DIETARY FIBRE:

Chemical and Biological Aspects

Edited by **D.A.T. Southgate, K. Waldron, I.T. Johnson and G.R. Fenwick**

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Preface

Whilst Hippokrates (460-377 BC) recommended the eating of wholemeal bread for its 'salutary effects upon the bowels' it is only in recent years that the potential health implications of dietary fibre have begun to be fully investigated. During this period, thousands of research papers, reports and articles have been published and many meetings organised.

The decision of the Food Chemistry Group of the Royal Society of Chemistry to hold a meeting on the chemical and biological aspects of dietary fibre (FIBRE 90) was a recognition both of the important role of chemistry in the study of fibre, and of the need for coherent, interdisciplinary research in this area. For these reasons, the meeting had the objective of bringing together workers in the chemical and biological fields, food technologists, representatives of industry and the regulatory authorities.

The advances of the last two decades have provided increasing insights into the chemical complexity of dietary fibre and have led to hypotheses being proposed to explain the resultant physiological effects of fibre-containing foods and products. The reasons why these hypotheses have been difficult to evaluate include the complexity and variability of the dietary fibre mixture, the difficulties of relating structure and physicochemical properties to physiological effects and, especially, the need to develop a proper conceptual framework to relate the composition of the dietary intake to indicators of health and disease.

This area, offering challenges to the scientist, opportunities to the food technologist and ingredient supplier, and benefits to the consumer, will continue to develop in the present decade. There will be a need, however, to facilitate the transfer of basic research data into the applied area, and to ensure that the public is kept fully informed of the real significance of fibre for health and wellbeing.

The editors are grateful to the Food Chemistry Group, to staff at the AFRC Institute of Food Research and Norwich City College who assisted in the planning and hosting of FIBRE 90. Particular thanks are due to the patience and support of Ms Catherine Lyall of the Royal Society of Chemistry. Above all the editors would like to thank all

those who attended FIBRE 90 - this volume is dedicated to
them.

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Foreword

The number of deaths in the UK directly attributable to food poisoning was over 200 and during the past year, the fear of food poisoning in the UK has almost overtaken sex and football in its extent of media exposure. Salmonella, listeria and poisonous shellfish are a few of the examples that have made the headlines.

This represents, however, only one part of a wider picture. Over the past few decades there have been profound changes in lifestyle and diet of individuals, especially in the Western World. This has come about through new technology and increased affluence that enables consumers to change their eating habits to meet individual tastes and choice.

It is therefore something of a paradox that relatively little publicity is devoted to the increasing numbers of people who now die each year from cancer and circulatory disease which can be directly attributed to diet and lifestyle. In England and Wales alone, the numbers amount to 250,000 each year! Only Ireland, Scotland, Finland, Czechoslovakia and Hungary have higher figures. In Japan the figure is lower by a factor of 10. Why is this? What are the biological mechanisms? How precisely does food control our health? Our understanding of these links is primitive in the extreme. This conference, which follows the highly successful meeting on Bioavailability in 1988, provides a platform for contributing to that understanding and making a real contribution to health, quality of life and the associated role of diet.

Professor P. Richmond, Royal Society of Chemistry, Food Chemistry Group.

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Dietary Fibre, Health and the Consumer

CONCEPTS OF DIETARY FIBRE

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Hellendoorn called dietary fibre an abstraction.[1]
Perhaps he went too far but it is certainly better to
think of fibre as a concept than as a substance. Indeed
it is a series of concepts, each with its own validity
and its own limitations. The term dietary fibre is
simply a convenient shorthand expression which covers
a wide variety of conceptions.

Trowell traced the history of the fibre hypothesis
and of fibre definitions in great detail.[2] His own 1972
definition of dietary fibre as "that portion of food
which is derived from the cellular walls of plants which
is digested very poorly by human beings" played an impor-
tant part in stimulating scientific thinking on the sub-
ject. The term dietary fibre had been coined in 1953 and
the idea of plant cell walls being indigestible was far
from new, as witnessed by the term unavailable carbohy-
drate, but the conjunction of a reasonably appropriate
name and a definition that seemed clearcut, physiological
and relevant was irresistible at a time when speculation
was running wild. The term and the definition have faced
no serious competition till 1990. But in 1990 the
British Nutrition Foundation's authoritative report will
advocate the term dietary fibre be dropped in scientific
discourse. Instead it will urge us to refer to the mat-
erial in question in precise terms like wheat bran, is-
paghula husk, citrus pectin, guar gum, carboxymethyl
cellulose etc.

This is fine in terms of describing experiments but
it is highly limiting in terms of hypotheses linking dis-
eases to eating habits. Yet it was the publication of
such hypotheses by Cleave[3] and Burkitt[4] in 1969 which set

off the modern wave of interest and research into diet-
ary fibre. The striking characteristic of these hypo-
theses was their enormous scope and potential. Perhaps
they were overambitious, and it is easy now to smile at
them as naive, but at least they widened people's hori-
zons. The new substance-orientated approach seems more
scientific but if it involves us putting on blinkers, we
could end up getting lost. Horses with blinkers don't
see road signs!

Cleave's concept - "what God hath joined together let no man put asunder"

Cleave was one of the first people to advocate raw
wheat bran as a laxative but it is quite wrong to talk
of his ideas as the bran hypothesis. What he advocated
was not that we add fibre to our food but that we stop
taking bran and other fibrous elements away from our
food.[3,5] In other words he indicted man-made fibre-
depleted foods, chiefly sugar and white flour, as caus-
ing disease. He called these foods refined carbohy-
drates but what he meant was foods from which a fibre-
rich fraction had been artificially split off. Such
foods, like broken marriages, cause all kinds of trouble.
Firstly, they rob the large intestine of the natural
stimulant to its activity and so cause constipation and
all the ills which go with constipation. Secondly, by
being too easy to eat and digest they encourage overnut-
rition or, as we might term it, excess energy intake and
so cause obesity and all the ills which go with obesity.
Thirdly, when refined foods are eaten, sugars enter the
bloodstream abnormally fast (hence his term saccharine
disease, meaning sugar-related) and this leads to hyper-
glycaemia, diabetes and coronary heart disease. Many of
Cleave's ideas were crude but they were supremely logi-
cal and fitted with the broad patterns of disease and of
eating habits in the world as far as they were known at
that time. Actually the epidemiology of chronic dis-
eases was then in its infancy and nutritional epidemiol-
ogy had not been invented. Both received a great stim-
ulus from the Cleave and Burkitt hypotheses. Too often
Cleave is not given credit but it was he who galvanised
Burkitt, Trowell, Kritchevsky and Avery Jones, and many
less famous people.

Burkitt's concept - "nature's laxative"[4,6,7]

Burkitt extended and popularised Cleave's ideas but
emphasised the protective value of fibre as opposed to
the harmfulness of fibre-depleted foods. This more

"positive" approach had great appeal to the public and provided scientists with apparently easy hypotheses to test, but it also distorted some people's perceptions. Bran came to be seen as a panacea and the malnutritious aspects of sugar and white flour were overlooked.

Burkitt produced a coherent and still attractive hypothesis linking large bowel cancer to small, concentrated, slowly moving colonic contents.[8] He also produced plausible arguments blaming straining at stool for hiatus hernia, haemorrhoids and varicose veins, though these ideas are still unproven and not widely held. Appendicitis "had to" be due to caecal stasis and blockage of the appendiceal lumen by inspissated contents;[9] but proof is still lacking and the epidemiology fits better with a "hygiene hypothesis"[10].

The central feature of Burkitt's message was the danger of a small faecal output, so characteristic of western societies. The essence of his concept of fibre was nature's laxative. Here he seemed to be on safe ground. No-one disputes that fibre bulks the stools. People have even published regression equations relating stool output to fibre intake, e.g. 24 h stool weight = 5.2 x dietary fibre intake + 48.[11]

Collecting and weighing stools may be simple in theory but it has seldom been done as an epidemiological exercise. As a result there are very few data relating the stool weights of populations to their risk of disease, indeed no prospective data whatsoever. So, Burkitt's hypothesis remains largely untested.

It has, however, emerged that within a British population variation in fibre intake accounts for only a small proportion of the variation in stool weight,[12] that within an American population, personality factors account for as much variation in stool weight as fibre intake,[13] and that some constipated people do not respond to fibre.[14] So it is not surprising that some iconoclastic people feel constipation is largely independent of fibre intake.[15] This may be true within a population eating generally small amounts of fibre but the fact remains that groups, like vegetarians, who eat more than the average amount of fibre, have bulkier faecal outputs[16] and it must be the case that, were Britons to double their fibre intake, the average British stool would be heavier and softer. Whether this would translate into fewer Britons complaining of constipation is less certain. Some of the elements of constipation are

symptoms of irritable bowel syndrome (abdominal discom-
fort relieved by defecation, unproductive calls to stool
and feelings of incomplete evacuation) and many cases of
IBS are stress-related, not diet-related.[17]

So, the concept of fibre as the answer to constipa-
tion and its associated ills is true but not the whole
truth.

Trowell's concept - "the colon's portion"

In 1972 a definition was what fibre needed to be
scientifically respectable but it soon became apparent
that Trowell's definition of indigestible polysaccharides
and lignin[18] was too restrictive. It had long been known
that starchy foods gave some people diarrhoea, colic and
excess flatus.[19] In 1981 it was reported that even
normal people malabsorb some of the starch in white bread
and macaroni[20] and, on direct measurement, this unabsorbed
fraction was found to amount to 10% on average, ranging
from 2 to 20%.[21] Since, even in the West which has
largely switched from starchy staples to fatty and sugary
foods, average starch intakes are ten to fifteen times
higher than average fibre intakes, it was immediately
apparent that of the polysaccharides entering the colon
half or more must be starch. At the same time it was
becoming accepted that the faecal bulking effect of fibre
(and other laxatives) was in large part due to the fer-
mentation of undigested polysaccharide in the colon with
consequent multiplication of colonic bacteria and an
increase in the bacterial component of faeces.[22,23]
Since starch too is fermented by colonic bacteria it is
likely that undigested starch has similar effects on the
colon as fibre. The scanty data available suggest this
is the case[24] but much more research is needed.

So indigestibility is no longer a secure foundation
for a definition of fibre. The Trowell definition has
had its day.

Various factors reduce starch digestibility - coarse
particle size with seeds like cereals and pulses,[25-27]
roasting and toasting, cooling after cooking (with pota-
toes)[28] and of course eating raw or unripe foods.[29,30]
All of these donate fibre-like properties to foods and
need to be put into the equation if one is trying to re-
late polysaccharide intake to human physiology and dis-
ease. It is a daunting task. In the meantime, knowledge
of these complexities should deter us from expecting
simple or close relationships between fibre intake and

disease incidence in different populations.

The structural concept - "prison walls"

Look at a photomicrograph of any plant food in its raw unprocessed state and what you see is a cellular structure. The nourishment is inside the cells. It is imprisoned within the cell walls. Plant-eating animals except herbivores have to break down these walls, that is disrupt the fibre, in order to obtain nourishment. The tools they have to do this are their teeth and the antrum of the stomach (the muscular distal third) which, respectively, chop up and churn up the food until it is reduced to particles of 1-2 mm or less. The wall-breaking is clearly incomplete but presumably this does not matter since teeth and antrum are all that is available to do the job - except in the case of man who has learned to cook roots and grind seeds, that is, to mill cereal grains. But milling was a crude process until at least the 17th century.

So man, like all other plant-eating animals has, until modern times, eaten his food with a largely intact cellular structure which is only partly destroyed by the time the food is discharged still in particulate form into the small intestine.

There is abundant evidence that the intact cellular structure of foods delays or impedes digestion and absorption. This has been shown in terms of reduced glycaemia and insulinaemia with apples,[31] oranges,[32] raisins,[33] lentils,[34] rice,[26] wheat,[25] maize,[25] and rye.[35] It has also been shown in terms of reduced fat absorption with peanuts.[36] There seems little reason to doubt this is a general law, though there are apparently odd exceptions, like grapes[32].

If slow or incomplete absorption and muted insulinaemia are the norm then the more rapid absorption and higher insulin levels which result from eating fibre-depleted <u>and</u> fibre-disrupted products must be abnormal.

Tearing down walls between nations is obviously a good thing. Tearing down the walls nature has put in our food may be less desirable. Insulin has wide-ranging effects on metabolism and hyperinsulinaemia is increasingly being linked with major western diseases - hypertension, atherosclerosis, gallstones and renal stones.[37]

Conclusion

Dietary fibre is not a substance or even a collection of substances. It is not a concept but a spectrum of concepts. In planning an experiment it is usually necessary to focus on one very restricted manifestation of fibre but in interpreting one's observations it is important to take off one's blinkers and look at fibre in as broad a way as possible. Undigested starch and intact cellular architecture may turn out to be more important than dietary fibre as normally defined. A diet which is naturally rich in starch and intact cell walls is likely to have other benefits - it will be rich in micronutrients and potassium and low in fat, sugar and sodium. Such a diet combines virtually all the internationally accepted guidelines to healthy eating.

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DIETARY FIBRE AND HEALTH

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1 INTRODUCTION

Many of the details of the physiological effects of
dietary fibre that are relevant to a discussion of the
relationships between the consumption of dietary fibre and
health have been discussed in earlier papers and I would
like to draw them together and try to present an overview
of the current evidence.

The topic of dietary fibre has raised a large number
of questions since it was first suggested that the intake
of dietary fibre was related to the aetiology of a range
of chronic disease states. In any discussion of the topic
I think that it is useful to start from the original
hypothesis that developed from the writings of Burkitt and
Trowell in the early 1970's[1].

This hypothesis contained two separate propositions;
First, that diets that were rich in plant foods containing
plant cell wall material were protective against a number
of diseases whose prevalence was higher in Western
developed communities. The diseases included obesity,
diabetes, coronary heart disease and some cancers. The
second proposition was that diets poor in these foods were
causative for some conditions particularly those that were
related to the development of excessive abdomenal
pressures: diverticular disease, haemorrhoids, and
appendicitis, and that for other diseases provided the
conditions where other aetiological factors were active[2].

The hypothesis therefore related to the effects of
types of diets and the differences in the dietary fibre
contents of these diets were identified as the key factor;

primarily because plausible subsiduary hypotheses could be
developed regarding possible modes of action.

Following this line dietary fibre research focussed
on the study of the specific component; dietary fibre and
many important physiological effects of dietary fibre
(which I am using in the sense of plant cell wall
material) have been identified as a result of this
research.

The focus on dietary fibre per se, while valuable in
itself, has diverted attention away from the central
"dietary" hypothesis. This is because the protective
diets which formed the basis for the observations that led
to the original hypothesis differed in many other
attributes in addition to the amounts of plant cell walls
they contained[3].

The observed physiological effects of a dietary
fibre-rich diet are an integration of the effects of the
diet itself and any specific effects due to the dietary
fibre component. It is this integration that I would like
to explore in discussing the relation between dietary
fibre and health.

This approach raises some fundamental nutritional
concepts that apply to all aspects of the relationships
between diet and health and the aetiology of disease.
This is not an obscurantist view that sees the diet as a
whole as a mystical concept but merely a reflection of the
fact that the nutritional/physiological effects of any
diet are an integration of the effects of many variables.
The composition of the diet varies in a multidimensional
domain; defined by a large number of food dimensions and a
range of dimensions that are defined by patterns of
consumption[4].

This is of particular importance in any discussion of
the differences between dietary fibre-rich diets and
dietary fibre-poor diets. The concentrations of plant
cell wall material in many plant foods are low and
therefore the dietary fibre is invariably associated, in
the foods, with many other components; starch in cereal
foods and potato, and a myriad of other components found
throughout the plant kingdom; pigments, vitamins,
inorganic constituents and a range of biologically active
components such as glucosinolates, glycoalkaloids and
flavonoids[5].

In epidemiological studies of populations there are

therefore many confounding variables that are not strictly
independent of dietary fibre since they are present in the
foods which provide the dietary fibre. Epidemiological
studies in themselves, can only form part of the
evaluation of the dietary fibre hypothesis[6].

Limitations of Epidemiological Studies

There are many limitations of epidemiological studies
of the relation between diet and disease that are of a
general nature. The most important of these is the
uncertainty concerning the validity of measurements of
food intake, especially habitual food intake over long
periods of time by free-living populations, where it is
probable that a nutritional "uncertainty" principle
applies since it is impossible to measure habitual food
intake accurately.

The second relates to the translation of food intake
data into nutrients especially by calculation from food
composition tables or nutritional databases where the
natural variability of foods places a limit on the
predictive accuracy of the calculated intakes[7]. This has
been particularly difficult in the case of dietary fibre
where the complexity of dietary fibre, the controversy
over its definition and the consequential debate over the
analytical measurement of dietary fibre have served to
delay critical studies of intake in populations with
differing patterns of disease incidence and prevalence[8,9].
Thus 20 years after the original hypothesis was proposed
we are only now in a position to compute intakes of the
non-starch polysaccharides (a good index of plant cell
wall material that characterises the composition of the
components)[10] in a few countries and total dietary fibre[11]
intakes in a few more. The studies that have been carried
out have been limited in number and in their capacity to
relate the types of dietary fibre consumed to the
incidence of the diseases in question.

At the present time the epidemiological evidence for
coronary heart disease and colorectal cancer shows
negative association between intake and incidence, that
is, one that is consistent with a protective effect[12]. The
crucial test at the epidemiological level requires the
demonstration of a high incidence of disease associated
with a high level of intake; such evidence would provide
refutation of the hypothesis. One must therefore seek
evidence for mechanisms of action and evidence from
intervention.

The Physiological Effects of Diets Rich in Dietary Fibre

The study of these effects and relating them to the aetiological mechanisms of specific disease states is a central part of the evidence for a relation between diet and disease. Many of the diseases linked to dietary fibre are strictly of unknown aetiology and the dietary fibre studies may, in themselves, be of great value in establishing the aetiological processes because dietary fibre provides a powerful experimental tool for modifying conditions in the small and large intestines and for modifying the metabolism of carbohydrates and lipids. The experimental studies with isolated polysaccharides as "models" of the components of plant cell walls and plant cell wall preparations have given new insights into the determinants of digestion in the small intestine and to the physiology of the colon, as the other papers in the symposium have shown. But in many cases, for sound experimental reasons, these studies are, de facto, studies of the pharmacology of polysaccharides; because isolation of the polysaccharide involves separating the polysaccharide from the food matrix and from the physical structures of the plant cell wall and there is substantial evidence that this alters the chemical and physical properties of the polysaccharides[13].

Modes of Action

There is substantial evidence that diets that are rich in dietary fibre exert effects throughout the intestinal tract and that some of these effects are specifically due to the dietary fibre present in the diets and others to the diet, the mixture of foods consumed.

Effects on Ingestion

These effects are related to the diet because the foods making up the diet possess specific and characteristic textural and physical properties that modify acceptability and patterns of ingestion. Fibre-rich diets are characteristically more bulky, that is their density is lower, and they may have lower energy densities, kcal per gram, because of the lower fat intake and the higher water content of many plant foods. The ingestion of isocaloric intakes of dietary fibre-rich and dietary fibre-poor diets will therefore require very different amounts of foods in total; this is especially true for the traditional diets eaten in the third world. Experimentally, using semi-synthetic diets and isolated fibre sources these dietary features may not be observed.

Dietary fibre rich diets containing relatively unprocessed foods also require considerable mastication before they can be swallowed attenuating the rate at which they can be eaten.

It is not established with certainty that these properties act to reduce voluntary food consumption; and experimentally the design of studies to test these effects are extremely difficult because of the difficulty of disguising the diets that differ in these properties.

Gastric Effects

The bulky diets, usually rich in starchy foods, hydrate with the gastric secretions and produce increased gastric bulking. The predominately insoluble plant cell wall materials are emptied more slowly from the stomach. Whether these effects are of physiological significance remains to be established with diets but the studies with some viscous polysaccharides suggests that a satiating effect may arise from these bulking effects.

Small Intestinal Effects

Studies with isolated polysaccharides show that some, particuarly the soluble, viscous polysaccharides, have effects on the rates of absorption of water-soluble nutrients such as glucose and amino-acids and in reducing fat absorption[14]. The effects of diets may thus be expected to depend on the proportion of the dietary fibre that can be solubilised in the small intestine and that has the capacity to modify the physical properties of the intestinal contents. Assessing the importance of this feature of the diets in epidemiological studies depends on a proper characterisation of the dietary fibre present in the diet. Most studies of the dietary fibre consumption in different population groups have not characterised the diets sufficiently to establish whether or not the diet contained sufficient of the particular fractions to elicit these types of effects. Some studies with a limited range of foods show that the integrity of the plant cell wall structures is important in determining the rates of sugar absorption[15], an effect that may be due to the steric effects of the cell wall structure in delaying difusion to the absorbing mucosal surfaces. Some starch digestion is also slowed or inhibited by the cell wall structures which have remained intact in the foods, thus legume starches are more slowly digested because of the thicker, more resistant, walls they possess in comparison with potato or cereals[16].

Other fractions of the starch may also be resistant to enzymatic hydrolysis in the small intestine because of physical changes or characteristics, especially those resulting from heat processing; retrograde amylose appears to be an important part of this resistant starch, although other forms of starch appear to escape digestion in the small intestine[17].

As small intestinal digestion proceeds the dietary fibre materials become more important quantitatively in the contents and binding to functional groups exposed during digestion or adsorption to the surfaces of the cell wall materials can occur. In vitro binding of many divalent cations and bile salts has been observed but these effects have been difficult to establish in vivo with high-fibre diets; this apparent anomaly may be due to the fact that the preparation of the fibre sources for the in vitro studies exposed functional groups not exposed during in vivo digestion or that the binding effects were dissipated by bacterial degradation in the large intestine. The studies with ileostomists suggest that the latter explanation is more probably correct[18].

Thus it is evident that the small intestinal effects will depend on the composition of the dietary fibre; the types of polysaccharides present and their physical properties. These will be determined by the mixtures of foods consumed. In addition there are effects of the integrity of the plant cell wall structures present which alter rates of solution and diffusion and therefore release of nutrients. The consumption of a high fibre diet will therefore produce effects over and above those seen in studies of isolated dietary fibre components.

Large Bowel Effects

Dietary fibre forms a major part of the organic matter entering the large bowel although it is accompanied by intestinal secretions including proteins and mucus, probably some amino acids and lipids. The traces of other indigestible substances present in the diet, salts, pigments, mineral oil and such like, will also be present[19]. These materials are mixed with the contents of the caecum and provide the microflora with substrates. A high dietary fibre intake will thus present the bacteria with carbohydrate substrate and may shift metabolism to a condition where the supply of nitrogen will be a limiting factor for bacterial growth. The fermentation will produce short chain fatty acids, carbon dioxide, methane and hydrogen and produce a substantial modification of the

physico-chemical environment. Undigested material will
contribute bulk, which together with the bacterial mass
arising from the fermentation of the carbohydrate
substrates, is responsible for the increased faecal matter
characteristic of an increased dietary fibre intake[20].

The extent of fermentation is dependent on the
composition of the dietary fibre ingested especially the
extent of lignification and cutinisation or suberini-
sation. The particle size is also an important
determinant because the bacterial attack is a surface
effect so the surface area of the dietary fibre particles
is a critical feature.

The physical properties and fermentability of dietary
fibre also have some direct effects on mucosal structure
and function where some isolated polysaccharides increase
cell turnover and the maturation of mucosal enzyme
systems.

Relation between Modes of Action and the Aetiology of Disease

The physiological effects of dietary fibre and some
specific polysaccharides are generally consistent with the
postulated protective effects although it is not possible
to construct a generalised hypothesis that applies to
dietary fibre as a whole. All the observed effects are
dependent on the composition and physical properties of
the polysaccharides present and most are modified by the
physical organisation of the polysaccharides into cell
wall structures[16]. It is not possible to predict with
certainty the relation between structure, properties and
physiological effects, although it is possible to make
some tentative statements, for example, the effects on the
rate of absorption of glucose and on serum cholesterol
levels seem to require soluble components that are capable
of forming viscous solutions. Faecal bulking effects
appear to be most strongly associated with dietary fibre
sources that are insoluble, poorly fermentable and with
good water-binding capacity. Although virtually all
sources produce some increases in stool weight the
increases with highly fermentable soluble forms are
usually small.

Direct evidence of prevention of diseases such as
cancer produced by chemical carcinogens is equivocal, and
some studies show enhanced tumour formation in the
presence of some polysaccharides. In these types of study
the tumour rates are very high and it is possible that any

protective effect can be overcome by very active
initiators, alternatively the studies using isolated
sources may not contain protective substances associated
with the dietary fibre in the plant.

High Dietary Fibre Diets : The Constraints Imposed by the Composition of Foods

The foods forming diets that are rich in dietary
fibre exert a number of effects that are relevant to the
discussion of the relation between intake and health or
the incidence of disease.

Firstly, the foods chosen determine the types of
polysaccharides present in the diet. Most plant foods
contain a range of polysaccharides. In general the
pattern of polysaccharides present is characteristic of
the major groups of plant foods and reflect the types of
tissues and especially the maturity of the cell walls
present[16]. Cereal foods are usually consumed in a mature
state so that the levels of pectic substances are low and
the soluble components are principally arabinoxylans in
wheat, rye and barley with substantial contributions of
beta glucans in oats. The insoluble components contain
arabinoxylans, xyloglucans and cellulose. In fruits and
vegetables the cell walls are usually less mature and the
soluble components are pectic substances and arabino-
galactans and the insoluble components include arabino-
galactans, galacto- and gluco- mannans, xyloglucans and
cellulose. The diets therefore contain a complex mixture
of soluble and insoluble polysaccharides and therefore the
effects of the diet will depend on the proportions of the
different foods present and not on the absolute amounts of
total dietary fibre present.

The concentrations of dietary fibre in the various
food sources vary considerably and the ratio of dietary
fibre to energy has implications for the amounts of
different foods present in high fibre diets[4]. Thus an
intake of say 30g per day requires a dietary fibre: energy
ratio of 1.2 g DF per 100 kcal in a intake of 2500 kcal
and this cannot be achieved with refined cereal foods
alone, the presence of vegetables providing between 13 and
3 percent of the energy is essential and this diet would
only meet the target if virtually no fibre-free foods were
eaten. Thus a high fibre diet for practical reasons
should include some high extraction cereal foods and a
substantial contribution from other plant foods, fruits
and vegetables. The implications of these quantitative
constraints are that a high fibre intake requires a

different choice of foods and that the inclusion of a
substantial proportion of the energy intake from vegetable
sources permits a greater choice of foods that are low in
fibre or fibre free. This also implies that a high-fibre
intake is invariably associated with intakes of a wide
range of other components of plants and of these the anti-
oxidant vitamins, vitamin C and the carotenoids and other
bioactive components in plants may be the principal reason
why these diets are protective against cancer. The
epidemiological evidence for many cancers strongly suggest
a protective effect of vegetable consumption[21].

The Protective Effects of Dietary Fibre

At the present time it is not possible to say that
the protective effects of a high intake of dietary fibre
have been established unequivocally, the observed
physiological effects are generally consistent with the
protective hypothesis and there is no real evidence that
refutes it. In addition to specific effects of the
polysaccharide components there are additional effects due
to their consumption in plant cell wall structures.

The diets that provide increased intakes of dietary
fibre are different in many ways from diets that are low
in dietary fibre and it is clear that dietary fibre may be
a "marker" that identifies diets that contain other
protective properties[22].

The ultimate test of the dietary fibre hypothesis
involves taking up the challenge of relating the
composition of the diet, as a whole, to the aetiology of
the disease processes and understanding how the various
effects are integrated; only from these studies can the
evidence be drawn to provide sound guidance on the choice
of diets to minimise the risks of chronic disease.

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1. Is there any evidence that existing 'fibre'
recommendations have had any effect up to now?

Minimal changes despite widespread, consistent, advice by
expert committees.
BUT REMEMBER
(i)   that total energy intake has declined during this
        time - so there has been a relative increase
(ii)   the types of food eaten have changed in the
        direction of 'fibre' rich alternatives e.g.
        wholemeal and bran-enriched breads, high fibre
        cereals
SO
        Our gradual change in eating habits and the food
        industry's response to them, might eventually show
        up in National statistics.

2. Does the scientific evidence justify giving any
'fibre' recommendations to the consumer at all? If so,
for what?

(a)   There are three expected benefits from consuming
        'fibre' rich foods:
(i)   improved large bowel function
(ii)   a strategy for lowering dietary fat intake
(iii)   slower utilisation of sugar and fat.

(b)   The very best evidence i.e. from epidemiology, human
        trials and animal studies for 'fibre' alone is for
        the prevention and treatment of constipation. Its
        role in the management of diabetes is fairly firm
        but epidemiological data are lacking. The role in
        the prevention of bowel cancer is better shown

epidemiologically than in trials. Its role in
cardiovascular disease is suggestive, but not
proven.

3. Does the scientific evidence justify giving a numerical target for desirable 'fibre' intake? If so, what should it be? How could it best be used?

(a)   Meta-analysis of stool weights in relation to
       constipation, bowel cancer and diverticular disease
       would suggest that target stool weight should be
       150g/day with a minimum of about 130g/day. This
       would correspond to a dietary 'fibre' intake of
       about 30g/day (or 18g NSP). Next step? Relate to
       energy intake?

(b)   Recommendations with even 'soft' quantitation (e.g.
       eat foods with adequate 'fibre') are too ambiguous
       and advice should be based on more food orientated
       guidelines e.g. choose a diet with plenty of
       vegetables, fruits and grain products.

(c)   Setting a quantitative target can only really be
       useful as 'part and parcel' of a major national
       campaign monitored with feedback to the consumer as
       part of the education plan.

(d)   Quantitative targets are more useful to the
       educators and policy makers than the consumer (cf.
       RDAs).

4. Are fibre recommendations applicable to all sections of the population?

(a)   Vulnerable groups are those with high energy
       requirements (e.g. children, pregnant women) or low
       energy intakes (e.g. the elderly, the slimmers) or
       those where plant materials form the major part of
       the diet. These groups are rarely addressed in
       standard recommendations and might be least
       receptive to the message anyway.

(b)   Micronutrients most at risk are calcium, iron, zinc
       and vitamin D because of 'fibre'-associated
       antinutrients such as phytate.
BUT
       These concerns could be counterbalanced by:
       (i)   the levels of many micronutrients 'per se' are
              higher anyway in 'fibre'-rich foods
       (ii)   the body's ability to adapt to the situation

e.g. long-term vegetarians?

and   (iii) many of the 'in vitro' effects of 'fibre' on
              micronutrients cannot be shown 'in vivo'.
so
        (i)   Ensure that 'vulnerable groups' are given
              advice in terms of real foods not 'ad hoc'
              supplements (e.g. not sprinkling bran on
              everything!)

and   (ii) Encourage, but don't force, children, to eat
              'fibre'-rich foods at an early age.

5. Should 'fibre' be given as a single value in nutrition
labelling on food products? If not, what should appear
and how should it be defined?

NO, it should not be given as a single value because:
(i)   there are many different types of 'fibre' which can
       have such a wide variety of properties (e.g.
       regulates bowel function, controls blood glucose
       levels, induces satiety, etc.). Different consumers
       have different needs.
(ii)   there are such difficulties with defining and
       analysing fibre in a consistent manner.
(iii) it could be compared with lumping all the vitamins
       together as a 'vitamin index' and not acknowledging
       that different vitamins have different roles.
(iv)   it is open to abuse by unscrupulous manufacturers
       who want to 'sell' useless 'fibre'.

YES, it should be given as a single value because:
(i)   consumers could not cope with the concept of
       different types of fibre
       e.g. bowel regulatory fibre
             cholesterol lowering fibre
             blood glucose controlling fibre
(ii)   too much detailed information on the food label
       could cause problems if it has to be changed at a
       later date. Scientific credibility could be lost.

FINAL DECISION
Leave as single value (reluctantly) until research is more
advanced.

6. Should 'fibre' health messages be allowed? If so,
where should the line be drawn? How can health messages
be regulated and monitored?

(a) YES, they should be allowed because
        (i)   they inform the consumer, whose awareness of

diet and health has never been higher
- (ii) they ensure the development of 'healthy' products
- (iii) they reach a much wider audience than is possible by any other means.

(b) NO, they should not be allowed because:
- (i) they can create a false distinction between 'good' and 'bad' foods and negate the importance of diet as a whole.
- (ii) they have the potential to mislead in the hands of less reputable manufacturers.
- (iii) they give a distorted image if only applied to packaged foods.

(c) Ideally, health messages would be more acceptable to the scientific community if they concentrated on specific physiological functions, e.g. the percentage decrease in the glycaemic index or the absolute increase in stool weight. Not surprisingly, industry and, we suspect, consumers would not be too enthusiastic!

(d) All agree the importance of obtaining 'peer' review and approval before media publicity or pressure groups make demands, but admit that this would be amazingly difficult to achieve!

Chemistry of Dietary Fibre

THE CHEMISTRY OF DIETARY FIBRE - AN HOLISTIC VIEW OF THE CELL WALL MATRIX

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1 INTRODUCTION

The role of dietary fibre (DF) in human nutrition remains a topical problem. DF was initially defined as the skeletal remains of plant cells that are resistant to the digestive enzymes of man[1] and was later extended to include polysaccharides used as food additives. This made the definition, 'all the polysaccharides and lignin in the diet that are not digested by the endogenous secretions of the human digestive tract'[2]. Accordingly, for analytical purposes, the term DF refers mainly to the non-starch polysaccharides (NSP) and lignin in the diet and methods have been developed to quantify these components in foods,[3-5] for use in food labelling and food composition tables. However, less attention has been given to the dynamics of the DF matrix as a structural and functional entity during gut transit, although this is an integral part of the DF hypothesis. The fibre hypothesis in outline states that, 'a diet rich in foods which contain plant cell walls (eg high extraction cereals, fruits and vegetables) is protective against a range of clinical disorders, in particular those prevalent in western society, and in some instances a diet low in sources of fibre is a causative factor in the aetiology of the disease and in other cases provides the conditions under which other aetiological factors are more active'[6]. The implication is that the protective effects of DF are due to the amount of cell wall material (CWM) in the diet and the protection is derived from the physicochemical properties of the fibre. Thus, methods which measure only the amount of DF in foodstuffs do not adequately explain the functional role of fibre in the diet since no account is taken of how the fibre matrix is affected by processing and has its

properties altered by the digestive environment during gut
transit[7,8]. For example, in the proximal gastrointestinal
tract (stomach and small intestine), although fibre is
resistant to digestion by endogenous secretions it may be
susceptible to changes in solubility. In the large intes-
tine a significant proportion of the fibre may be fermen-
ted and fermentation may be influenced by fibre 'condi-
tioning' in the proximal intestine[9]. Thus, it is apparent
that an understanding of the chemical composition of DF in
relation to the structure and function of the component
biopolymers in the cell wall of the various tissue types
common to plant foods is required for a better apprecia-
tion of the role of DF in the gastrointestinal tract. The
factors involved in the structure and function relation-
ships in DF are outlined in Figure 1 and will be elabor-
ated in the text. Particular attention is drawn to these
factors which have been previously only superficially or
inadequately considered.

Tissue Structure

Vegetable, fruits, legume seeds and cereals are all
sources of DF and are each composed of different tissue
types, as illustrated for de-seeded mature runner bean pod
and the outer layers of the wheat grain (mainly bran) in
Figure 2. The figure also indicates the major biopolymer
types that have been isolated from the cell walls of the
different tissues by detailed chemical fractionation and
illustrates the distinct differences between parenchymal
and lignified tissues. Cell wall biopolymers are pre-
dominantly NSP but also contain significant amounts of
glycoproteins and phenolics, including lignin in tissues
where secondary thickening has occurred. The chemical
composition and major structural features of cell wall
polysaccharides used as sources of DF have been discussed
extensively[4,8] but less attention has been given to the
types of bond and degree of cross linking between consti-
tuent biopolymers in the DF matrix. Each source of DF can
be considered to comprise epidermal, parenchymal, vascular
and occasionally sclerenchyma or supporting tissues.
Parenchymal tissues will predominate in most sources of DF
and only in cereal bran products will lignified tissues be
important as a foodstuff.

Detailed tissue separation and cell wall fraction-
ation techniques being developed now help us to appreciate
the complexity of events involved in understanding the
chemistry of DF and also to help target chemical behaviour
to dietary effect[8,10,11]. Although mature runner bean pods,
as illustrated, are not usually eaten at this developmen-

Function

Plant Organ Containing
Various Tissue Types

Tissue Types

Epidermal   Parenchymal   Vascular   Sclerenchymal

Wall Structure of Component Cell Types
[Matrix Formation]

Structure

Biopolymer Assembly
[Bonding and Cross-Linkage]

Hydrogen Bonding	Ionic Linkage	Sugar Esterification	Phenolic Ester and Phenolic Ether	Glycoprotein and Proteoglycan- Phenolic- Complexes
(e.g. Cellulose and xyloglucans)	(e.g. Calcium bridges between pectins)	(e.g. Between pectins and matrix polysacchardies)	(e.g. Between arabinoxylans and lignin)	(e.g. Between glycoproteins, pectins and cellulose)

Polysaccharides, Glycoproteins/Proteoglycans, (and Phenolics)

Composition

Celluloses   Pectic Polysaccharides   Hemicelluloses   Glycoproteins   Phenolic Esters   Lignins

Constituent Sugars   Constituent Phenolics

Figure 1 Plant cell wall composition and its relationship to biopolymer assembly, wall structure and cell function within plant tissues

Parenchyma

Pectic polysaccharides

Cellulose

Xyloglucans

Glycoproteins

Proteoglycans
(phenolics)

Parchment layer

Cellulose

Acidic-Xylans

Lignin

Pectic-xylan-lignin
complexes

Strings
(lignified)

Parchment layer
(lignified)

Epidermis
(cuticularised)

Parenchyma
(succulent)

Strings
(lignified)

TS mature runner bean (5 mm)

Beeswing bran

Highly branched acidic
arabinoxylans

Slightly branched acidic
arabinoxylans

Cellulose

Lignin

Phenolic esters

Beeswing bran
(lignified)

Cross cells
(lignified)

Aleurone layer

Slightly branched
arabinoxylans

Mixed-linkage β-glucans

Cellulose

Phenolic esters

Aleurone layer cells
(thick-walled non-lignified)

Endospermous tissue
(including starch granules)

Endosperm

Highly branched
arabinoxylans

Cellulose

Glucomannans

TS wheat bran and associated endosperm (50 μm)

Figure 2 Tissue distribution and compositional
characteristics in a vegetable (runner bean) and a cereal
(wheat grain) fibre source

tal stage, component tissues can be easily separated for
detailed fractionation and chemical analysis (Table 1).
In immature beans the cells corresponding to the parchment
layers and vascular tissues can also be easily identified
and their walls shown to contain cellulose, pectic poly-
saccharides and acidic xylan-pectic complexes, these com-
plexes corresponding to the acidic xylans in the lignified
mature tissues. The deposition of acidic-xylans and
lignin is coincident with rapid enlargement of the pod and
seed development. This can be inferred from the data on
runner bean pod cell wall tissue sugar composition and
also composition at different stages of maturity shown in
Table 1. The major polysaccharides of runner bean paren-
chyma that can be inferred from sugar analysis alone, in
decreasing order of abundance are : pectic polysaccharides
(GalpA, Ara, Gal), cellulose (Glc released on Saeman hyd-
rolysis) and xyloglucans (Glc and Xyl released by M H_2SO_4
hydrolysis). From the composition of the parchment layers
it can be inferred that the major polysaccharides present
are cellulose and acidic-xylans (Xyl released by M H_2SO_4
hydrolysis). The Klason lignin content of the parchment
layer is around 20%. This information in conjunction with
detailed fractionation studies can be used to infer the
changes in the cell wall polysaccharides which occur
during maturation of the pods (de-seeded). Thus, the very
immature pod (prior to significant development) contains
in decreasing amounts ; pectic polysaccharides, cellulose
and xyloglucans and in Stage 2 (when used as a vegetable)
rapid pod elongation has occurred but with little ligni-
fication or change in polysaccharide type. During Stage 3
the maturation of the pod results in extensive lignifi-
cation of the 'parchment' layers and 'strings' and corres-
ponding changes in the polysaccharide components (note the
increase in the proportion of xylose and glucose). The
relatively small increase in xylose content of pods during
Stage 2 indicates little secondary thickening at this
stage and by more detailed monitoring of bean pod develop-
ment it can be shown that after this stage rapid secondary
thickening occurs in parallel with seed development.

    The major cell wall polysaccharides of potato paren-
chyma and pea cotyledons inferred from compositional data
in Table 1 are comparable to those of the runner bean
parenchyma, except that the pectic polysaccharides in
potato are richer in galactose compared to runner bean and
in the case of pea cotyledons pectic arabinans predomin-
ate. Pea hulls contain significant amounts of acidic-
xylans in addition to pectic polysaccharides, cellulose
and xyloglucans. In beeswing wheat bran the major cell
wall polymers inferred are (highly branched) acidic

Table 1 Carbohydrate composition of DF from various
vegetable and cereal sources and at different
stages of vegetable development

Sugar composition (mg/g cell wall material)

Source	Deoxy[1]	Ara	Xyl	Man	Gal	Glc[2]	Uronic[3] Acid
Potato[29]	14	75	17	7	288	339	240
Runner Bean;							
–parenchyma[17]	35	160	48	50	134	358	196
–parchment[18]	4	5	288	4	4	386	77
–Pod[#];							
Stage 1	18	37	22	21	81	269	307
Stage 2	19	27	28	24	93	300	309
Stage 3	21	28	70	28	75	326	277
Pea[*];							
–hull[30]	16	27	118	3	11	570	154
–cotyledon[30]	9	132	12	5	28	58	60
Wheat bran[28]	4	176	286	8	12	195	63
Beeswing bran[14]	1	306	295	5	24	335	<30
–endosperm[12]	–	340	535	70	25	30	Tr
–aleurone[13]	–	170	480	10	30	310	Tr

[1]Deoxy = Rha + Fuc ; [2]Sugars are based on results of
Saeman hydrolysis and hence Glc would be derived from
cellulose and non cellulosic polysaccharides (~10% of
total Glc is non-cellulosic) ; [3]Uronic acid is mainly
galacturonic acid, except in parchment layers and wheat
tissues where glucuronic acid or its 4-OMe derivative
predominate ; [#](R.R. Selvendran and J.A. Robertson,
unpublished results) Stage 1 = pod at start of elongation
phase ; Stage 2 = pod at stage of vegetable use ; Stage
3 = pod during seed development and deposition of
parchment tissue but before pod maturity ; [*]carbohydrate
composition determined on starch depleted alcohol
insoluble residue in pea tissues. Tr = Trace.

arabinoxylans, cellulose and lignin, whereas in wheat
endosperm cell walls (highly branched) neutral arabino-
xylans predominate and cellulose content is very low. The
aleurone layer cells of bran contain (slightly branched)
neutral arabinoxylans and some β-D-glucans, with small
amounts of cellulose. The polysaccharides present in the
different tissues of the wheat grain have been deduced
from detailed fractionation studies[12-14].

The behaviour of the DF matrix will depend on the
ability of the constituent polysaccharides to cross link
and the maintenance of these cross links under different
physiological conditions. The persistance of cross links
for example will have an important influence on fibre sol-
ubility and ultimately on resistance to microbial degrada-
tion. The different types of cross link that prevail
within the DF matrix will be discussed briefly:
    <u>Cellulose</u>. Both crystalline and amorphous cellulose
can exist in the cell wall and the latter appears to pre-
dominate in parenchymatous tissues. Inter (and intra)
molecular hydrogen bonding between adjacent β-(1$\rightarrow$4)-linked
glucans which give rise to the cellulose microfibrils
account for the insolubility of cellulose in water.
Sulphonation of hydroxyl groups of C-6 disrupts H-bonding
and hence accounts for the solubility of cellulose in 12M
H_2SO_4. Similarly, converting cellulose to carboxymethyl
cellulose shows how cellulose can be rendered soluble.
    <u>Xyloglucans</u>. Two main types of xyloglucan, slightly
branched and highly branched, can be isolated from paren-
chymatous cell walls. The slightly branched xyloglucans
are the more strongly H-bonded to cellulose microfibrils
and require strong alkali (4M KOH) to be solubilised. In
primary cell walls this 'coating' of xyloglucan helps to
disperse the insoluble cellulose microfibrils within the
wall matrix. Xyloglucans and galactomannans are also pre-
sent as the predominant polysaccharides in some seed cell
walls, e.g. tamarind and guar respectively, where they act
as food reserves for the seed. H-bonding involving the
more highly branched storage xyloglucans and galactomann-
ans is more susceptible to disruption than for the slight-
ly branched xyloglucans and a large proportion of these
storage polysaccharides can be solubilised from de-hulled
seeds with hot water, and find application as food gums.
    <u>Pectins</u>. The pectins are a complex group of acidic
polysaccharides, characterised by a backbone containing
α-(1$\rightarrow$4)-linked-D-galacturonic acid residues with varying
degrees of methyl esterification interspersed by (1$\rightarrow$2)-
and (1$\rightarrow$2,4)-linked L-rhamnose. Associated oligosaccharide
side chains contain mainly D-galactose and L-arabinose
linked mainly to C-4 of rhamnose. The side chains may be

involved in ester cross linking within the wall matrix,
through ferulic acid linked to terminal Gal<u>p</u> or Ara<u>f</u> resi-
dues to form phenolic esters. These are common features
in cell walls of sugar beet, and account for the auto-
fluorescence of the cell walls. Calcium bridges between
non-esterified rhamnogalacturonan regions of pectic poly-
saccharides are also an important cross linking mechanism
between molecules and these cross links tend to predomi-
nate in the middle lamella region (between adjacent
cells). Removal of wall calcium by chelation can result
in solubilisation of middle lamella pectins and hence cell
separation. This phenomenon occurs during cooking, where
intracellular chelating agents, e.g. citrate, diffuse into
the cell wall, complex with the calcium and effect tissue
softening through cell loosening/separation[15]. The effect
is enhanced through trans-eliminative degradation (β elim-
ination) of methyl esterified pectins at cooking temper-
atures and/or mild alkaline conditions. Here the cooking
conditions disrupt chemical bonding adjacent to esterified
galacturonic acid residues and results in molecular frag-
mentation of the pectic material with solubilisation of
pectic polysaccharides of reduced molecular size.

     <u>Wall glycoproteins and proteoglycans</u>. Glycoproteins
present in cell walls can be classified as hydroxyproline
rich (Hyp- rich) or Hyp- poor. Hyp- poor glycoproteins are
mainly cell wall related enzymes whereas the Hyp- rich
glycoproteins appear to have a structural role within the
wall matrix. Hyp- rich glycoproteins which have been
found in relatively high concentration in runner bean
parenchyma, are strongly associated with cellulose and a
range of glycoprotein-pectic complexes and proteoglycans
have also been isolated from a range of parenchymatous
tissues[16,17]. Available evidence now strongly suggests that
Hyp- rich glycoproteins can form cross links between poly-
saccharides and are an important component in the deter-
mination of cell wall structure.
     <u>Acidic xylans</u>. Acidic xylans and associated acidic
xylan-pectic complexes are found mainly in dicotyledonous
plants and consist of β-(1→4)- linked xylose residues sub-
stituted at C-2 with 4-OMe glucuronic acid or glucuronic
acid residues (Glc<u>p</u>A). Ester linkage between phenolics
and Glc<u>p</u>A or 4-OMe Glc<u>p</u>A is possible and results in xylan-
lignin and xylan-pectic-lignin complexes, as have been
isolated from runner bean parchment tissues[18].
     <u>Acidic arabinoxylans</u>. Acidic arabinoxylans are
associated mainly with cereal bran and, as in the acidic
xylans, contain Glc<u>p</u>A or its 4-OMe derivative covalently
linked to the β-(1→4)- linked xylan backbone of the
arabinoxylans. The Glc<u>p</u>A residues are cross linked to

lignin and in addition the terminal arabinose residues of
side chains have associated phenolic residues, e.g. feru-
lic acid, which can be either ester cross linked or ether
cross linked to the terminal Ara$\underline{f}$ of adjacent arbinoxylans
within the wall matrix.
    <u>Lignin</u>. Lignins are composed of phenyl propane
monomers covalently linked to form a network through walls
which have undergone secondary thickening. Lignins may be
linked to polysaccharides through ester cross links and
through phenolic ethers. Lignins vary with plant source
as well as with developmental age but information on
lignin estimation and its chemical characterisation is
limited in comparison with other cell wall components[19].

    2 THE DIETARY FIBRE CONTENT OF PLANT FOODS – AS NSP

The NSP are the major constituents of the cell wall of
fruits and vegetables and comprise between 1.5 – 2.5% of
the plant fresh weight in most commonly used fruits and
vegetables[8]. This is typical for the cell walls of par-
enchymatous tissues. The water content of the cell wall
also varies with tissue type/fibre source and stage of
maturity. This important cell wall component can repre-
sent up to 90% by weight of the cell wall in parenchy-
matous tissue and contributes to the succulence of fresh
fruit and vegetables. In mature tissues the water content
of the cell wall is much lower and is very low in 'dry'
seed products, e.g. legume seeds. Legume seeds have a
variable NSP content, e.g. dried peas 18.6% ; haricot
beans 17.1% ; chick peas 9.9% ; butter beans 15.9%
measured on a dry weight basis[3,4].

    Cell wall composition in fruits and vegetables (% dry
weight) is typically ; cellulose 35% ; hemicellulose 15% ;
pectins 40% ; and proteins and phenolics each 5%. This is
comparable with the composition of cotyledon cell walls in
legume seeds. Legume seed hulls, however, are enriched
with acidic xylans. The NSP content of cereal grains is
variable, for example ; brown rice 2.1% ; porridge oats
7.1% ; pearl barley 7.8% ; and whole wheat 10.4%. In
cereal-based foods the extent of cereal grain refinement
is important. Thus, high extraction wheat products con-
tain more DF than low extraction products, for example ;
wheat bran 41.7% ; whole wheat flour 10.4% ; 72% extrac-
tion flour 3.3%. The differences in DF content reflects
the differential extraction of grain tissues during
milling and this also affects the DF composition of the
final product. Thus, white flour (72% extraction) con-
tains only endospermous tissue and has DF composition ;

cellulose 3% ; hemicellulose 85% ; protein 7% and phenolics 5%, but in wheat bran the composition is ; cellulose 30% ; hemicellulose 50% ; protein 8% and phenolics/lignin 12%. For further details of composition and fractionation see Refs 10 & 20. Pectic polysaccharides are absent in most cereal product, one major exception being rice.

3 ANALYSIS OF DIETARY FIBRE AS NSP[4,19]

Analysis of DF involves 3 major steps :

1. Preparation of an extractive free residue (usually alcohol insoluble residue (AIR)).
2. Removal of starch from the residue by gelatinisation and treatment with amylolytic enzymes.
3. Analysis of the de-starched residue for component neutral sugars, usually as alditol acetates, and uronic acids by colorimetry or by decarboxylation.

Minor variations have been developed for the preparation of extractive free residues in different foods, usually depending on the lipid content of the food. For starch removal two major procedures have been developed.

1 Starch in the AIR is gelatinised in buffer at 85°C (30 min), followed by amylolysis with Termamyl also at 85°C for up to 45 minutes. Subsequently the residue, (preferably after precipitation in alcohol to separate low molecular weight starch degradation products), is treated with amyloglucosidase at 60°C (16h) to complete starch digestion and provide the material for detailed fibre analysis[5,21,22].

2 The AIR is finely milled prior to starch gelatinisation in acetate buffer at 100°C (1h). The resultant suspension is treated with α-amylase and pullulanase at 42°C (16h)[23]. (Digestion at 42°C is preferable to lower temperatures to avoid the association and precipitation of partially degraded starch – fragments containing ~30-40 Glc residues). This procedure removes all but the 'resistant starch' and a subsequent modification to disperse the AIR in dimethyl sulphoxide prior to gelatinisation can effect complete starch removal by the enzyme treatment[3].

In both procedures the analytical scheme has also been used to provide an estimate of the 'soluble' and 'insoluble' fibre components in the food, by analysing separately the NSP in the insoluble residue and in the supernatant after amylolytic treatment and alcohol precipitation. However, traditional methods used to frac-

tionate cell wall polysaccharides, using hot water and oxalate to solubilise pectic polysaccharides, have been shown to result in a significant breakdown of these polysaccharides. Analysis of pectic material solubilised from potato cell walls using hot water and oxalate revealed material rich in galactose[24]. More recent fractionation using improved and non-degradative techniques has shown that the bulk of the galactose rich pectic polysaccharides remain associated with the α-cellulose residue and relatively little galactose rich material is solublised using chelators or mild alkaline non-degradative conditions[17] (Table 2). Hence, the use of hot water and oxalate must result in substantial degradation of pectic material, by β-elimination. Cooking may mimic this eliminative degradation of pectins and result in fibre solubilisation or indeed fibre loss in the cooking liquor. The methods developed for DF analysis which require high temperature to gelatinise starch will result in the partial degradation and subsequent solubilisation of pectic polysaccharides and hence could give misleading results in terms of soluble and insoluble fibre contribution from the original food under more physiological conditions. In terms of soluble and insoluble fibre it should be emphasised that cell structure, the profile of cross linking between constituent biopolymers and their ability to persist in the cell wall will be paramount in determining fibre solubility and hence the behaviour of the matrix under the physiological conditions encountered during digestion.

Fractionation of cell walls under non-degradative conditions

Cell wall fractionation involves solubilisation of polysaccharides under non-degradative conditions to determine how biopolymers are cross linked within the cell wall matrix, as outlined in Table 2 for fruit and vegetable parenchyma cell walls. The fractionation protocol can be considered in relation to 4 diverse tissue types :

1. Potato parenchyma cell walls to represent succulent vegetable tissue.
2. Runner bean parchment layer to represent secondary thickened vegetable tissue.
3. Wheat grain endosperm cell walls to represent cereal parenchyma.
4. Beeswing wheat bran to represent tissue with wall polymers cross linked by both phenolic esters and lignin.

Table 2   Scheme for the sequential extraction of polysaccharides from the plant cell wall (Fruits and Vegetables)[17,29]

Sequential Treatment	Effect	Polysaccharide Solubilised
CDTA ; 50mM 20°C (twice)	Abstraction of calcium from pectins and disruption of ionic cross links. May result in cell separation.	Slightly branched polysaccharides (mostly from the middle lamellae)
Na₂CO₃; 50mM (twice) (+20mM NaBH₄) 1st Extraction 1°C 2nd Extraction 20°C	Abstraction of calcium within the wall matrix and disruption of ionic cross links. Cleavage of some sugar-ester links	Highly branched pectic polysaccharides from the middle lamella and primary cell walls
KOH ; 1M (+20mM NaBH₄)	Cleavage of phenol-sugar ester and protein-carbohydrate bonds	Small amounts of highly branched pectic polysaccharides, glycoproteins and proteoglycans
	Disruption of some H-bonds	Highly branched xyloglucans
KOH ; 4M (+20mM NaBH₄)	Swelling of cellulose and disruption of H-bonds	Mainly slightly branched xyloglucans
[RESIDUE] α-Cellulose	— —	[INSOLUBLE] [Mainly cellulose and cross linked highly branched pectic polysaccharides and Hyp-rich glycoproteins]
Chlorite/Acetic acid (0.3% w/v: 0.12% v/v) @ 70°C	Disruption of phenolic cross links. – mild delignification	Hyp-rich glycoproteins and some highly branched pectic polysaccharides

The solubility of potato parenchyma cell wall bio-
polymers and types of cross link disrupted to effect sol-
ublisation are outlined in Table 2. The scheme involves
use of a powerful chelating agent, such as CDTA at 20°C,
and alkali of successively increasing strength to disrupt
sugar ester, phenolic ester and H-bonding between poly-
saccharides. In potato the α-cellulose residue contains
little Hyp-rich glycoprotein but in runner bean parenchyma
a significant amount of Hyp-rich glycoprotein is found.
This requires mild treatment with chlorite/acetic acid for
solubilisation and probably depends on disruption of   [17]
phenolic cross links involving the Hyp-rich glycoproteins.
Fractionation of runner bean parchment layers according to
the outlined scheme results in negligible polysaccharide
solubilisation and delignification (chlorite/acetic acid)
is required, i.e. production of holocellulose, prior to
fractionation. Little solubilisation of material from the
holocellulose occurs before exposure to M or 4M KOH and at
this stage significant amounts of hemicelluloses (acidic
xylans) are released. This is indicative of acidic xylans
in the holocellulose linked through phenolic ester bonds
(to degraded lignin) and in the original parchment tissue.
The acidic xylans are linked to lignin through glucuronic
acid esters[18].

In the case of wheat grain tissues a significant pro-
portion of endosperm cell walls, mainly neutral arabino-
xylans,can be solubilised with hot water and the remainder
solubilised in M KOH to leave only a trace of α-cellulose.
This is analogous to the solubilisation of β-glucans from
oat bran using hot water and yielding a viscous solution
containing β-glucans. However, in beeswing bran signifi-
cant solubilisation of polysaccharides (mainly acidic
arabinoxylans) only occurs when M or 4M KOH is used and,
with delignification after alkali treatment, further poly-
saccharide extraction with alkali is possible[14]. This
indicates that the cross links in the endospermous cell
walls are much weaker compared with those in beeswing
bran. In beeswing bran phenolic ester (e.g. ferulic acid
type) cross links predominate and these are much more per-
sistant than the sugar ester cross links found in potato
parenchyma, but the former are more alkali labile than the
lignin ester cross links in runner bean parchment tissue.

Through the development of these non-degradative
fractionation schemes it is possible to understand better
the associations between cell wall biopolymers[10]. They
also allow the interpretation of analytical data in terms
of cell wall matrix structure rather than just composi-
tion. Hence functional models of the cell wall can be

formulated as a necessary stage in understanding the beha-
viour of the fibre matrix during gastrointestinal transit.

4 DIETARY FIBRE BEHAVIOUR DURING GASTROINTESTINAL TRANSIT IN RELATION TO CELL WALL STRUCTURE AND ITS FRACTIONATION

During transit DF is exposed to different environments,
notably stomach acid, mild alkaline conditions in the
small intestine and fermentation in the hind gut. In the
stomach the acid (pH ≃ 2.5) might be expected to displace
some of the calcium from the cell wall matrix and this may
influence the solubility of pectic polysacharides and
hence cell loosening. In the small intestine the alkaline
endogenous secretions of bile salts and pancreatic fluid
will neutralise excess stomach acid but result in mild
alkaline conditions in the ileum. At body temperature
these conditions may be sufficient to result in some β
eliminative degradation of pectic polysaccharides with
their concomitant solubilisation and tissue softening.
Degradation of pectic polysaccharides during cooking and
possibly during transit in the upper gut also presents the
possibility that the physiological effects of 'pectin' may
be a consequence of their eliminative degradation in addi-
tion to the effect of 'intact' polysaccharides or their
fermentation products. Tissue softening whether by cook-
ing or conditioning in the upper gut will enhance the con-
tribution of soluble fibre to the diet and the generation
of soluble material will weaken cell structure such that
mechanical action in the gut will lead to tissue disinteg-
ration (particle size reduction).

The more severe conditions used for cell wall frac-
tionation, strong alkali, will not apply in the gut but an
appreciation of how matrix polysaccharides behave using
strong alkali also helps in understanding how the matrix
might behave under fermentative conditions. To be fermen-
ted the polysaccharide must first be solubilised and weaker
cross links will be preferentially attacked by micro-
organisms to effect solubilisation. Thus lignified tissues
persist under fermentative conditions as well as during
wall fractionation.

Evidence for polysaccharide solubilisation and tissue
degradation other than by fermentation has been noted from
experiments using the pig as an experimental model to in-
vestigate fibre behaviour during gut transit[9]. When fed a
minced vegetable (swede) diet or cereal bran diet a major
reduction in median particle size was found on the swede

diet anterior to the terminal ileum[11]. The reduction was
not due to mastication (stomach particle size distribution
being similar to the feed) but occurred in the small in-
testine and was associated with a reduction in the propor-
tion of insoluble uronic acid in the digesta recovered
from the terminal ileum. It should be noted that in the
case of 'wholefoods' mastication will be important to
determine particle size distribution in the stomach. A
further small reduction in particle size occurred in the
hind gut, along with the rapid disappearance of cell wall
material (87.8% apparent digestibility). No corresponding
reduction in the median particle size of bran was found
although some solubilisation of arabinoxylans may occur in
the small intestine and a large proportion of polysacchar-
ide (mainly arabinoxylans) was fermented (41.5% apparent
digestibility). However, particle shape did change in
bran, from flake to 'coiled cylinder', consistent with the
selective fermentation of thick-walled aleurone layer
cells[25]. This will alter the mechanical properties of the
particle. From particle size analysis it would appear that
tissue softening can occur in the stomach and pectic poly-
saccharides are solubilised in the small intestine. There,
mechanical conditions promoting transit leads to tissue
breakdown. This will not only increase the proportion of
soluble fibre present in the digesta but also the surface
area of particulate material. Thus, there exists the
possibility of further pectic polysaccharide solubilisation
and also an increase in the porosity of the residual cell
wall as polysaccharides are removed. Measurement of poten-
tial digestibility of insoluble fibre in the pig caecum,
using a nylon bag technique to suspend fibre in the
caecum[9,26], also showed swede fibre to be fermented almost
to completion within 24h and the uronic acid component
(representative of pectic polysaccharide) to diminish pre-
ferentially. This was in contrast to bran where the uron-
ic acid, as GlcpA, is associated with lignification and
hence is poorly fermentable. In bran the neutral non-
cellulosic sugars, predominantly from arabinoxylans and β-
glucans from aleurone cells, were preferentially fermented
but potential digestibility was less than 50% for bran
even after 48h in the caecum. Similar observations have
been made using in vitro fermentation of wheat bran with
faecal inocula[27,28]. This pattern of fermentation is con-
sistent with the fractionation scheme devised to investi-
gate cell wall cross link structure discussed previously
and also illustrates that even after prolonged exposure to
fermentation, cross linking within the wall matrix can
withstand microbial attack.

Therefore, although the chemistry of DF has been

studied extensively in recent years and methods developed
for its quantification in a range of fruits, vegetables,
cereals and legume seeds only now are the complexities of
the cell wall polysaccharide structure and function begin-
ning to be unravelled and hence the possible significance
appreciated in their nutritional context as DF. In devel-
oping methods to fractionate CWM for structural analysis
it has become apparent that the gastrointestinal tract may
also be actively involved in fractionating DF during gut
transit and hence it may be more advantageous to investi-
gate DF chemistry in line with this apparent fractionation
rather than persist with correlating physiological effects
with only quantitative intake as total fibre or perhaps
misleadingly as 'soluble' fibre by chemical analysis.
This can also be extended to overcome the assumption that
all cereal fibres constitute a distinct fibre source and
similarly all fruit and vegetable fibres are another dis-
tinct source since it is now becoming more apparent that
within cereals, as indeed within fruits and vegetables,
definite structural and compositional cell wall differ-
ences exist. These differences may be as responsible for
the reported dietary effects of fibre than the generally
assumed differences between subjects.

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CHANGES IN DIETARY FIBRE POLYMERS DURING STORAGE AND COOKING

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1 INTRODUCTION

The major components of dietary fibre (DF) are derived from cell wall polymers (1), and the amounts and relative proportions of these can affect the fibre quality (2). There is little definitive information concerning the changes in such components during maturation, storage and processing of edible plant organs. In this paper, we report changes in DF polymers that occur during (a) maturation and storage of asparagus stems, and (b) cooking of potatoes.

2 MATERIALS AND METHODS

Maturation and Storage of Asparagus Stems

Asparagus stems were harvested and marked, at 5, 10 and 15cm from the apex with indian ink. Spears, in groups of 12, were cut at the marks either immediately or after storage for 3 days at 21°C in darkness in punnets overwrapped with perforated auto-RMFA packaging film. Florets and scale leaves were removed and alcohol insoluble residues (AIRs) were prepared from the cut sections of both fresh and stored stems. The AIRs were analysed for the component neutral sugars and uronic acids as described (3). The alkali-soluble cell wall phenolics were estimated using Staffords (4) adaptation of the method of Bondi and Meyer (5) as described below. This method utilises the pH-dependant absorbance properties of the phenolics. The AIR was extracted 0.5M NaOH for 16h at 70°C, and the extract neutralised. Aliquots were diluted in buffer (pH 7 and pH 12) and the difference between the absorbance at pH 12 and 7 was determined at 340 nm.

   Whole asparagus stems (15 cm in length), either fresh
or stored for 3 and 7 days under similar conditions, were
also extracted for AIR which was analysed similarly. As in
the previous experiment, 12 stems were used per sample.

Fibre degradation caused by cooking

   Potato parenchyma cell wall material (CWM) was
prepared free of intracellular components including starch
(6). The pectic polysaccharides were extracted by 2
methods. One, which caused little or no degradation using
CDTA and mild alkali, and the other, using hot water and
hot oxalate which caused significant degradation of the
pectic polysaccharides, particularly the highly branched
ones.

3 RESULTS AND DISCUSSION

   The results reported in this paper show the same
trends in changes of DF and cell wall components as those
of preliminary experiments performed during two previous
growth seasons. Although the values obtained are from
single determinations on pooled samples of 12 comparable
stem sections, the consistent changes shown in Figure. 1
and Table 1 allow for comparison in the wall components
during maturation and storage. Furthermore, conclusions
drawn from this data are supported by the analysis of AIRs
from fresh and stored whole asparagus stems shown
subsequently in Figure 2 and Table 2.

Changes in DF during maturation of asparagus stems

   Since no starch could be detected in the AIRs, levels
of dietary fibre are calculated (on a fresh-weight basis)
from the values of the cell wall sugars. In fresh
asparagus stems dietary fibre decreased with distance from
the apex (Figure 1). This was due to a decrease in cell
number per unit fresh weight: examination by light
microscopy showed that in the immature tissues of the
apical 5 cm, the cells were small and closely packed. In
the second section, the majority of cells were elongating
whilst in the basal section, extension had ceased and some
secondary thickening and associated lignification was
evident. The carbohydrate composition (Table 1) showed
that in immature tissues the main sugars, in decreasing
order of abundance, were uronic acids, glucose, arabinose,
galactose, mannose and deoxy-sugars. From these values,
the main polysaccharides could be inferred to be pectins,
cellulose and hemicelluloses. Maturation was accompanied
by a decrease in arabinose and an increase in xylose and

Table 1 Changes in the composition of dietary fibre
         during maturation and storage of asparagus stems

Section	Rha	Fuc	Sugars mol% Ara	Xyl	Man	Gal	Glc	UA	g/100g AIR	Phenolic content AOD_{340}/mg
F1	1	1	18	6	3	12	29	30	33	1.7
F2	1	1	13	7	4	15	33	26	40	2.0
F3	1	1	9	9	3	14	35	27	53	5.5
S1	1	1	17	8	3	9	30	32	35	4.0
S2	1	1	11	9	4	7	36	32	47	5.5
S3	1	1	8	13	3	6	37	31	57	8.0

glucose. There was little change in the levels of other
sugars.

Changes in dietary fibre during storage of asparagus stems

     The trends shown during maturation in fresh asparagus
spears were also shown in those that had been stored for 3
days. However, there were significant differences between
the composition of AIRs of stored asparagus stem sections
and their corresponding fresh controls. Storage resulted
in a decrease in the total dietary fibre level (relative
to the fresh 'control' tissue) in the apical tissues, no
change in the mid section, and an increase in the basal
section (Figure 1). These changes were accompanied by
changes in the carbohydrate composition. Storage resulted
in a decrease in the relative levels of galactose and
arabinose in all sections. In the basal section, storage
resulted in an increase in the levels of xylose, glucose
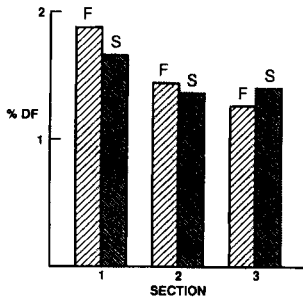
Figure 1 Changes in DF during
maturation and storage of
asparagus stems.
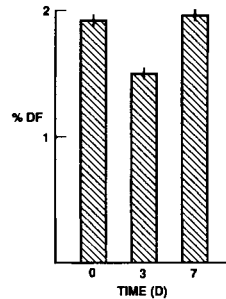
Figure 2 Changes in total
asparagus stem DF during
storage for up to 7 days

<u>Table 2</u> Changes in the composition of dietary fibre during
         storage of asparagus spears

			Sugars						g/100g	Phenolic content
Days of			mol%							
Storage	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	AIR	AOD340/mg
0	1	1	14	9	3	12	35	25	42	3.2
3	1	1	13	9	4	7	42	22	38	5.7
7	1	1	9	12	3	5	36	33	41	22.3

and phenolics (Table 1). These changes are in accordance
with secondary thickening of the sclerenchyma and vascular
tissues, and would account for the increase in DF in the
basal section.

