removal of protein bound solutes. Sorbents, including activated charcoals, have been used in patients with acute hepatic failure and coma with some degree of success (Gazzard *et al.*, 1974). In general, however, the results of these treatments show a broad range of effectiveness, probably due to the aetiology of the disease, level of hepatic failure, degree of cellular damage, and technical procedures employed. Such treatments, which are designed primarily for the removal of specific circulating toxins, do not provide the synthetic activity needed in hepatic failure. Chronic hepatic failure remains outside the scope of these treatments.

Therefore, nonconventional biological treatments have been proposed and employed, including exchange blood and plasma transfusions, cross circulation and cross dialysis. Treatments based on exchange transfusions and plasma exchange have been found to be of only transient help. Cross circulation is a more reasonable approach; however, the potential dangers to the donor and immunological problems have limited its employment. Cross dialysis minimises the problems associated with cross circulation, but the limitations of mass transfer associated with the semipermeable haemodialysis remain. The utilisation of healthy liver tissue, either homologous or heterologous, which could be expected so supply the more complete spectrum of liver functions, has also been considered. Liver perfusion and transplantation are two techniques employing the liver as an organ. Studies have been reported concerning their experimental and clinical uses, but procurement, preservation and the presence of immunological reactions remain as problems to be solved. Thus far, clinical results of liver perfusion are rather disappointing. It is difficult to maintain proper microcirculation of liver for an extended period of time. Therefore, the utilisation of liver other than the whole organ may be considered. Tissue, cells or cell components all perform at least some hepatic functions, and so hepatic assist devices with biological liver tissue were utilised by many investigators.

One important consideration in the design of a system for support of hepatic function is the fact that the liver has the capability of regeneration in most cases, and that the support need be only temporary until the liver can regain its capacity to take over total function. Although the chronic application of an hepatic support device remains the eventual goal, the necessity and applicability of a device today for even acute cases would be a major step toward this goal.

The uses of cellular components as enzymes and microsomes, as a means of hepatic assist, have been studied but there is insufficient knowledge about their function or lack of necessary functions to make them likely for use in total hepatic support. The use of normal cell cultures has been studied, but early reports (Soyer *et al.*, 1973) showed low activity yields and difficulties in preparations. Recently, hepatoma cells were grown on synthetic capillaries to achieve rapid growth rate, but the applicability of this concept to hepatic support is questionable because of the frequent occurrence of biochemical and morphological transformations. Liver tissue with the cellular structure intact

has been the primary form for studying isolated liver function and characteristics since the work by Krebs in 1932, and is believed to be the most promising form of liver tissue for hepatic support device. The utilisation of liver tissue for hepatic assist in the form of slices or granules as well as frozen and lypholised (Mikami *et al.*, 1959) has been studied by investigators over the past two decades. Although some of these liver preparations were utilized for clinical trials, the functions of these preparations were not well characterised.

Based upon this information, preliminary investigations (Nosé *et al.*, 1975 and Koshino *et al.*, 1975) were carried out on the application of liver slices for hepatic assist. A study of the previous work indicated that failures experienced in early devices stem from the insufficient knowledge and/or inadequate techniques employed for procurement and preparation of the liver tissue and not specifically from the devices themselves. It was thought that the application of liver slices for hepatic assist could be improved if optimal conditions for the procurement and preparation of fresh liver slices were established.

In summarised form the results of these studies to investigate the procurement and preparation of liver slices and their incorporation into an extracorporeal device are presented here. The accompanying review describes the further optimisation of the design and evaluation of an extracorporeal device which could be used with liver tissue or sorbents or other biological reactors in providing metabolic support.

## 39.2 Liver slice procurement and preparation

Adult mongrel dogs of both sexes, 15 - 25 kg, were fasted for 24 h preoperatively. Anaesthesia was induced by thiamylal sodium (20 - 25 mg/kg) and maintained with oxygen 2 l/min, nitrous oxide 2 l/min, and Fluothane (0.5 - 0.75%) under controlled respiration. The portal trunk was cannulated with a poly(vinyl chloride) tube ($\frac{3}{16}''$) ID via the superior mesenteric vein, passing the splenic vein up to the portal trunk proximal to its branching. The portal trunk was ligated around the tubing and the superior mesenteric and splenic veins were tied. The hepato-duodenal ligament, exclusive of the portal vein, was tied en masse, including the hepatic artery simultaneous to the initiation of the perfusion. Using a specially prepared solution, the liver was perfused by gravity feed at 20 - 30 cm of water until the hepatic parenchymal temperature dropped to 20 °C or less. The perfusate consisted of Waymouth's medium MD 705/1 (Grand Island Biological Co, Grand Island, New York) to which was added 2% dextran (20 000 mol. wt.), 5 mg% cephaloridine and 8 mg% gentamycin. This solution was saturated with oxygen and kept at 4 °C until use. The perfusion was initiated approximately 1 h after induction of anesthesia.

The liver was removed by sharp dissection of the hepato-duodenal ligament, the inferior vena cava and other attachments to the surrounding tissue. The temperature of the liver parenchyma dropped to 20 °C after approximately 10 min of perfusion, in which approximately 2 l of perfusate were used. The liver was removed and placed on a sterile drape and cut into 1.5 mm thick slices

using a specially designed cutter. These slices, being trimmed to the appropriate dimensions, were then weighed and rinsed in fresh perfusion solution at 4°C, and placed in incubating chambers for evaluation of metabolic activities, or loaded into devices for their evaluation in perfusion. The total time spent from removal of the liver to initiation of the incubation was 7 - 10 min or 12 - 15 min to the start of perfusion.

### 39.3 Evaluation of Slices by Incubation

Special incubation chambers were constructed. To each of six test chambers, 10 - 15 g of liver slices were added with 200 ml of oxygenated modified Waymouth medium (MWM). No liver slices were introduced into the three control chambers. The chambers were placed in a 38°C bath and mild agitation provided by shaking the chambers. Oxygen was delivered to each chamber at a flow rate of 300 ml/min and ammonia was periodically added at 1.65 mg% at 0, 1, 2, 3, 4, 5, 6, 10, 14, 18 and 22 h. Metabolic activities of the liver slices were followed for 7 and 24 h in two individual experiments. Oxygen and carbon dioxide partial pressures, hydrogen ion concentration, ammonia nitrogen, urea, glucose, lactic acid, amino acids, phosphate, calcium and the enzymes glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH) were measured. Bacteriology analyses for aerobic and anaerobic species were made.

For the test chambers the pH showed a moderate decrease (7.6 to 6.95 at 24 h) despite ammonia loading. In the control chambers the pH reached a stable level of 9.0. The test chambers showed evidence of respiration by exhibiting lower $pO_2$ and higher $pCO_2$ levels compared to the control. Ammonia concentration was always higher in the control chambers and its utilisation by the liver slices was evident. The test chambers showed increased urea concentration with time; 3.6, 4.5, 6.3 and 7.2 mg/g liver tissue at 6, 10, 14 and 24 h of incubation. No urea was present in the control chambers. The urea concentration is higher than the value calculated from ammonia consumption alone. The amino acid analysis indicated the utilisation of amino acids as a nitrogen source, as is shown by the complete disappearance of arginine. In addition, glutamic acid, histidine, phenylalanine, cystine and arginine decreased while tyrosine, 3-methyl-histidine and alanine increased in the test chambers. The glucose concentration was maintained stable in the test and control chambers. However, the test chambers exhibited the production of 7 mg lactic acid/g liver tissue, indicating the active regulation of glucose. The test chamber revealed the production of 0.44 $\mu$g catecholamine/g of liver in 7 h. The test chambers showed a marked increase in inorganic phosphate, indicative of chemical energy utilisation. Increased levels of GOT and LDH occurred after 14 h of incrubation indicating the maintenance of cellular integrity for at least up to this period of time. Only one sample from the test chambers showed contamination (*pseudomonas*) after 24 h.

### 39.4 Evaluation of Liver Slices in Perfusion Devices

In an effort to study the activities of this liver preparation in a flow-through device preliminary studies were carried out. A device was designed which incorporated frames positioned to allow serial flow. The individual frames allowed for sandwiches of the liver tissue to be made with porous polyester nets (nominal pore diameter of 1 $\mu$m). In operation the perfusion medium is pumped into the device and directed to flow across the plates. Under the pressure of pumping, plasma is forced into the liver compartment, interacts with the tissue and is forced into the mainstream. Thus, continuous perfusion can be carried out without whole blood interaction with the liver tissue and vice versa. In one system, modified Waymouth medium (MWM) was used as the perfusate. The device and circuitry had a priming volume of 3400 ml for nine plates containing 180 g of liver tissue. The pumping rate was 200 ml/min. A continuous supply of oxygen was provided by bubbling directly into the device. This unit being bulky, a second system was designed of 1200 ml priming volume for ten plates containing 230 g of liver. The flow rate was 100 ml/min. Homologous blood was used as the perfusate.

The general trends of metabolic activity were seen in these flow-through systems and compared well to the incubation chambers. However, they differed qualitatively. In the blood perfused device it was necessary to adjust pH with sodium bicarbonate. Urea was shown to increase linearily for the MWM perfused system for 14 h and then level off, but continued to increase in the blood perfused system. Amino acid changes in the MWM perfused system were comparable to those in the incubation system. Glucose remained constant in the flow through systems and lactic acid production at 24 h in the blood perfused system was approximatley 18 mg/g of liver tissue. Based on urea production alone, the perfused systems exhibited 40 - 60% the efficiency of the liver tissue in incubation.

### 39.5 Discussion

The results indicate that metabolic activities can be maintained for prolonged periods of time and most effectively up to 14 h. It is considered essential that to employ live tissue the proper medium be selected, adequate oxygen be supplied, temperature control and pH be maintained near physiologic level, and the anoxic period for liver tissue procurement be minimised. In the employment of liver tissue in the flow-through device, several device-related problems were encountered. The procurement of the liver tissue and mounting it into the device is still considered time consuming. Flow dynamics in the device were not optimised along with liver tissue packing and/or exposure to the perfusate; therefore, the decreased performance noted compared to incubation studies. Even so, activity noted was higher than that previously reported (Soyer *et al.*, 1973). Conservative calculations based on urea production alone indicate that 600 g of liver slices

could support a 15 kg dog on a normal diet. Based on further optimisation of the liver procurement and preparation procedures and improved design of the device, a biological extracorporeal metabolic device for clinical hepatic support can be a reality.

## 39.6 References

Fischer, J. E. (1974). Hepatic coma in cirrhosis, portal hypertension and following portacaval shunt. *Arch. Surg.*, **108**, 325.

Gazzard, B. G., Weston, M. J., Murray-Lyon, I. M., Flax, H., Record, C. O., Portman, B., Langley, P. G., Dunlop, E. H., Mellon, P. J., Ward, M. B. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet* **1**, 1301.

Kiley, J. E., Welch, H. F., Pender, J. C. and Welch, C. S. (1956). Removal of blood ammonia by haemodialysis. *Proc. Soc. Exp. Biol. Med.*, **91**, 489.

Koshino, I., Castino, F., Yoshida, K., Carse, C., Kambic, H., Scheucher, K., K retz, A. P., Malchesky, P. S. and Nosé, Y. (1975). Biological extracorporeal metabolic device for hepatic support. *Trans. Amer. Soc. Artif. Int.* Organs **21**,492.

Mikami, J. and Nosé, Y. (1959). Surgical treatment of acute liver failure. An experimental study of extracorporeal metabolism in the artificial liver using slices of canine liver. *Jap. J. Gastroenterol.*, **56**, 1022.

Nosé, Y., Koshino, I., Castino, F., Yoshida, K. Carse, G., Kambic, H., Scheucher, K., Kretz, A. P. and Malchesky, P. S. (1975). Further assessment of liver tissue materials for extracorporeal hepatic assist, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London.

Schechter, D. C., Nealon, T. F. and Gibbon, J. H. (1958). A simple extracorporeal device for reducing elevated blood ammonia levels. Preliminary report. *Surgery*, **44**, 892.

Schenker, S., Breen, K. J. and Hoyumpa, A. M. (1974). Hepatic encephalopathy. Current status. *Gastroenterology*, **66**, 121.

Soyer, T., Lempinen, M. and Eiseman, B. (1973). *In vitro* extracorporeal liver slices and cell suspensions for temporary hepatic support. *Ann. Surg.*, **177**, 393

# 40

# HEPATIC ASSIST 2: DEVICES FOR USE WITH SORBENTS AND BIOLOGICAL REACTORS

Y. NOSÉ, P. S. MALCHESKY, I. KOSHINO, F. CASTINO and K. SCHEUCHER

**Summary**

Present research has indicated the applicability of reactors such as sorbents and biological agents for hepatic insufficiency; but problems of blood incompatibility, blood cellular adsorption on the reactors, low efficiencies, and reactor particle carry over into the vascular system have limited their use. The technique of the separation of plasma from whole blood by filtration through microporous membranes, the removal of solutes from the plasma by means of sorption or metabolic transformations with reactors, and the reinfusion of the purified plasma into the mainstream through membranes, preventing the carryover of the reactor, is believed to overcome these problems. This technique is particularly applicable in the removal of those solutes that are protein bound or those high molecular weight species that are not appreciably transferred by other techniques such as dialysis. Membranes and reactors for this purpose have been selected. Devices have been constructed and evaluated, demonstrating the feasibility of this concept. Future work will concentrate on demonstrating the feasibility of this concept in the treatment of hepatic failure.

## 40.1 Introduction

In the treatment of metabolic diseases where large molecular weight or protein bound toxin removal is necessary, the use of haemodialysis is membrane limited. Even the use of higher flux membranes, as employed in haemodiafiltration (Henderson *et al.*, 1973), is limited in that such molecules are not appreciably transferred. Haemoperfusion over beds of sorbents has the disadvantage of adsorption of blood components or blood damage and the release of particles that could be embolising or toxic to the patient (Chang, 1972). These problems become critical when the use of such systems are considered for chronic support. Recognising the advantages of the use of membranes permeable to high molecular weight solutes and the need for specific reactors as sorbents or biological materials in the treatment of metabolic disorders, a system was conceived which would allow the passage of plasmatic fractions, yet prevent interaction of blood cellular elements with the reactor and avoid reactor carryover (Nosé *et al.*, 1975). As depicted in figure 40.1, whole blood enters the device and is perfused over appropriate microporous membranes. Under the force of a flow induced pressure

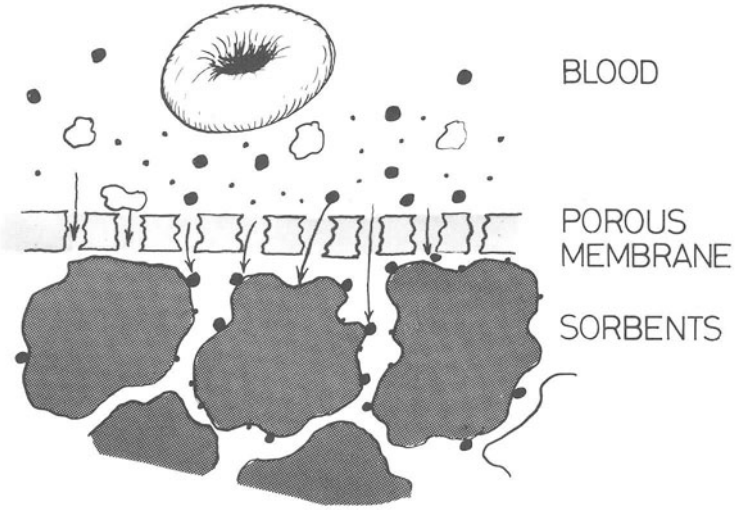



Figure 40.1 Principle of plasma filtration haemoperfusion. Whole blood enters the device. Due to the flow-induced pressure gradient, plasma is filtered through the microporous membrane and enters the reactor compartment. In the reactor compartment sorption or metabolic transformation of the solutes of interest takes place. The plasma then passes through another microporous membrane which prevents the passage of reactor particles. The plasma is reunited with the bulk flow. By proper selection of the flow conditions the process can take place without blood cellular deposition on the membranes and maintain a high efficiency

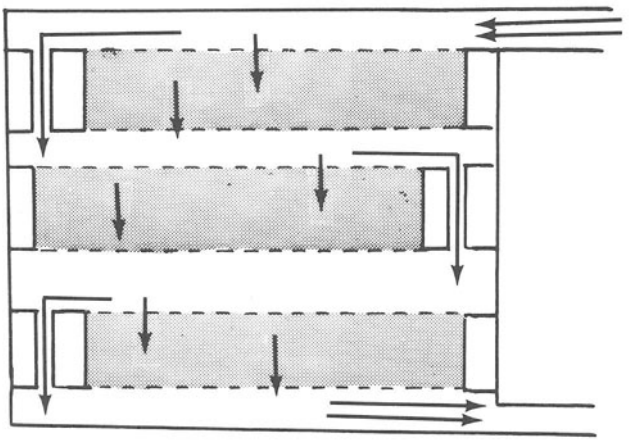


Figure 40.2 Schematic diagram of multilayer plasma filtration system

gradient, plasma is filtered through the microporous membrane and enters the reactor compartment. In the reactor compartment, sorption or metabolic transformation of the solutes of interest takes place. The plasma then passes through another microporous membrane which prevents the passage of reactor particles, and the plasma is thus reunited with the bulk blood flow. By stacking plates, as shown in figure 40.2, multilayer devices can be built housing the same or different reactor systems and of sufficient membrane filtration surface to meet the need. It is recognized that the above concept may have several applications, but based upon the inadequacies of present treatment schemes in hepatic failure, and the promise that sorbents offer even with their present shortcomings, design criteria were established for its application in hepatic failure. To apply this concept, several specific requirements must be met: a membrane must be selected which allows for the passage of the solutes of interest; the appropriate reactors must be chosen, which will selectively interact with such solutes, and device designs incorporating such membranes and reactors must be made safe and efficient.

## 40.2 Selection of Membrane

For a membrane to be studied in this concept for use in hepatic failure, the following criteria must be met.

(1) It must be hydrophilic or be able to be made hydrophilic, yet allow the ready passage of plasma.
(2) It must have a pore size large enough to allow the transfer of the molecules of interest at a reasonable rate, and yet small enough to prevent the passage of blood cellular components or embolising reactor particles. In consideration of its application to hepatic failure, a pore diameter of 0.02 to 0.5 $\mu$ appears suitable.
(3) The membrane must be of adequate mechanical strength to be assembled into devices and to maintain integrity under operational conditions.
(4) It must be biocompatible and lend itself to standard sterilising methods.
(5) To be practical, the membrane must be available or producible in quantity at a reasonable cost.

Based upon these criteria, 16 membranes from six different manufacturers have been obtained and evaluated (Castino *et al.*, 1976). By studying the filtration of deionised water at 22 °C, several membranes were discarded due to decreases in ultrafiltration rate versus time (up to 5 h studied). Other membranes were rejected based on uneven pore diameter distribution or surface roughness, as detected by scanning electron microscopy. Based on filtration studies with canine blood at 38 °C up to 5 h, three membranes were selected for further study: the Celanese polypropylene membrane 0.04 by 0.2 $\mu$ pore and two Nuclepore polycarbonate membranes 0.2 and 0.4 $\mu$ pore diameter. Obeying haemodynamic criteria (Forstrom *et al.*, 1975) necessary to prevent blood cellular deposition (deposition parameter $< 0.15$ on filtering walls),

**Table 40.1**

Sieving coefficient of blood solutes in microporous membranes

| *Solute* | *Molecular weight* | *Sieving coefficient* | | |
|---|---|---|---|---|
| | | *Polycarbonate 0.2* | *Polypropylene 0.2* | *Polycarbonate 0.4* |
| $Ca^{2+}$ | 40 | 0.95 | 0.95 | 1.00 |
| Urea | 60 | 0.90 | 1.00 | 1.00 |
| $PO_4^{3-}$ | 95 | 0.95 | 0.95 | 1.00 |
| Creatinine | 113 | 1.00 | 1.00 | 1.00 |
| Uric acid | 168 | 0.70 | 0.70 | – |
| Cholesterol | 387 | 0.15 | 0.15 | 0.80 |
| Bilirubin | 585 | 0.05 | 0.20 | 0.70 |
| Albumin | 70 000 | 0.25 | 0.35 | 0.90 |
| Lactic dehydrogenase | 130 000 | 0.10 | 0.05 | 0.60 |

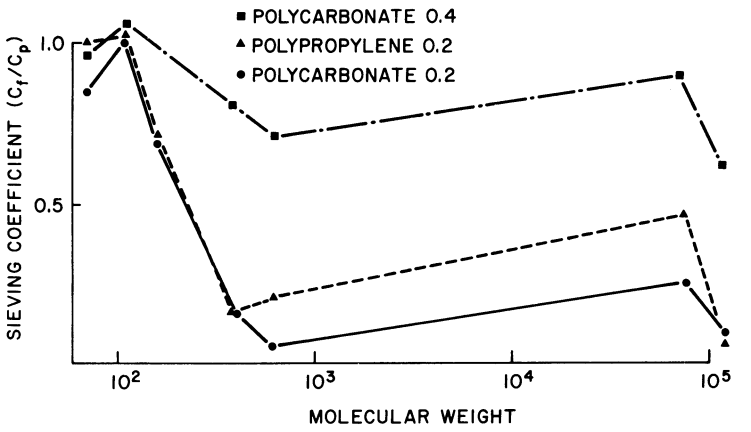



Figure 40.3 Variation of sieving coefficient versus molecular weight

single layer test cells (56 and 168 $cm^2$ surface area) were constructed. The sieving properties of these membranes were evaluated with heparinised canine blood at 38 °C for flow rates of 120 and 240 ml/min over 5 h periods. Table 40.1 and figure 40.3 outline the data in terms of the sieving coefficients for various solutes in blood. (Sieving coefficient equals concentration of solute in filtrate divided by its concentration in plasma.) Based upon the higher flux rate of the polycarbonate 0.4 $\mu$ membrane and its lower rejection coefficient for protein and protein bound solutes (bilirubin), this membrane was considered the most likely candidate for further study in an hepatic assist device.

