Current Topics in Microbiology and Immunology

Ergebnisse der Mikrobiologie und Immunitätsforschung

60

Edited by

W. Arber, Basle · W. Braun, New Brunswick · R. Haas, Freiburg · W. Henle, Philadelphia · P. H. Hofschneider, Munich · N. K. Jerne, Basle · P. Koldovský, Philadelphia · H. Koprowski, Philadelphia · O. Maaløe, Copenhagen · R. Rott, Gießen · H. G. Schweiger, Wilhelmshaven · M. Sela, Rehovot · L. Syruček, Prague · P. K. Vogt, Seattle · E. Wecker, Würzburg

With 16 Figures

Springer-Verlag Berlin · Heidelberg · New York 1973

ISBN-13:978-3-642-65504-3 e-ISBN-13:978-3-642-65502-9
DOI: 10.1007/978-3-642-65502-9

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically those of translation, reprinting, re-use of illustrations, broadcasting, reproduction by photocopying machine or similar means, and storage in data banks
Under § 54 of the German Copyright Law where copies are made for other than private use, a fee is payable to the publisher, the amount of the fee to be determined by agreement with the publisher
© by Springer-Verlag, Berlin · Heidelberg 1973. Library of Congress Catalog Card Number 15-12910.
Softcover reprint of the hardcover 1st edition 1973

The use of general descriptive names, trade names, trade marks, etc. in this publication, even if the former are not especially identified, is not to be taken as a sign that such names, as understood by the Trade Marks and Merchandise Marks Act, may accordingly be used freely by anyone. Universitätsdruckerei H. Stürtz AG, Würzburg

Table of Contents

KOCHAN, I., The Role of Iron in Bacterial Infections, with Special Consideration of Host-Tubercle Bacillus Interaction. With 4 Figures . . . 1

RAMSEIER, H., Antibodies to Receptors Recognizing Histocompatibility Antigens . . . 31

ACHTMAN, M., Genetics of the F Sex Factor in *Enterobacteriaceae*. With 6 Figures . . . 79

TAUSSIG, M. J., Antigenic Competition. With 6 Figures . . . 125

Author Index . . . 175

Subject Index . . . 187

The Role of Iron in Bacterial Infections, with Special Consideration of Host-Tubercle Bacillus Interaction

IVAN KOCHAN [1]

With 4 Figures

Table of Contents

I. Introduction 1
II. Effect of Iron on Bacterial Fate in Humoral Fluids 3
A. Alleviation of Bacteriostasis in Normal Serum with Iron 3
B. Alleviation of Bacteriostasis in Immune Serum with Iron 8
C. Alleviation of Serum Bacteriostasis with Iron-Binding Bacterial Products 10
III. Effect of Iron on the Fate of Bacteria in Tissues 15
A. Fate of Bacteria in Macrophages 16
B. Fate of Bacteria in Extracts of Macrophages 17
IV. Iron-Induced Susceptibility to Bacterial Infections 21
V. Summary and Conclusions 24
References 26

I. Introduction

Various bacteria can invade dead or weakened tissues of an animal but only pathogenic parasites are able to establish themselves in viable host tissues and cause disease. To survive in a host and to invade its healthy tissues, a bacterial parasite has to possess mechanisms to neutralize or resist the natural defenses of the host and to use tissues and fluids of the infected body as sources of energy and materials essential for growth. Some pathogens secrete exotoxins which produce symptoms of disease, and, if not neutralized by antitoxins, may cause death. The active or passive acquisition of antibodies to exotoxins usually eliminates toxemia but not infection. Direct destruction of bacteria by specific antibodies and complement occurs only in a few bacterial species. Many pathogens do not produce exotoxins but nevertheless are able to invade tissues of the host, to multiply, and to spread. There is no single characteristic which could account for the pathogenicity of the non-

1 Department of Microbiology, Miami University, Oxford, Ohio.

Dedication: It is with a great sorrow that I have learned about the sudden death of Dr. WERNER BRAUN. His interest in my research contributed greatly to the writing of this review. I will remember his friendship and I dedicate this review to his memory.

toxigenic bacteria. Some of them survive in the host by being able to multiply rapidly in its tissue fluids and to resist engulfment by his phagocytic cells; once phagocytized, these bacteria are readily digested by intracellular enzymes. Other nontoxigenic parasites are unable to multiply in tissue fluids of the host but survive and multiply in its phagocytic cells. Because of this ability to persist in the intracellular environment, these nontoxigenic bacteria are called facultative intracellular parasites. The diseases caused by facultative intracellular parasites tend to be chronic and their nature is certainly not as acute as that of diseases caused by other bacteria.