These results facilitated the interpretation of
changes in DF during prolonged storage of whole asparagus
stems for up to 7 days (Figure 2). Over this period, the
DF content of the fresh stems, which was 1.9%, decreased
to 1.5% after 3 days, and then increased to 2% by day 7.
Storage was accompanied by a significant decrease in
galactose and arabinose, and a notable increase in
phenolics and xylose, particularly by day 7 (Table 2).
These changes are probably due to breakdown of pectic
polysaccharides in the parenchymatous tissues, and
deposition of lignin and xylans, possibly in the
sclerenchyma and vascular tissues.

The results above show that pectic polysaccharides
can become metabolised and depolymerised during storage.
Pectins are also subject to depolymerisation as a result
of heat-catalysed degradation. The following section
reports on such changes during cooking.

Changes in dietary fibre during cooking

Cooking is usually accompanied by tissue softening
and changes in textural characteristics. This occurs as a
result of abstraction of calcium from the middle lamella
region by suitable chelating agents e.g. citrate (7) and
also due to β-eliminative degradation of pectins (Figure
3). Both of these processes encourage cell separation.
Degradation of apple pectins by β-elimination has been
studied during heating in phosphate buffer at pH 7 (8),
and these conditions are similar to those which occur in
vegetables during boiling. The above and related studies
(9) show that significant breakdown of the pectins can
occur during boiling of vegetables.

Table 3   Effects of cooking on the extraction of CW
polymers

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	g/100g
CWM	2	-	9	2	t	29	35	22	95
Cold treatment									
CDTA x2*	1	2	14	1	1	9	7	66	64
Alkaline solvents to solubilise hemicelluloses									
α-Cellulose	1	t	6	t	1	36	44	11	97
Hot treatment									
Hot water	2	-	14	-	-	53	t	32	98
Hot oxalate	2	-	5	-	-	21	t	72	97
Alkaline solvents to solubilise hemicelluloses									
α-Cellulose	1	-	2	1	2	3	72	18	96

Column header note: Component sugars (mol%)

* values refer to the first CDTA estraction.

In order to highlight the effects of β-eliminative
degradation on DF polysacharides, the effects of
extracting purified potato CWM with hot water and hot
oxalate were compared with extracting with CDTA at 20°C.
The results (Table 3) show that CDTA-extraction released
polysaccharides were rich in uronic acids with some
arabinose and galactose. In contrast, the polysaccharides
released by both hot water and hot oxalate contained
considerable amounts of galactose. The α-cellulose
residues remaining after subsequent (non-degradative)
extraction with alkali were also compared. Whilst the
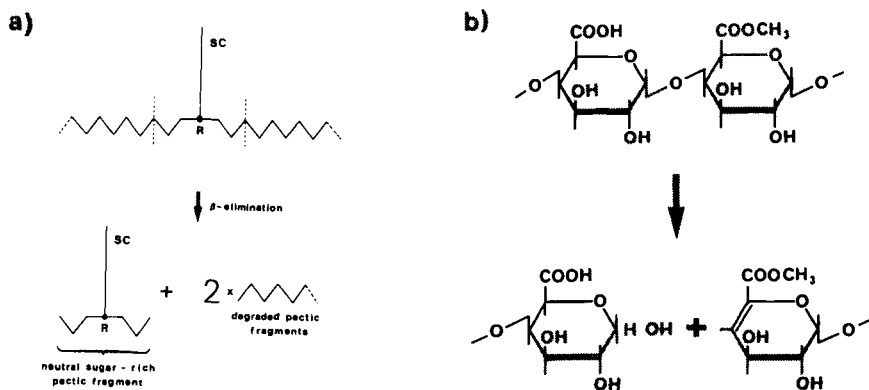
Figure 3 β-eliminative degradation of pectins.
(a) diagram of depolymerisation, (b) bond cleavage

α-cellulose from CDTA-extracted cell walls was rich in galactose, little galactose was found in the hot water and hot oxalate-extracted α-cellulose. It can be concluded that the release of galactose by heat was due to depolymerisation, since under non-degradative conditions it remained in the α-cellulose fraction.

4 CONCLUSIONS

This study has focussed attention on the metabolism of cell wall polyaccharides in succulent growing tissues during storage. The changes in pectic polysaccharides, particularly the breakdown of the galactan and arabinan side chains, are comparable to those in ripening tomato (10) and apple (11) although the tissues of ripening fruits are physiologically distinct from those of fast growing stems of asparagus. The increase in xylose-rich hemicelluloses and phenolics are indicative of secondary thickening, and correlate with an increase in DF. Finally, depolymerisation of DF polysaccharides have also been highlighted during cooking. Since the physicochemical characteristics of DF are dependant on the cell wall polymers and their interactions, it is likely that the changes discussed above are likely to affect the DF quality.

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HETEROGENEITY OF MONOMERIC COMPOSITION IN GRAMINEAE, MAIZE AND WHEAT, LIGNINS.

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I - INTRODUCTION

Even if the definition of crude-, detergent-, or dietary-fibres is not a simple task[1], plant cell wall phenolics such as lignins and linked phenolic acids have a great importance in connection with the indigestibility and thus the definition[2] of these fibres.

Several levels of heterogeneity of lignins, which include molecular, macromolecular and super-molecular levels, have been described in addition to associations with polysaccharides and phenolic acids[3,4]. Furthermore, biochemical variations mainly due to differences in plant species, cytological origin and stage of development could change the structure and the reactivity of these phenolics[5], and thus the digestibility of the corresponding fibres. Heterogeneity and inhomogeneity in angiosperm lignins has been, until now, particularly studied in the case of woods[6,7]. Only few studies have been published about the heterogeneity and the formation of grass lignins[8-10]. Evidences of heterogeneity in monomeric composition in wood lignin have been previously reported by comparison of the composition of lignin fractions isolated from different species[11,12]. However in the case of wheat- and of rice- straw lignins, clear occurrence of heterogeneity in monomeric composition were not found[8,9,13].

Evidence of this phenomenon was however recently obtained, after isolation of lignin fractions from maize culm internodes. A publication being in preparation (TOLLIER, LAPIERRE and MONTIES), these results are summarized here, with emphasis on data previously published in the laboratory.

II - MATERIAL AND METHODS

Extractive free cell wall residues (CWR) were prepared by successive Soxhlet extractions with toluene-ethanol (2-1, vol), ethanol and water from roughly ground (0.1 to 0.5 mm particle size) internodes of straw from wheat (<u>Triticum</u> sp.) and of culm from maize (<u>Zea</u> sp.) grown under field or greenhouse. Milled-stem lignin, LM, and enzyme lignin, LE, were successively isolated from ultraground extractive free CWR, according to standard procedure[7,13]. Alkali lignin, LA, was isolated after N sodium hydroxide extraction at 35°, with stirring under nitrogen[14], with recovery of the insoluble final residue, saponification residue, SR, by filtration and washing in dilute HCl and water[8]. Monomeric composition of these fractions was characterized by nitrobenzene oxidation with reversed phase HPLC identification of p-hydroxybenzaldehyde (h), vanillin (v) and syringaldehyde (s)[7,8] and by thioacidolysis with GC determination of the TMS derivatives of the tri-thioethylether monomeric units of phenyl (H), guaiacyl (g) and syringyl (S) types with identification by mass-spectrometry[7,15]. Ester- and ether- bound p-coumaric-(PC)- and ferulic-(FE)-acids were analyzed after successive alkaline and acid hydrolysis of the CWR and lignin fractions with HPLC determination[8,14]. Total lignin, KL, contents were obtained according to Klason type procedure without prehydrolysis[16]. Mean standard deviations for Klason lignin, nitrobenzene oxidation, thioacidolysis and phenolic acids were respectively, about : 10, 10, 5 and 5 per cent for independent and repeated series of determinations.

III - RESULTS

Dry plant stems were harvested about two weeks after maturity of the grain in the case of wheat, grown in field conditions and in the case of maize, grown in greenhouse. As previously reported[17], a normal line of Maize was compared to the corresponding brown-midrib mutant (b.m₃), mainly characterized by a low lignin content and the occurrence of an additional type of monomeric unit with a 5-hydroxyguaiacyl substitution pattern[17,18].

Total lignin contents in wheat straw, in normal- and in mutant-maize lines were respectively : 19.5 - 15.2 and 11.3% CWR. Corresponding lignin contents in saponification residue were respectively : 13.8 , 9.2 and 3.8% CWR. These last results agree with the well established high alkaline solubility of gramineae lignins and indicate a higher solubility in the case of b.m.-mutant. The molecular origin of this higher solubility (etherification by phenolic acids[19] and/or larger content in free phenolic hydroxyl groups[20]) is under study.

According to yields in non condensed monomeric units of phenyl, guaiacyl and syringyl type recovered after nitrobenzene

oxidation (b,v,s respectively) and after thioacidolysis (H,G,S respectively), maize culm lignins appeared as typical H-G-S lignins, as shown in table 1.

Table 1 : Monomeric   composition   of   cell   wall   (CWR)-   and saponification (SR)- residues of normal maize stem as shown by nitrobenzene oxidation and thioacidolysis.

$\mu M.g^{-1}KL$	Nitrobenzene			Thioacidolysis		
	b	v	s	H	G	S
CWR	1300	750	830	21	256	365
SR	459	491	693	trace	188	246

The relative contents in phenyl (b,H) compounds was dramatically decreased after alkali extraction. Such behaviour has been previously reported for other monocots[21], suggesting that a significant part of b and v recovered after nitrobenzene oxidation arose from p-coumaric- and from ferulic-acids and not from lignin. The same type of variations were obtained in the case of wheat straw[15] and b.m maize mutant[17,22]. In each case, thioacidolysis yields were lower than for nitrobenzene, confirming[7,15] that, in addition to aryl-alkyl-ether linkages, other structures, such as diaryl-propane, were degraded. As however the characteristic phenylpropane structure of phenolic acids and of lignin monomers is not degraded by thioacidolysis, thioacidolysis data shown in fig. 1 provide the more exact figure of the monomeric composition of the total stem lignin.

Significant contents of p-coumaric- and ferulic-esters and ethers were found linked to the three types of lignin fractions, obtained from wheat- and maize-stem CWR (data not shown). In all cases, p-coumaric ester contents were about ten times higher than the corresponding ferulic ester content with PC-ester contents ranging from about 150 to 600 $\mu M.g^{-1}$ of LM and LE fractions with relatively lower contents in wheat. Phenolic esters were not significantly found in LA fractions. Again, in each cases, p-coumaric-and ferulic-ether contents were found in the range of 10 to 70 $\mu M.g^{-1}$ of LM, LE and LA fractions with a characteristic relative higher content of ferulic ethers in LA fractions of both wheat and maizes. These data agree with previous results[3,14,15,19], indicating a preferential association of p-coumaric esters to lignin-rich fractions of monocot plant cell wall, and a higher content in phenolic ethers in alkali lignins. Large differences in absolute contents in linked phenolic acids were found between independent experiments confirming the importance of environmental and genetic factors on these contents[17,23].

Very significant differences of contents in non condensed guaiacyl (G) and -syringyl (S) monomeric units were found by thioacidolysis between LM and LE lignin fractions isolated from normal maize, but not from b.m-mutant line (table 2).

Table 2 : Monomeric composition of LM and LE lignin fractions isolated from normal- and mutant-maize shown by thioacidolysis.

μMole.g^{-1}	Normal		Mutant	
	LM	LE	LM	LE
H	12	9	12	9
G	169	144	197	293
S	201	220	90	130
H/G	0.07	0.06	0.06	0.03
S/G	1.19	1.53	0.45	0.44

Differences in monomeric composition, also indicated in table 2 by H/G ratio, appear only in the case of mutant and require confirmation. Further, in agreement with previous data[17], a lower relative content in syringyl units in LM and LE lignin fractions of the mutant, appears associated with a significantly higher content in guaiacyl units. Biosynthetic correlations between these variations are very likely[17,18].

Variations in monomeric composition of lignin fractions isolated from wheat and maizes are shown in table 3, with two independent series of samples[22,24], in the case of normal- and mutant-maize.

Table 3 : Monomeric composition of LM and LE lignin fractions isolated from wheat, normal- and mutant-maize as shown by thioacidolysis (data : S/G molar ratio with two independant experiment for maizes : years 1988 and 1989).

	Wheat[a]	Normal-maize		Mutant-maize	
		exp.1[b]	exp.2[c]	exp.1[b]	exp.2[c]
LM	0.84	1.19	1.01	0.45	0.46
LE	0.92	1.53	1.42	0.44	0.49
LA	1.04	1.80	1.70	0.47	0.50

a, b, c : according respectively to ref. : 19, 22 and 24

Differences in syringyl to guaiacyl ratio reported in the case of normal-maïze unambiguously shows the occurrence of an heterogeneity of monomeric composition in gramineae lignin which compares to other cases previously reported in angiosperm woods lignin fractions[7,11]. In the case of wheat, weak differences in S/G ratio may thus, tentatively, indicate the occurrence of lignin heterogeneity[12,19]. Furthermore, higher S/G values reported in table 3, for LA lignin fractions, have to be discussed cautiously as some water-soluble-colloïdal lignin fractions may be selectively lost during the isolation steps.

IV - CONCLUSION

Occurrence of heterogeneity of monomeric composition between lignin fractions, isolated from extractive free cell wall residue from internodes of the culm of maize, has been reported here for the first time. Due to its selectivity, thioacidolysis only allowed an unambiguous evidence of this phenomenon previously reported, in the case of angiosperm woods, for related guaiacyl-syringyl lignin. Gramineae lignin, however, appears as more condensed and intermediate in its molecular structural scheme[4] between angiosperm- and gymnosperm-lignins. Furthermore, lignins of monocotyledons examined by radiotracer methods were not so intensively heterogeneous in structure with regard to corresponding tree lignins[9,10,26]. It is likely that the differences in heterogeneity found between wheat and maïze may be related to the fact that, in the stem of these gramineae, seen in trans-section, widely spaced vascular bundles are not restricted in one circle but appear either in two circles in wheat, or scattered throughout the section in Maize[27,28].

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INTRODUCTION

Rye and wheat are widely grown cereals and the grains are
mainly used in bread and other products for human
consumption and in animal feeds. The starchy endosperm of
common cereals contains predominantly starch and protein
but significant amounts of dietary fibres and other
components are also present. In wheat and especially rye
these fibres are mainly composed of pentosans, including
arabinoxylans and arabinogalactan-proteins.[1] Arabinoxylans
are partly soluble in water and it has been shown that the
average molecular weight of soluble arabinoxylans in rye
flour is about 2-3 times greater than that of soluble
arabinoxylans isolated from wheat flour.[2]

     The influence of pentosans on the milling behaviour of
grain,[3] the quality of flour,[4] rheological properties,[5]
bread volume and staling[6] have been reported but little is
known about the molecular mechanisms of these influences.

     This paper will deal with the structure of pentosans
in wheat and rye, including methods for their determination
and effects of baking on pentosan structure and solubility.

STRUCTURE OF WATER-SOLUBLE PENTOSANS

Soluble arabinoxylans in the cell-walls of starchy
endosperm in rye and wheat are composed of 4-, 3,4- and
2,3,4-linked β-D-xylopyranosyl residues together with
terminal α-L-arabinofuransoyl residues (Figure 1).[7-9] The
major water-soluble arabinoxylan in rye grain contains
arabinose and xylose residues in a ratio of about 1:2

together with only traces of other components.[8] Methylation
and NMR analysis revealed that this polysaccharide
(arabinoxylan I) consists of a main chain of 4-linked
β-D-xylopyranosyl residues of which about 50 % are
substituted at position 3 by terminal α-L-arabinofuranosyl
residues. Small amounts of xylose residues are substituted
at both position 2 and 3 also by terminal α-L-arabino-
furanosyl residues. However, it is not known if these
double-branched residues are a part of arabinoxylan I.
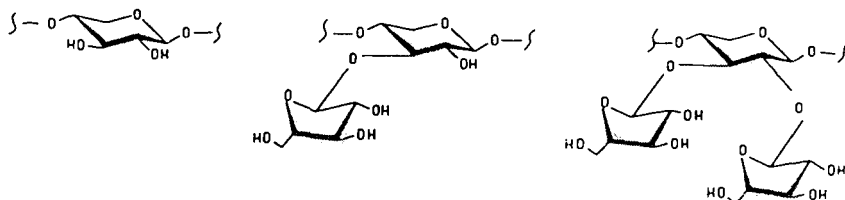
Figure 1 Structural units in soluble rye and wheat
arabinoxylans

Xylopyranosyl residues branched at position 3 are
stable to periodate oxidation. A sequential oxidation and
reduction procedure was used for complete oxidation of the
arabinose and unbranched xylose residues in arabinoxylan
I.[10] Quantitative analysis of products formed after mild
acid hydrolysis revealed the presence of glycerol-xylosides
with one, two or three xylose residues in the molar ratio
of 1.0; 0.86; 0.02. The xylose residues in these
oligosaccharides originated from the branched residues in
the main chain of the polysaccharide. It was concluded that
these branched residues are predominantly present as
isolated units or small blocks of two residues in arabino-
xylan I.

Arabinoxylan I

$$-[X]_m-X-X-[X]_n-X- \quad \begin{matrix} m \geq 1 \\ n \geq 1 \end{matrix}$$
$$\uparrow \quad \uparrow \qquad \uparrow$$
$$A \quad A \qquad A$$
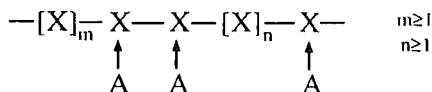
Figure 2 Structural features of arabinoxylan I, the major
water-soluble polysaccharide in rye grain

A water-soluble arabinoxylan fraction from rye grain with a structure similar to arabinoxylan I but containing about 4 % of double-branched residues, was hydrolysed with different amounts of a semi-purified xylanase from <u>Trichoderma reesei</u>.[11] The double-branched xylose residues became enriched in the residual polymeric fraction. After extensive hydrolysis with the enzyme, the residual polymeric fraction contained a main chain of 4-linked xylose residues in which about 70 % of the residues were substituted at both 0-2 and 0-3 with terminal arabinose units. It was evident that the semi-purified enzyme contained high xylanase and arabinosidase activities. These activities rapidly degraded un- and monosubstituted xylose residues in arabinoxylan I while the double-substituted xylose residues were very resistant. The results show that a new polysaccharide structure, arabinoxylan II, is present in rye grain as a separate polymer or as a region in a complex macromolecule. Since the double-branched xylose residues were slowly degraded by the enzyme it is evident that arabinoxylan II contains longer regions with double-branched xylose residues.
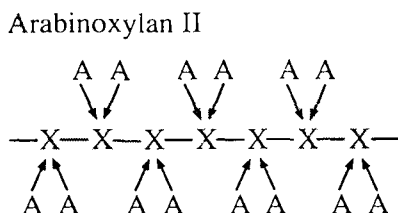
Arabinoxylan II

Figure 3 Structural features of arabinoxylan II, a minor water-soluble polysaccharide in rye grain.

Water extracts of wheat flour contain an arabino-galactan-protein with a hydroxyproline-rich polypeptide backbone.[7] This polymer has been reported to contain branched β-galactans substituted with arabinofuranosyl substitutents. Our preliminary results indicate that all three water-soluble pentosan structures are present in both rye and wheat grain, although the contents differ significantly.

EFFECTS OF BAKING ON POLYSACCHARIDES IN WHITE BREAD FRACTIONS

Bread baked with white flour at 210°C for 22 or 35 min was divided into outer crust, inner crust and crumb.[12] The content and composition of water-soluble and insoluble dietary fibre polysaccharides were determined in these fractions and the dough by using a gas-liquid chromatographic method. It was shown that the content of pentosans (arabinose, xylose and galactose residues) decreased during baking, probably due to thermal degradation of sugar residues or losses of low-molecular weight fragments. This trend was particularly noticeable in the outer crust fractions. The content of Na-acetate buffer insoluble arabinoxylans also decreased significantly during baking. The decrease was evidently related to the extent of heat-treatment as it was largest for fractions of outer crust and also became more apparent with the use of longer baking time. It can not be taken for granted, however, that the noted decrease in arabinose residues arises solely from effects on arabinoxylans, since arabinogalactan-proteins also are present in wheat flour. An increasing content of buffer-extractable arabinoxylans in the order crumb to outer crust showed that a redistribution from insoluble to extractable arabinoxylans had occurred. The amount of extractable galactose residues decreased substantially in the order crumb to crust and was also lower in bread fractions than in dough. This finding suggested that a soluble polymer containing galactose residues (probably the arabinogalactan-proteins) was rendered progressively insoluble as a result of more severe thermal treatment during baking. This was also confirmed by a higher level of insoluble galactose residues in the bread fractions compared with dough. It is possible that denaturation of the protein part of arabinogalactan-proteins is responsible for these effects.

Polysaccharides in the white bread fractions were extracted with water and fractionated on DEAE-cellulose.[9] Fractions isolated were characterized by sugar and NMR analysis, revealing the presence of arabinoxylan mixtures with widely different degrees of arabinose substitution. Arabinogalactan-proteins isolated, however, showed little variation in galctose/arabinose ratio (1.5-1.8). Water-soluble arabinoxylans had a higher degree of arabinose substitution in bread fractions than in flour and dough, probably due to increased solubilization of highly substituted arabinoxylans during baking, assuming that originally insoluble arabinoxylans have a more highly substituted structure than their soluble counterparts. It

is noteworthy that arabinoxylans of outer crust need
stronger borate concentrations for elution, possibly
reflecting some modifications in the polysaccharide
structure caused by the more severe heat-treatment of this
fraction during baking.

From these studies it is evident that the structure
and solubility of pentosans are affected by baking. These
polysaccharide modifications may have nutritional
implications and significant effects on bread properties.

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THE EFFECTS OF EXTRUSION COOKING ON THE PHYSICAL AND
CHEMICAL PROPERTIES OF DIETARY FIBRE IN SNACKFOODS

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1. INTRODUCTION

The importance of the intake of dietary fibre in relation
to health and disease is sustained by scientific and
clinical evidence for a number of physiological effects[1].
This has brought about the interest shown by the food
manufacturing industries in the nutritional aspects of
dietary fibre.

Today, a number of food manufacturing companies
produce DF enriched snackfoods. These snackfoods are
obtained by extrusion, i.e. HTST[2] (high temperature
short time) processing under high pressure evolving
important shear forces.
The impact of high temperature combined to high
pressure, which prevails during extrusion, has already
been studied for maize starch, the essential component
of snackfoods[3]. In the present investigation the
impact of these same parameters is examined for DF
enriched snackfoods. It is indeed logical to assume
that possible   structural and/or chemical
alteration of DF resulting from the extrusion process
might affect the functional properties of the DF and
the importance to assess such alterations is self
evident.

The evaluation of possible modifications of the
dietary fibre is carried out by measuring some physico-
chemical properties such as the water and oil absorption
capacities as well as the water activity of the samples
which are also examined by SEM(scanning electron
microscopy). Furthermore, the chemical composition
of the DF is also assessed in view to ascertain whether

the extrusion process is likely to produce any
analytical modifications of the DF conponents.

2. MATERIAL AND METHODS OF PROCESSING AND ANALYSIS

Material

The samples are prepared from pre-gelled maize
starch (Cerestar, Belgium) added with fibre-rich products
such as hulls from maize (Cerestar, Belgium) or from pea
(DDS, Denmark), or sugarbeet fibre (British Sugar, UK).
The blends are prepared from 80 % or 90 % maize starch
and 20 % or 10 % fibre-rich material.

Prior to extrusion all samples are powders and
these are analyzed as such. The snackfood obtained by
the extrusion process is suitably prepared for analysis
(see hereunder).

Extrusion

Extrusion is carried out in a twin   screw extruder
CLEXTRAL bivis BC 45, operating under temperatures of
120°, 160° or 200°C with moisture contents of 14 % or
24 %. The rotating speed is 152 RPM (this represents
optimum conditions of expansion and viscosity for
samples made from maize starch only[3]). The nozzle
diameter is 8 mm. The resulting pressures lay between
15 and 22 bars. It should be pointed out that besides
the high temperature and high pressure, the high shear
forces prevailing during the extrusion process are
also likely to contribute to possible modifications of
the polymer network of the DF.

Physico-chemical measurements

The absorption capacities of oil and of water by the
product are assessed by a centrifuge technique, wherein
the water absorption is measured according to the AACC
(American Association of Cereal Chemists) method N°88-04,
and the groundnut oil absorption is measured according
to the method by Caprez et al.[4]. All the samples are
ground to less than 0,5 mm prior to analysis.

For SEM the samples prior to extrusion are examined
as such. The extruded cylindrical shaped snackfood
samples are cut transversally to offer the inner
surface to the SEM. Each sample is glued on an aluminium
stub with TEMPFIX resin and then sputtered with 20 nm
gold-palladium (30 mA, dist. 30 nm for 120 s.).

SEM is carried out with a JEOL 840 A, operating at 15 kW or 20 kW and 10^{-11} to 6.10^{-11} amperes.

Chemical analysis

Total water soluble and water insoluble fibre content is measured according to the AOAC (Association of Official Analytical Chemists) method of Prosky et al.[5].

RESULTS AND DISCUSSION

The following summarizes the results obtained and highlights the most significant features.

Oil absorption

The extrusion process increases the oil absorption capacity (x 2) for each kind of sample (i.e. 10 % or 20 % fibre content, and extruded with 14 % and 24 % moisture contents at 160°C and 200°C).

The experimental date reveal that differences in extrusion temperatures do not affect the increase of the oil absorption capacities for the extruded samples. However, the oil absorption capacities are significantly higher for samples extruded at lower moisture contents (14 % INSTEAD OF 24 %). Fig. 1 shows the results obtained for extrusion with 14 % moisture.

<u>Figure 1</u> Oil absorption capacities of samples before and after extrusion. Extrusion conditions : 14 % moisture content at 160°C
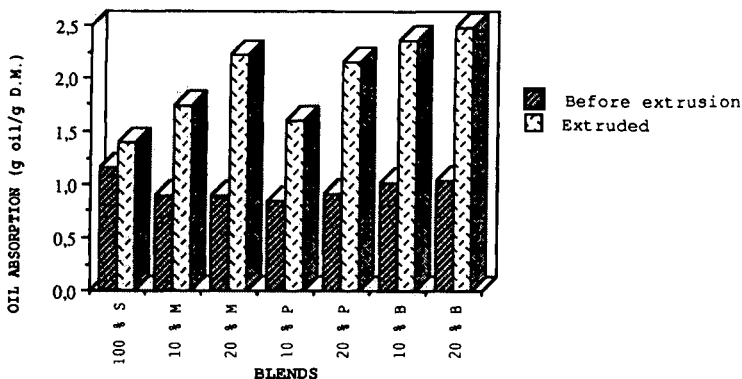
S : Starch ; M : Maize ; P : Pea ; B : Sugarbeet

WATER ABSORPTION

     The assessment of the water absorption capacity
is carried out on duplicate samples from the same
extrusion tests as those used for the oil absorption
assessments.
The results show an increase of the water absorption
capacity (x 2 to x 6)which is of about the same
magnitude as the increases recorded for the oil absorp-
tion capacities (see Fig. 2).

Figure 2 Waterabsorption capacities of samples before
          and after extrusion.   Extrusion conditions :
          24 % moisture content at 160°C.
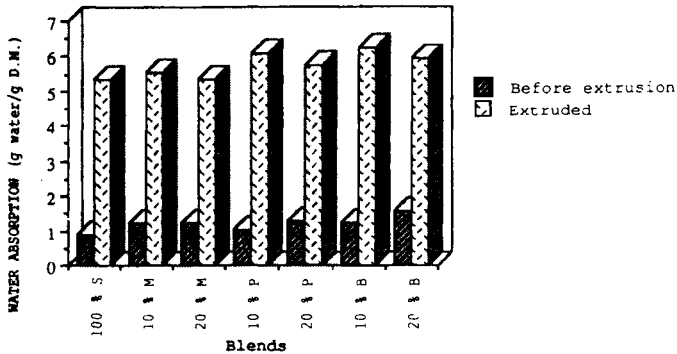
S : Starch ; M : Maize ; P : Pea ; B :Sugarbeet

As for the results of the oil absorption capacity, the
extrusion temperature does not influence the water
absorption capacity either. On the other hand, however,
for all the samples moisture contents of 24 % lead to
higher water absorption capacities than moisture
contents of 14 % during extrusion. But the fibre
content itself has little impact on the results of
the water absorption capacity. Hence the alterations
in water absorption capacities seem rather due to
modifications of the starch fraction and not to those
of the fibre fraction of the extruded snackfood.
It is noticeable that Chinnaswamy[3] recorded maximum
expansion ratio for maize starch extruded with 13.4 %
moisture content.

Hygroscopicity

     Only one type of extrusion parameter has been
tested : 14 % moisture content at 200°C. As shown in

Fig. 3, the extrusion process lowers the hygroscopicity at 0.75 a_w for all the blends (10 % or 20 % fibre and 90 % or 80 % starch content) with each type of fibre (maize, pea or sugarbeet).

<u>Figure 3</u> % Moisture content at 0.75 a_w for samples before and after extrusion (a_w : water activity)
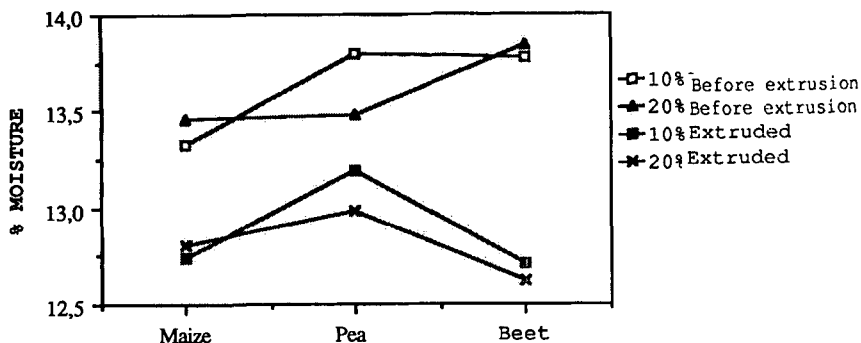
SEM

Under conditions applied SEM has the advantage of great magnification but cannot differentiate between the different fibre components. Thus it is not possible to identify visually cellulose, pectins, etc..., though SEM has been applied not only to the snackfood blends before and after extrusion but also to the fibre residues obtained in the analytical fibre assessment according to Prosky et al. Also, in spite of substantial magnification (x 15.000) SEM reveals no significant alteration of the DF network.

Chemical analysis

As shown in Fig. 4 a loss of 10 % of total DF content is recorded for all the samples extruded with 14 % moisture content at 200°C. Furthermore, as summarized in fig. 5, from the analysis of water soluble and water insoluble DF content, it appears that the ratio soluble/insoluble fibre content changes after extrusion. This seems due more specially to modifications of the insoluble fibre content. It should be kept in mind also that for very small amounts of soluble dietary fibre contents Prosky's analytical method shows poor reproducibility and consequently the differences of soluble DF recorded before and after extrusion are not significant.

Figure 4 Total dietary fiber content (%) for samples
          before and after extrusion. M : maize,
          P : Pea
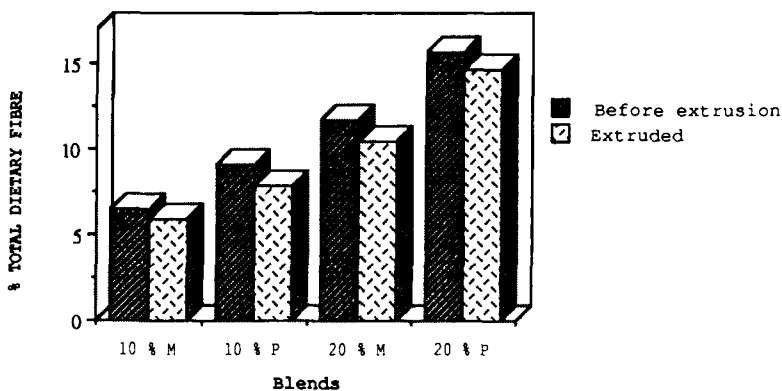
Figure 5 % water soluble and water insoluble dietary
          fibre contents before (first value) and
          after (second value) extrusion
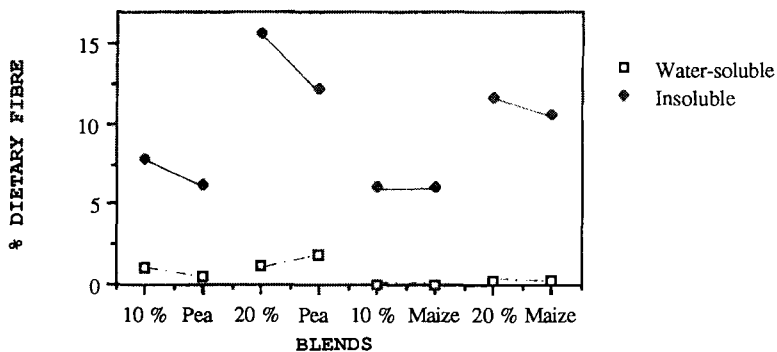
4. CONCLUSION

DF enriched maize starch based snackfoods produced by
extrusion process loose 10 % of their total DF content.
Water and oil absorption capacities are only slightly
modified by the extrusion process.

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CHEMICAL AND PHYSICO-CHEMICAL PROPERTIES OF FIBRES FROM ALGAL
EXTRACTION BY-PRODUCTS

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INTRODUCTION

   Certain species of marine algae are sources for the industrial
production of gelling and/or viscosifying polysaccharides used as
food additives (alginates, carrageenans, agars). By-products
from these extractions are not or poorly valorized and in a
project aiming at differentiating the sources and types of
dietary fibres such materials were investigated. The present
study focus on the chemical and physico-chemical characterization
of by-products from *Eucheuma cottonii* used as raw material for
the production of the gelling polysaccharide kappa-carrageenan[1]
(Fig.1).
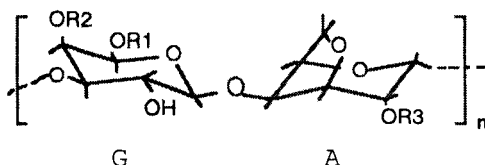
G: ß-D-galactopyranose; A: 3,6-anhydro-∝-L-galactopyranose
R1 = H, R2 = OSO3, R3 = H: kappa-carrageenan
R1 = H, R2 = OSO3, R3 = OSO3: iota-carrageenan
R1 = H or CH3, R2 = H or OSO3, R3 = H or OSO3: 'deviant' carrageenan

Figure 1. Chemical structures of carrageenans

MATERIALS AND METHODS

Eucheuma cottonii industrial residues were kindly provided as
dried pieces from Copenhagen Pectin Fabrik. These pieces were
ground to particle size $\leq$ 0.5 mm and washed extensively with 80%
ethanol (sample EC), or washed with 80% ethanol acidified with
HCl (0.01N) followed by 60% ethanol containing NaCl (0.1M), 60%

ethanol and 80% ethanol (sample ECNa). Preparation of the
calcium form (ECCa) was obtained by suspending ECNa in 60%
ethanol containing $CaCl_2$ (1.0M) followed by 60% and 80% ethanol
washes. These preparations were dried by solvent exchange and
over P_2O_5 at 40°C *in vacuo*. All results are expressed on a dry
weight basis.

Dietary fibre yields are obtained gravimetrically[2]. Soluble
fibres were precipitated in 4 vol. ethanol from the hot buffer
used for enzymatic digestions and after subsequent re-extractions
with water at 90-95°C. Hot-water insoluble residues represent
the insoluble fibres.

Chemical determinations. Neutral sugar contents were
determined by gas liquid chromatography of their alditol acetates
obtained after sulfuric acid hydrolysis[3]. Anhydrogalactose (36AG)
and sulfate (SO_4) were determined by colorimetry[4] and
turbidimetry[5], respectively. Ashes were measured gravimetrically
after mineralization. Protein concentrations were determined by
the Kejdhal method (N x 6.25).

Physico-chemical determinations. Infrared transmission spectra
were recorded on films prepared as described[6] using a Fourier
transform Nicolet 10-DX.

Water-absorption was determined using a Bauman apparatus
consisting of a fritted glass covering a thermostated (25°C)
solvent reservoir to which a graduated pipette is connected. The
volume of solvent absorbed by 50-100 mg sample on the fritted
glass is read on the pipette after 30 minutes.

Water-retention is the moisture content (1h at 120°C) of the
fibre pellet recovered after overnight agitation in the solvent
at room temperature, 1h centrifugation (14 000g), and removal of
excess solvent over fritted glass G1 for 2 h.

Water-swelling was determined after 16 or 48 hours incubation
at room temperature of samples in solvent using a graduated
cylinder.

RESULTS AND DISCUSSION

Eucheuma cottonii extraction by-products (EC) are rich in
soluble (57%, ECsol) and insoluble fibres (32%, ECins), or 41.5%
soluble and 29.2% insoluble dietary fibres (after subtracting ash
and protein contents).

Chemical characteristics of the polysaccharides found in the
soluble and insoluble fractions are shown in Table 1. The water
soluble polysaccharides are rich in anhydrogalactose, galactose
and sulfate, whereas galactose and glucose are the major sugars
of the insoluble fractions. Anhydrogalactose content in the
latter fraction could not be determined by colorimetry due to the
presence of interfering sugars. From these results a carrageenan
structure for the soluble polysaccharides and a mixed cellulose-
galactans population in the insoluble fibres are proposed. The
infrared spectrum of the soluble fibres allowed for the location
of the sulfate and confirmed the carrageenan structure of these
polysaccharides[6] (Fig. 2). From the sulfate content and the lower
absorbance observed at 820 cm^{-1} as compared to iota-carrageenan
(theoretical 37.3% sulfate), it can be concluded that this

fraction consists either of a mixed population of iota- and kappa-carrageenan and/or 'deviant' (hybrid) iota-carrageenan molecules.

WAVENUMBERS (cm⁻¹)
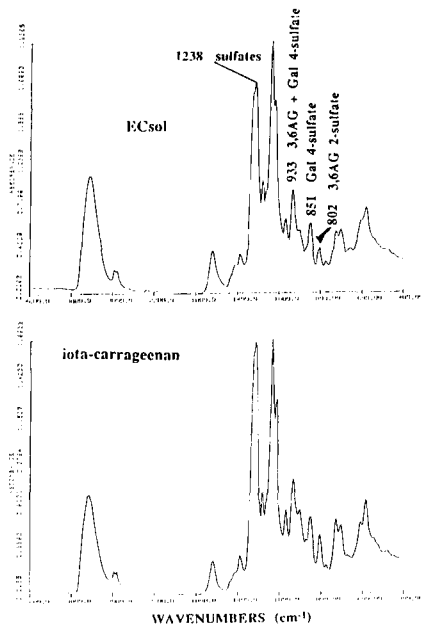
Figure 2.   Infrared spectrum of ECsol and iota-carrageenan

The physico-chemical properties of *E. cottonii* fibres reflect the ionic nature and the high proportion of the water-soluble fibres. Kappa- and iota-carrageenan are known for their selectivity for potassium and calcium, respectively, in their physico-chemical and rheological characteristics. All sodium forms of carrageenan are soluble in cold water whereas their calcium form will swell as a function of the type and concentration of cations present in the solution and temperature[7]. Being a mixed population and/or hybrids of both carrageenan types, the soluble fibres demonstrated differences in physico-chemical properties in relation with the salt solution and their ionic form.

Water-absorption values for EC, ECsol, and ECins with regard to the type of ions present in the solution are shown in Table 2. Although absorption obtained with water was higher in cases of EC and ECsol, reproducible results were only obtained with salt solutions. Water-absorption is higher with NaCl than with KCl and is very close for the EC and ECsol fractions. For these latter samples, water-absorption is related to the ionic strength of the solution (Fig. 3). No such marked variation is observed for ECins nor for commercial iota- and kappa-carrageenan   Other

chemical and/or physical particularities of EC and ECsol have therefore to be taken into account to explain this phenomenon.

The ionic form of fibreis affected water-absorption, water-retention, and water-swelling values (Table 3). Thus, fibres in sodium form absorb more water than those in calcium form at both ionic strength and for the three salt solutions investigated. At low ionic strength, absorption of both forms of fibres increases in the order K > Ca > Na, whereas at high ionic strength only the calcium form show a small increase in absorption in the order Ca > K > Na.

Water-retention values are very low and relate most probably to the insoluble fibre fraction as most of the soluble fibres were expected to be dissolved in the solvents. For this reason, the values are very close for both ECCa and ECNa and do not show marked variations between salt solutions. They show slightly higher retention at low ionic strength than at I = 0.5.

Water-swelling is higher for both fibre forms at low ionic strength than at I = 0.5 and values are close for both forms of fibres. Measurements were complicated for fibres in sodium form by the soluble polysaccharides that formed viscous solutions retarding the settling of the insoluble fibres. Water-swelling values increase in the order Ca > K > Na for fibres in calcium form at both ionic strength. Fibres in sodium form swell less in calcium solutions as compared to sodium or potassium solutions.

Table 1. Composition of fibre fractions from *E.cottonii* by-products (% weight)

	Rha	Fuc	Ara	Xyl	MeGal[1]	Man	Gal	Glc	36AG[2]	SO$_4$[3]
ECsol				2.0	0.4	0.3	30.6	1.7	38.3	27.5
ECins	0.3	0.1	0.6	2.1		3.3	10.9	55.2		6.1

[1] 6-O-methyl galactose, [2] 3,6-anhydrogalactose, [3] sulfate

Table 2. Water-absorption of fibre fractions from *E. cottonii* by-products (g / g)

	H$_2$O	NaCl (100mM)	KCl (100mM)
EC	20.0	17.2	14.1
ECsol	24.8	18.5	14.1
ECins	8.1	10.7	6.5

Table 3. Physico-chemical characteristics of fibres in the sodium and calcium form of *E. cottonii* by-products

FORM		SOLUTION					
		NaCl		KCl		CaCl$_2$	
	Ionic strength	0.01	0.5	0.01	0.5	0.01	0.5
	Absorption (g/g)	17.8	9.4	13.4	9.9	18.3	9.5
Na	Retention (g/g)	2.0	1.3	2.1	1.1	2.3	1.0
	Swelling (ml/g)	22.5	19.1	24.4	17.3	18.8	15.2
	Absorption (g/g)	15.2	9.2	8.4	8.5	12.9	7.3
Ca	Retention (g/g)	2.0	1.3	1.8	0.9	1.8	0.8
	Swelling (ml/g)	27.4	18.2	25.5	15.3	21.2	13.7

CONCLUSION

Extraction by-products of *E.cottonii* are potentially useful as they are specially rich in soluble dietary fibres. Being essentially composed of ionic polysaccharides, the physico-chemical properties of these fibres can be tailored by changing their ionic form and ionic environment.
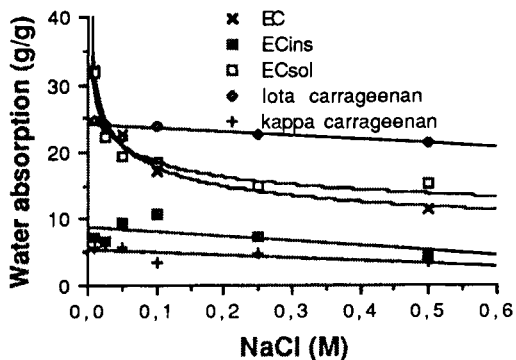
Figure 3 Effect of ionic strength on water-absorption

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THE CONTRIBUTION OF MICROBIAL NON-STARCH POLYSACCHARIDES (NSP) TO THE TOTAL NSP CONTENT OF FAECES

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1   INTRODUCTION

There is increasing interest in the use of alternatives to cereals as energy sources for pigs, because cereals are relatively expensive and can be consumed directly by man. There are, by contrast, many foods which are unpalatable for humans but which are readily eaten by pigs. These are often by-products of the food or feed industries and often have a high content of non-starch polysaccharides (NSP), which are fermented to varying degrees by the gut microflora. Thus, measurements of the digestibility of the NSP fraction are of importance when evaluating their potential as energy sources. Digestibility values are usually obtained by determining the difference between NSP intake in the feed and NSP output in the faeces. However, in addition to undigested diet residues, faeces contain gut microbes and endogenous secretions, and therefore NSP present in these fractions of non-dietary origin will contribute to the total faecal NSP content. This will result in an under-estimate of the digestibility of the NSP in the diet, the extent to which will clearly depend on the amount of NSP of non-dietary origin in the faeces. Such digestibility measurements are therefore approximate and are referred to as the 'apparent' digestibility of a diet. However, correction for the contribution made by non-dietary components will give a more accurate 'true' digestibility value.

It is known that microbial components in the faeces of pigs fed conventional diets may account for up to 75% of faecal dry matter (DM). As faecal microbes contain some of the same NSP constituents as those in diets, it is important to estimate the contribution of microbial NSP to

the faecal total, because NSP from such a major non-dietary fraction of the faeces could cause substantial under-estimates of the digestibility of NSP in a feedstuff.

The aim of this study was to determine both the types and amounts of NSP in cultures of faecal microbes, so that the extent of their contribution to apparent digestibility measurements could be assessed. This will be discussed in conjunction with an estimate of the contribution of NSPs present in endogenous material to faecal NSP.

2   MATERIALS AND METHODS

A mixed microbial population was obtained from a 36 kg Large White x Landrace boar pig, which had been fed a diet containing 32% cereals, 45% molassed sugar beet feed, 19% soya bean meal, 2.5% fishmeal and a vitamin and mineral premix.

One gramme of fresh faeces was introduced into a 250 ml screw-capped bottle, containing 100 ml of yeast-dextrose broth (YDB). After a 24 h incubation at 37°C without agitation, 1 ml of the culture fluid, free from non-microbial debris, was sub-cultured into each of 10, 250 ml bottles containing YDB and incubated for a further 24 h. The resultant cultures were centrifuged at 3,000 x g for 20 min., and the supernatants discarded. The pellets were then resuspended in 100 ml saline and centrifuged as before. This washing procedure was repeated twice, and the washed pellets were pooled and lyophilised.

The Englyst and Cummings[1] method was used to measure the constituent NSP monomers in feeds and faeces. This required boiling of samples in dimethylsulphoxide, followed by enzymic de-starching and washing in 85% ethanol. The NSP residue was then hydrolysed, and alditol acetates were prepared from the hydrolysates for analysis of neutral sugars by gas liquid chromatography. A color-imetric technique was used to quantify the uronic acid content of hydrolysates. These methods were also used to determine the NSP content of the lyophilized microbial material.

3   RESULTS

NSP accounted for 4.33% microbial dry matter. The con-stituent monomers were, in descending order of importance,

glucose, galactose, rhamnose and uronic acids (Table 1).

4   DISCUSSION

Dietary NSP is largely derived from the structural components of plant cell walls. Although plant cell walls differ in their composition depending upon their botanical source, those used in pig diets typically contain little rhamnose, some mannose and galactose, large amounts of glucose, and intermediate levels of arabinose or xylose. Quantities of uronic acids, however, vary greatly depending on their botanical origin.[2,3,4]

In this study, faecal microbes contained low levels of rhamnose, galactose, glucose and uronic acids. Theoretically, therefore, these individual NSP components of microbial origin could result in slight under-estimation of the digestibility values for their dietary counterparts. However, dry pig faeces frequently contain in excess of 35% NSP, and even if faecal microbes represented all of the remaining faecal DM, the maximum possible contribution of microbial NSP to the faecal total would be very small (<2.9%, i.e. 1.6%, 0.6%, 0.4% and 0.19% of DM in the form of glucose, galactose, rhamnose and uronic acids respectively).

A further potential source of non-dietary NSP is the glycoprotein in endogenous secretions. However, the only NSP constituent found in mucus from the mouth, stomach and colon of the pig was a small amount of galactose.[5] As galactose is usually well digested by pigs from a variety of diets[2,3,6] any effect of endogenous galactose on overall galactose digestibility is likely to be small.

Values obtained for the excretion of non-dietary non-cellulose NSP by pigs fed diets with cellulose as the sole NSP source are given in Table 2 (Longland et al., unpublished). These values are considerably less than the low, maximum theoretical values for microbial NSP quoted above. In either case, such small amounts of non-dietary NSP are unlikely to have much effect on NSP digestibility measurements. Therefore the 'apparent' digestibilities obtained for NSP monomers by pigs can probably be regarded as near to 'true' digestibility values. This is certainly the case for the pentosans arabinose and xylose which have not been detected in either faecal microbes or endogenous secretions.

In the light of the above results, it would seem

likely that the only occasion when a non-dietary NSP sugar
monomer could cause a major under-estimate of its apparent
digestibility would be when it was a very minor component
of the diet.

Table 1   The NSP content of the lyophilised faecal
           microbes (% of dry matter)

Rhamn- ose	Arab- inose	Xyl- ose	Mann- ose	Galact- ose	Gluc- ose	Uronic Acids	Total NSP
0.71	0	0	0	0.96	2.36	0.30	4.33

Table 2   NSP constituents of non-dietary origin in the
           faeces of pigs fed a semi-purified diet with
           cellulose* as the sole NSP source

		NSP (% faecal DM)			
Rhamnose	Arabinose	Mannose	Galactose	Uronic Acids	Total NSP
0.02	0	0	0.12	0.20	0.34

* contained glucose and xylose
(Longland *et al.*, unpublished results)

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DISTRIBUTION OF SOLUBLE FIBER IN VARIOUS MILLSTREAMS OF WHEAT

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1 INTRODUCTION

Fiber, described today as Total Dietary Fiber (TDF), can be broadly divided into water Insoluble Fiber (IF) and Soluble Fiber (SF). Whereas IF appears to be helpful in various disorders of the intestinal tract, SF has variously been reported[1,2] to lower elevated blood Cholesterol (CH) levels, a risk factor in heart disease.

Cereal-based foods made with white flour, although not high in TDF, do contain a good portion of the fiber as SF. This fiber (source: bread products) has been shown to lower blood CH in animals[3].   A recent study with hypercholesterolemic men has also shown a CH-lowering effect of SF present in refined cereal-based products[4]; cereal-based products tested in this study included white and variety bread, hamburger buns, saltine crackers, biscuits and waffles.

White flour contains about 2.5% TDF; two-fifths or more of this is SF. The study reported here was undertaken to both develop information on the distribution of SF in various millstreams of wheat and to identify a stream(s) that might be a more significant source of SF than white flour itself, and thus, could be used in food products to add more SF to the diet.

2 MATERIALS AND METHODS

A properly tempered hard red winter wheat was milled in the pilot mill of Kansas State Univeristy. The procedure used 19 grinding steps and yielded 23 flour streams and some by-products (bran, shorts, red dog and the resultant millfeed stream). Patent and clear flours are made up of blends from selected flour streams; straight grade flour is the blend of all flour streams.

All millstreams, including wholewheat flour, were analyzed for fiber -- IF and SF[5]. Samples were also analyzed for other components (information not included here).

3 RESULTS AND DISCUSSION

In the milling procedure followed, combining all flour streams yielded 76.4% straight grade flour (66.4% patent flour and 10% clear flour). The germ fraction accounted for 0.5% of the yield. The millfeed stream accounted for the remaining 23.1%.

Although millstreams have been extensively characterized by chemical and physical measurements, this characterization has not included an assessment of the distribution of IF and SF. The results of this study show that bran contained more SF (content, 2.1%) than any other millstream except the red dog fraction, a minor fraction of wheat milling. However, by virtue of its high IF content (41.9%), bran remains primarily a source of IF. Wholewheat flour and the germ fraction each contained about 10% TDF; however, only about one-tenth of this amount is SF. SF in flour streams that make up white flour ranged between 1 and 1.9%; IF in these streams ranged between 0.9 and 4.9%. These ranges would be much narrower, and IF and SF values much closer, if a few minor streams (bran and shorts duster, sixth middling, etc.) were excluded from consideration.

No one flour stream emerged as exceptionally high in SF; the fraction sixth middling is a little higher (SF content, 1.9%), but it is also higher in IF (content, 4.9%). A fraction exceptionally high in SF could be manipulated, through modification in the milling procedure, to increase its yield. In the absence of such a revelation, patent and straight flours remain the flours of choice to be used in products to simultaneously increase our intake of both IF and SF. Patent flour contained 1.3% SF and 1.2% IF while straight grade flour contained 1.1% SF and 1.4% IF. Clear flour contained about the same amount (content, 1.4%) of SF as the other two flours, but it was substantially higher in IF (content, 3.3%).

Products made with 150 g of patent flour, the current level of flour consumption in the U.S.A., would add about 2 g SF (4 g TDF) to our diet. A 50% increase in flour consumption would add another 1 g SF; this will also help increase the level of utilizable complex carbohydrates in the diet to the levels now recommended[6]. Other plant-derived foods can easily add another 4 to 5 g SF to our diet. This way SF would represent about one-third of the recommended intake of 20 to 35 g fiber a day in the U.S. diet. A more dramatic change in food habit (including specially formulated foods, for example) would be required to increase SF intake well above 8 g a day.

In conclusion, no single flour stream from the traditional wheat milling procedure could be identified as being exceptionally high in SF. Studies are in progress, however, to air-classify white flour which may yield a subfraction noticeable higher in SF.

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INFLUENCE OF AUTOCLAVING ON PHYSICOCHEMICAL PROPERTIES OF THE BEET PULP FIBRES AND ON SEVERAL OF THEIR PHYSIOLOGICAL EFFECTS

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1 INTRODUCTION

Beet pulp fibres represent an important by-product of the sugar industry which has been used up to now in animal feeding. Their physico-chemical properties suggest their possible utilization as dietary fibres in human nutrition and possibly in drug industry. Technological treatments have been shown to be able to optimize several of the properties of the fibres and thus, may be used to improve their physiological effects. For instance, extrusion-cooking of wheat bran is able to increase its soluble fibre content and its water absorption capacity [1]. This same process has been used to induce physico-chemical transformations in the fibres of sugar-beet pulp, citrus peels and apple marcs [2].
In the present work, the physico-chemical characteristics of these fibres have been compared to those of soluble (apple pectins) or mainly insoluble (wheat bran) fibres. Some physiological effects of these products have been evaluated on rats.

2 MATERIAL AND METHODS

Material: Sugar beet fibres, wheat bran and apple pectin (Rapid set pomme) were respectively purchased from S.R.D. Society (Sucre-Recherche-Développement, Compiègne, France), Breteau-Aubert (LaVarenne, France) and Unipectine (Redon, France).

2 Kg of beet fibres (average particle size: 570 μm) were swollen during 1 hour in a sodium acetate buffer (0.03 M) pH4. Half of the fibres were then autoclaved at 126°C during 1 hour. Both lots of fibres were freeze-dried without elimination of the unabsorbed solution and coarsely grounded using a Braun mixer.

<u>Analytical methods and water holding capacity determination</u>:
Analytical methods for crude protein, neutral sugars, uronic
acids, volatile fatty acids and chromic oxide were previously
described [3]. Hydrogen and methane were analysed as described by
Champ et al. [4]. Dietary fibre content was determined
gravimetrically after enzymatic removal of starch and proteins [5].

The water holding capacity was determined by the centrifugation
method according to the procedure of Mc Connell et al. [6] and
results expressed as g of solution (0.15M NaCl) per 100 g of dry
residue.

<u>In vivo experiments</u>
Animals: 4 male Wistar rats (mean weight: 320 g) were placed
individually in separate metabolic cages. The animals were
successively adapted to each experimental diet during 9 days; the
different nutritional and physiological parameters were
determined at the end of each adaptation period. The animals were
allowed free access to water and feed. Their feed intake was
controlled and the animals were weighed 4 times during each
experimental period. Transit time measurement has been estimated
on a 48 hours period. Feed containing 2% chromic oxide was
distributed during 2 hours (from 3:00 to 5:00 p.m.); food without
chromic oxide was then distributed (*ad libitum*). Faeces were
hourly collected during 48 hours and were pooled during the next
20 hours. The faeces were freeze-dried and kept at room
temperature until analysed for chromic oxide. The procedure for
quantification of excreted gases using a respiration chamber has
been described previously [4]. At the end of the experiment, the
rats were killed at 11:00 a.m. by intra peritoneal injection of
Nembutal, the caecum was quickly collected; its content was
controlled for pH, frozen at $-20°C$ and kept for volatile fatty
acids analysis.
Diets: A casein-DL-methionine mixture (about 50:1, w/w) was used
as protein source, supplying, with the fibre sources, 20 g
(N*6.25)/100 g, dwb, in the diets. All diets contained, 4.5 %
mineral mixture, 0.5 % vitamin mixture and 5 % maize oil. The
composition of the mineral and vitamin mixtures has been given in
full previously [3]. Autoclaved (BFa), unautoclaved (BFua) beet
pulp fibres, wheat bran (WB) and apple pectins (AP) were
respectively incorporated to BFa, BFua, WB and AP diets at the
following levels: 9.1, 8.8, 10.2 and 6.8 %. These diets contained
respectively 58.6, 58.9, 59.0 and 60.6 % instant mashed potatoes
(dwb).
Statistical evaluation: The *in vivo* data were expressed as the
mean ± the standard deviation. Student's t-test was used to
determine the significance of the differences between groups.

3 RESULTS AND DISCUSSION

Analytical and physicochemical characteristics of the fibre sources

Table 1 Chemical and physical characteristics of the fibre
sources

Fibre	BFa	BFua	WB	AP
Soluble fibre[1]	27.4	12.0	n.d.	92.7
Insoluble fibre[1]	57.0	80.3	n.d.	0
Total fibre[1]	84.4	92.4	44.0	92.7
Sugars (%/DM)[2]				
Rha	0.98	0.98	0.00	0.63
Ara	17.51	18.60	6.49	5.41
Xyl	1.30	1.20	12.35	1.01
Man	1.33	1.35	0.43	0.26
Gal	4.38	4.35	0.63	4.53
Glu	20.01	20.15	8.43	16.76
Uronic ac.(%/DM)[3]	20.41	21.60	2.70	59.30
W.H.C.(g. H_2O/g.MS)				
1h	11.15	10.71	4.25	nd
24h	12.71	11.23	4.67	nd

[1] Prosky et al.[5]; [2] Hoebler et al.[7]; [3] Thibault[8].

Dietary fibre content (total, soluble and insoluble) of the
different fibres sources is reported in Table 1. The total beet
dietary fibre content, was relatively high (92.4-84.4%) within
the range reported by other authors [9-13]. A shift from insoluble
to soluble fibres was observed when autoclaved. Since the
autoclave conditions were severe, some of the dietary fibres
were made soluble and degraded; therefore, they did not
precipitate in alcohol. That explained the slight decrease in
total dietary fibre for the BFa. This phenomenon was also
reported by Guillon [13]. She had shown that soluble fibres were
mainly constituted of pectic material and arabinans. Apple
pectins were soluble fibres, mainly composed of galacturonic acid
(59.3%), glucose (16.8%), arabinose (5.4%) and galactose (4.5%)
as neutral sugars. Wheat bran contained 44.4% of total dietary
fibres; these data are in agreement with the values reported in
the literature [1,9]. Xylose, glucose and arabinose were the major
sugars of these fibres. The water holding capacity obtained for
wheat bran were lower than those of the beet fibres. These values
are difficult to compare with data of the literature   because
they depend on the technique, the experimental conditions and the
preparation of the substrates [6,14,15]. However, values obtained
on several industrial by-products [16], (vegetables) [17] are higher

(2.6-35.1 g.g-1)   than for cereals (1.5-3.0 g.g-1). These
differences could be related to the individual properties and
structures of the various components of the substrates. Cereals
often contained some residual starch in a crystalline form and
lignin, an hydrophobic substance, while non cereal products are
rich in pectins which are very absorbent. Values for autoclaved
and initial fibres were not significantly different and did not
vary with the duration of the hydratation. They are lower than
those reported by other authors [10,11,13]. Pectins act as a cement
between the different cell wall components; their removal should
have caused a decrease in the water holding capacity.

Nutritional and physiological properties of the fibre sources
(Table 2)

Food consumption and weight gain were slightly lower (NS) for AP
diet whereas the best food efficiency is obtained for WB diet.
Total transit time is not deeply affected by the source of
fibres. However, if the parameter "excretion of 80% of the
marker" is considered, the tendancy appeared to be in agreement
with the literature [18]: their is a positive correlation between
the soluble content of the fibre and the transit time. Faecal
excretion was lower with the AP diet which contained highly
fermentable pectins; the same observation has been made by
Cummings et al.[18]. Slightly higher dry matter of the faeces and
caecal content observed in rats fed wheat bran may be explained
by a higher excretion of dietary residue and/or by a smaller
bacterial mass [20]. Most of the characteristics of the caecal
content agreed with the data of the literature [3,21]. Caecal pH
was higher for AP group and the VFA profile of this same group
revealed a low formation of butyric acid. Both diets containing
beet fibres had intermediate VFA profiles between WB and AP
diets. Hydrogen and methane excretions determined in a
respiration chamber indicated a poor production of methane for
the WB group compared to the other groups. Diets with PFa, BFua
and AP induced a high excretion of methane which may be due to
the methyl groups of their highly methylated pectins.

4 CONCLUSION

Physico-chemical characteristics of beet pulp fibres (with
or without autoclaving) have been compared to those of soluble
(apple pectins) or mainly insoluble fibres (wheat bran). These
fibres have then been incorporated to a semi-synthetic diet to
evaluate their effect on food consumption, transit, faecal
excretion and fermentability in the large intestine (gases and
short-chain fatty acids formation) in the rat.

Dietary Fibre: Chemical and Biological Aspects

It appears from this study that beet pulp fibres have physiological effects which are due to their soluble fibre fraction (hydratation of the faeces, flatus), or to their insoluble fibres fraction (faecal excretion). However, these effects are not influenced by an hydrothermic treatment as it has been applied in the present study (autoclaving in a buffered medium, pH 4).

Table 2 Nutritional and physiological effects of the fibres
(mean ± SD)

Diet	BFa	BFua	WB	AP
Food consumption[1]				
(g DM/day)	22.0±3.2[a][3]	21.6±1.4[a]	21.3±5.0[a]	18.3±4.2[a]
Weight gain[2]				
(g/day)	2.16±0.59[a]	2.06±2.21[a]	2.66±2.52[a]	1.79±3.25[a]
Transit time (hr:min)				
maximal conc. of the marker	13:15±2:04[a]	14:00±8:21[a]	14:00±8:26[a]	13:53±7:12[a]
excretion of 80% of the marker	27:50±5:17[a]	26:22±2:11[a]	24:46±10:29[a]	28:56±1:43[a]
Fecal excretion				
g DM/day	1.99±0.20[a]	1.89±0.18[a]	2.02±0.71[a]	1.41±0.10[a]
% DM	42.6±2.9[a]	42.0±1.1[a]	46.5±3.0[a]	42.0±2.8[a]
Caecal content characteristics[4]				
caecal wght/ body wght (%)	0.95–0.61[ab]	0.93–0.86[a]	0.71–0.67[b]	0.66–0.13[ab]
% DM	15.2–14.4[a]	12.8–14.5[a]	22.1–21.6[a]	18.1–10.0[a]
pH	6.60–6.36[b]	7.03–6.19[ab]	6.61–6.70[b]	7.32–7.33[a]
VFA				
μmoles/200g rat	260–136[a]	196–263[a]	164–145[a]	117–28[a]
molar ratio (%)				
C2	65.4–75.6[ab]	62.5–64.6[a]	59.0–56.8[b]	65.9–76.9[ab]
C3	18.1–14.8[a]	12.0–22.4[a]	16.8–13.8[a]	23.6–15.7[a]
C4	16.5–9.6[b]	25.5–13.1[ab]	24.2–29.4[a]	10.4–7.4[b]
H_2 and CH_4 excretion (cm^3/24hr/g. ing. fibre)				
H_2	4.80±5.47[a]	3.23±0.91[a]	4.57±3.81[a]	5.53±6.79[a]
CH_4	13.78±5.79[a]	18.07±6.04[a]	7.34±2.92[b]	12.09±8.21[ab]
total	18.58±7.13[ab]	21.31±5.15[a]	11.92±5.60[b]	17.63±3.67[ab]

[1] during the last 3 days of the adaptation period; [2] during the 14 days of the adaptation period; [3] means in the same line bearing common superscripts are not significantly different ($p \leq 0.05$); [4] data obtained from 2 rats

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BIOSYNTHESIS OF THE HEMICELLULOSE, GLUCURONOXYLAN, IN PLANT FIBRE

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1 INTRODUCTION

Glucuronoxylans are the main hemicelluloses of secondary walls of dicots, and hence are major constituents of fibre derived from these plants. They contain a backbone of $\beta(1-4)$-linked xylose residues, to about 10% of which 4-O-methylglucuronic acid residues are linked by $\alpha(1-2)$-linkages. About half the xylose residues are acetylated on C-2 or C-3, and some arabinose residues may also be present as side chains[1](Fig.1). Similar molecules, with the same backbone but with a higher proportion of arabinose side-chains, are found in the primary walls of dicots and in monocot hemicellulose.

The biosynthesis of this group of polysaccharides is carried out by glycosyltransferases, which are probably located in the Golgi apparatus[2]. Methyl- and acetyl-transferases are also thought to be involved. This paper summarises what is known about the biosynthetic system, and focusses on the functional interactions of the enzymes.

2 MATERIALS AND METHODS

Epicotyls (50g) from 7-day-old etiolated pea (*Pisum sativum*, var. Alaska) seedlings were homogenised with 150ml Tris/HCl (10mM, pH 7.5) at 4°C in a Polytron homogeniser. The homogenate was filtered through muslin and centrifuged at 97,000g for 30 min. The pellets were re-suspended in 50mM Tris/HCl, pH 7.5, to form the enzyme preparation.

Incubations were carried out at 25°C with 50μl enzyme

MeGlcA   Ara   MeGlcA
   | | |
...-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-Xyl-...
      | | | | |
     Ac   Ac Ac   Ac   Ac
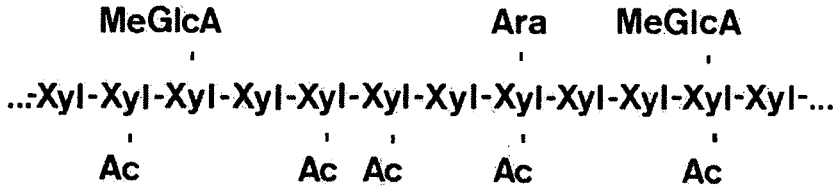
Figure 1: Structure of glucuronoxylan
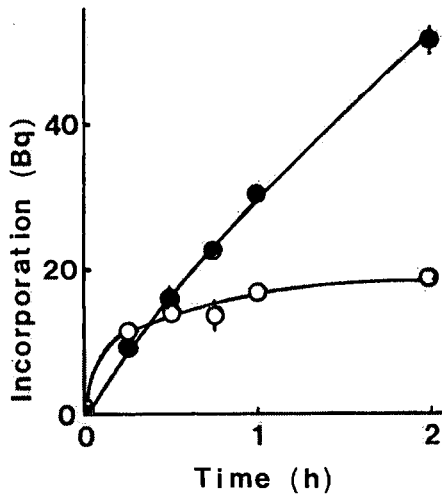
Figure 2: Incorporation of (^{14}C)GlcA into hemicellulose
          in the presence (●) and absence (○) of UDPXyl.

preparation in a total volume of 100μl. The standard
incubation media were as follows. For glucuronyltransferase:
UDP-(^{14}C)glucuronic acid (460 Bq, 0.5μM), $MnCl_2$(10mM),
± UDP-xylose (UDPXyl) (1mM). For xylosyltransferase:
UDP-(^{14}C)xylose (8.3 kBq, 1mM); $MnCl_2$(10mM). For methyl-
transferase: S-adenosyl-L-(methyl-^{14}C) methionine (6.5k
Bq, 0.3μM); $CoCl_2$(1mM). All incubations were terminated
by addition of 70% ethanol. Particulate material was
washed twice with 70% ethanol, extracted twice with EDTA-
NaH_2PO_4 buffer (50mM, pH 6.8) at 100°C for 15 min., and
washed with water. The radioactivity in the insoluble,
polymeric material was then estimated by scintillation
counting.