Just as it is important to prevent the passage of blood cellular elements into the reactor compartment, it is equally important to prevent the passage of embolising reactor particles into the blood stream. Using activated charcoal (Takeda 14 - 28 mesh), particle release was studied. Using commercial sterile saline solution as the perfusate and various loads of charcoal, no carbon particle release was detected (Nosé *et al.*, 1975) by observation of filters of submicron pore size after 3 to 5 h of perfusion. To better quantify particle release, a device containing 50 g of charcoal sandwiched between two membranes of polycarbonate 0.4 $\mu$ of 56 $cm^2$ surface area were perfused at a flow rate of 100 ml/min using 1 l of saline for 1 h, 2 h and 3 h consecutively. Thus, 1 l of saline was perfused in a single device over a 6 h period. The perfused saline at the end of each time period was filtered at a flow rate of 60 ml/min through preweighed 0.22 $\mu$ Millipore filters. The filters were then dried and weighed and a comparison made between the pre and post-weights. Weight changes for the filters were + 0.9 mg, + 1.0 mg and + 1.0 mg at 1, 2 and 3 h perfusion periods. Control filters processed the same way, except that the saline was not perfused through the device, showed a change of + 1.2 ± 0.2 mg. Therefore, by weight changes

alone, the change in the test filters was comparable to that of the control. Scanning electron microscopy of the filters revealed no carbon particles. Two commercial charcoal cartridges were treated in the same way and showed total weight changes (in mg) of + 1.8, + 1.8, and + 1.1 and + 1.0, + 2.2, and + 1.1 at 1, 3 and 6 h respectively. Visual inspection indicated the presence of charcoal debris.

### 40.3 Selection of the Reactor

The selection of a reactor for treating the plasma is qualitatively and quantitatively dependent upon the toxin(s) accumulating in the bloodstream. Since activated carbon has proven effective as a nonspecific sorbent in the removal of organic metabolites associated with kidney or liver disfunctions, initial emphasis is placed on its incorporation into prototype hepatic assist devices. Based upon batch studies of the adsorption at 37°C for creatinine (a small marker molecule known to be removed by activated charcoal), Takeda activated charcoal (Takeda Co, Osaka, Japan) 14 x 28 mesh was chosen for use. In 8 h Takeda charcoal removed 9.3 mg creatinine/g of carbon compared to 7.8 and 7.0 mg for Pittsburgh and Witco carbons respectively. In addition to having a high efficiency for short-term removal of creatinine, the Takeda charcoal has already been used clinically in the regeneration of dialysate and has been proven atoxic. The attention is also focused on the use of the polystyrene macroreticular adsorbents (Amberlite XAD-2 and XAD-4, Rohm and Haas Co, Philadelphia) which have been shown clinically effective in the adsorption of metabolites associated with drug intoxication and hepatic failure. Batch determinations of efficiency in the removal of bilirubin from jaundiced human plasma at 37°C with XAD-2 indicated that 0.34 mg of bilirubin/g of resin was removed in 2 h, 0.44 in 4 h, 0.46 in 6 h and 0.46 in 24 h. The capacities of these sorbents allow the construction of practical size devices for extracorporeal applications. As progress is made in the preparation and preservation of liver tissue or other biological agents, or as research points the direction to other sorbents useful to this purpose, these may also be readily incorporated.

### 40.4 Device Design and Evaluation

In the design of a device with a given membrane and reactor or reactor combinations, certain functional and operational requirements must be met. Functionally, the unit must have sufficient membrane flux and reactor capacity to carry out the required operation and it must be biocompatible and atoxic. Operational requirements such as flow rate, priming volume, and hydraulic pressures must be within allowable ranges for the patient. The unit must be easy to prepare for operation, easy to use, be without leakage, exhibit minimal clotting or thrombus formation, have a low residual blood volume, and preferably be disposable. With consideration of these requirements, both serial flow and parallel plate designs were conceived. The serial flow design was chosen since it was believed to provide for simpler construction and more uniform flow dynamics. Early proto-

type devices were constructed of circular plates but considerations of fabrication and flow geometry led to the present rectangular design. In an effort to maintain the blood channel geometry uniform throughout, but to prevent excessive mixing of the blood which could induce cellular deposition on the membrane, channel supports were initially introduced. The presence of such supports was found to induce stagnation in their vicinity, resulting in cellular deposition, even though correct haemodynamic considerations were adhered to and no deposition was noted in the filtration regions away from these supports. At the present time rigid membrane support screens are being employed, located adjacent to the flow channel, thereby eliminating the need for in-channel supports and allowing for blood perfusion over the porous membranes without cellular deposition on the filtration surface.

To exemplify the importance of matching the filtration properties of the membrane with that of the flow dynamics of the system, canine blood (Hct 30%, 37 °C) was perfused in single layer devices containing the polycarbonate membrane 0.4 $\mu$m pore diameter at deposition parameters of 0.10 and 0.33. At a maximum deposition parameter of 0.10, cellular elements are not deposited and the pores remain patent. At a maximum deposition parameter of 0.33, cellular deposit is evident. In particular the drawing of the red cells into the pores of the membrane is noted. In this test, filtration flux was shown to decrease from 18.8 to 3.2 l/m$^2$/h in a 2 h perfusion at an average transmembrane pressure drop of 25 mm Hg.

Several devices were evaluated for fluid dynamic and mass transfer characteristics. The construction was either as the single layer as previously described or multiples thereof. Tables 40.2 and 40.3 outline the characteristics and results obtained with these devices constructed with Nuclepore 0.4 $\mu$m membrane of 56 cm$^2$ filtering surface area per plate. For the charcoal containing devices, creatinine removal was in the range of 1.8 mg/g charcoal in 2 h, which is well below the sorbent's capacity. Analysis of the creatinine removal indicated that its removal by filtration and then sorption was accompanied by significant diffusion with sorption. This is to be expected for a low molecular weight solute, which is not protein bound. In contrast, removal of bilirubin was found to be highly dependent on filtration. Removal of bilirubin by the XAD-2 resign was 0.16 mg/g resin in 1 or 3 h studies. This was found to be the maximum removal capacity of the resin in the devices tested. In these devices, this saturation was reached within an hour after the start of perfusion.

An *in vivo* perfusion with a five-layer device was carried out on a male calf. The circuitry consisted of standard catheters and blood lines, a blood pump and the cartridge. The perfusion was carried out for 60 min at a flow rate of 240 ml/min. An initial dose of 3 mg/kg of heparin was administered. Pressure drop of the device during the run was 500 mm Hg. Pre and post-analyses of serum chemistries, including electrolytes, plasma haemoglobin and enzymes, and haematology, including cell counts, indicated no significant variations from perfusion without the device.

**Table 40.2**

Circuitry characteristics of devices (blood flow rate 200 ml/min, temperature 37°C)

| *Solute studied* | *Unit number* | *Number of plates* | *Reactor* | *Blood circulating volume (l)* | *Pressure drop mmHg* |
|---|---|---|---|---|---|
| Creatinine | C-7 | 1 | Activated | 1.5 | 60 |
| | C-15 | 1 | Charcoal | 1.5 | 150 |
| | C-18 | 5 | 50g/plate | 1.5 | 480 |
| Bilirubin | B-6 | 1 | XAD-2 | 1.07 | 400 |
| | B-7 | 1 | resin 60g/plate | 0.87 | 150 |

**Table 40.3**

Removal of solute versus length of perfusion

| *Unit number* | *C-7* | | *C-15* | | *C-18* | | *B-6* | *B-7* | |
|---|---|---|---|---|---|---|---|---|---|
| Time min | 50 | 110 | 50 | 110 | 50 | 110 | 55 | 55 | 115 |
| Total removed mg | 36 | 66 | 52 | 91 | 255 | 465 | 9.6 | 7.0 | 9.6 |
| Percent removal | 11.3 | 20.8 | 14.5 | 25.2 | 41.2 | 76.8 | 10.2 | 10.4 | 15.6 |
| Transfer rate* ml/min | 3.6 | 3.2 | 4.7 | 4.0 | 34.0 | 42.5 | 2.1 | 1.7 | 1.3 |
| Filtration pressure mm Hg | 30 | 30 | 75 | 75 | 400 | 400 | 200 | 75 | 75 |
| Theoretical filtration† ml | 36.4 | 80.1 | 91.0 | 200.2 | 485.3 | 1067.7 | 267.0 | 100.1 | 209.3 |
| Percent filtered | 2.4 | 5.3 | 6.1 | 13.3 | 15.2 | 33.4 | 25.0 | 11.5 | 24.1 |
| Filtration time ml/min | 0.73 | 0.73 | 1.82 | 1.82 | 9.7 | 9.7 | 4.9 | 1.82 | 1.82 |
| Ratio $\frac{\text{filtration time}}{\text{transfer rate}}$ | 0.20 | 0.23 | 0.38 | 0.45 | 0.29 | 0.23 | 2.3 | 1.07 | 1.40 |

*Transfer rate $= -\ln \frac{C_f}{C_i} \frac{V}{\Delta t}$

†Filtration coefficient 260 ml/m²/h/mmHg ± 10%

## 40.5 Conclusion

Extracorporeal devices containing sorbents have been designed which avoid blood cellular deposition and sorbent particle carryover. Membranes have been selected which allow for the passage of protein bound solutes with a high efficiency. Present work has concentrated on the use of conventional sorbents such as activated charcoal and nonionic resins; however, the concept is applicable to the use of a broad range of reactors including biological agents such as enzymes, cells or tissue.

## 40.6 References

Castino, F., Scheucher, K., Malchesky, P. S., Koshino, I., and Nosé, Y. (1976). Microemboli free blood detoxification utilising plasma filtration. *Trans. Amer. Soc. Artif. Int. Organs,* **22**, 637.

Chang, T. M. S. *Artificial Cells*, Thomas, Springfield, Illinois, 1972.

Forstrom, R. J., Bartelt, K., Blackshear, P. L. and Wood, T. (1975). Formed element deposition onto filtering walls. *Trans. Amer. Soc. Artif. Int. Organs*, **21**, 602.

Henderson, L. W., Livoti, L. G., Ford, C. A., Kelly, A. B. and Lysaght, M. J. (1973). Clinical experience with intermittent hemodiafiltration. *Trans. Amer. Soc. Artif. Int. Organs.*, **19**, 119.

Nosé, Y. Malchesky, P. S., Castino, F., Koshino, I., Scheucher, K. and Nokoff, R. (1975). Improved haemoperfusion system for renal-hepatic support. Presented at the *Symposium on Sorbents in Uraemia and Hepatic Failure.* Downstate Medical Centre, Brooklyn, N. Y., November 3 - 4.

# 41

# TOWARDS AN EXTRACORPOREAL DETOXIFICATION SYSTEM IN LIVER FAILURE. DETOXIFYING ENZYMES BOUND TO ARTIFICIAL CARRIERS AS MONO-AND MULTI-ENZYME COMPLEXES

G. BRUNNER and H. LOESGEN

**Summary**

In hepatic failure the enzymes of detoxification in the liver are greatly reduced and this results in an accumulation of toxins in the blood. The toxins which thus occur in hepatic failure in their turn inhibit many of the enzymes of the liver and brain metabolism. This review discusses the elimination of endogenous and exogenous toxins from blood and shows that this can be achieved *in vitro* and *in vivo* by extracorporeal enzymatic conjugation and hydroxylation using membrane bound UDP-glucoronyltransferase, cytochrome-p-450 and NADPH-cytochrome-p450 reductase.

## 41.1 Introduction

Coma is the most impressive clinical sign in acute liver failure. Coma can be experimentally produced by the same toxins which are found elevated in the serum of patients in liver coma. Among the toxins regularly found in the blood of comatose patients, ammonia, mercaptans, free fatty acids and free phenols play a major role (Zieve *et al.*, 1975; Brunner, 1975). Coma can be produced in rats and rabbits with each of these toxins and a synergistic and potentiating effect of these toxins in the production of coma can be demonstrated. This observation is strong evidence that toxins are involved in the aetiology of liver coma. However these toxins do not only interfere with the regulation of brain function by producing a state of unconsciousness but also inhibit vital enzymatic reactions in various other organs especially the liver. Free phenols inhibit a large number of enzymes in the liver. Mainly membrane bound enzymes are affected such as succinic dehydrogenase (up to 70%), monoamine oxidase (up to 97%), prolin oxidase and NADPH-cytochrome-C-reductase. Mitochondrial respiration is inhibited by free phenols more than 50% in those concentrations that occur in the serum of comatose patients (Brunner, 1976). Free fatty acids markedly inhibit the activity of the

enzymes glucokinase, hexokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Weber *et al.*, 1966). Among other effects ammonia inhibits oxygen utilisation in the brain (McKhann and Tower, 1961) and the metabolism of pyruvate. It lowers the concentration of pyridinnucleotides and disturbs the tricarbonic acid cycle (Bessman *et al.*, 1955, 1956; Bigler *et al.*, 1962). Mercaptans inhibit ATP-ases and phosphokinase (Foster *et al.*, 1974).

All these toxins pile up in the blood in patients with liver failure because the amount and activity of the enzymes of detoxification is greatly reduced. Already in precomatose stages of liver disease, enzymes of detoxification such as cytochrome-P-450, glucuronyltransferase and NADPH-cytochrome-P-450 reductase are found reduced between 50 - 80% as measured in liver biopsy specimen. In liver failure of animal or man often more than 90% of detoxification capacity is lost. Decrease of detoxification capacity leads to accumulation of toxins and as a result of this the remaining enzymes of energy production, synthesis, detoxification and regeneration are inhibited: in this way a vicious circle of destruction can develop in the late stage of fulminant liver disease.

One possible step of influencing liver regeneration could be removal of the endogenous toxins to brake the vicious circle. Besides physical techniques, biochemical methods can be applied. Since enzymatic reactions are the physiologically selective way of liver detoxification, we have started to develop an extracorporeal detoxification system for hydroxylation and glucuronidation by using detoxifying enzymes bound to artificial carriers.

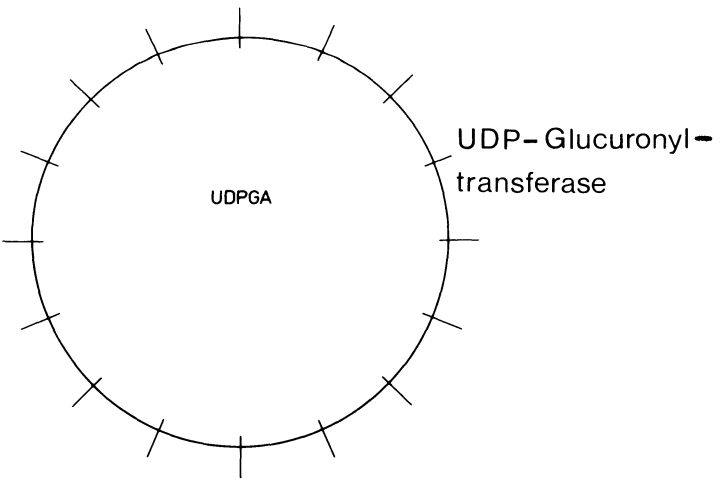



Figure 41.1 UDP-glucuronyltransferase bound to BrCN-Sepharose bead
UDPGA trapped inside the bead

## 41.2 Results

UDP-glucuronyltransferase was solubilised from microsomes by deoxycholate treatment. After solubilisation deoxycholate has to be removed before further purification can be attempted. In high molarity Tris buffer (0.7 - 1.5 M Tris pH 8.0) DOC precipitates leaving the enzyme in the supernatant. By use of ammonia precipitation, DEAE and Sephadex G-200 chromatography the enzyme can be purified 500 to 600-fold. This purified UDP-glucuronyltransferase was bound to a variety of artificial carriers. Best results could be achieved by binding the enzymes to copolymers of acrylamide and acrylic acid or to BrCN-Sepharose. The latter was more suitable and compatible for use with blood. Binding of the already stable enzyme to artificial carriers resulted in a more than 20-fold increase in stability.

For *in vivo* experiments Sepharose beads were produced approximately 3 mm in diameter (heating Sepharose in water to 80°C and pressing the liquid gel through a syringe into ice-cold chloroform) and UDP-glycuronyltransferase was bound to these beads by the method of Cuatrecasas (1970). UDPGA was trapped inside the beads (figure 41.1), diffusing out at a rate which allows maximum enzyme reaction for 50 - 60 min. The 'enzyme beads' can be reloaded with UDPGA by incubating them in concentrated UDPGA solution. Using this method of supplying UDPGA is much cheaper than infusing it into the circuit. For *in vitro* experiments beads as well as 'membrane bound enzymes' were used and UDPGA, in optimal concentration, was put directly into the reaction mixture. With membrane bound UDP-glucuronyltransferase, serum from patients in hepatic coma

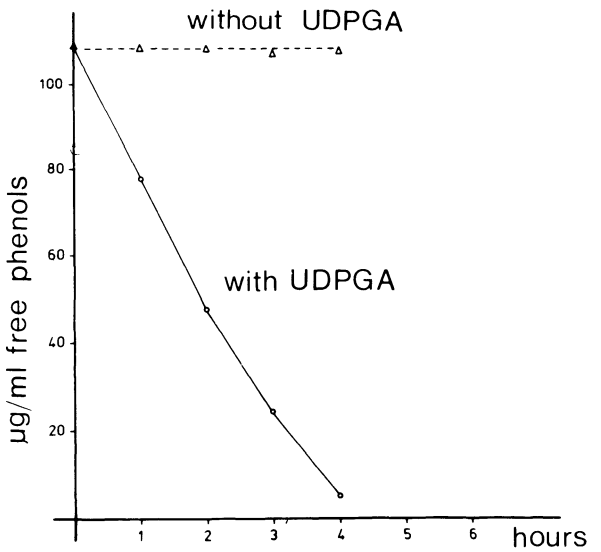



Figure 41.2 *In vitro* glucuronidation of free phenols in the serum of a patient in hepatic failure using 50 ml serum, 500 mg carrier enzyme, 50 µmol UDPGA at 37°C

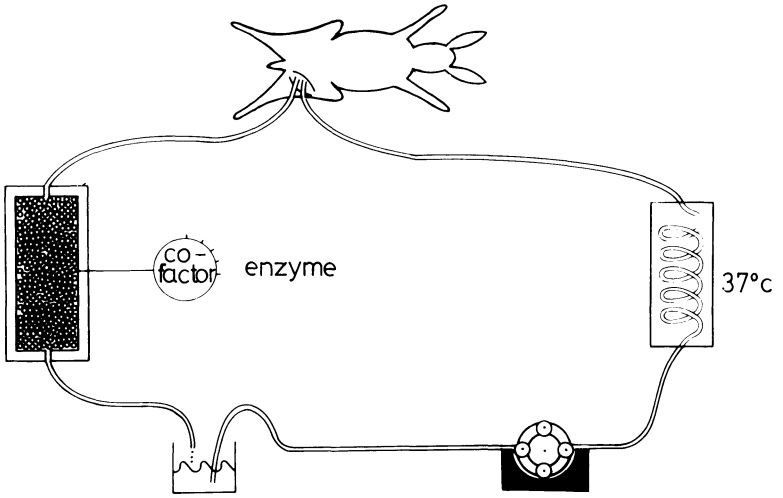



Figure 41.3 Extracorporeal circuit for enzymatic detoxification (glucuronidation of phenols)

could be freed from free phenols (figure 41.2) and *in vivo* the elimination of various phenols injected into rabbits could be significantly accelerated (figure 41.3 and 41.4).

For many toxins glucuronidation or sulphatation is only the second step of enzymatic detoxification. A large number of toxins has to be hydroxylated before conjugation can take place. On the other hand, hydroxylation is often sufficient in reaching enough hydrophility so that elimination through the kidney can take place. Two enzymes are necessary for many hydroxylation reactions: cytochrome-P-450 and NADPH-cytochrome-P-450 reductase. Cytochrome-P-450 was prepared and purified as described by Coon's group (Haugen *et al.*, 1975) and NADPH-cytochrome-P-450 reductase was prepared according to Vermilion *et al.* (1974). Both enzymes were bound to BrCN-Sepharose beads and activity could be demonstrated for both enzymes. Benzphetamine, hexobarbital, hexane and octanoic acid were readily hydroxylated by this bi-enzyme complex. Reaction mixtures were used according to van der Hoeven *et al.* (1974). To achieve a combination of hydroxylation and conjugation a multienzyme complex was formed by binding cytochrome-P-450, NADPH-cytochrome-P-450 reductase and UDP-glucuronyltransferase to BrCN-Sepharose beads. The necessary co-factors UDPGA, NADPH and 1,2-dilauroyl-3-phosphorylcholine were trapped inside the beads (figure 41.5). All three enzymes showed good activity on the carrier *in vitro*. *In vivo* experiments are being prepared.