Several observations suggest that the host has a poor chance of defending himself effectively against infection with facultative intracellular bacterial parasites. If immunity were effective, the presence of cell-mediated immune responses and of antibodies in vaccinated or infected hosts would eliminate the disease-producing parasites. However, animals frequently derive little benefit from the immunity induced by vaccination against such typical facultative intracellular pathogens as virulent tubercle bacilli (IZUMI and COSTELLO, 1971). Similarly, tuberculous infection itself does not seem to induce resistance; two-thirds of all new cases of tuberculosis in the United States develop in patients who have been previously infected with tubercle bacilli (MIDDLEBROOK, 1970). It seems that effective immunity persists for only as long as the protection-inducing bacteria survive in the vaccinated animal (COLLINS, 1968; COSTELLO and IZUMI, 1971).

The limited effectiveness of acquired immunity to facultative intracellular parasites stimulated investigation of the relationship between normal hosts and intracellular parasites. LURIE (1964) observed that differences in resistance between inbred strains of rabbits were quite often wider than differences between immunized and unimmunized groups of genetically heterologous rabbits. The existence of high levels of natural resistance to facultative intracellular parasites in normal animals suggests that it might be possible to achieve effective immunity against them if factors that play a role in host-parasite interactions were identified.

Irrespective of toxigenicity, phagocytosis, or the ability to persist in phagocytic cells, a crucial requirement for all pathogens is the ability to grow in body fluids and/or in phagocytic vacuoles. Little is known about the nutritional capabilities which enable pathogens to grow and survive in the animal body. Differences in predisposition of certain tissues of the same host to invasion by tubercle bacilli (LURIE, 1964) suggest that nutritional factors may be of considerable importance in determining the fate of the parasites in tissues of the infected host. Since serum and tissue extracts contain all nutrients necessary for bacterial growth, it is assumed that all materials required by the pathogen are available for its utilization. The intention of this review is to show that this is not so and that, in particular, the potentiality of various pathogens to survive in an animal hinges upon their ability to obtain sufficient iron for their metabolism. Iron is essential for bacterial growth (STEPHENSON, 1949; CLIFTON, 1957). Therefore, in many cases the

availability of the metal determines the fate of bacterial parasites in the body of a host (WEINBERG, 1966; WEINBERG, 1971).

Special attention will be given in this review to host-tubercle bacillus interaction. Extensive studies attempting to identify factors that enable virulent strains of tubercle bacillus to survive in an animal have been inconclusive. There is no satisfactory indication that a strain of tubercle bacillus is virulent because its bacilli have the ability to interfere with functions of phagocytes or with extracellular defense mechanisms. In view of this, RAFFEL (1961) suggested that, to propagate and spread through the body, a virulent tubercle bacillus may depend entirely upon its ability to utilize the cultural milieu supplied by the host better than the avirulent bacillus. Experimental support for this suggestion will be presented and discussed in this article.

II. Effect of Iron on Bacterial Fate in Humoral Fluids

A crucial question in the consideration of host-parasite relationships is whether or not the parasite can utilize materials encountered in the host's fluids and cells for its growth. In the past the question of bacterial nutrition in infected hosts received little attention. Tissue fluids, for example, were assumed to be good growth-supporting media. Recent findings in several laboratories show that this assumption was erroneous; many parasites are incapable of growing in tissue fluids because these fluids fail to provide parasites with the iron essential for their growth.

Iron is one of the essential metals needed for the growth of animals, plants, and microorganisms. The minimum iron requirements for bacterial growth have not been precisely established, because materials used in the preparation of bacterial media are usually contaminated with this metal. It has been determined that the quantity of iron needed for good growth of commonly studied bacteria varies between 0.02 μg and 0.2 μg of the metal per 1 ml of the medium (WEINBERG, 1971). We have reported (KOCHAN et al., 1971 b) that 0.09 μg of iron per 1 ml of a suitable medium is sufficient to support fairly good growth of tubercle bacilli. Since the amount of iron in mammalian sera varies between 1 μg and 3 μg per ml (KOCHAN et al., 1969), these quantities are more than sufficient for bacterial multiplication. It will be shown in the following discussion that, in spite of the presence of sufficient iron in mammalian sera, the latter cannot support bacterial growth unless supplemented by additional iron.

A. Alleviation of Bacteriostasis in Normal Serum with Iron

The effect of mammalian serum on the growth of tubercle bacilli has been studied more intensively than its effect on any other pathogenic microorganism. Nearly half a century ago, MEISSNER (1928) observed that sera of normal animals exert some inhibitory effect upon bacillary multiplication; she found even stronger antimycobacterial activity in sera of tuberculous animals. The bacteriostatic power of sera from certain cases of tuberculosis has been reported by KALLOS and NATHAN (1932), COURMONT and GARDERE (1933), PAGEL (1940),

and EMMART and SEIBERT (1945). COURMONT and GARDERE (1937) observed that the prognosis in tuberculosis was more favorable when the tuberculocidal power of the patient's blood was elevated. In spite of these findings, the study of the antimycobacterial activity of mammalian sera was limited mainly to superficial observations. Investigators were more impressed by the stimulatory effect of various sera on bacillary multiplication in iron-rich synthetic media devised for bacillary growth (KIRCHNER, 1932; DAVIES, 1938; BOISSEVAIN, 1940) than by the possibility of their antimycobacterial activity.