3 RESULTS AND DISCUSSION

The xylosyltransferase is found in the particulate fraction
sedimenting at 97,000g[1]. Under our assay conditions, 84%
of the radioactivity in the polymeric product was present
as xylose residues. The xylose-containing product is β
(1-4)xylan, as judged by its degradation by β(1-4)xylanase
but not by xyloglucanase. The remaining 16% is composed
of radioactive arabinose, as a result of epimerisation to
UDP-arabinose followed by the action of an arabinosyl-
transferase. It is not clear whether the arabinosyl is part
of the same polymer as the xylose, or whether it is part of
another polymer, such as arabinogalactan.

    The glucuronyltransferase is present in the same
membrane fraction as the xylosyltransferase. Only very
limited incorporation of glucuronic acid into polymeric
material occurs when UDP-glucuronic acid (UDPGlcA) is the
only sugar nucleotide present. However, in the presence
of UDPXyl, sustained incorporation of glucuronic acid can
occur for at least eight hours, though the initial rate
is lower as a result of competitive inhibition by UDPXyl
(Fig.2). This dependance of the glucuronyltransferase on
the presence of UDPXyl is thought to be due to the need
for xylan to be formed in the particulate preparation,
onto which glucuronic acid can then be transferred[3].

    To look more closely at the interaction between the
two enzymes, the ability of the glucuronyltransferase to
transfer GlcA onto pre-formed xylan was investigated.
First, it was shown that transfer onto added xylan or
short, β(1-4)-xylose-containing oligosaccharides did not
occur. Secondly, a preincubation experiment was carried
out. The particulate enzyme preparation was preincubated
either with non-radioactive UDPXyl (1mM) or with no added
sugar nucleotide. It was washed free of added sugar

Table 1 Effect of preincubation of the enzyme preparation
         with UDPXyl on incorporation of (^{14}C)GlcA from
         UDP(^{14}C)GlcA into glucuronoxylan.

Preincubation		Incubation with UDP-(^{14}C)GlcA		Incorporation of (^{14}C)GlcA (Bq)
Length	UDPXyl	Length	UDPXyl-	
30 min	+	4h	+	29.1 ± 2.0
"	+	"	−	9.9 ± 0.9
"	−	"	+	32.3 ± 1.2
"	−	"	−	5.1 ± 0.4
2h	+	"	+	22.2 ± 0.1
"	+	"	−	7.4 ± 0.2
"	−	"	+	22.7 ± 0.1
"	−	"	−	8.2 ± 2.0

nucleotides by centrifugation at 97,000*g*, and then incub‐
ated with UDP-(^{14}C)GlcA for 4 hours, either with or without
non-radioactive UDPXyl(1mM). The results are shown in
Table 1; each result is the mean of duplicate incubations.

     The incorporation can be-seen to be dependent only
on the presence or absence of UDPXyl during the incubation
in which UDP-(^{14}C)GlcA was present. Neither the presence
of UDPXyl in the preincubation, nor the length of the
preincubation, had any significant effect.

     Thus it appears that GlcA cannot be added to pre-
formed xylan. Instead, the GlcA must be added to the
xylan backbone either at the same time as, or shortly
after, that part of the backbone is formed. Since xylan
is formed in the absence of UDPGlcA[1], it would appear
that the proportions of Xyl and GlcA in the polymer are
dependent on the relative concentrations of the two sub-
stances present at the time of backbone elongation. This
implies a tightly regulated supply of substrates to the
biosynthetic system *in vivo*, since the ratio of xylose to
glucuronic acid is thought to be fairly constant in cell-
wall glucuronoxylan, and pure xylan is only found in
specialised tissues such as seed endosperms[4].

     In contrast to the addition of GlcA, the addition of
methyl groups to the 4-hydroxyl of GlcA appears to take
place after formation of the polymer. Incorporation of
(^{14}C)methyl groups from S-adenosyl-(^{14}C)methionine into
glucuronoxylan continues for at least 8 hours in the
absence of added sugar nucleotides. If UDPXyl and UDPGlcA
are added, the rate of incorporation of (^{14}C)methyl groups

is doubled. So the methyltransferase appears to be able
to transfer methyl groups onto both pre-formed and newly-
formed polymers[5].

The enzymes which add arabinose and acetyl groups
to the polymer have yet to be identified. The donors
are probably UDP-arabinose and acetyl-CoA.

The glucuronyl- and xylosyltransferases in pea epi-
cotyls appear to be involved in the synthesis of both
primary and secondary walls. The glucuronyltransferase
is present throughout the length of the 7-day-old epicotyls,
including both the youngest, rapidly-expanding region at
the top (primary wall synthesis) and the oldest region
(where probably only secondary wall synthesis is occurring).
Stimulation of the glucuronyltransferase by UDPXyl also
occurs throughout the epicotyl, indicating the presence
of the xylosyltransferase. Glucuronyltransferase has
been found to increase during xylem differentiation in
bean tissue culture[6], and xylosyltransferase also
increases[7,8].

Our recent work is concerned chiefly with the
solubilisation and purification of the xylosyl-,
glucuronyl- and methyltransferases. All three enzymes
are soluble in non-ionic detergents such as Triton X-100[5,9].
The functional interactions between the enzymes will be
investigated further using the solubilised and purified
enzymes.

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PHYSICAL PROPERTIES OF DIETARY FIBRE IN RELATION TO
BIOLOGICAL FUNCTION

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1 INTRODUCTION

The 'non-starch polysaccharides' which constitute dietary
fibre are also used widely for control of product texture
in the food and related industries, and their physical
properties have therefore been studied extensively. The
aim of this article is to give a brief overview of the
molecular understanding that has resulted from such
research and to consider the possible implications for
physiological activity as dietary fibre.

    The first crucial distinction to be drawn is between
insoluble materials (the traditional 'roughage'), whose
main importance is likely to be for lower-bowel function,
and soluble polysaccharides, which may be particularly
significant in controlling absorption of nutrients from
the small intestine. Between these two extremes,
polysaccharides often exist as hydrated networks in, for
example, soft plant tissues and many food products. The
factors determining which of these states will be adopted,
and the properties of each,[1] are outlined below.

2 ORDER AND DISORDER

In solution, polysaccharides normally exist as expanded,
fluctuating coils. Assembly into compact, unhydrated
forms, such as cellulose fibrils or starch granules, by
contrast, requires the chains to adopt regular, ordered
shapes which can pack together efficiently. Whether such
ordered assemblies remain stable on exposure to excess
water or dissociate into individual hydrated chains
depends on the interplay of a number of different factors.

The most obvious is charge. Many polysaccharides have
charged groups (e.g. COO^- or SO_3^-) which repel one
another, thus favouring expanded coil geometry in
solution, rather than compact ordering. Less obviously,
the presence of small co-solutes, such as sugars or
salts, can alter the solvent-quality of water and shift
the balance between polymer-polymer interactions and
polymer-solvent interactions, normally in the direction
of decreased solubility.

     Adoption of an ordered structure also entails
considerable loss of mobility (i.e. entropy), which
must be more than offset by favourable non-covalent
interactions between the participating chains.
Non-covalent bonds (such as hydrogen bonds, electrostatic
attractions and van der Waals forces) are individually
weak and are effective in stabilising polymer assemblies
only when they act *co-operatively* in extended arrays, so
that ordered structures have a minimum critical length,
typically of about 10 residues in each chain. Thus changes
in primary sequence (such as branching, irregularly-spaced
sidechains or anomalous residues in the polymer backbone)
can be an important drive to solubility if they occur at
spacings less than the minimum sequence length for ordered
association. The presence of sidechains can, in itself,
promote solubility by providing an additional entropic
contribution from freedom of rotation about the
sidechain-mainchain linkage. Similarly, polysaccharides
with flexible linkages in the polymer backbone are less
likely to form stable, solvent-resistant assemblies than
those that are inherently stiff.

     Finally, temperature is an important determinant of
solubility. The overall change in free energy (ΔG) on
going from a compact, ordered structure to a disordered
coil is related to the enthalpy change (ΔH) from loss of
favourable non-covalent interactions and the entropy
change (ΔS) from increased conformational mobility by:

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

Thus entropic effects, favouring solubility, become
increasingly important with increasing temperature (T).
Because of the simultaneous, co-operative involvement of
large numbers of weak bonds, the formation and melting of
polysaccharide ordered structures usually occur as sharp
processes (in many ways analogous to phase transitions of
small molecules), in response to comparatively small
changes in temperature (or other external variables such
as ionic strength or pH).

3 INTER-RESIDUE LINKAGE PATTERNS

A major difference between polysaccharides and most other types of polymer is that the same monomer unit can be linked together in different ways to give different macromolecular structures. Figure 1 shows four polymers of D-glucose (cellulose, amylose, laminarin and dextran). Despite being built up from the same monomer, these materials all have very different physical properties. Conversely, different sugars linked together in the same way often give polysaccharides with closely similar properties. For example cellulose, chitin, mannan and xylan all form mechanically-strong, solvent-resistant matrices, but are built up from different monomer units (glucose, *N*-acetylglucosamine, mannose and xylose). In each case, however, the component residues are linked together through equatorial bonds diagonally opposite each other across the sugar ring, so that the bonds to and from each residue are parallel and almost co-linear. This linkage pattern (Figure 2a) gives flat, ribbon-like structures that can pack into tough, fibrillar assemblies.
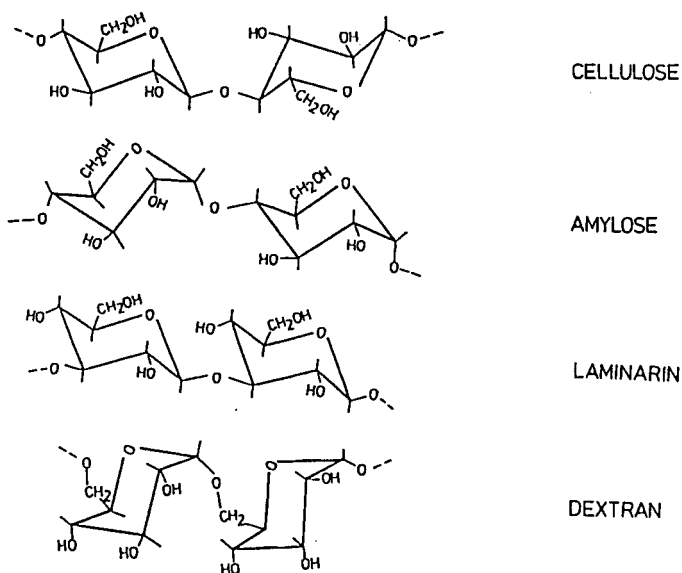
Figure 1 Polymers of D-glucose, linked β-1,4 (cellulose), α-1,4 (amylose), β-1,3 (laminarin) and α-1,6 (dextran).

Other types of bonding pattern also give rise to
characteristic ordered structures, with associated
characteristic physical properties, largely independent of
the nature of the component residues (unless gross
changes, such as the presence or absence of charge, are
also involved). For example, when the bonds to and from
each residue are once more directly opposite one another
but axial rather than equatorial, so that, although still
parallel, they are now offset by the full width of the
sugar ring, the resulting ordered structures are again
ribbon-like, but highly buckled (Figure 2b) and pack
together with cavities which, when the chains are charged,
can accommodate site-bound counterions.

When the bonds to and from each residue are no longer
parallel (Figure 2c), a systematic 'twist' in chain
direction is introduced, giving helical ordered structures
which are usually stabilised by packing together
co-axially. This situation can arise from bonds which are
no longer diagonally opposite each other (as in the
laminarin structure shown in Figure 1) or which are
diagonally opposite, but with one axial and one equatorial
(as in amylose). The ordered structures of amylose and
laminarin are double and triple stranded helices,
respectively. Linkage outside the sugar ring (as in the
dextran structure in Figure 1) joins the component
residues by three rather than two covalent bonds, giving
additional entropic stability to the disordered form.
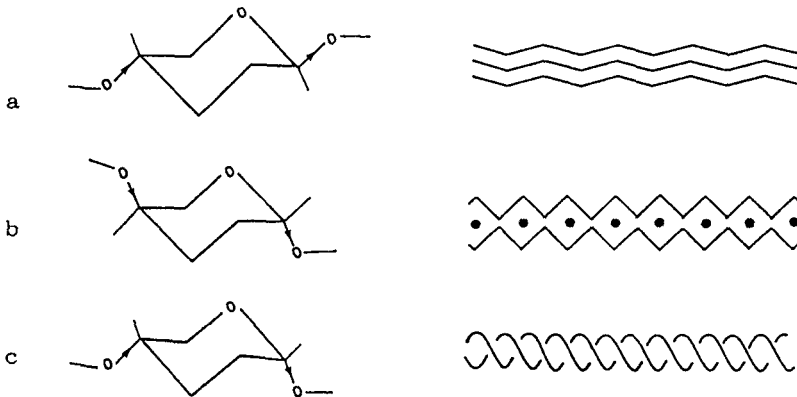
Figure 2   Inter-residue linkage patterns giving:
a) flat ribbons, b) buckled ribbons, c) hollow helices.

4 DISORDERED COILS IN SOLUTION

In contrast to the ordered structures of polysaccharides which, as outlined above, are critically sensitive to detailed primary structure, the properties of disordered chains in solution are dependent predominantly on molecular size,[2] which in turn is therefore likely to be a crucial determinant of biological function.

The most direct index[3] of the volume occupied by individual coils is the 'limiting viscosity number' or 'intrinsic viscosity', $[\eta]$. This is the fractional increase in viscosity per unit concentration of polymer, under conditions of extreme dilution (where there are no interactions between chains), and has units of reciprocal concentration (rather than units of viscosity). Intrinsic viscosity is related to molecular weight (M) by the Mark-Houwink relationship:

$$[\eta] = KM^{\alpha} \tag{2}$$

Theoretically, α has a value of 2 for fully-extended rigid rods and 0.5 for freely-jointed coils. For most real disordered polysaccharides α is close to 1, so that intrinsic viscosity (i.e. coil volume) is roughly proportional to molecular weight.

The constant of proportionality (K) is largely dependent on the nature of the linkages between adjacent residues in the polysaccharide backbone. Linkage patterns (Fig. 2) giving extended, ribbon-like ordered structures (flat or buckled) also give rise to expanded coil dimensions in solution (i.e. high values of K), whereas the non-parallel linkages that promote helical ordered structures give small, compact coils (low values of K).
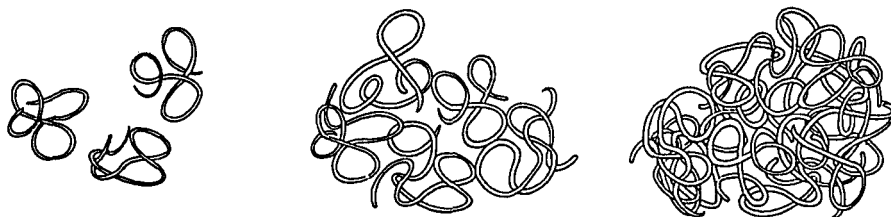
Figure 3   Space-occupancy by disordered polysaccharide coils.   Left: c < c*   Centre: c = c*   Right: c > c*.

Short sidechains (such as the single-residue galactose
substituents on plant galactomannans) have little, if any,
effect on coil dimensions; the intrinsic viscosity of
such materials is determined almost entirely by the length
of the polymer backbone.[4]   Extensive branching (as in
amylopectin), however, gives rise to very compact coils
(i.e. extremely low values of K) and to a decreased
dependence of coil volume on molecular weight (a < 0.5).

     As the concentration of a polymer solution is
increased, a stage is reached at which the individual
coils are forced to interpenetrate one another to form an
entangled network, as shown schematically in Figure 3.
The concentration at which this occurs is known as c*, and
is inversely proportional to coil volume, as characterised
by intrinsic viscosity (i.e. the larger the individual
coils, the fewer are required to fully occupy the space
available).   In general, c*[η] ≈ 4.

     Below c*, where individual coils are free to move
through the solvent, with little mutual interference,
viscosity is virtually independent of shear rate (i.e.
'Newtonian' behaviour).   Above c*, where chains can move
only by the much more difficult process of 'wriggling'
(reptating) through the entangled network of neighbouring
chains, viscosity (η) becomes highly dependent on shear
rate ($\dot{\gamma}$).   At low shear rates, where there is sufficient
time for entanglements pulled apart by the flow of the
solution to be replaced by new entanglements between
different chains, with no net change in 'crosslink
density', η remains constant at the maximum 'zero shear'
value, η_0.   With increasing shear rate, however, the rate
of re-entanglement falls behind the rate of forced
disentanglement, and viscosity falls (Figure 4).
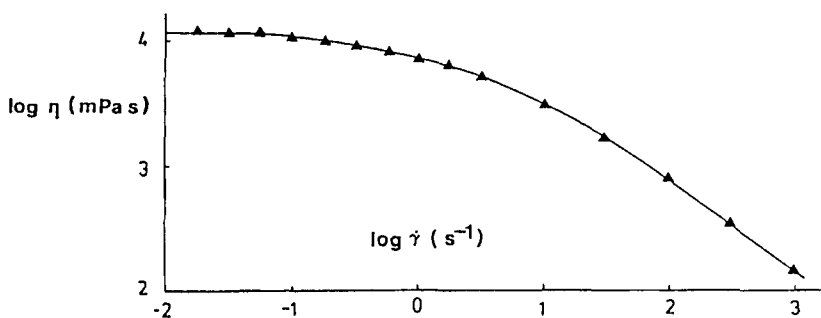
Figure 4   Typical shear-thinning for entangled coils.

The reduction in η with increasing $\dot{\gamma}$ is known as 'shear thinning' and can be very large, with viscosities often dropping by a factor of 100 or more over the shear-rate range available on most commercial viscometers.

The maximum viscosity (η_0) for disordered polysaccharides varies in a general, predictable way with concentration (c), irrespective of primary structure or molecular weight. At concentrations below c*, the increase in viscosity above that of the solvent (i.e. $\eta_0 - \eta_s$) is proportional to $\sim c^{1.3}$ (i.e. doubling concentration increases the 'excess' viscosity by a factor of ~ 2.5). Above c*, the concentration-dependence increases to $\sim c^{3.3}$, so that doubling concentration gives about a 10-fold increase in viscosity. The viscosity at c* is usually about 10 times that of water, a difference that would be difficult to detect visually. Thus almost all practical situations where solutions are noticeably 'thicker' than water involve the 'entangled' regime.

For samples with a high polydispersity of chainlength (which is invariably the case for food polysaccharides) at concentrations above c*, the form of shear-thinning is entirely general and can be fitted[5] with good precision by:

$$\eta = \eta_0 / [1 + (\dot{\gamma}/\dot{\gamma}_{\frac{1}{2}})^p] \qquad (3)$$

where $\dot{\gamma}_{\frac{1}{2}}$ is the shear-rate required to reduce viscosity to $\eta_0/2$, and p = 0.76 (the absolute value of the slope of log η *vs.* log $\dot{\gamma}$ at high shear rate). Rearrangement of Equation 3 gives:

$$\eta = \eta_0 - (1/\dot{\gamma}_{\frac{1}{2}})^p \eta \dot{\gamma}^p \qquad (4)$$

Thus plotting η against $\eta\dot{\gamma}^{0.76}$ gives a straight line of intercept η_0 and gradient $-(1/\dot{\gamma}_{\frac{1}{2}})^{0.76}$. For any given solution, viscosity ( ) at any shear-rate ($\dot{\gamma}$) can therefore be defined completely (Equation 3) by two parameters, η_0 and $\dot{\gamma}_{\frac{1}{2}}$, both of which can be derived from a simple linear plot. It should be noted that the common practice of fitting shear-thinning data to a 'power-law' of the form:

$$\eta = k\dot{\gamma}^{(n-1)} \qquad (5)$$

where n is the so-called 'pseudoplasticity index', is equivalent to treating double-logarithmic plots of η *vs.* $\dot{\gamma}$ (such as that shown in Figure 4) as straight lines, and is therefore totally invalid for entangled coils.

5 HYDRATED NETWORKS

One of the most characteristic features of many
polysaccharides *in vitro* is their ability to form
cohesive gels at low concentrations (typically ~1% w/v,
although some polysaccharides gel at concentrations of
0.1% or less). The same behaviour often occurs *in vivo* in
formation of hydrated tissue structure. Crosslinking of
such networks (Figure 5) involves extended 'junction
zones' in which the participating chains pack together
into ordered assemblies, as in the solid state (Figure 2).
Formation of a hydrated network rather than an insoluble
precipitate, however, requires the junctions to be linked
by interconnecting sequences that are conformationally-
disordered (as in solution), providing an entropic drive
to solubilise the network. Co-existence of ordered and
disordered regions within the same polysaccharide system
can occur in a number of ways. As a general mechanism,
the presence of a small amount of residual disorder in an
otherwise fully ordered polymer can lower the overall
free-energy by an entropic contribution that more than
outweighs the enthalpic advantage of further ordering.
In most polysaccharides, however, ordered association is
limited by primary structure.[1]

Covalent Features Limiting Ordered Association

The simplest situation is where the polymer has a
block structure, with regions that are soluble under the
prevailing solvent conditions interspersed by regions
capable of forming stable associations. A well
characterised example is the calcium alginate gelling
system, where sequences of one type (polyguluronate) adopt
a 'buckled-ribbon' conformation and form junctions
stabilised by site-bound calcium ions (Figure 2b) and
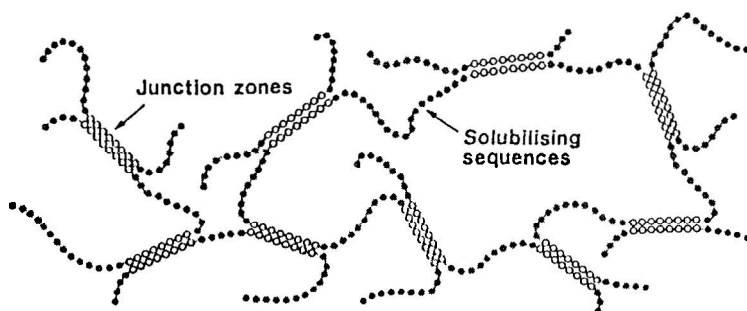
Figure 5 Hydrated polysaccharide network (schematic).

other sequences which cannot bind calcium in this way
confer solubility (polymannuronate and heteropolymeric
blocks incorporating both mannuronate and guluronate).
In other systems, such as the plant galactomannans (which
include guar gum and locust bean gum), pendant sidechains
limit association of the polymer backbone.

Interchain junctions can also be terminated by the
occurrence in the primary sequence of anomalous residues
that are geometrically incompatible with incorporation in
the ordered structure. The structural properties of agar
and carrageenan polysaccharides in algal tissue are
regulated in this way. 'Kinking' residues of rhamnose
have a similar role in pectin, and are spaced such that
the polygalacturonate sequences of the polymer chain have
a regular length of ∿25 residues. These sequences form
calcium-mediated junctions in the same way as the
polyguluronate regions of alginate, but the extent and
stability of association may be modified by the occurrence
of a proportion of the galacturonate residues as the
methyl ester.

Polysaccharide 'Weak Gels'

Certain polysaccharides, notably the bacterial
exopolysaccharide xanthan, have properties intermediate
between those of solutions and gels. The origin of this
behaviour is that xanthan exists in solution in a rigid,
ordered conformation and forms a tenuous three-dimensional
network by weak, side-by-side association of the ordered
chains.[6] Other polysaccharides with soluble ordered
structures behave similarly. Such 'weak gel' networks are
often sufficiently cohesive to hold particles in
suspension or stabilise emulsions over long periods of
time. They are not, however, strong enough to support
their own weight under gravity, and therefore flow like
normal polymer solutions.

The shear-thinning behaviour of 'weak gels' is quite
different from that of entangled coils. In particular,
since a finite stress is required to rupture the network,
the viscosity (defined as the ratio of stress applied to
shear-rate generated) increases progressively with
decreasing shear rate, rather than reaching a maximum
equilibrium value (as in Figure 4 for entangled coils).
Indeed, for most 'weak gel' systems, double-logarithmic
plots of η *vs.* $\dot{\gamma}$ are linear (so that in this case the
'power law' analysis of Equation 5 is valid), and have a
slope greater than the maximum value of ∼0.76 for
disordered polysaccharides.

6 IMPLICATIONS FOR STUDIES OF BIOLOGICAL FUNCTION

Since the physical properties of polysaccharides are
determined more by the way in which the component residues
are linked together than by the nature of the residues,
analysis of dietary fibre for the types and proportions of
sugars present is unlikely, in itself, to give much
indication of function. Perhaps the only useful
generality is that charged polysaccharides are usually
more readily soluble than neutral chains, although even
here the presence of a sufficient concentration of
appropriate counterions can promote stable association of
polyelectrolytes into solvent-resistant assemblies.

        Determination of the linkages between adjacent
residues in the polysaccharide repeating sequence is, of
course, a substantial improvement, but is still an
incomplete description since, as detailed above, minor
departures from structural regularity (e.g. branching,
anomalous residues, sidechains) can have a major influence
on physical properties. When the spacing of these
interruptions is larger than the critical sequence length
for ordered association, their effect is to promote
formation of hydrated networks rather than insoluble
aggregates. At shorter spacings they may completely
abolish interchain association and solubilise an otherwise
insoluble primary sequence.

        Characterisation of the distribution of structural
irregularities along the polymer chain (i.e. determination
of 'fine structure') has been achieved for some
polysaccharide systems, by selective chemical or enzymic
degradation, but this is a highly skilled, lengthy
undertaking that could certainly not be adopted for
routine analysis of dietary fibre. Progress in
understanding the mechanism of action of insoluble fibre
or swollen, hydrated networks is therefore more likely to
come from relating physiological effects to the
macroscopic properties of these materials rather than to
their chemical composition.

        An obvious exception to this rather sweeping
assertion is the susceptibility of fibre to bacterial
degradation in the lower bowel. However, although the
potential susceptibility of polysaccharides to cleavage by
specific enzymes is, of course, dependent on their primary
sequence, the extent of hydrolysis which occurs in
practice can be drastically reduced by conformational
ordering and packing (as in 'resistant starch').

Physiological Effects of Soluble Fibre

The ability of soluble fibre to retard postprandial release of nutrients from the gut almost certainly arises from increased viscosity reducing rates of mixing (rather than, for example, to any further suppression of much slower transport processes such as diffusion). As detailed previously, the viscosity generated by disordered coils is dependent on the degree of space-occupancy by the polymer, characterised by the (dimensionless) product of concentration (proportional to the number of chains present) and intrinsic viscosity (proportional to the volume which each occupies). It is therefore just as essential when documenting studies of physiological activity to give an indication of molecular size as it is to report concentration.[7] A particular danger is that apparent differences in the effectiveness of different soluble fibres may be due simply to differences in the molecular weights of the particular samples used, rather than reflecting a genuine dependence of physiological activity or chemical composition.

The most direct index of molecular size is intrinsic viscosity, whose determination is described in detail elsewhere.[3] An acceptable alternative would be to measure viscosities at a few concentrations in the range of practical usage. In view of the extreme shear-thinning of polysaccharide solutions, it is virtually useless to report viscosity at a single shear rate (particularly if the rate used is not specified). Measurements at a few different shear rates, however, can be used to construct a linear plot of η *vs.* $\eta\dot{\gamma}^{0.76}$ and hence derive the values of η_0 and $\dot{\gamma}_{\frac{1}{2}}$ which define completely[5] the viscosity at all shear rates. For 'weak gel' systems the equivalent parameters are k and n (Equation 5) from a linear plot of $\log \eta$ *vs.* $\log \dot{\gamma}$.

A complication in interpreting results of clinical studies of soluble fibre is that concentrations in the lumen may be quite different from those ingested. In particular, recent studies using a pig model[8] have shown a substantial increase in the total volume of digesta passing through the small intestine with increasing concentration and molecular weight of soluble polysaccharide in the feed (thus partially offsetting the initial differences in viscosity). The mechanism of this feedback process is not yet fully understood, but it is clearly of importance in defining optimum levels and types of soluble fibre for clinical use.

A further obvious consideration is that
polysaccharides can confer viscosity only once they have
dissolved. This may explain conflicting reports on the
effectiveness of guar gum in reducing postprandial
hyperglycaemia, since different commercial guar granulates
dissolve at quite different rates.[9]

Finally, increased dietary intake of charged
polysaccharides may have implications for the availability
of essential mineral nutrients. Tight site-binding of
counterions within the ordered structure of charged
polysaccharides such as pectin has been detailed above.
Weaker 'atmospheric' binding can occur around disordered
polyelectrolyte chains in solution or at the surface of
insoluble fibres. In all cases, electrostatic binding
will, of course, occur only when the ionic substituents of
the polysaccharide retain their charge. Sulphate groups
(as in carrageenan) remain charged to very low pH, but the
pKa of carboxyl groups in, for example, pectin and
alginate is around 3.4. Thus at gastric pH these materials
may become partially un-ionised and shed their bound
counterions, while at higher pH in the small intestine
they will regain their charge and once more participate
fully in electrostatic binding, perhaps with different
ions from those with which they were initially associated.

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CHARACTERIZATION OF SOME PHYSICAL PARAMETERS OF SOLUBLE FIBRES

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1 INTRODUCTION

Gels are an intermediate state of matter between solid and liquid. The gelation process of a polymer solution consists of the formation of cross-linkages involving extensive segments of polymer molecules - junction zones - to form a three-dimensional network holding water. As a consequence of the gelation process, the polymer solution becomes a viscoelastic medium in which the mobility of the different components (gelling molecules and water molecules) is considerably reduced.

Nuclear Magnetic Resonance (NMR) is a suitable technique for studying both gelation process and gel structure. Investigation on gels can be based on measurements of longitudinal (T_1) and transverse (T_2) relaxation times, these parameters being related to the dynamic state of a system. Since T_1 and T_2 are inversely related to the correlation time of a molecule, the decrease in the mobility of the gel components leads to a corresponding decrease in T_1 and T_2 values. Consequently, lower T_1 and T_2 values can be observed in relation to more extended junction zones and/or to a better efficiency of the intermolecular forces.

In this paper we report an NMR study of high-methoxyl pectin gels. High-methoxyl pectins are linear polymers of

D-galacturonic acid in which more than 50% of the acidic groups are esterified with methanol. At pH values below 3.5 and in the presence of a cosolute, such pectins form gels. The structure of pectin gels is stabilized by both hydrogen bonding and hydrophobic interactions between the ester methyl groups.[1] The nature and the concentration of the cosolute affect the contribution of hydrophobic interactions to the stability of gels.[2]

Aim of the present study was to characterize pectin gels obtained with different cosolutes - sucrose, ethanol, tert-butanol and dioxane - by measuring the NMR parameters T_1 and T_2.

2 MATERIALS AND METHODS

Pectin from apples, 70–75% degree of methylation (Fluka), was used without further treatment.

The dry pectin was mixed first with the cosolute and thoroughly dispersed. The appropriate weight of water was added and the pH adjusted to about 3.5 with 0.1 M citric acid. The mixture was placed in a water bath (95°C for samples containing sucrose as cosolute; 60°C for samples containing ethanol, tert-butanol and dioxane as cosolutes) for 1 hr under stirring.

Gel preparation was carried out directly in NMR glass tubes (18 mm external diameter). Concentrations were measured as % (w/w). Gel samples were stored at 25°C for 24 hr before measurement.

The empirical gel threshold was determined by inversion of the NMR tubes: a sample that did not flow was considered to have gelled.

NMR measurements were carried out on a pulse low resolution spectrometer Minispec pc120 (Bruker Spectrospin, Germany) operating at a frequency of 20 MHz for protons.

The spin-lattice relaxation times were measured by the inversion recovery technique,[3] sampling 10 points with delays

spanning between 0.002 and 6 s and using a repetition rate in the range between 1 and 8 s (in any case larger than 5 T_1). Signal enhancement was achieved by averaging over 4 acquisitions using a repetition delay ranging between 1 and 8 s.

The spin-spin relaxation times were measured by the Carr-Purcell-Meiboom-Gill (CPMG) technique,[3] sampling up to 500 echoes; 4 FIDs were detected, using a repetition delay in the range between 1 and 8 s, and the signal averaged in order to obtain signal enhancement.

Samples were thermostated for 30 min at the temperature of measurement (40°C).

3 RESULTS AND DISCUSSION

The variation of T_1 and T_2 values with pectin concentration in gels containing 55% sucrose as cosolute is reported in Figure 1. The figure points out a decrease in both T_1 and T_2 values as pectin concentration increases, as expected since T_1 and T_2 values are inversely related to solution viscosity.
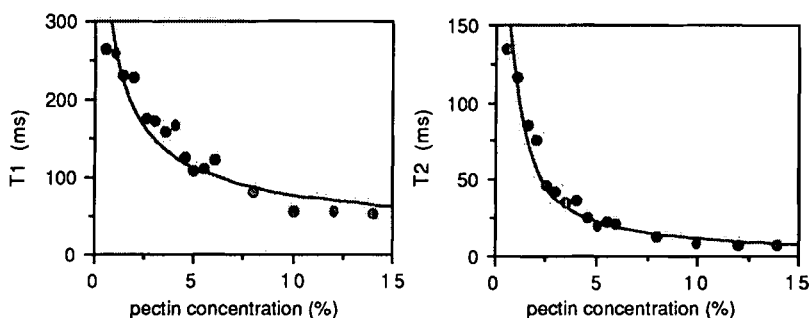
Figure 1   Variation of T_1 and T_2 with pectin concentration in gels containing 55% of sucrose as cosolute.

At the pectin concentration at which the gel is formed - evaluated as described in the previous section - T_1 and T_2 become constant and their absolute values depend on the rigidity of the molecular network. The measurement of physical- chemical parameters can thus replace an empirical evaluation in the determination of the gel threshold.

The variation of T_1 and T_2 values with sucrose concentration in 5% pectin gels is reported in Figure 2. A behaviour of the relaxation times curves analogous to the previous one can be observed, so confirming the reliability of relaxation times measurements to determine the gel threshold for gelling agent as well as for cosolute.
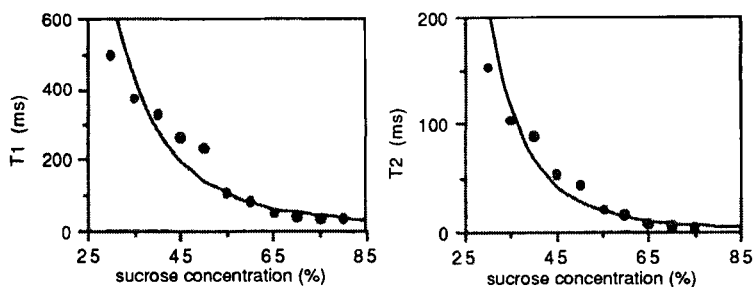
Figure 2   Variation of T_1 and T_2 with sucrose concentration in 5% pectin gels.

In order to investigate the role of hydrophobic interactions in gel formation, sucrose was replaced by other cosolutes, such as ethanol, tert- butanol and dioxane. The variation of relaxation times with cosolute concentration in 5% pectin gels is reported in Figure 3.

It is evident that the behaviour of relaxation times is different when using cosolutes other than sucrose. In fact, in the case of ethanol, tert-butanol or dioxane the T_1 and T_2 curves pass through a minimum which corresponds to the cosolute concentration at which the gel is formed. That
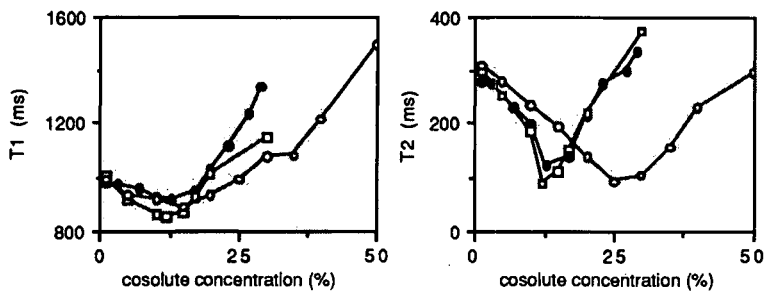
<u>Figure 3</u>   Variation of T_1 and T_2 with cosolute concentration in 5% pectin gels:● - ethanol;□ tert-butanol; ○ - dioxane.

indicates a critical cosolute concentration in the gel formation which depends on the nature of the cosolute. The T_2 curve has a more pronounced minimum than the T_1 curve, thus indicating that T_2 relaxation time is a better parameter to describe gel properties. That follows from the fact that spin-spin relaxation time is sensitive to low-frequency molecular motions while spin-lattice relaxation time depends on high-frequency molecular motions.

The relaxation time curves measured for pectin gels prepared with ethanol, tert-butanol and dioxane closely match the curves for rupture strength (RS) versus cosolute concentration of similar gels.[4] These authors find that RS passes through a maximum at the cosolute concentration at which the gel is formed. These results can be explained by considering the effect of the different cosolutes on the interactions that stabilize gel structure. Solutions of 55% sucrose, 15% ethanol and 14% tert-butanol (these percentages corresponding to cosolute concentrations at the minimum in T_2 curves) have a dielectric constant of 57.5, 65.9 and 61.5, respectively, as calculated by interpolating data reported by Åkerlof et al.[5] A solution of 30% dioxane has a dielectric constant of 49.0. Thus, sucrose, ethanol and tert-butanol seem to stabilize hydrophobic interactions between

pectin methoxyl groups, while dioxane is likely to stabilize polar interactions between pectin unesterified polar groups.

In conclusion, T_1 and T_2 relaxation times are reliable parameters in the description of gelation process and gel structure; moreover, relaxation times can be related to the rheological properties of gels. Thus, besides being non-invasive and rapid, the NMR technique provides very reproducible parameters, that can favourably replace empirical evaluations.

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1 INTRODUCTION

The term dietary fibre (DF) includes all the plant polysaccharides and lignin which are resistant to enzyme digestion[1]. Several enzymatic-gravimetric methods are given to determine DF as a whole, e.g. the new AOAC method[2]. Other insoluble substances not included in the DF definition may be found in the DF residues[3].

Cell wall protein could be regarded as part of DF, because of lower apparent digestibility. On the other hand, many vegetable foods contain phenolics which may inhibit proteases and form complexes with protein[4,5] thereby increasing the amount of indigestible protein.

Recently, Trowell[6] suggested that resistant protein must be considered in the DF definition.

The presence of resistant protein and condensed tannins (polyphenols) in DF residues is considered in the present article.

2 MATERIALS AND METHODS

White and red grapes (Airen and Garnacha varieties) were used. The juice was extracted and the remaining pulp was freeze-dried and milled to pass 0.5 mm sieve.

Insoluble dietary fibre (IDF) was determined by the AOAC method[2]. One gram of sample was successively treated with heat-stable α-amylase (100º C, pH=6, 30 min), protease (60º C, pH=7.5, 1 hr) and α-amyloglucosidase (60º C, pH=4.5, 30 min).

Then, filtration and washing with destilled water, 95% ethanol and acetone were carried out. The residue corresponded to IDF.

The residue obtained after acid hydrolysis of IDF residues (12M H_2SO_4, 20ºC, 3 hrs; 1M H_2SO_4 refluxing 2 hrs) was determined as Klason lignin (KL).

Klason Lignin (KL) residues were treated with HCL-activated triethyleneglycol at 125º C, 1 h[7]. The solubilized lignin was measured by the Morrison procedure[8]. Kjeldalh and Biuret determinations of protein were made.

Condensed tannins were spectrophotometrically quantified in the anthocyanidin solutions obtained by sample treatment with HCl-BuOH[9].

Other samples rich in condensed tannins have previously been studied with similar procedures and purpose: carob pods[9], apples[10] and Spanish sainfoin[11].

3 RESULTS AND DISCUSION

The samples studied are rich in condensed tannins (proanthocyanidin polymers). The contents of CT and total protein are listed in Table 1.

The IDF values shown in Table 2 correspond to the gravimetric residues obtained by the AOAC method, after substracting the ash content. Significant amounts of protein were found in these residues representing between 25 and 80% of total protein in the original samples. These amounts must include both cell-wall and intracellular protein.Nevertheless, after the protease treatment usually only very low percentages of protein remained in the IDF residues. The high retention of protein in these samples is related with the presence of CT. A double effect of CT on protein was reported: inhibition of protease and formation of tannin-protein complexes[8]. Both

TABLE 1.- PROTEIN AND CONDENSED TANNINS (CT) IN THE SAMPLES (% DRY MATTER)

SAMPLES	PROTEIN	CT
Carob pod	2.7 ± 0.1	17.9 ± 0.4
Cider wastes	5.4 ± 0.2	3.1 ± 0.1
Spanish sainfoin	8.2 ± 0.4	7.0 ± 0.6
White grape pulp	10.7 ± 0.2	14.5 ± 0.5
Red grape pulp	13.8 ± 0.3	36.4 ± 0.9

Mean value ± standard deviation

TABLE 2.- INSOLUBLE DIETARY FIBRE (IDF) AND PROTEIN IN IDF (%
         DRY MATTER OF ORIGINAL SAMPLES)

SAMPLES	IDF RESIDUE	PROTEIN IN IDF
Carob pod	32.8 ± 0.6	1.8 ± 0.1
Cider wastes	51.3 ± 0.4	3.0 ± 0.1
Spanish sainfoin	54.6 ± 0.4	2.1 ± 0.1
White grape pulp	62.5 ± 1.1	7.5 ± 0.2
Red grape pulp	65.7 ± 0.6	12.2 ± 0.3

Mean value ± standard deviation

factors contribute to the non–digestion of protein (resistant
protein).

Klason lignin was determined in IDF residues and the
results are indicated in Table 3. A significant part of the
resistant protein is not solubilized by the strong acid
treatment performed to obtain KL. The KL residues include
lignin, protein and CT.

Condensed tannins resist acid hydrolisis, yielding a red
solution of anthocyanidins and remaining an amorphous residue of
phlobaphenes[12]. Klason Lignin treatment was performed on CT
standard and only 9% was dissolved.

Finally, the KL of grapes and lignin standard residues were
treated with HCL/triethyleneglycol[7]. A lignin standard was
completely dissolved while only a partial solution of KL was
observed. The composition of the supernatant, expressed as
percentage of original dry matter was: 2.6% lignin and 7.6% CT
in red grape pulp and 2.5% lignin and 2.2% CT in white grape
pulp. Protein was not detected in the supernatants.

The presence of protein and tannin in IDF and KL residues
may indicate these compounds are quite resistant to enzymatic

TABLE 3.- KLASON LIGNIN (KL) AND PROTEIN IN KLASON LIGNIN (% DRY
         MATTER OF ORIGINAL SAMPLE)

SAMPLES	KL	PROTEIN KL
Carob pod	15.7 ± 0.1	0.8 ± 0.05
Cider wastes	20.0 ± 1.0	1.9 ± 0.1
Spanish sainfoin	n.d.	n.d.
White grape pulp	41.2 ± 1.2	2.6 ± 0.1
Red grape pulp	39.7 ± 0.6	2.8 ± 0.05

Mean value ± standard deviation
n.d.= not determined

and acid hydrolysis, and subsequently indigestibles. Both substances, condensed tannins (polyphenolic polymers) and resistant protein could be considered constituents of DF. Condensed tannins are common in many vegetables of the diet.

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WORKSHOP REPORT: PLANT SUBSTANCES ASSOCIATED WITH DIETARY FIBRE

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The workshop addressed 8 broad questions while recognising that there were very many important issues that could not be accommodated because of time:

Question 1 What (a) makes a fibre polysaccharide and (b) how does the plant produce them?

(a) Fibre polysaccharides are essentially carbohydrate chains with various carbohydrate and non-carbohydrate side chains. (b) There is no template, control of synthesis is vested in the enzymes that add on or split off units. We concluded that compounds shorter than 10 polymer units were not fibre polysaccharides.

Question 2. What is lignin?

Lignin is a 3-dimensional phenolic network associated with different compounds. Although lignin consumption is only some 200-300mg/person/day in Britain, these small quantities are important because they modify the effects of other food components, e.g. phenolics. Lignification can also affect the mechanical properties of food.

Question 3. Is phytate important?

Mineral availability can be greatly increased by phytase although some foods (e.g. legumes) lack this enzyme and in oats phytase is destroyed by processing. Because increased consumption of plant foods raises mineral supply, phytate is most probably not a problem in advanced countries.

Question 4.   Protease and other inhibitors.

Trypsin inhibitors in ingested soya greatly stimulate pancreatic flow and (acting through cholescystokinin) clearly affect gut function. Cooking destroys a large fraction of the inhibitor but the residue may still be significant. Lipase inhibitors (e.g. in wheat) and amylase inhibitors may also be beneficial by slowing digestion. This area was identified as requiring further research.

Question 5.   What are the effects of Maillard Products?

Maillard products at low levels have pleasant taste properties and may be functionally similar to fibre. Although laboratory Maillard products bound bile acids in vitro they had no effect on cholesterol in vivo. Because some products are potentially carcinogenic more research is urgently required.

Question 6.   Starch

Under some circumstances starch may behave like fibre although quantities of dietary resistant starch are probably small. Processing may increase this fraction especially through heating. The original source of raw material is important in this respect as starch digestibility can be influenced by environment and genetics. Good analysis for starch is essential and the quantities entering the large bowel needs to be established by experimentation. There was no consensus on a definition of resistant starch except that it was a functional property of food.

Question 7.   Are oligosaccharides fibre?

Although certain di- and oligo- saccharides enter the large bowel in significant quantities and are fermented, they are not fibre. The term 'fibre' should be reserved strictly for plant cell wall and (possibly) other related structural materials. Oligosaccharides are fermented rapidly and give widely different products depending on structure. Their effects and those of sugar alcohols are urgently in need of investigation.

Question 8.   How should we regard antinutrients and related compounds?

Numerically these greatly exceed synthetic additives. They have been investigated to a very limited extent and

many studies are of dubious significance as purified compounds only have been used. Vegetarians have exposures higher than average but their health status seems unimpaired and resistance to adverse effects is probably well established. Exposures in the general population are set to increase due to nutritional and agronomic trends. Concern was expressed that plant genetic manipulation may increase adversely the level of some components. In people with eccentric practices (e.g. eating potato peel or drinking certain herbal infusions) toxic reactions are possible. More research and monitoring was recommended.

Analytical Techniques

EVALUATION OF DIETARY FIBER METHODS AND THE DISTRIBUTION OF ß-GLUCAN AMONG VARIOUS FIBER FRACTIONS

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1   SYNOPSIS

Total dietary fiber (TDF) values for fruits, vegetables, processed foods and cereal grains were determined by the urea enzymatic dialysis (UED) method. For 12 oat-based cereal products, the ß-glucan levels of the TDF residues were determined by a NaOH enzymatic assay. The insoluble dietary fiber and soluble dietary fiber values were determined for an oatmeal cereal, an oat bran cereal and rolled oats. The insoluble and soluble dietary fiber residues were analyzed for ß-glucan. The results obtained using the UED method were compared with the AOAC procedure and the neutral-detergent fiber method. Crude protein and ash contamination was usually lower with the UED method compared with the AOAC method. The UED method was effective in removing starch even at the relatively low temperatures of the assay (50°C). The TDF values were comparable using the UED method and the AOAC method, but these methods gave significantly different insoluble and soluble dietary fiber values. The insoluble dietary fiber values determined by the AOAC method were larger than values obtained using the UED method and the neutral detergent fiber procedure. In the evaluation, the UED method quantitatively recovered, with less variation, more of the ß-glucan in the insoluble and soluble dietary fiber residues. The data indicate that the UED method is more precise and accurate than the AOAC method.

2   MATERIALS AND METHODS

Locally purchased cereal-based food products representing national brands and in-store bakery products were

used. These products were air-dried, ground to pass a
20 mesh sieve and then stored at -20°C in polyethylene
bottles until needed for analyses.

The water-soluble ß-glucan and total ß-glucan content
were determined for the cereal-based products using a
ß-glucan enzyme assay (Carr et al., in press).

Total dietary fiber (TDF), soluble (SF), and in-
soluble dietary fiber (IF) contents were analyzed by the
modified AOAC method[1] and by the urea enzymatic dialysis
(UED) method[2]. All dietary fiber residues were analyzed
and corrected for protein (Kjeldahl N x 6.25) and ash
(ignited at 525 °C for 8 hours).

Neutral detergent fiber (NDF) content was analyzed
by the modified method described by Jeraci et al.[3]. All
NDF residues were analyzed and corrected for protein
(Kjeldahl N x 6.25) and ash (ignited at 525 °C for 8
hours).

Fiber residues from each of the three runs were used
to determine the ß-glucan content of the various fiber
fractions. These fiber fractions generated were col-
lected on Whatman No. 54 filter paper and air dried. A
filter paper blank was carried through the analysis for
each set of samples and consistently yielded a value of
0.0 mg ß-glucan.

3   RESULTS AND DISCUSSION

The cereal-based products exhibits a wide variation
in their composition which is attributable to the
proportion of ingredients and to the processing proced-
ures used in the manufacture of the product[4].

In terms of TDF for the food products analyzed, there
was close agreement between the two TDF methods. Ad-
vantages of the UED method have been discussed[2]
(Table 1). These advantages include (1) removal of
essentially all of the starch, (2) generation of smaller
crude protein and ash correction factors, (3) better
quantitative recovery of semi-purified dietary fiber
products, (4) more efficient in terms of labor, equipment
and assay time, (5) milder assay conditions ($\leq$50°C) and
(6) fewer enzymes and reagents.

The NDF values were consistently lower than the TDF
values. The NDF method determines an "insoluble" dietary

Table 1. Composition of Various Cereal-Based Food Products (% dry weight)[a].

Food Product[e]	NaOH[b] β-Glucan,%	Urea Enzymatic Dialysis[c] TDF,% ± SD	Urea Enzymatic Dialysis[c] β-Glucan,%[f]	AOAC-Prosky[d] TDF,% ± SD	AOAC-Prosky[d] β-Glucan,%[f]
"Quick" rolled oats	4.3	11.2 1.8	3.3	11.3 1.1	3.1
Rolled oats	4.6	9.5 1.6	3.1	12.6 1.2	3.5
Oat RTE cereal	2.5	8.4 1.2	2.2	9.0 0.5	1.7
Oat bran RTE cereal	1.9	17.4 0.7	1.6	17.2 1.5	1.6
Oat bran flaked RTE Cereal	2.1	8.4 1.4	2.1	9.4 2.7	1.6
Oat bran hot cereal	8.9	19.3 2.1	6.9	21.3 2.6	7.1
Oat bran	7.3	18.8 2.7	5.6	21.1 1.2	5.8
Granola bar	1.4	8.5 1.8	1.1	6.8 2.8	1.2
Oat bran muffins	0.6	5.7 0.8	0.4	7.3 1.7	0.2
Oatmeal bread A	0.9	5.5 0.7	0.5	7.4 1.7	0.6
Oatmeal bread B	1.1	7.2 0.6	1.0	7.9 1.1	0.4
White bread	0.4	3.4 0.8	0.9	5.4 0.5	0.48

[a] Values corrected for water content. Total dietary fiber (TDF) values are corrected for ash (525°C, for 8 hr) and protein (Kjeldahl nitrogen x 6.25).

[b] 1.0 N NaOH extracted β-Glucan values adapted from Carr et al. (in press).

[c] TDF determined by the method described by Jeraci et al. (in press). Mean and standard deviation (SD) for TDF from two replicated experiments, n=6.

[d] TDF determined by the method described by Prosky et al. (1988). Mean and standard deviation (SD) for TDF from two replicated experiments, n=6.

[e] RTE: ready to eat.

[f] β-Glucan content of TDF residues expressed as percent of the original sample dry matter using a modification of the NaOH method described by Carr et al. (in press). Mean for β-Glucan from two replicated experiments, n=2.

Table 2. Composition (Insoluble Fiber, and Soluble Fiber) for Three Ready to Eat Cereals Using the Urea Enzymatic Dialysis (UED) Method, the AOAC Total Dietary Fiber Method, and the Neutral-Detergent Fiber Method [a,b,c,d].

	% Dietary Fiber ± SD				
Method:	UED		AOAC		NDF
Cereals	Insoluble	Soluble	Insoluble	Soluble	Insoluble
Oat bran hot	9.6 ± 0.0	10.2 ± 0.7	23.8 ± 0.2	1.6 ± 0.9	14.0 ± 0.3
Oat bran	16.8 ± 1.1	2.9 ± 0.7	18.2 ± 1.5	4.2 ± 2.1	14.9 ± 0.2
Rolled oats	3.5 ± 0.4	6.8 ± 1.2	12.8 ± 2.1	1.0 ± 1.0	4.9 ± 0.8

a Corrected for water.
b The AOAC Total Dietary Fiber Method (Prosky et. al., 1).
c The Neutral-detergent fiber described by Jeraci and Van Soest (5) modified to calculate insoluble fiber corrected for ash and crude protein.

Table 3. Composition (Insoluble Fiber, and Soluble Fiber) for Three Ready to Eat Cereals Using the Urea Enzymatic Dialysis (UED) Method and the AOAC Total Dietary Fiber Method [a,b].

	% ß-Glucan					
Method:	UED		AOAC		UED	AOAC
Cereals	Insoluble	Soluble	Insoluble	Soluble	Sum[c]	
Oat bran hot	0.71	3.50	1.42	0.75	4.21	2.17
Oat bran RTE	0.32	1.04	0.28	0.12	1.36	0.40
Rolled oats	0.32	1.65P	1.42	0.75	1.97	0.99

a Corrected for water.
b The AOAC Total Dietary Fiber Method (Prosky et. al., 1).
c Sum of ß-glucan in the insoluble (I) and soluble (S) dietary fiber fractions.

fiber. With the samples used in this study, high NDF values relative to TDF values may reflect elevated NDF values caused by difficult filtration or less ß-glucanase activity in the amylase use in this modified NDF method[3,4,5]. Previous reports that NDF does not recover ß-glucan[6] are not confirmed by the present study and the amount of ß-glucan (0.2-7.0% ß-glucan) in NDF may be due to the heat stable α-amylase used in the present study.

The UED method had relatively better recovery of ß-glucan for the majority of the products than the 1988 modification of AOAC method for TDF. Both of the TDF methods, however, only recovered from 39 to 89% of the ß-glucan (Table 1).

The content of soluble and insoluble dietary fiber for three cereal-based products was determined (Table 2) by the modified AOAC method[1] and the UED method[2]. There was better agreement between the determined and calculated (sum) values for TDF using the UED method than the TDF values using the modified AOAC method. There is a large difference in the distribution of the ß-glucan between soluble and insoluble fiber fractions as measured by the two methods. The UED method gave much higher proportions of soluble fiber than the modified AOAC method for two of the three products. Both of these methods had lower ß-glucan recoveries in the soluble and insoluble dietary fiber fractions than in the TDF (Table 3). The UED method, however, was significantly better than the modified AOAC method in the recovery of ß-glucan for the soluble and insoluble dietary fiber fractions (Table 5).

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SOME PROBLEMS ASSOCIATED WITH THE DETERMINATION OF DIETARY FIBRE IN CITRUS BY THE A.O.A.C. METHOD

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1 INTRODUCTION

The official A.O.A.C. method for dietary fibre (DF) determination was recently modified[1,2]. The authors reported that "the precision of the method for the individual fractions especially for SDF, is not yet satisfactory" and "further studies of fruits, vegetables, and leguminous seeds are needed".

On this basis, citrus samples were chosen to study some problems associated with the determination of DF by this method.

Some aspects related with precipitation of soluble constituents and retention of several substances in the fibre matrix are considered.

2 MATERIAL AND METHODS

Both orange and lemon pulp and peel were used. Raw samples were dried and ground to particles of <0.5 mm.

Analysis of starch in the samples by a UV-method[3] were performed after free sugars extraction.

A brief scheme of the methodology used is shown in Figure 1.

The fibre content was determined by the enzymatic-gravimetric A.O.A.C. method[2] omitting α-amylase and amyloglucosidase treatment, because the samples did not contain starch. Corrections of blank, protein and ash