## 41.3 Discussion

The results validate the concept of using enzymes bound to artificial carriers for detoxification. Enzymes are more selective and physiological than adsorbents.

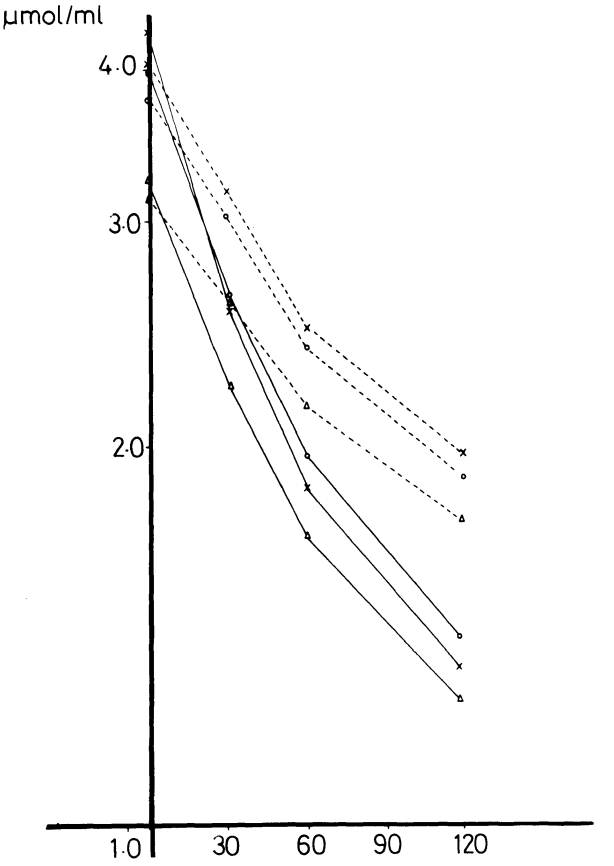



Figure 41.4 Extracorporeal glucuronidation in rabbits after paracetamol intoxication using 2 g carrier bound UDP-glucuronyltransferase, 200 µmol UDPGA, with flow rate 5 ml/min at 37°C; —— with UDPGA, – – – without UDPGA

Binding the enzyme covalently to a carrier overcomes the problem of immune reactions and allows multiple applications with the same enzyme preparation. More enzymes will have to be prepared not only for detoxification but also synthetic steps of high energetic cofactors should be aimed at. These results are only the tip of an iceberg which lies ahead of us to be discovered.

## 41.4 Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft Grant Br 302/7.

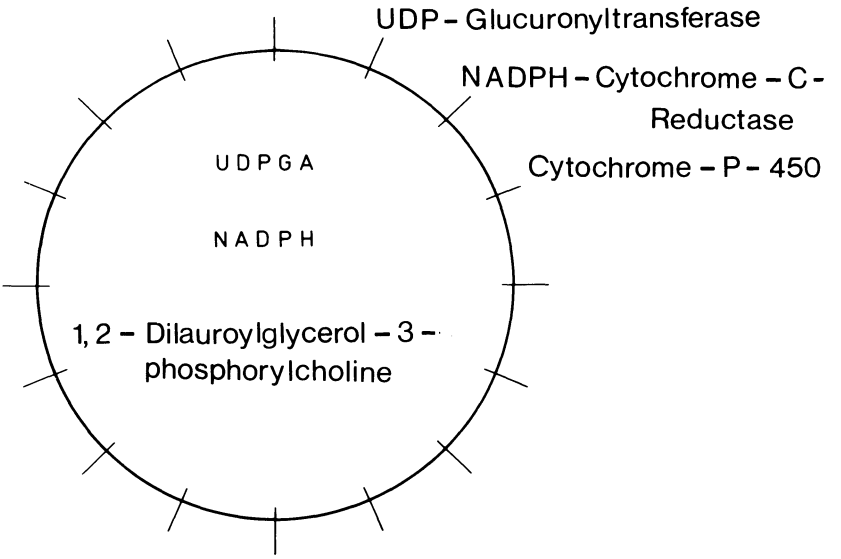



Figure 41.5 Multienzyme complex: binding of three enzymes to BrCN-Sepharose beads. Cofactors included inside beads

## 41.5 References

Bessman, S. P. (1956). Ammonia metabolism in animals, in W. D. McEtroy and B. Glass, B. (Eds.), *Symposium on Inorganic Nitrogen Metabolism*, Baltimore 408 - 437.

Bessman, S. P. and Bessman, A. N. (1955). The cerebral and peripheral uptake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma. *J. Clin. Invest.*, **34**, 622 - 628.

Bigler, F., Thölen, H. and Staub, H. (1962). Zur Pathogenese des Urämiesyndroms. IV. Mitteilung über den Einfluß von pH, Sauerstoff und Coenzym A auf die Acetoinsynthese aus Brenztraubensäure in Leber und Hirn der Ratte. *Schweiz. Med. Wschr.*, **92**, 746 - 748.

Brunner, G. (1975). Microsomal enzymes bound to artificial carriers. A new approach towards an extracorporeal detoxification in liver failure, in R. Williams and I. M. Murray-Lyon (Eds.) *Artificial Liver Support*, Pitman Medical, London, pp. 153 - 157.

Brunner, G. (1976). Habilitation thesis. University of Göttingen.

Cuatrecasas, P. (1970). Protein purification by affinity chromatography. *J. Biol. Chem.*, **245**, 3059 - 3065.

Foster, D., Ahmed, K. and Zieve, L. (1974). Action of methanthiol on $Na^+$, $K^+$-ATPase: implications for hepatic coma. *Ann. New York Acad. Sci.*, **242**, 573 - 576.

Haugen, D. A., van der Hoeven, I. and Coon, M. J. (1975). Purified liver microsomal cytochrome-P-450. *J. Biol. Chem.*, **25**, 3567 - 3570.

McKhann, G. M. and Tower, D. B. (1961). Ammonia toxicity and cerebral oxidative metabolism. *Amer. J. Physiol.*, **200**, 420 - 424.

Van der Hoeven, T. A. and Coon, M. J. (1974). Preparation and properties of partially purified cytochrome-P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome-P-450 reductase from rabbit liver microsomes. *J. Biol. Chem.*, **249**, 6302 - 6310.

Vermilion, J. L. and Coon, M. J. (1974). Highly purified detergent-solubilised NADPH-cytochrome-P-450-reductase from phenobarbital-induced rat liver microsomes. *J. Biol. Chem.*, **249**, 1315 - 1322.

Weber, G., Convery, H. J., Lea, M. A. and Stamm. N. B. (1966). Feedback inhibition of key glycolytic enzymes in liver: action of free fatty acids. *Science*, **154**, 1357 - 1360.

Zieve, L., Doizaki, W. M., and Zieve, F. J. (1974). Synergism between mercaptans and ammonia or fatty acids in the production of coma: a possible role for mercaptans in the pathogenesis of hepatic coma. *J. Lab. Clin. Med.*, **83**, 16 - 28.

# 42

# HEPATIC SUPPORT SYSTEM UTILISING HIGH-POROSITY PROTEIN-PERMEABLE MEMBRANES IN CONJUNCTION WITH DIALYSATE REGENERATION

R. MAINI, H. BAILLIE and M. STARK

**Summary**

A system for application as an artificial liver support is proposed. A haemodialyser constructed with high porosity membranes allows the use of large quantities of mixed sorbents for toxin removal. The advantages afforded by this approach are discussed.

## 42.1 Introduction

The identity of specific toxins causing hepatic coma remains unknown. Ammonia, mercaptans, fatty acids and phenols have been implicated and hepatic coma has been experimentally produced with these chemicals (Zieve, 1975; Brunner, 1975). Of these, mercaptans, fatty acids and phenols are known to be protein bound. In addition, protein bound anions such as bilirubin and bile acids, normally excreted by the liver, accumulate in liver failure. These substances may serve to exacerbate the effects of other protein bound toxins by displacing them from their protein binding sites and allowing their migration across the blood-brain barrier.

Attempts have been made to support the liver by removing some of these toxins by haemoperfusion over sorbent materials (activated carbon, ion-exchange resins, uncharged polymeric adsorbents). It is felt that an artificial liver support system should have the capability of removing a wide spectrum of substances, both free and protein bound, as well as the ability to be applied for protracted periods. Two criteria have to be considered in this context: biocompatibility and capacity.

*Biocompatibility*

Haemoperfusion over sorbents is known to produce blood platelet and white cell loss. The thrombocytopenia already present in liver failure makes biocompatibility requirements much more stringent than for other haemoperfusion applications, e.g. acute poisoning.

In an attempt to reduce platelet loss Weston *et al.* (1975) have perfused

Amberlite XAD-2 resin with plasma separated from blood by a celltrifuge, but without success. This is because the celltrifuge produces a platelet drop of its own accord and the separated plasma contained platelets.

*Capacity*

Gazzard *et al.* (1974, 1975) and Chang (1975) have used a 300 g charge of active carbon for their clinical haemoperfusions. Willson *et al.* (1974) have used 500 g quantities of anion-exchange resin in canine experiments while Weston *et al.* (1975) have used the same weight of uncharged polymeric adsorbent (XAD-2) in theirs. Juggi (1971, 1973) has used a 200 g charge of cation-exchange resin for canine experiments and clinically.

It is evident that a sorbent based system for hepatic support comprising all the sorbents listed above, each sorbent with its own special purpose, would necessarily require a very large sorbent charge in order to be effective over any length of time. This would necessitate impossibly large priming volumes.

With these criteria in mind the following design for a hepatic support system has been proposed by Maini (1975).

## 42.2 Proposed Hepatic Support System

The core of this design is a haemodialyser constructed with very high porosity membranes. These membranes would be porous enough to allow serum proteins through while being impervious to cellular constituents of blood. Dialysate composed of banked human plasma would be recirculated through a column containing a large quantity of mixed sorbents. Toxins of small and middle molecular weight would diffuse rapidly across the membrane to be removed by the sorbents, the transport of high molecular weight protein bound toxins being

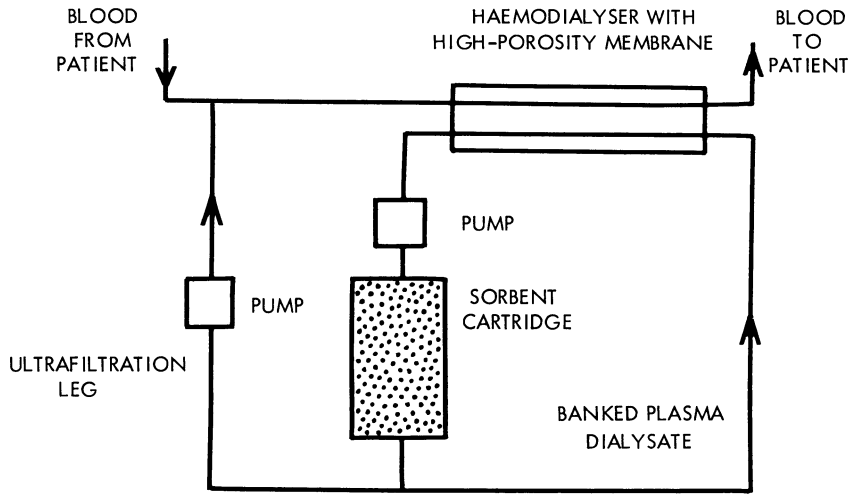



Figure 42.1 Proposed hepatic support system

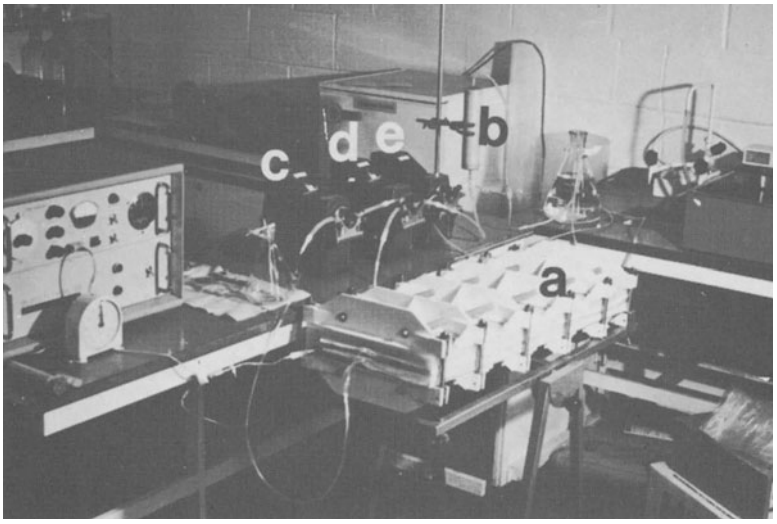



Figure 42.2 Prototype system. a: haemodialyser with high porosity membrane, b: sorbent cartridge, c: blood pump, d: dialysate pump, e: ultrafiltrate pump

increased by the use of convective flux provided by the inclusion of an ultrafiltration leg in the circuit. Figure 42.1 is a schematic diagram of the system and figure 42.2 is a photograph of a recently constructed prototype.

The following advantages are afforded by this system.

(1) Large quantities of sorbent may be used as the sorbent column is decoupled from the blood circuit. Circulatory volume changes should be comparable to conventional haemodialysis.
(2) Small particle sizes may be used in the column as high pressure drops and fine retaining meshes should pose no major problems away from the blood stream.
(3) The sorbents do not require coating with a 'biocompatible' polymeric membrane as they are not in contact with white blood cells or platelets. Since platelets would not be able to cross the dialyser membrane, removal should be lower than that produced by haemoperfusion over a large sorbent bed.
(4) The spent sorbent column could be replaced by a fresh one without disturbing blood line connections.

*Suitable membranes*

For this application membranes must have a pore diameter of less than about 2 $\mu$m (diameter of blood platelets) while being porous enough to allow serum proteins through (pore diameter $> 0.02$ $\mu$m).

Examples of membranes fulfilling these conditions are commercially available in the Nuclepore, Gore-Tex and Celgard ranges. The pore structure of Nuclepore membranes (figure 42.3) is produced by exposure of polycarbonate film to

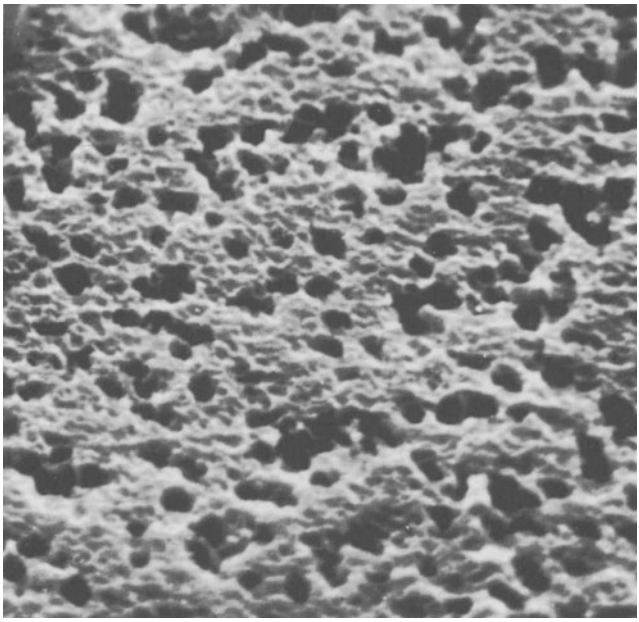


Figure 42.3 Photomicrograph of Nuclepore membrane

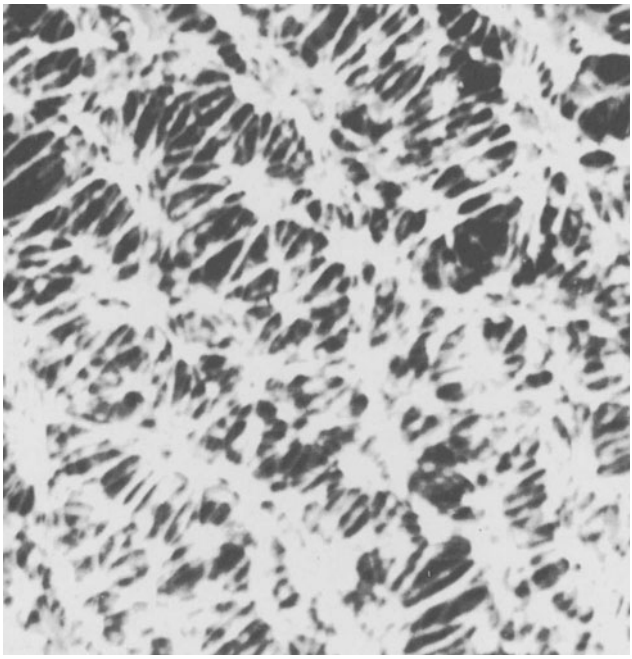


Figure 42.4 Photomicrograph of Gore-Tex membrane

Figure 42.5 Photomicrograph of Celgard membrane

charged particles in a nuclear reactor, the tracks left by these particles being etched into uniform cylindrical pores. Gore-Tex membranes are porous structures of PTFE (figure 42.4) while Celgard membranes are microporous polypropylene structures (figure 42.5). The Gore-Tex and Celgard membranes are hydrophobic and would therefore require a wetting procedure prior to use, whereas Nuclepore membrane is hydrophilic.

The Kiil dialyser in the prototype system (figure 42.2) was loaded with 50 $\mu$m thick Gore-Tex membrane of 1 $\mu$m pore size which was wetted with ethanol prior to use. Initial results indicate that a sustained ultrafiltrate flow rate of 40 ml/min at a 200 ml/min blood flow rate can be achieved.

*Sorbents*

Sorbents to be used in this system would comprise activated carbon, anion-exchange resin, cation-exchange resin and uncharged polymeric adsorbents.

The work of Chang and Migchelsen (1973), Chang (1975) and Gazzard *et al.* (1974, 1975) has raised the hope that haemoperfusion over *activated carbon* may be effective in removing cerebral toxins from blood and reversing hepatic coma. Hypotheses have been advanced that the unknown substances removed are of middle molecular weight or are protein bound or both. In this context it is interesting to note the work of Gottlieb and Ludwig (1937) who found that the anionic dye Azorubin S was readily removed by active carbon from the serum of normal subjects but was not removed from the serum of patients with liver failure. The conclusion can be drawn that the adsorption sites for the dye were blocked by some preferentially adsorbed substance present in the serum of liver failure patients. Jetzer *et al.* (1974) have reported the removal of mercaptans by active carbon.

The ability to use uncoated, finely divided activated carbon in the system may be particularly advantageous in reference to endotoxin removal. Endotoxins, lipopolysaccharides from gram-negative organisms in the gut, have been implicated in the renal failure often associated with hepatic failure (Wilkinson *et al.*, 1974). Hepatic failure patients with endotoxaemia and renal failure complications have an extremely poor prognosis and some method of removing endotoxin from blood may improve their chances of recovery. Wilkinson (1975) has been unable to demonstrate endotoxin removal by coated activated carbon granules while Lees and Levvy (1940) have shown endotoxin to be efficiently removed by finely divided, uncoated active carbon and have effected the depyrogenation of infusion solutions by this method.

Willson *et al.* (1974) have demonstrated removal of bilirubin from plasma using *anion-exchange resin* haemoperfusion. The ability to use small particle size anion-exchangeresin would lead to better kinetics and more efficient removal of protein bound anions. The effect of particle size on the removal rate of bilirubin from plasma is evident from the data presented in figure 42.6.

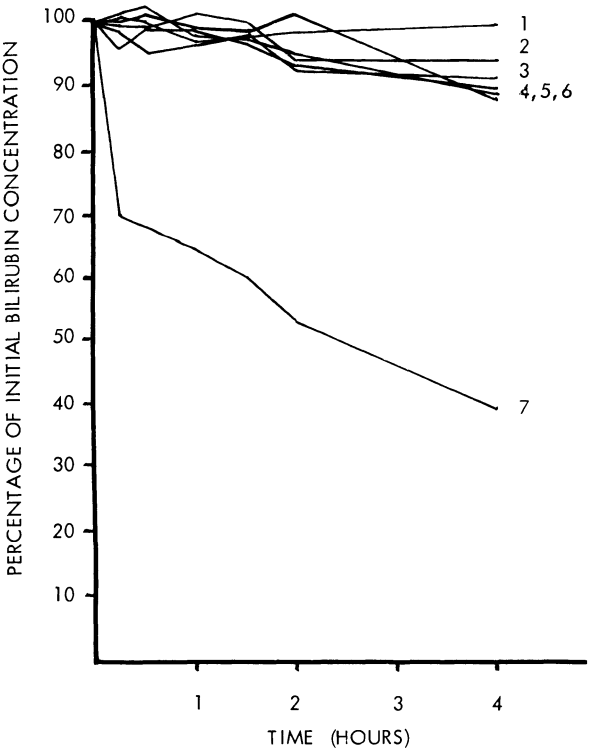



Figure 42.6 Sorption of bilirubin from plasma. Figures in brackets indicate particle size. 1: Amberlite XAD-2 (300 μm), 2: Amberlite XAD-4 (300 μm), 3: Dowex 21K-50 (300 μm), 4: Dowex 1x4-100 (150 μm), 5: Dowex 1x4-50 (300 μm), 6: Amberlite XAD-7 (300 μm), 7: Dowex 1x2-400 (40 μm)

Juggi (1973) has used *cation-exchange resin* haemoperfusion to remove ammonia from the blood of patients in hepatic oma.