Clinical observations were more pertinent; CANETTI (1955) observed the absence of tubercle bacilli in cell-free tuberculous exudates and wondered about the possibility of an antimycobacterial effect of body fluids. Since plasma and blood were frequently incorporated into media to increase their growth-supporting quality for tubercle bacilli, CANETTI, despite his perceptive observations, dismissed the possibility of the existence of antimycobacterial effect in animal fluids. Experimental results obtained by LURIE (1936, 1939) showed that the acellular body fluids of immune animals were markedly tuberculostatic. In his elegant experiments, LURIE exposed tubercle bacilli to fluids of normal and immune animals by incorporating the parasites in agar blocks or within collodion-impregnated silk bags. In the complete absence of phagocytic cells, the body fluids exerted a pronounced tuberculostatic effect. It was probably the inability to protect animals against tuberculosis by the administration of antibody-containing serum that caused LURIE to belittle these observations and to direct his attention to the role of phagocytic cells in tuberculous immunity (LURIE, 1942).

The concept of immunity to tuberculosis based upon the antimycobacterial activity of antibodies has little support (EFFORT, 1954; KOCHAN, 1958; RAFFEL, 1960). In the nineteen-fifties several groups of investigators reported that sera of immune animals exert tuberculostatic activity by non-antibody inhibitory factors. Thus, MYRVIK and WEISER (1951) attributed serum tuberculostasis to the presence of a lysozyme-like substance, and ZITRIN and WASZ-HÖCKERT (1957) found an antituberculous factor was present in various Cohn fractions obtained by fractionation of tuberculous human serum. Serum tuberculostasis was also attributed to the absence of a hypothetical growth-promoting factor which might be present in normal but absent in immune rabbit sera (TSUJI et al., 1957 and 1958; OSHIMA et al., 1958). A strong inhibitory effect on tubercle bacilli was found by KOCHAN and RAFFEL (1960) in sera of tuberculous and BCG-vaccinated guinea pigs. In infected animals, this effect was present throughout the disease period. In vaccinated animals it lasted for about three months. Since this tuberculostatic effect was not diminished after the adsorption of serum with tubercle bacilli nor after heating the serum at 56° C, KOCHAN and RAFFEL concluded that the tuberculostatic mechanism in the serum was not dependent on the activities of antibodies or of complement.

The role of iron in the survival of serum-exposed tubercle bacilli emerged only slowly. The conflicting reports on the antimycobacterial activity of

normal and immune sera suggested that technical experimental variations used in tests could account for the contradictory results. We observed that most investigators who were unsuccessful in the demonstration of serum tuberculostasis diluted the tested sera in various media devised for bacillary growth. The suitability of various media for the study of serum tuberculostasis was determined in our laboratory by evaluating the growth of BCG cells in serial dilutions of human serum after a 14-day incubation at 37°C (KOCHAN et al., 1963a). Results presented in Table 1 show that serum diluted in the growth-supporting media did not inhibit bacillary multiplication while serum was inhibitory when diluted in saline or Hanks' solution. Good bacillary growth occurred in higher but not in lower dilutions of serum in control medium, suggesting that N-Z amine and Vegex provided a substance which either neutralized an antimycobacterial factor or promoted bacillary growth in a mixture of serum and Hanks' solution. Since serum heated at 65°C and diluted in control medium or in Hanks' solution did support bacillary growth in all dilutions, we concluded that serum possesses a tuberculostatic factor which can be inactivated either by an ingredient present in various media or by heat. A beta-globulin fraction with antimycobacterial activity was subsequently separated from human serum by the use of continuous flow-curtain electrophoresis. The immunoelectrophoretic analysis showed that the active fraction was composed of beta-lipoprotein, beta-2A globulin, heat-labile beta proteins, transferrin, or ceruloplasmin (KOCHAN et al., 1963b).

Bacillary multiplication in serum diluted 1:16 or higher in control medium, and tuberculostasis in dilutions made in Hanks' solution alone (Table 1) seemed to offer an approach to the identification of the antagonist to the tuberculostatic factor. In contrast to Hanks' solution, the control medium contained N-Z amine and Vegex. We found that the addition of untreated or ashed Vegex to tuberculostatic serum promotes bacillary multiplication (KOCHAN, 1969). Since Vegex is "fortified" with iron by the manufacturer and since our previous results suggested that transferrin (Tr) could be the

Table 1. Effect of diluents on the tuberculostatic activity of human serum. Modified from KOCHAN et al., J. Immunol., **90**, 711–719 (1963a)