were carried out in the IDF and SDF residues.

```
                    ┌─────────────┐
                    │ DRY SAMPLE  │
                    └─────────────┘
                           │ Enzymatic treatment
          ┌────────────────┴───────────────────┐
       IDF AOAC                            Supernatant
    ┌──────┴──────┐                       ┌──────┴──────┐
Solubilization    │                   Dialysis        78%
   treatment      │                                 Ethanol
                  │                      │              │
  ┌─────┴─────┐   │                      │          SDF AOAC
rIDF        sIDF  │                      │              │
  │           │   │                      │              │
Acid          │  Acid                    │            Acid
hydrolysis    │  hydrolysis              │          hydrolysis
 ┌──┴───┐     │   ┌──┴───┐               │              │
KL  Supernatant│  KL  Supernatant        │              │
      │        │       │                 │              │
    S+UA     S+UA    S+UA              S+UA           S+UA
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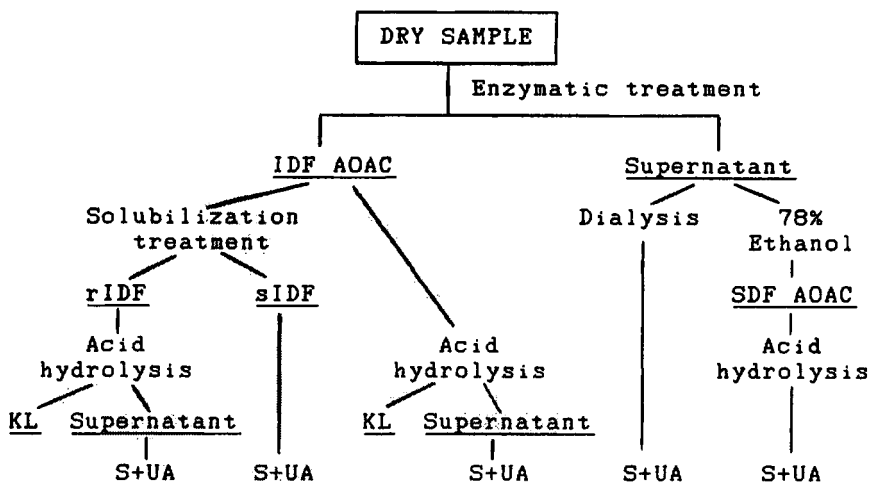
S+UA = Sugars + Uronic Acids ; KL = Klason Lignin

Figure 1   Scheme of the methodology

Some IDF residues were solubilized in water ($100^\circ C$, 30 min twice). Both supernatant (sIDF) and IDF residue (rIDF) were collected.

Additionally to the determination of SDF by 78% ethanol precipitation, some SDF values were obtained by dialysis. After the protease treatment of the raw samples, the supernatant liquid and washings were combined and transferred quantitatively into a dialysis bag (Dialysis Tubing Visking 9-32/36 mm. Medicell International, Ltd.) and dialysed against distilled water for 48 h.

Acid treatments of IDF, rIDF and SDF were carried out following conditions previously tested (IDF, rIDF: 12M H_2SO_4, 1 h, $30^\circ C$ + 1M H_2SO_4, 1.5 h, $100^\circ C$; SDF: 1M H_2SO_4, 1.5 h, $100^\circ C$). The residue was determinated as Klason Lignin (KL).

Neutral polysaccharides and pectines, expressed as neutral sugars (S) and uronic acids (UA) respectively, were spectrophotometrically determined[4,5]   on the acid hydrolysates and solutions as it is shown in Figure 1.

3 RESULTS AND DISCUSSION

Results shown in the tables are expressed as percent of
dry material of the original samples (mean values of at
least three determinations ± standard deviation).

        SDF AOAC values are expressed in Table 1. The major
portion   of   the   gravimetric   residues   correspond   to
inorganic components   precipitated   in   the   78%   ethanol
solution   (Ash=   62-82   %).   The   high   value   of   the
relationship Residue/SDF may be a source of error in the
determination of SDF.

        On   the other hand,   the polysaccharide   content of
this residue was only a part of the SDF AOAC value. That
is to say, other non-fibre constituents precipitated in
the   78% alcoholic solution. This could be due to the
retention of non-fibre components in the polysaccharide
matrix of SDF.

Table 1   Content and composition of SDF obtained by
          precipitation (AOAC procedure) (% dry material)

	ORANGE		LEMON
	Pulp	Peel	Pulp
Residue	37.94±1.62	46.16±2.39	56.75±0.43
Ash	30.23±1.70	37.47±1.54	36.03±0.35
Protein	0.69±0.07	0.73±0.07	0.93±0.00
SDF AOAC	7.02±1.70	7.96±2.39	19.79±0.43
Sugars	1.55±0.06	1.54±0.10	1.85±0.10
Uronic acids	0.93±0.04	0.97±0.06	7.24±0.28
*SDF	2.48±0.06	2.51±0.10	9.09±0.28

Table 2   SDF obtained by dialysis (% dry material)

	ORANGE		LEMON
	Pulp	Peel	Pulp
Sugars	2.19±0.27	2.17±0.27	3.44±0.11
Uronic acids	3.41±0.41	4.25±0.63	16.25±2.08
SDF	5.60±0.41	6.42±0.63	19.69±2.08

SDF values obtained by dialysis (Table 2) were always higher than the actual value of AOAC method (*SDF) (Table 1). This indicated that some hemicelluloses and pectins remain solubilized in the 78% ethanol solution and are lost when the AOAC method is used.

Gravimetric and spectrophotometric IDF values are listed in Table 3. Gravimetric results were about three units higher than the spectrophotometrics.

Solubilization treatment of the IDF AOAC yielded an rIDF 8-10 units lower than the first one (Table 4).

From the data on Table 5, it can be deduced that only a part of the IDF solubilized fraction (sIDF) was actually fibre (sSDF). This part could be considered as SDF while the remaining may correspond to non-fibre constituents retained in the IDF polysaccharide matrix.

In summary, both IDF and SDF values obtained by the AOAC method in citrus are higher than the actual content.

Table 3 Content and composition of IDF obtained by AOAC method (% dry material)

	ORANGE		LEMON
	Pulp	Peel	Pulp
IDF AOAC	32.08±0.30	32.64±0.69	26.04±0.19
Lignin	7.23±0.27	6.88±0.07	2.93±0.59
Sugars	13.70±0.22	14.20±0.34	15.57±0.01
Uronic acids	9.59±0.05	8.30±0.85	4.78±0.03
*IDF	30.52±0.27	29.38±0.85	23.28±0.59

Table 4 Content and composition of IDF after solubilization treatment (% dry material)

	ORANGE		LEMON
	Pulp	Peel	Pulp
rIDF	23.71±0.37	24.18±0.06	16.17±0.56
Sugars	12.27±0.29	11.62±0.45	13.07±0.34
Uronic acids	6.62±0.30	4.22±0.08	0.79±0.03

Table 5   Content and composition of solubilized fraction
          (% dry material)

	ORANGE		LEMON
	Pulp	Peel	Pulp
sIDF	12.33±0.37	11.51±0.69	12.72±0.56
Sugars	1.70±0.19	2.27±0.30	1.95±0.10
Uronic acids	1.51±0.04	4.73±0.24	2.73±0.30
sSDF	3.21±0.19	7.00±0.30	4.68±0.30

4 SUMMARY

Determinations of insoluble and soluble dietary fibre
(IDF and SDF) were carried out in orange and lemon pulp
and peel by the A.O.A.C. enzymatic-gravimetric method
and by spectrophotometric procedures (as the sum of
sugars and uronic acids in the acid hydrolysate of the
IDF and SDF residues).

In the precipitation residue, ash weight was higher
than SDF values. The spectrophotometric results were
appreciably lower than the gravimetric values.
Alternatively, dialysis was employed and yielded the
highest SDF values.

On the other hand, an important fraction of IDF
residues was solubilized in boiling water. Only a part
of the solubilized fraction corresponded to fibre
constituents. This indicate that other soluble compounds
were retained in the insoluble polysaccharides matrix.

The results suggest that the A.O.A.C. method yields
higher IDF and SDF values than the actual content. Some
modifications of the A.O.A.C. method to avoid the
problems associated with precipitation and retention of
soluble substances in the insoluble fraction are
considered. These considerations could be applied to
other fruits and vegetables as well.

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DETERMINATION OF DIETARY FIBRE IN VEGETABLES BY HPLC

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1 INTRODUCTION

Several works[1-3] for hydrolysis of polysaccharides from
Dietary Fibre and quantification of monosaccharides have
been published. These procedures differ, mainly, in hydro-
lysis conditions and chromatographic techniques (GLC and
HPLC) used to identify and quantify the monosaccharides.

The objective of this work is to study the method
of Englyst[1], but HPLC was used instead of GLC. Preparation
of samples for HPLC was carried out following Slavin and
Marlett[2] method.

2 MATERIALS AND METHODS

In the Englyst method, hydrolysis of polysaccharides
consists of the following steps: primary hydrolysis with
H_2SO_4 12M at 35°C during 1h and secondary hydrolysis with
H_2SO_4 1M at 100°C during 2h. Preparation of samples for
HPLC analysis implies a neutralization step with $Ba(OH)_2$
or $BaCO_3$.

To study primary hydrolysis microcrystalline and
crystalline cellulose was used. Two conditions of the
procedure were changed: temperature (35°C and room tem-
perature) and neutralizing agent ($Ba(OH)_2$ and $BaCO_3$).
Two different kind of columns were used and resolution
in both cases was compared.

3 RESULTS AND DISCUSSION

In Table 1 recoveries obtained for microcrystalline and
crystalline cellulose are shown. Though Slavin and Marlett

TABLE 1   Percent Recovery of Glucose from Cellulose Using Various
          Chemical Hydrolysis Procedures

Cellulose	Primary Hydrolysis (Temperature)	Neutralizing Agent	%R
Microcrystalline	35ºC	$Ba(OH)_2$	44.89
"	"	$BaCO_3$	44.92
"	25ºC	"	71.79
Crystalline	35ºC	$BaCO_3$	84.01

method[2] is carried out with $Ba(OH)_2$ as neutralizing
agent, neutralization step is long and tedious. It was
tried to use instead $BaCO_3$ which made the procedure easier
and quicker. Percentage of recovery was similar with both
agents (44.89 and 44.92). Other modifications were related
to temperature used during the primary hydrolysis. Results
show that when microcrystalline cellulose was hydrolyzed
at room temperature percentage of recovery increased
(71.79). This was possibly due to a lower loss of glucose
at that temperature in strong acid conditions. However,
when analyzing crystalline cellulose keeping 35ºC of tem-
perature during primary hydrolysis, it was noticed that
percentage of recovery was higher (84.01). Once efficacy
of hydrolysis was studied with cellulose, separation of
neutral monosaccharides was studied in an amine-bonded
column (Carbohydrate Analysis, Waters Associates). Reso-
lution was not good; mannose, glucose ahd galactose elute
at the same time. For this reason, this column was subs-
tituted by an aminex HPX-87P heavy metal cation exchange
carbohydrate column (Bio-Rad, Richmond Laboratories,CA).
Separation was excellent. Carrots were analyzed following
Englyst method. Neutralization was carried out with
$BaCO_3$ and analysis was done using both columns. It was
confirmed that HPX-87P column gave better resolution.

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1 INTRODUCTION

Cassava is an important staple for some 450-500 million people in tropical regions where, in some cases it provides up to 60% of the total calorie intake. (Cock, 1982). The crop is highly productive even under adverse conditions. Approximately two thirds of the annual production is used for human consumption, around 20% for animal feed and the remainder for industrial processing.

Cassava, whilst being an excellent source of highly digestible starch (30-40% fresh wt.; 85% DM), contains little fat and protein and in some instances, has appreciable quantities of cyanogenic glycosides. (Cock, 1982). These glycosides seriously restrict the utilisation of fresh cassava for human consumption.

Thus, if cassava is to be utilised efficiently in human food and animal feed, a detailed knowledge of its composition is essential. Information on the composition of the dietary fibre of cassava is scarce. Work was initiated in this Department to characterise this component in cassava as part of a broader study to appraise the nutritional importance of all carbohydrates and the anti-nutritional factors.

2 MATERIALS AND METHODS

Cassava, cultivars HMC1 and MCol 1684 containing different levels of cyanogenic glycosides, was obtained from CIAT Columbia through ODNRI. Both cultivars underwent two drying procedures - tray and floor dried in the sun - resulting in four different samples for analysis. Dietary fibre analysis was carried out on the ground material (1mm) according to the procedure of Englyst and Cummings (1984)

3 RESULTS
TABLE 1

COMPOSITION OF THE DIETARY FIBRE OF CASSAVA.

SAMPLE	TOTAL SUGAR mg/g DM	SOL[1]	INSOL	NCP	CELL	LIGNIN[2]
1684 T	90.03	32.94	67.12	52.75	44.73	4.52
1684 F	91.85	30.93	69.87	68.21	29.82	4.64
HMC T	78.37	29.96	72.81	62.40	35.20	4.07
HMC F	90.37	27.12	74.33	60.09	33.37	4.76

1 SOL (Soluble NSP); INSOL (Insoluble NSP); NCP (Non-cellulosic polysaccharides); CELL (Cellulose). All values are expressed as total sugars (% Total NSP - Non-starch polysaccharides)

SOL NSP is calculated as TOTAL NSP - INSOL NSP
CELL is calculated as TOTAL NSP - NCP

2 LIGNIN is expressed as mg/g DM and is determined gravimetrically.

In all samples INSOL NSP and SOL NSP accounted for 70% and 30% of the TOTAL NSP respectively (Table 1). Cellulose amounted to between 30-44% of the TOTAL NSP. NCP contents varied between 53-68% TOTAL NSP. The monosaccharide composition of the TOTAL NSP of cassava, variety HMC1 Tray, is presented in Table 2 along with that for potato obtained by Englyst, Wiggins and Cummings (1982) and Graham and Aman (1988).

4 DISCUSSION
The content of soluble and insoluble NSP in cassava were similar to those obtained for raw potato (Graham and Aman, 1988) (Table 2). The monosaccharide composition of the total NSP of cassava, however, differed to that of potato with respect to rhamnose and xylose, being much higher in the former and mannose, and galactose being present to a greater extent in the latter (Table 2).

In contrast, Anderson and Bridges (1988) and Englyst and Cummings (1982) obtained much higher values of 57.2% and 53.7% respectively for the soluble NSP in potato. Also, the total NSP value for potato obtained by Englyst and Cummings (1982) was considerably lower than that reported by Graham and Aman (1988). Equally significant were the differences in the monosaccharide composition of the total NSP obtained by the two authors (Table 2).

TABLE 2

MONOSACCHARIDE COMPOSITION OF THE TOTAL NSP OF CASSAVA AND POTATO.

SAMPLE	RHA[1]	ARA	XYL	MAN	GAL	GLC	GalUA	LIGNIN[2]	TOTAL[2]
CASSAVA (HMC T)	5.07	5.68	9.15	2.64	16.71	38.19	22.54	4.07	81.82
POTATO[a]	2.3	4.9	2.03	4.06	19.8	38.9	21.54	6.46	78.90
POTATO[b]	2.5	6.6	1.95	1.17	31.12	36.19	20.43	N/A	51.40

1 Individual sugars expressed as a % of the total.
2 Total sugars and lignin expressed as mg/g DM.

a Graham and Aman, 1988
b Englyst and Cummings, 1982.

 Cellulose contents of approximately 30% of the total NSP were obtained for potato by both Anderson and Bridges (1988) and Englyst and Cummings (1982) which is consistent with that obtained for cassava in this study. However, Holloway, Monro, Gurnsey, Pomare and Stace (1985) only obtained a value of 14% for cellulose.

 It may be speculated therefore, that the dietary fibre of cassava will behave in a similar manner to that of potato in the gastro-intestinal tract. This aspect is currently being investigated.

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ANALYSIS OF THE DIETARY FIBRE FROM Olea europaea (Gordal and Manzanilla var.).

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1 INTRODUCTION

The olive (Olea europaea) is a foodstuff equilibrated in fibre, especially those varieties used as pickling fruits (Manzanilla and Gordal var.) which have good organoleptic characteristics and a considerable nutritive value.

The purpose of the present study has been to carry out the isolation of the different fibre fractions, using chemical and enzymatic methods, and to analyse the sugars obtained from their hydrolysis. Methodologies for fibre included neutral detergent fibre (NDF), acid detergent fibre (ADF), insoluble fibre (IF), soluble fibre (SF) and total fibre (TF).

2 MATERIALS AND METHODS

Materials.- Olives of Manzanilla and Gordal varieties were harvested in the Province of Seville (Spain). The fruits were stored at 4°C for no longer that 24 hours until used.

Fibre determination. Neutral detergent fibre were determined from the de-fatted flesh, using the procedure of Van Soest et al (1). Insoluble and soluble fibre were isolated by the method of Asp (2).

Acid hydrolysis. The polysaccharides of ADF, NDF, IF and SF were subjected to sequential hydrolysis with concentrated sulphuric acid and then more dilute.

Neutral sugars determination. The analysis of the sugars resulting from hydrolysis was carried out by GC. The alditol

The percentages of SF are close to 1% , and those of TF close to
5%. The fibre values obtained by system of Van Soest (NDF and
ADF) are lower than those of IF and TF.

Acid hydrolysis of the fibre fractions. After carrying out
a study of the optimun conditions, a primary hydrolysis was
established with H_2SO_4 (72 %) for two hours at 40°C, and a
secondary hydrolysis with H_2SO_4 (2N) for two hours at 40°C. Table
III show the composition of neutral sugars (Gordal Var.) Glucose
is the main major sugar in all the fractions except in SF, in
which it is arabinose. The highest percentages of galactose are
found in IF and SF, whereas this sugar is partially solubilized
in NDF, and only traces appear in ADF. The mannose content is
notably equal in IF, ADF and NDF, and much lower in SF. Part of
the xylose is solubilized in the ADF treatment, only traces
appear in SF, while the highest percentages are those in IF and
NDF. An important fraction of arabinose is lost in the NDF
treatment, practically disappears in the ADF, it is found in high
percentage in IF, and in SF it is much lower. Ramnose is present
in small quantities in all the fractions.

Table III. Hydrolyzates composition of the fibre fractions (%)
in Gordal var.

	IF	SF	ADF Original	ADF Delignified	NDF
Glucose	26.04	6.090	54.160	39.350	21.560
Galactose	0.78	2.950	1.120	0.030	0.430
Mannose	0.085	0.400	1.490	0.890	0.680
Xylose	6.76	1.770	4.210	2.520	4.460
Arabinose	5.86	2.040	0.190	0.130	2.590
Ramnose	0.57	1.530	traces	traces	0.140

The values are the means of two determinations; coefficient of
variation 10%

Cellulose. The results obtained are shown in Table IV.
Significant differences between fractions, but not between
varieties were observed. There aren't significant differences
between the cellulose content of ADF, NDF and IF fractions, but
higest values are obtained by Van Soest's method. This could be
explained by the fact that sugars corresponding to hemicellulose
have been detected in the de-lignified ADF fraction.

Hemicelluloses. The results showed that very low precision
was got with the Van Soest's method (C.V. 40%). In relation with
the hemicellulose content of ADF, NDF and IF, the higest value
was found for IF. It's important to remark that an important
quantity of hemicelluloses was present in ADF fractions.

acetates were prepared by the method of Englyst et al (3), with little modifications.

3 RESULTS AND DISCUSSION

Fruit composition. The fruit had been picked in the best conditions of ripeness for pickling (ripe green fruit) The results obtained for moisture, fat, proteins and free sugars are shown in Table I.

Table I Composition of Olives (Olea europaea, var. Gordal and Manzanilla) (%):

	Gordal	Manzanilla
Moisture	71.62 ± 0.06*	69.66 ± 0.04*
Dry mather	28.38	30.34
Fat	15.19 ± 0.30	16.03 ± 0.44
Protein	1.29 ± 0.14	1.20 ± 0.07
Free sugars	3.94 ± 0.55	3.75 ± 0.54
Ash	0.58 ± 0.007	0.47 ± 0.006
Free sugars		
Sucrose	0.30 ± 0,03	traces
Glucose	2.97 ± 0.35	2.33 ± 0.23
Fructose	0.37 ± 0.03	0.90 ± 0.17
Manitol	0.31 ± 0.07	0.37 ± 0.00

* Percentages refered to: fresh flesh
All values are means of four analysis ± standard desviation

Fibre determination. Table II summarizes the results of NDF, ADF, SF and IF for both varieties. The main major is IF, with very similar values for Gordal and Manzanilla.

Table II. Fibre fractions, Gordal and Manzanilla var. (%).

	Gordal	Manzanilla
ADF	2.08 ± 0.08	2.41 ± 0.08
NDF	2.82 ± 0.23	3.19 ± 0.10
IF	4.03 ± 0.09	4.26 ± 0.15
SF	1.01 ± 0.13	0.65 ± 0.32
TF	5.04	4.91

Percentages refered to fresh flesh

Table IV. Fibre fraction composition (%).

	IF	SF	ADF	NDF
		(GORDAL VARIETY)		
Cellulose	26.04 ± 2.01*	6.09 ± 0.2*	54.16 ± 5.40* 59.72 ± 0.41**	21.56 ± 0.75*
Hemicelluloses	14.82 ± 1.20	18.69 ± 5.75	5.95 ± 0.20	8.30 ± 0.03 26.54 ± 14.01**
Lignin	20.59 ± 0.20	–	39.97 ± 0.38	29.32 ± 0.3
Protein	18.06 ± 1.06	3.81	3.62 ± 0.09	17.06 ± 0.8
		(MANZANILLA VARIETY)		
Cellulose	18,69 ± 1.02	1.70 ± 0.11	3.4 ± 1.5 46.6 ± 3.57	17.02 ± 2.4
Hemicelluloses	8.44 ± 0.30	5.36 ± 0.20	3.12 ± 0.03	7.57 ± 1.19 24.44 ± 0.34**
Lignin	–	–	48.49 ± 0.46	–
Protein	18.66 ± 1.55	2.62	16.24 ± 1.8	14.65 ± 0.22
Ash	2.79	–	0.47 ± 0.06	–

Percentages to: * fibre; ** Determined by Van Soest method.

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ISOLATION AND CHARACTERISATION OF DIETARY FIBRE IN WHITE ASPARAGUS

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1. INTRODUCTION

The production of white asparagus (Asparagus officina-lis) in Andalucia (Spain) has undergone a very important expansion in recent years. The commercialization of white asparagus for its consumption is very scarce in Spain, with almost all going to the conserve industry.

The objetive of the present work has been to establish the general composition of processed white asparagus, with special attention in the fibre fraction because of its nutritive, organo-lectic and industrial characteristics.

2. MATERIALS AND METHODS.

The asparagus are processed by the traditional system in the conserve industry and differentiated by three zones: the apex, middle, and base, which are triturated and then individually frozen.

The fibre determinations are carried out by different methods: acid detergent fibre (ADF), and neutral detergent fibre (NDF), both described by Van Soest (1), and the total fibre (TF), insoluble fibre (IF) and soluble fibre (SF), developed by Asp (2).

The different fibre fractions are subjected to the acid hydrolysis, and the resulting sugars analysed by gas chromatography as alditol acetates (3).

The pectic substances are similarly analyzed by the method of Blumenkrantz (4).

In each fibre fraction obtained, the residual content of proteins and uronic acids is determined.

Results analysis have been made by ANOVA techniques. Means comparison have been made by Duncan's method.

3. RESULTS AND DISCUSSION

The distribution of the chemical constituents of the different zones of white asparagus (apex, middle and base) is studied, as well as that of the whole product. Table I shows the percentages of the main components, expressed as fresh product. No significant differences are seen in the composition of the different zones of this vegetable.

TABLE I.- Composition of different zones or white asparagus (%).

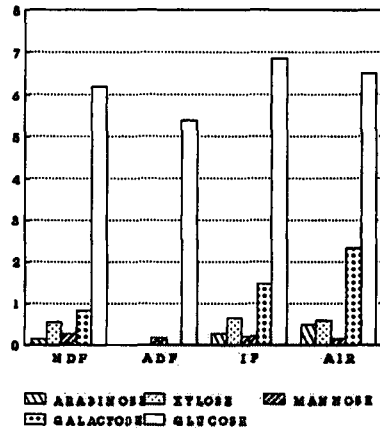|	APEX	MIDDLE	BASE	WHOLE
Moisture	93.49±0.41	94.70±0.01	94.83±0.14	94.25±0.19
Dry Matter	6.51±0.41	5.31±0.01	5.17±0.14	5.76±0.19
Proteins	3.26±0.11	2.56±0.10	2.39±0.17	2.65±0.16
Free Sugars	22.34±1.83	22.73±0.86	24.94±1.07	24.35±1.24
Uronic Acids	0.39±0.01	0.28±0.01	0.31±0.04	0.34±0.01
Ash	1.13±0.08	1.00±0.08	1.14±0.01	1.14±0.04

The results obtained for the fibre (NDF, ADF, IF, SF, TF) are shown in Table II, expressed as dry matter. No significant differences (= 0.05) are found between the various parts of the asparagus. Significant differences between the fibre fractions have been found. The great superiority of NDF over ADF is indicative of the high hemicellulose content of asparagus. Ratio ADF: Hemicelluloses 1:1

It is interesting to remark that the NDF values are higher than IF ones.
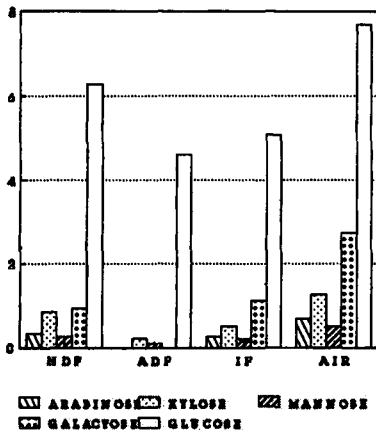
APEX ZONE
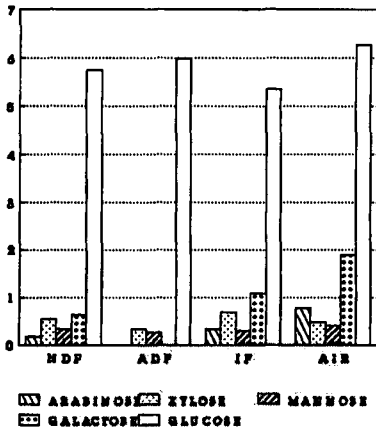
MIDDLE ZONE
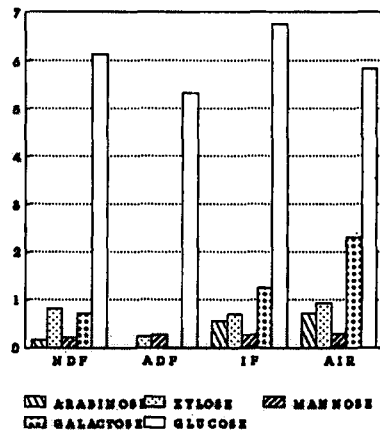

BASE ZONE

WHOLE ASPARAGUS

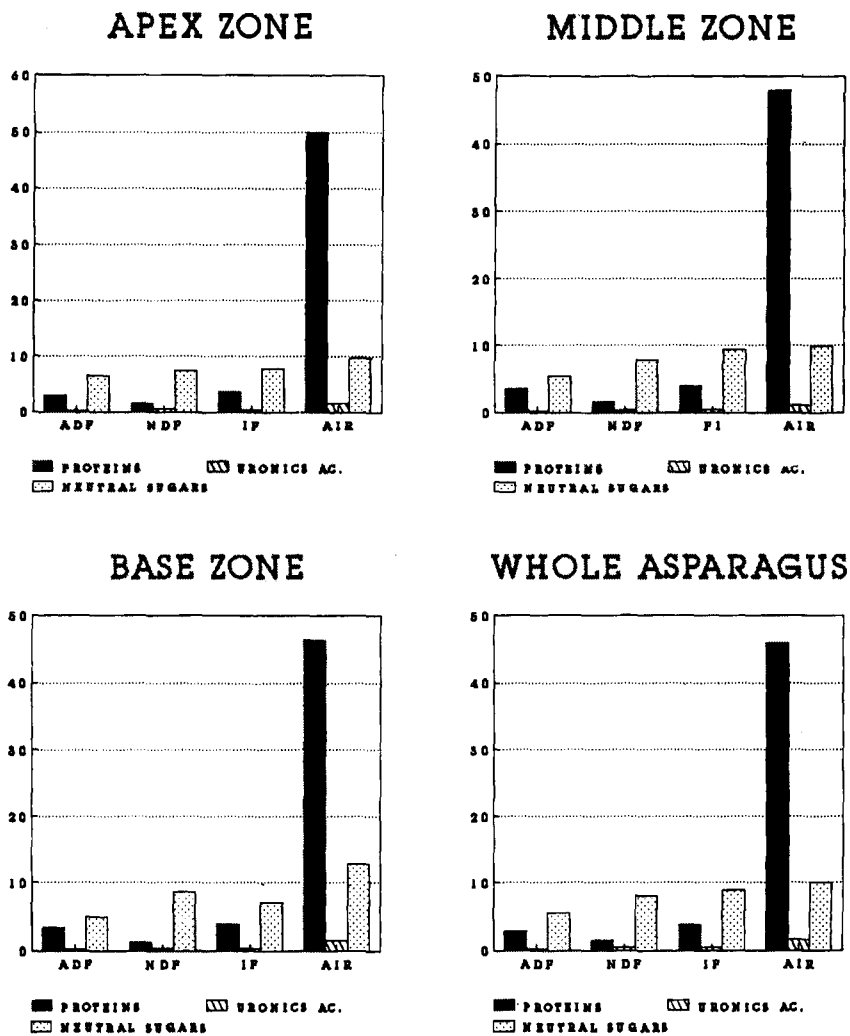Fig 1.- Hydrolyzates composition of fibre fractions (% dry matter)

Fig. 2.- Fibre fractions composition (% dry matter)

TABLE II. Fibre fractions of white aparagus (%)*.

	APEX	MIDDLE	BASE	WHOLE
ADF	10.17±0.06	12.26±0.42	12.77±0.12	11.35±0.13
NDF	24.79±0.62	20.74±0.40	19.92±2.26	23.65±0.27
FI	20.97±0.04	18,87±1.44	21.60±0.27	19.38±0.15
FS	6.35±0.43	6.01±0.23	5.81±0.51	5.05±0.33
FT	27.31±0.47	24.88±1.21	27.41±0.24	24.43±0.47

* refered to dry matter

Figure 1 shows the composition of the hydrolyzates of the fibre fractions. In all fractions, except ADF, the main sugar in the different parts is glucose, followed by galactose and xylose, and in lower concentration, mannose and arabinose. In fraction ADF small quantities of hemicellulosic sugars are detected (mannose and xylose).

The alcohol insoluble fraction (AIR) composition is also showed as a measure of the recovery of each monosaccharide.

Figure 2 shows the composition in proteins, uronic acids and neutral sugars of the different fibre residues.

Significant differences between fibre fractions in uronic acids, but not in protein content, are found. Uronic acids in NDF and IF are statistically equal and higher than ADF. The protein solubilisation has been very good in all treatments.

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WORKSHOP REPORT: ANALYSIS OF PLANT CELL WALLS IN RELATION
TO FIBRE

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The original hypothesis was related to plant cell-wall
material and it is suggested by Dr. Eastwood, Dr. Englyst
and Professor Southgate that the measurement of dietary
fibre (DF) should represent an index of this. Non starch
polysaccharides (NSP) are a measure of cell-wall
polysaccharides and therefore represent a good index of
cell-wall material.

It was shown that for some types of unprocessed foods
similar DF values are obtained by both the gravimetric
AOAC-Asp technique and by the measurement of NSP by the
Englyst procedure. However, for many types of processed
foods, quite different values are obtained because the
AOAC-Asp procedure includes part of the starch retrograded
during processing, Maillard reaction products and other
lignin-measuring substances.

Either type of method can possibly be used for some
unprocessed foods, especially cereals, as an index of
cell-wall material. For processed foods, the inclusion of
lignin-measuring substances and retrograded starch makes
the AOAC-Asp procedure unsuitable as an index of plant
cell-wall material. Furthermore, the inclusion of
substances formed during food processing, as in the AOAC-
Asp procedure, make it impossible to calculate the DF
content using the recipe and food table values for the DF
content of the raw foods.

Dr. Asp suggested that starch retrograded during
processing should be regarded as a legitimate component of
DF. However, Dr. Cummings and Dr. Englyst have shown that
the amount of starch included in the measurements by the
AOAC-Asp procedure represents only a small proportion of

that escaping digestion in the small intestine, and see no reason for its inclusion as DF. They propose that all the starch escaping digestion is important and that it should be measured separately.

Dr. Englyst presented a rapid colorimetric technique allowing total, soluble and insoluble dietary fibre (NSP) to be measured within a normal 8 hour working day, whilst analysis by the AOAC-Asp procedure and by the Englyst GLC procedure requires 1.5 days.

It was mentioned by Professor Asp that the gravimetric AOAC-Asp procedure could be modified so that it would not include retrograded amylose. Dr. Jeraci presented a gravimetric technique where all starch, including retrograded amylose, was dispersed by urea and it was pointed out that in Canada three methods - the gravimetric AOAC-Asp, the Englyst NSP and the gravimetric procedure developed by Dr. Mongeau, Ottawa - were accepted as official methods for the measurement of dietary fibre.

It was generally agreed that it was advantageous to have more than one type of technique for the measurement of DF provided that the various methods measure the same components of the diet.

Professor Southgate and others agreed that methods giving information about the properties of the different types of DF are required for research purposes. For routine analysis including food labelling the detailed information is not always required, but it is essential that the overall values obtained by research methods and the more rapid methods for routine analysis are in agreement.

Effect of Fibre on the Small Intestine: Implications for Digestion and Nutrient Absorption

THE BIOLOGICAL EFFECTS OF DIETARY FIBRE IN THE SMALL INTESTINE

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Most of our present knowledge of nutrient absorption has been achieved using tightly controlled experimental conditions, and relatively simple physiological media. However, during normal nutrition, the intestinal lumen provides a much more complicated environment. The dietary fibre hypothesis has done much to focus attention on the fact that nutrients are usually absorbed from complex food materials that must be physically degraded and digested before transport processes can take place. In most human diets, plant cell wall polysaccharides are an essential component of the intraluminal milieu, and it is now becoming clear that they can play an important regulatory role in many aspects of intestinal physiology.

Barrier Effects of Cell Walls

The cellular organisation of plant tissues depends upon the presence of non-starch polysaccharides which comprise the structural components of cell walls. There is now extensive evidence to show that the physical characteristics of fruits and lightly processed cereal products can influence the rate of digestion and assimilation of carbohydrate (1,2). Furthermore, the cellular structure of particularly robust plant tissues can survive processing, cooking and mastication, and hence moderate the rate of digestion of starch and other nutrients in the small intestine. Jenkins et al (3) have systematically investigated the variation in human glycaemic response to carbohydrate foods, and have shown that many legumes give particularly small blood glucose excursions following a test-meal. Some have ascribed this effect to the supposed presence of viscous "gel-

forming" fibre in such foods (4). In fact viscosity
appears not to be relevant in this particular context.
(5,6). Wursch et al (7) studied the relationship between
cellular integrity and the rate of starch hydrolysis in
a variety of legumes, and confirmed that whereas cooking
alone would ensure the gelatinisation of starch granules
in white kidney beans, the starch remained encapsulated
within intact cell walls. The presence of an intact
diffusion barrier impeded the access of α-amylase and
significantly slowed starch hydrolysis in vitro.
Rigorous processing can disrupt cell walls however, and
increase the rate of starch digestion both in vitro and
in vivo.

Such studies suggest that for some diets one of the
most important functions of fibre is to maintain the
structural organisation of plant foods during digestion,
and hence to exert a moderating influence over nutrient
digestion and post-prandial metabolism (8,9). However
these effects cannot be described or predicted by any
currently used chemical assay for the routine
determination of total fibre in foods, nor can they be
reproduced by the addition of isolated polysaccharides as
a supplement to the diet. Failure to allow for this
important functional aspect of dietary fibre may explain
why the beneficial effects of legume-rich diets in the
management of non-insulin dependent diabetes (10) have
not always been observed when simple fibre supplements
have been used (11).

Binding Effects of Dietary Fibre

The factors which control the disintegration of plant
tissues and cell walls during digestion have not been
thoroughly investigated. The residual material in the
lumen of the small intestine after a meal containing
plant foods will include partially degraded cell walls
with a range of physical and chemical properties. Most
foods will yield insoluble but finely divided particles,
together with some soluble polysaccharides such as
pectins and β-glucans. There is a growing tendency to
distinguish between soluble and insoluble fibre in
physiological studies, and, in some cases even on food
labels. This is a potentially useful development but the
currently available techniques for the analysis of fibre
do not necessarily predict the solubility of fibre under
physiological conditions (12).

In many foods, the barrier effects of intact cell
walls will be lost as the result of disruption by

processing, cooking, and the mechanical effects of gastrointestinal motility. However the constituents of disrupted cell walls may continue to influence nutrient absorption by binding both enzymes and food components in the gut lumen. This topic has received a considerable amount of attention, both because of fears that binding of inorganic micronutrients may have adverse effects on mineral balance in persons consuming high fibre diets, and because the adsorption of bile salts and lipids has been proposed as a mechanism to account for the hypocholesterolaemic effects of some forms of fibre.

The classic studies of Widdowson and McCance (13) established that subjects consuming whole-meal bread showed reduced iron absorption in comparison to that from white-bread, and this observation has since been confirmed by other groups. Isolated components of fibre have been shown to bind iron _in vitro_, but the extent of such binding depends on the physical conditions of such studies and the presence of other ligands. Cereal brans are complex materials which contain high levels of phytic acid in addition to dietary fibre, and when wheat bran is dephytinised much of the iron-binding activity is lost (14). Therefore, although diets with high levels of fibre may lead to negative mineral balances, it is not necessarily the dietary fibre component of complex carbohydrate foods which is primarily responsible for the mineral binding effect.

Many components of dietary fibre have the capacity to bind bile salts and the lipid components of mixed micelles _in vitro_ (15), and this effect has long been thought to play some role in human lipid metabolism (16). Under normal circumstances bile salts are efficiently reabsorbed in the distal ileum. If a sufficiently high proportion of bile salts are bound to some non-absorbable phase in the small intestinal lumen, this process of entero-hepatic circulation is partially interrupted and bile salt excretion is increased, leading to a compensatory increase in hepatic bile salt synthesis. This is accompanied by a fall in serum LDL cholesterol, presumably as a result of increased LDL uptake by the liver and perhaps other tissues (17). The mechanism is exploited clinically in the treatment of hereditary hypercholesterolaemia by the use of ion-exchange resins such as cholestyramine.

Both soluble and insoluble forms of dietary fibre have been shown to bind bile salts _in vitro_. Lignin is particularly efficient, but the binding capacity depends

upon the presence of methoxyl and β-carboxyl groups, and
most importantly, on the ambient pH (18). Removal of
lignin substantially reduces the bile acid binding
capacity of alfalfa, but residual activity appears to be
associated with the hemicellulose fraction (19). Pectin
(20) and other soluble polysaccharides, such as the D-
mannopyrannose polymer of guar gum and the β-glucan of
oat bran, also exhibit bile-acid binding capacity *in
vitro* (21). Nevertheless the physiological implications
of these results are uncertain. Bile salt binding is
most efficient at acidic pH, and it declines markedly at
neutral pH, which is characteristic of the distal ileal
lumen.   Furthermore,   attempts   to   correlate   the
effectiveness of bile salt binding determined *in vitro*
with faecal bile salt excretion and hypocholesterolaemic
effects *in vivo* have not been consistently successful.
It is therefore unlikely that this mechanism provides
more than a partial explanation for the well established
cholesterol-lowering properties of some high-fibre diets
(22).

Some components of dietary fibre can bind to the
endogenous enzymes of the gastrointestinal tract.   *In
vitro* studies have shown that wheat bran, pectin and guar
gum reduce the activity of pancreatic amylase and lipase,
at least partly as a result of adsorption (23,24). There
are substantial reserves of digestive capacity in man and
it seems unlikely that this effect is a rate-limiting
factor during digestion. However there is some evidence
of an adaptive increase in pancreatic output in rats fed
wheat bran (25). If feedback regulation of pancreatic
function occurs in man it might be of some physiological
significance.

The Effects of Soluble Polysaccharides on Luminal Convection

Amongst various proposals for mechanisms whereby
dietary fibre could influence the energy content of the
human diet, Southgate suggested that the presence of the
fibre matrix in the gut lumen might reduce the diffusion
of solutes to the mucosal surface and thereby lead to
malabsorption of nutrients (26). This concept was first
tested experimentally by Jenkins and co-workers (27) who
showed that when isolated components of fibre were added
as supplements to test-meals, only soluble materials with
a high viscosity reduced the post-prandial blood glucose
response.   The mechanism underlying this observation
remained conjectural however until *in vitro* experiments
carried out by Elsenhans *et al* (28), and by Johnson and

Gee (29) established that dilute solutions of the highly viscous hydrocolloid guar gum could markedly reduce the rate of absorption of monosaccharides. The use of in vitro methods enabled quantitative kinetic studies to be undertaken which showed that the reduction in transport rate was greatest at intermediate substrate concentrations, implying an increase in the kinetic parameter Km, the apparent substrate affinity of the mucosal carriers (30). This was subsequently shown to be true also for other soluble gums such as carboxymethylcellulose (31) and β-glucan (32).

The epithelial surfaces of transporting tissues are overlain by a poorly stirred boundary-layer or "microclimate" in which solute concentrations differ from those in the well-stirred aqueous "bulk phase". This phenomenon is well known to biochemists wishing to measure transport kinetics in vitro because the presence of an unstirred water leads to a reduction in the substrate concentration at the cell surface, and hence to an over-estimate of the true Km value. There is no doubt that such a layer also exists in the gut in vivo, although both the complex morphology of the mucosa and the irregular fluid movements induced by the peristaltic motion of the gut wall make its dimensions and physiological significance uncertain (33). The concept of the unstirred water layer provides a useful model to describe the effects of viscous media on nutrients transport in the small intestine. By measuring the time-course for the development of transmural electrical potentials, induced by imposing an osmotic load on isolated mucosal tissue incubated with polysaccharide-enriched media, it has been possible to demonstrate that the apparent thickness of the unstirred layer increases in a viscosity-dependent manner (34,31). Moreover this critical range of viscosities is consistent with that observed in the small intestinal contents recovered from rats after dietary supplementation with guar gum (35).

In isolated loops of intestine, a retardation of sugar and water absorption due to intraluminal guar gum has been demonstrated by Blackburn and Johnson (36), using a two-stage perfusion of rat jejunum. Similar results were obtained by Rainbird et al using a single-stage perfusion in the conscious pig (37), and by Blackburn et al using intubated human jejunum (38). In general the effect has not been observed in closed intestinal loops where both flow and peristalsis are absent (39), or in preparations where perfusion at high viscosity has led to distension of the gut, with a

consequent increase in mucosal surface area (40). In an
elegant paper, Anderson et al have recently shown that
these various observations are consistent, and in the
intact intestine, a viscosity-dependent reduction in the
rate of transport only occurs when the lumen is well
stirred by peristalsis (41).

The combined evidence obtained from these various
approaches to the problem seems to confirm that viscous
forms of dietary fibre do indeed impede access of solutes
to the mucosal surface, and other mechanisms, such as a
reduction in the rate of gastric emptying, are probably
only of secondary importance (38). The increased
resistance to transport appears to be due primarily to
viscous damping of convective fluid movement in the
vicinity of the mucosa, so that the path-length for
solute diffusion is increased. The presence of a
dispersed hydrocolloid must also impede free diffusion to
some degree, but the reduction in the diffusion
coefficient appears to be negligible for small solutes
and low concentrations of polymer (42,43). Precise
values for the viscosity of guar and other polymers
during digestion in vivo are difficult to estimate
because their rheological behaviour is non-Newtonian.
Viscosity will vary with the intra-luminal shear-rate,
and this itself is an unknown quantity during
peristalsis. There is no doubt however that intraluminal
viscosity can be increased in man after dietary
supplementation with guar or similar polysaccharides, and
perhaps after eating oats (44). In practice, the precise
values achieved in vivo are probably not of great
significance.

Guar gum (45) and oat gum (32) both reduce the rate
of uptake of cholesterol from mixed micelles in vitro.
Apart from the mechanism already discussed, there is also
some evidence from in vitro experiments to suggest that
these polysaccharides may directly impede the diffusion
of mixed micelles which are very much larger than sugar
molecules (46). Furthermore, the rate of cholesterol
uptake is proportional to that of water absorption in
perfused preparations of small intestine (41). Viscous
polysaccharides reduce the rate of water uptake, and this
effect probably also favours a reduction in the rate of
lipid absorption.

Secondary Effects of Delayed Nutrient Absorption

All the various mechanisms which have been outlined
so far have the effect of delaying nutrient absorption,

but it is not clear to what extent they also cause losses
of nutrients to the large bowel. An important
distinction needs to be drawn between the barrier and
binding effects of fibre, which probably prevent
absorption of some nutrients entirely, and the convective
effects which probably only delay absorption, but in so
doing modify the normal intraluminal concentration
profile (47). This latter effect is important because
the small intestinal mucosa shows an adaptive response to
exposure to nutrients. Both types of mechanism can lead
to important metabolic consequences through a reduction
in the rate of uptake of carbohydrate as glucose, and
perhaps because of an increase in the quantity of
carbohydrate absorbed as bacterial fermentation products.
A full discussion of these effects is outside the scope
of the present paper, but mention will be made here of
the possible impact on gastrointestinal endocrinology.

The relatively low post-prandial glycaemic response
seen after carbohydrate meals containing large quantities
of legumes, or those enriched with viscous gums, are
associated with a reduced insulin response in normal
subjects or non insulin-dependent diabetic patients (48).
Morgan et al observed a statistically significant
reduction of about 70% in post-prandial gastric
inhibitory peptide (GIP) levels in healthy volunteers
given a guar-enriched mixed meal (49), and a smaller
reduction in glucagon-like immunoreactivity (GLI). More
recently Trinick et al reported that the relatively low
glucose and insulin response to a guar-enriched liquid
meal was associated with a statistically insignificant
reduction in GIP, but observed no effect on glucagon, C-
peptide and pancreatic polypeptide (50). The reduction
in GIP secretion after a test-meal containing guar gum is
probably a reflection of the reduced rate of glucose
absorption in the proximal small bowel. The reduced GLI
levels reported by Morgan et al (49) are more difficult
to explain because enteroglucagon is released by
specialised mucosal cells in the distal small bowel and
colon. A delay in absorption would therefore be expected
to increase GLI release. However, much may depend upon
the duration of the experiment and the design of the
sampling procedure. Paganua et al have reported raised
enteroglucagon levels in diabetic children given diets
supplemented with guar gum and fructose (51), but this
increase may have been due to stimulation of
enteroglucagon secreting cells by volatile fatty acids,
following fermentation of guar. It is unclear what
proportion of enteroglucagon in normal human subjects is
derived from the large bowel in man, but it has been

observed that ileostomists have lower enteroglucagon
levels than control subjects (52).

Adaptation to Fibre in the Small Intestine

Most of our knowledge concerning the adaptive
response of the small intestine to high fibre diets is
derived from studies with the rat, which exhibits
morphological adaptation to plant materials in the diet
(53). Plant foods are highly complex in composition
however, and substances such as protein and
pharmacologically active plant cell constituents can
influence small intestinal growth. Only recently have
the effects of cell wall materials begun to be studied in
isolation.

Several groups have observed an increase in small
intestinal length in growing rats given diets enriched
with viscous dietary fibre. This gross morphological
response is accompanied by an increase in mucosal mass
and DNA content (54,55,56). The intestinal mucosa is a
highly proliferative tissue in a constant state of cell
renewal, and it seems probable that the morphological
changes are a reflection of an increased rate of cell
proliferation. Such an effect was reported by Jacobs
(56) and confirmed by other groups using the metaphase
arrest technique to measure absolute rates of crypt cell
proliferation (57,58). An enhancement of cell
proliferation is seen throughout the small intestine of
rats given pectin, guar gum and sodium
carboxymethylcellulose (59), but it does not occur in
rats fed gum arabic which is soluble in the small
intestine but non-viscous (60).

Several groups have reported changes in mucosal
enzyme activity in rats fed isolated sources of fibre,
but the reports are not entirely consistent. For example
whereas Brown et al (54) and Thomsen and Tasman-Jones
(61) observed decreased mucosal disaccharidase activity
in rats fed pectin or galactomannan-supplemented diets,
other groups have reported unchanged or slightly elevated
levels (62,63). In the present author's experience,
polysaccharides which stimulate cell proliferation are
associated with reduced alkaline phosphatase and lactase
activity compared to rats fed a fibre-free diet, whereas
maltase displays a complex change in the distribution of
activity throughout the small intestine (58). It is
probable that mucosal enzyme activities reflect changes
in the average maturity of mucosal cells in the
epithelium, complicated by the induction of specific

enzymes, perhaps in response to luminal nutrient concentration.

If the reduced mucosal enzyme activity seen in response to some forms of dietary fibre is one expression of a less "mature" population of mucosal cells, then a similar reduction in carrier transport capacity might also be expected. The maximum jejunal transport rate for 3-O-methyl glucose in rats maintained for 4 weeks on a semi-synthetic diet containing guar gum was approximately 40% lower than in rats fed a similar diet containing insoluble cellulose (57). Schwartz et al (64) reported changes in enzyme activity and a reduction in glucose and leucine transport in animals fed diets containing pectin for 6 weeks. There is also evidence that lipid absorption in the rat is modified by an adaptive response to prolonged feeding with various components of dietary fibre (65).

Several mechanisms have been proposed to explain the changes in cell proliferation which seem to underlie the adaptive response to fibre in the small intestine. Short-chain fatty acids are produced in the colon by fermentation of carbohydrates, and Sakata has proposed that these are growth-promoting factors for the small intestine (66). However this does not provide an entirely satisfactory explanation for the trophic effects of dietary fibre when the responses to particularly well-defined polysaccharides are considered. Sodium carboxymethylcellulose is a highly viscous gum which stimulates cell proliferation (60) but it is almost entirely resistant to fermentation in the rat colon (67). Conversely, gum arabic, which is non-viscous but highly fermentable, has no trophic effect in the rat small intestine (60). An increase in the level of enteroglucagon in the blood usually accompanies trophic stimuli such as massive resection of the small bowel, and this peptide has long been regarded as a probable mucosal growth hormone (68). The high levels of enteroglucagon seen in rats fed guar gum and other viscous polysaccharides seemed to corroborate this hypothesis. However enteroglucagon levels are also increased in rats given gum arabic, suggesting that there is no obligatory trophic response to this peptide (60). Germ-free rats also have high basal levels of enteroglucagon with no corresponding increase in cell proliferation compared to normal controls (69).

It seems probable that the increase in cell proliferation seen in animals given certain soluble

polysaccharides is an adaptive response to high intraluminal viscosity. The increased pre-mucosal diffusion resistance which occurs under these conditions may be assumed to delay nutrient clearance, thus displacing the intra-luminal concentration profile in such a way that the more distal mucosa is exposed to unusually high concentrations of nutrients, and of lipids in particular. Fatty acids have recently been shown to elicit a particularly significant trophic response (70).

Conclusions

The presence of dietary fibre in the gut lumen exerts a number of primary effects on the digestion and absorption of nutrients which have important metabolic consequences, some of which can be usefully exploited in man. The barrier effect of fibre depends upon the cellular structure of plant tissues. It is perhaps the most important small intestinal effect in relation to the dietary management of diseases such as diabetes, but, being a structural attribute, it cannot be predicted by the analysis of total fibre-content, nor can it be achieved simply by adding fibre supplements to manufactured food products. The secondary effects of fibre on the structure and function of the small intestine which are observed in laboratory animals seem to reflect an adaptive response to alterations in the distribution of nutrients in the small bowel, as a result of high luminal viscosity. There are few sources of viscous polysaccharides in conventional western diets, but the pharmaceutical use of guar gum and other polysaccharides, and perhaps high intakes of oat bran, may lead to similar effects in man. The long-term consequences of small bowel adaptation to dietary fibre are unknown.

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INFLUENCE OF WHEAT BREADS CONTAINING GUAR FLOUR SUPPLEMENTS OF HIGH AND LOW MOLECULAR WEIGHTS ON VISCOSITY OF JEJEUNAL DIGESTA IN THE PIG

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1 INTRODUCTION

Guar gum, a high molecular weight galactomannan, is
known to be of therapeutic value in the treatment of
diabetes largely because of its capacity to reduce post-
prandial blood glucose and improve lipid metabolism[1].
The mechanism by which guar improves glycaemic control
is not, as yet, fully understood but current evidence
indicates that a primary factor is the way in which guar
slows the rate of carbohydrate absorption in the small
intestine by modifying the rheological behaviour of
digesta[2]. There is however a lack of information about
the levels of viscosity induced by guar in vivo and how
this relates to changes in blood nutrients and hormones.

We have recently demonstrated in pigs that peak
viscosities of guar-containing digesta (collected 2.0m
distal to the pylorus) occurred within the first 60 min.
of the post-prandial period corresponding with the time
during which the greatest decreases in glycaemia and
insulinaemia have been observed in previous studies[3].
The test meals used in our study were fully-hydrated
solutions of guar gum added to a low-fat, semi-purified
diet. However, guar-containing foods, which are more
palatable than pure solutions, contain the polymer in a
relatively unhydrated form. Hence the aim of this study
was to determine whether a guar-containing wheat bread,
when administered to pigs, elicits comparable rheological
changes in digesta to those achieved with fully-hydrated,
pure guar solutions[3].

2 METHODS

Four Large White x Landrace boar pigs of initial weight
28–30kg were fitted with re-entrant cannulae 2.0m distal
to the pylorus in the mid–jejunum. The pigs were fed
twice daily at a level of 4% body weight. Each meal con-
sisted of a low fat, semi-purified (SP), wheat starch-
based diet and/or white wheat bread (based on the recipe
of Apling and Ellis[4]). The test bread meals contained
60g/kg of either M150 (high viscosity and high molecular
weight) or M60 (low viscosity and low molecular weight)
guar flour (Meyhall Chemical UK Ltd., Wirral). Each pig
received a total of seven diet combinations (3 control
and 4 test) as follows: 100% semi-purified (SP) diet,
100% control bread, 100% M60 bread, 100% M150 bread, 50:50
SP diet:control bread, 50:50 SP diet:M60 bread and 50:50
SP diet:M150 bread. Each diet was fed to each pig for 7
days.

Samples (2 x 30 ml) of jejeunal digesta were
collected immediately following the morning feed (time 0
min.) and at 10, 20, 30, 40, 50, 60 ,90, 120, 150, 180,
210 and 240 min. Apparent viscosity was measured immed-
iately at 39°C (Brookfield DV–II rotoviscometer,
Stoughton, USA) across a range of shear rate conditions.
'Zero shear' viscosity[5] was then calculated.

3 RESULTS AND DISCUSSION

The maximum viscosities for all treatments occurred within
90 min. of feeding (Table 1) and this is consistent with
our earlier studies using fully-hydrated guar solutions[3];
in both cases peak viscosities corresponded with maximal
falls in glycaemia and insulinaemia in similar studies in
man[6]. The viscosity of jejeunal digesta was considerably
higher for all guar bread treatments than for the controls
(Figures 1 and 2). The viscosities were considerably
lower for the 100% guar bread treatments than for our
previous study with pure guar solutions[3]. In the latter
case, although the concentration of guar in the diet was
much lower, it was fully hydrated and this induced a
higher initial viscosity. The much more sustained high
levels of viscosity when the guar bread meals are fed were
of interest in relation to between-meal control of glyc-
aemia; this effect may be the result of progressive hydr-
ation of guar within the bread matrix. It is also notable
that the viscosity profile induced by the low and high
viscosity guar in the 100% bread meals was very similar
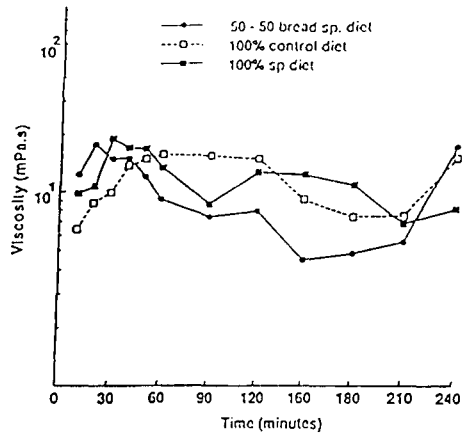and may explain why these two grades of gum elicit similar

Figure 1   Zero shear viscosity of jejeunal digesta
            following control treatments.
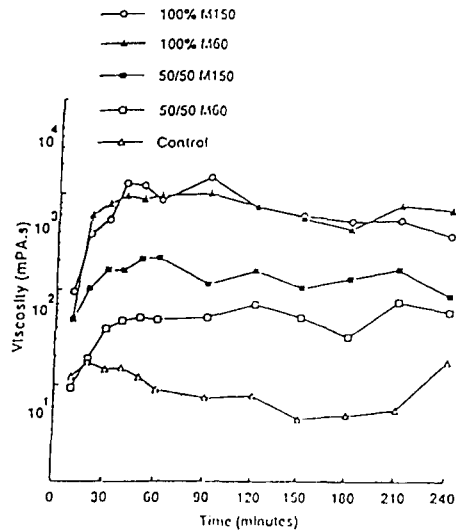

Figure 2   Zero shear viscosity of jejeunal digesta
            following guar bread treatments.

<u>Table 1</u> Maximum 'zero shear' viscosity (mPa.s) of
        jejeunal digesta ($\bar{x} \pm$ se)

	Control	M60	M150
100% SP	18 (1)	---	---
50:50 SP Bread	17 (1)	70 (3)	257* (10)
100% bread	16 (1)	1005** (11)	1454** (16)

significantly different from control; * $p > 0.05$; ** $p > 0.01$

falls in post-prandial plasma insulin in healthy human
subjects (Ellis et <u>al</u>., unpublished observations).

4   CONCLUSIONS

1. Bread containing guar flour induced higher jejeunal
    viscosities than either control bread or semi-
    purified diet.

2. The longer period of high viscosity seen with bread
    meals, compared to meals containing fully-hydrated
    guar solutions, may be the result of progressive
    hydration of the guar over several hours.

3. Similar increases in viscosity were seen for 100%
    bread meals whether high or low viscosity guar was
    incorporated, and may explain why low viscosity guar
    reduces post-prandial insulinaemia in man.

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EFFECTS OF SOME SOLUBLE NON-STARCH POLYSACCHARIDES ON FOOD INTAKE, CELL TURNOVER AND GASTROINTESTINAL PEPTIDE LEVELS IN THE RAT

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1 INTRODUCTION

In the growing rat, the consumption of some soluble non-starch polysaccharides leads to an enlargement of the small intestine, increased mucosal cell proliferation, and reduced levels of blood cholesterol compared to control groups[1]. These changes, which are frequently accompanied by increased circulating levels of the small intestinal peptide enteroglucagon, may be an adaptive response to a high luminal viscosity in the proximal small intestine, associated with a reduced rate of nutrient transport[2]. However, the interpretation of the results from such experiments is frequently complicated by differences in food intake. In the present study we have compared the effects of two soluble non-starch polysaccharides on the release of gastrointestinal peptides and intestinal mucosal cell proliferation, in rats, with and without food restrictions.

2 METHODS

In the present study three groups of weanling rats were fed _ad libitum_ on a fibre-free semisynthetic diet, (FF), or diets containing guar gum, (GG), or gum arabic, (GA), both of which are readily fermentable, but only one of which, (GG), is highly viscous in solution. Three other groups received either the fibre-free diet, a cellulose-supplemented diet, (C), or a starch-supplemented diet, (S), at levels paired to the intakes of the guar-fed rats. The composition of the diets is given in Table 1; food intakes were monitored throughout the experimental period. After 28 days the animals received injections of the anti-mitotic drug vincristine sulphate ($1mg. kg^{-1}$, Sigma, Poole,

UK), and were subsequently sacrificed at approximately
equal intervals between 12 and 120 minutes thereafter.
Each animal was deeply anaesthetised by an intraperitoneal
injection of sodium barbiturate (Sagatal; M & B Dagenham,
UK), and, after laparotomy, venous blood samples were
collected, followed by cervical dislocation. The small
intestine was removed and its unstretched length measured.
Segments (1cm) of distal ileum (95% of length) were
removed and placed in fixative (75% ethyl alcohol : 25%
acetic acid) for subsequent microdissection and
determination of crypt cell production rate by the
Stathmokinetic method[3]. Serum and plasma samples were
prepared from the collected blood. Glucagon-like
immunoreactivity (GLI) and neurotensin in plasma were
determined by radioimmunoassay (Novo Biolabs and
Immunodiagnostics UK, respectively. Gastrin was measured
in serum by radioimmunoassay (Immunodiagnostics, UK).

Table 1   Composition of diets (g.kg^{-1})

Constituent	FF	GG	C	S	GA
Starch	330	330	330	430	330
Sucrose	330	330	330	330	330
Casein	200	200	200	200	200
Corn oil	80	80	80	80	80
Mineral mix[+]	40	40	40	40	40
Vitamin mix[+]	20	20	20	20	20
D-L-methionine	2.5	2.5	2.5	2.5	2.5
Guar gum	-	100	-	-	-
Cellulose (Solka Floc)	-	-	100	-	-
Gum arabic	-	-	-	-	100

[+]   Johnson, et al.[2].

3 RESULTS

Total food intake by the GG-fed rats was approximately 15%
lower than that of the FF and GA groups fed ad lib (Fig
1). GG-fed rats had serum gastrin and neurotensin levels
significantly lower than those fed the FF diet ad lib, but
not compared to the pair-fed animals (Fig 2). In
contrast, GLI activity was approximatley 3 fold higher in
both the GG and GA fed groups compared to all others (Fig
2).

Cell proliferation in the distal ileum was significantly
increased in the GG-fed rats compared to all other groups,
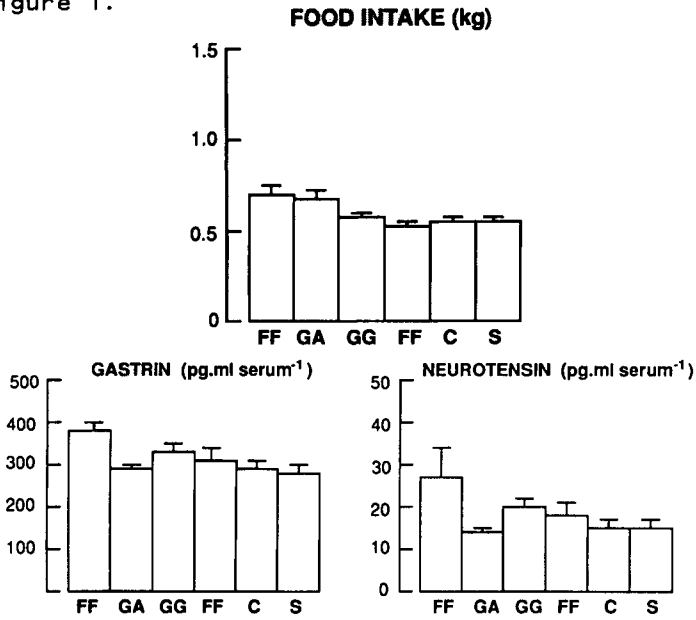and the total small intestinal length was greater (Fig 3).

Figure 1.

FOOD INTAKE (kg)

Figure 2.

GASTRIN (pg.ml serum^{-1})

NEUROTENSIN (pg.ml serum^{-1})

Figure 3.

GLUCAGON + ENTEROGLUCAGON
(ng.ml plasma^{-1})

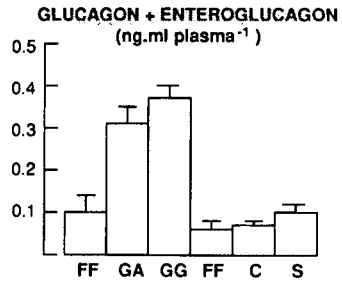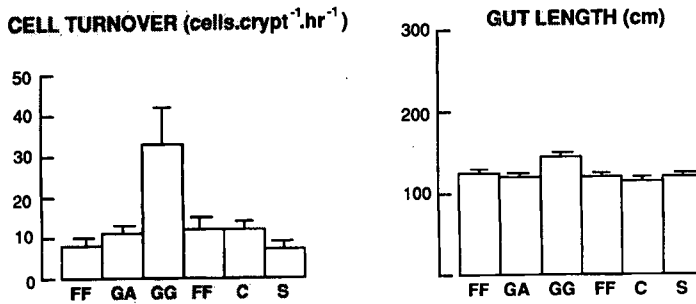**CELL TURNOVER (cells.crypt^{-1}.hr^{-1})**

GUT LENGTH (cm)

4 CONCLUSIONS

Soluble non-starch polysaccharides lead to an increase in plasma-GLI activity, which, as we have shown elsewhere, is accounted for primarily by increased enteroglucagon[2]. The present study illustrates that this is independent of any effect of food intake, and does not depend on increased viscosity in the small intestine. However, faster cell proliferation and greater small intestinal length were observed only in rats fed guar gum, which suggests that neither the provision of a fermentable substrate, nor high levels of enteroglucagon in the plasma provide sufficient stimuli for this form of small intestinal adaptation. Increased intraluminal viscosity may however be an essential pre-requisite for this effect.

Serum levels of both gastrin and neurotensin were reduced by the modest food restriction imposed in this study, but there was little evidence of any effect of guar gum. However the GA-fed rats, which showed no significant decline in food consumption, had lower levels of both peptides compared to FF-fed controls. The reason for this is unclear but it may indicate an effect of soluble polysaccharides on nutrient flow or luminal concentration of nutrients in the small bowel which is independent of viscosity.

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INTRODUCTION

The gastrointestinal tract has one of the most rapid rates
of cell renewal in the body,[1] intestinal epithelial cell
proliferation and intestinal absorption[2] are profoundly
influenced by food intake, and a considerable atrophy of
the gut is seen when it is deprived of food or 'luminal
nutrition',[3] this is perhaps best seen when the animal is
fed intravenously.[4] A similar atrophy is seen in the colon
of animals fed a fibre-free 'elemental' diet[5].

The Effects of Dietary Fibre

The colonic atrophy associated with fibre-free diets
can be reversed by the addition of dietary fibre.[6] This
phenomena was further investigated using the starvation-
refeeding model, as this is one of the best ways of
potentiating a proliferative response.[7] Refeeding starved
rats with an elemental diet supplemented with a variety of
dietary fibres provoked a marked proliferative response in
the colon, and to a lesser extent in the small intestine,
the magnitude of the response being greater in rats fed
more fermentable fibres, with no response to inert bulk
observed[8].

The effects of the intestinal microflora

Starvation Refeeding Experiment The starvation-
refeeding model, (in which the rats were starved for three
days and then refed for two days), was also utilised to

investigate the contribution of the intestinal microflora to the proliferative response to dietary fibre. Crypt cell production was assayed using vincristine to arrest cells as they entered metaphase, and measuring the rate of accumulation of arrested cells in microdissected crypts.[9] Refeeding conventional rats with an elemental diet or with the elemental diet supplemented with inert bulk had little effect on intestinal proliferation. Crypt cell production in the colon increased sixfold when the diet was supplemented with a fermentable fibre mixture.[10] A smaller response was also observed throughout the small intestine. This response was not observed in germ-free rats. See Figure 1.
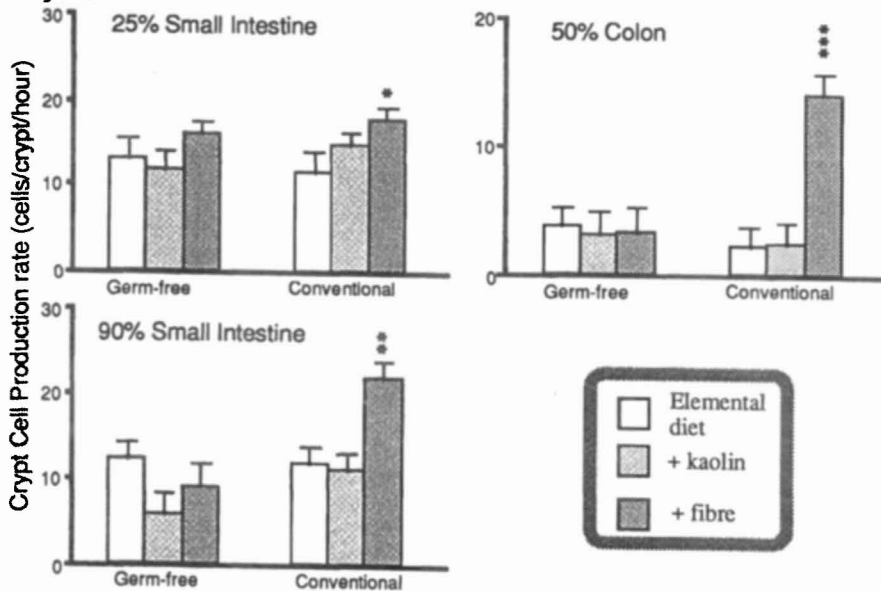
Figure 1. The effects of refeeding starved rats with a fibre-free elemental diet supplemented with inert bulk or a dietary fibre mixture.

Steady State Experiment The marked response to refeeding indicated that the response to fibre may also be seen in rats fed continuously. The rats were fed the elemental diet plus the fibre mixture (1 part ispaghula mucilage (Reckitt & Coleman Ltd) to 9 parts wheat grain fibrous extract (Labaz Sanofi Ltd). Crypt cell production rate was more than doubled in the colon of conventional rats, a small response was noted in the distal small intestine, but not in the proximal small intestine (Figure

2). Administration of the fibre-supplemented diet to the germ-free rats again had no proliferative effect.
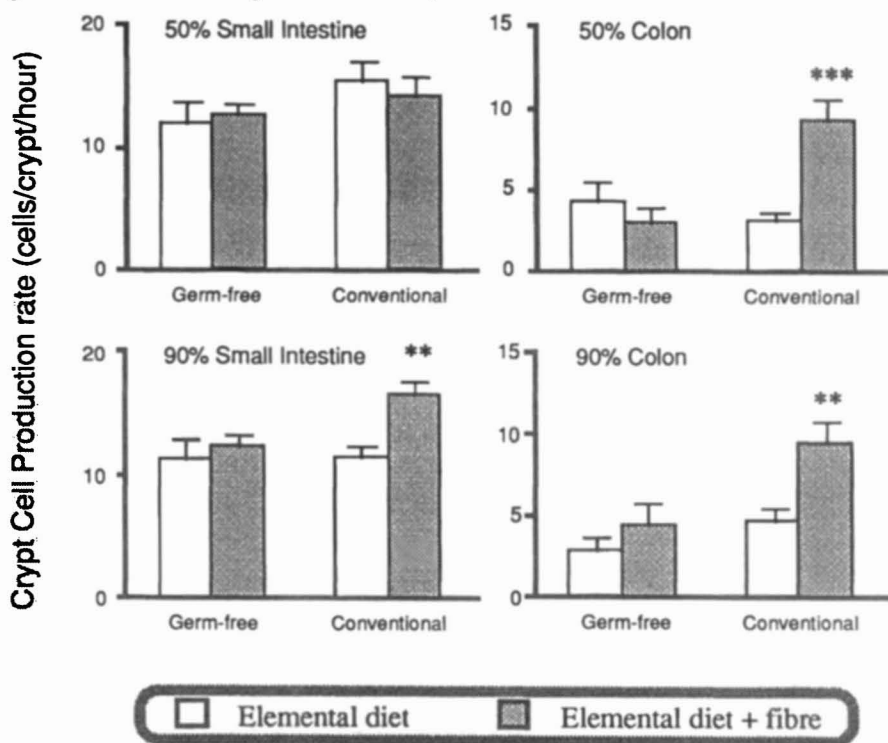

Figure 2. The effects of 2 weeks feeding with a fibre-free elemental diet or with the same diet supplemented with a dietary fibre mixture.

The wet weights of the various sections of the gastrointestinal tract are shown in Table 1. Two-way analysis of variance showed that diet had a significant effect (P<0.001) on the stomach, small intestine, caecum and colon weights. The microflora had significant (P< 0.001) effects on the caecum and colon weights only.

Table 1 Tissue weight (as percentage of total body weight) mean ± standard error

	Germ-Free		Conventional	
	Flexical	**Flexical + Fibre**	**Flexical**	**Flexical + Fibre**
Stomach	0.366 ± 0.004	0.473 ± 0.011***	0.390 ± 0.006	0.459 ± 0.011***
Small Intestine	2.056 ± 0.037	2.140 ± 0.033	1.944 ± 0.034	2.120 ± 0.036**
Caecum	0.537 ± 0.023	0.630 ± 0.014**	0.276 ± 0.006	0.353 ± 0.013***
Colon	0.360 ± 0.014	0.561 ± 0.015***	0.336 ± 0.008	0.498 ± 0.015***

Significantly greater than respective elemental diet** $P<0.01$, *** $P<0.001$.

DISCUSSION

We have shown that dietary fibre can stimulate epithelial cell proliferation in the colon, and (to a lesser extent) in the small intestine. This effect is dependent on the presence of the colonic microflora, indicating that it is the products of hind-gut microbial breakdown of fibre that has the trophic effect. The effect of diet on the colonic and caecal weights of the germ-free rats suggests that there is also an effect on the muscle layers, as was observed in the mouse.[6]

Fibre is broken down to short chain fatty acids, which can stimulate cell proliferation in ruminants[11] and in rodents.[12] This could either be a direct effect or moderated by some systemic influence.

Our own preliminary results also indicate a trophic effect of the direct infusion of short chain fatty acids. Sakata[12,13] has shown that this effect is systemic, which has been confirmed by Rombeau et al.[14]

The implications of a proliferative response to fibre are wide ranging, and sound a note of warning. Stimulation of intestinal proliferation can act as a promoter of carcinogenesis, as demonstrated by Jacobs.[15] Most animal carcinogenesis experiments use very powerful agents to initiate the process, thus these studies may concentrate excessively on the end stages of what is a complex and multistage process.

Dietary fibre has a multitude of effects on the gut, and proliferation is just one of these. Its other effects may well outweigh the proliferative risk, but this must be established, especially in the light of the rather dubious nature of most of the much publicised data used to advocate the health benefits of a greatly increased intake of dietary fibre.

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THE INFLUENCE OF DIFFERENT DIETARY FIBRE SOURCES ON DIGESTIBILITIES OF PROTEIN AND DRY MATTER IN RAT

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1 INTRODUCTION

With increase in awareness of the importance of consuming more fibres in the human diet due to its protective role against a number of diseases of modern societies, its nutritional importance has become increasingly recognised. It is now evident that not only the amount of fibre in diet but also the type and source of dietary fibre is important. Inclusion of guargum in an otherwise complete diet causes a significant decrease in apparent protein digestibility and a significant rise in faecal dry matter[1]. Less common fruits found in semi-arid zones of India have been reported to affect the biological utilization of the diet[2]. This paper presents the result on comparative affects of different sources of dietary fibre on digestibilities of protein and dry matter, and their relationship.

2 MATERIALS AND METHODS

Fibre Sources

Cellulose was procured from Patel Chest Institute, New Delhi, Isabgol (Plantago ovato) and cabbage (Brassica oleracea) from local market and fresh guave fruit (Psidium guajava) from Horticulture Farm of Haryana Agricultural University, Hisar, India.

Cabbage leaves were cleaned, cut into small pieces, spread under fan at room temperature (40-42°C) to make it dry and then ground to fine powder using 0.5 mm sieve in a cyclotec mill. After cleaning guava fruit, the pulp (free from seeds) was prepared by using an electric pulper, dried at 50°C in dehydrating chambers and ground to a fine powder (0.5 mm sieve). Both plant foods, cabbage and guava were analysed for netural detergent fibre (NDF), acid detergent fibre (ADF), cellulose and lignin by the method of Van Soest and Wine[3]. Hemicellulose was determined as the difference between NDF and ADF. Pectin was determined as calcium pectate[4]. Total dietary fibre was calculated as a sum of hemicellulose, cellulose, lignin and pectin.

Cellulose, pectin, cabbage and guava were incorporated in the diets so as to provide 5 and 10 per cent dietary fibre. As Isabgol has very high water holding capacity (about 40 times) and it is used in very small amounts for medical purposes it was fed at low levels (1 and 2 per cent). The fibre free diet was employed as control diet. The protein content of diets was adjusted to 10 per cent with egg albumin after taking into account the true protein content of plant foods. Other dietary ingredients were added as per standards for nutritional studies[5].

Feeding Experiment

Eighty eight male weanling rats, weighing 27-35 g were randomly divided into eleven groups and housed in individual cages in an air-conditioned room maintained at 22±2°C with 12 hours dark and light period.Food and water were provided ad libitum. Feeding lasted for 37 days. Nine days before the expiry of the feeding, the rats were transferred to metabolic cages for estimations recorded in Table 2. Diet residue, faecal matter and urine were collected daily. The rats were weighed twice a week. The faeces and urine were collected separately from each rat and pooled at the end of experiment. Pooled samples of faeces and diet residues were dried in hot air oven at 80°C for 12 hr, cooled in a desiccator and weighed. To the urine a few drops of H_2SO_4 were added and kept under frozen condition. Nitrogen in urine and faeces was determined by the micro-kjeldahl method[6]. Endogenous N in the faeces and urine was determined by feeding a group of rats on protein free diet containing cellulose (5 per cent) as dietary fibre for five days.

Apparent protein digestibility (APD) and true protein digestibility (TPD) were determined by the method of Chick et al[7]. Dry matter digestibility (DMD) was worked out as the proportion of dry matter intake appearing in the faeces i.e. the indigestible dry matter. In vitro protein digestibility was determined by incubating sodium caseinate (Sigma) as reference protein with different fibres at protein weight to fibre weight basis (Casein:fibre, 1:1 and 1:2) following the modified method of Singh and Jambunathan[8].

Statistical Analysis

The data were subjected to analysis of variance and correlation coefficients between APD, TPD, DMD and in vitro protein digestibility were worked out[9].

3 RESULTS

Apparent protein digestibility and true protein digestibility receiving different dietary fibres had significantly ($P < 0.05$) lower intake of nitrogen compared to control (fibre free group) (Table 2).

The group fed on fibre diet showed significantly ($P < 0.05$) higher nitrogen absorption as compared to that of those fed on different dietary fibres. Further, increase in amount of dietary fibre from different sources significantly ($P < 0.05$) lowered the nitrogen absorption except Isabgol fed groups. This decrease in nitrogen absorption is due

to increased nitrogen loss in faeces.

Apparent and true digestibilities of protein of diets containing
different dietary fibres varied from 64.4 to 88.2 per cent and 68.4 to
93.7 per cent, respectively being the lowest in diet containing 10
per cent cabbage and the highest in fibre free diet. Increasing dietary
fibre (either from cabbage or guava or pectin) in the diets caused a
significant decrease in APD whereas TPD decreased significantly only
in case of plant foods. Apparent as well as true digestibilities of all
the diets containing different fibres varied significantly (P < 0.05)
among themselves except cellulose and pectin diets.

Dry Matter Digestibility

Inclusion of dietary fibre in the diet resulted in a significant
(P< 0.05) decrease in dry matter digestibility of diets (Table 2). DMD
of different diets varied from 82.6 (cabbage at 10 per cent level) to
96.9 per cent (fibre free diet).

With the increase of dietary fibre in the diets there was a
decrease in dry matter digestibility. However, significant (P< 0.05)
reduction was found only in case of diets containing cabbage and
guava. DMD of diets containing cellulose, pectin or Isabgol (at both
levels) did not differ significantly (P < 0.05) among themselves but had
significantly (P < 0.05) higher DMD than that of diets containing
cabbage and guava.

In vitro Protein Digestibility

The in vitro protein digestibility of casein (fibre free) was 87.7
per cent but the addition of various dietary fibres caused significant
reduction. The maximum decrease was observed when guava fibre and
casein were incubated at the ratio of 2:1. When pectin and Isabgol
were incubated with casein there was a slight decrease in protein
digestibility at ratio 2:1 but in case of cellulose, cabbage and guava,
protein digestibility decreased significantly (P < 0.05).

4 DISCUSSION

Low APD observed on inclusion of dietary fibre in the diets seems to
be because of increased nitrogen losses in faeces. Total faecal N
tended to increase with increased levels of fibre (cellulose and pectin)
in the diets resulting in   decreased apparent digestibility of protein[10].
The decreased absorption of N following ingestion of diets containing
different fibres may lead to increased faecal N excretion which is
probably because of interference of these polysacchrides with protein
digestion. Apparent as well as true digestibility of protein decreased
with increase in level of plant foods as source of dietary fibre[11-12].
Increasing the level of cellulose to influence dietary protein use by
decreasing its apparent digestibility has also been observed earlier[13].
This decrease in APD and TPD by causing increased excretion of
faecal as well as urinary N may be due to a number of factors.
Increased secretion of digestive enzymes has been shown to occur when
pectin was added to the diet[14]. Reabsorption of endogenous amino
acids secreted into the gut has been found to be low on feeding a fibre-
supplemented diet[15]

Table 1 Effect of different dietary fibres on nitrogen absorption, apparent protein digestibility, true protein digestibility and dry matter digestibility

Dietary fibre (per cent)		Nitrogen consumed (mg)	Nitrogen absorbed (mg)	Apparent protein digestibility (per cent)	True protein digesti- bility (per cent)	Dry matter digestibility (per cent)
Cellulose	5	498	425	85.3	91.9	93.2
	10	462	379	82.0	88.0	92.5
Pectin	5	485	420	86.6	90.9	95.8
	10	433	336	77.6	88.0	93.2
Isabgol	1	502	438	87.3	93.7	95.5
	2	497	424	85.3	90.0	93.4
Cabbage	5	453	338	74.6	80.5	86.9
	10	450	290	64.4	68.4	82.6
Guava	5	457	369	80.7	86.3	92.0
	10	450	340	75.5	79.6	84.5
Fibre free		527	465	88.2	92.2	96.9
SE(m)		±8.0	±10.1	± 1.7	±1.3	±1.2
CD(P < 0.05)		22.6	28.6	4.7	3.7	3.5

Table 2 Effect of different dietary fibres on in vitro protein digestibility

Fibre: Protein		Protein digestibility (in vitro) (per cent)
Cellulose	1:1	85.4(2.6)
	2:1	82.7(4.6)
Pectin	1:1	83.6(4.8)
	2:1	82.9(5.5)
Isabgol	1:1	83.4(4.9)
	2:1	83.2(5.1)
Cabbage	1:1	83.1(5.3)
	2:1	81.6(7.0)
Guava	1:1	82.3(6.2)
	2:1	81.2(7.4)
Casein (no fibre)		87.7
SE(m)		±0.32
CD (P < 0.05)		0.9

Table 3   Correlation coefficient (r) among apparent protein
          digestibility, true protein digestibility, in vitro
          protein digestibility and dry matter digestibility
          of different diets

	Per cent digestibility			
	APD (In vivo)	TPD (In vivo)	Protein digestibility (In vitro)	DMD (In vivo)
Apparent protein digestibility (APD) (in vivo)	–	0.982**	0.719*	0.714*
True protein digestibility (TPD) (in vivo)	0.982**	–	0.672*	0.708*
Protein digestibility (in vitro)	0.719*	0.672*	–	0.519
Dry matter digestibility(DMD)	0.714*	0.708*	0.519	–

Based on 9 degrees of freedom

*   Significant at 5 per cent level
** Significant at 1 per cent level

Low APD and TPD observed by feeding composite dietary fibre may be attributed to their relatively high lignin content in addition to hemicellulose, cellulose and pectin[16-17]. Any reduction in intestinal transit time associated with fibre-containing diets could also leave less time for digestion and absorption of dietary protein[18]. More pronounced effects of plant foods in lowering either APD or TPD in this study and that cabbage too which also contributed some of its N to diets was most effective, it can be inferred that it is rather the protein-N associated with the fibre source that is poorly digested and excreted in the faeces. Variations in DMD of different food groups may be explained on the basis that different constituents of dietary fibre are fermented by microbial flora to varying extent[19]. Higher dry matter digestibility of cellulose and pectin containing diets may be due to higher fermentability rates of these fractions in the large intestine of rats. Cellulose can be fermented upto 50 per cent whereas water soluble polysacchrides like pectin is completely fermented, thus resulting in decreased faecal bulk excretion[20]. Analysis of faeces reveal that lignin in all diets is indigestible but hemicellulose or cellulose is partially digested or fermented in certain diets[21].

Lower dry matter digestibility of cabbage and guava may be due to presence of lignin, the undigestible constituent of dietary fibre of these foods. As lignin is known to inhibit bacterial activity, it may be responsible for lowering the DMD of food. In general, the more lignified a cell was on plant structure, the less liable it is to complete fermentation in the gut.

In vitro protein digestibility of casein was markedly reduced when incubated with guava followed by cabbage and gums (Pectin and Isabgol) and the decrease was even greater when ratio of fibre to protein was increased. Higher levels of fibre especially pectin, xylan and Karaya gum were more active in interfering with casein digestibility as compared to lignin and hemicellulose and cellulose generally considered to be non-reactive[22]. Low protein digestibility in case of guava and cabbage may be due to presence of pectin and lignin which together exert greater effect than when pectin alone is present. Lignin may exert an effect on casein hydrolysis through physical/or hydrogen bonding since it contains numerous internal ether and ester linkages within a phenylpropane polymer structure.

Protein digestibility in vitro was positively and significantly related with APD and TPD. DMD was positively and significantly correlated with APD and TPD but non-significantly with protein digestibility (In vitro). This shows that even in the presence of various dietary fibres, the in vitro and in vivo protein digestibilities are positively and significantly associated. Thus in vitro protein digestibility can be used to assess the protein quality of various foods which are even rich in fibres without going into tedious and time consuming in vivo methods. A positive correlation between in vitro and in vivo methods reflects that the animal assays (rats) and commercial proteolytic enzymes used in vitro digestibility are identical in their abilities to digest food protein which will allow eventually to develop a rapid assay that would predict protein nutritional quality

as measured by man. Four–enzyme in vitro assay for protein digestibility was able to predict in man and rat the in vivo protein digestibility[23].

Therefore, it can be concluded from this study that with increase in amount of dietary fibre especially composite dietary fibre from 5 per cent to 10 per cent the different parameters of protein digestibility were adversely affected to varying extent indicating thereby that higher levels of dietary fibre lowers the absorption of protein, which is usually low in the diets of low socio-economic groups. In vitro and in vivo protein digestibility were positively and significantly correlated which reveals that in vitro methods can be used to assess the protein quality.

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EFFECTS OF TWO INCORPORATION RATES OF GUAR GUM ON DIGESTIBILITY,
PLASMA, INSULIN AND METABOLITES IN RESTING DOGS.

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1. INTRODUCTION

In a previous work (De Haan et al., 1989) three different fibres
(cellulose, pectin and guar gum) were incorporated at 3.5% on a dry
matter (DM) basis in diets for resting dogs. Guar gum, the most
viscous fibre, induced a decrease of gastric emptying, a reduction of
digestibility mainly of proteins and lipids, a reduction of the
postprandial peak of glucose, α-amino-N, urea, triglycerides,
cholesterol and non-esterified fatty acids (NEFA).

The present experiment was designed to compare two incorporation
rates of guar gum on digestibility and blood metabolites in the
resting dog.

2. MATERIALS AND METHODS

Animals and diets.

Six adult Beagle dogs were used in 2 (3 x 3) latin square design.
They were offered a control diet or a diet supplemented with fibre.
The control diet was based on steam-treated rice (475 g/kg DM),
minced meat (419 g/kg DM) maize oil (84 g/kg DM) and minerals +
vitamins (22 g/kg DM).   The diet was calculated to supply 550 kj of
metabolizable energy/kg 0.75 body-weight.

Measurements.

Water and DM intakes were measured.   The apparent digestibility was
determined by total collection of faeces on a one-week period.
During the following week, blood samples were collected before
feeding and during 6 hours after the meal.

3. RESULTS AND DISCUSSION

Composition of the faeces.

The DM content of the faeces decreased with the level of guar gum:
46.61, 34.47 and 24.82% (P<0.001) with 0, 3.5 and 7% of guar gum,
respectively (Table 1). These results are in agreement with
previous observations.[1]   The concentrations in ash and insoluble ash
decreased in the faeces with the incorporation rate of fibre.   By
contrast, the proteins, ether extract (EE), and nitrogen free extract
(NFE) contents of the faeces increased.   Such findings were
previously reported in humans with whole bread, fruits and
vegetables [2], and with cellulose [3].   The increase in proteins could
partly be associated with a microbial fermentation in the large
bowel, as indicated by an increase of volatile fatty acids in the
caecum of rats offered guar gum and arabic gum [4].   The content of Na
and K in the faeces increased with the addition of guar gum, while
that of P, Ca, Cu, Zn and Mn decreased.   It was with the inclusion
of 7% that the largest changes were observed for all the chemical
components of the faeces.

Table 1. Composition of the faeces.

Guar gum		0%	3.5%	7%	SED
DM(%)		46.61	34.47	24.82	1.76
Ash	(g/kg DM)	467.88	374.33	295.55	14.55
Insoluble ash	(")	26.96	21.63	17.55	2.64
Proteins	(")	277.95	274.27	317.46	11.41
EE	(")	101.20	117.87	201.50	16.44
NFE	(")	206.46	221.47	232.62	12.84

Digestibility coefficients.

The digestibility of DM was 9%, 31% with the control diet (Table 2).
This value, along with the high digestibility of the organic
components, indicated the high quality of the diet offered to the dog
(steam-treated rice and minced meat).   The incorporation of fibre
reduced the digestibility of all organic components, the difference
being significant for proteins (P<0.001 with 3.5% and P<0.001 with
7%).   The incorporation rate of 7% induced the largest reductions
mainly for EE (91.74 vs 96.71%).   The digestibility of Na and K was
high (95.33 and 96.24% respectively) and was reduced by the fibre.
By contrast, the digestibility of P was relatively low at 41.04% and
was increased with the two levels of guar gum (P<0.05 or 0.001).
The digestibility of Ca was very low.

Table 2. Digestibility coefficients (%)

Guar gum	0%	3.5%	7%	SED
DM	92.31	91.21	90.73	0.52
Crude proteins	94.33	91.86	89.68	0.01
EE	96.71	95.51	91.74	0.01
Ca	4.42	1.43	10.63	0.05
P	41.04	47.00	55.58	0.02
Na	95.33	91.77	82.33	0.01
K	96.24	92.20	87.53	0.01

Plasma insulin and metabolites.

The pattern of plasma insulin was quite similar between the 3 diets (Figure 1a). However, the extent of the post prandial rise was smaller with 7% of guar gum. No significant differences were observed in plasma glucose before and after feeding (Figure 1b).

The concentration in α-amino-N (Figure 1c) was lower than the control ($P<0.01$ from 2 till 5 hours after feeding with 3.5%, and $P<0.1$ from 1 h 30 till 6 h with 7% of fibre). Amino acids are bound on the digesta in the small intestine [5], and therefore, the utilization of dietary proteins is reduced, as indicated by the reduction of protein digestibility and the lower plasma α-amino-N concentrations. Plasma urea (Figure 1d) was reduced by addition of guar gum at the 2 incorporation rates: such effect could be associated with the delay in the absorption of amino acids and their catabolism by the liver.

The incorporation of guar gum did not change to a large extent the post prandial pattern of plasma triglycerides (Figure 1e), although there was a trend for lower concentrations with 7% of fibre. Guar gum also significantly reduced plasma concentrations of cholesterol (Figure 1f). These reductions have to be related to different mechanisms such as a reduction in transit time, a lower absorption or a higher faecal excretion [6].

4. CONCLUSION

From this experiment, it appeared that proteins and lipids metabolisms mainly were influenced by increasing doses of guar gum. Various mechanisms were involved: reduction of digestibility and absorption of the nutrients, decrease of gastric emptying, gelification of the digesta [7], binding of amino acids [5] or bile acids [8], changes in the activity of enterocytes or in the mucosal thickness [9].

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WORKSHOP REPORT: RELATION BETWEEN PHYSICAL
PROPERTIES/PHYSIOLOGICAL EFFECTS OF UNAVAILABLE
POLYSACCHARIDES

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1.   How does structure relate to physical properties?

Soluble polysaccharides are often charged. Viscosity is
related to the entangling of long chains as ribbons,
rather than densely packed clumps. Formation of a gel
involves chemical bonding of adjacent chains.

Pectin can change from a viscous sol to a gel at
acid pH. This can alter physiological effects. When a
mixture of pectin and glucose gels in the stomach,
postprandial blood glucose levels are not reduced.

Gums with densely packed side chains resist
bacterial attack, but bacteria are crafty and can adapt
to break down even densely packed molecules like xanthan
gum.

Application of heat in cooking can markedly alter
physical properties; different effects can be seen with
different gums.

2.   How can viscosity alter absorption?

Viscous solutions alter the convection of luminal
contents by inhibiting digestion and by decreasing the
access of products of digestion to the cell surface. The
latter can be regarded as having two components, but both
are probably different manifestations of the same
physiological effect. They are: a decrease in the mixing
in the bulk phase and an increase in the dimensions of
the poorly stirred layer immediately adjacent to the
epithelium. Some polysaccharides, particularly the
substituted celluloses, may stick to the epithelium.

3. **Is it possible to predict the effects of viscous polysaccharides in vivo from their physical properties in vitro?**

The viscosity of some gums will change when they are exposed to solutions of differing pH and ionic composition. It is not possible to predict the exact ionic environment of different regions of the gut. Viscosity of a solution is related to the shear characteristics; these will also vary in different parts of the gut and are impossible to predict. Dr Morris has shown how to define the viscosity of a polysaccharide over the whole range of shear rates by two values; maximum viscosity at theoretical zero shear rate and the shear rate that gives half the maximum viscosity.

        Dr. Edwards suggested that in theory, shear characteristics could be determined by studying the relative behaviour of a range of polysaccharides that do not alter their viscosity with changes in ionic strength, and reading off the point at which the gums rank as they do *in vivo*..

4. **Are effects of polysaccharides on absorption in the small bowel related to their viscous properties?**

They probably are. Dr Heaton showed that the plasma insulin response of wheat and rice but not oats was related to the size of the particles fed. Presumably the reason that oats decreases insulin response independent of particle size relates to the fact that only oats produce viscous solutions. In another study, Dr Schneeman treated oats with β-glucanase and abolished the reduction of plasma cholesterol.

        Although we eat few viscous polysaccharides, cooking can produce viscous mixtures and gut contents can be quite viscous.

5. **What is the physiolgical basis of laxative action**

There are several factors. Bacterial cell mass is probably less important than some of the others. Dr Eastwood felt that totally fermented fibre gave no increase in stool weight even though it stimulated bacterial proliferation.

        Water holding capacity after fermentation is a much more important factor and Dr Tomlin's experiments

indicate that retention of polysaccharide structure is
essential for stool bulking. Stool bulking is however
not necessarily associated with an acceleration of
transit time. Some bacterial degradation of the
polysaccharide structure appeared to be necessary to
induce an acceleration of transit time. It is not yet
clear what fermentation products do to transit time and
colonic motility.

Another recent study showed that inert particles of
polyvinyl tubing or polystyrene have a similar laxative
action as bran, reintroducing the concept of roughage.
Particles may stimulate multimodal receptors to induce
secretion and propulsion, but there are other
possibilities. Dr Berry suggested that it would be
interesting to investigate the effect of coating bran
with silicon.

MINERAL ABSORPTION OF DIETARY FIBRE FROM PROCESSED VEGETABLES

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1 INTRODUCTION

Some studies have shown that vegetables usually contain soluble and insoluble dietary fibre (DF)s, and the amount of both DFs vary separately depending on cooking method.[1,2]   The variation in the amount of DFs by cooking probably is a result of the diversity of chemical composition. Many studies have indicated that the physiological effects depend on the kinds of DF, such as pectin, hemicellulose or cellulose. Thus it was shown by our studies that the effect of vegetable DF on the availability of Ca and Mg in rats was changed by cooking.[3] These results may be partly due to mineral absorption, since McConnel et al[4]   reported that cooking had a varied effect on the cation capacity. Therefore, the present study was undertaken to determine the effect of cooking on the Ca and Na absorption of DF separated from carrot, spinach and the root of edible burdock (burdock) in vitro.

2 METHODS

Preparation of dietary fibre. Samples of sliced carrot and burdock, and cut spinach were either raw, boiled for 10, 15 and, one and a half minutes, or fried for 3, 3 and 2 min. respectively. Experiment 1 : The raw, boiled and fried vegetables were homogenized in acetone, and dehydrated and defatted with acetone and ether(fibre A). The dehydrated vegetables were boiled in 85% methanol for 30 min., and filtered. The residues were washed with boiling water, acetone and ether (fibre B). Experiment 2: The dehydrated vegetables were extracted by boiling in a solution of 85% methanol three times.

The residues were washed with acetone and ether (fibre C
). A part of the residue was washed with boiling water 5
times and then washed with acetone and ether (fiber D).
All air-dried DFs were got through a 32-mesh sieve.
Determination of Ca and Na absorption. Two vasking
tubings containing 5 ml water were hung in a 300 ml tall
beaker and 200 ml 0.1% $CaSO_4$ or 0.05% NaCl solution (pH
5. 4 or 8. 0), and 0. 5g DF were entered in the beaker and
stirred at once. 0. 1 ml of solution was taken out from
the water in the tubings at 30, 60, 120 and 180 min.
after stirring. The solution was diluted with 0. 1N HCl.
The amount of Ca or Na in the solution was determined
by atomic absorption spectrophotometry. The concentra-
tion of Ca and Na in the tubing became maximum at 120
and 90 min. after stirring respectively, and were in a
state of equilibrium to the concentration of Ca and Na
in the beaker. The Ca and Na absorption of DFs were
estimated from the concentration of Ca and Na in the
tubing in the state of equilibrium.

   3 RESULTS AND DISCUSION

Experiment 1: Table 1 shows the Ca and Na absorption
of fibre A and B at pH 5. 4 and pH 8. 0. The Ca absorption
of carrot fibre A at pH 5. 4 was significantly increased
over that of the fibre A of raw carrot by boiling. It
was further increased by frying, but the difference
between the values of boiled and fried fibre A was not
significant. The Ca absorption of carrot fibre B was the
most in the boiled DF, but that was not significantly
chnged by cooking. In spinach fibre A and B the Ca
absorption was more than that of carrot and burdock. But
that of fibre A was increased by cooking, whereas that
of fibre B was decreased by cooking. That part of fibre
B which probably did not contain soluble fraction was
less than that of fibre A, with no relation to cooking.
This result suggests that a part of the mineral
absorption in spinach DF may be ascribed to the soluble
fraction. Fried fibre A and fibre B absorbed signifi-
cantly more or less Ca than the raw fibre. In burdock
fibre A absorbed almost no Ca, whereas fibre B absorbed
some Ca. Cooking had almost no effect on the Ca absorp-
tion of both fibre A and B. DF of burdock was mostly
inulin so the difference in Ca absorption of fibre A and
B might be ascribed to inulin content. In spinach DF the
amount of Na absorption was   much   less than the
amount of Ca absorption. The Na absorption was changed
only by frying significantly. The Ca absorption at pH
8. 0 tended to be less than that at pH 5. From this
result it seemed that the effect of vegetable DFs on Ca

availability was probably lower in small intestine than in stomach. The fibre A of raw carrot absorbed more Ca than the cooked fibre. Ca absorption of carrot fibre A

Table 1   Ca and Na absorption of fibre A and B separated from raw and cooked carrot, spinach and the root of edible burdock. (mg/g of dry matter)

Cooking	Fibre A or B	Carrot	Spinach	The root of edible burdock
		Ca absorption at pH 5.4		
Raw	A	3.37±0.02^{*ab}	20.92±2.66	−1.65±1.89^a
	B	8.42±5.99	17.81±0.68^a	8.36±2.21^a
Boiling	A	7.04±1.65^{ac}	23.04±5.66	0.73±2.81^b
	B	11.87±0.02^c	14.27±4.42	8.47±2.83^b
Frying	A	11.68±4.25^b	25.83±2.17^b	−1.13±1.40^c
	B	9.93±2.55	11.19±2.15^{ab}	8.69±4.55^c
		Ca absorption at pH 8.0		
Raw	A	9.45±0.35^{abc}	17.91±1.51^{ab}	4.34±0.35
	B	2.97±1.89^a	12.52±0.71^{ade}	3.94±1.98
Boiling	A	1.69±0.07^b	15.87±1.05^b	2.89±0.00
	B	1.27±0.60^d	6.02±2.88^{bd}	2.91±1.02
Frying	A	2.93±1.27^c	22.38±1.01^{cb}	3.13±1.23^a
	B	3.81±0.00^d	7.29±3.13^{ce}	1.46±0.69^a

Spinach		*Na absorption*		
		pH 5.4	pH 8.0	
Raw	A	5.41±2.45	2.50±0.00	
	B	5.41±1.79^b	2.40±1.12^b	
Boiling	A	5.75±2.57	1.10±1.57	
	B	6.87±2.37	2.60±1.36^c	
Frying	A	3.34±0.98^a	0.42±1.56^a	
	B	10.18±3.64^{ab}	6.33±2.40^{abc}	

* Mean ± SD (n=3 or 5)   Same superscript are significantly different from each other.

and B was significantly decreased by boiling, and the change in Ca absorption at pH 8.0 by cooking was different from that at pH 5.4. In spinach DF the difference of pH did not have much effect on the Ca absorption. The fried fibre A absorbed more Ca than the raw fibre A, whereas the fried fibre B absorbed less Ca than the raw fibre did. The Ca absorption of burdock fibre A and B was low at pH 8.0, and was mostly uneffected by cooking.
Experiment 2.   Fibre C was purified 'more than fibre A,

since acetone dehydrated vegetables were treated with
boling 85% methanol, whereas fibre D was similar to
fibre B, since the preparation methods of both fibres
were almost the same. Table 2 shows Ca and Na absor-
ption of fibre C and D.

Table 2 Ca and Na absorption of fibre C and D separated
from raw and cooked carrot, spinach and the root of
edible hurdock. (mg/g of dry matter)

Cooking	Fibre C or D	Carrot	Spinach	The root of edible burdock
		Ca absorption		
Raw	C	9. 33±0. 44[a b]	30. 66±1. 87[a d f]	9. 07±1. 28
	D	9. 94±1. 13[c]	11. 27±1. 07[a c g]	9. 18±1. 42[b]
Boiling	C	10. 83±0. 76[b]	17. 53±1. 06[b d]	6. 72±1. 64
	D	11. 03±1. 09[c d]	7. 78±0. 53[b e]	6. 11±1. 70
Frying	C	10. 14±0. 82[a]	25. 86±1. 51[c f]	5. 39±0. 21[a b]
	D	7. 87±1. 32[a d]	5. 86±2. 81[c g]	8. 23±0. 70[a]
		Na absorption		
Raw	C	10. 91±0. 84[a]	1. 49±0. 33[a b]	7. 62±0. 00[a c]
	D	9. 77±1. 69[b]	1. 51±0. 00[c]	4. 33±1. 42[a d]
Boiling	C	5. 62±0. 85[a c]	3. 35±0. 80[a]	5. 02±0. 00[b c]
	D	6. 83±0. 84[d]	1. 49±0. 00	2. 18±0. 84[b d]
Frying	C	18. 71±3. 36[c]	6. 09±1. 50[b]	7. 18±0. 55[e]
	D	20. 09±3. 33[b d]	4. 64±1. 17[c]	4. 48±1. 04

* Mean ± SD (n=3 or 5)   Same superscript are
  significantly different from each other.

In carrot, the boiled fibre C and D absorbed more Ca
than raw fibres, while the Ca absorption of fried fibre
D was less than that of raw and boiled fibre D. In
spinach fibre C, raw and fried fibre especially absorbed
significant amounts of Ca. THe Ca absorption of fibre D
was less than that of fibre B, but the change of fibre
D by cooking was similar to that of fibre B.
In burdock, fibre C and D absorbed Ca, but the effect of
cooking on Ca absorption was slight. The Na absorption
of fibre C and D was more than the Ca absorption , and
the change in the Na absorption of fibre C by cooking
was similar to that of fibre D. Boiled fibre C and D
absorbed significantly less Na than raw fibres, whereas
fried fibres significantly more Na than the raw fibres.
In spinach, the Na absorption of fibre C and D was
less than the Ca absorption. In burdock, the Na
absorption of fibre D was less than that of fibre C,

but the changes resultant from cooking method on both fibres tended to be the same.

The above results suggested that the effects of cooking on the Ca and Na absorption of the vegetable fibre differed in the cooking methods, the kinds of vegetable involved and the methods of fibre separation.

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BINDING OF MINERALS BY NEW SOURCES OF HIGH DIETARY FIBRE CONTENT

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1 INTRODUCTION

A major negative effect to increased consumption of dietary fibre may be a reduction in the bioavailability of trace minerals.[1] There are many 'in vivo' and 'in vitro' studies concerning this subject.[1,2] In vitro studies of mineral bioavailability in the presence of dietary fibre are useful because they are faster and less expensive than 'in vivo' studies and because they offer better control over experimental variables, such as pH, type(s) of fibre, mineral concentration, etc.[3]

Certain by-products from Spanish food and agricultural processing plants have been shown to have high dietary fibre content.[4,5] The interactive effect of some of these subproducts with Fe^{3+} and Ca^{2+} have been investigated 'in vitro', in order to know the percentage of these minerals bound in an insoluble form and to understand the possible mechanisms of these interactions.

2 MATERIALS AND METHODS

Materials

Citrus wastes (lemon peel, LP), cider wastes (CW) and olive pomace (OP), were obtained from Spanish food processing plants. Alcohol-insoluble solids (AIS) of these ground materials (≈ 0.5 mm) were prepared and dried. The standard fibre components were: lignin (Lg) (Therapharm Ltd., Dowhan Market, Norfolk), and cellulose (C) (Aldrich).

Procedure

Solutions containing the metal ions Fe^{3+} and Ca^{2+} at gastric pH (pH 2-4) and pH of the small intestine (pH 5-8), respectively, were added to centrifuge tubes which contained ca. 100 mg of sample. The suspensions were shaken for 3 h (time of an 'in vivo' digestion) at room temperature. Supernatants were discharged after centrifugation at 3000 g for 15-30 min. The equilibrium metal ion concentrations were determined by A.A.S. The amount of mineral elements bound in an insoluble form (BC, $\mu g\ ml^{-1}\ g^{-1}$ of sample) was calculated from the residual equilibrium concentration in the supernatant, corrected by the endogenous metal ion concentration, (EC), the initial metal ion concentration (IC) and the mass of AIS or standard fibre components.

Analyses were performed at least twice. Results of bound concentrations are expressed as the difference between mean values of two determinations.

3 RESULTS AND DISCUSSION

Dietary fibre composition and other constituents of the food by-products, determined in earlier investigations,[4,5] is described in Table 1.

Mineral binding properties of AIS and standard fibre components were studied as a function of sample mass, the initial metal ion concentration and the pH of the medium.

Effect of Sample Mass on Binding

Figure 1 shows the relationship between sample mass and the extent of mineral binding by the AIS of two food by-products and standard components of fibre, when other variables are held constant. In all cases, bound mineral

Table 1   Dietary Fibre Content and Main Constituents of food by-products (% dry matter), determined by the AOAC method

Sample	TDF	IDF	SDF	TP	KL	I-UA
Cider waste	62.5	48.3	14.2	5.4	18.2	5.75
Olive pomace	69.4	65.7	3.7	5.3	37.2	2.30
Lemon peel	47.7	28.2	19.5	1.3	5.5	5.33

TDF: Total dietary fibre; IDF: insoluble dietary fibre; SDF: soluble dietary fibre; TP: total protein; KL: Klason lignin; I-UA: insoluble uronic acids.
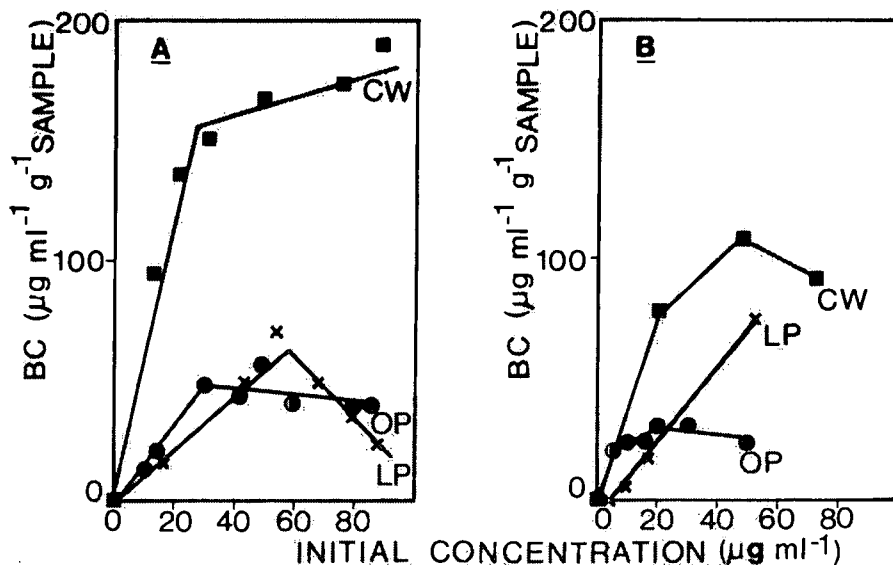
<u>Figure 1</u>   Influence of sample mass on Ca^{2+} (A) and Fe^{3+}(B) binding
IC ($\mu g \; ml^{-1}$) and pH values were: (A) LP: 32.3, 5.9; Lg: 45.25, 6.3; C: 44.26, 5.6; and (B) CW: 48.4, 2.5; Lg: 45.67, 2.5

concentration decreased as the sample mass increased, up to a mass greater than 200 mg, when almost a constant bound concentration value is reached. This behaviour may indicate that most of the metal ions were bound at the sur- face, as was previously reported.[6]

Effect of Metal Ion Concentration on Binding

The effect of initial solution concentration, at a constant pH value, of both Ca^{2+} and Fe^{3+} on calcium and iron binding is shown in Figure 2. From this figure, it is obvious that binding of both ions increased with increa- sing solution concentrations, up to a certain value of ma- ximum binding, which varies with the nature of sample and the mineral considered.

The binding affinity to the by-products investiga- ted and the maximum amount of metal ion bound per 100 g of sample ($\mu mol \; g^{-1}$ of sample) can be ranked as

Ca^{2+} :   CW (>118.2) > OP (31.2) > LP (14.1) and
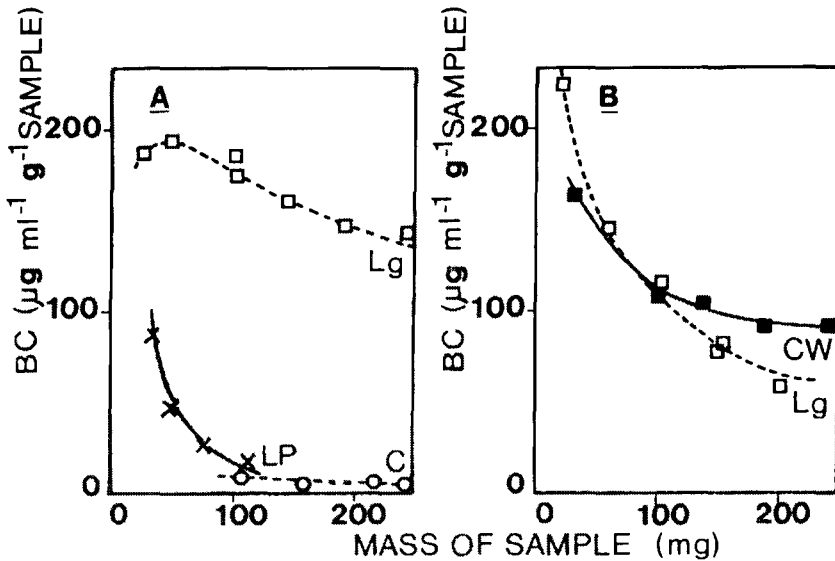
Figure 2 Influence of initial Ca^{2+} (A) and Fe^{3+} (B) con-
centration on binding
Sample mass (mg), and pH values were: (A) CW: 100,
5.5; OP: 100, 5.8; LP: 50, 5.7; and (B) CW: 100,
2.0; OP: 100, 1.8; LP: 100, 1.9

Fe^{3+} :   CW (48.3) > LP (>33.6) > OP (13.9)

The contribution of lignin and cellulose to Fe^{3+} and
Ca^{2+} binding was also studied. The order of affinity was:

Ca^{2+} :   Lg (>114.7) $\gg$ C (9.4)   and
Fe^{3+} Lg (145.5) $\gg$ C (18.0)

As shown, cellulose has essentially no ability to
bind either ferric or calcium ions, while lignin interac-
tions with both elements are very strong. On the basis of
these results, the presence of both carboxyl and hydroxyl
groups in the phenylpropanoid units of lignin, together
with the great content of this fibre component (Klason lig-
nin) in CW an OP may indicate the contribution of these
functional groups to cation binding by fibre. Moreover,
the significant content of pectic substances, with carbo-
xyl groups in their structure, in a sample with a low con-
tent in Klason lignin, LP, corroborates this conclusion.
On the other hand, contribution to mineral binding of solu-

ble uronic acids was not considered since recent 'in vitro' studies showed the release of cations bound to soluble fibre in a pH range of 6-8.[7]

Scatchard analyses of Ca^{2+} and Fe^{3+} binding by the AIS of food by-products, lignin, and cellulose, showed the existence of one specific binding site for Ca^{2+} in CW (log $K_{eff.}$ = 4.13) and one specific binding site for this ion in Lg (log $K_{eff.}$ = 4.34). No specific binding sites were observed either in the OP-Ca, LP-Ca, and C-Ca systems or in the binding of ferric ions by the by-products, lignin and cellulose. This fact helps us to explain the great interaction of lignin and cider waste with calcium.

Effect of pH on Mineral Binding

The effect of pH on binding, at a constant metal ion concentration (Table 2) showed that mineral binding increased with increasing pH; the increase was much more marked in the case of Fe^{3+} (which tends to hydrolyse in aqueous systems, with the formation of insoluble polynuclear species at pH $\Rightarrow$2.0). The small increase of Ca^{2+} bound to Lg and CW in the pH range 4-7.5 may suggest involvement of groups with high pK_a, probably phenolic groups, while the greater increase of Ca^{2+} bound to LP and OP in this pH range may indicate involvement of carboxyl groups (of lignin and/or uronic acids).

Table 2   Mineral binding ($\mu g\ ml^{-1}\ g^{-1}$) as a function of pH

Ion	CW		OP		LP		Lg		C	
	pH	BC	pH	BC	pH	BC	pH	BC	pH	BC
Ca	5.5	154.8	5.2	35.3	5.9	50.2	3.9	152.0	3.3	7.7
Ca	6.7	172.4	6.6	55.5	6.8	57.9	6.3	185.5	5.1	11.2
Ca	7.4	188.4	7.4	93.0	7.4	81.6	7.1	196.8	7.0	17.0
Fe	2.1	56.6	1.9	24.5			2.0	8.9	2.6	34.7
Fe	2.5	128.6	2.9	62.6			2.7	298.6	3.1	156.7
Fe	2.9	198.1	3.9	28.5			3.0	445.3		

IC $\sim$50 $\mu g\ ml^{-1}$ in all cases, except for OP-Fe ($\sim$10 $\mu g\ ml^{-1}$)

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EFFECTS OF DIETARY FIBER ON MINERAL BALANCES IN HUMANS

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1. INTRODUCTION

Although there were reports that fibers from cereals
and also from fruits and vegetables can reduce the bio-
availability of minerals and trace elements [1,2,3], the
subject is controversial. It has been shown that cations
will bind to dietary fiber in vitro [4,5] . In vivo, the
binding will be influenced by several factors in foods
like phytic and oxalic acid, vitamin C , protein and
other minerals and the pH of the gastrointestinal tract.
Those factors may enhance or inhibit binding. The effects
of fiber and/or other agents on cation availability may
be studied by test meals. However, test meals do not
give a picture of the utilization of minerals for a
longer period of time. It was the objective of our
studies to measure by balance experiments the influence
of various kinds and levels of dietary fiber on mineral
availability. The fiber containing foods under study
were constituents of mixed diets as were consumed in
the Federal Republic of Germany.

2. MATERIALS AND METHODS

Subjects and experimental design. Four studies were per-
formed with healthy female students (21-27y). Each study
was divided in a low fiber control and one or more high
fiber experimental periods. All food eaten during the
experiments was prepared in the institute kitchen and
weighed exactly. As beverages various kinds of tea,
coffee and water were allowed. The beverage consumption
was recorded. Each experimental period lasted 21 d,
between the periods there was a break of at least 3 wk.

Table 1   Fiber sources and increase in fiber intake on
           the high fiber diets

Study	Fiber source	Increase in DF intake (g/d)	Phytic acid (mg/d)
1	**a.** Bread enriched with barley fiber (High protein intake)	15	low
	b. Bread enriched with barley fiber (Medium protein intake)	15	low
2	**a.** Whole grain bread and rolls	29	1200
3	**a.** Raw carrots	15	n.d.
	b. Blanched carrots	15	n.d.
	c. Canned carrots	15	n.d.
4	**a.** Various fruits and vegetables	33	n.d.

Diets. In each study, the basal diet (15 to 19 g
DF/d) was the same during the control and the experimen-
tal periods. The basal diet consisted of two 1 d menus,
which were fed in rotation. During the high fiber ex-
perimental periods, high fiber breads were substituted
for low fiber breads (studies 1 and 2) or vegetables
and fruits were added to the basal diet (studies 3 and
4) (Table 1).
Balance technique and analytical methods. From d
15 to 20 duplicates of all foods and beverages, from d
16 to 21 all feces and urine were collected. Dietary
fiber was analyzed by the AOAC procedure [6]. Ca, Mg, Zn
and Fe were determined by AAS.

3. RESULTS AND DISCUSSION

When the intake of minerals and trace elements was ele-
vated due to the consumption of the fiber sources, the
absolute excretion of those cations by the feces was in-
creased. This could be measured with whole grain bread,
carrots and fruits/vegetables. These observations are in
agreement with other reports [3,7] and may be the result of
the increase in fiber and/or phytic acid. Balances were
not decreased by the elevated fecal excretion. Concern-
ing Zn and Fe, balances were improved due to the higher
intake with the whole grain bread and the fruit/veget-
able diets.

Table 2   Intake and balance of calcium (mean values)

Study	Period	Intake (mg/d)	Balance (mg/d)	n
1	Control	979	+ 11	12
	a.	1020	+ 86	12
	b.	916	− 32	12
2	Control	1190	− 7	6
	a.	1259	+ 20	6
3	Control	1098	+ 84	12
	a.	1273	+ 87	12
	b.	1311	+ 41	12
	c.	1284	+ 25	12
4	Control	1094	+ 27	8
	a.	1009	− 18	8

Table 3   Intake and balance of magnesium (mean values)

Study	Period	Intake (mg/d)	Balance (mg/d)	n
1	Control	252	+ 8	12
	a.	245	− 5	12
	b.	243	− 11	12
2	Control	221	− 15	6
	a.	434	− 12	6
3	Control	261	+ 3	12
	a.	316	+ 10	12
	b.	305	+ 1	12
	c.	303	− 6	12
4	Control	232	− 9	8
	a.	368	− 8	8

Table 4   Intake and balance of zinc (mean values)

Study	Period	Intake (mg/d)	Balance (mg/d)	n
1	Control	10.8	+ 0.2	12
	a.	10.9	- 0.3	12
	b.	7.8	- 1.2	12
2	Control	10.1	0	6
	a.	15.8	+ 1.1	6
3	Control	10.8	- 0.6	12
	a.	12.1	- 0.7	12
	b.	11.9	- 0.7	12
	c.	11.8	- 0.8	12
4	Control	9.6	- 0.3	8
	a.	14.1	+ 0.8	8

Table 5   Intake and apparent absorption of iron (mean values)

Study	Period	Intake (mg/d)	Apparent absorption (mg/d)	n
1	Control	8.6	+ 0.9	12
	a.	10.4	+ 0.3	12
	b.	8.6	- 1.3	12
2	Control	7.3	+ 1.0	6
	a.	17.0	+ 3.6	6
3	Control	7.5	+ 0.8	12
	a.	8.8	+ 0.8	12
	b.	9.0	+ 0.8	12
	c.	9.7	+ 0.2	12
4	Control	6.8	+ 0.3	8
	a.	14.8	+ 2.7	8

In this study, the processing of carrots did not influence the balances of the elements studied. Compared to the control, no differences were observed. In an investigation with rats, raw carrots decreased the balances of Ca and Mg compared to sauted carrots [8].

When the intake of minerals was not changed due to the increase in fiber intake, fecal excretion and also the balances were not significantly affected. However, a lower consumption of animal protein decreased Zn and Fe balances.

4. CONCLUSIONS

Concerning mixed diets with a relative high amount of animal foods, our studies indicate that dietary fiber intakes up to 50 g/d have no adverse effects on mineral balances. However, a decrease of animal products in the diet may influence trace elements balances.

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Dietary Fibre in the Large Intestine: Implications for Colorectal Function and Energy Metabolism

Function of Dietary Fibre in the Large Intestine

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1 INTRODUCTION

The colon is one of the least understood organs in man
and animals. Problems of nomenclature arise as knowl-
edge accumulates.

   The colon is tubular, capacious and contractile,
and a reservoir which conserves water, electrolytes,
anions and cations. One end is in continuity with,
though functionally separated from, the ileum. The
left side controls the orderly evacuation of faeces.
The right side, or caecum, contains a large bacterial
population which is metabolically active within the
constraints of a nutrient starved environ. The
sources of nutrition to the caecal bacterial flora are
dietary, exfoliated cells, intestinal and biliary
secretions[1]. The nutrients include polymeric car-
bohydrates (eg fibre, resistant starch)[2], proteins[3],
lipids[4] and small molecular weight molecules secreted
in bile which have been conjugated with glucuronic
acid, amino acids or sulphate moieties[5].

   An important element in colonic physiology is
dietary fibre. Hugh Trowell defined dietary fibre as
plant cell wall material resistant to the intestinal
secretions of the host[6]. A problem of nomenclature
has arisen with dietary fibre[7]. This problem is,
however, indicative of a more basic dilemma concerning
fibre on the one hand and caecal bacterial metabolism
on the other. Implicit in Trowell's definition is the
bacterial breakdown of fibre in the colon. In the
caecum there is a large number of bacteria, some 500

or more separate species. These caecal bacteria can
however be regarded as a functional single metabolic
entity, a bacterial mass or even an organ.

Chemical and Physical Properties of Dietary Fibre

There is a poor correlation between the chemistry
(total amount and the constituent sugars) and the
functions of various fibres[8,9]. Different fibre types
may initiate a different response from bacteria within
the colon.   There may be a generalised increase in
bacteria leading to an increase in bacterial mass and
metabolism.   It is not known whether increases in
bacterial populations occur uniformly in all species
or are restricted to certain bacterial types that
benefit from and are implicated in the metabolism of
the fibre that has stimulated the increase.

Physical properties that influence function along
the gastrointestinal tract are a combination of the
colligative properties of the water soluble fibre
components and the surface properties of the water
insoluble fibre components.   These properties include
water holding, cation exchange, organic absorption and
gel filtration. These may be influenced by particle
size distribution. These physical properties are
interrelated. It has been suggested that dietary fibre
can act as an ion exchanger and molecular sieve
chromatography system throughout the gastrointestinal
tract.   The plant cell wall provides a water soluble
or insoluble matrix or phase through which the soluble
content of the gut can variably penetrate and parti-
tion with[8].

The biological potency of dietary fibre depends
upon its relationship with water along the gastroin-
testinal tract.   Holding water as a solvent affects
the biological availability of solutes.   In the colon
water is distributed in three ways[10]

1. free water that can be absorbed from the colon
2. water that is incorporated into bacterial mass
3. water that is bound by a fibre.

The measurement of the amount of water in fibre
can be followed in a number of ways by centrifugation
or by suction pressure methods [10,11].   A quick useful
comparative method is using flow characteristics of
insoluble fibre and this enables the effect of cooking

to be identified[12].

Caecal Fermentation. The process whereby a
compound is bacterially dissimilated in the caecum
under anaerobic conditions is complex and varied,
leading to partial or complete decomposition with the
end products being either absorbed from the colon to
be utilised as nutrients, or absorbed and re-excreted
in the enterohepatic circulation or to be excreted in
stool. Many compounds are variably and simultaneous-
ly decomposed in the mixture and apparently unrelated
compounds may well influence the metabolism of each
other.

Recent work has demonstrated that dietary fibre
can be extensively degraded to volatile fatty acids
and other metabolic products[13] including methane,
hydrogen and carbon dioxide. The degree to which
this occurs depends on the type of fibre and also the
amount of fibre[14]. The duration of fibre feeding is
important. Experiments feeding gum arabic[15], or
carrot[16], show that a period of ingestion is required
to develop evidence of fermentation of these fibres.
This is in contrast to acute exposure when there may
be no evidence of fermentation[17]. The proportion of
the major fatty acids, acetate, butyrate and propion-
ate varies with diet and other conditions. Gum arabic
results in an increase of production of short chain
fatty acids in the rat colon[14]. The relative amounts
of acetate and butyrate resulting from the fermenta-
tion of gum arabic in the rat, is dependent upon the
amount of gum arabic fed and also whether the gum
arabic is fed with an elemental diet or standard rat
pellet diet[14]. The metabolic products maybe absorbed
from the colon and utilised by the colonic mucosa or
more remotely in the body. It has been suggested in
man that on a control diet, only 20 per cent of the
overall ingested fibre is recovered in the stools. It
has been suggested that approximately 20g of cell wall
polysaccharides and other carbohydrates are fermented
in the human colon each day, so that approximately
200mmol of short chain fatty acids are produced[13].
Whilst a considerable amount of short chain fatty
acids are produced, only 7 - 20mmols per day are
excreted in the stool, therefore there must be sub-
stantial absorption and metabolism of short chain
fatty acids in the colon. The residual fibre, some
of the metabolic products and bacteria are excreted in
the faeces[8].

Hydrogen, methane and carbon dioxide constitute flatus. The volume of flatus passed per rectum has been estimated to range from 200 to 2,400 mls per day[18]. Certain foods produced flatulence, particularly beans. Hydrogen production in the colon is dependent upon the delivery of ingested non-absorbable fermentable substrates to a high concentration of bacteria[19]. Normally these substrates consist of non-absorbable oligosaccharides such as stachyose, raffinose and possibly more complex polysaccharides such as starch[20]. The evolution of breath hydrogen has a regular pattern through the day, with a comparatively high volume in the morning, falling until midday and rising during the early afternoon. The mean hydrogen excretion at any time in the day is $\leq$ 0.5μmol/L and individual breath samples rarely contain more than 0.9μmol/L[21]. The average daily breath hydrogen excretion by an individual is of the order of 50-200mls per day. In acute studies few of the different components of dietary fibre, cellulose, pectin and lignin resulted in an increased production of hydrogen[22]. The single dose administration of unprocessed bran results in a modest increase in breath hydrogen[17], though it may be that there is contamination of this wheat bran by starch. If a fibre preparation is taken for 3 weeks, then there is an increase in breath hydrogen[15]. Therefore the breath hydrogen is a useful indicator of caecal fermentation activity.

There are wide variations reported in the proportion of individuals who exhale methane in their breath. This ranges from 33 - 55 per cent in North America, 40 - 61 per cent in Britain and 75 - 80 per cent in Nigeria[18]. The methane excreting status of an individual has been shown to remain stable over periods of up to 3 years. The fermentation of faecal material from both methane and non methane breath excretors leads to methane production. This suggests that methane is produced by all subjects but in varying concentrations. That only when the production reaches a threshold level does methane appear in the breath[23]. In vitro fermentation studies have shown methane production to be stimulated by added amino acids and glycoproteins[24].

It has now been shown that a proportion of dietary starch may be resistant to pancreatic enzymes and passes to be fermented in the caecum[2,20]. The question now arises, is this resistant starch, which is

dietary plant material, a fibre? The term fibre im-
plies structural plant cell wall composed of polysac-
charides and lignins. By narrowing the concept of
fibre to caecally degraded polymeric substances the
potential for understanding the role of fibre and
other substances along the gastrointestinal tract is
reduced. Mucoprotein and biliary excretion compounds
are also metabolised in the caecum[1,5,24].

Caecal metabolism and enterohepatic circulation.

Water soluble conjugates arriving in the caecum
usually biliary excretion products which have not been
absorbed in the intestine, e.g. bile acids and biliru-
bin, are modified by bacteria to less water soluble
compounds. Such modified metabolites may be adsorbed
to bacteria or fibre, or be absorbed into the circula-
tion through the colonic mucosa. This enterohepatic
circulation contributes to the conservation of com-
pounds in the body, e.g. bile acids and possibly other
compounds of intermediate metabolism. This process is
of physiological importance. It has been shown that
bile acids can be adsorbed to dietary fibre[25]. Howev-
er, those fibres which are most important in increas-
ing faecal bile acids excretion are those which are
extensively fermented in the colon, e.g. pectin and
gum arabic[9].

Bile acids are 7α-dehydroxylated in the colon
yielding the secondary bile acids, lithocholic and
deoxycholic. It has been suggested that the amount
of faecal bile acids excreted may be of importance in
the aetiology of colonic neoplasm[26]. There is an in
verse relationship between stool weight and the litho-
cholate-deoxycholic acid ration in faeces. It is
possible that primary bile acids reaching the colon
are more efficiently converted to secondary bile acids
when there is a longer transit time and a smaller
stool weight. Populations with an increased litho-
cholic to deoxycholic ratio and low stool weight may
be more likely to develop neoplastic change. Alterna-
tively however it may be that low stool weight is
characterised by a high lithocholic acid to deoxychol-
ic acid ratio and is a feature of a normal population
and not primarily a predictor of colon cancer[27] .

Colonic Fermentation

Dietary fibre can usefully be regarded as plant

cell wall material, consisting of polysaccharides and
lignin passing as a hydrated sponge, modulating intes-
tinal function along the foregut to the hindgut where
the fibre may be degraded by bacteria[8].

The factors that affect the fermentation of poly-
saccharides in the colon include the inherent charac-
teristics of fibre; water solubility, lignification,
particle size and molecular structure[8]. The interplay
of water, bacteria and fibre must mean that no single
factor stands on its own, so that bacteria which get
ready access to the interior of a fibre, such as
pectin, by virtue of water solubility will also be
influenced by particle size and molecular structure.
Similarly, the resistance to fermentation of wheat
bran is influenced by particle size which is in part
mediated through chemistry, the availability of bacte-
ria and also water holding capacity.

There is also a range of chemicals which pass to
the caecum providing bacteria with an energy source[5].
Such compounds of exogenous and endogenous origin
hormones, mucoproteins, metabolic breakdown products
will be completely, partially or minimally metabolised
by bacteria.

Faeces

There is no simple logic available based on the
reaction of complex carbohydrates which predicts
biological activity in the colon. The disparate
actions with each dietary fibre source can only be
defined by complex and time consuming experiments[9].

Faeces are complex and consist of 75% water, the
dry weight being composed of bacteria, the residue
being fibre, and excreted compounds[28]. There is a
wide range of individual and mean stool weights[29].
In a study in Edinburgh the variation in stool weight
was between 19 to 280g for 24 hours. Faecal constitu-
ents, bile acids, sterols, fat, electrolytes, corre-
lated strongly with faecal mass.   There was no rela-
tionship between age and faecal weight and transit
time.   Of the dietary constituents, only dietary
fibre influenced stool weight[30]. The most important
mechanism whereby stool weight increases is through
the water holding capacity of unfermented dietary
fibre, e.g. wheat bran. The greater the water holding
capacity of the bran, the greater the effect on stool

weight[8].   Fibre may influence faecal output by another
mechanism.   The stimulation of colonic microbial
growth is the result of ingestion of such fermentable
fibre sources as apple, guar or pectin.   This is an
uncertain method as there is not always an increase in
stool weight as a result of eating these fibres[28].

Action of Dietary Fibre on Human Colon and Faeces

    Human studies suggest that the physiological
action of dietary fibre in the colon may be in one of
three ways[9] -

An ingested fibre may

1. Increase stool weight with no effect on serum
   cholesterol, faecal bile acids or caecal
   fermentation as demonstrated by hydrogen excretion
   (e.g.   wheat bran, gum tragacanth).

2. No effect on stool weight, but with a reduction
   in serum cholesterol, a change in faecal bile
   acid excretion and an increase in breath hydrogen
   excretion (e.g. gum arabic, pectin as raw carrot).

3. No effect on stool weight, serum cholesterol,
   faecal bile acids or breath hydrogen (gum karaya).

<u>Table 1</u> Summary of Results from experiments
                      wherein a specific fibre
                      source is added to the diet[9]

Fibre Source and daily intake production g	Stool weight production initial/after 21 days g/day mean ± s.d.
16g Wheat bran	120 ± 18* 183 ± 22*
10g Gum tragacanth	125 ± 40* 188 ± 48*
25g Gum arabic	147 ± 40 161 ± 43
200g Raw carrot (pectin)	142 ± 83[+] 177 ± 74[+]
10g Gum karaya	134 ± 25 139 ± 39

*p ≤ 0.01; [+]p ≤ 0.05

Table 2 Summary of results from
experiments wherein a specific fibre
source was added to the diet[9]

Fibre source and daily intake g	Breath-hydrogen mean production increase ppm mean ± s.d.
16g Wheat bran	no change
10g Gum tragacanth	no change
25g Gum arabic	6 to 17
200g Raw carrot(pectin)	17 to 34
10g Gum karaya	no change

Table 3 Summary of results from experiments
wherein a specific fibre source was
added to the diet

Fibre source and and daily intake g	Total faecal bile acids	
	initial	after 21 days
	mmol/d	
16g Wheat bran	0.55 ± 0.33	0.53 ± 0.25
10g Gum tragacanth	0.92 ± 0.09*	1.88 ± 0.28*
25g Gum arabic	1.08 ± 0.55	0.99 ± 0.41
200g Raw carrot(pectin)	0.81 ± 0.10	0.95 ± 0.10
10g Gum karaya	1.01 ± 0.41	0.79 ± 0.22

Summary of Action of Fibre on Colon and Faeces

In an attempt to symbolise the action of any
dietary fibre or in the colon, the following sugges-
tions are made.

1. Colonic metabolism is dependent upon nutrient
   availability to the caecal bacterial mass,
   therefore colonic metabolism will be inversely
   proportional to the efficiency of upper gut
   digestion and absorption (ingestion).

   Protein and fat are 95% absorbed in
   the upper intestine, 5 – 10% starch evades
   the digestion as retrograde starch[2, 20] and
   fibre passes virtually intact to the caecum[6].

2. Stool weight is affected by the extent of fibre
   fermentation and hence is influenced by the water
   distribution as a result of