Both types of ion-exchange resin should be in the mixed ion form: the cation-exchange resin in the sodium/potassium/calcium/magnesium form and the anion-exchange resin in the chloride/bicarbonate form. This can be achieved through a resin pretreatment procedure such as described by Maini *et al.* (1975).

The removal of bile acids from plasma using the *polymeric adsorbent* Amberlite XAD-2 has been demonstrated by Willson (1975). Amberlite XAD-2 and XAD-4 are known to have good adsorption of fatty acids and phenols.

## 42.3 Discussion

The proposed design should allow the efficient use of sorbents in a hepatic support device with biocompatibility which should be of the same order as that afforded by conventional haemodialysis.

The ability of the system to remove the protein bound anion, bilirubin, could extend its application to the treatment of bilirubin encephalopathy (kernicterus). This grave condition is a complication in the newborn and 70% of affected infants die within 7 days of the onset (Sherlock, 1971). Scharschmidt *et al.* (1975) have removed bilirubin from blood using haemoperfusion over albumin-conjugated agarose beads but report high losses of formed elements.

In addition to toxin removal it is desirable to be able to correct the electrolyte disturbances and fluid overload which occur in hepatic failure (Keynes, 1968). Conventional haemodialysis could accomplish this task and it may be useful to include a conventional dialyser in the circuit. By placing this haemodialyser after the porous membrane unit any electrolyte abnormalities produced by the use of banked human plasma could be rapidly corrected. Ammonia would also be removed by the conventional dialyser and would obviate the need for cation exchange resin in the sorbent column.

## 42.4 Acknowledgement

We would like to thank Dr A. C. Munro of Law Hospital for kindly supplying the human plasma.

## 42.5 References

Brunner, G. (1974). In R. Williams I.M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 26.

Chang, T. M. S. (1975). In R. Williams I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 229.

Chang, T. M. S. and Migchelsen, M. (1973). *Trans Amer. Soc. Artif. Int. Organs*, **19**, 213.

Gazzard, B. G., Portmann, B., Weston, M. J., Langley, P. G., Murray-Lyon, I. M., Dunlop, E. H., Flax, H., Mellon, P. J., Record, C. O., Ward, M. B. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet*, **1**, 1301.

Gazzard. B. G., Weston, M. J., Murray-Lyon, I. M., Record, C. O. and Williams, R. (1975). In R. Williams, I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 234.

Gottlieb, H. and Ludwig, H. (1937). *Z. Klin Med.*, 358.

Jetzer, T. C., Merino, G. E., Shous, A. R., Moberg, A. W., Simmons, R. L. and Nariah, J. S. (1974). *Abstracts Trans. Amer. Soc. Artif. Int. Organs*, **3**, 34.

Juggi, J. S. (1971). *In vivo* studies with a cation-exchange resin mixture in the removal of excessive ammonium from the extracorporeal circulatory system. *Aust. N.Z.J. Surg.*, **41**, 194.

Juggi, J. S. (1973). Extracorporeal cation-exchange circuits in the treatment of hyperammonaemia of hepatic failure. *Med. J. Aust.*, **1**, 926.

Keynes, W. M. (1968). Haemodialysis in the treatment of liver failure. *Lancet*, **2**, 1236.

Lees, G. and Levvy, G. A. (1940). *Brit. Med. J.*, **1**, 430.

Maini, R. (1975). Ph.D. Thesis, University of Strathclyde, Glasgow.

Maini, R., Courtney, J. M., Gilchrist, T., Winchester, J. F. and MacKay, J. M. (1976). Paraquat removal by ion-exchange haemoperfusion. *Proc. Eur. Soc. Artif. Organs*, **2**, 222.

Scharschmidt, B. F., Martin, J. F., Shapiro, L. J., Plotz P. H., Berk, P. D., Gordon, J. K., Waggoner, J. G. and Vergalla, J. (1975). *Clin. Res.*, **33(3)**, 256a.

Sherlock, S. (1971). *Diseases of the Liver and Biliary System*, Blackwell, Oxford.

Weston, M. J., Mellon, P. J., Langley, P. G., Dunlop, E. H. and Williams, R. (1974). In R. Williams, I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 127.

Wilkinson, S. P. (1975). Discussion, in R. Williams, I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 109.

Wilkinson, S. P., Arroyo, V., Gazzard, B. G., Moodie, H. and Williams, R. (1974). Relation of renal impairment and haemorrhagic diathesis to endotoxaemia in fulminant hepatic failure. *Lancet*, **1**, 521.

Wilson, R. A. (1974). In R. Williams, I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 109.

Willson, R. A., Hofmann, A. F. and Koster, G. G. R. (1974). *Gastroenterology*, **66**, 95.

Zieve, L. (1974). In R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, p. 11.

# 43

# APPROACHES TO THE DEVELOPMENT OF ARTIFICIAL LIVER SUPPORT

R. WILLIAMS

**Summary**

Clinical experience with support systems in hepatic failure, gathered during the past 3 years is presented. Comments are offered on the various techniques employed. The distillation of this experience is that only a completely artificial system of liver support and not one based on animal or cadaveric tissue, can provide the patient assistance required.

## 43.1 Perfusion Over Activated Charcoal

On 23 September 1973 we first connected a patient to an artificial liver support system. He was a man of 26 years in deep coma from fulminant hepatic failure. For 4 h on that day, and for similar periods on two subsequent occasions, his blood was perfused at a rate of 300 ml/min through a column containing activated charcoal. Following the third perfusion his conscious level lightened and (as can be seen from figure 43.1) he subsequently made a complete recovery.

The use of charcoal haemoperfusion in this patient had, of course, been preceded by a long series of experimental studies, and one to which particular attention should be drawn was a controlled trial in the dog with liver failure, in which the charcoal perfused group showed a statistically significant prolongation of survival (Weston *et al.* 1974).

Charcoal was used in the haemoperfusion system because it is a very effective adsorbent for a wide range of water-soluble substances, including molecules in the middle molecular weight range (300 - 2000). Early attempts to use charcoal haemoperfusion were hampered by severe loss of platelets and by the liberation of small particles of charcoal into the circulation. To improve biocompatibility, Smith and Nephew Research Ltd, with whom we have worked closely during the past few years, coated the charcoal with an acrylic hydrogel, identical to the material used for soft contact lenses. The exact thickness of the coating applied is a compromise between biocompatibility and the reduction in adsorpitive capacity caused by the coating (Gazzard *et al.*, 1974a).

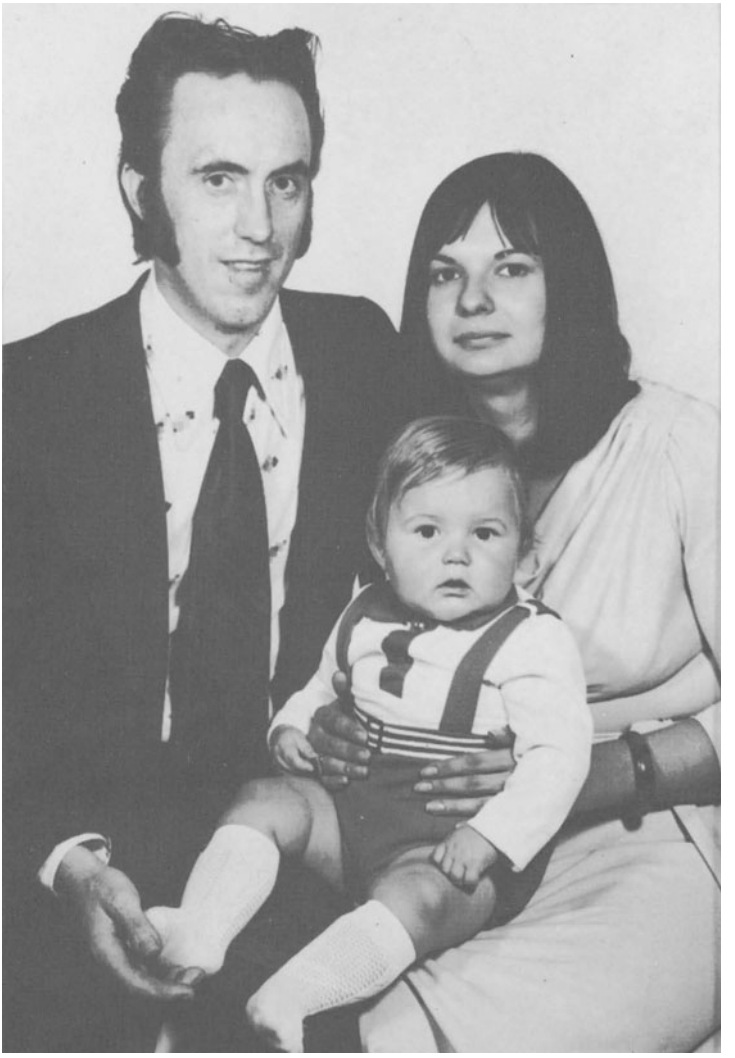


Figure 43.1 Survivor with his wife and child

## 43.2 Biochemical Aspect

The recovery of consciousness from deep coma, being the most striking feature in our initial series of 31 patients treated by charcoal haemoperfusion (Gazzard *et al.*, 1974b), led to a consideration of the *biochemical abnormalities* underlying hepatic encephalopathy. These are exceedingly complex with many interactions. Zieve *et al.* (1974), for instance, have shown that the coma-producing potential of toxic substances, such as ammonia, fatty acids and mercaptans, may be multiplied

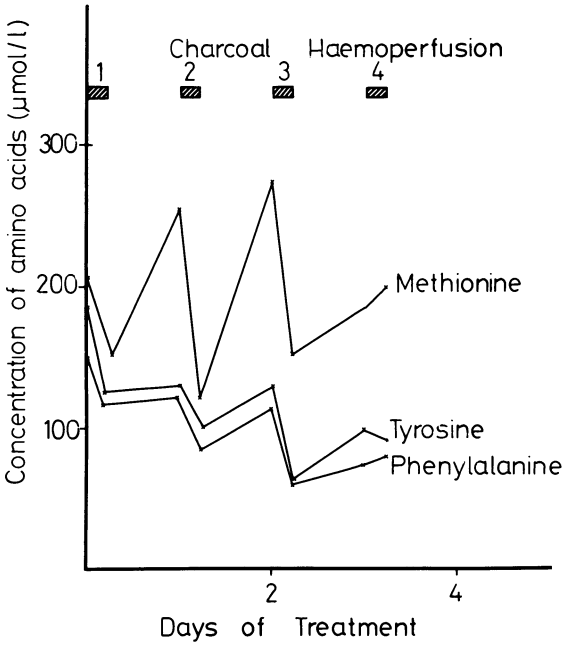



Figure 43.2 The fall in plasma levels of three amino acids during four periods of haemoperfusion in a patient with hepatic necrosis from *Amanita phalloides* poisoning (Gazzard *et al.*, 1974b)

severalfold when they are present together. With respect to the possible correction of such abnormalities by charcoal haemoperfusion, there is evidence from various experimental studies that mercaptans and ammonia may be adsorbed. In addition, the brain may be damaged secondarily by anoxia, so common in hepatic failure, or by hypoglycaemia. Progression of these various lesions leads eventually to brain death with the development of cerebral oedema in some 36% of the cases in our series.

Currently there is also a great deal of interest in the relationship between *changes in the plasma amino acid pattern*, which is known to be grossly deranged in hepatic failure, and the synthesis of false neurotransmitters in the brain. These various precursor amino acids are very efficiently removed by charcoal haemoperfusion. Serial measurements in a 32-year-old woman with *Amanita phalloides* poisoning, who was perfused on four successive days before recovering consciousness, are shown in figure 43.2. On each occasion the plasma levels of phenylalanine and tyrosine fell as did methionine, another amino acid which is increased in the blood of these patients and which may be important in the pathogenesis of the encephalopathy.

The importance of *cerebral oedema* in the progressive cerebral deterioration of some of these patients cannot be disputed. Fortunately we have now an experimental model in which to study the underlying mechanism and the effectiveness

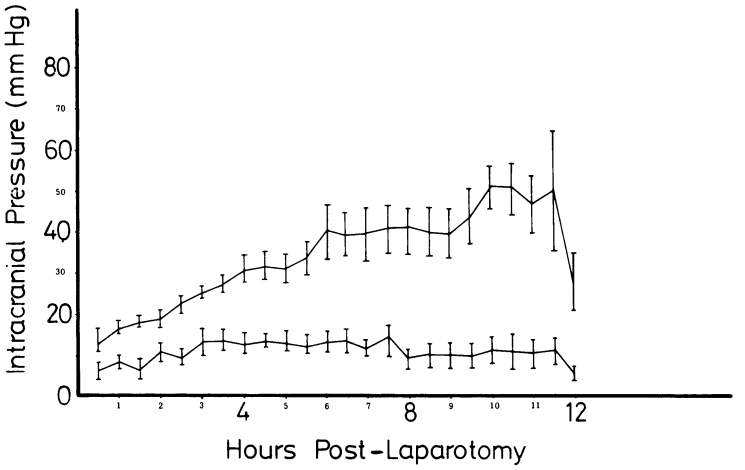



Figure 43.3 Intracranial pressure measurements in test and sham operated pigs. Mean values ± SEM are shown (Hanid *et al.*, 1976)

of possible treatment measures, such as dexamethasone. In the pig with liver failure induced by surgical devascularisation, we have recently shown that there is no sudden change in intracranial pressure but a steady and progressive rise (figure 43.3). Along with the rise in intracranial pressure there was progressive deterioration in EEG appearances with the tracing becoming flat just before death occurred. Although the development of cerebral oedema in man is related, at least in part, to the severity of liver failure, we have seen a number of patients die from this complication at a time when their liver function was improving (Gazzard *et al.*, 1975).

### 43.3 Inhibitory Factors

An undue susceptibility to *bacterial infection* characterises the patient with fulminant hepatic failure and some of our haemoperfused patients have died tragically of pulmonary infections and septicaemias at a time when their liver function was recovering. At this point another series of studies may be considered (Bailey *et al.*, 1976) in which we have determined the activity of the hexose monophosphate shunt pathway in leucocytes as an index of bactericidal capability. $^{14}CO_2$ production by the white cells is measured in the presence of normal serum and kills *Candida albicans.* The mean value obtained for the patients' leucocytes was identical to that of controls, so there is no intrinsic abnormality in the polymorphs. However, when in the assay normal serum was replaced by fulminant

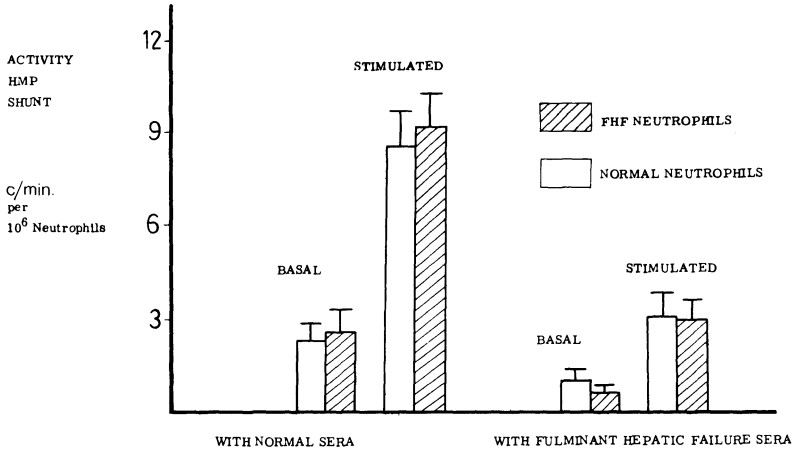



Figure 43.4 HMP shunt activity of normal neutrophils and FHF patient neutrophils in the presence of normal serum and FHF serum (mean ± 1 SEM) (Bailey *et al.*, 1976)

hepatic failure serum, there was a marked drop in activity (figure 43.4). Characterisation of this inhibitory substance in the serum is currently underway but one other interesting finding is that preincubation of fulminant hepatic failure serum with the same coated charcoal as that used in our perfusion system will increase leucocyte metabolic activity back to normal.

Whilst on the subject of inhibitory factors, I will also touch briefly on another study in which plasma from patients with fulminant hepatic failure has been shown to be cytotoxic to isolated hepatocytes in a tissue culture system (Hughes *et al.*, 1975). This *cytotoxic factor* differs from the leucocyte inhibitor in certain respects but resembles it in that it is also reduced by charcoal haemoperfusion. The presence of such a factor in the serum may explain why regeneration often appears to be delayed in these patients and is clearly a most important area for further research.

Returning to the overall results obtained with charcoal haemoperfusion, at the time of our first publication (Gazzard *et al.*, 1974b), some 31 patients with fulminant hepatic failure had been treated, the 39% survival rate comparing most favourably with our previous figure of 13% in 93 patients with grade IV coma managed by standard supportive measures. But from then on the results got steadily worse with no survivals in the last 18 patients perfused, and with a much greater frequency of severe hypotensive reactions (50% of cases), the cause of which I shall return to shortly. At that stage perfusions were stopped and since then the biocompatibility of the charcoal preparation has been actively reconsidered.

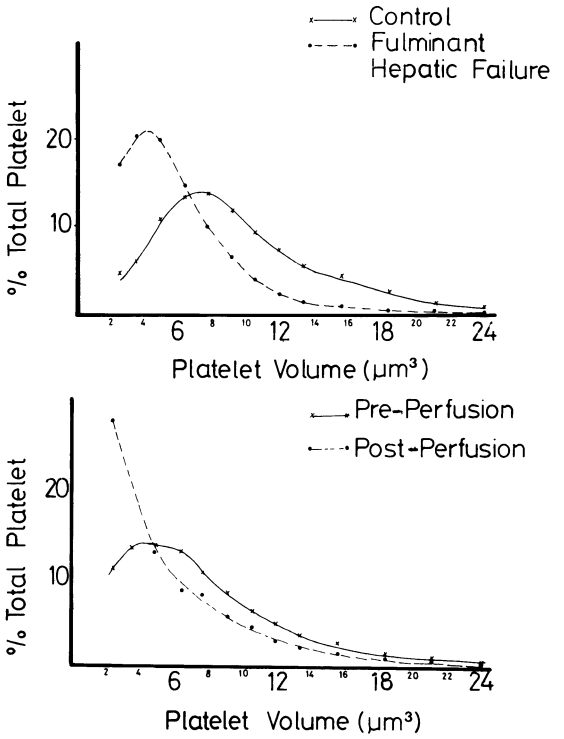



Figure 43.5 Platelet population curves from a normal control and patient with fulminant hepatic failure (top) and showing effects of charcoal haemoperfusion (bottom) (Weston *et al.*, 1976)

### 43.4 Platelet Function

Although the hydrogel coating of the charcoal reduces platelet loss, percentage reductions in platelet count over the 4 h period of perfusion were greater than 30% of the pre-infusion level in over a quarter of the perfusions. Another effect of charcoal haemoperfusion is shown in figure 43.5. Platelet sizes in the peripheral blood vary as can be seen in the distribution curve obtained with blood from a normal subject. In patients with liver failure we found that the curve shifted slightly to the left even before perfusion indicating a greater percentage of small platelets in the total count. Following perfusion, the curve shifted even further to the left, presumably because of selective adherence of the remaining larger and stickier platelets to the charcoal. The loss of these larger platelets which are known to be haemostatically more active than the smaller ones accounts for another observation, namely, a disproportionate prolongation of bleeding time in relation to the measured reduction of platelet count.

During haemoperfusion we have also shown that platelet aggregates may form within the column and are then released into the circulation. To detect these we

have used the Swank screen filtration pressure technique, in which an aliquot of the outflow blood is pumped through a metal screen containing small square pores and the pressure generated is recorded. When aggregates are present in the blood, there is a sharp rise in filtration pressure. Those perfusions in which aggregates have been detected were associated with a greater than average reduction in platelet count but, more importantly, by a profound hypotensive reaction. These were presumably due to liberation of vasoactive amines from the platelets as a result of aggregate formation. The formation of these aggregates may be related to an increase in shear stress building up at the inlet manifold of the column but it is not yet clear why this only occurred in the later series of perfusion. During the period of these investigations, the preparation and coating of the charcoal has also been under critical evaluation, and hopefully we will now have less problems with biocompatibility in these very sick patients with liver failure.

## 43.5 Perfusion Over Uncoated Charcoal

We have also been evaluating the completely different type of column manufactured by Becton, Dickinson in which the carbon particles are immobilised on a support polyester film. This is then wound into a spiral coil and packed into the polycarbonate housing. Blood flows between the coils of the membrane plates. The advantages of this immobilised charcoal is that there is no coating of the charcoal, and therefore none of the constraints on its adsorptive capacity. It presupposes, however, that damage to the platelets during haemoperfusion occurs as a result of agitation with loose particles, which may not necessarily be correct.