Diluent	Generations[a] in serum diluted				
	1:2	1:4	1:8	1:16	1:32
Dubos medium	11.3	13.0	12.0	12.9	12.1
Kirchner medium	10.0	9.5	9.8	10.9	11.3
Sauton medium	9.0	12.0	11.7	12.3	10.7
Proskauer-Beck medium	7.8	11.1	12.3	12.0	12.5
Saline	0.2	1.8	1.2	1.5	2.4
Hanks's solution	0.0	0.1	0.9	2.0	1.2
Control[b] medium	0.2	1.0	2.8	13.0	13.0

[a] The growth of BCG was determined after a 14-day incubation period.
[b] Control medium consisted of Hank's solution enriched with N-Z amine and Vegex at the same concentration as used in Dubos medium.

tuberculostatic factor, we decided to study the effects of iron and Tr on bacillary growth. Iron, as a solution of ferric chloride, and iron-free Tr were added in various concentrations to tuberculostatic human serum diluted 1:4 in Hanks' solution. Results presented in Table 2 show that tuberculostasis in 1 ml of a 1:4 dilution of human serum can be neutralized by iron in concentrations between 1.0 μg and 0.5 μg. Subsequent titrations showed that as

Table 2. Iron neutralization of tuberculostasis in 1:4 dilution of human serum and the reconstitution of tuberculostasis in iron-neutralized serum with transferrin. Modified from KOCHAN, J. Infect. Dis., 119, 11–18 (1969)

Transferrin (mg/ml)	Generations[a] in serum with iron[b] (μg/ml)						
	0.0	0.5	1.0	2.0	4.0	8.0	16.0
0.00	0.0	2.3	11.0	12.5	12.9	13.2	12.7
1.25	0.0	0.0	2.7	9.6	9.1	12.2	13.0
2.50	0.0	0.0	0.5	4.5	10.2	13.1	12.1
5.00	0.0	0.0	0.0	0.3	2.6	9.4	12.3
10.00	0.0	0.0	0.0	0.0	0.0	5.0	10.7
20.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] The growth of BCG was determined after a 2-week incubation period at 37°C.
[b] Iron added as $FeCl_3 \cdot 6H_2O$ salt.

little as 0.8 μg of iron vitiates tuberculostasis in 1 ml of 1:4 diluted serum. The addition of Tr to iron-neutralized serum reconstituted tuberculostasis. The data show that the amounts of Tr which were effective in the reconstitution of tuberculostasis in iron-neutralized serum correlated closely with the binding capacity of the protein for iron (1 mg of Tr can bind 1.2 μg to 1.4 μg of iron, LAURELL, 1960).

This study of the iron-Tr interplay in serum tuberculostasis suggested two possibilities that may account for the suppression of bacillary growth in human serum: (1) Serum tuberculostasis could be attributed to iron binding. According to this suggestion, a serum can support bacillary growth only when excess iron is added to saturate the iron-binding Tr to a point at which free iron is available. Since Tr exists in human serum at one-third saturation, it becomes an indirect but effective antimycobacterial agent. (2) Tuberculostasis could also be attributed to the direct inhibitory action of unsaturated Tr. If this were so, the bacillary growth in serum-iron mixture would not be due to the growth-stimulatory effect of the metal but rather to the neutralization of Tr by iron. Such a possibility has been suggested by LANDAU et al. (1964) to explain the antifungal activity of Tr for *Candida albicans*. If Tr were to possess a direct effect on tubercle bacilli we would expect that (a) it would be adsorbed onto the bacilli, (b) since zinc and cupric copper can be chelated by Tr, these metals, like iron, would neutralize its tuberculostatic activity, and (c) the replacement of Tr by another iron-binding chelator would not induce tuberculostasis in the iron-containing medium. We found that the

amount of Tr does not decrease after adsorption with tubercle bacilli and that the activity of Tr cannot be lowered by the presence of large amounts of zinc and cupric copper (KOCHAN, 1969). It is also possible to produce antimycobacterial activity in Dubos Tween-albumin-dextrose medium by the use of iron-binding Desferal (desferrioxamine B methane sulfate; Ciba Pharmaceutical Products, Inc., Summit, N. J.). We concluded from these results and from the

Table 3. Correlation between levels of iron-saturated Tr and degrees of bacillary growth in mammalian sera in the absence and presence of exogenous iron. Modified from KOCHAN et al., J. Bact., **100**, 64–70 (1969)

Number and source of serum samples	Amount of iron (μg) [a]		Tr saturation [b] %	Number of generations [c]	
	TIBC	SI		no iron	2 μg iron ml
10 Human	327	97	30.0	0–1	10–14
4 Bovine	490	191	39.0	0–1	10–14
10 Mouse	382	230	60.2	1–5	9–15
8 Rabbit	317	204	64.3	1–5	10–15
20 Guinea pig	323	273	84.4	9–14	9–14

[a] Individual determinations of TIBC and SI fell within 10% variation of the mean values shown in the table. Both values show amounts of iron present in 100 ml of untreated serum.
[b] Percentage of iron-saturated Tr in serum sample equals (SI/TIBC) × 100.
[c] Growth of BCG was determined after a 14-day incubation period at 37°C.

fact that tubercle bacilli do grow on suitable media when removed from inhibitory solutions of Tr or Desferal that Tr does not inhibit tubercle bacilli by direct antibacterial activity but rather by depriving the bacilli of iron which is essential for their growth (KOCHAN et al., 1969).