```
        fermentation
        bacterial mass
        water holding capacity of the residual fibre
        transit time
        chemical or osmotic water from fibre
        fermentation
```

Figure 1
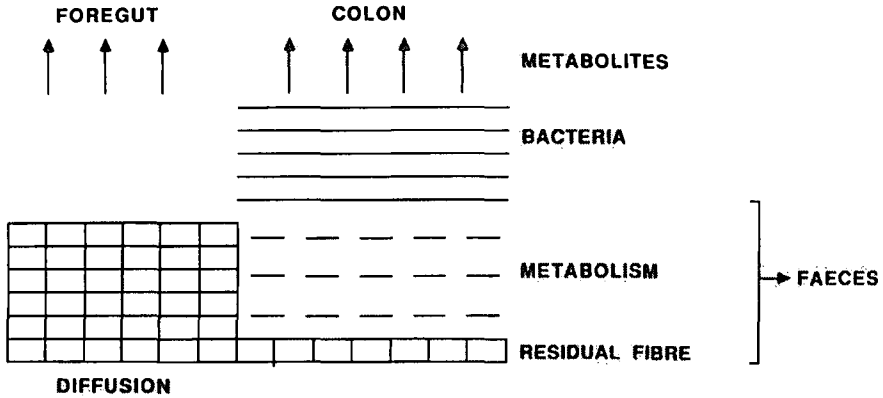
Legend

```
Action of dietary fibre along the intestine
(1) A sol modulating upper intestinal physiology and
    absorption
(2) A nutrient for bacteria in the caecum
(3) Key contributor to stool weight and constituents