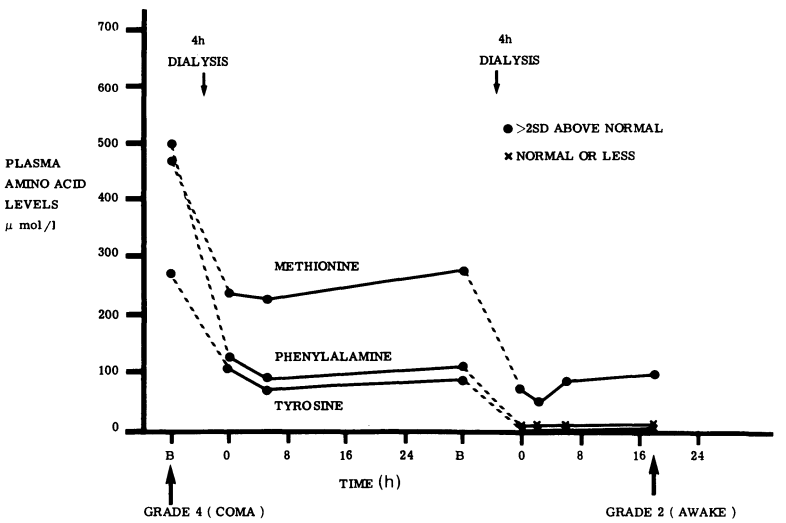



Figure 43.6 Serial measurements of plasma amino acid levels during dialysis with the Rhone-Poulenc machine on two occasions in a patient with fulminant hepatic failure (Silk *et al.*, unpublished observation)

### 43.6 Treatment by Dialysis

Another recent and very exciting development here, for which some preliminary data can be shown, is the Rhône-Poulenc dialysis machine. This has a novel membrane made of polyacrylonitrile which, unlike the ordinary Cuprophane dialysis membrane, allows passage of middle molecular weight substances. It is anticipated that its effect in fulminant hepatic failure would be similar to that of charcoal haemoperfusion. Opolon *et al.* (1974) in reporting their experience with this technique describe consistent improvements of conscious levels, although, rather surprisingly, none of the patients treated recovered to leave hospital. Our own experience with it has shown that the removal of amino acids is extremely fast and complete (figure 43.6) and two of the patients treated have made a complete recovery. Biocompatibility studies so far would suggest that it is well tolerated.

So far we have been considering only the toxicity of water soluble substances which can be absorbed to charcoal and we have not touched another group of potentially toxic substances, namely bilirubin and bile acids which are bound to the plasma proteins.

### 43.7 Removal of Protein Bound Substances

Although such protein-bound substances can be efficiently adsorbed *in vitro* to certain resins of the Amberlite series, their biocompatibility is poor and it is not possible to use them for haemoperfusion. We therefore decided to try a different approach in which the celltrifuge was used to continuously separate plasma from the formed elements of the blood, the plasma being passed through the columns of XAD-4. The components of the blood are then reconstituted before return to the patient. Unfortunately plasma becomes completely free of platelets only when a very high centrifugal force is used. Even with the lower centrifugal forces that we used and which only partially separated platelets from the plasma, some damage to platelets occurs (Weston *et al.*, 1975).

The development of biocompatible and efficient adsorbent/perfusion systems for protein-bound substances is also being pursued by a group at the National Institute of Health who have developed a very interesting technique in which human serum albumin is bound to agarose beads (Plotz *et al.*, 1974). The theory behind this is that toxic substances in plasma will dissociate from their binding sites there and bind instead to the albumin covering inert agarose. Our own results with this preparation were rather disappointing, but similar techniques of affinity chromatography can also be applied to other support materials. Currently we are looking at the use of albumin-coated XAD-7. This latter resin, unlike Sepharose, has a high affinity for protein-bound metabolites so that both supporting material and coating should contribute to the binding capacity.

### 43.8 Design Aspects of Perfusion Columns

Finally, I wish to consider various aspects relating to the design of the column as distinct from the nature of its contained adsorbent. While the equilibrium capacity

of any of these adsorbent substances for the toxic metabolites to be removed may be high, the time required to achieve equilibrium may be longer than can be tolerated clinically. Adsorption of metabolites from blood takes place in several stages. Flow of blood brings the metabolite into the vicinity of the adsorbent. It must then diffuse through the relatively stagnant layer of fluid adjacent to the adsorbent particle, the so-called boundary layer. Then the metabolite has to diffuse through whatever coat has been used and through pores in the adsorbent itself to the surface where binding takes place. By measuring the removal of substances at various flow rates, an index of overall effectiveness, the mass transfer coefficient, can be calculated. This can be resolved into its component parts mathematically by plotting the reciprocal of mass transfer coefficient against the reciprocal of velocity. When extrapolated to zero or infinite velocity the intercept on the $y$-axis represents the residual resistance to adsorption once the boundary layer effects are removed. This is what happens with bilirubin removal by XAD-7 where blood flow and other resistances (e.g. pore diffusion) are important, whereas with methionine and paracetamol removal by charcoal there is no intercept, the process being almost entirely controlled by boundary layer diffusion. Finally, there is the extreme case where boundary layer diffusion is unimportant, for example, in removal of tryptophan from plasma by charcoal. Knowledge at such perfusion/adsorption characteristics is of course vital in designing the best systems for particular metabolites. For instance, the thickness of the boundary layer may be reduced by increasing the local velocity around the particles either by mechanical oscillation or by the use of long thin columns.

## 43.9 Conclusion

Undoubtedly we learnt much from our first attempts at artificial liver support and the view to which I still adhere, despite all the tribulations that have ensued, is that only a completely artificial system of liver support, and not one based on perfusion of animals or cadaver human liver, can provide the type of reproducible and repeatable support that these patients need.

## 43.10 Acknowledgements

In presenting this review I have drawn heavily on the current work of Drs Bailey, Hanid (1976), Mackenzie, Silk, Trewby, Weston, and Woolf. These studies form part of a long-term programme of research into liver failure supported by the Medical Research Council, the Wates Foundation and Smith's Charity. The National Research Development Corporation and Smith and Nephew Ltd are also involved in the development of a liver support system, and to all these I am most grateful.

## 43.11 References

Bailey, R. J., Woolf, I. L., Cullens, H., and Williams, R. (1976). Metabolic inhibition of polymorphonuclear leucocytes in fulminant hepatic failure. *Lancet*, (in press).

Gazzard, B. G., Langley, P. G., Weston, M. J., Dunlop, E. H. and Williams, R. (1974a). Polymer coating of activated charcoal and its effects on biocompatibility and paracetamol binding. *Clin. Sci. Mol. Med.*, **47**, 97.

Gazzard, B. G., Weston, M. J., Murray-Lyon, I. M., Flax, H., Record, C. O., Portmann, B., Langley, P. G., Dunlop, E. H., Mellon, P. J., Ward, M. B. and Williams, R. (1974b). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet*, **1**, 1301.

Gazzard, B. G. Portmann, B., Murray-Lyon, I. M. and Williams, R. (1975). Causes of death in fulminant hepatic failure and relationship to quantitative histological assessment of parenchymal damage. *Quart. J. Med.*, **176**, 615.

Hanid, M. A., Mackenzie, R. L., Jenner, R. E., Mellon, P. J., Davis, M. and Williams, R. (1976). Raised intracranial pressure and development of cerebral oedema in an animal model of fulminant hepatic failure. *Gut* (in press).

Hughes, R., Cochrane, A. M. G., Thomson, A. D., Murray-Lyon, I. M. and Williams, R. (1975). Plasma inhibitory factors, in R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London.

Opolon, P. and Caroli, J. (1974). Etudes des facteurs du traitement des atrophies hepatiques aigues d'origine virale. *Medecine et Hygiene*, **32**, 159.

Plotz, P. H., Berk, P. D., Scharschmidt, B. F., Gordon, J. K. and Vergalla, J. (1974). Removing bilirubin and other albumin-bound substances from plasma and blood with albumin-conjugated agarose beads. *J. Clin. Invest.*, **53**, 778.

Weston, M. J., Gazzard, B. G., Buxton, B. H., Winch, J., Flax, H., Machado, A. and Williams, R. (1974). Effects of haemoperfusion through charcoal or XAD-2 resin on an animal model of fulminant hepatic failure. *Gut*, **15**, 482.

Weston, M. J., Mellon, P. J., Langley, P. G., Hughes, R. D., Dunlop, E. H. Gazzard, B. G. and Williams, R. (1975). Resin column perfusion with whole blood or plasma separated by the continuous flow celltrifuge. *Clin. Sci. Mol. Med.*, **48**, 187.

Zieve, L., Doizaki, W. M. and Zieve, F. J. (1974). Synergism between mercaptans and ammonia, or fatty acids in the production of coma: a possible role for mercaptans in the pathogenesis of hepatic coma. *J. Lab. Clin. Med.*, **83**, 16.

# 44

# REMOVAL OF BILIRUBIN FROM THE BLOOD OF JAUNDICED INFANTS

S. SIDEMAN, L. MOR, I. ROUSSEAU, J. M. BRANDES and D. BEN-ARIE

**Summary**

Removal of bilirubin by haemoperfusion is suggested to replace exchange transfusion of blood of jaundiced infants. Anion exchangers, coated with biocompatible polymers, were tested *in vitro* with human blood for bilirubin binding capacity and blood compatibility. Dowex 1 × 2 coated with poly-HEMA and an acrylic polymer crosslinked with albumin removed 50 – 75% of the initial bilirubin in 2 – 3 h. Platelet count fell by some 25%. Additional work is still required to develop a small, efficient and safe haemoperfusion system.

## 44.1 Introduction

The main effort of obstetrics nowadays has shifted to the so-called high risk pregnancy. Premature babies represent 10% of the newborns, exhibiting a high perinatal mortality and a much higher morbidity rate. One of the crucial problems is neurological and 'minimal' brain damage. Jaundice, which appears in about half of the prematures, is one of the major factors which contributes to these sequelae. Out of 3855 newborns in the Rambam Hospital in Haifa in 1973, 1.7% were affected by jaundice, whereas out of 234 admissions to the Premature Unit (weighing less than 2 kg) 45% were affected.

In haemolytic diseases the serum bilirubin (BIL) concentration may reach toxic levels, penetrating cerebral tissues and impairing cellular function by blocking the oxidative phosphorylation, inhibiting respiration and resulting in the development of brain damage, kernicterus and death. Currently, symptoms of mild hyperbilirubinaemia are treated by phototherapy or by the administration of human serum albumin (HSA) solution. In cases of severe hyperbilirubinaemia an incremental exchange transfusion is necessary. In some clinics the administration of drugs is used to induce hepatic activity of bilirubin UDP glucoronyl transferase (Radzialowski, 1973).

The objective of this investigation is to develop a small cartridge capable of removing BIL from bilirubinaemic whole blood of newborn babies in a closed haemoperfusion cycle, thus replacing exchange transfusion and reducing the dependency on the application of phototherapy, of which the long-term effects are still undetermined.

Carbon haemoperfusion was suggested by Yatzidis (1965) as a means of removing creatinine, uric acid, phenolic compounds, guanidines and barbiturates from blood adsorption studies of sera BIL on active materials, i.e. charcoal (Barakat and MacPhee, 1971; Dunlop *et al.*, 1975), uncharged resins (Rosenbaum *et al.*, 1971) and ion exchangers (Sawchuk and Bairn, 1968; Evenson and de Vos, 1972; Willson *et al.*, 1972, 1974; Lopukhin *et al.*, 1975) were reported. Affinity chromatography over albumin conjugated agarose beads was used to remove BIL from a patient with Crigler-Najjar Syndrome (Plotz *et al.*, 1974), from jaundiced rats (Scharschmidt *et al.*, 1974) and from Rhesus monkeys (Scharschmidt *et al.*, 1975). Counter-current dialysis has also been attempted (Willson *et al.*, 1972). This report concentrates on *in vitro* haemoperfusion tests with a packed bed of anion-exchange beads which have shown good binding capacity for BIL. However, unlike the earlier related work (Willson *et al.*, 1974; Lopukhin *et al.*, 1975), an attempt is made here to render biocompatibility to the ion exchanger by coating the particles with appropriate blood compatible, but BIL permeable, polymeric coatings.

Chang (1972) suggested the application of coated (microencapsulated) carbon granules in order to minimise blood damage and eliminate the carry-over of fine particles into the blood during haemoperfusion. A coated activated carbon column has been commercially developed by Smith and Nephew Research Ltd, England. Unfortunately, activated carbon columns are ineffective in removing BIL (Dunlop *et al.*, 1975). An evaluation of the various coatings is presented by Andrade *et al.* (1975). A detailed review on the biocompatibility of the various coatings and the application of coated activated carbon to remove various metabolites, toxins and overdoses of various medications is presented by Chang (p. 164 these Proceedings) and will not be repeated here.

Particular difficulties exist due to the small scale apparatus required for the infants and the relatively large amount of BIL to be removed. This dictates the development of a highly efficient small scale haemoperfusion system.

## 44.2 Materials and Methods

A number of *ion-exchange beads* were tested for their BIL binding capacity. These include Dowex 1 x 2 (50 - 100 mesh), Dowex 1 x 4 (20 - 50 mesh), Dowex 21 K (16 - 20 mesh), Dowex macroporous (20 - 50 mesh), Amberlite IRA-400 (20 - 50 mesh) and Amberlite XAD-2 (20 - 50 mesh).

*Solutions*

A model solution was used in the preliminary screening of the ion-exchange resins. It consisted of PBS (NaCl 0.14 M and phosphate buffer 0.01 M, pH of 7.4) with 15 mg % bilirubin (sigma) and 1.5 g % (human) albumin (sigma) (1 : 1 molecular ratio of B/A). The solution was prepared and maintained in the dark. An anticoagulant, acid citrate dextrose (ACD), was used in runs employing fresh blood. The ACD consisted of 0.8% disodium citrate, 2.2% trisodium citrate and 2.45% dextrose.

*Plasma*

*Fresh plasma* was supplied from the Rambam Hospital (Haifa). The plasma was kept for up to 2 weeks at 4 °C, and was filtered before use. 15 mg% bilirubin and 1.4 I.U./ml heparin were added before the experiments. Additional heparin solution of 2.8 I.U/ml was added during the fixed bed tests, at a rate of 3.2 ml/h.

*Coating materials and techniques*

A number of coating materials have been tested. These include hydroxyethyl methacrylate (HEMA), an acrylic AG-1039 polymer (Hydrophilics Ltd, Haifa) and (human) albumin.

Poly-HEMA is extensively used for carbon coating. The coating of the particles is achieved by polymerisation *in situ* of the pre-polymer solution in a fluidised bed (Andrade and Kolff, 1973), utilising a redox initiator system. The *acrylic AG-1039 polymer* is applied from 0.7% solution in dioxan in a fluidised bed. The precipitation of the polymer on the particles was accomplished by replacing the dioxane solution first by alcohol and then by a nonsolvent solution. *Albumin* showed good blood compatibility when adsorbed on collodion (Chang and Malave, 1970; Chang *et al.*, 1971). Here, the adsorbed albumin was bound to the various surfaces by crosslinking with glutaraldehyde. All the coated particles were stored in water.

*Preliminary screening*

The tests, aimed to determine the BIL adsorption capacity of the various resins, bare or coated, were carried out with either the model solution or with plasma. All tests were carried out in the dark. 4 g resin in a 15 mm diameter glass column and 100 ml solution (or plasma) were used throughout. The resin was pre-equilibrated overnight in PBS, when the model solution was used, and in PBS + heparin 1.4 I.U/ml when the plasma was used. The solution (or plasma) was circulated through the column at a flow rate of 3 ml/min, utilising a peristaltic pump (LKB 2115 Multiperspex) and 3 mm I.D. silicone tubes. Samples for BIL determination were taken from the column exit and the reservoir, and BIL concentration was assessed spectrophotometrically by the Jenderassik-Gróf procedure (Doumas *et al.*, 1973) using a Beckman Model 25 spectrophotometer.

*In vitro haemoperfusion tests*

The two-facet conical Perspex column, shown in figure 44.1, was 5 cm in height and 3 cm wide. The inlet and outlet entry leads were tapered Perspex cones with 1.5 cm diameter connections. The (empty) volume of the system, including tubings, was 29 ml. 20 ml wet resin (50 - 60 mesh) were used for each run. 70 mesh stainless steel grids were placed in the inlet and outlet of the column. The whole column was 'siliconised' with Dow Medical Fluid no. 360 (Plotz *et al.*, 1974).

Fresh human blood was supplied by the Maccabi Haematological Laboratory nearby. It included 15% acid citrate dextrose (ACD) as an anticoagulant. Soluble

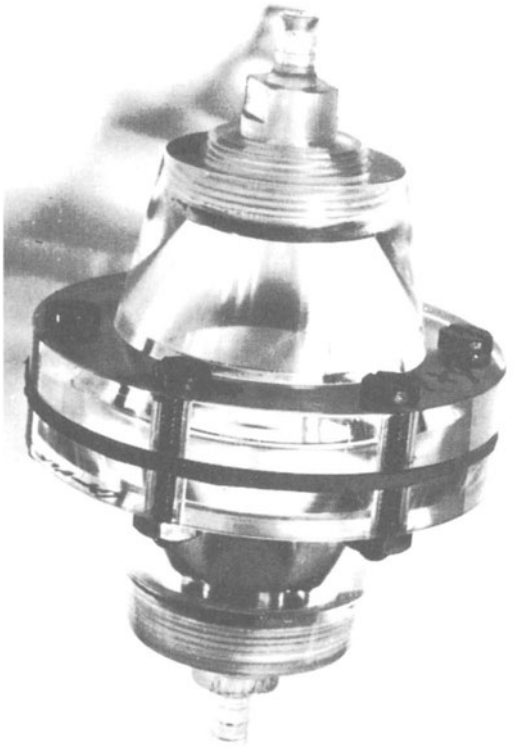


Figure 44.1 *In vitro* haemoperfusion test column

BIL was added to the blood, before the test. The blood volume in each run was 50 ml. The resin was equilibrated inside the column with a solution of 15% ACD in PBS for 15 h prior to each run. This priming volume was slowly replaced by blood and discarded. Circulation at a flow rate of 12 ml/min was effected by the peristaltic pump. Blood samples were taken at the column outlet and, at the end of the run, from the blood container.

*Blood tests*

Platelet, erythrocyte and leucocyte counts were taken during the various runs, utilising a Coulter counter Z-B. The counts were intermittently checked by microscopic counts, and only insignificant deviations were noted.

*Surface morphology*

The particles were examined with a JSM-U3 scanning electron microscope. Pictures of the resin particles to show surface morphology were taken before and after the blood runs. The latter were taken after fixation with glutaraldehyde. The samples were coated with gold-palladium before the scanning.

**Table 44.1**

Percent bilirubin in solution at various time intervals

| *Time* | *Dowex 1 x 2 50-100 mesh* | *Dowex 1 x 4 20-50 mesh* | *Dowex 21 K 16-20 mesh* | *Dowex macro-porous 20-50 mesh* | *Amber-lite IRA-400 20-50 mesh* | *Amber-lite XAD-2 20-50 mesh* |
|---|---|---|---|---|---|---|
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 30 min | 62.1 | | | | | |
| 60 min | 42.1 | 65.8 | 76.5 | 63.7 | 70.4 | 82.9 |
| 120 min | 25.7 | 59.4 | 69.2 | 62.7 | 64.2 | 81.9 |
| 180 min | 17.9 | 49.7 | 65.2 | 60.2 | 61.2 | 82.0 |
| 24 h | | 31.1 | 69.2 | | 37.7 | 74.7 |

**Table 44.2**

Effect of the initial bilirubin concentration. Percent BIL in solution

| *Time min* | *Initial concentration* | | |
|---|---|---|---|
| | *7.5 mg%* | *15 mg%* | *20 mg%* |
| 0 | 100 | 100 | 100 |
| 60 | 46.6 | 42.1 | 44.4 |
| 120 | 29.2 | 25.7 | 28.9 |
| 180 | 19.8 | 17.9 | 22.6 |

## 44.3 Results and Discussion

The resin screenings were carried out by circulating the model solution through the small glass column. The results are summarised in table 44.1.

As seen from table 44.1, Dowex 1 x 2 is most efficient, as it removes some 75% of the original (15 mg%) BIL in 2 h and over 80% in 3 h. The results are consistent with the reported (Sawchuk and Nairn, 1968) high binding capacity of Dowex 1 x 2. The complete time history for the Dowex 1 x 2 is shown in figure 44.2.

The dependence of the BIL removal rate on the initial concentration of BIL in the test solution is shown in table 44.2. As seen, this effect is rather small, indicating that the operating line in the range of operation is approximately parallel to the equilibrium saturation line. In other words, because of the relatively large binding capacity of Dowex 1 x 2, the concentration driving forces in this range of concentrations are practically constant. This is rather fortunate as it allows simple scale-up of the system (the quantity of the resin and the circulating fluid).

The comparison of BIL removal rate from the model solution and plasma is summarised in table 44.3. The resins were uncoated.