KOCHAN and RAFFEL (1960) reported that guinea pig serum, in contrast to some other mammalian sera, supports a good growth of tubercle bacilli. This difference, as well as the interplay between iron and Tr in the mechanism of serum tuberculostasis, suggested that mammalian sera should be investigated in terms of serum iron (SI), total iron binding capacity (TIBC), and serum tuberculostasis. Table 3 shows the correlation between degrees of saturation of Tr with iron and differing degrees of bacillary growth in mammalian sera. Human and bovine sera with around 30% saturated Tr were tuberculostatic, whereas guinea pig serum with 84.4% saturated Tr supported bacillary multiplication. Limited bacillary multiplication was present in mouse and rabbit sera which contained 60% saturated Tr. The addition of 2 μg of iron per 1 ml of 1:2 dilution of any mammalian serum neutralized tuberculostasis. Other experiments showed that the quantities of iron required for the neutralization of human and rabbit serum samples were proportional to the amounts of iron-free Tr in the respective sera (KOCHAN et al., 1969). Since BCG cells multiplied in the presence of guinea pig serum, we suggested that in sera with a high content of iron-saturated Tr enough ionic iron is present to support bacillary

growth. Probably the dissociation balance between iron-Tr, Tr, and iron determines the amounts of ionic iron whose availability defines the growth-supporting quality of a serum sample. According to this, the ratio between iron-saturated and iron-free Tr is of crucial importance in determining the fate of tubercle bacilli in mammalian sera.

It seems that the percentage of iron saturation of Tr in animal sera determines the fate of not only tubercle bacilli but other pathogens. Schade and Caroline (1946) observed the bacteriostatic activity of Tr against *Shigella dysenteriae* and suggested that the protein exerted its effect by binding iron. Since this initial observation, several investigators have found that the iron-Tr interplay determines the fate of various parasites in normal mammalian sera. It has been observed that the addition of iron to normal human serum greatly enhances the growth of *Staphylococcus aureus*, *Salmonella typhimurium* and *Pasteurella pestis* (Jackson and Morris, 1961). Schade (1961, 1963) observed a correlation between the staphylococcal growth rate in human serum and the level of iron-saturated Tr. Similarly, growth- and respiration-inhibiting effects of rabbit sera for *Bacillus subtilis* appeared to be related to levels of unsaturated Tr (Bornside et al., 1964); these investigators attributed the serum-neutralizing activity of iron to its inactivating effect on an antibacterial serum globulin and not to the growth-stimulatory effect of the metal. In contrast to this conclusion, Rogers (1967) showed that the small amount of iron, sufficient to abolish the inhibitory effect of horse serum for *Clostridium welchii*, is insufficient to saturate Tr. On the basis of this and similar observations, Rogers suggested that the metal interacts directly with the bacteria and that bacterial growth in the presence of serum may be related to the degree of iron-saturation of Tr. This conclusion was reinforced by later studies in which Bullen and Rogers (1969) demonstrated that antibacterial activity of rabbit sera for *Pasteurella septica* and for *Escherichia coli* can be neutralized by the addition of iron.

B. Alleviation of Bacteriostasis in Immune Serum with Iron

It has been observed by Kochan and Raffel (1960) that in contrast to the growth-supporting nature of normal guinea pig serum, serum of immune animals exerted a pronounced suppression of growth for the $H_{37}Rv$ strain of tubercle bacillus. This initial observation of acquired antimycobacterial activity of immune sera and subsequent findings on the subject of the Tr-iron interplay in serum tuberculostasis suggested that immune sera should be investigated in terms of levels of iron-saturated Tr and the tuberculostatic activity. Such studies have been carried out using sera from BCG-vaccinated, lipopolysaccharide (LPS)- and tuberculous cell wall (TCW)-treated, and saline-injected guinea pigs (Kochan et al., 1969). Results presented in Table 4 show that a brief period of hypoferremia developed in animals treated with LPS and TCW materials. In BCG-vaccinated guinea pigs, hypoferremia developed within about 2 weeks after the vaccination and lasted for more than 4 weeks. The hypoferremia was accompanied by tuberculostasis which was neutralized by

Table 4. Iron neutralization of tuberculostasis in sera of LPS- or TCW-treated and BCG-vaccinated guinea pigs[a]. Modified from KOCHAN et al., J. Bact., **100**, 64–70 (1969)