3.  It is possible to symbolize the biological effect
    of a fibre in the caecum, colon or faeces as
```

$$\alpha \quad \frac{F_R}{F_O} \qquad \frac{WHC}{WHC}_O \ F \qquad \frac{B_F}{B_O} \qquad \frac{M_F}{M_O}$$

F   Fibre (g)

WHC   Water holding capacity
B   Bacterial mass
M   Metabolic product

                       O original
                       F after fermentation
                       R residue

    A more direct symbolizing of the potency of a fibre on stool weight might be -

    Effectiveness of 1g ingested fibre to influence stool weight

$$\alpha \quad (F_R \ WHC) \ + \ (B_F \ WHC) \ + \ M_F$$

Water holding capacity (WHC)
Bound water to
F_R fibre residue after fermentation
B_F bacterial mass after fermentation
M_F

This chemically or osmotically active water could be created following the fermentation of dietary fibre. For example, short chain fatty acids.

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THE EFFECT OF 9.5 g/d RESISTANT STARCH ON COLON FUNCTION

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1 INTRODUCTION

Retrograded amylose is a type of starch which resists
hydrolysis with pancreatic amylase. It forms in foods
which have been baked or boiled and subsequently cooled
(RS_3). Starch that resists breakdown in the small
intestine will pass into the large intestine where it may
act in a similar manner to the unabsorbed non-starch
polysaccharides of dietary fibre. One of the proposed
mechanisms for their effects on the colon is that the
carbohydates act as substrate to the bacteria, stimulate
bacterial cell growth and increase bacterial cell mass[2]
and generate fermentation end-products such as short-chain
fatty-acids (SCFA) and gases which may affect colonic
motility and secretion.[3] As human colonic bacteria are
capable of fermenting RS in in-vitro incubations,
increasing bacterial cell mass and producing SCFA,[4] RS
may be involved in maintaining normal colon function.
The relative importance of RS is difficult to tell as
there are no published figures for the amount normally
present in the diet, and the effect of ingestion of RS
on stool output and gas production in humans has not been
studied before.

The aims of this experiment were to investigate
whether RS can influence colon function in humans by
monitoring whole-gut transit-time, stool mass, frequency,
consistency, flatus production and the ease of
defaecation, and to test whether RS affects breath levels
of one of the fermentation end-products, hydrogen, as this
may indicate the amount of substrate being fermented in
the colon.[5]

2 METHODS

Eight healthy male volunteers were asked to supplement their normal diets with commercial breakfast cereals containing different amounts of RS. The dietary supplements were administered in a random order during two 7 d study periods which were separated by at least a week. One of the supplements contained 350 g/d Kellogg's Cornflakes and the other contained 380 g/d Kellogg's Rice Krispies (providing 10.33 and 0.86 g/d RS respectively).[6] The major nutritional components of the two supplements were balanced to within 1% of each other by including small amounts of other substances (Table 1) using food table data.[7] The volunteers were encouraged to maintain their normal diets as far as possible and were encouraged to eat similar foods during the two study weeks, although it was accepted that the cereal supplements and accompanying milk and sugar (equivalent to six large bowls /d) would displace some of their normal food intake. They kept diaries of food intake using approximate portion sizes and alcohol consumption was restricted to no more than 15 units per week and no more than 4 units per day (1 alcohol unit = $^1/_2$ pint beer, = 1 glass wine, = 1 measure spirits).

During the study the volunteers took 15 small plastic radio-opaque markers at around the same time each day and noted the exact time in a diary. During the last 5 d of

Table 1 Composition of the two dietary supplements.

	RS (g)	Starch (g)	NSP (g)	Energy (kJ)	Pro (g)	Fat (g)	CHO (g)
DIET A							
350g Cornflakes	10.33	262.1	2.3	5.39	30.1	5.6	298
33g Boiled sweets	0	0.1	0	0.45	0	0	28
2.4g Wheat-bran	0	0.3	0.9	0.02	0.3	0.1	6
2.3g Butter	0	0	0	0.07	0	1.9	0
TOTAL	10.33	262.5	3.2	5.93	30.4	7.6	332
DIET B							
380g Rice Krispies	0.86	262.1	3.2	5.79	22.0	7.5	327
9g Casilan	0	0	0	0.14	8.1	0.1	0
TOTAL	0.86	262.1	3.2	5.93	30.1	7.6	327
DIFFERENCE (A-B)	9.47	0.4	0	0	0.3	0	5

Abbreviations used in table; RS = resistant starch, NSP= non-starch polysaccharides, Pro = protein, CHO = carbohydrate.

each study-week the volunteers collected all stools passed
into individual plastic bags and labelled them with the
time and date of defaecation. The stools were then weighed
to yield the stool mass and X-rayed to visualise the
radio-opaque markers. The whole-gut transit-time was
calculated using the continuous method,[8] and the median
value of the 5d period was used in the calculations.

      Breath hydrogen measurements were made at 15 min
intervals for 8h on the first day of each supplement.
End-expiratory samples were obtained using a modified
Haldane-Priestley tube, and the hydrogen concentration
was measured on a breath hydrogen monitor (GMI, Renfrew,
Scotland). The area under the hydrogen curve was
calculated (with no base-line correction), and this was
used to give an indication of the amount of substrate
fermented. The amounts and timings of the cereals and
other food and drink was identical during the two weeks.

      The volunteers noted in the diary the ease of each
defaecation on a visual analogue scale with the 0 and 100
points labelled 'no effort' and 'much straining'. They
assessed the form of the resultant faeces on a scale from
1 to 8 by comparison with a set of standard descriptions
and photographs, based on a linear scale of stool consist-
ency.[9] They also noted all episodes of flatus passage per
rectum. The volunteers assessed the effects of the two
supplements compared with their usual bowel habits in
questionnaires completed at the end of the study weeks.

      Paired results from the two weeks were compared using
Wilcoxon's matched pairs rank sum test.

      3. RESULTS

There was no significant difference between the total mass
of faeces collected during the last 5 d of the two study
weeks (p>0.05; Table 2). Whole-gut transit-times were not
significantly different between the two periods (p>0.05;
Table 2). There were also no significant differences in
the stool frequency, the mean stool form or in the ease
of defaecations recorded by the volunteers (p>0.05; Table
2).

      Breath hydrogen excretion, measured as the area under
the 8h breath hydrogen curve, was significantly higher
on Cornflakes than on Rice Krispies (p<0.05; Table 2).
There were no significant differences in the number of
flatulent episodes recorded (p>0.05; Table 2), although

<u>Table 2</u> Results of bowel function measurements (mean values, n=8).

	CORNFLAKES	RICE KRISPIES
Faecal Output (g/5d)	893	982
Stool frequency (/5d)	5.0	5.8
Mean consistency*	5.0	5.0
Mean ease of defaecation£	28	30
Whole-gut transit time (h)	43.4	39.5
Total flatulent episodes (/wk)	35	27
Total breath hydrogen (/8h)	12.1	7.5

* - rated on a scale from 1 (liquid) to 8 (hard pellets)
£ - rated on a scale from 0 (no effort) to 100 (much straining)

Cornflakes produced 63 more episodes in total than Rice Krispies.

Volunteers reported that both supplements reduced flatus frequency compared to their normal diets. The questionnaires also revealed that both supplements seemed to reduce the amount of faeces produced, to make the stools firmer, reduce the frequency of defaecation and to make defaecation more difficult compared to normal bowel habits. There were no significant differences in the subjective assessments of the two supplements ($p > 0.05$).

The order in which the supplements were fed had no effect on the results.

4. DISCUSSION

The normal intake of RS in the UK was calculated to be 2.76 g/d, using data on the RS contents of a number of foods and food ingredients,[6,10,12] and the average weekly consumption of these foods (and foods prepared from the food ingredients) by the UK population.[13] The Cornflake supplement should therefore have provided over three times the amount of RS in the UK diet to the colonic bacteria.

The difference in RS calculated to be supplied by the two supplements (9.47 g/d) was not sufficient to cause any change in faecal output, stool frequency, consistency or whole-gut transit-time. This may have been because the background diets of some volunteers contained relatively high amounts of dietary fibre (between 4.4 and 27.1 g/d),

so that any effect of the RS was swamped, however the significant difference in breath hydrogen excretion on the first day of the supplements suggests that more substrate **was** reaching the bacteria from the Cornflake supplement.

It is possible that the RS in Cornflakes was primarily converted to gas rather than being used for bacterial cell growth. Another explanation is that the bacteria adapted to the steady input of RS, so that any initial impact on colon function could not be detected after measurements began on day 3.

In conclusion our data suggest that whilst on a normal Western diet, a tripling of the normal intake of RS would not cause dramatic changes in colon function.

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USE OF THE WISTAR RAT AS A MODEL FOR INVESTIGATING THE
FIBRE-DEPLETION THEORY OF COLONIC DIVERTICULOSIS,
1949-1988.

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1   INTRODUCTION

Diverticular disease is one of the degenerative conditions
that have been blamed upon chronic consumption of a fibre-
depleted diet. It is characterised by outpouchings of the
mucosal lining through the muscle wall of the colon, and
is common in the UK and other Western countries,
particularly in older people. A substantial body of
epidemiological evidence offers strong support to the view
that dietary fibre is protective against diverticular
disease[1], though other factors, notably age-related changes
in connective tissue have been implicated[2]. There is also
a straightforward conceptual link between fibre and
diverticulosis, based on the idea that the lumen of the
colon diminishes when deprived of bulking agents, as a
consequence of adaptive changes in colonic muscle. In
this contracted state it becomes more prone to complete
constriction during normal or increased activity of
colonic circular muscle. Raised pressure in these
segments allows the mucosa to herniate through weak points
in the muscle coat.[3]

    This paper summarises attempts to obtain experimental
proof of a causal link between fibre deficiency and
diverticular disease using the Wistar rat as an animal
model.

2   CARLSON AND HOELZEL EXPERIMENTS

Carlson and Hoelzel reported in 1949 the findings of two
lifespan trials using Wistar rats.[4] In the first
experiment, results of which are summarised in Table 1,

Table 1 Incidence of diverticulosis
          Carlson and Hoelzel (1949).

	Diet	Male Rats	Female Rats
(a)	Control	8/19 (42%)	14/26 (54%)
(b)	10% alfalfa	4/9 (44%)	3/14 (21%)
(c)	As (b) plus 5% kapoc and 5% psyllium husk	3/11 (27%)	4/12 (33%)
(d)	"Vegetarian diet" inc. 50% wholewheat flour	1/21 (5%)	3/25 (12%)

137 rats were fed diets with a variety of fibre
supplements for their entire lifespan.

Colonic diverticula began to be seen after the 20th
month of the trial in animals dying of natural causes.
The overall incidence of diverticulosis was abut 30%, with
a preponderance in animals fed a control diet described as
"low residue, omnivorous, and containing 35% meat protein
and 28% fat". However, the fibre content of the diets was
not determined (even as "crude fibre"). Moreover, animals
were allowed free access to "trimmings of head lettuce".
(Fortunately lettuce leaves consist mainly of water (96%)
and contain only about 1.5% dietary fibre).

The diverticula described by Carlson and Hoelzel were
either solitary of multiple (as in human clinical
presentation), and were generally situated close to the
caecum. In the latter respect they differed from typical
Western diverticular disease, which affects the more
distal ("left-sided") sigmoid region of the colon.
However, right-sided diverticular disease is not unknown
in human populations, and is the favoured site of
presentation in Oriental populations[5]. The reason for the
different distributions is unknown.

Carlson and Hoelzen also described in their 1949
paper a remarkable experiment involving 115 rats divided
between what appears to be 15 (or maybe 17) dietary
regimen, 10 of which involved crossovers between 2 diets,
and a further 2 involving crossovers between 3 diets. In
some instances the group size was reduced to 1 (or zero),
and there were many groups with just 2 or 3 animals. This
prolific scattering of animals between treatment groups

clearly precluded any kind of statistical analysis.
Nevertheless, the data appeared to suggest that a rat that
was raised initially on a high-fibre diet and then
subsequently switched to a low-fibre diet was especially
prone to diverticulosis.

3 FMBRA LIFESPAN TRIAL (WHEAT FIBRE)

Our first and largest trial at Chorleywood used a total of
1800 Wistar rats. A range of levels of fibre, supplied as
white or wholemeal bread, or as unprocessed wheat bran,
were incorporated into isocaloric low-fat semisynthetic
diets. In addition, we tested a non-isocaloric high
fat/low fibre diet and a commercial high-fibre stock diet.
The first diverticulum was seen at around 18 months. The
results, which we published[6] in 1985, showed a
statistically highly significant inverse relationship
(p<0.001) between total DF, measured by the Southgate
procedure, and incidence of histopathologically-confirmed
colonic diverticulosis (Fig. 1).

Only 27.8% of rats fed a diet containing 34% dried
wholemeal bread (Diet E) developed diverticulosis compared
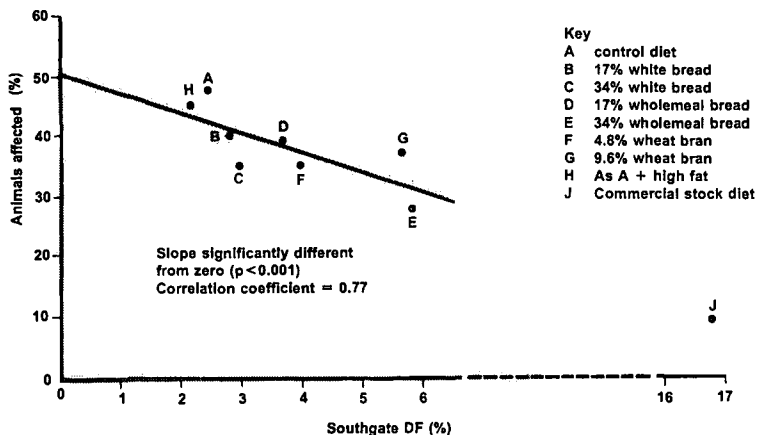
Figure 1 Effect of cereal DF on incidence of colonic
diverticulosis (900 male, 900 female rats).

with 47.5% on Control diet A in which pregelatinised wheat starch was the main component. Diet G, with the higher of two levels of unprocessed wheat bran, produced an anomalously high incidence (37.2%) of diverticulosis, despite having about the same level of wheat fibre as the wholemeal bread diet E. These data give no grounds for thinking that heat processing reduces the protective effect of wheat bran; in fact, the opposite appears to be true. The lifespan trial also showed that fibre associated with starchy endosperm in white bread (Diets B and C) was at least as effective in protecting against diverticulosis as that associated with the branny outer layers (pericarp, testa and aleurone) of wheat, when compared on a 1:1 basis, despite its greater fermentability.

Further evidence of the efficacy of cereal fibre was the exceptionally low incidence of diverticulosis (9.4%) in the group of animals fed a commercial stock diet containing 47.5% whole wheat and 40% Sussex ground oats (run primarily as a check on animal husbandry).

4   SECOND FMBRA TRIAL (WHEAT VERSUS NON-WHEAT FIBRE AND RESISTANT STARCH)

The first FMBRA trial, summarised above, ran for the entire lifespan of the rat, and did not end until almost all the animals had become moribund, or had died of natural causes (almost 3 years). In the second trial a shorter feeding time (approx. 15 months) was achieved by starting with 306 ex-breeder female rats purchased at 8-9 months of age having previously been maintained on a commercial high-fibre breeding stock diet. Table 2 lists the diets tested in the trial and their fibre contents. Dried white bread was this time tested at its highest practicable level (80% of the diet). In addition, autoclaved, amylomaize starch, shown previously[7] to be a

Table 2   Diets fed in second FMBRA trial.

Diet	Source of fibre	A.O.A.C. Total DF %	Enzyme-resistant Starch
A	None (sucrose control)	0	0
B	80% dried white bread	3.4	1.4
C	80% rolled oats	6.6	0.5
D	37% extruded peas	5.5	0.7
E	15% amylomaize starch	3.4	4.0
F	15% wheat bran	7.5	0

rich source of enzyme-resistant starch (ERS), was tested
at a level which gave an identical analytical value for
"apparent" dietary fibre in a AOAC gravimetric procedure.
In addition, three other sources of fibre, two of cereal
origin (wheat bran and rolled oats) and one non-cereal
(legume) were tested at the same estimated level of
Southgate total fibre (6.3%). Actual levels were
subsequently determined retrospectively on pooled batches
using FMBRA's currently available AOAC and Englyst
methodology.

Once again, a highly significant linear inverse
relationship was seen between total apparent fibre and
incidence of diverticulosis (Fig. 2). The rolled oats
diet was as effective as the wheat bran diet, despite
having a slightly lower level of AOAC total fibre, and a
greater proportion of soluble fibre. In addition, the
amylomaize diet, supplying apparent fibre primarily as
ERS, was as effective as the "isofibrous" white bread diet
in which both NSP and ERS were present in a ratio of
approximately 1.8:1. The legume diet was associated with
a lower incidence of diverticulosis relative to zero fibre
(sucrose) control, but the difference failed to reach
significance at the 5% level.

These data confirm the findings of the previous
trial, namely that the protective effect of fibre in the
rat model is not confined to insoluble, poorly fermentable
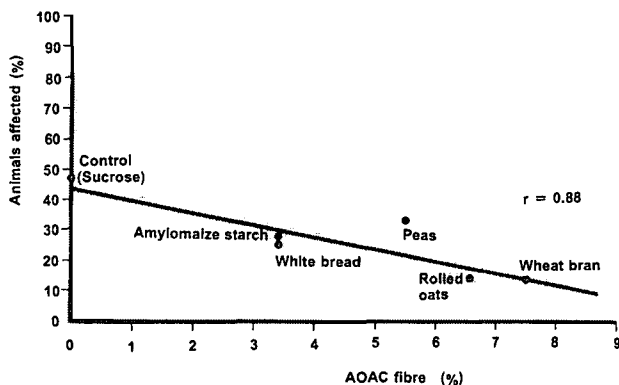
__Figure 2__ Effect of sources of cereal and non-cereal fibre
and enzyme-resistant starch on incidence of diverticulosis
(306 female rats).

wheat bran fibre, despite epidemiological evidence that
the latter is more protective in Western populations.
However, the caecal site of presentation of diverticula in
the rat makes it likely that most if not all bulk-forming
agents in the diet may be protective in this model, since
bacterial degradation will be less complete than at more
distal sites. It is possible that the low incidence of
the predominantly right-sided diverticular disease in
Oriental populations reflects an ability of all forms of
NSP and ERS to protect the proximal colon. Wheat bran, on
the other hand, may have a special role to play in
protecting the susceptible sigmoid region of the Western
bowel against this condition, by virtue of its exceptional
resistance to anaerobic degradation.

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ENHANCEMENT OF COLONIC CRYPT PROLIFERATION IN MAN BY SHORT-CHAIN FATTY ACIDS (SCFA)

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INTRODUCTION

The major constituents of dietary fibre(DF) are non-starch polysaccharides(NSP)[1]. Together with certain fractions of starch[2] they resist enzymatic digestion in the small bowel and reach the caecum. Depending on chemical composition and physical properties most NSP are rapidly degraded under anaerobic conditions by the colonic microflora[3]. End products of this fermentative process are short-chain fatty acids(SCFA). The predominant SCFA are acetic(AC), propionic(PROP) and n-butyric(BUT) acids which are generated in an approximate molar ratio of 60 : 25 : 15[4]. They are rapidly absorbed from the colonic lumen[5]. BUT serves as a fuel for energy metabolism of colonic epithelia. Moreover, isolated colonocytes of man and rat prefer BUT to other substrates like glucose, glutamine, lactate and ketone bodies[6,7]. The metabolism of the other SCFA by colonocytes seems to be less extensive which allows AC and PROP to enter the portal circulation. At the hepatic level PROP is taken up by the hepatocytes and may affect cholesterol metabolism[8]. AC alone is recovered in peripheral venous blood[9] to be oxidised to CO_2 by many tissues including skeletal and heart muscle[10].

While the extraintestinal effects of SCFA[11] are not well understood there is evidence that these acids play a major role at their production site in the caecum. The interactions between bacterial metabolites and the colonic epithelium seems to be of special interest. As BUT is a preferred energy yielding substrate to colonocytes[6,7] the hypothesis was put forward that SCFA may also affect cell replication of the colonic mucosa in man.

METHODS

During routine colonoscopy 6 biopsies were taken in 31
patients from normal caecal mucosa. Pathologic findings
elsewhere in the colon included diverticula or
hemorrhoids but not inflammatory bowel disease or tumors.
The samples were immersed in Eagle's medium and incubated
for 3 h in the presence of SCFA (3 biopsies) or equimolar
NaCl (3 biopsies, control). Then pulse labelling of
proliferating cells was achieved by addition of [3]H-
thymidine for 1 h[12]. The samples were fixed in formalin,
embedded in paraplast, section-cut in 4 um slices and
stained with acid Schiff's reagent (Feulgen reaction).
Sections were dipped in Ilford K2 emulsion and exposed
for 15 days following standard techniques of autoradio-
graphy[12].

The incubation medium contained sodium salts of SCFA at
the following concentrations:
    (a) AC = acetate, 60 mmol/l, n = 7;
    (b) PROP = propionate, 25 mmol/l, n = 8;
    (c) BUT = n-butyrate, 10 mmol/l, n = 8;
    (d) SCFA = AC(60 mmol/l) + PROP(25 mmol/l) + BUT
       (10 mmol/l, n = 8)

The labelling frequency of colonocytes was estimated in
15-20 longitudinally sectioned crypts of each run by
light microscopy.The number of labelled(l) and unlabelled
(u) cells per crypt column was determined and the whole
crypt labelling index (LI, l/l+u) calculated. Crypts were
divided into 5 equal compartments, compartment 1
representing the crypt base and compartment 5 the crypt
surface; in this way a LI for every crypt compartment was
calculated. Mean LI values of 15-20 crypts per run were
compared by the Wilcoxon rank sum test for paired data or
by the Mann-Whitney U-test for unpaired data where
appropriate. Differences were considered significant at
$p < 0.05$. All values were expressed as mean $\pm$ SEM.

RESULTS

Under control conditions (NaCl 10 - 95 mmol/l) the whole
crypt LI values (Figure 1) ranged from 0.062 ± 0.008 to
0.074 ± 0.012 without a significant difference between the
groups. AC raised crypt labelling by 30% (LI: 0.084 ± 0.005,
$p<0.05$), PROP by 70% (LI: 0.124 ± 0.014, $p<0.05$), BUT by
89% (LI: 0.140 ± 0.016, $p<0.05$) and SCFA (AC+PROP+BUT) by
103% (LI: 0.126 ± 0.012, $p<0.05$). Due to interindividual
variation there was no difference between PROP, BUT, and
SCFA, but labelling in these 3 groups was higher than in

Whole Crypt Labelling Index
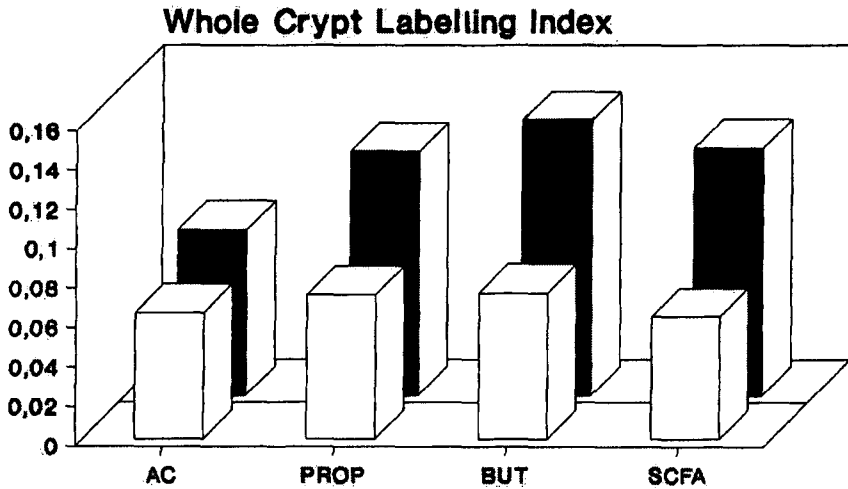
Figure 1 Whole crypt labelling index for incubation
experiments with acetate(AC), propionate(PROP), butyrate
(BUT) and the combination of SCFA(AC+PROP+BUT).
■SCFA, □NaCl(control).

Labelling Index / Crypt Compartment
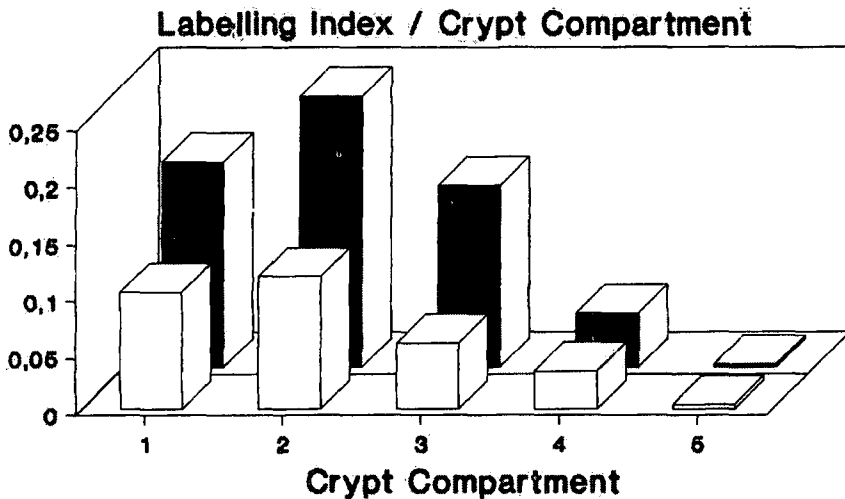
Crypt Compartment

Figure 2 Labelling index per crypt compartment
crypt base, comp.5 = crypt surface) for incubation
experiments with the combination of SCFA(AC+PROP+BUT).
■SCFA, □NaCl(control).

the AC group.

When the individual crypt compartments were evaluated
separately, a typical pattern was observed: PROP, BUT and
SCFA stimulated proliferation significantly ($p < 0.05$) in
compartments 1 - 3 and AC in compartments 1 - 2.
Thymidine labeling was not enhanced in compartments 4 and
5 (Figure 2).

DISCUSSION

SCFA stimulate cell proliferation in biopsies taken from
the normal human caecum. This part of the colon was
chosen because carbohydrate fermentation and SCFA
production is highest in the caecum and ascending colon
due to substrate availability. In this study PROP and BUT
were as effective as a combination of SCFA, while AC had
a minor impact on cell proliferation. These data are in
good agreement with Sakata's results obtained in rat
experiments[13,14]. He reported a 52 - 99% increase of cell
proliferation by SCFA; butyrate exerted a stronger effect
than propionate or acetate. Kripke et al.[15,16] perfused
the rat colon with SCFA (AC 70 mmol/l, PROP 35 mmol/l,
BUT 20 mmol/l) and found a rise of mucosal weight,
protein and DNA contents; BUT alone was as effective as
the SCFA combination. Thus, in man (in vitro) and in rat
(in vivo) colonic crypt proliferation is stimulated by
SCFA of which BUT seems to be most important. BUT is a
preferred fuel for colonocytes[6,7] which may also explain
a higher proliferative activity of these cells in the
presence of BUT. Other mechanisms, e.g. hormonal, may
play a role as well.

It was shown in experimental animals that feeding of
fermentable dietary fibre components raised colonic crypt
proliferation to a higher degree than cellulose[17]. Fibre
but not inert bulk (kaolin) prevented mucosal atrophy in
the colon of rats given a fibre-free diet[18]. In germ-free
rats fibre had no effect on colonic proliferation whereas
in conventional rats fibre caused a marked proliferative
response[19]. With regard to these data, it is likely that
the stimulation of colonic proliferation by dietary fibre
is at least partly due to the generation of SCFA.

The enhancement of crypt proliferation by SCFA may be
clinically important. In long-term artificial enteral
nutrition it may be desirable to avoid colonic atrophy by
adding fermentable carbohydrates to liquid formula
diets[20]. The functional capacity of the colon may be
improved by SCFA in the short-bowel syndrome; in the rat

model intestinal adaptation to massive small bowel
resection was enhanced by addition of pectin[21] which is
fermented in the colon to give rise to SCFA. - The
stimulation of caecal proliferation by SCFA suggests
close interactions between the microflora and the host
organism which merit further scientific attention.

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LABORATORY ASSESSMENT AND EFFECTS OF INCREASED
COLONIC FERMENTATION INDUCED BY UNABSORBABLE
CARBOHYDRATES.

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1 INTRODUCTION

Dietary fiber has been suggested to exert metabolic
effects either by altering small intestinal absorption or
possibly by increasing colonic fermentation[1,2] However,
discriminating between the two mechanisms is difficult
since the fibers that delay absorption are generally
easily fermented. Moreover, data on fiber fermentability
are mainly derived from in vitro studies, and only
indirect evidence of the fate of the fermentation products
exist for fibers mixed in actual diets. We wish to pre-
sent the sequential changes in breath H_2 and circulating
Short Chain Fatty Acids (SCFA) as a possible marker of
colonic microclimate adaptation to fermentable substrates.
The carbohydrate source used was lactulose, a non-absor-
bable readily fermentable sugar widely used as a laxative
and for therapy of hepatic encephalopathy.

2 METHODS

SCFA analysis

SCFA were determined by HPLC in serum after sample
preparation involving acid deproteinization or ultra-
filtration, followed by vacuum distillation.

Sample preparation. One-hundred ul of serum or whole
blood were added to 150 ul of an ice-cold standard solu-
tion containing 2 mmol/l butyric acid (internal standard)
and 5% perchloric acid, into a 500 ul eppendorf centrifuge
tube. After careful mixing, the tubes were centrifuged 10
minutes at 3000 x g, and 150 ul of clear supernatant were
transferred to a 5x75 mm glass tube. Alternatively, 500 ul

of serum were deproteinized by ultrafiltration at 1500 x g
for 70 minutes at 4 °C using an MPS-1 system with a
30,000 MW cutoff (Amicon, Danvers, MA USA). To 400 µl of
ultrafiltrate, 100 µl of 0.4M perchloric acid containing 2
mmol/l butyric acid were added, and an aliquot of 150 µl
transferred to a glass tube. The solution was wall-frozen
in liquid nitrogen on one side of the tube, and the sample
was distilled at low (<5 mm Hg) pressure using an Y-shaped
device similar to that described by Tollinger et al.[3].

Chromatographic conditions. The apparatus was a
Hewlett-Packard Chemstation (Hewlett-Packard, Palo Alto,
CA, USA) including an isocratic 1050 series pump (Hewlett-
Packard), a diode-array 1040A detector (Hewlett-Packard),
a Column Heater (Bio-Rad, Richmond, CA, USA), a Wisp 710B
autoinjector (Waters Ass., Milford, MA, USA). The column
used was a Bio-Rad HPX-87H Organic Acid Analysis Column
(Bio-Rad) on line with a Cation-H Micro-Guard Column (Bio-
Rad). Mobile phase was 0.005 N H_2SO_4 with a flow rate of
0.6 ml/min at a column temperature of 60 °C.
For routine analysis detection was made at 210 nm, while
for purity analysis the whole spectra from 190 to 280 nm
were acquired.

Breath Hydrogen analysis

Breath Hydrogen was measured on expired alveolar air
samples collected by mean of a modified Haldane-Priesly
tube[4] into 30 ml plastic syringes with a 3-ways luer lock
tap. The subjects were not allowed to smoke during the
test, and kept their normal activity during the day of the
test. The samples were analysed by gas chromatography
using a dedicated gas analyzer (model 12i MicroLyzer,
Quintron instruments Co Inc, Milwaukee, Wi, USA).

Study design

Eight healty volunteers, mean age 37.5y±3.0y were
randomly placed on two 2-week metabolic diets of identical
composition, one of which was supplemented with lactulose
(1g per 100 kcal, maximum 25 g/day), following a crossover
design. Diets provided 30%, 30% and 40% of the calories at
breakfast, lunch and dinner respectively. Diet composition
was 15% protein, 33% fat and 52% carbohydrate, and apport-
ed 15g fiber per 1000 kcal, according to food composition
tables. Breath H_2 was measured hourly on days 1, 7 and
13 of the lactulose period, and on day thirteen of the
control period. Serum SCFA was measured every two hours on
day thirteen of each phase, and, in a supplementary study,
on the first day of lactulose supplementation.

3 RESULTS

The only SCFA detectable in periferal blood was ace-
tate, identified by its retention time and UV spectrum.
Mean±SD recoveries of formate, acetate, propionate and
butyrate, from 100 ul of pooled serum spiked with 100 µl
of standard solution, were 105±5, 100±3, and 101±1%
respectively. When the perchloric acid procedure was used
on pooled fasting serum, the calculated coefficent of va-
riation for acetate analysis was 25% . To this value, dis-
tillation contributed 8% CV (10 replicates), and chromato-
graphy 2% CV (10 replicates). Thus, the coefficent of va-
riation for deproteinization was about 15% , a quite high
value possibly deriving from acetate esters hydrolysis
operated by the concentrated acid at various extent.
However, when samples were deproteinized by ultrafiltra-
tion, the coefficent of variation for duplicates dropped
to 5% ,and for the whole procedure to 12%, due to a better
reproducibility of both the deproteinization and the dis-
tillation steps. Moreover, the SD for replicates remained
constantly low (about 5 to 10 umol/l) independently from
the acetate concentration in blood.

During the day profile, serum acetate levels increased,
though not significantly, in the latter part of the day on
lactulose diet by about 22% on day 1, and 24% on day 13,
(Figure 1).

A much higher difference was noted for breath H_2. The mean
day-long difference in breath hydrogen levels on the lac-
tulose diet on day 13 was 8.0±1.1 ppm (p<0.001, Figure 2).
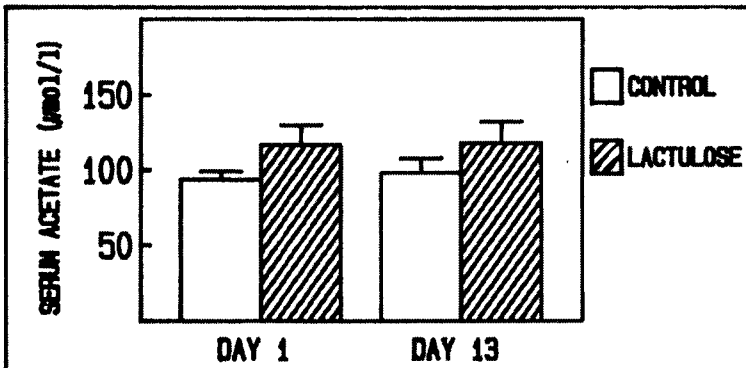
Figure 1   Mean acetate levels on the second half of the
day profile (14.00 to 20.00)
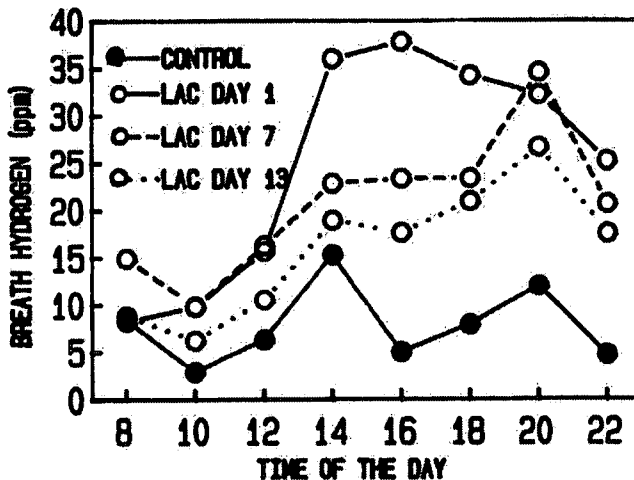
Figure 2 Mean breath H_2 levels over the day.

Considering the areas under the curve, values for lactulose on day 1, 7 and 13 were 310%, 268% and 206% of the control respectively (Figure 3).

4 DISCUSSION

The most common markers of colonic fermentation are, together with colonic pH, breath hydrogen and circulating SCFA, usually determined by Gas Liquid Chromatography. High Performance Liquid Chromatography of SCFA has been successfully used in acute studies showing colonic absorption of infused acetate and propionate[5]. This method permits good sensitivity even at the low circulating acetate levels found in fasting blood.
In this study we observed no difference between the one day and the two week acetate levels. Thus, if the microflora adaptation toward higher production of SCFA into the lumen[6] exist, it is not reflected by higher circulating levels *in vivo*. However, the progressive reduction of the amount of H_2 expired may reflect a modification of bacteria metabolism over the time, possibly due to a decrease in colonic pH, with consequent inhibition of hydrogen production[7,8]. As a consequence of increased colonic fermentation, due to addition of lactulose to otherwise identical diets, we observed an effect on FFA levels over the day and in fasting serum total and LDL cholesterol and apolipoprotein B. The results of this part of the study will be published in detail elsewhere[9].
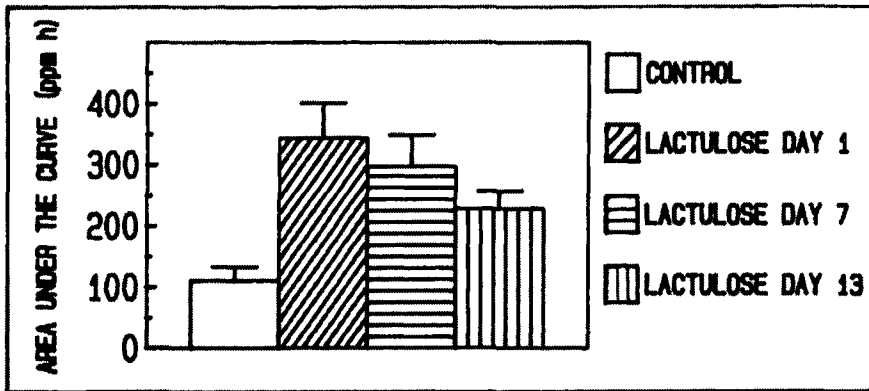
<u>Figure 3</u> Area under the breath H_2 day-long curve.
Lactulose values are all significantly different from
control (p<0.001).

It can be concluded that, in assessing colonic fer-
mentation using only breath H_2 as fermentation marker, the
lenght of the study is a major factor of variability that
should be taken into consideration.

ACKNOWLEDGEMENTS

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DIETARY FIBRE AND COLONIC MUCIN CHANGE IN GOLDEN HAMSTER

A Morphometrical and Histochemical Study

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1. INTRODUCTION

Dietary fibre has been shown to have a number of impacts on physiology and pathology.[1-8] However, the influence of dietary fibre on intestinal mucins has been paid less attention to.[1-4] Gastrointestinal mucins are complex glycoproteins that are secreted from mucin-producing or goblet cells, and that form a viscoelastic gel covering the mucosal surface of the gastrointestinal tract. They have a range of known or potential functions,[2,10] such as lubrication, protection of mucosa, waterproofing of mucosa, antibacterial (viral, parasitic) action, adsorption of cholesterol, cooperation with sIgA, interaction with enzymes, and action as an enzymatic substrate. In physiological conditions, the mucin secreting cells of the gastrointestinal canal consistently produce their own specific mucin type(s) both in humans and in animals.[9-13] Qualitative changes appear in pathological conditions such as inflammation and neoplastic transformation.[10-13]

2. MATERIALS AND METHODS

Twenty-five male Syrian golden hamsters (age of 4-6 weeks) were randomly divided into a control group fed fiber free diet, a rye bran and a soybean fiber supplemented group. The mean body weight at the beginning of the study was 71 ± 2 g. All animals were individually housed with a 12 hr light/dark cycle, and had free access to their respective diets (Table 1) and to tap water. In each diet, 25% of the energy came from protein, 35% from fat and 40% from carbohydrate.

Table 1. NUTRITIONAL COMPOSITION OF THE DIETS   (g/100g)

	DIET		
	Control	Rye Bran	Soybean Fibre
Milk protein	37.6	23.9	29.0
Corn oil	15.9	11.9	13.1
Wheat starch	38.2	26.2	32.6
Vitamins	1.2	1.0	1.0
Minerals	6.1	4.9	5.1
Gelatine	1.0	1.0	1.0
Rye bran	0	31.1*	0
Soybean fibre	0	0	18.2*

* Fibre source; fibre composition is shown in Table 2

Table 2.   FIBRE COMPOSITION*   (g/100 g diet)

	Control	Rye Bran	Soybean Fibre
Soluble Fibre	0	3.0 (20.5%)	1.6 (10.8%)
Insoluble Fibre	0	11.9 (79.5%)	13.2 (89.2%)
Total Fibre	0	14.9 (100 %)	14.8 (100 %)

* Analysed according to Asp[14];

After six weeks of feeding, all hamsters were sacrificed by heart puncture under anesthesia. From each animal, transverse circular gut specimens were taken from the proximal colon (at 1 cm distance from the daecum) and the distal colon (at 1 cm from the rectum). All samples were fixed in 10% neutral formalin, dehydrated and embedded in paraffin. Five serial sections (5 μm) were cut from each sample. One section was stained with hematoxylin and eosin (H & E); the other sections were treated by the alcian blue pH 2.5/periodic acid-Schiff (AB/PAS),[11] high-iron diamine/alcian blue pH2.5 (HID/AB),[13] and periodic acid-borohydride/potassium hydroxide/ periodic acid-Schiff/hematoxylin (PB/KOH/PAS/H)[11] methods. The AB/PAS method discriminates neutral mucin (red) from acidic mucins (involving N-acetyl sialomucin, O-acetyl sialomucin, and some sulphomucin as well)(blue). The HID/AB method stains sulphomucin (brown-black) and sialomucin (blue). By the PB/KOH/PAS/H procedure, only O-acetyl sialomucin is stained red.

For quantitatively morphological evaluation, stereological techniques[15,16] were applied. For this purpose,

all histochemically stained sections were light microsco-
pically projected on a paper with a printed quadratic
lattice with 1 cm of line space. Total magnification (M)
was calibrated by a 10^{-2} mm scale. Volumes of goblet cell
mucin (**Vg**), mucosa (**Vmc**) and muscular layer (**Vms**) were
estimated by a formula:

$$V = Z^2 * L * P$$

V = volume; **Z** = distance between lines of the lattice (10
mm in the study) divided by the total magnification (M)
(131 in the study); **L** = given length of a gut tube speci-
men (10 mm in this current study); **P** = counted points of
the lattice cross falling on target. From these figures
obtained, volume density of goblet cell mucin (**Vg/Vmc**)
was also calculated. A Student t test was used for statis-
tical data.

3. RESULTS

Most of the mucin-producing goblet cells were located in
the crypts, while only a small number were seen in the
surface epithelia towards the intestinal lumen. The dis-
tribution of the histochemically defined mucin types dif-
fered between the proximal and the distal colon. Proxi-
mally, goblet cells of the mucosa produced preponderantly
N-acetyl sialomucin, sulphomucin and O-acetyl sialomucin,
and a minor amount of neutral mucin. Distally, a medium
amount of N-acetyl sialo- and sulphomucin, small amounts
of neutral mucin as well as a trace of O-acetyl sialomu-
cin were observed. However, rye bran or soybean fiber

Table 3. VOLUME ALTERATIONS OF GOBLET CELL MUCINS OF
COLONIC MUCOSA IN HAMSTERS AFTER SIX WEEKS OF FEEDING

	No.	Vg (mm^3/cm)	Vmc (mm^3/cm)	Vg/Vmc (mm^3/mm^3)
Proximal Colon:				
Control	(9)	3.41±0.45	21.62±3.26	0.17±0.02 *
Rye bran	(8)	3.98±0.61	15.01±1.60	0.27±0.02 *
Soybean f.	(8)	4.77±0.91	17.85±1.87	0.27±0.03 #
Distal Colon:				
Control	(9)	1.06±0.17 *	13.02±1.45	0.08±0.01 *
Rye bran	(8)	2.03±0.29 *	10.57±1.05	0.19±0.03 *
Soybean f.	(8)	1.57±0.21	10.69±1.21	0.15±0.01 *

Mean value ± SE; *P < 0.01, #P < 0.05; AB/PAS staining

supplementation did not induce any qualitative changes
of goblet cell mucin either in the proximal or the distal
colon.

Vg of the distal colon was significantly larger in
the rye bran group compared with the fibre-free group.
Vg/Vmc was significantly increased both in the proximal
and the distal colon and both in the rye bran and the
soybean fibre group as compared to the fibre free control
group (Table 3). The muscular and mucosal volumes per cm
colon did not differ between the fiber free and the two
fibre groups.

4. DISCUSSION

Previous studies on the effect of dietary fibre on the
intestinal mucins have mainly concerned the small intes-
tinal mucosa.[1,3,4] Using scanning electron microscopy and
autoradiographic analysis, an increased goblet cell se-
cretory activity has been observed after dietary supple-
mentation with cellulose or wheat bran.[1,3,4] Quantitati-
vely, enhancement of the relative number of goblet cells
due to dietary fiber has been reported[3,4] but also lack
of alteration or even a decrease.[3,4] In the colon, scan-
ning electron microscopical observations[1,4] have indica-
ted that the mucosal surface of rats fed wheat bran exhi-
bits a larger amount of mucous producing cells compared
to rats fed a fibre free diet.

In the present study, the increase in volume density
of goblet cell mucin (Vg/Vmc), seen both in proximal and
distal colon after both rye bran and soybean fibre sup-
plementation, indicate that dietary fibre also induces a
relative increase in the goblet cell mucin volume of the
large intestine. A significant enhancement of total gob-
let cell mucin volume (Vg) was noted in the distal colon
when the diet was supplemented with rye bran. This could
be the result of an increase in either goblet cell size
(hypertrophy) and/or number (hyperplasia). The mechanism
seems complicated and can not be evaluated from the pre-
sent data. It should be noted, however, that a close cor-
relation between goblet cell mucin volume and number of
goblet cells was observed in the small intestine from the
same animals (unpublished data).

Water insoluble or poorly fermentable components of
dietary fibre (e.g., cellulose and lignin) are related to
the enhancement of fecal volume with dilution of toxic
and carcinogenic substances.[1-8] Increased amounts of se-
creted mucins might also contribute to fecal bulk enhan-
cement.[2] The increased goblet cell volume found in this
study after rye bran supplementation may be related to

such a possible mucous influence on fecal bulk. In this respect, the effect of both water insoluble and water soluble fractions of dietary fibre could be considered.

In conclusion, dietary supplementation with rye bran or soybean fiber did not result in qualitative alterations of goblet cell mucin in the hamster colon. Quantitatively, however, there was a general relative increase and in part a total enhancement of goblet cell mucin volume. Further studies are needed to demonstrate the influence of dietary fibre on mucin secretion, its significance and the possible mechanisms involved.

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THE DIETARY FIBRE MATRIX DURING GUT TRANSIT - MATRIX SOLUBILITY, PARTICLE SIZE AND FERMENTABILITY.

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1 INTRODUCTION

The physiological effects of dietary fibre (DF) are related to the behaviour of the DF matrix under conditions prevailing during gastrointestinal transit. Whilst methods have been developed for the analysis of DF in foods [1,2] less attention has been focused on its analysis from digesta during gut transit, although measurements of physicochemical properties and their relationship to physiological effect have been attempted [1,3,4]. To determine how fibre is affected by the environmental conditions in the gut lumen, samples of feed and its digesta recovered from specific points along the gastrointestinal tract of the pig, were analysed. A vegetable-based and a cereal-based diet were analysed for DF content and composition, particle size distribution and fermentability.

Materials and Methods

Pigs were individually housed and fed a weight maintenance diet based on either swede (Brassica napus) or cereal wheat bran to provide 240g/day vegetable fibre or 440g/day cereal fibre. Feed was presented twice daily and intakes recorded[5]. Digesta for analysis was recovered from animals sacrificed 3 weeks after acclimatization to diet. Potential fermentability and rate of fermentation were measured using pigs cannulated at the mid point in the caecum, and involved the suspension of a known weight of water-insoluble fibre in a nylon bag (5μm mesh) in the caecum for set time periods. Digesta samples recovered and residues from caecal fermentation were freeze dried prior to analysis.

Table 1   Potential Fermentability of Water-Insoluble Swede Fibre and Wheat Bran in Feed and Ileal Digesta Samples Measured in the Pig Caecum and in Relation to Apparent Digestibility.

	SWEDE			BRAN		
	*Potential		#Apparent	*Potential		#Apparent
	Feed	Ileal	Faeces	Feed	Ileal	Faeces
'Fibre'[1]	93.1	90.5	87.8	45.2	45.3	41.5
Cellulose	93.5	94.0	91.7	23.1	29.6	24.1
Non-cellulosic neutral sugars	87.5	80.1	78.7	58.5	61.5	46.5
Uronic acid	96.5	81.3	88.6	45.0	27.9	39.2

[1]Non-starch polysaccharides and lignin.   *Potential fermentability (% loss of component within 48hr) of feed and ileal digesta in the caecum. #Apparent digestibility measured in faeces (% loss of component during transit).

Table 2 Fractional Rate of Fermentation of Swede Water-Insoluble Fibre and its Ileal Digesta and Bran Water-Insoluble Fibre and its Ileal Digesta.

	SWEDE				BRAN			
	Feed		Ileal		Feed		Ileal	
	k	t 1/2	k	t 1/2	k	t 1/2	k	t 1/2
Fibre[1]	14.4 ±0.3	4.8	19.1 1.8	3.6	9.5 1.4	7.3	6.7 1.7	10.3
Cellulose	13.0 ±0.8	5.3	19.4 1.8	3.6	*8.8 4.8	7.9	*4.4 1.6	15.8
Non-cell. n. sugars	14.8 ±0.7	4.7	19.6 4.9	3.5	8.6 1.2	8.1	9.9 1.7	7.0
Uronic acid	18.4 ±1.6	3.8	25.8 2.7	2.7	*5.9 1.2	11.7	*2.9 1.2	23.9

[1]Non-starch polysaccharides and lignin. *No significant linear relationship. k = – Rate of fermentation (%h) (mean +/–standard error). $t\frac{1}{2}$ = half life (h), i.e. time taken to ferment to 50% original (LN_e 2/K).

Chemical analysis was by standard methods and particle size analysed by wet sieving (stacked sieves, size range 4000 - 5μm; 10g sample dry weight; water flow rate 600 ml/min applied via spray nozzle; amplitude 1.5mm; time 30 min). Material retained on each sieve was recovered and represents water insoluble material of specific particle size.

Results

Estimation of potential fermentability (proportion of material lost during prolonged caecal incubation) showed swede fibre and its ileal digesta were rapidly fermented, with a potential fermentability over 90% achieved within 24h[5]. Similarly, bran samples were rapidly fermented but each had a potential fermentability of fibre of only around 45%[5]. Fermentability of fibre components varied within each fibre source and between swede and bran (Table 1). For both swede and bran samples apparent digestibility measured during gut transit agreed with component potential fermentability measured in the caecum. Rate of fermentation (Table 2) showed swede samples to be fermented faster than bran samples and swede ileal digesta to be fermented consistently faster than the feed. In bran, for only the non-cellulosic neutral sugars, was a linear and significant rate of fermentation found. Half life calculation from fermentation rates confirmd potential fermentability would be achieved within 24 hours. From chemical analysis of each sample (Figure 1) a proportionate decrease in water insoluble uronic acid was observed in swede during ileal transit. However, in faecal material uronic acid was still detectable despite the preferential fermentation of uronic acid in the caecum. In cereal bran no corresponding change in chemical composition was found during ileal transit and in faecal material each fibre component persisted, although apparent digestibility was 41.5%. Particle size analysis of feed and digesta (Figure 2) showed that little change in particle size distribution occurred in bran but in swede feed, particle size was reduced in the proximal gut. Stomach particle size and distribution was similar to that in feed and hence particle size reduction and change in distribution were not the result of mastication. A further reduction in swede particle size occurred in the large intestine and was accompanied by a loss of particulate material (87.8% apparent digestibility). Apparent digestibility of bran (41.5%) was not accompanied by a significant particle size reduction.
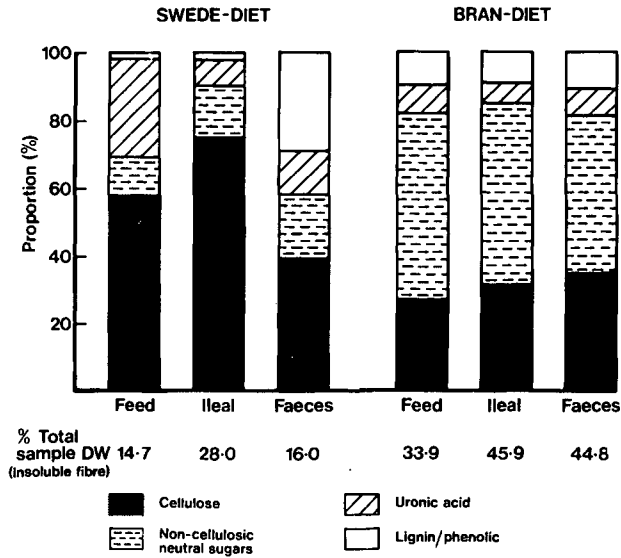
Figure 1. Proportionate composition of water-insoluble dietary fibre components recovered during gut transit in the pig.

Discussion

Swede and wheat bran comprise distinct tissue types and constituent cell wall biopolymers, which are cross linked to form the fibre matrix [1,6,7]. Persistence of cross links affect the solubility and structural integrity of the fibre matrix. Acid in the stomach may influence solublisation of pectic polysaccharides through displacement of calcium from constituent uronic acid residues involved in ionic cross link formation. Similarly, the mild alkaline conditions in the small intestine may encourage B-eliminative degradation of methyl esterified pectic polysaccharides[8]. Both processes will result in cell loosening/separation and hence particle size reduction, with a corresponding decrease in associated water insoluble pectic polysaccharides (uronic acid). The absence of pectic polysaccharides in bran
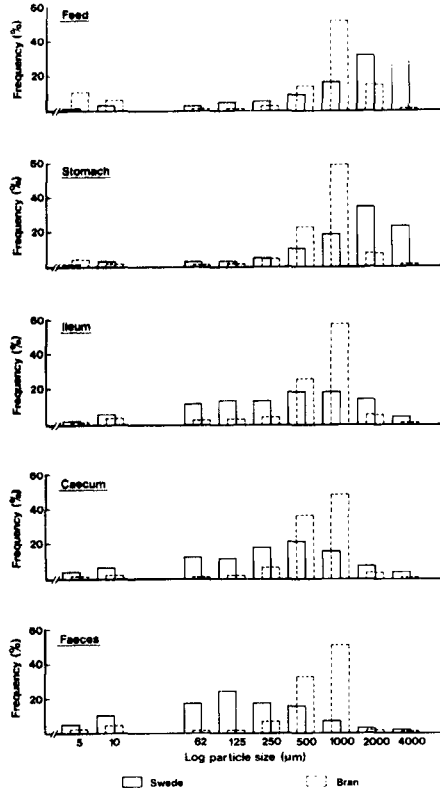
Figure 2. Particle size distribution (% insoluble organic material) of a cereal (Bran) – and a vegetable (Swede) – based diet measured during gut transit in the pig.

MEDIAN PARTICLE SIZE (μm)

	Feed	Stomach	Terminal Ileum	Caecum	Faeces
SWEDE	2398	2222	560	459	228
BRAN	1197	1110	1041	970	907

and the existence of a highly cross linked polysaccharide network involving phenolic esters and lignin will preclude a similar particle size reduction. However, selective fermentation of arabinoxylans, which can be attributed to be from aleurone layer cells[8] may result in a mechanical 'weakening' of the bran particle and a change in shape[9]. In swede, mainly parenchymatous tissue is found, also with selective fermentation of component polysaccharides, [10]. This tissue is readily fermentable and the result is the rapid and effective disappearance of swede cell wall material, ie particle destruction, during transit. In bran, fermentation is limited mainly to non-lignified tissues (endosperm and aleurone layer[8]) and hence rate and extent of fermentation is also limited. The resistance to fermentation of cross links in the lignified tissues of bran, maintains particle structure in bran during transit.

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ESTIMATION OF THE DIGESTIBILITIES OF NSP FOR WHOLEMEAL BREAD AND HARICOT BEANS FED IN MIXED DIETS

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1   INTRODUCTION

It is now accepted that dietary non-starch polysaccharides (NSP) which escape small intestinal digestion are fermented within the large bowel of man,[1] that the extent of this fermentation varies considerably between foods and that the rat appears to be a reliable model for man in this respect. [2]

Most studies of NSP digestibility have investigated individual foods or food components and where mixed diets were eaten it has seldom been possible to obtain separate estimates of digestibility for each food. This study used a multiple linear regression (MLR) approach to test the hypothesis that the digestibility of NSP in foods is influenced by the dietary presence of other NSP sources using mixed diets containing wholemeal bread and cooked haricot beans which are commonly-eaten, rich sources of NSP in the UK diet. Separate estimates of digestibility for bread and beans were obtained and we found little evidence that beans affected the digestibility of bread NSP.

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2   METHODS

Four test diets were prepared each containing 500 g freeze-dried, ground wholemeal bread and 0, 150, 300 or 450 g cooked, freeze-dried and ground haricot beans (<u>Phaseolus</u> <u>vulgaris</u>)/kg diet with alterations in sucrose and casein to maintain similar energy and protein contents. Each diet was fed to groups of 6 male Wistar rats for 21 d with measurement of food intake and complete faecal collection over the final 7 d. NSP in diets and faeces were measured by the Englyst method.[3]

The digestibilities of NSP and its components in the bread and beans were calculated using a MLR model in which NSP output in faeces (Y) was regressed against intake of bread NSP (X_1) and beans NSP (X_2) for each individual animal as follows:

Model 1 $Y = \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_1 X_3$ 1

where X_3 has the value 0 or 1 when beans are absent or present in the diet respectively. α_1 and α_2 are the indigestibilities of the component in bread and beans respectively and α_3 the additional effect of presence of beans on bread indigestibility.

Where α_3 is non-significant, a simpler model is appropriate:

Model 2 $Y = \beta_1 X_1 + \beta_2 X_2$ 2

where β_1 and β_2 are the coefficients of indigestibility for bread and beans respectively.

Data were analysed using the STATGRAPHICS package (STSC Inc., Rockville, Maryland, USA).

3   RESULTS AND DISCUSSION

Whilst the digestibility of whole diet dry matter (DM) decreased linearly that of the NSP increased curvilinearly with increasing inclusion of beans in the diet (Figure 1).

MLR analysis (Model 1) showed that the presence of beans had little effect on the digestibility of NSP or any of its components in bread (α_3, not significant; $p > 0.05$) so the simpler Model 2 was used to estimate digestibilities for bread and beans separately (Table 1).
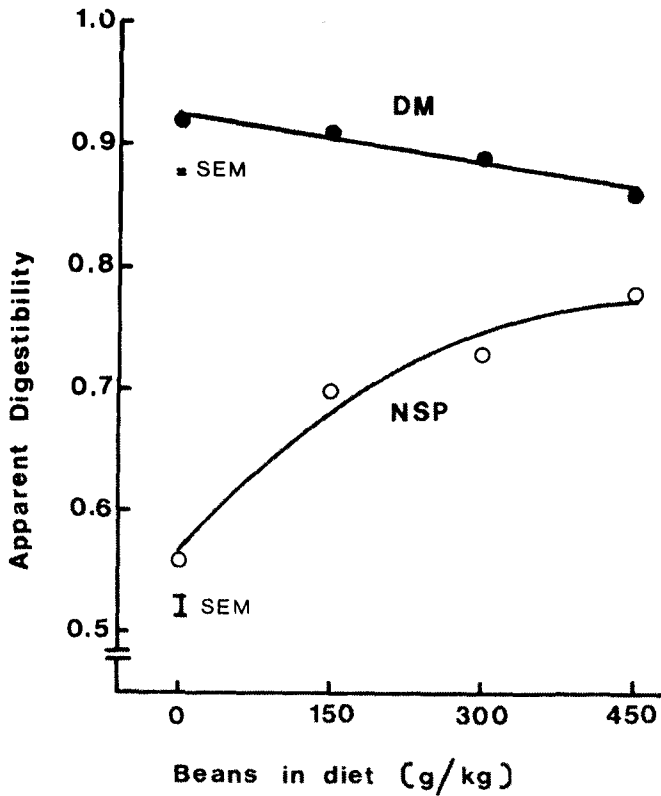
Figure 1   Digestibilities of whole diet dry matter (DM)
and non-starch polysaccharides (NSP).   Each
point is the mean for 6 rats.

Table 1   Estimates by MLR using Model 2 of the digestibilities of NSP and its components for wholemeal bread and haricot beans fed in mixed diets

Component	Bread	SE	Beans	SE	R^2
NSP	0.56	0.023	0.86	0.017	0.99
NCP	0.64	0.020	0.92	0.013	0.99
Cellulose	0.22	0.038	0.23	0.045	0.98
Arabinose	0.56	0.022	0.98	0.015	0.98
Xylose	0.67	0.022	0.88	0.046	0.97
Mannose	0.53	0.088	0.85	0.039	0.86
Galactose	-0.04	0.185	0.78	0.050	0.91
Glucose	0.40	0.040	0.74	0.023	0.99
Uronic acids	0.99	0.008	0.99	0.001	0.89

NCP = non-cellulosic polysaccharides

Model 2 provided a very satisfactory description of the relationship between intake and ouput of NSP and its constituents (R^2 0.85 - 0.99) in these mixed diets. It is clear that the digestibility of beans NSP is much greater than that of bread and similar to values for peas.[4]   Non-cellulosic polysaccharides (NCP) were considerably more digestible than was cellulose for both bread and beans.

Further studies should be carried out to determine to what extend food NSP digestibility is influence by other dietary components and we suggest that the MLR approach may have broad application in such studies.

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1. INTRODUCTION

Most of the Western diets are dietary fibre deficient. One way of curing this is the consumption of foods including added dietary fibre. Tegument of pea (pisum sativum) is a usable by-product. Nevertheless, the fibre must be reduced in small particles in order to make it untasted.

It is generally assumed that the particle size per se does affect the susceptibility to bacterial degradation of the fibre but earlier studies are not consistent. A decrease in the susceptibility to bacterial fermentation has been found in man given coarse bran compared with fine bran[1], whereas a slight increase in the fermentation of cellulose has been reported in pigs given coarse bran[2]. In the rat, the particle size per se of wheat bran did not affect the faecal dry weight or the fermentability of the fibre[3]. No significant differences in mean retention time were observed among coarse and fine bran in the pig[2].

The present study was undertaken to provide information on whether particle size could change the fermentability and the mean retention time of the pea fibre. A rat experimental model was used.

2. MATERIAL AND METHODS

Two forms of tegument of pisum sativum (Fibragel, CASI Co, Schoten, Belgium) were used: a coarse form (Fibragel as it is) and a milled one. The geometric mean diameter was respectively 0.411 and 0.187 mm for the coarse and the milled fibre.

Three lots of 6 male Wistar rats (250 g mean live weight) were housed individually in metabolism cages. A control diet was composed of 4 % cellulose, 12.5 % casein, 40 % corn starch, 31 % sucrose, 7.5 % soja oil and 5 % of a mineral-vitamin mix. Two experimental diets were prepared by substituting 5 % sucrose by 5 % either coarse or milled pea fibre. The animals were fed 15 g of their respective diet once daily.

The pea fibre, the diets and the faeces were analysed for ash, Kjeldahl N, total dietary fibre (TDF)[4], neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin[5]. In addition diaminopimelic acid (DAPA) was analysed in the faeces[6]. The apparent digestive utilization of dry matter (DM), organic matter (OM), crude protein (CP, N x 6.25), NDF and ADF and the total mean retention time (MRT) of the indigestible dietary particles were determined[7] using chromic oxide as marker (Cr_2O_3)[8]. The results were submitted to analysis of variance.

3. RESULTS AND DISCUSSION

Chemical Composition of the Pea Fibre and of the Diets (Table 1).

On a DM basis, the tegument of pea was composed of 2.7 % ash, 8 % CP and 77.7 % TDF including 12.7 % hemicellulose (NDF-ADF), 54.8 % cellulose (ADF-lignin), 0.3 % lignin and 9.9 % soluble fibre (TDF-NDF). The main constituent of the TDF was water-insoluble fibre. As a reference, ADF content of wheat bran average 14 %.

The chemical composition of the 3 diets was similar for OM and CP. The TDF content was higher in the pea diets (9.3 % and 9.4 % vs. 5.3 %)

Table 1 : Chemical composition of the pea fibre and of the diets fed to rats (% of DM).

	PEA FIBRE	DIETS		
		CONTROL	COARSE PEA	MILLED PEA
ash	2.7	5.2	5.5	5.2
OM	97.3	94.8	94.5	94.8
CP	8.0	11.9	12.2	12.6
TDF	77.7	5.1	9.3	9.4
NDF	67.8	5.3	9.2	9.2
ADF	55.1	4.2	7.3	7.4
Lignin	0.3	0.4	0.7	0.9

Table 2: Chemical composition of the faeces

	CONTROL	COARSE PEA	MILLED PEA
Water (%)	47.7	43.4	46.4
Ash (%)	18.2	14.9	14.2
CP (%)	7.1	6.9	6.7
NDF (%)	20.4	28.6	27.5
ADF (%)	19.0	25.4	24.8
DAPA (mg/kg)	110.4	126.6	111.4

Chemical Composition of the Faeces (Table 2)

The addition of pea fibre to the diet was accompanied by a lower ash content and a higher fibre content. DAPA was used as bacteria marker. The lack of a significant increase of DAPA in stools demonstrated the resistance to fermentation of the pea fibre used.

The particle size had little effect on the faecal chemical composition. Only water content tended to be higher (46.4 vs. 43.4 %) with milled fibre when compared with coarse fibre.

Apparent Digestibility of the Diets (Table 3)

The apparent digestibility of both DM and OM in pea diets was 4 points lower than in control diet (P<0.001). The digestibility of CP in pea diets was 3 points lower (P<0.001). On the other hand, digestibility of NDF and ADF was not affected by the pea fibre and was low (respectively 16 % and 5 %). In rats, digestibility coefficients for DM and gross energy declined significantly with each increase in the ADF content from 0 % to 15 %[9]. On the other hand, fibre consumption causes a faecal N increase. Substitution of 10 % wheat bran in rats decreased apparent N digestibility from 93 % to 87 %[10]. Suggestions include a decrease in the true digestibility of protein, increased losses of protein from the tissues of the intestinal tract and increased microbial protein synthesis[11]. This latest suggestion can not be retained for our trial since DAPA was not increased (table 2). Two factors of considerable importance affecting the digestibility of dietary fibre are the chemical composition of the fibre and the transit time for passage through the alimentary tract. In our study, the main component of pea fibre was cellulose which is known to

Table 3: Apparent digestibility of the diets (%)

	CONTROL	COARSE PEA	MILLED PEA
DM	88.8[a]	84.8[b]	84.5[b]
OM	92.3[a]	88.2[b]	87.8[b]
CP	88.3[a]	85.5[b]	85.4[b]
NDF	17.5	17.2	13.5
ADF	4.2	6.2	3.9

[ab] : means with different superscripts differ significantly (P<0.001)

have a slow fermentative breakdown, and MRT was very rapid (table 4).

The particle size did not change significantly the fermentability of the fibre. This is in agreement with observations concerning wheat bran[3].

Faecal Bulk and Mean Retention Time of Chromium (Table 4)

Faecal bulk was increased in the rats fed the pea diets (3.7 and 4.0 vs. 3.0 g/d; P<0.001) and MRT was decreased from 18.4 h to respectively 14.1 h and 17.7 h for the coarse and the milled pea diets. When compared to coarse fibre, fine fibre leads to a higher faecal bulk (4.0 vs. 3.7 g/d) and to a decreased MRT (17.7 h vs. 14.1 h). It is generally assumed that higher faecal bulk shortens the retention time. This conviction is supported by the difference in MRT observed among control and pea diets but not when comparing both pea diets. In pigs, no significant differences in MRT were observed among coarse and fine bran[2].

Table 4: Faecal Bulk and Mean Retention Time of Chromium

	CONTROL	COARSE PEA	MILLED PEA
Faecal bulk (g/d)	3.0[a]	3.7[b]	4.0[c]
MRT (h)	18.4[a]	14.1[b]	17.7[ab]

[ab]: values with different superscripts differ significantly (P<0.05).

4. CONCLUSION

Composed of more than 50 % cellulose on a DM basis, tegument of pea, as the other water insoluble dietary fibres decreased the apparent digestibility of OM and CP, increased the faecal bulk and shortened MRT when 5 % were substituted in the diet of rats. When compared to coarse pea fibre, fine pea fibre tended to increase the faecal water content, the faecal bulk and the MRT. As a decreased MRT is the wanted effect of insoluble dietary fibre in order to prevent constipation and to shorten the contact of carcinogens with the bowel inner side, more information is needed about the physiological properties of dietary fibre at reduced particle sizes.

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DIETARY FIBRE PRODUCTS, THEIR CHARACTERISTICS AND
GASTROINTESTINAL IMPLICATIONS

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1 INTRODUCTION

Dietary Fibre (DF) comprises a very complex and hetero-
geneous group of substances whose functional proper-
ties and nutritional effects vary with composition,
structure and treatment of the fibre source[1,2,3]

In the present study 3 commercially available fi-
bre products were examined: wheat bran, a long known
fibre source used in numerous studies; oat bran , a
rediscovered cereal fibre which has been claimed to
have cholesterol-lowering effects, and pea fibre , a
"novel" food ingredient whose nutritional relevance
has only been studied in relation to blood glucose re-
sponse[4].

The fibre sources were analysed chemically, and
their physiological effects along the gastrointestinal
tract examined in rats.

2. MATERIALS AND METHODS

The content of dietary fibre (DF), defined as non-
starch polysaccharides (NSP)[5], was measured in
wheat bran, oat bran, pea fibre, and a white wheat
flour (control product).

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The DF sources were mixed into diets with a total of 13% NSP and along with the control diet (low fibre, wheat flour diet) fed <u>ad. lib.</u> to rats with an initial body weight of about 280 g. Each diet was fed to 12 rats during a 2 weeks adaptation period and a 16 days balance period.

The faecal bulking defined as gram faecal dry matter (DM) per day was measured during the balance period.

The effects of the diets on the development of the gastrointestinal (GI) tract were measured on 6 rats per diet. After a total of 30 days on the diet the rats were killed, the entire GI tract removed and the length measured. The length of the small intestine (SI) and the large intestine (LI) was measured separately.

The passage rate of digesta was measured in 6 rats per diet after the termination of the balance period. Glass beads (diameter of 50 μm) were used as markers and administered on a single day over a 6 hour period. Total faecal collection followed over the subsequent 96 hours.

3 RESULTS

The total content of DF and the monomeric constituents of the DF sources differed widely. Wheat bran characterized by its high content of cellulose (6.4% of DM) and insoluble arabino-xylans (22% of DM) while oat bran contained a high amount of soluble betaglucans (9% of DM). Pea fibres comprised soluble and insoluble arabinans are (20% of DM) and insoluble cellulose (6% of DM) (Fig. 1). The solubility properties of the experimental diets varied from 65% in the oat bran diet, to 43% in the pea fibre diet, and to 19% in the wheat bran diet. The bulking effect of the diets differed significantly. In decreasing order the faecal bulking (g DM/d) of the diets were: wheat bran diet > oat bran diet > pea fibre diet > control diet (Fig. 2).

The length of the GI tract of the rats which were fed the experimental diets differed significantly. The elongating effects of oat bran were more pronounced than those of the wheat bran. Both cereal brans elicited effects on the small intestine (SI) as well as the large intestine (LI). The pea fibre had little effect on the length of the GI tract (Table 1).

<u>Table 1</u> Length of gut segments (mean values
         and SD)

Dietary Group	Small Intestine		Large Intestine		Total Gut	
Control	111[b]	7	15.3[c]	1.4	130[c]	7
Wheatbran	114[b]	9	17.9[ab]	2.0	138[b]	7
Oatbran	128[a]	12	19.1[a]	1.5	147[a]	13
Peafibre	114[b]	16	17.2[b]	2,9	135[ab]	15

Column mean values with different superscript letters
were significantly different (P<0.05).
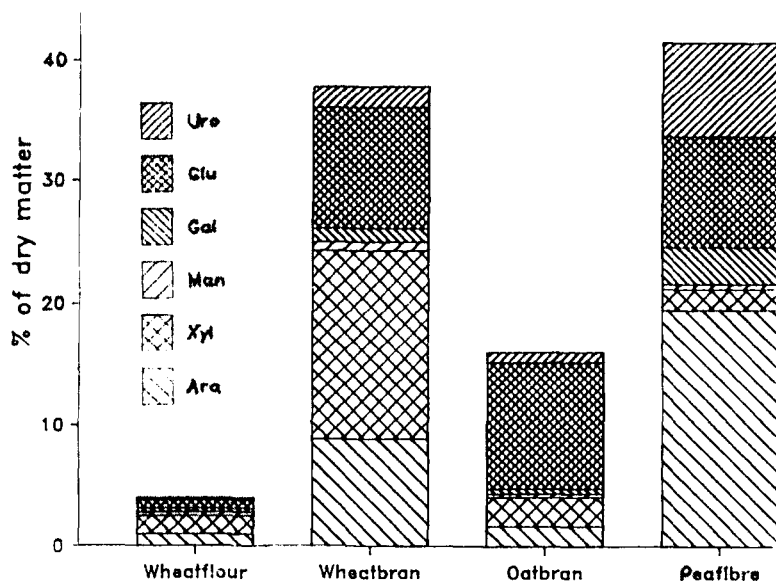
<u>Figure 1</u>   Content and compositions of non-starch
          polysaccharides (NSP).
          Uro=Uronic acids, glu=glucose,
          gal=galactose, man=manmose, xyl=xylose,
          ara=arabinose
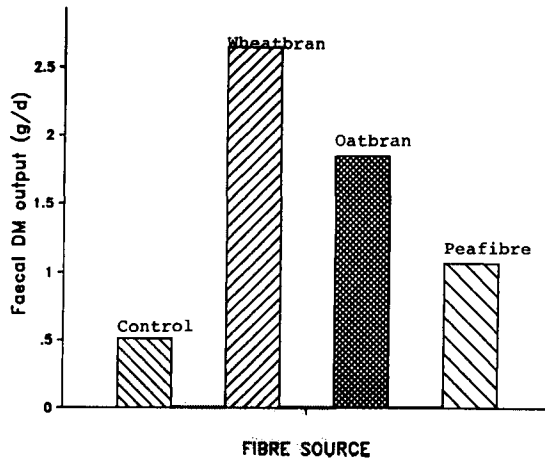
Figure 2 Faecal bulking (faecal DM/d) of diets
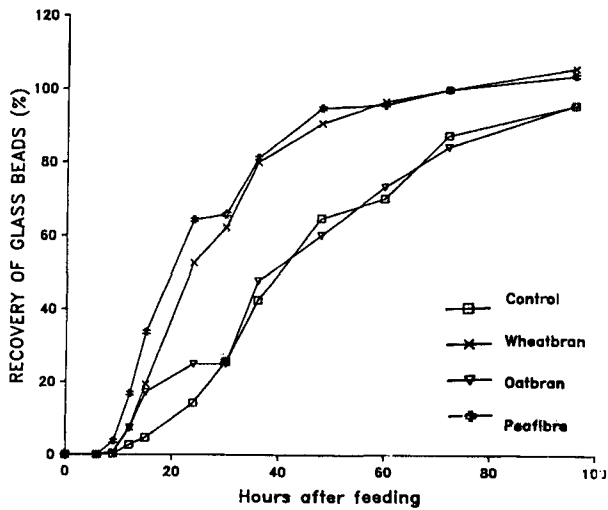
Figure 3 Rate of passage of digesta in rats fed different diets

The rate of passage of digesta differed significantly. The time for 50% of the markers to appear in the faeces was 22 hrs. for the pea fibre diet, 25 hrs. for the wheat bran diet, 37 hrs. for the oat bran diet, and 38 hrs. for the control (low fibre) diet (Fig. 3).

4. CONCLUSIONS

Analysis of three dietary fibre (DF) sources: wheat bran, oat bran and pea fibre showed considerable variation with regard to chemical composition and solubility properties. *In vivo* studies with rats confirmed that the mode of action of the DF sources along the gastro-intestinal tract differed greatly: bulking effects as well as rate of passage of digesta (transit time) were influenced to various degrees by the individual fibre sources. Also the direct effects of the DF on the development of the GI tract differed significantly which may be of importance in explaining long-term effects of DF.

In conclusion, the study provided evidence that dietary fibre sources of different origin elicit a range of effects along the GI tract. The mechanisms of action are complex, the knowledge of which is much needed to provide the consumer as well as the food producer with a soundly based guideline for the choice of acceptable and effective types of dietary fibre.

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DIGESTIBILITY OF RAW LEGUME STARCH

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1 INTRODUCTION

Legume seed starches have been found to exhibit considerable variation with respect to bioavailability (El-Harith et al 1976, Fleming and Vose 1979, Hoover and Sosulski 1985). No satisfactory explanation of this phenomenon has been forthcoming. A study has been initiated in this Department to assess the bioavailability of the starches of certain legume seeds and ultimately to define the physiochemical properties of the indigestible starch.

2 MATERIALS AND METHODS

The starch content of all food and faecal samples was estimated using a modification of the AOAC method 14.073/4 (1984).
Field bean (Vicia faba: varieties Maris Bead and Albinette), Lima bean (Phaseolus lunatus) and lentils (Lens culinaris) were obtained from the University of Nottingham Department of Agriculture or local health food stores. All were ground to pass through a 2mm sieve.
In all studies male Wistar rats were used (4 per group). Test animals were housed individually and allowed a 5 day adaptation period prior to the balance. Diets and water were supplied ad libitum. Feed intake, faecal output and weight gain were recorded in all cases.
Trial 1: Adult rats (250g) were offered diets containing 50% by weight of the above legumes. For comparative purposes one group of rats received a diet based on maize starch.
Trial 2: Weanling rats received diets containing field bean meal (variety, Maris Bead) at incorporation levels ranging from 340g/kg to 780g/kg. The diets were isonitrogenous (protein content = 195g/kg) and of comparable metabolisable energy (ME) values. The balance period lasted for 9 days.
Trial 3: Weanling rats received a series of diets containing 780g of Field bean meal (variety, Maris Bead) per kg of diet but with a

range of ME values. At the expense of oil, silicic acid was included in the diets to give a range of ME values between 10.03 and 15.29 MJ/Kg.

3 RESULTS

All the legume seed starches tested were found to have lower apparent digestibilities (p<0.001) than the control diet (Trial 1 and Table 1). Although a considerable variation in the digestibility of Lima bean starch was obtained, all the faeces excreted were soft and pale. When viewed under the light microscope numerous intact starch granules could be seen.

The level of starch included in the diet did not significantly affect the proportion of starch digested overall or throughout the balance period(Trial 2 and Table 1). There was a significant difference (p<0.05) in food intake between those rats receiving low and high energy diets but this did not affect starch digestibility in any group (Trial 3 and Table 1).