**Table 44.3**

Comparison of BIL removal from model solution and plasma.
Percent BIL in fluids

| *Time* | *Dowex 1 x 2* | | *Amberlite IRA-400* | |
|---|---|---|---|---|
| | *Solution* | *Plasma* | *Solution* | *Plasma* |
| 0 | 100 | 100 | 100 | 100 |
| 30 min | 62.1 | 78.1 | 79.0 | 79.0 |
| 60 min | 42.1 | 72.6 | 70.4 | 78.5 |
| 120 min | 25.7 | 61.2 | 64.2 | 76.4 |
| 180 min | 17.9 | 52.8 | 61.0 | 74.3 |
| 24 h | | 33.9 | | |

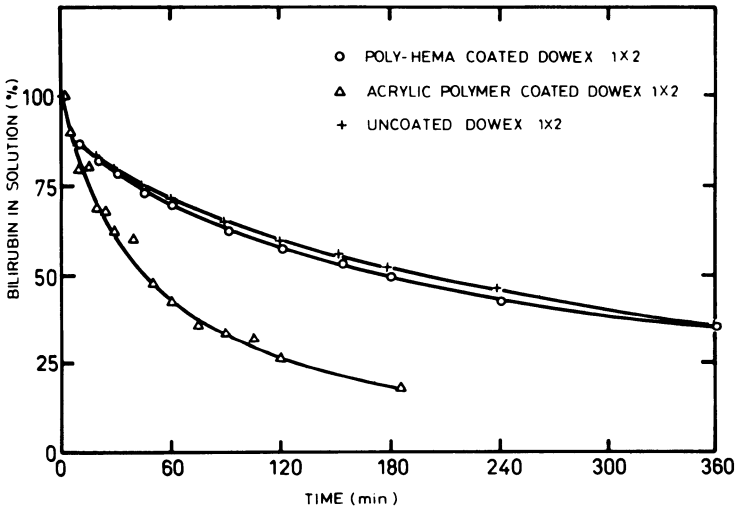



Figure 33.4 Bilirubin removal by coated and uncoated Dowex 1 X 2 in a closed cycle with model solution

As seen from table 33.4, the removal capacity of the Dowex 1 x 2 resin is greatly affected by the presence and eventual settling of the various proteins in the plasma. These results are consistent with prior adsorption studies of blood proteins (Morissey and Stromberg, 1974) which show that the amount of albumin settling on the surface after 30 min is quite significant, reaching a maximum after 80 min. As the binding of BIL by anion exchange resins is most probably diffusion controlled (Sawchuk and Nairn, 1968), the reduction in the bilirubin adsorption may be attributed to the added resistance of the protein layer to mass transfer. Indeed, electron microscope pictures show a fairly thick layer of protein surrounding the resin particle (figure 44.5a). Obviously, the hindering effect of this layer on the BIL transfer rate of the Amberlite resin is less pronounced, since

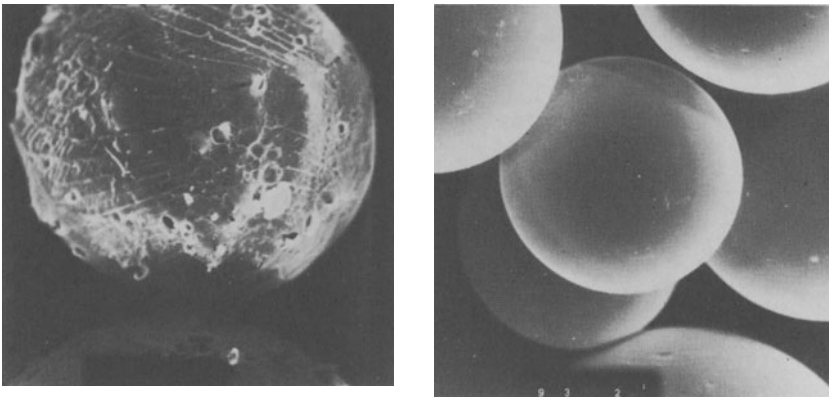


Figure 44.3 Electron microscope pictures (X 300) of Dowex (a) uncoated and (b) 2% HEMA coated

its binding capacity, or the diffusion rate constant, is of the same order of magnitude as the diffusion rate through the albumin layer.

The effect of the polymeric coatings of Dowex 1 x 2 on the removal rate of BIL from the model solution is presented in figure 44.2. A comparison of the electron microscope pictures of uncoated and HEMA coated Dowex 1 x 2 is shown in figure 44.3a and b. It is noted that the resistance to the BIL transfer rate added by the coating is similar to that presented by the above-mentioned

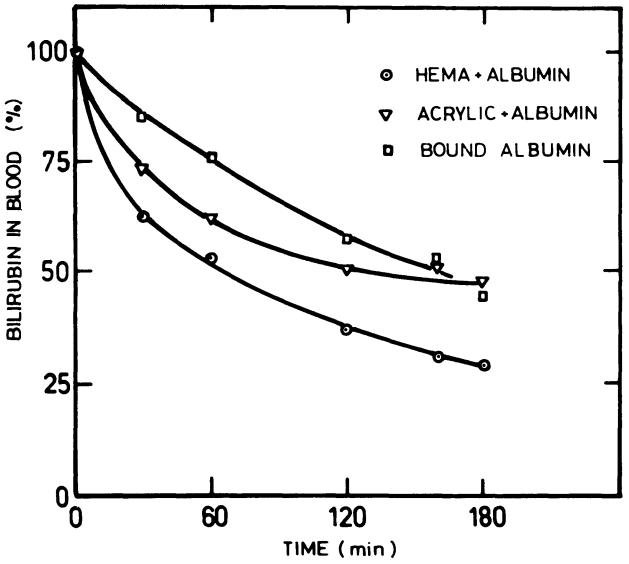
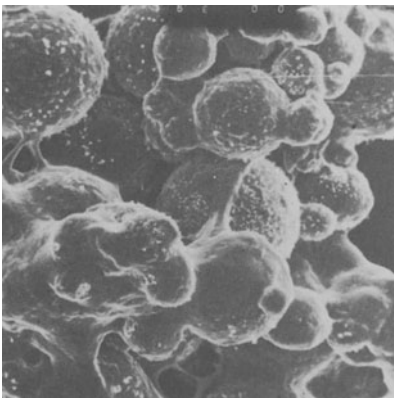
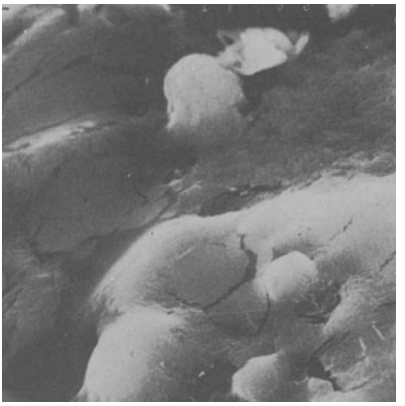


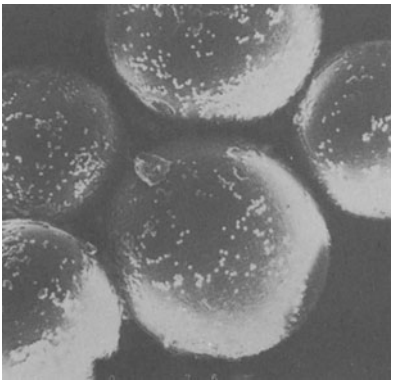

Figure 44.4 Bilirubin removal from blood by Dowex 1 X 2 with different coating



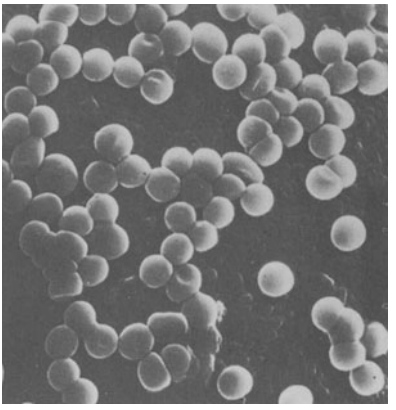
(a)



(b)



(c)



(d)

Figure 44.5 Comparison of uncoated and acrylic + albumin coated Dowex 1 × 2 particules after contact with blood in *in vitro* tests: (a) uncoated Dowex 1 × 2 (× 100), (b) uncoated Dowex 1 × 2 (× 3000), (c) coated Dowex 1 × 2 (× 180), (d) coated Dowex 1 × 2 (× 1800)

adsorbed protein layer. However, as shown in figure 44.5c and d, the adsorption of proteins on the coated particles is quite small.

The removal rate of BIL from (human) blood by the coated particles is presented in figure 44.4. The data are based on samples taken at the column outlet but the concentrations in the reservoir after 2 h were practically the same. No final conclusions can yet be drawn as the blood was quite different for each run, and the initial BIL concentrations were 19.6 mg% (for HEMA), and 13.8 mg% for the other two runs.

The blood compatibility of the various particles, tested in the *in vitro* closed cycle tests with fresh blood, is summarised in table 44.4.

As seen from this table the erythrocyte count in all cases is practically unaffected by the surface treatment. However, as seen in figure 44.5b, the protein layer which is formed on the uncoated Dowex traps the erythrocytes. On the

other hand, the proteins do not significantly adhere to the coated particles, and the erythrocytes are only loosely attached to the surface. Indeed, practically all the erythrocytes counted as missing from the blood were easily recovered by washing the packed coated resin bed with saline. It could, therefore, be returned to the blood stream.

It is evident from inspection of table 44.4 that the uncoated Dowex is unsuitable for haemoperfusion, as it removes some 70% of the platelets and 60% of the leucocytes. The HEMA coating improves the platelet count in the blood, and only some 35 to 40% of the platelets adhere to the surface. Similar results were obtained with albumin coating, which seems to have a better resistance to leucocyte adhesion. Evidently, the best performance is obtained with the acrylic + albumin coating, where platelet count falls only 25%, and the leucocyte count falls by some 40% during the course of operation. The latter is a relatively minor drawback, as leucocyte count returns to the original level within 1 h of discontinuing the haemoperfusion (Brookings and Ramsey, 1975).

The results are better than those obtained in dog haemoperfusion (Brookings and Ramsey, 1975) on activated carbon coated by an acrylic hydrogel (2% w/w, Smith and Nephew Research Ltd), where platelet count reached some 50% after some 30 min, and leucocyte count fell by 40 to 60%. Platelet count fall of some 50% was also observed in haemodialysis (Lindsay *et al.*, 1972), haemoperfusion in dogs (Gazzard *et al.*, 1974) and humans (Vale *et al.*, 1975). It is interesting that platelet count fall of up to 50% is considered safe, provided that the total count remains greater than $0.5 \times 10^5$ per $\mu$l (Brookings and Ramsey, 1975; Hin *et al.*, 1972). This notion, however, should not discourage the quest for better coatings which produce a smaller fall of the platelet and leucocyte counts in the blood.

## 44.4 Concluding Remarks

The work reported herein represents a preliminary effort to develop a small but efficient cartridge capable of removing excess BIL in a closed haemoperfusion cycle from the blood of jaundiced infants. Haemotransfusion, by haemoperfusion, is considered as more convenient and more reliable than heterotransfusion by presently used exchange transfusion. Haemolytic reactions, serum hepatitis and allergic and febrile reactions will be minimised. Also, the system, being closed, can be monitored and safeguarded against overloading and air embolism. The operation will be facilitated by utilising ready-made sterile apparatus, thus minimising undesired reactions. BIL levels lower than those attained by exchange transfusion may be achieved. Bank blood, which sometimes is not available, will not be necessary.

The essence of the proposed device is a cartridge which contains a resin with high binding capacity for BIL and which is made biocompatible by a suitable coating. Dowex 1 x 2, coated with albumin-treated polyacrylic membrane, is quite promising. However, additional work is still required to develop a better and still safer system.

**Table 44.4**

Results of haematological tests of fresh (human) blood. *In vitro* haemoperfusion with uncoated and coated Dowex 1 x 2 particles.

| *Coating* | *Time* min | *Erythrocytes* | | *Leucocytes* | | *Platelets* | |
|---|---|---|---|---|---|---|---|
| | | $\times 10^6/\mu l$ | % | $\times 10^3/\mu l$ | % | $\times 10^5/\mu l$ | % |
| Uncoated Dowex 1 x 2 | 0 | 6.27 | 100 | 6.81 | 100 | 1.215 | 100 |
| | 30 | 5.59 | 89.1 | 3.81 | 56.0 | 0.52 | 43.6 |
| | 60 | 5.03 | 80.1 | 3.54 | 51.9 | 0.34 | 28.0 |
| | 120 | 5.06 | 80.5 | 2.49 | 36.5 | 0.39 | 32.1 |
| | 150 | 5.34 | 85.1 | 2.33 | 34.2 | 0.43 | 35.4 |
| Albumin | 0 | 3.29 | 100 | 4.23 | 100 | 2.57 | 100 |
| | 30 | 2.74 | 83.3 | 4.05 | 95.7 | 1.75 | 68.0 |
| | 60 | 2.83 | 85.9 | 3.55 | 84.0 | 1.57 | 60.9 |
| | 120 | 2.81 | 85.4 | 2.89 | 68.3 | 1.23 | 47.8 |
| | 150 | 2.75 | 83.7 | 2.65 | 62.7 | 1.28 | 49.8 |
| | 180 | 2.73 | 83.0 | 2.49 | 58.9 | 0.94 | 36.4 |
| | end | 2.69 | 81.8 | 2.41 | 56.9 | 1.41 | 54.9 |
| HEMA | 0 | 6.27 | 100 | 6.81 | 100 | 1.215 | 100 |
| | 30 | 5.33 | 84.9 | 3.77 | 55.4 | 0.895 | 73.7 |
| | 60 | 4.99 | 79.5 | 3.48 | 51.1 | 0.83 | 68.3 |
| | 120 | 4.54 | 72.3 | 3.40 | 49.9 | 0.835 | 68.7 |
| | 150 | 5.16 | 82.2 | 2.72 | 39.9 | 0.69 | 56.8 |
| HEMA + albumin | 0 | 3.90 | 100 | 3.37 | 100 | 1.77 | 100 |
| | 30 | 3.47 | 88.9 | 2.19 | 64.9 | 0.51 | 29.0 |
| | 60 | 3.37 | 86.4 | 2.13 | 63.2 | 0.47 | 26.6 |
| | 120 | 3.40 | 87.0 | 2.15 | 63.9 | 0.49 | 27.9 |
| | 150 | 3.31 | 84.7 | 2.14 | 63.4 | 0.53 | 30.0 |
| | 180 | 3.35 | 85.9 | 2.06 | 61.1 | 0.52 | 29.7 |
| | end | 3.41 | 87.5 | 2.14 | 63.5 | 0.62 | 35.4 |
| Acrylic | 0 | 3.90 | 100 | 3.37 | 100 | 1.77 | 100 |
| | 30 | 3.53 | 90.4 | 2.42 | 71.8 | 0.57 | 32.2 |
| | 60 | 3.56 | 91.2 | 2.15 | 63.7 | 0.67 | 37.7 |
| | 120 | 3.59 | 96.8 | 1.99 | 59.0 | 0.75 | 42.5 |
| | 150 | 3.53 | 90.4 | 1.89 | 56.1 | 0.85 | 47.9 |
| | 180 | 3.49 | 89.4 | 1.67 | 49.5 | 0.79 | 44.9 |
| | end | 3.54 | 90.6 | 1.55 | 46.0 | 0.82 | 46.6 |
| Acrylic + albumin | 0 | 3.29 | 100 | 4.23 | 100 | 2.57 | 100 |
| | 30 | 3.0 | 91.1 | 3.15 | 74.4 | 1.92 | 74.5 |
| | 60 | 3.07 | 93.3 | 2.72 | 64.3 | 1.30 | 69.8 |
| | 120 | 2.84 | 86.3 | 2.49 | 59.0 | 1.93 | 74.8 |
| | 150 | 2.92 | 88.8 | 2.54 | 60.0 | 1.97 | 76.5 |
| | 180 | 2.84 | 86.3 | 2.37 | 56.0 | 1.86 | 72.4 |
| | end | 2.77 | 84.2 | 2.22 | 52.6 | 2.12 | 82.5 |

## 44.5 Acknowledgements

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## 44.6 References

Andrade, J. D. and Kolff, K. J. (1973). Development of more efficient, less costly artificial kidneys: adsorbent haemoperfusion. Progress Report 4, No. Ak-4-43-68-1027.

Andrade, J. D., Coleman, D. L., Kim, S. W. and Lentz, D. J. (1975). In R. Williams and I. M. Murray-Lyon (Eds.), *Artificial Liver Support*, Pitman Medical, London, pp. 84 - 92.

Barakat, T. and MacPhee, I. W. (1971). Bilirubin and alkaline phosphatase clearance from blood plasma by perfusion through activated charcoal. *Brit. J. Surg.*, **58**, 355 - 358.

Brookings, C. H. and Ramsey, J. D. (1975). Salicylate removal by charcoal haemoperfusion in experimental intoxication in dogs: an assessment of efficacy and safety. *Arch. Toxicol*, **34** (3), 243.

Chang, T. M. S. and Malave, N. (1970). The development and first clinical use of semipermeable microcapsules (artificial cells) as a compact artificial kidney. *Trans. Amer. Soc. Artif. Int. Organs*, **14**, 141.

Chang, T. M. S., Gonda, A., Dirks, J. H. and Malave, N. (1971). Clinical evaluation of chronic, intermittent and short-term haemoperfusions in patients with chronic renal failure using semipermeable microcapsules (artificial cells) formed from membrane-coated activated charcoal. *Trans. Amer. Soc. Artif. Int. Organs*, **17**, 246.

Chang, T. M. S. (1972). *Artificial Cells*, Thomas, Springfield, Illinois.

Doumas, B. T., Perry, B. W., Sasse, E. A. and Straumfjord, J. V. (1973). Standardisation in bilirubin assays: Evaluation of selected methods and stability of bilirubin solutions. *Clin. Chem.*, **19** (9), 984.

Dunlop, E. H., Weston, M. J., Gazzard, B. G., Langley, P. G., Mellon, P. J. and Williams, R. (1975). Artificial liver support based on haemoperfusion of adsorbents. *Biomed. Eng.*, **10**, 213 - 218.

Evenson, M. A. and de Vos, D. (1972). Direct-contact (membraneless) haemoperfusion through oils. *Clin. Chem.*, **18** (6), *554.*

Gazzard, B. G., Langley, P. G., Weston, M. J., Dunlop, E. H. and Williams, R. (1974). Polymer coating of activated charcoal and its effects on biocompatibility and paracetamol binding. *Clin. Sci. Mol. Med.*, **47**, 97 - 104.

Hill, J. D., de Leval, M. R., Mielke, C. H., Bramson, M. L. and Gerbode, F. (1972). Clinical prolonged extracorporeal circulation for respiratory insufficiency: haematological effects. *Trans. Amer. Soc. Artif. Int. Organs,* **18**, 546 - 552.

Lindsay, R. M., Prentice, C. R. M., Davidson, J. F., Burton, J. A. and McNicol, G. P. (1972). Haemostatic changes during dialysis associated with thrombus formation on dialysis membranes, *Brit. Med. J.*, **11**, 454 - 458.

Lopukhin, U. M., Shurkalin, B. K., Leykin, U. A., Evseev, N. G., Molodenkov, M. N., Kuznetzov, V. N., Gorchakov, V. D. and Blagosklonov, A. S. (1975). Bilirubin removal by anion-exchange resin. *Lancet*, 2, 461.

Morrissey, B. W. and Stromberg, R., (1974). The conformation of adsorbed blood proteins by infrared bound fraction measurements. *J. Colloid Interface Sci.*, **46** (1), 152.

Plotz, P. H., Berk, P. D. and Scharschmidt, B. F. (1974). Removing substances from blood by affinity chromatography. I. Removing bilirubin and other albumin-bound substances from plasma and blood with albumin-conjugated agarose beads. *J. Clin. Invest.*, **53**, 778.

Radzialowski, F. M. (1973). Effect of Spironolactone and Pregnenolone-16 $\alpha$ Carbonitrile on bilirubin metabolism and plasma levels in male and female rats. *Biochem. Pharma.*, **22**, 1607.

Rosenbaum, J. L., Kramer, M. S., Raja, R. and Boreyko, C. (1971). Resin haemoperfusion: A new treatment for acute drug intoxication. *New Engl. J. Med.*, **284** (16), 874.

Sawchuk, R. J. and Nairn, J. G. (1968). Rate studies on the binding of bilirubin by ion-exchange resins. *J. Pharm. Sci.*, **57**, 1896.

Scharschmidt, B. F., Plotz, P. H., Berk, P. D., Waggoner, J. G. and Vergalla, J., (1974). Removing substances from blood by affinity chromatography. II. Removing bilirubin from the blood of jaundiced rats by haemoperfusion over albumin-conjugated agarose beads. *J. Clin. Invest.*, **53**, 786.

Scharschmidt, B. F., Martin, J. F., Shapiro, L. J., Plotz, P. H., Berk, P. D., Gordon, J. K., Waggoner, J. G. and Vergalla, J. (1975). Haemoperfusion through albumin-conjugated agarose beads (ACAB) for the treatment of neonatal jaundice in premature Rhesus monkeys. *Clin. Res.*, **23**, 256A.

Vale, J. A., Rees, A. J., Widdop, B. and Goulding, R. (1975). Use of charcoal haemoperfusion in the management of severely poisoned patients. *Brit. Med. J.*, **1**, 5 - 9.

Willson, R. A., Webster, K. H., Hofmann, A. F. and Summerskill, W. H. J. (1972). Toward an artificial liver: in vitro removal of unbound and protein-bound plasma compounds related to hepatic failure. *Gastroenterology*, **62**, 1191.

Willson, R. A., Hofmann, A. F. and Kuster, G. G. R. (1974). Toward an artificial liver. II. Removal of cholephilic anions from dogs with biliary obstruction by haemoperfusion through charged and uncharged resins. *Gastroenterology,* **66**, 95.

Yatzidis, H. (1965). A convenient haemoperfusion microapparatus over charcoal for the treatment of endogenous and exogenous intoxication. 1. Its use as an effective artificial kidney. *Proc. Eur. Dial. Transpl. Assoc.*, **1**, 83 (1964); *Lancet,* **2**, 216.