Days after Treatments[b]	Tr Saturation	Generations in serum with iron (μg/ml)				
	%	0	1	2	4	8
LPS-1	17.5	0.0	0.0	0.4	8.0	9.2
LPS-2	42.1	0.0	10.6	11.3	10.7	12.2
LPS-3	60.2	0.0	11.8	11.9	9.5	11.1
LPS-5	74.7	8.7	12.0	11.4	10.7	11.3
LPS-10	93.8	12.5	11.6	12.0	12.1	11.3
TCW-1	26.5	0.0	5.1	9.5	9.5	9.5
TCW-2	42.1	0.5	10.2	10.3	10.0	10.6
TCW-3	59.3	1.2	9.7	9.9	10.7	11.0
TCW-5	66.3	0.7	7.3	12.0	11.3	12.3
TCW-14	78.8	11.8	12.6	12.1	11.9	12.7
BCG-3	86.2	10.5	11.5	11.9	11.0	11.2
BCG-7	79.4	9.1	10.6	11.3	12.1	11.7
BCG-14	75.0	4.3	9.5	10.2	12.3	11.1
BCG-21	75.3	0.4	9.1	10.3	9.9	10.7
BCG-28	68.6	0.0	9.7	10.2	10.9	11.3
Saline-1	85.6	11.5	11.8	11.6	11.5	12.1

[a] Tests for the presence and neutralization of tuberculostasis were performed in a 1:4 dilution of serum samples. Bacillary fate determined after a 14-day incubation period.
[b] On day "O", animals were injected intraperitoneally with 0.05 mg of LPS, 1 mg of TCW preparation, or 1 mg of BCG cells per 100 g of body weight.

the addition of iron. The effectiveness of this iron-neutralization indicates that the mechanism of tuberculostasis in sera of vaccinated or endotoxin-treated animals is the same as that present in normal animals' sera with tuberculostatic activity.

The hypoferremic effect of endotoxin has been observed by BAKER and WILSON (1965) who found a close correlation between the amounts of endotoxin injected and the levels of hypoferremia which developed in the treated mice. A decrease in plasma iron levels was observed in mice infected with *Listeria monocytogenes* (SWORD, 1966) and in humans infected with *Francisella tularensis* (PEKAREK et al., 1969). The bacteriostatic effects of immune sera for *Cl. welchii* (ROGERS et al., 1970) and for *P. septica* (BULLEN et al., 1971) were abolished by a variety of iron-containing compounds. Although the levels of hypoferremia or degrees of iron-saturation of Tr were not determined in these studies, various experiments clearly indicated that iron binding by Tr was essential for bacteriostasis. The bacteriostasis in antisera was also abolished by heme compounds, which are not bound to Tr. This strengthens the conclusion that the iron binding capacities of normal or immune sera determine their antibacterial effect.

Although the degree of tuberculostasis in normal or immune sera is directly proportional to the level of unsaturated Tr, BULLEN et al. (1971) observed

that the bacteriostatic system against *P. septica* also involves specific antibody and complement. The results obtained by Bullen and associates indicate that bacteriostasis results from the combined action of Tr, antibody and complement. Nevertheless, the latter two components of the system cannot exert antibacterial activity in the absence of Tr. They suggested that the role of antibody and complement might be to neutralize the activity of a hypothetical iron-chelating bacterial product which transports iron from Tr into bacterial cell. It will be shown later that such a chelating product, mycobactin (M), has an iron-transporting function for the tubercle bacillus (KOCHAN et al., 1971 a). Attempts to produce antibodies to M or to detect the presence of such antibodies in serum of BCG-vaccinated animals were not successful (KOCHAN and GOLDEN, unpublished data). The possibility exists that the suggested iron-chelating product of *P. septica* is more antigenic than the small molecular weight M.

Thus there is ample evidence that the addition of iron to normal or immune sera neutralizes their antibacterial effect for several bacterial species. Results suggest that the mechanism which interferes with iron metabolism may vary slightly with different bacteria but in all cases it is dependent upon the level of unsaturated Tr. For example, the bacteriostatic mechanism against *P. septica* requires the presence of iron-free Tr, specific antibody, and complement (BULLEN et al., 1971); the mechanism against *Cl. welchii* requires no complement (ROGERS, 1967); and that against *Mycobacterium tuberculosis* requires neither antibody nor complement (KOCHAN, 1969). Although some potentiating effect of antibody and complement in the antibacterial activity of serum cannot be excluded as yet from such considerations, results obtained in our laboratory indicate that the intensity of tuberculostasis in normal and in immune sera is directly proportional to the level of unsaturated Tr (KOCHAN et al., 1969). The development of hypoferremia in endotoxin-treated or vaccinated animals and the associated increase in serum tuberculostasis suggest that the ability of the immune host to limit or to prevent bacterial growth may depend largely on a mechanism that interferes with iron-availability for potential pathogens.

C. Alleviation of Serum Bacteriostasis with Iron-Binding Bacterial Products

The results discussed in the previous section show that many bacteria cannot grow in mammalian sera because the sera fail to provide the parasites with the iron essential for growth. In spite of serum bacteriostasis, pathogenic bacteria can infect and cause disease in experimental animals. This ability to escape or to overcome the effect of growth-preventing serum can be explained in two ways: (1) some bacteria are facultative intracellular parasites and find growth-essential iron inside cells of the infected host, (2) in certain conditions, bacteria may produce a substance which makes Tr-bound iron available for their utilization. The first possibility will be discussed in sec. III of this paper.