4 DISCUSSION

It has been shown that the bioavailability of starch in raw legume seeds varies considerably. At this time the reason for the poor digestibility of these starches is obscure. Microscopic examination of the faeces from those rats which received a Lima bean based diet revealed large numbers of starch granules,some of which were intact while others appeared to have been partially digested. Thus it would appear that the somewhat lower digestibility of the Lima bean starch cannot be entirely ascribed to physical properties and chemical factors may be involved.

Studies on the bioavailability of starches of raw legume seeds may, in some cases, be complicated by the presence of anti-nutritional factors (ANF) (Gupta 1987). Such effects cannot, at this time, be completely overlooked in the Lima bean.

Although field bean starch was found to be more digestible than Lima bean starch, a constant fraction proved resistant to digestion irrespective of the amount consumed or the energy status of the diet.

The native starch escaping digestion or fermentation in the GI tract is termed refractive starch. Refractive starch can be distinguished from resistant starch which is formed during the processing of food by virtue of the fact that it can be assayed by conventional enzymatic procedures (Berry 1986). Refractive starch in raw or undercooked legume seeds can therefore be included in the fraction termed 'dietary fibre' since it should have similar physiological effects to other unavailable components of this fraction (Cummings and Englyst 1987). Refractive starch may, in the future, become of greater significance in this respect since legume consumption is increasing in both developed and less developed countries. This is particularly significant in many less developed countries where the beans are consumed uncooked.

TABLE 1

EFFECT OF DIETARY INCLUSION LEVEL AND ME CONTENT ON DIGESTIBILITY OF LEGUME STARCHES

LEGUME	% LEGUME STARCH IN DIET	ME VALUE OF DIET (MJ/kg)	% STARCH UNDIGESTED
LENTIL	24.6	15.42	1.34 $\pm$ 0.8
LIMA BEAN	21.5	15.22	9.38 $\pm$ 2.56
FIELD BEAN			
VARIETY ALBINETTE	17.5	15.19	2.05 $\pm$ 0.42
VARIETY MARIS BEAD	14.22	15.26	2.05 $\pm$ 1.05
	23.43	15.18	2.21 $\pm$ 0.77
	32.63	15.29	2.77 $\pm$ 1.14
	32.63	12.15	3.75 $\pm$ 0.91
	32.63	10.03	4.17 $\pm$ 1.22

5 CONCLUSION

A variable proportion of the starch of different raw legume seeds has been found to be resistant to digestion <u>in vivo</u>. This fraction has been termed refractive starch and may be regarded as an additional component of the dietary fibre fraction of plant foods.

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ACKNOWLEDGEMENTS

The Effect of Isolated Complex Carbohydrates on Caecal and Faecal Short Chain Fatty Acids and Stool Output in the Rat.

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The mechanisms by which complex carbohydrates increase faecal output, and in particular faecal water, are not fully understood. Carbohydrates which are resistant to fermentation by bacteria in the colon are the most effective faecal bulkers probably because they retain their water holding capacity[1]. The extent to which a complex carbohydrate is fermented in the colon of an animal is dependent not only on the ease of fermentation but also the time it remains within the colon[2]. The factors which determine colonic transit time are not clear but propulsion may be increased by distension[3], bile acids[4] or by the stimulation of the mucosa with the edges of particulate matter[5]. The effect of fermentation products on motility has not been investigated thoroughly and short chain fatty acids (scfa) have been shown to inhibit motility in the caecum of the sheep[6] and the isolated rat colon[7], but to stimulate contractions in mid and distal rat colonic strips[8]. The major sites of fermentation are thought to be the caecum and proximal colon but it may be possible, if propulsion is stimulated before fermentation is complete, for fermentation of a complex carbohydrate to continue further along the colon. The absorption of scfa in the distal colon although very rapid may be less efficient than absorption in the proximal colon[9,10] and this could have important implications for colonic pH and the absorption of water. In this study, we have compared the caecal and faecal scfa of rats fed a variety of complex carbohydrates with a range of in vitro fermentabilities and have related this to the effects of the carbohydrates on faecal output and faecal water.

51 male Wistar rats (weight approx. 150g) were fed a fibre deficient diet (2.5% dietary fibre) for 28d. They were then divided into groups of 7 or 8 and their diet supplemented with 5% of guar, karaya, tragacanth, gellan, xanthan or ispaghula gum for a further 28d. At the end of this period faeces were collected over 2 days and the animals were killed and caecal contents obtained. Caecal and faecal wet and dry weight were measured and short chain fatty acid content determined by gas liquid chromatography[1].

Each carbohydrate had a different effect on caecal and faecal wet and dry weight and scfa content. However, they appeared to fall into three groups when compared with the fibre deficient group.

<u>Group 1</u>

Guar gum (G) was the only carbohydrate to increase the caecal scfa concentration (47% increase, p<0.01) but had no effect on faecal scfa or faecal output.

<u>Group 2</u>

Tragacanth (T) and karaya (K) significantly increased the total amount of caecal scfa (T 36% and K 89% p<0.05, p<0.001). Xanthan (X), however, decreased the total amount of caecal scfa (44%, p<0.01). All of these three gums significantly increased faecal scfa concentration (T 53%, K 55%, X 100%; p<0.01) and faecal wet weight (T 39%, K 47% and X 41% p<0.05) but had no effect on faecal dry weight.

<u>Group 3</u>

In contrast, ispaghula (I) and gellan (G) had very little effect on caecal scfa. Gellan increased scfa concentration per gramme caecal content dry weight but this was related to a decrease in dry weight and in fact resulted in a decrease in scfa concentration per gramme caecal content wet weight. Ispaghula and gellan also had no effect on faecal scfa concentration but did increase total daily scfa output (G 72%, I 114%). These carbohydrates significantly increased both faecal wet (G 88%, I 75% p<0.001) and dry weight (G 62%, I 33% p<0.01, p<0.05) and were the most effective faecal bulkers.

The caecum and proximal colon of the rat are thought to be the major sites of carbohydrate fermentation. If we can assume that comparison of caecal and faecal contents gives an indication of events occuring in the colon, these results indicate that the fermentation of some complex carbohydrates has a significant effect on luminal scfa content in more distal colon and this may be related to continued fermentation in more distal regions of the colon. In addition, an increase in the scfa concentration of faeces appeared to be related to an increased output of faecal water which may suggest that under some circumstances scfa absorption is less efficient and may play a role in determining faecal output.

Continued fermentation of complex carbohydrates in the distal colon of the rat has been indicated by pH measurements in other studies[12,13] and may explain the effects of some carbohydrates on the cellular proliferation of distal colonic epithelium[14].

In conclusion, although the knowledge that scfa are rapidly absorbed in the colon[9] has led us to believe that they play no role in determining faecal output, these results suggest that in some cases where carbohydrates are slowly fermented and increase faecal scfa the role of the scfa may need to be reassessed.

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TIME COURSE OF THE EFFECTS OF DIETARY FIBRE ON ENERGY INTAKE AND SATIETY

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INTRODUCTION

Satiety has previously been described as 'the state of inhibition over eating'[1]. The state of satiety may exist for a variable length of time which may be partitioned according to the mechanisms which are responsible for its maintenance. Figure 1 illustrates the proposed contribution of different processes to the intensity and time course of satiety. Thus, in the early phase of satiety, sensory and cognitive aspects of the food consumed may be responsible for activating and maintaining satiety. Post-ingestive properties such as the capacity of food to distend the stomach, its potential to evoke the release of gut hormones and the rate at which it is emptied from the stomach, are likely to be involved in prolonging the state of satiety in its mid and late stages. Late stages of satiety may be extended by the properties of food which decrease the rate of digestion and/or absorption. It follows therefore, that an assessment of the time course of satiety after a meal will reveal something of the underlying mechanisms responsible for its genesis and maintenance.

High fibre foods have been tested in relatively few short-term studies, and there is little information on how fibre influences the development and endurance of satiety. In this paper the results of two studies will be outlined which reveal something of the time course of action of high fibre meals on energy intake, appetite and satiety.
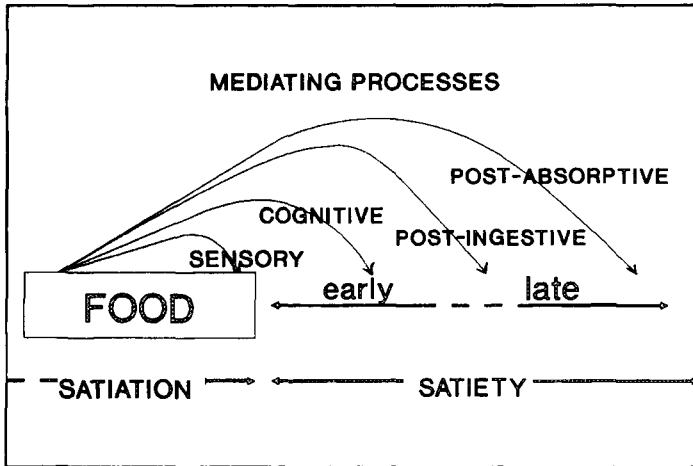
Figure 1   Conceptualization   of   the contribution of different processes to the intensity and time course of satiety

The Effects of a High and Low Fibre Lunches on Energy Intake and Satiety
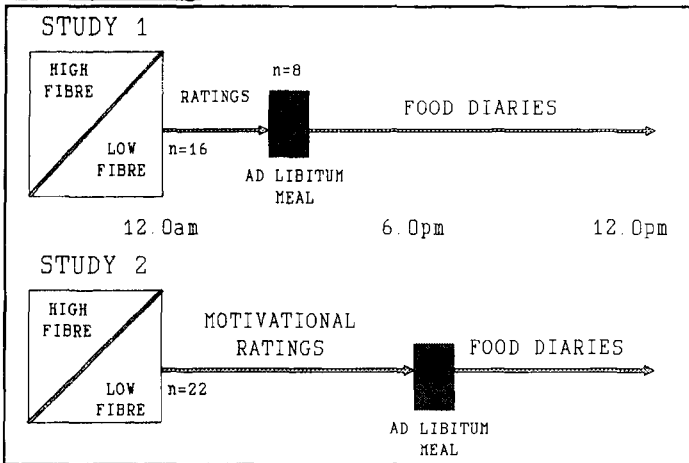
Figure 2 Experimental   strategies   used   to assess the time course of action of dietary fibre

Using   a   within-subjects   design,   non-obese   female volunteers consumed high and low fibre lunches after an

over night fast. Lunches were prepared to be as similar as possible in terms of macronutrient content and palatability. Table 1 lists the components of each lunch, and Table 2 shows their calculated nutrient and fibre content.

Visual analogue ratings (100mm lines anchored with descriptors) of motivation to eat were completed by the subjects before and for 2½ hours after each meal in study 1, and for 6 hours after lunch in study 2. After 2½ hours, in study 1 half the subjects were offered an afternoon meal of sandwiches, biscuits and a beverage for the direct assessment of satiety. Following this, all subjects left the feeding area and maintained weighed diet records for the rest of each test day. Energy intakes were calculated from the afternoon meal intakes and at each meal recorded in the food diaries after each lunch by the use of a computerised version of British Food Tables. In study 2, after 6 hours a similar ad libitum meal was offered to all subjects, consisting of bread, spreads, ham, cheese, biscuits, salad and fruit. Energy intake was assessed with reference to food table values. Following this meal, subjects kept a food diary until 12.00 pm.

Table 1 Composition of the high and low fibre test lunches

HIGH FIBRE	LOW FIBRE
Lentil Soup	Chicken Soup
Wholemeal Bread	White Bread
Butter	Butter
Wholemeal Pasta	White Pasta
Spiced Red Kidney Beans	Bolognese Sauce
Cheddar Cheese	
Tinned Black currants	Fruit Yoghurt
Fruit Yoghurt	

Table 2 Energy (kcal) and Nutrient Content (g) of High and Low Fibre Lunches (* Southgate values)

	Study 1		Study 2	
	High	Low	High	Low
Energy kcal	795	790	762	764
Protein	33	33	34	34
Fat	28	29	25	26
Carbohydrate	106	106	106	106
Fibre*	30	3.3	30	4.1

Study 1 - Results. Analysis of the motivational ratings data showed that for 2½ hours following each lunch, there was no significant difference between the meals in terms of their effect on motivational ratings. Moreover, at the afternoon meal, consumed by half the subjects, energy intake did not differ significantly according to whether high or low fibre lunches had been consumed. However, energy intake calculated from food diaries, from 2½-3 hours after lunch until 12.0 pm was significantly lower after the high fibre lunch (967±532 vs 1559±930 kcal). Figure 3 illustrates cumulative energy intake following high and low fibre test lunches from food diaries (including energy intake of subjects who consumed the afternoon meal). This figure shows that until approximately 5-6 hours after lunch no effect of fibre on energy intake was apparent.
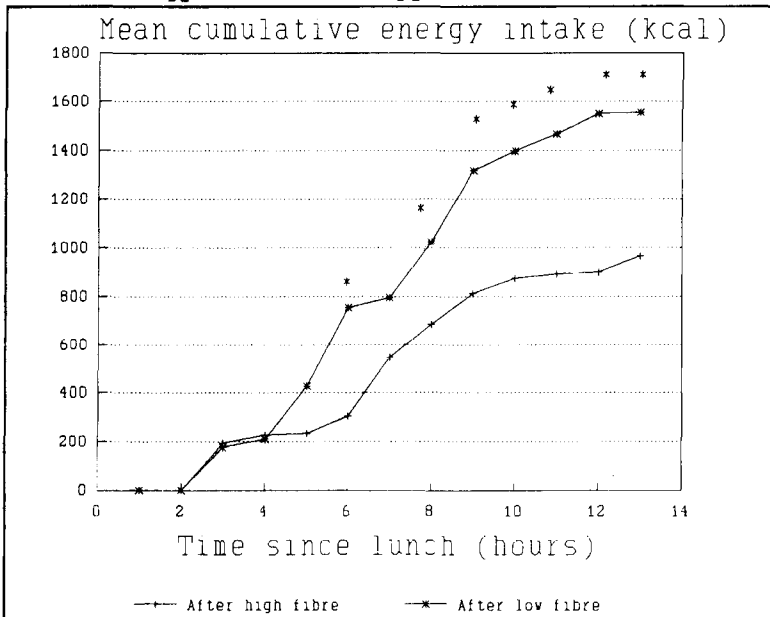
Figure 2 Effect of High and Low Fibre Lunches on Cumulative Energy Intake (from food diaries) for 13 Hours After Consumption. *Means Significantly Different p<0.03

Study 2 - results. Analysis of visual analogue scale data revealed a greater reduction in motivation to eat following the high fibre lunch compared to the low fibre meal, a difference which persisted from 30 minutes

to 6 hours after eating. Figure 4 illustrates the mean prospective consumption rating (on a scale of 0-100mm asking the question, 'How much food do you think you could eat? Nothing at all - A large amount) following both meals.
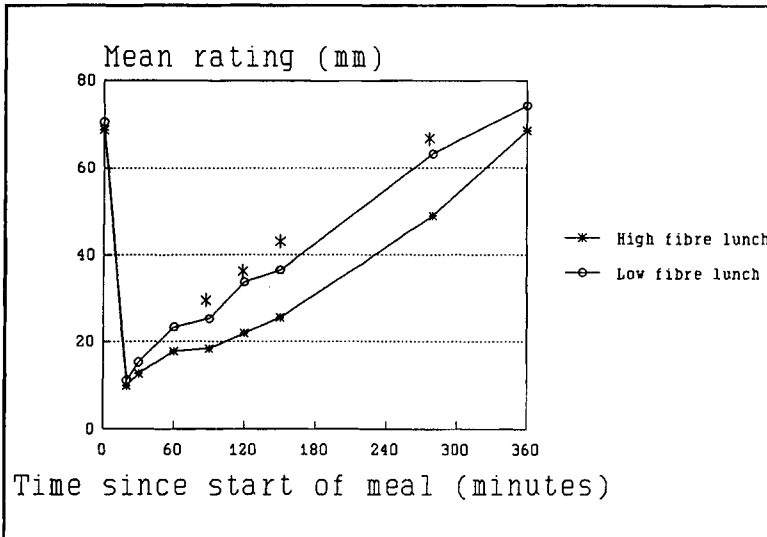
Figure 4 Effect of High and Low Fibre Lunches on Post-prandial Prospective Consumption Ratings. *Means Significantly Different p<0.01

Energy intake at the ad libitum meal presented 6 hours after lunch was lower by 86±187 kcal after the high fibre meal (p<0.04), and intake after this meal, obtained from food diary analysis was also lower by a further 112±528 kcal after fibre, though this difference failed to reach statistical significance.

Both these studies indicate that although there was some suggestion of increased satiety in the first 3 hours following the high fibre lunch in study 2, the primary impact of this meal was on later energy intake (5-6 hours after consumption). It is possible therefore that fibre consumed as high fibre foods reduces energy intake by an action on post-absorptive satiety.

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WORKSHOP REPORT: DIETARY FIBRE AND ENERGY BALANCE

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A wide range of topics was covered, aimed at
understanding the extent of body weight regulation by
'fibre' and 'high-fibre' diets.

     The question: ''Fibre', an adjunct to the treatment
of obesity or not?' tended to be answered affirmatively
but proof was lacking.   Thirteen studies addressing this
problem were reviewed by G. Livesey.   These showed 3 with
no effect and 10 each with an enhanced weight loss of
about 2kg.   Oddly the effect on absolute weight loss
appeared independent of overall weight loss, duration of
weight loss or the cumulative intake of 'fibre'.
Methodological problems might explain the 2kg effect.   No
studies included 'high-fibre foods'.   A 14th study, a 2yr
coronary heart disease follow-up in men who had been
advised to increase 'fibre' intake showed no effect on
body weight, although fibre intake was assessed as
increased.   A potential problem was dietary advice from
the media or from the researchers indirectly.   One
thought was that a study with 'high-fibre foods' is near
impossible, on the other hand this undermined the
original observations of Cleave, who must have considered
subjects not eating fibre supplements.   It was supposed
fibre may behave like a drug, in which case there would
be responders and non-responders.   A study investing
'high-fibre foods' affecting weight loss was thought
beyond the means of most (all) research groups.   In the
absence of such a study the discussion followed the
approach that if all (or most) effects on the physiology
were consistent with enhanced weight loss, the
probability of an effect could be established on
theoretical grounds.

'Caloric compensation with 'high- or higher-fibre diets' or satiety accompanied with lowered food intakes?' was the question addressed by V. Burley. Plenty of evidence was available for effects of fibre on satiety, but information linking 'fibre' with effects on calorie intake or intake of individual macro-nutrients was limited. High-fibre foods enhanced satiety at about the time of the next meal. This coincided with the onset of fermentation. It was suggested the effects of short-chain fatty acids on satiety be examined. Long-term studies of effects of fibre on food intake were absent and some thought these more important than studying the mechanisms in short-term effects. Methodology for assessing intake in the long-term was poor however, while in animals adaptation occurred so fibres eventually had no effect on intake. Short-term effects could be useful, e.g. when reintroducing subjects to conventional foods after procedures for severe body weight reduction. Furthermore there may be critical periods in life when a short-term effect would help, e.g. with dangerously obese subjects.

Are there significant energy losses from the terminal ileum other than 'dietary fibre'? was addressed by N. Read who gave evidence from ileostomists, intubated subjects and breath hydrogen studies. Ileostomists fed a control diet with and without 10g guar gum in an acute study showed terminal losses increasing from about 100 kcal to 180 kcal daily with only about a half of the increase that could be due to guar. The losses with the control diet and the increased losses due to guar were due to fat, protein and glucose (G_n). The elevated losses due to guar were thought by the workshop as an upper limit to what might be expected with other fibres which show less effects and adaptation might occur. It was considered valuable to obtain more information on the ileal losses of energy substrates.

John Mathers gave an account of an empirical model which can be used to calculate losses and gains of energy from fermentation of materials entering the colon from the small bowel. The major factor influencing energy salvage is the extent of fermentation of the organic materials supplied in ileal effluent. Assuming a value of 0.8 for the latter and making reasonable assumptions about i) the yield of bacterial biomass, ii) colonic SCFA pattern and iii) the proportion of SCFA absorbed he calculated a salvage of only 34-37% of energy from carbohydrate entering the colon of man. No single experiment emerged that could test the calculated result.

Information on the effects of protein and fat entering the colon was lacking and studies were needed to assess this.

Species differences in the extent of fermentation in man, pig and rat was addressed by I. Hansen. It was concluded that rat was similar to humans in the extent of fermentation of a whole range of materials from poorly fermented Solka floc up to easily fermented soluble fibres. Possibly greater variation occurred with poorly fermented fibres and between dosage with fibre within the same species. The similarity between man and rat in this respect made the rats a useful model for quantitative studies.

The energy values of 'dietary fibre' was a subject addressed by the British Nutrition Foundation Task Force and there being no time to continue with discussions individuals were suggested to consult the Task Force report when this becomes available. Briefly, here it can be stated that the Task Force concluded a value of 2 kcal/g was appropriate if a caloric conversion factor was needed for a mixed meal, with 'unavailable carbohydrate' having a fermentability of about 70%. The same could be true for supplements except it could be as high as 3 kcal/g and as low as 0 kcal/g when fermentability is about 100% and about 0% respectively. With diets high in cereal grain problems remain with quantification of carbohydrate that is unavailable in vivo.

Dietary Fibre and Lipid Metabolism

DIETARY FIBER IN LIPID METABOLISM

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There are many facets of research involving lipid
metabolism and dietary fiber. Data have been
collected concerning the influence of fibers on serum
or plasma lipid levels in man or animals; usually the
emphasis is on cholesterol and often on cholesterol
and its principal metabolites, the bile acids. Since
bile acids are related closely to cholesterol absorp-
tion, effects of fibers on bile acid binding or tur-
nover are also of interest. As an extension of
interest in cholesterol metabolism, there have been
investigations into the effects of fibers on experi-
mental atherosclerosis. Studies of fiber effects in
animals almost always involve the use of purified or
defined sources of fiber. In man, effects of both
identifiable fibers and fiber-rich foods have been
studied. In a space constrained exposition such as
this, it is not feasible to offer in-depth discussion
or exhaustive citations of the literature, but rather
one is limited to high-lighting studies which have
been of a pioneering nature or which have influenced
research directions.

Lipid Metabolism in Animals

Portman observed that diet affected bile acid
turnover and excretion in rats. Compared to a com-
mercial ration, a semipurified diet increased cholic
acid turnover by 110% and reduced the body pool by
38%.[1,2] These experiments represent one of the
earliest studies of metabolic effects of fiber.

Ershoff and his colleagues carried out studies in
which rats were fed fiber-free diets containing cho-

lesterol and compared the effects with those observed
in rats fed the cholesterol diet with added fiber.
Pectin, guar gum, locust bean gum and carageenan all
lowered serum and liver cholesterol, but agar and
cellulose did not.[3,4] Pectin has also been shown to
be hypocholesterolemic in rabbits[5,6], chickens[7], and
swine[8]. Cellulose appears to be hypercholesterolemic
in rats[3,9] and rabbits[6] and also increases total body
cholesterol in rats.[10] Soluble fibers such as pectin
or guar gum also exert beneficial effects on plasma
lipoproteins and apolipoproteins in rats.[11,12] A
comprehensive compilation of fiber effects on blood
and liver cholesterol levels in a number of animal
species has been published.[13]

     Since bile acids are a metabolites of cholesterol
and bile salts are required for the micelle formation
which is necessary for lipid absorption, it is logi-
cal to study fiber effects on bile acid metabolism.
Eastwood and Hamilton[14] studied the binding in vitro
of cholic and taurocholic acid to a number of plant
substances at pHs of 3.9 or 8.0. They concluded that
the binding was a hydrophobic phenomenon most pro-
bably due to the lignin content of the plant
materials used. Story and Kritchevsky[15] studied
binding of cholic, chenodeoxycholic, deoxycholic
acids and their taurine and glycine conjugates to
alfalfa, bran, cellulose and lignin. Each substrate
was bound to a different extent by each of the fibers
used. Cellulose bound very little of any of the
substrates. Studies with lipid micelles show that
different fibers can also bind cholestesrol and
lecithin.[16] Taurocholic acid is bound to a number of
spices such as curry powder, cloves and oregano.[17]

     A study in which a variety of fibers were fed to
rats for four weeks showed that almost all of them
increased fecal steroid excretion (mg/day) over that
seen in rats fed a fiber-free diet but fecal steroid
concentration (mg/gm dry weight) was usually lower.[18]
The insoluble fibers caused a consistent increase in
fecal neutral steroids and a variable increase in
acidic steroid excretion. The percentage of fecal
acidic steroids present as primary bile acid is
increased in almost all cases suggesting reduced
metabolic activity by colonic bacteria. Results of
the fecal steroid analyses are summarized Table 1.
Eastwood and Boyd[19] had observed earlier that addi-
tion of cellulose to a stock diet increased excretion
of trihydroxy bile acids at the expense of dihydroxy

Table 1. Fecal Steroids in Rats Fed Fiber*

Fiber (%)	Fecal Dry Weight	Total	Steroids Neutral (OL/ONE)[a]		Acidic (P/S)[b]	
None	(100)	(100)	(100)	(3.65)	(100)	(0.26)
Cellulose (10)	349	161	151	(3.65)	186	(0.42)
Wheat Bran (10)	218	146	154	(3.10)	125	(0.38)
Alfalfa (10)	245	149	145	(4.03)	157	(0.53)
Pectin (7)	77	68	86	(7.33)	66	(0.31)
Guar Gum (5)	196	180	161	(6.14)	113	(0.32)
Psyllium (10)	220	188	182	(3.55)	204	(0.41)

* After ref. 18

a Cholesterol + cholestanol + coprostanol/coprostanone
b Primary/secondary bile acids

bile acids. The various fibers also affected the
liver phospholipid spectrum; principal differences
were in levels of lecithin (7-25% above control
level) and sphingomyelin (20-53% below control
level).[20]

Investigation of mechanisms of action of fiber in
rats has shown that recovery of cholesterol in lymph
four hours after feeding a test meal is significantly
lower in rats fed either soluble or insoluble fiber,
but at 24 hours lymphatic absorption in rats fed
insoluble fibers begins to approach that of the
fiber-free control but recovery in lymph of rats fed
soluble fibers is still reduced significantly.
Recovery of oleic acid is unaffected in 4 hour lymph
of rats fed insoluble fiber and at 24 hours there are
no significant differences in recovery except for
rats fed psyllium.[21]

Recovery of cholesterol (endogenous or exogenous)
from serum and tissues of rabbits fed a semipurified
diet is significantly higher than in those fed a com-
mercial preparation; recovery from the feces is
lower.[22] The data are consistent with enhanced tran-
sit and excretion of cholesterol in rabbits fed a
diet high in crude fiber.

Experimental Atherosclerosis

In the late 1950s, Lambert et al.[23] and Malmros
and Wigand[24] reported that they had produced
atherosclerosis in rabbits by feeding them a
cholesterol-free semipurified diet containing high
levels of saturated fat. These findings appeared
paradoxical since other investigators[25-27] had found
that addition of saturated fat to commercial ration
did not render that diet atherogenic. A summary of
existing literature[28] showed that addition of
saturated fat to a semipurified diet (in which the
fiber was usually cellulose) rendered that diet
atherogenic but addition of the same fat to a stock
diet was without effect. It was hypothesized[28] that
a factor determining a diet's atherogenic potential
was its fiber content. Another view was that the
small amount of polyunsaturated fat present in com-
mercial ration was sufficient to negate the effects
of saturated fat.

To compare these hypotheses, an experiment was
conducted in which rabbits were fed a semipurified

diet containing coconut oil, the same diet plus 2% of the fat (iodine value 115) extracted from commercial ration, or the extracted residue plus 14% saturated fat. Addition of the unsaturated fat did not affect cholesterolemia or severity of atherosclerosis. The extracted residue plus 14% coconut oil or stock ration plus 14% coconut oil led to significantly lower serum cholesterol levels and atherosclerosis of 54-71% lower severity.[29,30] In a somewhat similar study, Moore[31] fed rabbits 20% butter fat in diets in which the fiber (19%) was wheat straw, cellulose, cellophane or cellophane-peat 14:5. Cholesterol levels were highest and atherosclerosis most severe in the rabbits fed cellophane. Rabbits fed wheat straw exhibited significantly lower serum cholesterol values (by 47%) and 66% less severe atherosclerosis. When the diet contained corn oil rather than butter fat, cellophane was still significantly more cholesterolemic and atherogenic than wheat straw. Dilution of an atherogenic regimen by addition of alfalfa (1:9)[32] or stock diet (1:1)[33] will also reduce cholesterolemia and severity of atherosclerosis in rabbits.

Fiber may also influence the effects of other components of an atherogenic diet. Thus, when effects of casein or soy protein were compared in diets containing cellulose, the former was more cholesterolemic and atherogenic; when the dietary fiber was wheat straw, casein was still more cholesterolemic than soy protein but the atherogenicity was about the same; alfalafa in the diet rendered effects of the two proteins virtually equal.[34]

Aortic sudanophilia in Vervet monekys fed semi-purified diet containing wheat straw was significantly less severe than that seen in monkeys fed the same diet with cellulose.[35] When the diet contained 0.1% cholesterol, aortic sudanophoilia was similar in monkeys fed cellulose or pectin.[36] Chickens fed 0.6% cholesterol and 3% pectin exhibit lower cholesterol levels and less severe atherosclerosis than those fed cholesterol and cellulose.[37]

Human Studies

Walker and Arvidsson[38] suggested in 1954 that the low levels of cholesterol in black Africans and their low incidence of coronary disease could be due, in part, to the high levels of fiber in their diet.

Keys et al.[39] found that pectin lowered cholesterol
levels in man, whereas cellulose has no effect.   The
effects of dietary fiber on serum or plasma cho-
lesterol have been summarized by Kay and Truswell[40]
and by Schneeman and Lefevre.[41]   In general, inso-
luble fiber such as cellulose or wheat bran has no
effect on either cholesterol or lipoprotein levels.
Oat bran is hypolipidemic probably because it con-
tains oat gum.[42]   Soluble fibers have been shown to
have hypolipidemic properties particularly guar
gum[43], pectin[44], and locust bean gum.[45]   Diets rich
in fiber[46] or legumes[47,48] are hypocholesterolemic.
A thorough review of dietary fiber effects on lipid
metabolism has appeared recently.[49]

      Effects of a vegetarian life style on lipidemia
can be studied in Seventh Day Adventists, some of
whom are vegans and most of whom are lacto-ovo vege-
tarians.   Hardinge and his colleagues[50,51] showed
that vegans whose fiber intake was judged to be
significantly higher than that of the general popula-
tion had significantly lower levels of plasma cho-
lesterol.   A later study[52] confirmed this observation
and found that the major difference in fiber intake
between Seventh Day Adventist vegans, other Seventh
Day Adventist groups and the general population was
in pectin consumption which was almost twice as high
in vegans as in the other groups.   Intake of other
fibers were comparable.   Vegetarians exhibit lower
plasma levels of those apolipoproteins associated
with increased risk of atherosclerosis.[53]

      In summary, soluble (or gelling) fiber affect
lipidemia and atherosclerosis in animals and man.
The effect may be due, in part, to binding of bile
acids but this property is not always reflected in
bile acid excretion.   Fiber can affect intestinal
morphology[54] and activity of intestinal[54] and
pancreatic[55] enzymes but the precise relation of
these effects to lipid metabolism has not been
established.   Effects on lipid absorption and distri-
bution into lipoproteins have been demonstrated.[21]
The role of the products of colonic fermentation of
fiber, namely, the short chain fatty acids, remains
to be clarified.

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DO OATS LOWER BLOOD CHOLESTEROL?

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1 INTRODUCTION

Oats and oat bran have recently received considerable
attention due to their alleged cholesterol lowering
properties. The active constituent is generally assumed
to be the soluble fibre fraction or oat gum which is
predominantly β-glucans. We have previously shown that
rats fed <u>ad libitum</u> a diet based on 50% oats (ca. 2% gum)
have significantly lower cholesterol levels compared to
cellulose-fed controls[1]. Food intake remained the same in
the two groups and both diets contained less than 350 mg
of cholesterol per Kg of diet.

2 THE HYPOCHOLESTEROLAEMIC EFFECT OF OAT GUM IN RATS

Hypocholesterolaemic properties have been suggested not
only for oat gum but also for the lipid and protein
fractions, so we have now looked for a dose-response
effect of isolated oat gum. Figure 1 shows that no
significant effects on total, LDL or HDL cholesterol were
found when the same control diet as that used above, but
with ; 0.5, 1.0, 2.0 and 4.0 % gum substituted for
cellulose, was fed for 4 weeks. However the control
cholesterol level was already reduced by placing all rats
on restricted food intake (18g/day) to obtain equal energy
intake. A reduction in cholesterol in rats on restricted
intakes has been reported previously[2] but has largely been
ignored in studies on soluble fibre. Where rats are fed
10% guar gum or pectin, food consumption is reduced. This
in itself will lower their blood cholesterol level, and
may account for much of the hypocholesterolaemic affect of
these diets.[3]
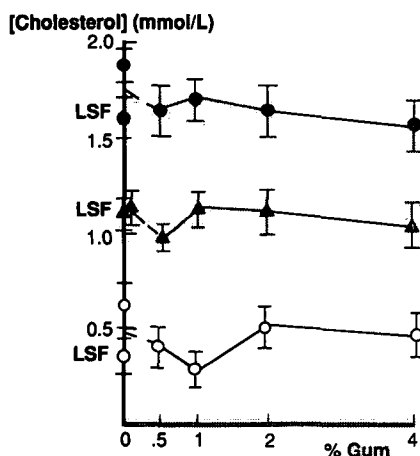
[Cholesterol] (mmol/L)

Figure 1   Plasma cholesterol levels vs. oat gum concen-
           tration. (LSF. 6% solkafloc control.
           o-Total Cholesterol   Δ HDL-Cholesterol
           o-LDL-Cholesterol   (mean ± SEM n=6)

3 CHOLESTEROL REDUCTION IN MAN

These problems, of separating the effects of reduced food
intake from any true hypocholesterolaemic value of oats,
are also relevant to human studies. We have recently
conducted a study on 35 healthy subjects mean age 36 (22-
54). They had a relatively low mean cholesterol level
(4.9 ± 0.2 m.mol/L) at the beginning of the trial which,
after eating 50g of oats/day for 42 days, dropped by 1.4%
to 4.8 ± 0.2 m.mol/L (p>0.05).   This statistically
insignificant reduction is consistent with many other
studies using volunteers with mean cholesterol levels
close to the accepted maximum desirable level of 5.2
m.mol/L[4-7].   When hypercholesterolaemic subjects have been
fed oats or oat bran, large statistically significant,
reductions in cholesterol levels have been reported[6-9].
Figure 2 shows that a dose-dependent effect of oats is
only apparent in hypercholesterolaemic groups of subjects.
In many of these studies a small weight loss was observed
and, therefore, as with rats, reduced food intake may have
been a complicating factor. This however seems unlikely
to account for all the hypocholesterolaemic effect.

% Cholesterol Reduction   % Cholesterol Reduction

Total soluble fibre/day (g/day)   Total soluble fibre/day (g/day)
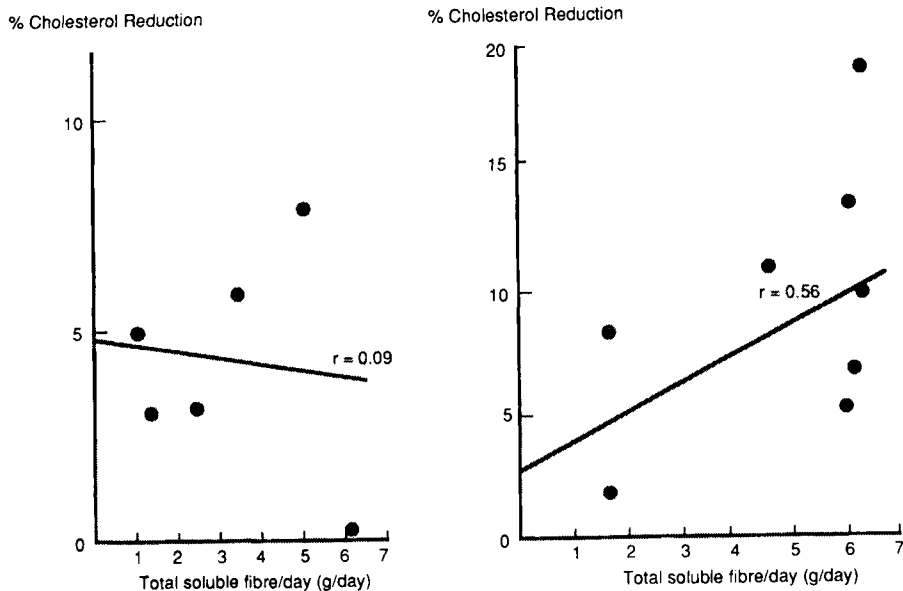
Figure 2 Percent reduction in mean plasma cholesterol in
normocholesterolaemic (a) and hypercholesterolaemic (b)
subjects from 14 studies.

3 DISCUSSION

Oats probably do reduce LDL-cholesterol when fed to
hypercholesterolaemic rats or human subjects but this is
hard to distinguish from effects due to reduced saturated
fat and total food intake. A further complicating factor
is that the percentage cholesterol reduction is dependent
on the baseline level. The correlation between %
reduction and starting cholesterol is stronger than that
between % reduction and the amount of gum fed (r=0.74 and
r=0.53 respectively, n=14). It is still not certain that
soluble fibre is the key factor in lowering serum
cholesterol but recent studies have shown that oat gum can
reduce plasma levels in cholesterol fed rats. Present
studies using hypercholesterolaemic rats but with no
cholesterol in the diet should resolve some of these
points.

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MECHANISMS WHEREBY FIBRE COULD LOWER PLASMA CHOLESTEROL

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1. INTRODUCTION

Fibre preparations such as oat bran, guar gum and pectin have gained
attention through their potential for lipid lowering with reported
reductions of 10-20% in plasma cholesterol in man.[1]   Oat bran has
been examined most thoroughly and recent reports generally have
confirmed more modest (but useful) reductions than earlier
studies.[2,3]   In an investigation of three cereal brans in mildly
hypercholesterolaemic men, oat bran reduced cholesterol by 6%
relative to wheat bran.[3]   We have screened eight commercially
available Australian oat brans in rats and found that they all lower
cholesterol.   These rat data have been confirmed in man[4] for one
product (The Uncle Toby Company) with similar reductions to the
earlier study of Kestin et al.[3]   Foods like oat bran and barley
flour are believed to lower cholesterol through their water-soluble
non-starch polysaccharide (NSP) content.[5]   This is because very
similar compounds (such as guar gum) in isolated form also lower
plasma cholesterol.[6]   Three mechanisms of action have been proposed:
alteration of digestion through viscosity in aqueous solution;
inhibition of hepatic cholesterogenesis by the propionate produced by
large bowel bacterial NSP fermentation; and, enhanced sterol
excretion through a specific binding by NSP.   However, none has been
established beyond doubt and one (inhibition of cholesterol
synthesis) seems incorrect.

2.   EFFECTS OF NSP VISCOSITY ON PLASMA CHOLESTEROL

Oat and barley β-glucans and gums such as guar are highly viscous in
aqueous solution.   This viscosity can effect cholesterol reduction
by slowing gut transit and reducing absorption of fat and
cholesterol.[5]   Delayed transit from the stomach to the small
intestine is a well-documented effect of NSP.[7]   High viscosity

Table 1.   Plasma cholesterol concentrations of rats fed a
            low-cholesterol diet containing different cereal
            preparations.

Diet	Plasma Cholesterol* (mM)
Wheat bran	3.22 ± 0.11^a
Oat bran	2.59 ± 0.10^b
Barley flour (unmalted)**	2.75 ± 0.13^b
Barley flour (malted)**	2.55 ± 0.10^b

*Mean+SEM of 6 observations per group.   Values with the same
superscript are not significantly different.
**From George Weston Foods.

guar gum lowers plasma cholesterol more than low viscosity guar gum
in hypercholesterolaemic men.[8]   This is consistent with an effect of
viscous drag on transit and absorption.   The viscosities of cereal
β-glucans and "high viscosity" guar gum are similar and both lower
cholesterol in rats fed hypercholesterolaemic diets.[5]   However, the
cholesterol loads in such diets are excessive compared to most human
diets and in rats fed a low-cholesterol diet, plasma cholesterol is
unaffected by NSP viscosity in the range up to and including that of
guar gum.[9]   Moreover, in rats fed malted barley (in which β-glucans
are greatly modified) plasma cholesterol is the same as with the
unmalted product. (Table 1).[10]

Malting has similar effects to treatment of cereals with β-glucanase
which in chickens abolishes the hypocholesterolaemic effects of
barley.[5]   Thus either the same mechanism does not operate in
different species or effects of NSP may be influenced by other
factors such as diet.   The latter seems to be a real possibility.
In the rat, NSP modify lymphatic fat and/or cholesterol transport,
depending both on the type of NSP and fat.[11]   We have found that in
rats fed fish oils with rice bran, plasma lipids were lower than with
wheat bran and the down-regulation of the liver low density
lipoprotein receptor seen with the latter bran was prevented.[12]
Finally, a recent study has shown that oat bran does not enhance the
cholesterol reduction of a "prudent" low-fat diet in
normocholesterolaemic men.[13]   Thus, the degree of cholesterol
lowering could depend on the type of fat as well as the source of NSP
and the cholesterol status of the subject.

    3. VFA AND HEPATIC CHOLESTEROL SYNTHESIS

The fermentation of NSP by the hindgut microflora of omnivores yields
VFA in significant quantities and it has been proposed that one acid

(propionate) mediates the cholesterol reduction by such NSP.[7]
Propionate at 1-5% of the diet lowers plasma cholesterol in animals.
Propionate also inhibits hepatic cholesterogenesis *in vitro* but at
concentrations 10-15 times higher than those found in the portal vein
in vivo during NSP fermentation.[14]   Moreover, propionate arising
from NSP fermentation in the hindgut is absorbed with a totally
different time course to that consumed in the diet.   In the pig,
portal venous concentrations rise and fall rapidly after propionate
feeding with a peak concentration far below that needed to inhibit
hepatic cholesterol synthesis.   The rise in propionate formed by
fermentation is delayed for several hours until food reaches the
hindgut.   In rats the effects of NSP on plasma cholesterol appear
unrelated to propionate production with some preparations raising
both caecal propionate levels and plasma cholesterol.[15]   On balance,
propionate does not seem to be the agency for cholesterol lowering by
NSP.

4. NSP AND BILE ACID AND NEUTRAL STEROL EXCRETION

The third alternative mechanism for cholesterol reduction is binding
of bile acids by NSP and is supported by observations in rats that
faecal bile acid excretion is greater with oat bran than with
cellulose.[16]   Neutral sterol excretion is also increased.   This
could simply be another aspect of effects of NSP on small intestinal
digestion but the changed molar ratios of the bile acids suggests
more specificity.   It is an attractive mechanism, resembling that of
therapeutic bile acid sequestrants such as cholestyramine.   NSP
preparations that lower cholesterol generally do increase steroid
excretion.[17]   Such binding also could account for the effects of NSP
on fat and cholesterol absorption and transport described by Ikeda
et al, especially as chitosan (an anion-exchange resin) was most
effective in reducing absorption.[9]

However, it is hard to discern how neutral NSP could effect such
specific binding.   Possibly, the NSP have domains where bile acid
micelles might become sequestered but this mechanism cannot apply to
acidic NSP (such as pectin) as both they and bile acids are ionized
at the mildly alkaline pH values of the small intestine and therefore
would be mutually repulsive.   Additionally, we have found no
relationship between steroid excretion and plasma cholesterol in rats
fed different oat brans.

5.   NON-FIBRE COMPONENTS AND PLASMA CHOLESTEROL:
POSSIBLE INTERACTIONS BETWEEN NSP AND LIPIDS

Investigations into the hypolipidaemic effect of plant foods precedes
the relatively recent interest in NSP.   For example, Judd and
Truswell concluded from their own work and earlier animal studies

that cholesterol reduction by oats in man was due to both the lipids and NSP.[18]   It is generally assumed that the important lipids are the unsaturated triacylglycerols present in oats at 8% (or more). More recently Qureshi et al have reported that minor components of barley, the tocotrienols, directly inhibit hepatic cholesterol synthesis in chickens.[19]   We have found that delipidation of oat bran and relipidation with polyunsaturated oils abolishes the cholesterol lowering in rats fed a low cholesterol diet.   These effects were unrelated to the tocotrienol content of the diet.

6.   CONCLUSIONS

NSP isolates may lower cholesterol by their physicochemical properties such as viscosity in solution.   For neutral NSP (e.g. cereal glucans) this could involve interaction in the gut either with dietary or biliary lipids.   Interaction with bile acids seems unlikely for acidic NSP under physiological conditions.   Cereals such as oats and barley, may lower plasma cholesterol through both their NSP and lipid components.   VFA production by NSP fermentation in the large bowel does not seem to relate to cholesterol reduction in animals.

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BENEFICIAL EFFECT OF WHEAT GERM ON PLASMA LIPIDS AND LIPOPROTEINS IN HYPERCHOLESTEROLEMIC SUBJECTS.

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1 INTRODUCTION

According to several epidemiological studies, the concept that a high dietary fibre intake may protect against coronary heart disease becomes more and more plausible (1,2,3). It is generally assumed that hypercholesterolemia and especially elevated LDL and VLDL-cholesterol , hypertriglyceridemia and hypertension are major risk factors for cardiovascular diseases.

In addition to wheat bran, another by-product of wheat milling is wheat germ, which contains about 8-12 % total dietary fibres, most of them being hemi-celluloses and cellulose as in wheat bran. We have already shown in the adult rat (4) that the addition of 7 % wheat germ to a high fat-cholesterol diet significantly decreased the VLDL-cholesterol and the VLDL-triglycerides and increased the HDL-cholesterol after 7 weeks feeding.

Given the beneficial effects previously observed in the rat on the liver and blood parameters, the present study was performed in hypercholesterolemic subjects in view to experiment the effects of a 30 g daily intake of wheat germ on various parameters of lipid metabolism.

2 SUBJECTS AND METHODS

Subjects

Ten adult volunteers participated in the study. Free-living subjects (8 females and 2 males) were from 35 to 68 years old. Based on body mass index values, none was obese. None was a

cigarette smoker. None had received hypolipidemic agents in the 3 months before the study.

All volunters had serum cholesterol concentrations exceeding normal value, from 6.5 to 9.46 mM cholesterol. Fasting serum triglycerides were variable, with normal values in 4 subjects and above normal values in 6 other ones (1.7 to 5.0 mM). Glycemia were in the normal range.

Experimental design and diets

The protocol consisted of 1 week basal diet, 4 weeks experimental diet (basal diet plus 30 g/d wheat germ) and a 4 weeks follow-up coming back to basal diet. The wheat germ used was Supergermes coming from Diepal, France and contained 27.5 % protein, 27.2 % carbohydrate, 11.0 % fat and 9.7 % total dietary fibre . Its pancreatic lipase inhibitory capacity was 497 inhibitory units per g, as measured in vitro (5).

The usual diet (basal diet) of each subject was monitored during the first week and for 3 days during the last week of the experimental period (basal diet plus wheat germ). During the control week, complex and refined carbohydrates, fat and proteins represented 41.0 %, 8.7 %, 35.9 % and 14.4 % of the total energy intake, respectively. The mean daily intake of dietary fibres was 13.6 g.

When comparing the basal diet plus wheat germ with the basal diet consumed during the week before supplementation, only the protein daily intake was significantly higher (p ≤0.05). The mean daily intake of dietary fibres increased from 13.6 to 15.1 g.

Plasma Analysis

Blood samples were taken after an overnight fast at baseline (first week of basal diet), after the 4 experimental weeks and after the 4 week follow-up period. The lipoprotein classes (VLDL: d≤ 1.006; LDL: 1.006 ≤ d ≤ 1.060; HDL: 1.060 ≤ d ≤ 1.21) were separated from 1.5 ml plasma by ultracentrifugation (38,000 rpm at 15 ° for 24 h in a Beckman SW41 rotor) on a KBr discontinuous density gradient. Total and free cholesterol, triglycerides, phospholipids and glucose were measured by enzymatic procedures.

Student's t test for paired values was used to assess the statistical significance of the differences observed between the experimental periods at the probability level of 95 %.
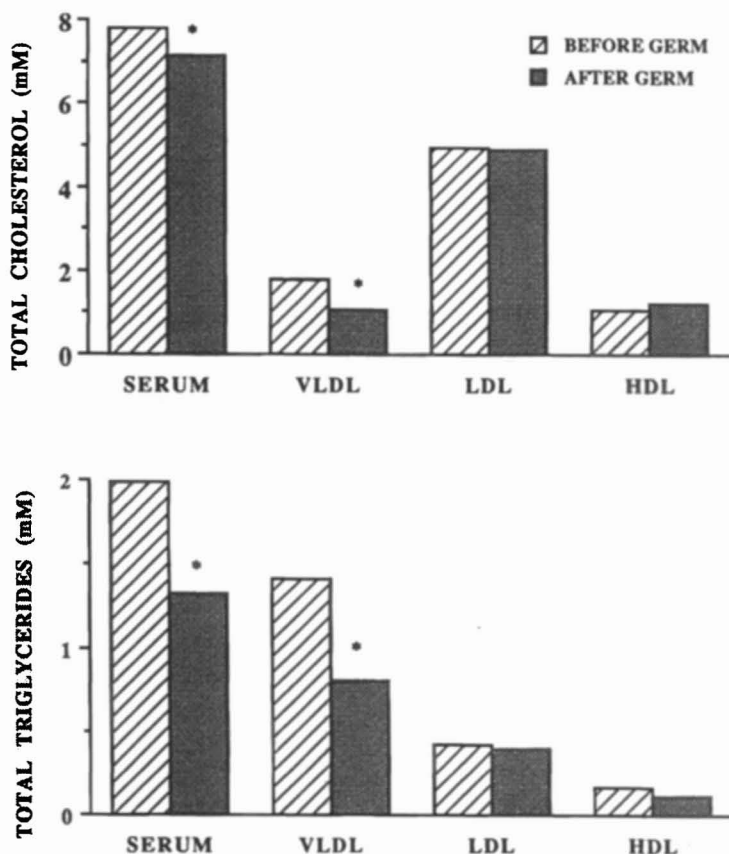
Figure 1 Changes in plasma or lipoprotein cholesterol and triglycerides after supplementing diet with 30 g/d wheat germ for 4 weeks. Paired columns (mean values of 10 subjects) bearing an asterisk are significantly different (p ≤ 0.05).

3 RESULTS

Normal glycemia did not change after wheat germ supplementation. The changes observed in plasma lipids and lipoproteins are shown in Figure 1. The intake of 30 g per day wheat germ significantly decreased plasma total cholesterol by 8.3 %. HDL cholesterol tended to increase moderately (+ 15.2 %) while LDL cholesterol did not noticeably change

(- 1.2 %). The VLDL cholesterol markedly decreased by 40.6 %.Thus, the plasma / HDL total cholesterol ratio significantly decreased from 8.74 to 7.24 after 4 week wheat germ intake.

As shown in Figure 1, mean plasma triglycerides of ten subjects markedly decreased (- 33.7 %) after one month intake of wheat germ. The LDL and the HDL-triglyceride concentrations were not modified over the experimental period, while VLDL-triglycerides significantly dropped by 43.3 %. Indeed, the mean triglyceridemia of the four normotriglyceridemic subjects did not change whereas that of the six hypertriglyceridemic subjects significantly decreased from 2.68 to 1.64 mM. VLDL-triglycerides were mainly affected.

4 DISCUSSION

The data obtained during this short-term study done on subjects with type IIa and IIb hyperlipoproteinemia are strikingly comparable to those previously obtained in the adult rat (4). Indeed, supplementing a high fat-cholesterol diet with 7 % wheat germ for 7 weeks resulted in significant decreases in VLDL-cholesterol, VLDL-triglycerides and in the plasma / HDL total cholesterol ratio in the rat.

Numerous studies have been already performed to investigate the effects of fibre-rich cereal fractions on lipid metabolism but the mechanisms involved in the alteration of lipid metabolism by dietary fibre-rich fractions are still debated(6). Concerning wheat germ, it is striking to observe a parallel lowering effect on both elevated plasma cholesterol and triglycerides in the rat and in humans; this can account for the drop in the Very Low Density Lipoproteins. In fact, we have already demonstrated in the rat (5,7) that addition of 10 % wheat germ into fatty test-meals lowers fat lipolysis and thus the intestinal uptake and the output in the blood stream of dietary lipids and cholesterol.

The respective role of different wheat germ components may be briefly discussed. The addition of 2.9 g dietary fibres provided daily by wheat germ did not significantly increase the fibre intake and it seems unlikely that this low amount of wheat germ fibres, mainly composed of cellulose and hemi-celluloses, plays a key role in lipid metabolism.(8,9).

The protein intake significantly increased because the high protein content of wheat germ. Various vegetable proteins

have plasma cholesterol lowering properties and proteins isolated from wheat germ have the ability to inhibit pancreatic lipase activity in vitro (8,10) and in vivo in the rat gut (5).

The total amount of phytosterols (98 mg/30 g) provided by wheat germ could also contribute to the lowering of plasma cholesterol.Phytosterols are well known to inhibit the intestinal absorption of cholesterol and indeed wheat germ was shown to decrease the intestinal uptake dietary cholesterol in the rat (7).

Since the accumulation of apo-B containing lipoproteins is well known to increase the risk for atherosclerothic lesions, wheat germ supplementation could represent a preventing factor.

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PRUNES AS A SOURCE OF DIETARY FIBER

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Prunes are a partially dried fruit derived from a vari-
ety of plums. We were interested in studying prunes as
a source of dietary fiber for several reasons. Analysis
of the fiber content of prunes (32% moisture) indicated
that they provide 6-7 g of total dietary fiber per 100
g, an amount that is typically higher than most fresh
fruits. An analysis of the carbohydrate components of
prunes conducted by John Labovitch, Ph.D. (Department of
Pomology University of California, Davis) indicated that
approximately 60% of the total dietary fiber in prunes
is pectin. Consumption of pectin has been associated
with a decrease in plasma cholesterol in human clinical
trials and in experimental animal studies (1-7). The
dose of pectin fed in the human studies ranges from 9-50
g per day with 15 g as a typical daily dose. The hypo-
cholesterolemic effect of pectin has been associated
with an increase in fecal bile acid excretion, although
this response has not been observed in all studies.
Stasse-Wolthuis et al. (7) have reported that increasing
the amount of fruits and vegetables in experimental
diets in order to provide an additional 8 g of pectin
daily lowers plasma cholesterol in human volunteers.
For modification of diets consumed by the population as
a whole it is clearly important to test the efficacy of
pectin-containing foods in lowering plasma cholesterol.

The objective of the study was to determine if
adding 100 g of prunes to the diet daily would be asso-
ciated with some of the potential benefits of dietary
fiber, including a lowering of plasma cholesterol and an
increase in fecal bulk.

Male subjects with plasma cholesterol levels of
5.7-6.0 mmol/L were recruited as volunteers. Each sub-
ject participated in the study for 10 weeks which in-
cluded a 2-week training period, 4 weeks of consuming
100 g of prunes and 4 weeks of consuming 240 mL of grape
juice. Grape juice (GJ) served as the control to pro-
vide a similar intake of energy from carbohydrate. A
plasma sample and 3-day fecal collection were taken at
the end of each block. Food records were kept by each
subject throughout the experimental period.

The consumption of either prunes or grape juice,
which supplied 240 kcal primarily from carbohydrate, was
associated with a slight reduction in the percentage en-
ergy from fat and a slight increase in the percentage of
energy from carbohydrate. Substitution of prunes for GJ
lead to an average increase in fiber intake of about 6 g
per day. Both plasma cholesterol and LDL-C tended to be
higher in the GJ period than the baseline. However,
consumption of prunes resulted in levels lower than the
GJ control.

There was no difference in fecal wet or dry weight
between baseline values and the GJ control. However,
both wet and dry weights were significantly higher after
the prune period than the GJ control. The increased fe-
cal weight due to prunes indicated that subjects com-
plied with the consumption of the dietary supplements.

In the feces total bile acid excretion did not dif-
fer among the experimental periods. However, the fecal
concentration of bile acids was altered by supplementa-
tion with prunes. After the prune period the fecal con-
centration of secondary bile acids, and specifically the
concentration of lithocholic and deoxycholic acid, was
lower than after the GJ period. There were no differ-
ences in fecal bile acid concentrations between the GJ
and baseline periods.

These results indicate that increasing fiber intake
by about 6 g from a dried fruit was adequate to increase
fecal weight and lower fecal bile acid concentration.
Although the changes in plasma and LDL-cholesterol lev-
els were not large, analysis of our results indicates
that the change in fiber intake due to the prune supple-
ment was associated with a change in plasma cholesterol
and suggest that a larger increase in fiber intake may
have a greater effect on plasma lipids. Given the over-
all design of our study, our results indicate that the
addition of sources of fiber to the diet has benefits

beyond that of simply lowering total fat intake and that prunes can contribute effectively to total dietary fiber intake.

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EFFECTS OF RYE BRAN, OAT BRAN AND SOY BEAN FIBRE ON LIPID AND BILE METABOLISMS, AND GALLBLADDER MORPHOLOGY IN MALE SYRIAN HAMSTERS

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1 INTRODUCTION

Food sources of fibre are complex, and their physiological responses are different. Oat bran which is rich in water soluble, viscous fibre has been shown to have hypocholesterolemic effects.[1,2] Soy bean fibre, alhough containing less water soluble fibre, has also cholesterol lowering effects.[3] Rye bran contains considerable amounts of water soluble, highly viscous fibre[4] but little is known about its physiological effects. Some isolated fibre components prevent cholelithiasis in animals by influence on the bile metabolism[5] but little information is available on the effects of natural fibre sources. A lithogenic diet causes changes in morphology of the gallbladder in mice,[6] but whether dietary fibre has an effect on the morphology of gallbladder is still obscure.

The purpose of this study was to investigate the effects of rye bran, oat bran and soy bean fibre on serum cholesterol level, bile composition, gallstone formation, and the morphology of the gallbladder in hamsters.

2 MATERIALS AND METHODS

Male Syrian hamsters (age 4-6 wk) were randomized into 7 dietary groups, 8-14 animals in each group. Two basal fibre free diets were used in this experiment: The O1 diet is a stone provoking diet based on glucose and casein,[7] and the non-stone provoking O2 diet is based on

Table 1 Diet composition and nutrient content (%,w/w)[a]

	O1	O-O1	S-O1	R-O1	O2	S-O2	R-O2
Glucose	72.7	31.5	61.9	54.4	0	0	0
Casein	20.2	8.6	17.1	15.1	0	0	0
Wheat starch	0	0	0	0	38.2	32.6	26.2
Milk protein	0	0	0	0	37.6	29.0	23.9
Corn oil	0	0	0	0	15.9	13.1	11.9
Vitamins[b]	1.0	0.4	0.9	0.7	1.2	1.0	1.0
Minerals[b]	5.1	2.2	4.3	3.7	6.1	5.1	4.9
Gelatine	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Oat bran	0	56.3	0	0	0	0	0
Soy fibre	0	0	14.8	0	0	18.2	0
Rye bran	0	0	0	25.1	0	0	31.1
Protein	18.0	17.9	16.9	17.5	26.1	22.1	21.6
Fat	0.2	4.6	0.2	1.0	16.4	13.6	13.3
Carbohydrates	73.3	56.9	63.8	62.0	40.4	34.1	33.1
Dietary fibre[c]							
soluble	0	3.4	1.3	2.5	0	1.6	3.0
insoluble	0	8.6	10.7	9.5	0	13.2	11.9

[a]fresh wt; [b]reference (7); [c]excluded from carbohydrates.

starch, milk protein and corn oil. The diets of O-O1, S-O1 and R-O1 were prepared by supplementation of the O1 diet with oat bran, soy bean fibre and rye bran. The S-O2 and R-O2 diets were the O2 diet supplemented with soy bean fibre and rye bran. Diet composition and nutrient content are listed in Table 1. The animals were fed respective diets for 6 weeks before sacrifice. The methods for sampling and analysis of bile and serum, and the evaluation of the gallstone formation were essentially performed as previously described.[8] The bile phospholipid was determined according to the method of Qureshi et al.[9] The lithogenic index was calculated according to Thomas et al.[10] The morphological analyses were carried out on 5 μm paraffin sections of the gallbladder wall stained with H & E or Van Gieson's method. The point counting method was used for the stereological volumetric analysis.[11]

For statistical evaluation the X^2-test was used for the frequency of gallstones and Student's t-test for determining the significance of difference of mean value. The results in tables 2-5 are reported as the mean ± S.E. The symbols *, ** and *** denote the following significance levels as compared with the fibre free control diets: P<0.05, P<0.01 and P<0.001.

3 RESULTS AND DISCUSSION

Oat bran and soy bean fibre have repeatedly been shown to exert hypocholesterolemic effects.[1,2,12,13] Results in this experiment are also consistent with these former findings (Table 2). The cholesterol lowering effect was also observed in the animals fed O1 and O2 diets supplemented with rye bran. The water soluble fraction of fibre in the oat is supposed to be responsible for the hypocholesterolemic effect. Although diets supplemented with rye bran and soy bean fibre contain less water soluble fibre than the oat diet it may still play a role in lowering serum cholesterol. In rye, the dominant part of dietary fibre is pentosans. The water soluble part of pentosans is known to have high viscosity.[4] Whether this plays a role in lowering serum cholesterol should be considered.

Some isolated dietary fibre components prevent cholesterol gallstone formation in animal experiments.[5,14,15] Although the mechanism is still obscure changes in bile metabolism and a lowered lithogenic index are supposed to be the main reasons. In this experiment, rye bran supplementation reduced the incidence of gallstone formation significantly (Table 3) as compared to the control, O1 diet group. The concentration of bile acids was significantly higher in groups fed the rye bran diets.(Table 4), but the

Table 2 Effect of dietary fibre supplementation on serum cholesterol level (mmol/L)

Dietary group	Serum cholesterol	Dietary group	Serum cholesterol
O1(14)	4.19±0.24	O2(9)	4.03±0.15
O-O1(10)	2.91±0.16***	S-O2(8)	3.10±0.29***
S-O1(10)	3.28±0.20*	R-O2(8)	2.90±0.15***
R-O1(10)	2.73±0.16***		

Table 3 Effect of dietary fibre supplementation on gallstone formation

Dietary group	Number of animals with gallstones	Dietary group	Numberof animals with gallstones
O1(14)	8	O2(10)	1
O-O1(10)	3	S-O2(8)	1
S-O2(10)	4	R-O2(8)	0
R-O2(10)	1*		

Table 4 Effects of dietary fibre supplementation on
bile composition (mmol/L) and lithogenic index

Dietary group	Bile acids	Phospho-lipids	Chole-sterol	Lithogenic index
O1(14)	10.6±0.4	5.6±0.4	1.5±0.2	1.1±0.1
O-O1(10)	11.6±0.4	5.5±0.4	1.0±0.2*	0.5±0.1*
S-O1(10)	9.8±0.7	5.3±0.4	1.3±0.1	0.9±0.1
R-O1(10)	14.5±1.1*	6.0±0.5	1.7±0.1	0.8±0.1
O2(9)	11.8±1.2	9.6±1.0	1.2±0.1	0.7±0.1
S-O2(8)	15.8±2.1	8.3±2.4	1.1±0.1	0.5±0.1
R-O2(8)	17.0±1.1**	9.3±2.1	0.8±0.1	0.3±0.0***

lithogenic index was lower only for the hamsters fed the
O2 diet supplemented with rye bran as well as in those
fed the O1 diet supplemented with oat bran. Most
gallstones found in this experiment were of the
cholesterol and pigment mixed stones. Their frequency
did not correlate well to the lithogenic index. This is
inconsistent with previous findings where the stone
provoking diet produced only cholesterol gallstones. The
reason for this may be the varying response in different
animal strains or some other unknown factors.

When mice were fed a lithogenic diet, an increase
in volume density of gallbladder epithelium was observed
(6). In this experiment the O1 diet seemed to have a
similar effect on hamsters. Whether this change is
associated with the formation of gallstones is not
clearly known. Rye and oat bran supplementation to the
lithogenic, O1 diet decreased the volume density of the
epithelium (Table 5). In addition, supplementation with
rye bran, oat bran and soy bean fibre to the O1 diet

Table 5 Effects of dietary fibre supplementation on
volume densities of epithelium and smooth muscle of the
gallbladder (volume percentage to gallbladder wall)

Dietary group	Epithelium	Smooth muscle
O1(13)	36.0±1.1	33.4±1.2
O-O1(10)	32.4±1.0*	40.8±2.4**
S-O1(9)	34.0±1.1	41.9±2.3**
R-O1(10)	32.8±0.8*	38.0±1.2*
O2(8)	33.5±1.7	32.7±1.8
S-O2(8)	33.8±1.0	38.7±2.3
R-O2(8)	29.8±1.2	34.2±1.9

increased the volume density of the smooth muscle layer. The mechanism for this is unclear and should be studied further.

In conclusion, rye bran as well as oat bran and soy bean fibre were found to have serum cholesterol lowering effects in hamsters. Rye bran increased the concentration of bile acids in the bile and prevented gallstone formation in the hamsters. These fibre sources also exert effects on the morphology of the gallbladder wall.

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INTRODUCTION

It is often stated that the processing of dietary fibre sources could induce physical and chemical changes in the dietary fibres and alter their physiological effects. However, very few human studies have actually addressed processing effects. With their comparatively thin and little lignified cell walls vegetables appear to be attractive study materials for this purpose. In addition, many vegetables can be easily consumed both as raw or processed foods. Yet, vegetables received little attention in comparison with cereal fibres or purified fibre sources.

Carrots were chosen for this study which followed a similar protocol as previously applied to assess the faecal bulking capacity and other properties of a number of fibre sources[1-4]. Two hundred grams of raw carrots per day have been reported to lower serum cholesterol by 11% after three weeks and to increase faecal weight, faecal bile acids and faecal fat[5], but fiber-mediated effects of processed carrots have not been investigated.

EXPERIMENTAL

Twelve healthy females (age 21-27) volunteered for this study which comprised four experimental periods each of three weeks in a latin square design. These periods were separated from each other by at least three weeks in order to avoid carry-over effects. During all periods the subjects consumed the same, strictly controlled, basic diet which provided about 15 g dietary fibre per day. During

three periods this diet was supplemented with either raw, blanched or canned carrot cubes of equal size, in amounts providing additional 15 g dietary fibre. Blood samples were drawn at the beginning and end of each period, all stools and urines were collected during the third week of the periods.

RESULTS AND DISCUSSION

Raw, blanched and canned carrots increased mean stool weights similarly, from 93 g/day during the basal periods to 129-151 g/day during carrot periods. Dry weights increased by about 30%, faecal water by 43-72%. The faecal bulking of carrots was comparable to fine wheat bran[2].

Faecal fibre increased from an average 6 g/day (basal diet) to 8 g (carrot periods). Thus apparent carrot fibre digestibilities were around 85%, with no difference between the different carrots. Such high carrot fibre fermentabilities have recently also been found in rats[6,7].

Serum cholesterol decreased during all carrot periods, by 14, 19 and 11% for raw, blanched and canned carrots, respectively. There were no significant differences between the different carrots, but a 12% decrease was also observed after the basal diet alone, making a mainly carrot-mediated cholesterol-lowering effect unlikely.

The mean daily faecal bile acid excretion and the extent of conversion of primary to secondary bile acids were similar in all periods. Faecal bile acids were more diluted after carrots than after the basal diet, but the interindividual variation was considerable.

CONCLUSIONS

a) Dietary fibres in carrots are highly fermentable and yet have good stool bulking ability in man.
b) In spite of appreciable effects of processing (blanching and canning) on carrot texture and microscopic structure (C. Schlienger & C. Probst, personal communication), the physiological effects of raw and processed carrots were very similar.
c) There appears to be good agreement between the faecal parameters measured in this human study and the results obtained with similarly processed carrots in rats[6,7].

This finding further strengthens the rat model for the predictive screening of dietary fibre sources.

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WORKSHOP REPORT: DIETARY FIBRE AND STEROL METABOLISM - WHAT SHOULD WE MEASURE APART FROM PLASMA TOTAL CHOLESTEROL?

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Suppose that a particular fibre has been shown to lower plasma total cholesterol (TC). What additional data should be gathered that is of potential benefit to the consumer at large, the physiologist or even the food manufacturer?

In his opening remarks the Chairman (Dr. C. Berry, Chorleywood) noted that lowering of TC per se cannot be assumed to confer reduced risk of CHD if we are ignorant of the fate of that cholesterol. Dr. David Kritchevsky supported this point by citing instances of 2 cholesterol-lowering strategies - colestipol/nicotinic acid and partial ileal bypass, which increased rather than reversed atherosclerosis in a minor but nevertheless substantial proportion of patients (e.g. ~14%). The Chairman suggested that monitoring of faecal total sterols (acidic and neutral) remained an important non-invasive method for confirming that surplus plasma cholesterol was indeed lost from the body, but that it should be done in a way that recognised the existence of steady-state constraints. Thus the decrement in plasma cholesterol may appear in stool as a short-lived pulse in faecal sterols; the latter may then settle back to their original value even though the diet remained supplemented with fibre. Failure to detect increased faecal sterol output at a single fixed arbitrary sampling time was not inconsistent with any of the suggested mechanisms of cholesterol-lowering, including sequestration by fibre of sterols in the gut. Additionally, careful measurement of the final steady state value of faecal sterol excretion, relative to its initial value before feeding fibre, may give important clues to effects of the fibre and associated substances on rates of de novo cholesterol synthesis. The latter may be

increased, for example, by homeostatic mechanisms (relief of feedback inhibition etc.).

If this is shown to occur, then a search might usefully be made for synergistic agents in diet that, when co-ingested with fibre, might prevent or attenuate the rebound effect.

Elizabeth Lund presented a survey of literature data showing that the hypocholesterolaemic effect of oat bran diminished with initial plasma TC concentration. Dr Kritchevsky and others considered that more weight should be given to fibres that lowered TC that was not initially greatly elevated.

Dr Gur Ranhotra (American Institute of Baking) reported that he had lowered plasma TC and apolipoprotein B in human subjects by increasing soluble dietary fibre supplied primarily in the form of refined cereal products. The precise mechanism of the effect is unknown.