# 45

# SUCCESSFUL MANAGEMENT OF STAGE IV HEPATIC COMA IN A CHILD BY HAEMOCARBOPERFUSION

M. C. GELFAND, A. COLON, J. H. KNEPSHIELD, Z. PAPADOPOULOU, P. SANDLER and G. E. SCHREINER

**Summary**

Hepatic failure and its encephalopathy (grade IV coma) carries a mortality rate of 60 to 70% in children under 15 years of age (Silverman *et al.*, 1971). Current treatment is predominantly aimed at curtailing known precipitants with anti-ammonia regimens, potassium repletion (Conn, 1973), amine replacement (Colon and Sandberg, 1973), and other supportive care. Recent attention has turned towards methods for removing accumulated toxic metabolites. Exchange transfusion, human, primate and pig cross-circulation, and dialysis have all been used with limited success. Recently, carboperfusion has been successfully employed to reverse the encephalopathy of hepatic failure (Gazzard *et al.*, 1974; Gelfand *et al.*, 1976). In this report we describe the successful treatment of grade IV hepatic encephalopathy in a 13-year-old boy. This patient represents the youngest reported case treated successfully with haemoperfusion.

## 45.1 Methods

After obtaining informed consent, an arteriovenous shunt was created in the patient's left ankle. As reported previously (Gelfand *et al.*, 1976) blood was pumped through a column containing 300 g of charcoal covered with a 2% by weight coating of an acrylic hydrogel polymer (Smith and Nephew Research Ltd, England, and Warner Chilcott, New Jersey). The charcoal was washed thoroughly with normal saline just prior to use. Blood was pumped at a constant flow rate of between 100 and 200 ml/min, and the patient was heparinised to maintain the Lee-White clotting time at between 10 and 20 min. Each haemoperfusion was performed for 4 h. At the termination of each haemoperfusion, 0.1 unit of fresh frozen plasma and 0.1 unit of platelet concentrate per kg body weight were infused.

## 45.2 Case Report

A 13-year-old black male underwent drainage of a right frontal cerebral abscess under halothane anesthesia. He did well for several weeks post-operatively and was ambulatory when fever and eosinophilia appeared. One month post-surgery, jaundice, confusion, persistent fever, abdominal right upper quadrant tenderness,

vomiting and lethargy appeared. Serum SGOT and SGPT levels were both greater than 2400 μg and the prothrombin and partial thromboplastin times were prolonged 100%. Serum ammonia was 460 μg/100 ml. Serum protein dropped to 4.2 g (albumin 1.5 g). Haematuria and upper GI bleeding ensued and renal failure supervened with a rising blood urea nitrogen of 90 and creatinine of 4 mg %. Bilirubin was 25.2 with a direct fraction of 19.2 mg %, and eosinophilia persisted between 16 and 20%. A clinical diagnosis of halothane hepatitis was made; however, because of the rapid onset of a severe coagulopathy, liver biopsy was not performed.

Despite an antiammonia campaign, renal failure regimen, and supportive care, the patient lapsed into stage IV hepatic coma. Exchange transfusion was carried out twice without change in status. Haemodialysis was performed twice without change. Informed consent was obtained from the patient's parents, and haemocarboperfusion was carried out twice on consecutive days.

Following the first perfusion, the patient went from a stage IV to a stage III coma. Following the second perfusion, the boy awoke. He remained fully conscious over the next ten days during which hepatic function returned to near normal. One month post-coma onset, the patient was discharged. Follow up studies at four months revealed no hepatic pathology.

## 45.3 Discussion

This patient represents the youngest patient with grade IV hepatic encephalopathy successfully treated by haemocarboperfusion. His recovery of consciousness after haemoperfusion was especially impressive in view of the failure of two attempts at total body exchange transfusion and two haemodialyses.

The two haemoperfusions were carried out using the Haemocol brand activated charcoal device without significant difficulty in spite of the paediatric age of the patient. No significant changes in blood electrolytes, $Ca^{2+}$, $Mg^{2+}$, glucose, or liver transaminases were seen. Table 45.1 shows changes in haematocrit, white blood count, platelets, prothrombin time, partial thromboplastin time, triglycerides and cholesterol. Little change was observed in these values. Our protocol entails the

**Table 45.1**

Changes in various blood parameters pre and post-perfusion

| | *Pre-first perfusion* | *Post-first perfusion* | *Pre-second perfusion* | *Post-second perfusion* | *Following day* |
|---|---|---|---|---|---|
| Haematocrit | 29 | 30 | 28 | 30 | 29 |
| WBC (× 1000) | 12 | 11 | 17 | 15 | 17 |
| Platelets (× 1000) | 95 | 65 | 62 | 80 | 83 |
| Prothrombin time | 19/12 | 22.5/12 | 16/12 | 21/12 | 15/12 |
| PTT | 113/37 | >120.0/36 | 47/37 | 96/37 | 46/37 |
| Cholesterol | 171 | 134 | 161 | 182 | 171 |
| Triglycerides | 105 | 77 | 116 | 180 | ND |

**Table 45.2**

Effect of haemoperfusion on depth of coma, plasma ammonia levels and concentrations of various amino acids

| | *Pre-first perfusion* | *Post-first perfusion* | *Post-second perfusion* |
|---|---|---|---|
| *Coma grade* | *IV* | *III* | *0* |
| Taurine | *13.45 | 0.20 | 0.46 |
| Threonine | 2.47 | 1.67 | 1.67 |
| Hydroxyproline | *4.51 | 0.82 | 1.45 |
| Serine | 1.42 | 1.07 | 1.02 |
| Glutamine | 3.12 | 3.15 | 3.14 |
| Proline | *6.88 | 3.68 | 2.88 |
| Glutamic acid | *9.17 | 2.55 | 1.45 |
| Citruline | present | trace | trace |
| Aspartic acid | present | present | present |
| Glycine | 3.80 | 2.85 | 2.79 |
| Alanine | *6.85 | 3.97 | 3.33 |
| α-amino-*n*-butrate | 0.53 | 0.34 | trace |
| Valine | 2.34 | 0.52 | 0.45 |
| Cystine | 2.20 | 1.83 | 1.92 |
| Methionine | *2.98 | 2.16 | 2.04 |
| Isoleucine | 1.03 | 0.63 | 0.81 |
| Leucine | 1.29 | 0.94 | 1.00 |
| Tyrosine | *2.34 | 1.25 | 1.00 |
| Phenylalamine | *2.33 | 1.41 | 1.25 |
| Ornithine | *2.40 | ND | 1.07 |
| Ethanolamine | *1.10 | ND | trace |
| Lysine | *6.30 | ND | 3.98 |
| Histidine | 2.00 | ND | 1.19 |
| Arginine | 1.80 | ND | 0.50 |
| Ammonia | 146 | 120 | 97 |

*Elevated amino acid level

infusion of fresh frozen plasma and platelets after each perfusion. This may be responsible for the prevention of significant changes in coagulation parameters and platelet levels.

Table 45.2 shows change in coma grade, blood ammonia, and plasma amino acid levels. As the coma grade fell to normal, blood ammonia fell to normal and the plasma concentration of taurine, hydroxyproline, proline, glutamic acid, alanine, methionine, tyrosine, phenylalanine, ornithine, ethanolamine and lysine returned to normal.

Although the exact cause of hepatic encephalopathy is uncertain, it is often associated with elevated blood levels of ammonia and certain amino acids. Therefore, it is reasonable that the lowering of blood ammonia and amino acid levels by haemoperfusion was at least in part responsible for recovery from coma.

Alterations in circulatory levels of other toxins not specifically measured, such as false neurotransmitters, free fatty acids, and other compounds, may have also played an important role.

Since it is probable that hepatic coma represents the accumulation of a variety of toxic wastes, the use of a procedure such as haemocarboperfusion presents an attractive method of removing a broad spectrum of compounds. If a specific aetiological factor is identified, a more specific modality may be more appropriate.

The complete recovery of this patient and patients reported elsewhere (Gazzard *et al.*, 1974) after charcoal haemoperfusion suggests an important potential role for this therapy in hepatic coma. Nevertheless, considerable clinical experience is required for the adequate understanding of the management of patients with hepatic insufficiency whose lives may be prolonged by this therapy.

## 45.4 References

Colon, A. K. and Sandberg, D. H. (1973). Hepatic encephalopathy treated with L-Dopa. *Pediatrics*, **51**, 1105 - 1106.

Conn, H. O. (1973). Current diagnosis and treatment of hepatic coma. *Hosp. Prac.*, **8**, 65 - 72.

Gazzard, B. G., Weston, M. J., Murray-Lyon, I. M., Flax, H., Record, C. O., Portman, B., Langley, P. G., Dunlop, E. H., Mellon, P. J., Ward, M. B. and Williams, R. (1974). Charcoal haemoperfusion in the treatment of fulminant hepatic failure. *Lancet*, **1**, 1301 - 1306.

Gelfand, M. C., Knepshield, J. H., Cohan, S., Ramirez, B. and Schreiner, G. E. (1976). Treatment of hepatic coma with haemoperfusion through polyacrylamide hydrogel-coated charcoal. *Kidney International* (in press).

Silverman, A., Roy, C. C. and Cozzetto, F. J. (1971). *Pediatric Clinical Gastroenterology*, Mosby, St. Louis, pp. 366 - 367.

# SESSION E DISCUSSION

*Hill* opened by suggesting a novel method of liver failure treatment in which the first step would be to perfuse a normal healthy donor whose blood group matched that of the liver failure patient and the second step to perfuse that patient with the same device. This would effect cross adsorption using the isotherm principle of simultaneous removal of high concentration substances from the patients' blood in return for depleted substances. *McClure* warned that in view of the known catalytic effect of charcoal, development of this type of system should at least be postponed until charcoal was more fully characterised, especially with respect to its effect on labile biochemicals. *Fischer* commented on some of his group's work on animal models of hepatic failure. The method used in the pig was that of surgical devascularisation (occlusion of the portal vein and hepatic artery following the construction of a portacaval shunt) since other techniques such as the injection of isolated mushroom poison (from the *Amanita phalloides* species) had been found to produce lesions in organs other than the liver (for example the kidney and pancreas). Controlled trials of such pig models of permanent hepatic ischaemia gave survival times of 12 - 14 h. These were different from results obtained using dogs and thus the effect of species individuality should not be overlooked. Pigs which had survived the procedure for liver ischaemia inducement and were awake within 6 h of the operation were perfused (pig to pig) for 8 to 10 h after devascularisation. From comparisons with control (untreated) groups it appeared that the treated animals lived 1 - 2 h longer, but since one untreated animal survived for 26 h in comparison to treated survival times of 12 - 14 h, these results were not significant. *Abouna* agreed that different species would present different models of hepatic failure. He had already shown that in the dog temporary occlusion of the portal vein and hepatic artery resulted in a mortality rate of only 45% and was therefore not a good model of hepatic failure. On the other hand, permanent occlusion of the portal circulation, followed by arterial occlusion only, resulted in a 70 - 80% mortality rate and was a more consistent method of producing hepatic failure. As for the use of drugs, the injection of phosphorus was considered because of its simplicity but was found to affect organs other than the liver. *Hughes* asked Abouna if the decreased survival time observed in the resin treated animals was due to a higher incidence of bleeding problems (for example retroperitoneal bleeding). *Abouna* agreed that bleeding along with platelet and white cell damage and haemolysis was more evident in the resin (Amberlite XAD-4) than the charcoal treated animals.

*Denti,* returning to drug-induced hepatic failure, commented that this was more predictable if it was preceded by phenobarbital administration. This system

had also been proved with dimethylamine promazine and was effective in the presence of toxins continually liberated by the activity of microsomal enzymes. Some experiments with this drug had been performed in the University of Utah, showing that only liver tissue was destroyed by the effect of such metabolites but, in contrast to this, there was a high incidence of *Amanita phalloides* poisoning in Italy with accompanied evidence of renal failure. For this reason a model using this toxin was not desirable. He went on to ask Abouna if (as he had found) there were any clotting problems in his shunt experiments. *Abouna* said that such problems had been completely avoided through the use of an extremely short Busselmeier shunt in conjunction with the largest available cannula.

*Brunner* commented that Nosé in using liver slices or granules as an adsorbent system had utilised a membrane for blood protection. How, therefore, were lipid soluble substances which would be unable to pass through this membrane detoxified? *Nosé* had not studied this particular problem, but the pore size of the membrane used was 0.4 $\mu$m and any substance which could pass through this could be metabolised. *Brunner* agreed that this would be acceptable when using adsorbents such as charcoal but that when liver slices were used, it was essential that *no part* of the serum be allowed contact otherwise immunological reactions would occur. Direct contact with pig or dog liver was therefore unacceptable and only partly usable. *Nosé* contended that the ideal membrane was one of a pore size which permitted the passage of molecules the size of albumin but not of globulins. Since it was known from pig perfusion studies that hepatic coma patients had impaired immunological reactions there was hopefully an application for this system. *Fischer* then asked Nosé if it would not be easier to perfuse an intact liver rather than slices which were technically more complex to produce. He pointed out that Eiseman in Denver had shown that the efficacy of perfused liver slices was 1/10th that of the intact organ. *Nosé* said that potency of the liver slices was dependent on preparation technique, source of tissue, slicing method and storage medium, and was greater than had once been estimated. He explained that adequate perfusion of the liver required the recruitment of the capillary system, a difficult achievement with an extracorporeal system. However, if the liver slices or granules were reduced to 50 $\mu$m in size, then cellular diffusion and metabolism could take place. Two problems of significance were evident in liver perfusion: the availability of operating staff and the preparation and availability of liver tissue for use in emergencies. Because of these problems, freeze-dried liver granules were used although their potency was one third that of fresh liver slices. The anticoagulant used in these experiments was heparin.

*Leber* questioned Brunner on the capacity of his enzyme preparations. Firstly, what did he think was the percentage enzyme activity in his column compared to the microsomal protein content? Secondly, was the reaction velocity limited by enzyme activity in the column, or by the cofactor concentration, or (as far as mixed function oxidation was concerned) by the oxygen pressure? Thirdly, how much liver was used to prepare one column and was storage a problem? *Brunner* replied that pretreated phenobarbital was used in the enzyme preparation and that

the activity of the preparation was about 30% that of the initial enzyme in the liver. A relatively pure enzyme extract contained about 30% of the initial microsome content. In answer to the second question, previous studies had shown that the reaction was slow because the enzyme and cofactor concentrations were critical. The problem of low NADPH levels was being investigated. Turning to the question of storage, he said that all microsomal enzymes were comparatively stable and therefore easily stored, and that once bound to a carrier they could be re-used.

*Maini* commented that toxin removal by sorbent was a desperate procedure for a desperate condition. It was, however, a practical approach and it was evident that some supplementation of essential substances removed by sorbents would be necessary for protracted application. He then went on to ask Brunner what weight of enzyme beads were required to remove phenols from the blood during a long period of treatment and what was the priming volume of his system? *Brunner* said that with the uridine diphosphate glucoronyltransferase (UDPG) 10 g were required for a 2-day treatment. He gave the column specification as: length 0.5 m, diameter 3 - 4 cm, and priming volume 600 ml. He added that the amount of enzyme that could be used was unlimited. *Martin* remarked that hypotension had been cited as a complication of haemoperfusion and that adrenalin removal might be responsible for this. However, he had not experienced any such blood pressure changes even with repeated daily coated carbon haemoperfusion in drug intoxication and renal failure. Did Brunner know of any perfusion system which was associated with the effect, and did he have any data on the central venous pressure (CVP) of his perfused liver failure patients? *Brunner* replied that a group in Germany had investigated the Smith and Nephew column in relation to blood pressure changes and that the CVP of his patients was between 2 and 4 cm mercury. *Martin* then asked Williams if hypotension could occur during extracorporeal circulation without the implication of platelet damage, since platelet aggregates were seen during perfusion and dialysis when there was no pressure change? *Williams* thought there was some correlation between platelet aggregation and hypotension, although such aggregation occurred less frequently in dialysis. In consequence he felt that any system developed should be tested in liver patients who had compromised platelet function. This was also the reason why he considered basic work on platelet function and structure important.

*Brunner* remarked that although the enzymes and carriers used in his system were available some 6 years ago, platelet damage had been so catastrophic as to delay progress. Soft beads were now used, which gave a platelet drop of 20 to 30% after 4 h circulation. The device had not yet been used in patient treatment. *Chang* agreed that enzyme immobilisation was a useful study but thought that they might not be in clinical practice for some time since their action was so specific as to necessitate identification of the toxin to be removed. Although adsorbents were less specific in their action, if some substance was depleted during toxin removal it could be replaced subsequently to ensure the patient's safety. *Brunner* said that the essential substances which were depleted by sorbent per-

fusion must be identified to permit their replacement since previous investigations had concentrated only on toxins whose removal was desired. Returning to his enzyme system he expressed regret that there had been no parallel enzyme studies since; although the nature of the work meant that progress was slow, the number of enzymes requiring study was limited and the detoxification mechanisms few. *Chang* did not agree that no parallel work was being done since he knew of 50 centres where work on immobilised enzymes for therapeutic application (including some for liver failure) was being carried out. He re-emphasised that the problem in this work was the identification of toxins which in liver failure were often unknown.

*Chang* asked Williams if, since he was concerned with platelet depletion, he had tried an albumin-coated collodion system. It had been found by many workers that such a system could prevent platelet depletion and aggregation. The data *Williams* had on this score showed negative results. He expressed his doubts that any coated system would prove fully biocompatible for every patient with liver failure. *Maini* then asked Williams if the principal drawback of the celltrifuge system was that platelets were present in the centrifuged plasma, and for how long could this system be used? *Williams* replied that recent evidence showed that minimal platelet drops occurred with the Haemonetic celltrifuge. The particular perfusion had been of 3 h duration but further development of the technique should permit more protracted operations. He thought that pure plasma perfusion might be the answer to the many problems of the liver failure patient.

Commenting on the substantial platelet drop in Williams' system, *Bjørnson* asked what heparin dosage had been used since it was known that heparin induced platelet aggregation including that potentiated by ADP and collagen through the release reaction. Patients with liver failure had an increased half-life for heparin since the heparin cofactor anti-thrombin III levels were lowered. *Williams* acknowledged that this was a complex subject but that plasma concentrations of heparin were monitored in order to avoid its over-administration. He added that the platelet aggregation studies mentioned by Bjørnson were carried out primarily *in vitro* and not in human liver failure and that there was evidence which suggested platelet aggregation could be reversed by heparin administration.

SESSION F

# REPORT OF WORKING GROUPS

*Chairman*
Professor R. M. Kenedi, B.Sc., Ph.D., A.R.C.S.T., C.Eng., F.I.Mech.E., M.R.Ae.S., F.R.S.E.

*Editorial note* An established pattern of the Strathclyde Bioengineering Seminars is the setting aside of the last session for a workshop activity. Hopefully this will result in reports which summarise for the designated areas the present state and trends, the gaps in knowledge and practice and the aspects which appear most urgently to require clinical and laboratory study.

Three working groups were convened, each consisting of a Chairman, Chairman-Associate and Reporter concerned with the following areas of enquiry:

| | |
|---|---|
| Working Group 1 | The clinical role of the membrane oxygenator |
| Working Group 2 | The clinical role of adsorbents in acute and chronic therapeutic medicine |
| Working Group 3 | Thrombogenesis in membrane oxygenators and adsorption devices |

Reports were prepared in draft form and distributed to all participants just prior to the commencement of the session. The Chairmen, Chairmen-Associate and Reporters then presented their reports in outline and introduced them for plenary discussion to which the following seminar participants (listed alphabetically and not in order of speaking) contributed: Abouna, Bjørnson, Black, J., Black, M. M., Brunner, Chang, Courtney, Denti, Ms Duncan, Edwards J., Fairweather, Feijen, Galletti, Gaylor, Ghadiali, Hill, Hughes R. D., Kenedi, Kim, Lindsay, McClure, Maini, Martin, Millington, Nathan, Olijslager, Radcliffe, Sharrock, Sparks, Tijssen, Veenhof, Wildevuur, Ms Wilkinson, Winchester, Wyatt.

The reports that follow on each area of the working groups have been compiled by the editors from the draft reports and the recorded discussion. They represent a concensus of the views that emerged.

## WORKING GROUP 1
# THE CLINICAL ROLE OF THE MEMBRANE OXYGENATOR

*Chairman*
Professor M. M. Black, M.Sc., Ph.D., F.Inst.P., C.Eng., M.R.Ae.S.

*Chairman-Associate*
P. E. Ghadiali, M.B., F.R.C.S.

*Reporter*
J. D. S. Gaylor, B.Sc., Ph.D., C.Eng., M.I.Mech.E.

## WORKING GROUP 2
# THE CLINICAL ROLE OF ADSORBENTS IN ACUTE AND CHRONIC THERAPEUTIC MEDICINE

*Chairman*
Professor R. E. Sparks, D.Eng.

*Chairman-Associate*
A. M. Martin, M.B., Ch.B., F.R.C.P.

*Reporter*
J. M. Courtney, B.Sc., Ph.D., A.R.C.S.T., C.Chem., M.R.I.C., A.P.R.I.