Results suggesting an important role of iron-binding bacterial products in the survival of parasites in the host's fluids will be discussed in this section.

It has been suggested by NEILANDS (1957) that microbes can be divided into two types on the basis of their capability to secrete iron-binding materials. The great majority of bacterial species (autosequesteric) produce sideramines (BICKEL et al., 1960; BURNHAM, 1963) which facilitate the transport of iron into the microbial cell. A few bacterial species (anautosequesteric) are unable to synthesize their own sideramine and, unless supplied with such a material, cannot grow even in the presence of available iron. The need of some bacteria for such preformed iron-transporting materials is well documented (LANKFORD et al., 1957; DEMAIN and HENDLIN, 1959; MORRISON et al., 1965; WANG and NEWTON, 1969; SEIDMAN and CHAN, 1970). A growth-promoting substance named enterobactin has recently been isolated from cultures of *S. typhimurium* and found to serve as an iron-carrier for this parasite (POLLACK and NEILANDS, 1970). A number of mutants of *S. typhimurium* were isolated that had lost the capacity to synthesize enterobactin and their growth was drastically inhibited unless the citrate-containing medium was supplemented with enterobactin or iron salts. POLLACK et al. (1970) concluded that the function of enterobactin is to promote the adsorption of iron from the medium by the bacteria. Transduction experiments suggested that the genes for enterobactin biosynthesis are closely linked and that they form an operon that is repressed by the presence of iron.

Probably the best known example of anautosequesterism is the dependence of *Mycobacterium paratuberculosis* on a product manufactured by other species of mycobacteria (TWORT and INGRAM, 1912). This growth factor has been extracted from *Mycobacterium phlei* and was named M (mycobactin) (FRANCIS et al., 1949; FRANCIS et al., 1953). Although the iron-chelating property of M has been recognized from the time of its isolation, it has been viewed as an essential growth factor principally for *M. paratuberculosis*. In a recent detailed review on M, SNOW (1970) considered the biochemical function of these bacillary products as poorly defined and indicated that circumstantial evidence suggests that M may be concerned with the transport of iron between medium and tubercle bacilli.

It has been reported by MARKS (1953, 1954) that M exerts its growth-stimulatory effect not only in *M. paratuberculosis* but also in other strains of mycobacteria. He observed that mammalian tubercle bacilli exposed to inhibitory human plasma require the presence of a lipid growth factor which appeared to be related to or identical with M. We have clarified the biochemical function of M by experiments in which M in various quantities was added to samples of tuberculostatic serum which received additional quantities of iron-free or iron-saturated Tr (KOCHAN et al., 1971a). The fate of BCG cells in tuberculostatic serum-Tr-M mixtures was determined and the results are presented in Fig. 1. In the analysis of these results it is important to remember that the tuberculostasis in serum is not caused by direct action of iron-free Tr on tubercle bacilli. Since at each concentration of M its growth-promoting effect

varied according to the amount of unsaturated Tr it is safe to conclude that M was not acting as an essential growth factor. This observation shows that M, an iron-chelating product of tubercle bacillus, promotes bacillary growth in tuberculostatic serum by removing iron from Tr-iron complexes and supplying it to tubercle bacilli. These findings indicate that the biosynthesis of M arms bacillary cells with a survival mechanism that may play a significant role in host-tubercle bacillus interaction.