Dr John Mathers (Newcastle) reported that including peas (a rich source of fermentable fibre and slowly digestible starch) in the diet of rats increased significantly the activity of hepatic HMG Co A reductase. Similarly, feeding guar gum increased *in vivo* hepatic cholesterol synthesis measured by the tritiated water incorporation method. However orally administered SCFA given in the amounts expected to be produced from the guar gum fermentation had no effect on liver cholesterol synthesis. Thus the data suggest that the rat liver readily compensates with increased synthesis when sterol excretion is raised and that propionate is unlikely to be responsible for cholesterol lowering.

Dr Linda Morgan (Surrey) reported studies in normal and diabetic subjects in which effects of guar on apolipoproteins were monitored. She recommends measurement of the ratio of apolipoproteins A_1/B as being of superior predictive value for CHD than total cholesterol, or ratios of its complexes with lipoproteins.

Dr David Topping (CSIRO Australia) presented data showing synergism between fish oils and dietary fibres (wheat and oat brans) on plasma TC. Whilst omega-3 fatty acids on their own are not hypocholesterolaemic in man, they are potentially valuable as synergists by their ability to prevent the down-regulation of the LDL-receptor. Effects of this nature will no doubt be of great potential for both food manufacturers and the general public.

Dr Zhang (Umea, Sweden) had been observing hypocholesterolaemic effects of oat bran and also, interestingly, a source of insoluble fibre (spent brewers grains) in ileostomy subjects, which reinforces data from Dr Mather and others that SCFAs are not obligatory for cholesterol-lowering.

In the short time available at the end for discussion, it was agreed that fibre effects on sterol metabolism were so diverse and complex that it was not sensible at the present time to recommend rigid test protocols, even for routine documentation. This area must still be approached with an open mind.

Dietary Fibre and the Food and Pharmaceutical Industries

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RAW MATERIALS NATURALLY RICH IN FIBRE VERSUS FIBRE CONCENTRATES/ISOLATES IN FOOD 'PRODUCTION

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1 INTRODUCTION

The tremendous interest in dietary fibre, both among
nutritionists and in the general public, has stimulated
food producers to upgrade fibre rich waste products -
hitherto used mainly as cattle feed - to food ingredients.
The recommendations on dietary fibre intake issued
recently in several countries - 3 g/MJ in the Scandinavian
countries (1) - has reinforced the possibility to enrich
foods with high fibre ingredients, in order to help in
reaching these recommendations.

The use of gums and other soluble, gel-forming poly-
saccharides as consistency aids in foods preceeds the
dietary fibre era, but it is now realized that their use
may contribute in providing nutritionally beneficial
properties attributed to this type of dietary fibre.

This paper addresses the question to what an extent
physiological properties of foods naturally rich in fibre
can be restored by addition of fibre concentrates or
isolates.

2 CLASSIFICATION AND NUTRITIONAL PROPERTIES OF FIBRE PREPARATIONS

A large number of fibre concentrates or isolates are now
available. A recent overview (2) listed about 20 different
sources of fibre, many of them available from several
producers. Another special issue (3) listed 128 different
food fibre preparations available in the United States in
1987.

Table 1 attempts to classify various fibre sources.

As for protein preparations, it might be useful to
distinguish between concentrates that contain appreciable
amounts of non-fibre material, and isolates of more or
less pure fibre polysaccharides.

A number of raw materials with very high fibre
content are available, that have not earlier been used in
foods. These include oat hulls, straw, and cellulose. The
dietary fibre in these materials is almost completely
insoluble and difficult to ferment in the intestine,
resulting in a good fecal bulking capacity (4). Their
content of available nutrients is negligible.

Cereal bran from wheat, rye and oats can be regarded
nutritionally as flours, that are enriched in dietary
fibre and nutrients, roughly parallelly. However, the
phytic acid, located mainly in the aleuron cells that
contain high concentrations of nutrients as well, is also
enriched.

<u>Table 1</u> Classification of fibre concentrates and isolates

<div align="center">

<u>Concentrates</u>

</div>

        1. Husk, straw etc
        2. Bran - wheat, oats, rye, barley,
                  maize, rice, soy, pea
        3. Cotyledon - soy, pea
        4. Tuber pulp - beet, carrot, potato
        5. Fruit pulp - orange, apple etc.

<div align="center">

<u>Isolates</u>

</div>

        1. Purified, natural polysaccharides
           - pectins, gums, alginates,
           carragheenan etc

        2. Processed, natural polysaccharides -
           microcrystalline cellulose
           - carboxymethyl cellulose etc
           - modified food starches
           - resistant starch (retrograded amylose)

        3. Related undigestible poly- or
           oligosaccharides
           - polydextrose
           - inulin, fructo-oligosaccharides
           - soy α-galactosides

Brans are used either raw or processed. In the case of oat bran, heat processing to inactivate lipases is necessary for an acceptable shelf life. Other enzymes, including the phytase naturally occurring in oats as well as in other cereals (5), are also inactivated. Therefore, enzymic degradation of phytate is less likely during processing of oat bran containing foods. This problem needs further consideration in view of the present emphasis on the consumption of large amounts of oat bran. The bioavailability of zink in oat porridge has been demonstrated to be very low (6).

As illustrated in Fig. 1, rye and barley contain as much soluble fibre as oats. Rye bread gave lower post-prandial glycemic response than the corresponding wheat bread (7), an effect most probably due to the soluble fibre content of rye. A further glucose lowering effect is obtained by baking with whole grains of rye (8).

Fibre preparations from leguminous seeds are available both from the outer layers (bran) and from the cotyledon, with distinctly different composition and properties. The bran preparations contain mainly insoluble fibre, whereas the cotyledon fibre preparations are rich in pectins and hemicelluloses, partly soluble. The considerable amounts of protein in these preparations must be considered when evaluating their physiological effects, for instance on serum cholesterol levels.

Fibre preparations derived from the pulp of sugar beets, carrots or potatoes have a relatively high soluble fibre content. Therefore, they have been regarded as promising concerning effects on serum cholesterol levels and postprandial glucose response.

The fibre isolates can be divided into 1) Purified, natural polysaccharides traditionally used as food additives - pectins, gums, alginates, carrageenan etc and 2) Modified materials, e.g. microcrystalline cellulose and carboxymethyl cellulose. Chemically modified food starches and resistant starch (retrograded amylose) should also be regarded in this context. The physiological properties vary widely due to the different physico-chemical properties. A third group, not analysed as dietary fibre with any current method, include polydextrose, inulin and fructo-oligosaccharides, and soy a-galactosides. These are not absorbed in the small intestine and can therefore be expected to have physiological properties generally attributed to dietary fibre. The reason why they are not analysed as dietary fibre is their solubility in 78 - 80 %
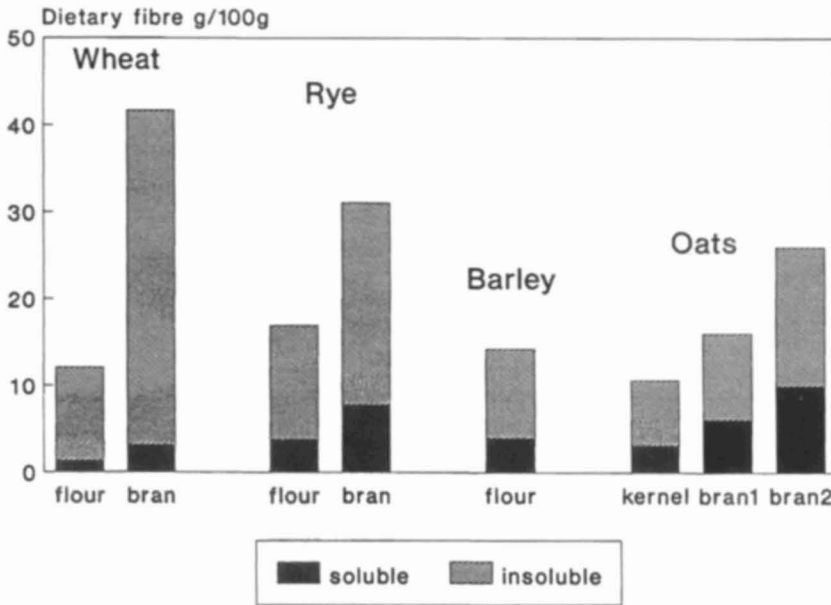
Figure 1 Soluble and insoluble dietary fibre in cereals
analysed according to Asp et al. (9). Data from
4,10-12. The extraction rate of the barley flour
corresponds to about 90 %.

ethanol. Polydextrose, and also chemically modified
food starches (13) are alcohol soluble after degradation
to low molecular weight fragments by amylase used in
dietary fibre analysis.

3 USE OF FIBRE CONCENTRATES AND ISOLATES

Dietary fibre concentrates or isolates can be used in
different ways (Table 2): 1) As functional ingredients at
a low concentration (usually 0.5 - 2 %) or 2) To increase
the dietary fibre content. In the first instance,
physiological effects of the fibre per se can hardly be
expected. However, the fibre addition can have important
indirect effects, enabling the formulation of foods with
reduced fat and energy content.

Only a few types of foods are suitable for incorpo-
ration of large amounts of fibre: Bread, breakfast

<u>Table 2</u> Fibre concentrates/isolates can be used in different ways

	<u>Functional ingredient</u>	<u>Dietary fibre enrichment</u>
Typical food	Meat prod Jam Ketchup Bread	Bread Breakfast cereals Bars
Typical amount	0.5 - 2 %	5 - 20 %
Dietary fibre/ serving	< 1 - 2 g	2 - 10 g

cereals, other cereal products, bars etc. Only foods containing appreciable amounts of fibre should be marketed with claims on fibre content. The Association of Swedish Food Industries recommends (14) that only foods containing at least 10 g dietary fibre/1000 kcal or 2 g/serving should be claimed as "containing fibre". The corresponding limits for claiming "rich in fibre" are 15 g/1000 kcal or 3 g/serving.

4 PHYSIOLOGICAL EFFECTS OF ADDED FIBRE

The physiological effects of dietary fibre in foods are related partly to properties of the fibre polysaccharides themselves, and partly to structural features of plant cell walls, attained by the fibre. Addition of fibre preparations can restore some, but not all of these effects. Recent studies with fibre preparations, especially sugar beet fibre (Fibrex[R]), will be reviewed to illustrate this point.

<u>Glycemic response</u>

A number of food properties - in addition to the content of soluble, viscous types of dietary fibre - influence the glycemic reponse (15,16), as shown in Table 3. Gross and cellular structure, as well as degree of starch gelatinisation (17) seem especially important.

Incorporation of 12 g dietary fibre (about 3 g soluble fibre) from Fibrex into a breakfast meal decreased postprandial glucose response in type 2 diabetics significantly (18). In healthy controls, however, only the insulin response was decreased (19). In spite of a sizable increase in soluble and total fibre, these effects were

Table 3 Food properties influencing the rate and extent
        of carbohydrate digestion and absorption

- Gross and cellular structure
- Dietary fibre
- Starch gelatinisation
- Amylose/amylopectin ratio
- Starch/lipid complexes
- Starch/protein interactions
- Starch retrogradation
- Amylase inhibitors

rather small compared to the large variation in glycemic
indices reported for different foods (16). A similar
experiment with a potato fibre preparation containing
comparable amounts of total and soluble fibre gave no
significant effect on the glycemic response in type 2
diabetics (Hagander et al., to be published).

An 8+8 weeks cross-over study in well controlled type
2 diabetics with 30 g sugar beet fibre incorporated into
various foods in a conventional diabetic diet (percent of
energy from protein 20, fat 35, and carbohydrates 45;
dietary fibre in basic diet g/day) failed to show any
significant improvement of the glycemic control. The blood
pressure, however, was lowered significantly (19).

Two studies have been reported in which guar bread
was given to type 2 diabetics providing 5 or 6 g guar
gum/day with improvements of serum cholesterol and
glycosylated hemoglobin values (20,21). The palatability
of guar bread can be questioned, however.

In summary, incorporation of suitable dietary fibre
preparations into bread and other cereals may help to
reduce the glycemic response with possible favorable long
term effects. However, other factors such as the form of
the food, its cellular structure and the degree of starch
gelatinisation seem to be more important in this respect.

Serum cholesterol level

It is well documented that soluble, gel-forming types
of dietary fibre lowers the serum cholestrol, and that the
decrease is due to a fall in LDL-cholesterol. Pectin and
guar gum have been most extensively studied in doses
generally around 10-15 g/day, i.e. considerably more than
present in a normal diet. In studies with guar gum, 5 g/
day is the lowest dose reported to lower the serum
cholesterol significantly (for review see 22).

Sugar beet fibre (22-26 g/day) has shown significant lowering effect on total and LDL cholesterol in two different studies (23,24), whereas the 8+8 weeks study on diabetics (19) did not show any significant influence on plasma lipids.

The cholesterol lowering effect of oat bran has been much emphasized recently and attributed to its ß-glucan content. Several studies have demonstrated a 3 - 5 % cholesterol lowering effect of oat bran in addition to that obtained by a fat modified diet acording to current recommendations. The recent study by Swain et al (25) is compatible with ealier studies in that it does not exclude a 4 % cholesterol lowering effect of the oat bran fibre, although the fat displacement effect of adding a large amount of any cereal is emphasized.

The contributions to the serum cholesterol lowering effect of various dietary changes in a typical Western diet according to current dietary guidelines suggested by Kay and Trushwell (26) still seem valid. These authors expected about half of the effect to be due to fat modifications, whereas decreased dietary cholesterol, increased dietary fibre, and increased ratio vegetable/ animal protein was judged to be of roughly equal importance in explaining the rest of the change. Thus, a lowering of the fat content by incorporation of dietary fibre may be more important in influencing plasma lipids than specific effects of the fibre in itself.

Fecal bulking
The fecal bulking capacity of dietary fibre is inversely related to the fermentation. For example, oat hulls are very difficult to ferment and have a high bulking capacity, whereas oat bran is more extensively fermented with a lower bulking capacity (5). There is no evidence that added fibre preparations have different bulking effect than corresponding naturally occurring fibre, although particle size and heat treatment have been reported to influence the bulking effect of wheat bran.

Sugar beet fibre increased fecal dry matter in rats by 0.6 g/g fibre (27) - a figure similar to that obtained for both raw and variously processed vegetables (28). Human studies also show similar fecal bulking capacity of fibre from sugar beet and carrots (W. Feldheim, personal communication; E. Wisker, W. Feldheim, T.F. Schweizer: Metabolic effects of raw and processed carrots in humans, this symposium).

Table 4 Relationship between properties of dietary fibre and physiological effects

Main effects	Solubility	Viscosity	Monomeric comp.	Lignification	Functional groups/associated compounds
Fecal bulking	- - -			+ + +	
Fermentation	+ + +			- - -	
Glycemic response	+	+ + +	+		+
Lipoprotein levels	+ +	+			+
Mineral binding					+ + +

5 PREDICTION OF PHYSIOLOGICAL EFFECTS BY IN VITRO ASSAY

In view of the highly variable physiological effects of different dietary fibre preparations, methods capable of predicting such effects are desirable. Table 4 summarizes properties of dietary fibre related to physiological effects.

Fecal bulking and fermentation are properties highly related to solubility and lignification, and glycemic response is influenced by viscous fibre. Solubility is also related to effects on plasma lipids. Monomeric composition has some relationship to effects on glycemic response and plasma lipids, in that pectins with galacturonic acid as main monomeric constituent is one class of dietary fibre polysaccharides with such effects. In general, however, the monomeric composition is a poor predictor of physiological effects. There is a great need for further method development for prediction of physiological response.

6 CONCLUSIONS

Dietary fibre concentrates or isolates can be used as functional components in small amounts, or as dietary fibre enrichment/fortification. Structural effects of dietary fibre may be nutritionally important and cannot be restored by addition of fibre concentrates or isolates. Indirect effects due to lowered fat and energy content may be more important nutritionally than physiological effects of the fibre per se. Cereal brans can be regarded nutritionally as enriched flours in that nutrients are concentrated roughly proportionally to the fibre. Even detailed chemical analysis of fibre polysaccharides does not predict physiological function. So far, physiological effects have to be documented in vivo.

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DIETARY FIBRE ENRICHMENT, A SIGN OF QUALITY:
POSSIBILITIES IN BREAD.

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1. INTRODUCTION

The aim of this research project was to find out the limitations of
the enrichment of bread with dietary fibres, maintaining still a good
sensorial quality of the products. To meet this goal we tried to
find out new sources of dietary fibre which give a better acceptance
and possibly higher content of dietary fibre than wheat bran.

Table 1:   Contents of dietary fibre of some materials.

	dietary fibre (% dry matter)		
	soluble	insoluble	total
Soya bran	9.6	59.6	62.2
Pea bran	9.3	74.1	83.4
Cacao hulls	22.6	47.2	69.8
Coffee hulls	9.8	54.2	64.0
Sugarbeet fibre	26.1	26.0	52.1

We analyzed a great amount of different materials (some are given in
the table) for their content of dietary fibre and their rheological
properties with respect to bread making.

It turned out that soja bran and especially pea bran can be employed
for this purpose. Their acceptance was tested also regarding their
rheological properties in dough as well as their baking properties.

The breads were analyzed for their sensorial quality and their
volume. The results pointed out that an enrichment up to more than
20% dietary fibre (on dry matter basis) does not result in breads
with acceptable sensorial quality.

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1   INTRODUCTION

The main theme of the workshop was the practical, rather
than nutritional, aspects of high-fibre foods; i.e. what
could be produced commercially and accepted by the
consumer at large, taking account of legislative
requirements around the world.

Fibre concentrates or isolates are generally by-
products of the food industry. A wide range of sources
mentioned included cereal and legume brans (husks), sugar
beet fibre and by-products of fruit processing, e.g. from
citrus fruits, apples, grapes and olives. Other
potential sources from waste products of vegetable
preparation were identified; from a compositional point
of view, these could be good sources of dietary fibre,
but need to be studied further with respect to commercial
applications. The nature of the non-starch
polysaccharides in the different sources of fibre varies
widely, e.g. in solubility, degree of substitution,
cross-linking etc. Even in a given class, (e.g. pectins)
chemical and physical properties can be very different.
Thus, apart from nutritional considerations, the
resultant textural and organoleptic characteristics of a
food product will determine the type of fibre
incorporated.

Some constraints on the food manufacturer producing
fibre-enriched products were discussed; these included:-

- the cost, availability and potential variability in raw
  materials.
- functional effects on product quality; e.g. sugar beet

fibre can be used in pasta and some types of bread, but not in others.
- palatability; since the population at large regard eating as a pleasurable and social activity. Fibre-enriched foods must be enjoyable (not medicine!). It was suggested that palatability and nutritional studies on new types of food should be run in parallel.

The processes used to produce beet fibre and fruit pectin were described. For many types of fibre, processing is necessary for food safety, product shelf-life or organoleptic reasons. However, the type of processing can have important effects on the fibre quality. Examples quoted were the particle size of cereal brans and the type of hydrothermal treatment used; for instance, conventionally cooked cornflakes have much higher levels of resistant starch than do extruded cornflakes. Soluble fibre imparts high viscosity and, whilst this may be beneficial nutritionally, it limits applications in, e.g. drinks; partial hydrolysis reduces viscosity but raises questions about efficacy and definition of fibre.

Finally, legislative and labelling constraints in different countries were discussed. There are wide discrepancies, even within Europe. In the U.K. and Sweden, "wholemeal" means 100% and 70% whole grain, respectively; in Norway, food containing fibre isolates cannot be labelled as 'fibre-enriched'. Some rationalisation is needed, but this was outside the remit of this workshop!

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Purified soluble complex polysaccharides have been advocated as medicines to treat diabetes mellitus, hypercholesterolaemia and dumping syndrome. Some have been used as slimming agents, others, particularly isphagula and methyl cellulose, are used as bulk laxatives in patients with constipation and Irritable Bowel Syndrome.

1   DIABETES MELLITUS

The administration of viscous polysaccharides with a meal or with a drink of glucose reduces postprandial hyperglycaemia [1-6] and reduces the plasma insulin response. Does this matter? It is generally believed that the microvascular and macrovascular complications of diabetes are directly related to hyperglycaemia. Stabilising the blood glucose should help to delay the onset of these complications.

Viscous polysaccharides can also reduce reactive hypoglycaemia that is thought to occur when the 'too rapid' absorption of glucose causes and excessive secretion of insulin pushes the plasma glucose concentration down below the fasting level [7]. Thus, viscous polysaccharides may help to prevent hypoglycaemic episodes in patients taking a high carbohydrate, low fat diet especially with brittle 'type 1' diabetes. It has even been suggested that the increased plasma levels of acetate following colonic fermentation of polysaccharides may reduce the effects of hypoglycaemia by providing an alternative energy source for the brain [8].

Mode of action

Viscous polysaccharides are thought to reduce plasma glucose and insulin by delaying absorption of glucose in the small intestine. This action may have three components; 1: delay of the delivery of carbohydrate from the stomach to the small intestine, 2: delay in the ingestion of starch and 3: delay in the access of the products of digestion to the epithelial surface [9]. Viscous polysaccharides appear to work better when they are mixed more intimately with the food material than when they are given in a capsule form or even as a drink before the meal [3]. It is not always possible to predict the effect of these agents on blood glucose levels in vivo from measurements of viscosity in vitro, since dilution with acid and alkaline digestive juices can radically alter the viscous properties of some polysaccharide solutions [10].

Ingestion of viscous polysaccharides do not just reduce plasma glucose and insulin levels after the meal in which they are incorporated. They also influence postprandial glycaemic responses after the following meal [4]. There are at least two possible mechanisms; the first is that the delay in absorption of nutrients, particularly fat, causes an increased load to the ileum which then slows the emptying of the subsequent meal from the stomach and reduces the glycaemic response [11]. The second is that the short chain fatty acids released into the peripheral blood as a result of fermentation of the polysaccharide, may reduce hepatic glycogenolysis and enhance insulin sensitivity [12].

Is the prolonged use of viscous polysaccharides useful in treatment of type 2 diabetes?

Although the acute administration of viscous polysaccharides with a meal may reduce postprandial glycaemia, this does not mean that chronic administration will necessarily influence the disease process. Aro and his colleagues [13] have shown a reduction in fasting plasma glucose on long term guar with little effect on postprandial measurements. The success indices in long term management of type 2 diabetes are fasting plasma glucose, glycosylated haemoglobin levels, plasma lipids and weight. Studies by Simpson and his colleagues showed that a diet high in carbohydrate caused lower plasma glucose levels even when fibre intake remained the same [14]. But when

leguminous fibre was added, there were improvements in
both fasting and postprandial blood glucose levels [15-
17]. How does fibre work long term? Is the effect due
to a direct pharmacological effect in delaying
absorption or is it caused by a compensatory reduction
in other components of the diet? We attempted to
answer this question by treating a group of 24 obese
'type 2' diabetics with a sensible high carbohydrate,
low fat reducing diet, either by itself or together
with supplements of guar gum or bran [18]. Our results
showed that all three groups of subjects lost weight
and showed an improvement in glycoslated haemoglobin
levels and fasting plasma glucose and there was no
significant difference between each group of subjects.
We concluded that if the energy content of the diet was
carefully controlled, the addition of viscous
polysaccharide did not contribute significantly to
diabetic control. This did not necessarily mean that
the viscous polysaccharide is not effective in the
overall management of diabetes. On the contrary, it may
be effective but when taken ad libitum its mode of
action may be to assist the patient in reducing energy
and losing weight.

    Type 2 diabetes is very much related to the obese
state. Anything that causes weight loss in these
patients is likely to result in improved diabetic
control. Several studies have demonstrated that the
administration of viscous polysaccharides does cause a
modest weight loss in the diabetic patient and this can
be associated with an improvement in diabetic control.

2   HYPERCHOLESTEROLAEMIA

    Despite the doubts that blood cholesterol levels
may not be the best discriminators of risk of coronary
artery disease, there is nevertheless a very marked
association between mortality from coronary artery
disease and cholesterol levels [19]. A large number of
studies have demonstrated that viscous polysaccharides,
in particular guar gum [20], cause a reduction in total
cholesterol and LDL-cholesterol, most of the studies
have been conducted over a short term, but two recent
studies have shown that the effects of guar gum on
reducing cholesterol could be sustained for over 12
months [21, 22].

Mode of action

    It is unlikely that the hypocholesterolaemic

action of viscous polysaccharides occurs by direct
reduction in cholesterol absorption. Some complex
polysaccharides bind bile acids, impairing their
absorption. Under these circumstances, cholesterol
could be used to synthesise the bile acid lost in the
faeces or the bile acid pool could be reduced to such
an extent that insufficient bile acid could be
available for lipid solubilisation and absorption.
This hypothesis was not supported by Gallagher and
Schneeman [23], who recently showed that although guar
gum bound significant amounts of bile acid in the small
intestine it did not reduce the amount of solubilised
lipid. Other studies have suggested that viscous
polysaccharides could trap fat in the intestinal lumen
or delay the diffusion of micelles to the cell surface
[24]. Another theory is that hepatic cholesterol
synthesis is reduced by reduced postprandial insulin
and glucose levels; hyperinsulinaemia may be a primary
risk factor for the development of hypotrigliceridaemia
and coronary artery disease [25]. Plasma cholesterol
may also be reduced by increased portal blood levels of
propionic acid produced by bacterial fermentation of
polysaccharide in the colon. Finally, there are
significant correlations between obesity and
hypercholesterolaemia and between obesity and
arteriosclerosis. Several studies have shown that
reduction in plasma cholesterol induced by viscous
polysaccharides is related to a loss in weight [26].

3   OBESITY

When concentrates of viscous polysaccharides like
guar gum and pectin are added to test meals, they slow
down gastric emptying and increase feelings of satiety
[27]. When taken before a meal, however, the reduction
in hunger levels by guar gum is very brief [personal
observations]. Ingestion of crackers rich in
isphaghula immediately before a meal caused a small but
significant reduction in total energy intake over 2
week period [28]. Can this effect extend over a much
longer time period? Most controlled studies have shown
an advantage of supplements of dietary fibre over
placebo if a sufficiently high dose is taken [29-34].

4   DUMPING SYNDROME

Some patients experience severe postprandial
symptoms of nausea and faintness after gastric surgery.
These symptoms are thought to be explained by two
mechanisms. The early symptoms are thought to be

related to the rapid entry of food material into the
small intestine. This can distend the small intestine
and also cause profound osmotic shift of fluid from the
extracellular fluid space into the gut and consequent
hemoconcentration. The more delayed symptoms are
thought to be related to the rapid absorption of
glucose which causes an excess insulin secretion and a
reactive hypo-glycaemia. Supplements of viscous
polysaccharides taken with meals have been shown to
reduce the reactive postprandial hypoglycaemia and to
depress dumping symptoms [35].

5   IRRITABLE BOWEL SYNDROME

The popularity of wheat bran as a natural way to
manage IBS has made it the first line treatment for
this condition for over 10 years [36]. But is it an
effective treatment for IBS? Manning and Heaton showed
that the addition of 7 grams of fibre in the form of
wheat bran for 6 weeks to the diet of patients with IBS
resulted in significant improvement in symptoms [37].
The possibility that the control diet in Manning and
Heaton's study had little placebo effect [38] make the
results of the bran diet difficult to interpret in a
condition that shows such a marked response to placebos
[39]. Other placebo controlled trials of bran in IBS
[39, 40, 41, 42]. have failed to show any convincing
effect of the fibre on overall symptom patterns. The
most recent study [42] compared the effect of
supplementing the daily diet of 44 IBS patients with
either 12 bran biscuits (12.8 grams fibre) or 12
placebo biscuits (2.5 grams fibre). Patients were
randomly allocated to receive either high fibre or
placebo biscuits for 3 months and then to cross over to
the alternative biscuit for another three months. The
patients were not told which type of biscuits they had
been given, though some may have guessed! The results
showed that both placebo and bran groups experienced
similar improvements in overall symptom scores.
Moreover, the beneficial effects of bran were
independent of any change in stool weight. The paucity
of constipated patients may have influenced the
conclusions from this study.

Cann and his colleagues [39] compared the
independent responses of a number of typical IBS
symptoms to bran with the responses to placebo tablets
in 38 patients. Eighteen patients (47%) said they had
improved on bran treatment, but only five of these said
that they were entirely satisfied and did not require

further treatment. Eleven patients (29%) experienced
no change in their symptoms, and nine patients (24%)
found that their symptoms were exacerbated by a high
fibre diet. When the characteristics of the patients
who said they had improved on bran were compared with
those who said that their symptoms had not changed or
become worse, the bran responders had smaller, harder,
less frequent stools and longer colonic transit times
upon entering the trial than the non-responders. In
other words, it was the more constipated patients that
responded to bran. This impression was confirmed by
analysis of the responses to individual symptoms;
constipation was the only symptom that showed a
significantly greater response to bran than placebo.
This doesn't mean that none of the other symptoms
responded to bran. They did, but the responses were no
greater than the responses to placebo. This study
failed to confirm the suggestion that ingestion of
wheat bran in patients with diarrhoea delays rapid
intestinal transit [43, 44]. Instead, wheat bran
tended to accelerate transit in everybody and
exacerbate symptoms of diarrhoea.

So is there any point in advising patients to take
bran for their irritable guts? It would, in my
opinion, be a mistake to reject the idea completely.
What is needed is a sense of realism. Some IBS
patients, particularly those who are constipated,
undoubtedly obtain symptomatic relief from taking bran
in their diet and should continue to do so. Others
find that bran induces or exacerbates symptoms of
distension, flatulence, diarrhoea and abdominal pain.
Their hypersensitive and irritable guts are probably
irritated by the particulate nature of the bran, its
bulk and the products of its fermentation by colonic
bacteria. In the current climate of bran for
everything, judicious reduction in fibre intake in
these patients can prove very useful. For the
remainder, then bran is a less toxic 'placebo' than
many drugs and is better than placebo for constipation
associated with IBS.

Viscous polysaccharides

Are other forms of dietary fibre more effective in
IBS? Ritchie and Truelove [45] showed that ispaghula
was better than wheat bran when both were given in
combination with a psychotropic agent and an
antispasmodic. A large controlled trial, published
last year [46], Prior and Whorwell reported overall

symptomatic improvement in 82% patients, who had been
taking ispaghula compared with 53% patients who had
been taking placebo. Many of their patients had
constipation and in these ispaghula accelerated transit
time and increased stool weight. There was no evidence
that ispaghula improved patients with diarrhoea.

Kumar and his colleagues [47] carried out a dose
ranging study in a group of male Asian patients to
determine the optimum dose of ispaghula husk in IBS and
to assess the correlation between the relief in
patients' symptoms and colonic function. Thirty grams
of ispaghula caused a significant improvement in
patients symptoms, accompanied by an increase in stool
weight but no significant change in transit time.
Surprisingly, patients reported improvement in
diarrhoea, even though stool weight increased.

6   CONSTIPATION

Meta-analysis of the data from 20 studies [48]
showed that (i) stool output and transit times
responded less to fibre supplementation in constipated
patients than in normal subjects, and (ii) that
treatment with fibre of constipated patients often
failed to return transit time and stool output to
normal. There is therefore little evidence to support
the contention that constipation in all patients is
wholly caused by fibre deficiency and constipated
patients should not be blamed for non-compliance if
dietary advice fails. Constipation should probably be
regarded as a disorder of colonic or anorectal motility
that may respond to the mild laxative action of complex
polysaccharides rather than simply the result of a
fibre deficient diet [49].

How do complex polysaccharides work on the colon

Wheat bran and other bulk laxatives containing
complex polysaccharides are not digested in the small
intestine, but act on the colon to increase stool
weight and frequency to make the stool softer and
bulkier and to reduce whole gut transit time [36, 50].
The mechanism of the laxative action of bran and other
types of fibre on the colon is not established. The
most popular theory relates to their bulk. Fibre
contains plant cell walls that may resist breakdown by
bacteria; and the associated complex polysaccharides
adsorb and retain water. Complex polysaccharides also
stimulate microbial cell growth, resulting in a greater

faecal bacterial cell mass. Bannister and his colleagues [51] recently showed that small hard spheres mimicking faecal pellets are much more difficult to expel from the rectum than large spheres. Fermentation of fibre release gases which may be trapped in colonic contents, contributing to their bulk and plasticity. The increased colonic bulk promote colonic propulsion, which leads to reduced water absorption by the colon and the easier passage of bulkier and softer stools. In a recent study comparing the action of a number of complex polysaccharides with their fermentation characteristics, Tomlin [52] suggested that polysaccharides that resisted breakdown increased stool weight, while those that were fermented, accelerated colonic transit. The best bulk laxatives appeared to be those, like bran and ispaghula, that retained their structure but were also fermented. An increase in their volume of colonic contents will distend the colon, stimulating colonic propulsion and secretion [53].

There may, however, be other mechanisms of action. It is possible, for example, that the lignified particles of bran irritate the colonic epithelium, activating nervous reflexes that cause colonic secretion and propulsion. This may explain why coarse bran is a more potent laxative than the same amount of finely milled bran [54]. In support of this idea, Tomlin has recently observed [55] that the addition of 15g/day of small segments of polyvinyl tubing to the diet increases stool mass and frequency, improves stool consistency and accelerates whole gut transit to the same extent as the addition of an equivalent weight of coarse wheat bran.

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BEET FIBRE PRODUCTS: NEW TECHNOLOGIES AND PRACTICAL APPLICATIONS TO DIET THERAPY

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1 INTRODUCTION

As an alternative to guar and its products, already successfully experimented in some diabetic foods [1,2] but of limited palatability and acceptability, sugar beet pulp appears an attractive source of dietary fibre (DF).[3,4] It is in fact available in large amounts and contains high levels of fibre. Unlike guar, beet fibre is obtained from a local crop, of common use in Italy, more economical and also well accepted. This aspect was found of particular interest. Only with a good compliance it can be hoped, in fact, that diabetics will respect dietetic recommendations not merely for a few days but rather for the rest of their life, as needed for these patients.

The Aim of the present study was to investigate the metabolic impact of pasta enriched with beet fibre, natural or enzymatically modified, in comparison with commercial pasta or rice, traditional Italian dishes. Furthermore, the opportunity of integrating the DF intake with other beet fibre-enriched products (extruded and baked products), and its limitations, was evaluated.

2 MATERIALS AND METHODS

Ten non insulin-dependent diabetic (NIDDM) patients, mean age 59.8 ± 2.2 years, were recruited from the metabolic day care unit at the Ospedale Forlanini. The patients, all treated by diet alone, were in good metabolic control and homogeneous for weight (67.0 ± 3.5 kg), BMI (24.0 ± 1.7 kg/m^2), basal blood glucose (117.3 ± 3.8 mg/dL) and HbA_{1c} (5.9 ± 0.1 % Hb). All the patients were free from other major illness and not treated with any drug for the whole duration of the experiment.

After giving their informed consent to the study, the patients were summoned to the metabolic day care unit for a basal control and kept resting until lunch time. At that time, over a 30-min period each patient ate one of the three test meals, received in random order during three test periods at least 2-wk apart, under the supervision of dietitians.

The test meals, calculated to be isoglucidic and isocaloric, consisted of commercial pasta (100 g), beet fibre enriched pasta[*] (15% fibrex in the dough, 130 g) and rice (regular long grain white rice, 95 g), all served with the same tomato sauce. The ingredients of the sauce were the following: fresh ripe tomatoes, 250 g; celery, carrot and olive oil, 20 g each; onion and basil leaves, 10 g; salt. Duplicate samples of each meal were sent to the Food Chemistry Unit of the I.N.N. for composition analysis. Official AOAC methods were utilized for the major nutrients. DF was determined according to the method of Prosky *et al.*[5] As shown in Table 1, the actual meals were not isoglucidic, as calculated from tabular data.[6]

In vitro digestibility of starch was evaluated on the test meals, with and without sauce, and also on some other fibre-enriched products according to the method of Wong *et al.*[7] Sugars in the enzymic digesta were quantified by ionic chromatography.

Table 1   Composition of test meals (g/ration)

Nutrient	Pasta + F	Pasta	Rice
Protein (Nx5.7)	16.9	16.1	9.8
Lipid*	18.5	22.5	23.9
Soluble carbohydrates	9.9	11.8	11.8
Starch	76.5	65.4	51.6
Ash	6.0	5.1	5.9
Dietary fibre	14.3	7.5	4.8
soluble	3.8	2.8	1.5
insoluble	10.5	4.7	3.3
Energy Kcal	587	581	511
Kj	2456	2430	2139

*acid hydrolysis

Venous blood was collected *via* an indwelling needle before eating and 15, 30, 45, 60, 90, and 120 min after the beginning of the meal. Blood glucose was determined by a glucose oxidase method and serum insulin by RIA. Glycosylated Hb was tested by ion-exchange chromatography. Results are expressed as mean+SEM. Significance of differences between means was determined by the paired *t*-test · (two-tailed).

3 RESULTS

The changes in plasma glucose concentration after the oral ingestion of the three meals are presented in Figure 1. As it can be observed, the post-prandial glucose response to the pasta + fibrex meal was lower than those of the other two meals. However, a statistically significant difference (p<0.03) could be observed only when comparing fibre-enriched pasta with rice.
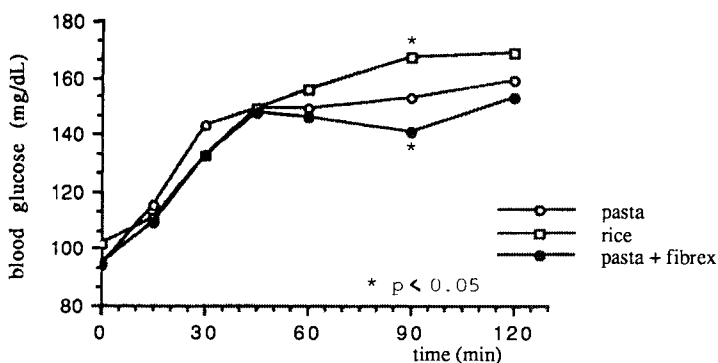
__Figure 1__   Mean (± SEM) blood glucose responses to the meals

Regarding hormonal changes the fibre-enriched pasta meal tended to produce a mean insulin rise intermediate between those of rice and pasta, the difference being statistically not significant.

The *in vitro* starch digestibility confirmed the results of *in vivo* absorption: both pasta meals showed lower digestibility (and consequently a more modulated absorption) than rice (Figure 2). The difference in starch digestibility was significant when comparing rice vs pasta (p<0.05), while in the case of rice vs fibre-enriched pasta more significant differences were observed in absence than in presence of the sauce (p<0.01 vs p<0.05, respectively).
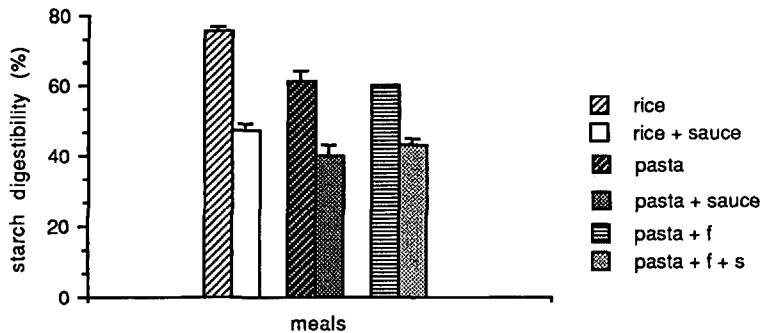
Figure 2   In vitro starch digestibility of the meals

4 DISCUSSION

Our results confirm that the rate of starch hydrolysis *in vitro*, as already reported,[8] is a useful mean to assess the degree of gelatinization of the starch in a product and to predict bioavailability of starch *in vivo*.

In vivo, Although the fibre-enriched pasta had a total DF content twice that of pasta (14.3 vs 7.5 g, respectively), their glycemic responses were not significantly different. It is well known however that only, or mainly, soluble fibre is metabolically active. Thus, probably the increment in soluble fibre of the fibre-enriched pasta (1.0 g) was not sufficient to induce a postprandial glycemic response significantly different from that of pasta. On the other hand, its increment vs rice (2.3 g) was sufficient to induce a significant difference.

Consequently, in view of the need of increasing the soluble DF content of fibre sources, a study has been undertaken to modify the soluble/insoluble DF ratio of beet pulp fibre by enzymatic treatment. The modified beet fibre has then been utilized in the formulation of extruded products (pasta and cracotte). Such products appear very promising, in terms of processing, DF content and *in vitro* starch digestibility, but their palatability still needs improvement.

To maximize the effect of soluble fibre on metabolic parameters without affecting patients' acceptability, it would in fact be helpful to have other fibre-enriched products. In the present study the fibre-enriched pasta and consequently there was very much liked by the patients and consequently there was no problem at all with

compliance. For daily use, traditional products such as for example pasta in Italy, are preferable to pharmacological fibre supplements (guar, xantham gum), particularly since the fibre incorporated in a food is more effective than fibre administered separately.[10]

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1 INTRODUCTION

During the last decades various epidemiological studies suggested a correlation between decreased ingestion of dietary fibres and some diseases mostly affecting Western countries (1,2).

These clinical conditions are represented by gastrointestinal diseases (constipation, diverticulosis, irritable bowel syndrome, cholelithiasis) and dismetabolisms (dislipidemia, diabetes, overweight and obesity) and cardiovascular pathologies (1,3).

The clinical research has recently confirmed the precise role of dietary fibres in the prevention and therapy of many of these diseases, and it has furthermore identified specific treatments with particular fibre types, such as the hydrosoluble ones (guar gum, glucomannan, pectin) for dismetabolical conditions and non hydrosoluble ones for gastrointestinal pathologies (4).

In fact, the efficacy of non soluble fibres, and in particular of wheat bran, in the treatment of diverticulosis chronic constipation and irritable bowel syndrome is well proved, due to their marked bulk forming effect (5).

The aim of the present experiment is to evaluate the efficacy, the safety and the compliance of the sugar beet fibre, which contains in high proportions soluble and non soluble components.

Materials and Methods

Twenty-seven patients (18 females and 9 males) ranging in age 21 - 42 years (mean age 42 years), complaining of chronic constipation, in absence of organic pathologies potentially responsible for stool alterations, entered the study. All the patients were treated with sugar beet fibre (Fibrex; SIRC, Rome) integrated by organic salts of iron, zinc and copper, according to the FDA requirements (Table 1, 1 bis) (8).

The fibre preparation obtained by means of drying and grinding the sugar beet pulp after sugar extraction, was pressed in tablets containing 750 mg of product.

The daily dose was 9 tbs divided in three equal consumptions at the main meals. This dosage was reached after 4 days of a progressive consumption (4tbs at the 1st and 2nd day, 6 tbs at the 3th and 4th day), for the purpose of obtaining a patient's adaptation to the dietetic integration.

All the patients were invited to avoid any variation of their alimentary habits and to quit drugs or dietary products active on constipation and previously consumed.

Before starting therapy and after 15 and 30 days from full dose consumption (9tbs/d) the stool frequency and

Table 1 Pulp Composition of Sugar Beet (after Saccharose Extraction)

Alimentary fibre	80±5 g
Proteins	10±1 g
Saccharose	3±2 g
Mineral substances	1 g
Lipids	0.5 g
Water	5±1 g

Table 1 bis. Percentage Composition of Sugar Beet Fibre

Hemicellulose	41.5%
Pectin	25 %
Cellulose	31 %
Lignin	2.5%

<u>Table 2</u>   Stool Frequency Following Sugar Beet Fibre
                      Treatment

	Beginning		15th day		30th day	
Stools	N°	%	N°	%	N°	%
Severe const.	11.0	42.3	2.0	7.7	1.0	3.8
Mod. const.	15.0	57.7	8.0	30.8	3.0	11.5
Normal	0.0	0.0	16.0	61.5	22.0	84.7
Total	26.0	100.0	26.0	100.0	26.0	100.0

composition were assessed according to precise criteria
(severe constipation: 1 evacuation every >5 days;
moderate constipation: 1 evacuation every 3-4 days;
normality: 1 evacuation every <2 days). Moreover, stools
were classified as hard, semi-hard, soft and liquid ones.
In 7 patients, who presented serum cholesterol levels
higher than 230 mg/dl, a check up was performed after 30
days of sugar beet fibre treatment.

The statistical analysis of the obtained data was
assessed by means of the student's T test for paired data
and chi square test.

<u>Results</u>

The study was completed in 26 out of 27 cases. The
drop out case was a 69 years old male, who was admitted
to hospital for a hip fracture after 12 days of treatment
with sugar beet fibre.

The stool behaviour evaluation, reported in Table 2
and Figure 1, shows a marked decrease of severe and
moderate constipation at both the 15th and the 30th day
of treatment; with a significant increase in faecal
frequency normalization, which reached the 61.5% after 15
days and 84.7% after 30 days (P<0.001 when compared to
time 0, and P<0.05 when compared to the 15th day of
treatment).

Moreover, it is observed that stool composition
turned in better with the appearance of soft stools in
69.3% (P<0.001) and semihard stools in 26.9% of patients,
at the 30th day. The cholesterol study performed in the
7 patients, at the 30th day. The cholesterol study
performed in the 7 patients with more than 230 mg/dl at
the starting moment, demonstrates a significant reduction

after 30 days of treatment (P<0.05) (Table 4).

The tolerance and compliance of patients to sugar beet fibre were good. No case presented collateral effects which induced therapy suspension. In two cases the clinical efficacy was exaggerated: multiple liquid stools appeared respectively after 12 and 14 days of treatment: the problem recovered after reducing the dose from 9 to 6 tbs per day, with a complete stool normalization.

Discussion

The analysis of the obtained data proves the efficacy of sugar beet fibre in the therapy of chronic constipation. This is confirmed by the increase in evacuation frequency, with persistence of severe constipation in only 3.8% after 1 month of therapy (Table 2, Figure 1).

The pharmacological effect of sugar beet fibre is associated to the increase in faecal bulk, which subsequently stimulates colon activity, as it happens with other bulk forming products (5,7).

Another relevant parameter is the modification of faecal consistence, with the crossing over from hard and semi-hard stools to soft ones, which is observed in significant percentage in comparison to the total cases (69.3%) (P<0.01) (Table 3, Figure 2). Therefore, the present research confirms the possible role of sugar beet fibre in the clinical management of chronic constipation and, possibly, of all the GI diseases treatable by non hydrosoluble fibres (diverticulosis, IBS, haemorrhoids, etc.). These results show a particular clinical interest by considering their high frequency at any age and sex.

The clinical interest is also stressed by the possibility of using a dietetic integration with a vegetable product such as the sugar beet, which presents a particularly high fibre content (Table 1). In fact, the alimentary fibre percentage in dried sugar beet pulp ranges from 75 to 85% of the whole weight, with a mean protein content of 10±1% and a minimal water (5±1%) and saccharose (3±2%) content.

The fibre content in sugar beet appears much higher than other commonly used fibre sources, such as wheat bran, which presents a fibre concentration of 35-40% of total weight.
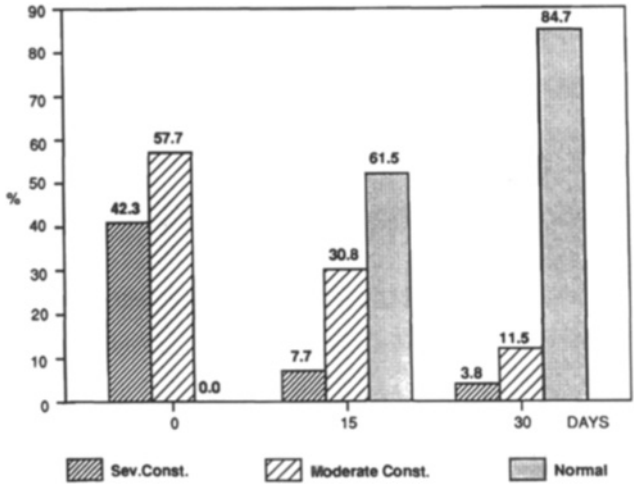
Figure 1. Stool Frequency Variation (percentual values)
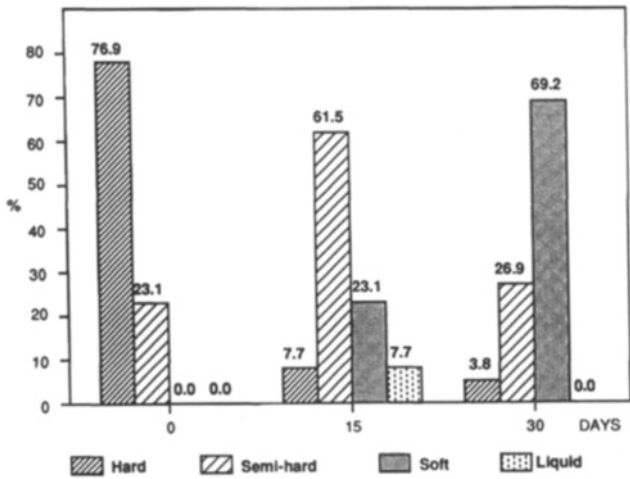
Figure 2.
Stool Consistence Variation following Sugar Beet Fibre (percentual values).

Table 3   Variation in Stool Consistency Following
                 Sugar Beet Fibre Treatment

                 Beginning   15th day   30th day

Stools   N°   %   N°   %   N°   %

Hard   20.0   76.9   2.0   7.7   1.0   3.8
Semihard   6.0   23.1   16.0   61.5   7.0   26.9
Soft   0.0   0.0   6.0   23.1   18.0   69.3
Liquid   0.0   0.0   2.0   7.7   0.0   0.0
Total   26.0 100.0   26.0 100.0   26.0 100.0

The sugar beet fibres, besides their elevated concentration, are characterized by a contemporary presence of both non soluble (hemicellulose: 41.5% of the whole fibre pool, cellulose: 31%, lignin: 2.5%) and soluble (pectin: 25% of the whole fibre pool) components.

The high content in global fibres and in the soluble component allows the clinical use of sugar beet fibre in dismetabolic conditions and particularly in glucose intolerance and dislipidemia. This is confirmed by multiple literature data and by the present experience, which proves a significant decrease in serum cholesterol in hypercholesterolic patients treated for one month (6).

Sugar beet fibre has not presented any collateral effects except the need of reducing the daily dose in 2 out of 26 patients, who complained of multiple liquid stools after the consumption of 6.75 gr/d of fibre (9 tbs).

In conclusion, sugar beet fibre appears to be useful in the clinical management of many gastroenterological diseases (first of all constipation and its associated pathologies) and dismetabolical conditions (glucose intolerance and dislipidemia). Furthermore, this fibre may be used in the preparation of many common dishes, since it appears to be particularly palatable also when it is cooked.

The ordinary use of sugar beet fibre could enter therefore in "healthy life" programs, because it finds a precise placement in prevention strategy for many diseases of our civilization.

Table 4   Serum Cholesterol Behaviour Following Sugar
               Beet Fibre in 7 Cases with Cholesterolemia
               > 230 mg/dl

Time	0	30
Cholesterol (mg/dl)	258±14	220±18

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ENRICHMENT OF ETHNIC FOODS WITH EXOGENOUS SOURCE OF DIETARY FIBER TO IMPROVE THE PLASMA GLUCOSE CONTROL IN NIDDM

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1 INTRODUCTION

Ample evidence exists indicating that plasma glucose response to equivalent amounts of carbohydrate varies in the function of the specific carbohydrate-rich food consumed (1). This led to the ranking of individual carbohydrate foods according to their postprandial glucose excursions, termed, glycemic index (GI) (2,3). Although notable differences in the glycemic responses to carbohydrate foods solely consumed have been reported, experiments delineating the effect of regular meals composed of various carbohydrate foods with GI on postprandial glucose in NIDDM subjects have yet to be satisfactorily defined. The purpose of the present research is to examine the short-term effect of representative major Israeli ethnic foods on postprandial glucose levels. In addition, we evaluated incorporation of dietary fiber into ethnic foods with high GI on postprandial plasma glucose patients with NIDDM.

2 MATERIALS AND METHODS

Seventeen NIDDM volunteers, aged 42 - 77 years of age, BMI - 28.6 $\pm$ 0.9 were recruited for this study. Informed Consent from all subjects and approval of The Committee for the Protection of Human Subjects, "Meir" Hospital, Kfar Saba, Israel were obtained. Six subjects were treated by diet alone and eleven, by oral hypoglycemic agents. Each subject consumed at

least four test meals in randomized order on separate mornings, at least one week apart, following an overnight fast. The test meals were composed of four different Jewish ethnic origins: Melawach (Yemenite) - a fried bread; Majadrah (Syrian) -lentils and rice; Kugel (Polish) - egg noddles, cheese and raisins and Couscous (Moroccan) - a stew of semolina and vegetables. The Melawach was enriched with 15 g of dietary fiber derived from carbohydrates and offered in the same manner. Aside from the four meals, each subject also consumed a Standard Meal, consisting of white bread, cheese and milk. Each meal provided 50 g carbohydrates and were compared to a glucose load of 50 g.

Blood samples were drawn before and at 30, 60, 120 and 180 min following completion of the test meal. Plasma was removed and glucose level was determined.

From area under the glucose curve, observed and predicted Glycemic Index were calculated. Results are expressed as Mean ± SE.

3 RESULTS AND DISCUSSION

The effect of the various ethnic foods on the glycemic responses in NIDDM subjects is shown in Figure 1.
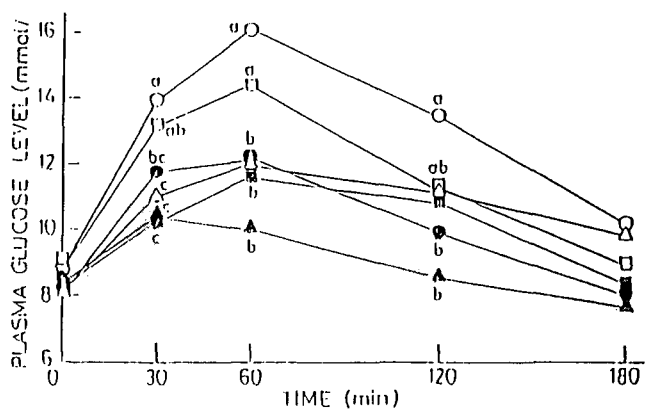
Figure 1 Glucose response to various kinds of ethnic foods compared to glucose given to NIDDM subjects. The meals ingested included: (●) Standard Meal (Δ) Melawach (▲) Majadra (□) Kugel (■) Couscous (O)

Glucose.   Values are Mean $\pm$ SE.   For each time,
values not sharing the same letter were significantly
different (P < 0.05)

   No significant differences were seen in the
fasting glucose levels among subjects.   The peak
glucose levels following the meals was reached at 60
min with the exception of Majadrah which peaked at 30
min.   Ingestion of glucose and Kugel led to a sharp
rise in glucose levels while the Standard Meal,
Melawach, Couscous and Majadra only moderately
affected glucose response.   The plasma glucose levels
following glucose loading was significantly higher
than that of the Yemenite, Moroccan, Standard Meal and
Majadra at 30, 60 and 120 min.   Majadra elicited the
lowest glucose response curve.   These results indicate
that glucose response of NIDDM subjects differed
according to the various meals.   These findings are
consistent with those of Chew et al. (3) in which
significant differences were noted in glycemic
responses.   The integrated area under the glucose
curve in NIDDM is shown in Figure 2.
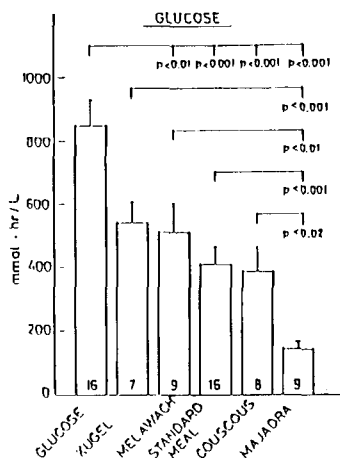
Figure 2 Area under the curve of NIDDM subjects after
consumption of different meals.   Bars indicate Mean
$\pm$ SE.   The number of subjects studied following each
meal is indicated in the bars.

All the meals provided a lower incremental area under the glucose curve compared to glucose (P < 0.05). Moreover, ingestion of Majadra also led to a lower incremental area under the glucose curve compared to all the other foods (P < 0.01). The glycemic indices in NIDDM subjects are presented in Figure 3.
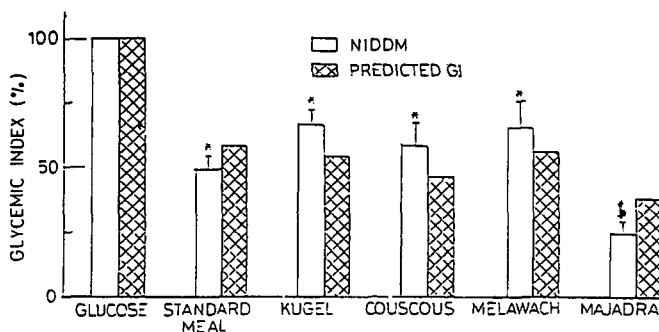
Figure 3 Observed and predicted glycemic index of various ethnic foods given to NIDDM subjects. Bars indicated Mean ± SE. *P < 0.05 compared to glucose † P < 0.05 compared to the other meals.

Although carbohydrate quantities were equal in all ethnic foods, the glucose response curves were different. The low response following Majadra consumption indicate the nutritional components of each meal might contribute to the glucose response following the meals. Majadra is a dish rich with dietary fiber and other anti-nutrients, such as phytic acid which attributed to the reduction in glucose response (6).

The high content of lentils in Majadra (containing a relatively high amylose content) may contribute to the low glycemic response seen in NIDDM subjects (7). This finding confirmed previous reports stating that various sources of the same amount of carbohdyrate led to varied glycemic responses (4,5). Based on our results, we have suggested the main nutritional factor in the mixed meal influencing the glycemic response is the type and composition of the starch (8). The observed and predicted GI were closely correlated.

Differences in the GI may be attributed, in part, to the dietary fiber shown with Majadara. While Melawach (low in dietary fiber) exhibited high GI, Kugel, made of egg noddles, is known to be a weak provocative for glycemic response and also presented an elevated GI. We believe the other components, i.e. sugar and the interaction between the components are to blame for this discrepancy.

In conclusion: (1) The GI concept is valid and potentially useful in diet planning. (2) The GI of food enriched with legumes should serve as a carbohydrate source when diets are formulated for NIDDM patients.

Since Melawach (white flour and fat, mainly butter) demonstrated a high GI, two types of dietary fiber (15 g) were incorporated into this dish and given to NIDDM subjects to determine whether the dietary fiber, in fact, can decrease the high GI of this mixed meal. The glucose response is shown in Figure 4.
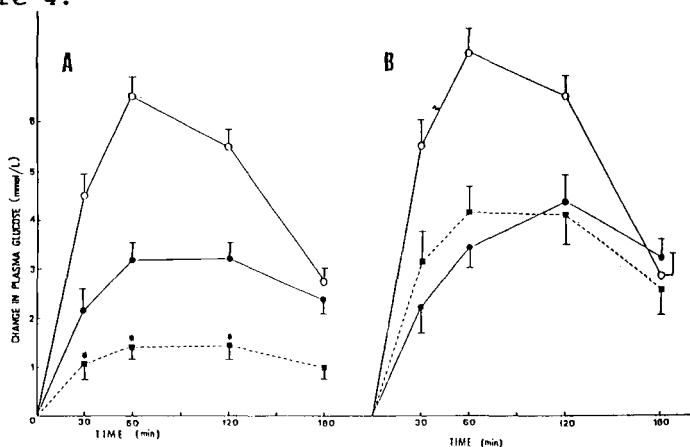
Figure 4. **A.** Plasma glucose response of diabetic patients following ingestion of Melawach with (▣) or without (●) locust bean fiber. *P < 0.05. ○ – glucose. **B.** Plasma glucose response of diabetic subjects following ingestion of Melawach with (▢) or without (●) corncob fiber. (◐) – glucose.

Note that locust bean (carob) significantly decreases the plasma glucose at 30, 60 and 120 min following the meal test when compared to Melawach

without fiber whereas corncob did not affect plasma glucose levels.

The effect of dietary fiber of GI has been evaluated. Incorporation of locust bean significantly decreased the GI from 60 to 30%. However, the GI remained unchanged when corncob was included into the Melawach. These results indicate that incorporation of soluble dietary fiber into foods with high GI may reduce the glucose response and GI index as shown previously (9) and might be beneficial as a means of controlling plasma glucose.

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EFFECTS OF SUGAR-BEET FIBRE ON BLOOD GLUCOSE, SERUM LIPIDS AND APOLIPOPROTEINS IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

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1.   INTRODUCTION

Diabetes is often associated with abnormalities in lipid and lipoprotein metabolism[1]. In non-insulin-dependent diabetes mellitus (NIDDM) these can include elevated triglyceride and total-cholesterol and reduced HDL-cholesterol. In long-term diabetics, apolipoprotein AI (apo AI), and apo AI to apo B ratio are also lowered. These changes have been associated with coronary heart disease [2].

Some soluble fibres have hypoglycaemic and hypocholesterolaemic effects in normal hypercholesterolaemic and diabetic subjects[3-5]. Sugar-beet fibre (SBF) contains approximately 40% soluble fibre (soluble non-starch polysaccharide determined according to Englyst et al[6]). When incorporated into the diet of healthy subjects it lowers total- but not HDL-cholesterol[6] with reduction in apo B but not apo AI. This study assesses the effect of SBF supplementation on glycaemic control and serum lipid, lipoprotein and apolipoprotein levels in subjects with NIDDM.

2   METHODS

Seven NIDDM subjects (4 male and 3 female) participated. Mean age was 66 years (range 42-84 years) and average body mass index was $26.7 kg/m^2$ (SEM $1.61 kg./m^2$). All subjects were treated with oral hypoglycaemic agents. Mean duration of diabetes was 9 years. All subjects gave their informed consent before participating in the study. Approval for the study was

given by the Ethical Committees of the University of Surrey and SW Surrey District Health Authority. The subjects maintained their usual diet for a two-week basal period. During that time two fasting blood samples were taken and the subjects' height and weight recorded. Nutritional intake was assessed by a three-day dietary record using estimated weights. The subjects were provided with bread, seed thins and lemon cookies, containing SBF (British Sugar plc, Peterborough) at approximately 2g/slice or per biscuit. By using a combination of bread and biscuits, they were asked to consume the equivalent of 18g dietary fibre from SBF per day for six weeks. Dietary advice was given to minimise changes to total energy and fat intake during the SBF period. Three fasting blood samples were taken at two-week intervals during the SBF period and each subject completed a further one or two dietary records. A final fasting blood sample was taken two weeks after the end of the supplementation period.

Plasma glucose, serum total- and HDL-cholesterol, triglyceride and apo AI and B were measured as described previously[8]. Plasma fructosamine was measured with an automated colorimetric assay[9].

Dietary records were analysed using Compeat Nutritional Analysis Program, based on food composition tables prepared by Paul and Southgate[10]. Results were compared using Student's t-test for paired values (one-tailed).

3   RESULTS

Body weights of the subjects did not change during the study. Intakes of energy, carbohydrate, fat and dietary fibre tended to be higher during the SBF period; however only energy intake differed significantly from basal values. The percentage of energy intake as protein was reduced ($p<0.01$), possibly due to a non-significant ($p>0.05$) increase in the percentage of energy intake as fat (See Table 1). Five of the seven subjects found the SBF supplemented bread and biscuits less palatable than normal products. This led to a reduction in compliance during the last two weeks of SBF supplementation. An average of measurements taken after two and four weeks of SBF supplementation was taken for comparison with basal values. No adverse abdominal symptoms were reported.

Fasting plasma glucose and fructosamine levels were similar throughout the study. During the SBF period,

serum total-cholesterol, triglyceride and apolipoprotein B levels showed reductions (p<0.05) of 6.2% ± 2.81, 10.6% ± 5.09 and 6.0% ± 1.72 (mean ± SEM), respectively (see Table 2). Serum apo B was correlated (p<0.001) with both total cholesterol (r = 0.9173) and triglyceride (r = 0.8666). The change in apolipoprotein B was correlated with both the change in total cholesterol (r = 0.8301, p<0.001) and in triglyceride (r = 0.8200, p<0.001). HDL-cholesterol and serum apolipoprotein AI levels were not altered during the study (p>0.05). Apo AI concentrations were correlated significantly with HDL-cholesterol (r = 0.9225, p<0.001).

4   DISCUSSION

Inclusion of SBF in the diet of NIDDM subjects had no effect on glycaemic control, in agreement with other studies[7,11]. This may be because, despite the high soluble-fibre content, the fibres did not form solutions of sufficient viscosity to impede glucose absorption in the small intestine[11].

Reductions in serum cholesterol of between 3.6% and 7.8% have previously been reported[7,11,13]. In these studies, the dietary fibre intake of the participants was significantly increased compared to their normal diets. In the present study a decrease in serum cholesterol was observed when subjects attained only a modest increase in total dietary fibre intake, as they mainly substituted SBF for other mixed fibres in their diet. In accordance with previous results[7,11], SBF had no effect on HDL-cholesterol levels, implying that the predominant cholesterol fractions lowered were the atherogenic LDL and VLDL fractions.

Serum triglyceride levels were lowered by SBF as has been reported previously[11]. This may represent some reduction in the formation of triglyceride-rich particles by the liver.

In the fasted state, virtually all serum apo B will be in the LDL and VLDL fractions[14]. The tendency for SBF supplementation to lower serum apo B is, therefore, consistent with the assumption made above that the circulating levels of these fractions were reduced.

Inclusion of SBF into the diet of our NIDDM subjects was achieved largely by substitution for other mixed dietary fibres. The effects of SBF were therefore

<u>Table 1</u>   Composition of diet and mean daily nutrient intakes, calculated from three-day records before and during SBF supplement periods for seven NIDDM*.

Constituent	Basal Period	SBF Period
Energy, total kcal	1643 ± 110	2058 ± 184 I
MJ	6.9 ± 0.46	8.6 ± 0.77 I
Protein g	80 ± 4.2	77 ± 5.1
% E	20 ± 1.2	15 ± 1.0 II
Carbohydrate g	168 ± 10.7	197 ± 21.9
% E	41 ± 3.5	39 ± 3.7
Total dietary fibre		
g	25 ± 2.7	32 ± 2.2 I
from SBF g	–	18 ± 1.6
Fat g	72 ± 10.4	105 ± 16.1
% E	38 ± 3.5	45 ± 4.0
Saturated % E	11 ± 1.8	14 ± 1.7
Monounsaturated % E	11 ± 1.3	15 ± 2.1
Polyunsaturated % E	5 ± 0.6	6 ± 2.0
P:S ratio $	0.5 ± 0.11	0.5 ± 0.09
Cholesterol mg	256 ± 64	252 ± 22

*   Values are presented as means ± SEM
I   Significantly different from value for basal period I ($p<0.05$) and II ($p<0.01$)
$ Ratio of polyunsaturated to saturated fatty acids

<u>Table 2</u>   Effect of SBF supplementation on plasma glucose and fructosamine and serum lipids and apolipoproteins in seven NIDDM *

	Basal Period	SBF Period
Glucose mmol/l	10.8 ± 1.79	10.6 ± 1.77
Fructosamine mmol/l	2.5 ± 0.26	2.5 ± 0.23
Total Cholesterol		
mmol/l	6.5 ± 0.57	6.1 ± 0.46 I
HDL-Cholesterol		
mmol/l	1.1 ± 0.26	1.0 ± 0.21
Triglyceride mmol/l	2.3 ± 0.50	1.9 ± 0.33 I
Apolipoprotein B		
mg/dl	116 ± 18	107 ± 14 I
Apolipoprotein A I		
mg/dl	148 ± 16	144 ± 14

*   Values are presented as means ± SEM
I $P<0.05$ for comparison with basal period

assessed in free-living subjects following realistic dietary regimens. Under these circumstances the SBF had no adverse side-effects, tended to reduce serum total cholesterol, triglyceride and apo B without altering HDL-cholesterol and apo AI levels. These changes represent an improvement in the lipid and apolipoprotein risk profile for CHD.

5   ACKNOWLEDGEMENTS

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