## WORKING GROUP 3
# THROMBOGENESIS IN MEMBRANE OXYGENATORS AND ADSORPTION DEVICES

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# GROUP 1. THE CLINICAL ROLE OF THE MEMBRANE OXYGENATOR

## Introduction

Historically oxygenators began, in the form of the lung component of the heart-lung bypass device, as an attempt at total replacement in 'acute' instances such as cardiac surgery. In common with other artificial organs (kidney and heart for example) its development has been characterised by shifts of emphasis from short to long-term assistance and *vice versa*. Unlike the artificial kidney, where until recently primary developments have been consolidation of the long-term extra-corporeal replacement, the artificial lung problem called more emphatically for varieties of short-term assistance. The membrane oxygenator lagged somewhat behind, becoming only recently a reliable and preferred device for routine cardiac surgery. While such oxygenators may prove the means by which long-term respiratory assistance is accomplished, there is still a long way to go before a 'permanent' booster or total extracorporeal replacement of the lung, for clinical use, is achieved.

Research areas responsible for progress include: improved membrane materials and manufacturing techniques, modelling of the gas transfer process, development of high efficiency devices and improved anticoagulant therapy and design of the extracorporeal circuitry. Recent forward moves in several of these areas have been reported at this seminar in addition to clinical experience on both short and longer-term applications.

## Oxygenator Design

Reliable theoretical models of $O_2$ and $CO_2$ transfer in membrane lung channels with laminar flow have been developed, and it is possible to predict performance for a wide range of operating conditions without recourse to extensive experiment. Of particular interest is the Advancing Front Theory (AFT) in which the $O_2$ transfer is represented by the progressive movement of a saturated blood zone (e.g. that which exists in the region of the membrane boundary) into a zone of blood at 'desaturated' or inlet condition. The theory permits a simplified approach to the analysis of $O_2$ transport in well-defined geometries. It has also been shown to apply for $CO_2$ transport enabling correlation of data for a range of inlet blood values. Extension of AFT to oxygenators which employ convective mixing for enhancement of gas transfer has been demonstrated for secondary flows in coiled tube geometries. Where vortex motion is more vigorous or more complex such

that a geometric analogue of the convection effect on diffusion cannot be readily visualised, the AFT approach is limited. This situation applies to the Taylor-vortex oxygenator and instead an empirical correlation between dimensionless groups has been used to predict performance. This method, also adopted for the oscillating curved channel membrane lung, precludes any physical description of the mass transfer process but has proven useful for design and feasibility purposes.

The characteristics of secondary flows as observed in large-scale models of the oxygenator flow channel may be altered when the actual scaled-down membrane channel is used. Thus, development of mass transfer modelling techniques is to be encouraged in this area if only to assess how the expected performance might be influenced by altered flow patterns.

The Oxford oxygenator demonstrates the extremely high transfer rates obtainable when strong convective flows are employed with high permeability microporous membranes. An adult capacity unit with about 0.5 $m^2$ surface area would appear feasible and would produce low blood trauma.

Microporous membranes bring benefits of increased permeability, mechanical strength and reduced cost but their high water vapour permeability may impair gas exchange and cause dehydration problems during long-term respiratory support. Largely unexplained is the physical nature of the blood - gas interaction at the microporous interface and the question has been raised whether microporous membrane lungs behave as solid membrane lungs or as sophisticated micro-bubble oxygenators.

## Clinical Application

The primary reason for the clinical use of *membrane* based oxygenation is that it offers better blood handling characteristics. The present clinical applications in the *short-term* relate almost exclusively to cardiac surgery; in the *longer-term* (days to weeks duration) to instances of severe but reversible lung damage and finally in the *long-term* (that is in excess of weeks) to cases such as circulatory failure. The experience in this last area has been disappointing in that when the lesions prove irreversible in nature, the patient deteriorates once the therapy is ceased.

In *short-term* applications such as routine cardiac surgery, the blood trauma produced by the lung component may not be significant compared to that caused by coronary suction systems and by other components in the bypass circuitry. Thus, while it may be difficult to demonstrate directly superiority of the complete systems with membrane as opposed to bubble oxygenators, specific improvements have been reported in the control of $O_2$ and $CO_2$ exchange, reduction in post-operative enzymes release, increase in urinary output during bypass and reduction in platelet loss when a *membrane* lung was employed in cardiac surgery.

Assessment of the *longer-term* treatment of respiratory insufficiency by membrane oxygenation is extremely difficult owing to the complex nature of the procedure. Experimental models of the condition of respiratory insufficiency in

which either the degree of hypoxaemia can be controlled or the time course of decreasing arterial saturation and increasing pulmonary oedema can be reproduced are valuable, and permit oximetric and therapeutic effects of long-term membrane oxygenation to be studied. Likewise, information on haemodynamic, haematological and gas-transfer changes *during* extended clinical perfusions is necessary to define more closely the role of the oxygenator and to provide treatment rationale.

Components of the extracorporeal circuitry which are relatively insignificant in terms of blood trauma during cardiac surgery may assume prominent roles in extended perfusions. A system has therefore been described in which the conventional roller pump is replaced by a ventricle type with ECG-actuated control.

One of the main problems is the difficulty of identifying the subtle changes which occur in blood (particularly in plasma proteins and cellular elements) flowing in an extracorporeal circuit. Present haematological tests are crude and are mostly indicative of gross damage only. It is suggested that the nature of these subtle changes might best be examined under *in vitro* conditions followed by the application of quantitative techniques to comparative studies. The culmination of this testing sequence should be *in vivo* assessment of the effect of these blood changes.

Another aspect is the thrombogenic nature of the present bypass circuitry which demands anticoagulation therapy and thus produces problems of bleeding. The advance towards nonthrombogenic circuitry requires identification and separation of the various factors involved, including mechanical forces, foreign surfaces and biochemical components. Since these effects may be interrelated, their detailed examination is difficult and results must be interpreted cautiously. An important aspect of these interacting effects is the unsatisfactory present state of the patient/machine interface. The current difficulties such as the development of tight, 'non-kinking' junctions in cannulation can still prove the limiting factors in clinical practice, despite the benefits of increased efficiency by membrane oxygenation.

## Economic Aspects

As far as it could be ascertained, studies of cost effectiveness and/or cost benefit have not been undertaken in the field of oxygenation. In this connection it was suggested that a major increase in the use of membrane oxygenation will occur only with reduction of the costs of the oxygenator units. Militating against such price reductions is the relatively small present oxygenator market. For example, world wide there are about 170 000 surgical open heart operations per year, implying in disposables the same number of units. Against this there are some 70 000 chronic renal patients in the world each dialysed around 100 times per year. This means some 7 million disposable dialyser units, a market some 40 times that for oxygenators in cardiac surgery. It is anticipated that market development will go hand-in-hand with development of 'simpler to use and cheaper to buy' membrane oxygenators.

**Summary**

Research at both clinical and laboratory levels oriented at concurrent advance is required on the following three areas.

*Basic medical understanding* of the problems initiating the need for oxygenation. This covers research into disease states including the problem of microemboli associated with long-term perfusion. Other aspects are the biochemistry of the lung and the mechanism of lung renewal to resolve, for example, the controversy of whether it is rest or controlled usage that enchances self-repair. The assessment and study of biocompatibility is of particular importance in extracorporeal circuitry, the development of standardised procedures of assessment being long overdue.

*Device development* calls firstly for system study aimed at identifying the components most damaging to blood in extracorporeal circuitry and secondly for the upgrading of all such components to equitable levels of effectiveness within, however, realistic cost limits corresponding to market demands. System elements requiring such critical evaluation and development are coronary suction devices, pumps and cannulae which provide the patient/machine interface links. In general, devices of lesser complexity and needing less anticoagulants than at present are the desirable goals to aim at.

Finally, *patient well-being* should be taken as the controlling criterion of therapeutic success, assessed by long-term (that is periods of months) monitoring in quantified terms following extracorporeal membrane oxygenation.

# GROUP 2. THE CLINICAL ROLE OF ADSORBENTS IN ACUTE AND CHRONIC THERAPEUTIC MEDICINE

## Introduction

Within the past 10 years, the clinical use of adsorbent materials has increased sharply. One factor influencing this increase has been the growing interest in blood purification by haemoperfusion over adsorbents and, at present, there are three commercially available haemoperfusion devices based on activated carbon granules and one based on resin particles. Other current interests include intestinal adsorbents, bound enzymes, portable and wearable artificial kidneys and hepatic support systems.

This report considers the acute and chronic clinical roles in three areas of application: drug overdosage, treatment of chronic renal failure, hepatic support.

## Drug Overdosage

This represents an increasingly frequent cause of hospital admission and a procedure for the rapid removal of drugs is desirable. Because of the ability of activated carbon to adsorb a wide range of materials and the availability of suitably prepared devices, haemoperfusion over granules of activated carbon or charcoal has received considerable clinical attention. The procedure is effective for the removal of many drugs, including barbiturates, ethchlorvynol, glutethimide, methaqualone, methyprylon and salicylate. When compared to other techniques for rapid detoxification, carbon haemoperfusion avoids the dangers and limitations of exchange transfusion and peritoneal dialysis and is superior to forced diuresis for the removal of barbiturates as well as the nonbarbiturate hypnotics. The cost effectiveness is greater than that of haemodialysis, since not only is haemoperfusion less complex and less expensive, but also, unlike haemodialysis, the technique is capable of satisfactorily removing drugs such as glutethimide, which are protein bound.

There are several factors governing the clinical use of carbon haemoperfusion for drug removal. The carbon must have a suitable affinity, and the haemoperfusion device sufficient adsorbing capacity, for a given drug. The technique will operate most efficiently when the drug is present in high concentration in the circulating blood or is in rapid equilibration with it. Carbon haemoperfusion is perhaps more problematical where a drug is mostly bound to tissue and equilibra-

tion with the circulating blood is low. Finally, it must be remembered that the majority of patients acutely poisoned by drugs recover physically without any special treatment, while most of the remainder are treated by standard supportive measures.

As an alternative to the use of activated carbon, there is resin haemoperfusion. One form of resin, Amberlite XAD-4, an uncharged, macroreticular, styrene-divinylbenzene copolymer, is particularly suited for the removal of lipid-soluble molecules. Haemoperfusion over this resin has given high removal rates for barbiturates, ethchlorvynol, glutethimide and methaqualone. As with haemoperfusion over carbon granules, the procedure shortens the interval of drug-induced coma and assists in restoring the vital functions of the body. Where charged resins are used and an ion-exchange capacity is introduced, suitable pretreatment of the device is necessary to prevent the loss of essential ions.

Haemoperfusion is capable of simplifying rapid detoxification. Even greater simplication would result from the successful clinical application of intestinal sorbents. However, competitive adsorption remains a major problem and the capacity of activated carbon is greatly reduced by the adsorption of lipids. The successful solution to this problem appears to depend on coating the adsorbent with a suitable, selective polymer membrane.

## Treatment of Chronic Renal Failure

While adsorbents appear to have a clear role in the acute situation of drug overdose, the role of adsorbents in the treatment of chronic renal failure is much less definite. The precise requirements for an adsorbent-based device cannot be specified since these are not known but, by analogy with haemodialysis, the device should be capable of removing a range of solutes and water while maintaining homeostasis.

Clinical experience of haemoperfusion over activated carbon granules has demonstrated that the procedure is as effective as that of haemodialysis for the removal of creatinine and uric acid and superior for the removal of solutes in the 'middle molecule' range, which may contribute to the symptoms of uraemia. Thus haemoperfusion might conceivably improve the condition of some patients who do rather poorly on dialysis. However, carbon haemoperfusion is unsatisfactory for the removal of water, urea, phosphate and ions. At present, in order to ensure satisfactory solute removal and to maintain acceptable water and electrolyte balances, haemoperfusion over carbon must be supplemented by the use of a dialyser or ultrafilter. The use of dialysis equipment, either in series with perfusion or as a separate, alternate treatment, militates against the inherent simplicity of the perfusion technique and reduces its cost effectiveness, particularly with respect to high efficiency, short duration haemodialysis. The use of carbon perfusion for chronic treatment as opposed to acute, rapid detoxification role means that even greater care must be given to potential blood damage or toxicity. With devices based on activated carbon granules, there is no common

approach to the problem of achieving the desired objectives of acceptable blood compatibility, limitation of small particle generation and adequate solute removal rates. Current procedures include coating the granules with a hydrophobic polymer which can tightly bind a coating of albumin; coating with a hydrophilic polymer and finally, the use of immobilised, uncoated carbon. Consideration must also be given to the fact that both creatinine and uric acid undergo chemical conversion in the presence of certain grades of activated carbon. In view of the fact that perfusion has to be combined with dialysis or ultrafiltration, it would seem appropriate to examine in more detail carbon in fibre or fabric form as a means of improving device design. With regard to the problem of urea removal, the successful application of an intestinal sorbent, such as oxystarch, would perhaps be preferable to more complex approaches involving enzymes and preconversion of urea into ammonia.

The role of adsorbents in the treatment of chronic renal failure is not limited to straightforward detoxification. Adsorbents offer the possibility of simplifying the problem of dialysate supply to give dialysate regeneration, portable and, ultimately, wearable artificial kidneys. There is, at present, one dialysate regeneration system commercially available. This utilises activated carbon, urease, zirconium phosphate and zirconium oxide. Less complex alternatives have been proposed based on activated carbon and alumina or activated carbon alone. A consequence of omitting urease and zirconium phosphate is an increase in predialysis urea concentration levels and patient well-being must influence the adoption of such an approach. As with haemoperfusion, the development of satisfactory adsorbents for urea and phosphate is very important and this development will determine the rate of progress from dialysate regeneration and a portable artificial kidney to a truly wearable device.

## Hepatic Support

The complexity of the liver rules out the possibility of a simple perfusion procedure providing long-term hepatic support. However, adsorbents appear to have a role to play in assisting the detoxification function, particularly in the early stages of hepatic failure. Activated carbon haemoperfusion has been shown to be capable of improving the consciousness of grade IV hepatic coma patients and in some cases prolonging their survival. Other adsorbents are required to extend this detoxification capability and possibilities include the use of ion-exchange resins, entrapped-gel systems, microencapsulated enzymes and enzymes covalently bound to an artificial carrier. To provide active metabolic activity, the utilisation of liver tissue has been proposed. Activated carbon granules comprise the most highly developed adsorbent material and future emphasis must be placed on extending the limited clinical role of carbon haemoperfusion in hepatic support. Since there is no standard hepatic support system comparable to that of haemodialysis for the treatment of renal failure, the cost effectiveness of introducing adsorbent-based systems is, in this case, difficult to estimate.

**Summary**

Research at both clinical and laboratory levels is shown to be necessary in the following three areas.

*Basic medical understanding* of the problems, the treatment of which may be assisted by haemoperfusion over adsorbents. To quote one of the discussion contributors, 'with haemodialysers we were trying to design membranes that would pass things that we *did* not know; now with haemoadsorbers we are trying to evolve sorbents that could adsorb things the nature of which we *do* not know'. Activated carbon or charcoal emerged as the preferred sorbent because it is a 'broad spectrum' material in the same sense as the cellulose based dialysis membrane range. Once basic data is obtained which permits functional specifications for organs such as the kidney and liver to be determined in biophysical and biochemical terms, custom-built sorbent systems of definite specificity will rapidly materialise.

*Assessment of treatment effectiveness.* Survival or death is clearly no longer either an acceptable or viable form of assessment of treatment efficacy. Patient well-being, as measured by improvements in the quality of life, requires to be quantified. It is anticipated that future forays into such life quality assays will be based, in addition to biochemical parameters, on studies of cellular type function such as platelet behaviour, red cell turnover, nerve conduction velocities and others. Concurrent effort is also required to evolve internationally accepted standards for evaluating therapeutic devices and techniques. Associated with this, it is highly desirable to designate (and provide the necessary funds to maintain) national and international test centres to carry out comparative investigations of devices and techniques and to highlight 'best value for money'.

*New concepts and developments* are clearly anticipated. These will firstly come in the shape of hybrid devices combining the best features of say haemodialysis and perfusion. A number of such 'mixed' systems are in development. Secondly, significant advances are anticipated through the use of substances such as bound enzymes, antibodies and antigens. Finally, there are indications at attempts to provide a primarily biological solution to a biological problem by means of purpose cultured cells.

# GROUP 3. THROMBOGENESIS IN MEMBRANE OXYGENATORS AND ADSORPTION DEVICES.

## Introduction

Thrombus formation on foreign surfaces is a complex process initiated by two main mechanisms: platelet retention and coagulation. The final result is fibrin formation and loss of formed elements. It is unlikely that there will ever be an extracorporeal circuit available which will be able to function with the same characteristics as the vascular system. Thus, the general aim must be to understand the mechanisms involved in order to minimise thrombogenesis either by modification of the surface or by influencing the body responses, or both. The areas being researched consist of the mechanisms of thrombogenesis, covering platelet adhesion, protein adsorption, the influence of blood flow conditions on cellular elements; biocompatibility of artificial surfaces, their assessment and clinical performance. Recent forward moves in several of these areas have been reported at this seminar and are highlighted in the following sections.

## Platelet Adhesion and Protein Adsorption

Blood flow over a foreign surface initiates the immediate formation of a proteinaceous layer which is then followed by platelet retention. There are a number of possible mechanisms and factors that influence platelet retention, for example the increase of exogenous cyclic adenosine monophosphate (AMP) reduces platelet adherence as against adsorbed fatty acids which may cause enhancement of their adhesion. Blood peptides and chemicals leaching out of plastics (for example the plasticiser in PVC) are considered capable of enhancing platelet adhesion. The volume of platelets retained on the surface will probably affect the degree of thrombus formation. The detailed mechanism of this is not fully understood but adhesion, *per se*, and retention of aggregates together with the release reaction causing secondary aggregation are important.

When plasma comes in contact with a foreign surface, activation of factor XII takes place which in turn sets off the coagulation enzyme cascade, resulting in fibrin formation. The interaction of factor XII with other adsorbed protein has been mentioned in this seminar but requires still further elucidation.

There is no doubt that the retention of other cells, white and red, by a surface will augment thrombus formation. Little mention has been made of the role of

white cells although they have tremendous affinity for foreign surfaces and generate procoagulant activity. In their behaviour in extracorporeal circuits the leucocytes are very similar to that of platelets. Blood contact with the artificial materials of the circuit appears to activate a reaction (perhaps that of the complement system) leading to the trapping of leucocytes in the body. Red cells, of course, are rich in adenine nucleotides which, if released, will also influence platelet retention.

## Influence of Flow Conditions

Different surfaces will influence each of the above mentioned events and by different mechanisms will cause thrombus formation. Several attempts have been made to relate surface properties to aggregation: microscopic factors, wettability, surface tension, parameters which describe morphology (domain structures), molecular activity (presence of specific groupings at the surface) and shape. Most work has been concerned with the microscopic surface parameters but more emphasis will have to be given in future work to the morphological and molecular factors.

The effect of flow conditions appears to be aggregation and disaggregation of platelets depending on the magnitude of the shear stresses generated in the flow pattern. Thus for platelet rich plasma (PRP), the aggregating and disaggregating shear stress magnitudes are respectively 0.25 and 0.3 $N/m^2$. Mechanical damage to the platelets in PRP occurs when the shear stress exceeds a value of around 15 $N/m^2$, and serotonin is released from a shear stress value of around 5 $N/m^2$.

Platelets adhere in each other's 'footsteps'; metabolite release from adhering platelets is transported by the flow to other platelets making them adherance prone. Eliminating such platelet interaction by the appropriate flow pattern design is important in order to assist avoidance of the cascadic affects culminating in a form of clotting.

Extrapolation of the PRP derived threshold values to the circulation in actual extracorporeal devices is tenuous, the relevant values probably being significantly higher in the latter. Additionally platelet behaviour is known to change depending on the disease state (for example in hepatic failure).

The ideal biocompatibility test system should allow precise analysis of the pathophysiological events produced by the foreign material of interest; the realistic simulation of the clinical environment and situation in which the device will be used; and finally data analysis and access in an acceptably reasonable time and at minimal expense.

Such test systems may be *in vitro* – controllable and cheap but may not correlate with the clinical situations; *in vivo* – difficult to control and also expensive in time and money; animal *ex-vivo* – allows flowing blood to be used with controlled measurements and turnover studies leading to best cost effectiveness.

In the range of *in vitro* tests the utilisation of the release reaction (which occurs on simple contact with the foreign surface) has been suggested as an effectively

direct means of biocompatibility assessment. Another proposal put forward is the determination of the degree of denaturation of the plasma proteins for biocompatibility comparisons.

### Comments on Coagulation

*In vitro* tests on heparinised PRP and whole blood simulating clinical conditions appear to indicate that coagulation factors II, V, IX, X and XII are reduced in haemoperfusion over uncoated charcoal. Only factors II and X were reduced, however, and factor VII was observed actually to rise when the charcoal was polymer coated. The results obtained are also open to the interpretation that heparin used as an anticoagulant agent may actually enhance platelet retention. Clearly the ideal anticoagulant for extracorporeal circuitry is yet to be discovered.

An associated aspect of primary importance is the effect of thrombogenesis on the health and well-being of a patient subjected to prolonged periods of extracorporeal circulation. This is virutally unexplored and requires at least the collection and analysis of available clinical data.

### Summary

One major requirement of urgency is envisaged: development of an internationally acceptable and cost-effective technique for biocompatibility assessment. It was emphasised to seminar participants that Health Authorities worldwide are now looking for such test methods. Unless the clinicians and researchers directly involved contribute their experience and pool their knowledge to evolve suitable test regimens as soon as possible, they may find less useful or acceptable methods thrust upon them.

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*In order to publish these proceedings as soon as possible following the Seminar it was not found practicable to include a detailed index. However, most of the main entries can be found by using this abbreviated index in conjunction with the contents list.

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