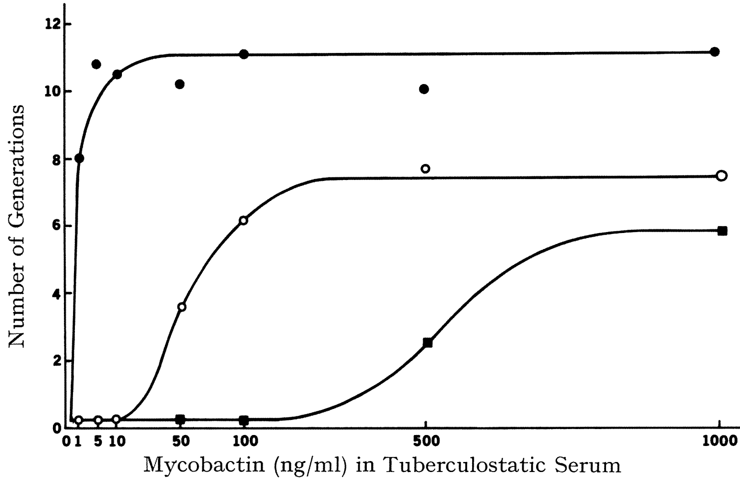


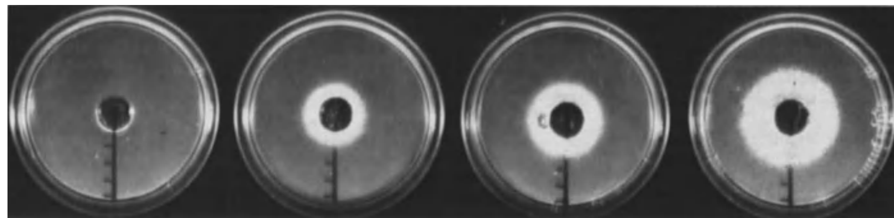
Fig. 1. Growth of BCG cells in tuberculostatic serum in the presence of different concentrations of mycobactin and 5 mg of transferrin per ml. Symbols: • iron-saturated Tr; ▪ iron-free Tr; ○ no Tr. [Reprinted from KOCHAN et al., Infec. Immun., **3**, 553–558 (1971a)]

Recently we developed a technique which significantly reduced the time and work required previously in the study of serum tuberculostasis and associated Tr-iron-M interplay (KOCHAN et al., 1971b). The test is performed in plastic Petri plates containing a sample of mammalian serum mixed in 1:4 dilution with iron-void Dubos agar medium (content of iron in the medium is 0.09 μg per ml). A well is made in the medium at the center of the plate with a glass cylinder. After solidification of agar, the surface of the serum-medium mixture is inoculated with tubercle bacilli. After the evaporation of the inoculum, the glass cylinder is removed and the well is filled with a solution of an antagonist to serum tuberculostasis. The inoculated plates with charged wells are incubated at 37°C in a high humidity chamber for 2 to 3 weeks. The degree of neutralization of tuberculostasis around the wells is determined by measuring the area covered by bacillary growth. Results are recorded by taking pictures of plates placed on a template which has a scale indicating 0.5 cm distances.

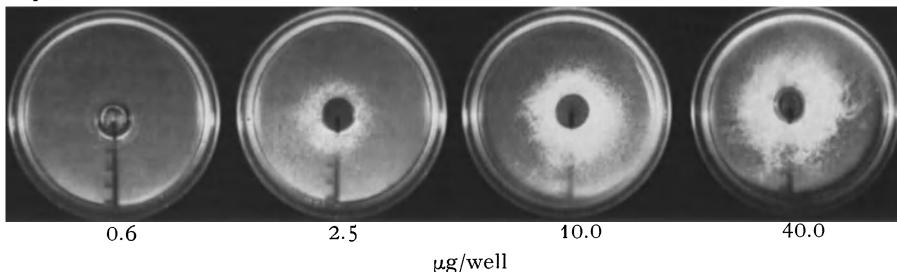
The antagonistic activity of iron and M against serum tuberculostasis was tested by the use of the agar-plate diffusion test. Fig. 2 shows that with each

increasing concentration of iron or M, an increased surface area covered by bacillary growth was observed around the wells. The growth of BCG cells promoted by iron was heavy and elevated with rather sharply defined edges, whereas the growth around M wells was more spreading and similar to that of cord-producing virulent strains. These and similar results (KOCHAN et al., 1971b) showed that this test is quite useful for the study of the competition

Iron



Mycobactin



0.6 2.5 10.0 40.0

μg/well

Fig. 2. Bacillary growth on tuberculostatic bovine serum-agar medium around wells which received 0.6, 2.5, 10.0, and 40.0 μg of iron or mycobactin. The unbound iron-binding capacity of serum was 380. [Reprinted from KOCHAN et al., Infec. Immun., 4, 130–137 (1971b)]

among various iron-chelating biological substances for iron. Since the metal serves as a growth factor not only for tubercle bacilli, but also for many other bacteria and yeasts (WEINBERG, 1971), the test should be suitable for the study of iron requirements of a variety of microorganisms.

It was of considerable interest to determine whether the agar-plate diffusion test was sensitive enough to evaluate the degree of tuberculostasis in various sera, and, if so, whether the degree of tuberculostasis could be predicted by determining the value for unbound iron binding capacity (UIBC) of a given serum. Two bovine sera, one with 410 UIBC and the other with 224 UIBC of iron per 100 ml, were tested by the agar-plate diffusion test for tuberculostatic activity in the presence of 0, 5, and 15 μg of iron (Fig. 3). Wells which received iron were surrounded by bacillary multiplication, and the areas covered by growth increased with increasing concentrations of iron. At each iron concentration, plates containing serum B, with the lower UIBC value, showed more growth than those containing serum A with a high UIBC value. On the basis of these and similar results, we concluded that the agar-plate diffusion test

offers a method which is suitable for the evaluation of the degree of tuberculostasis in various sera.

Much attention has been given to the production of M in amounts sufficient for chemical analyses (SNOW, 1970) and, therefore, bacilli were routinely grown in iron-poor media that stimulate production of iron-chelating products. There are no data pertaining to the amount or even the presence of M on bacillary

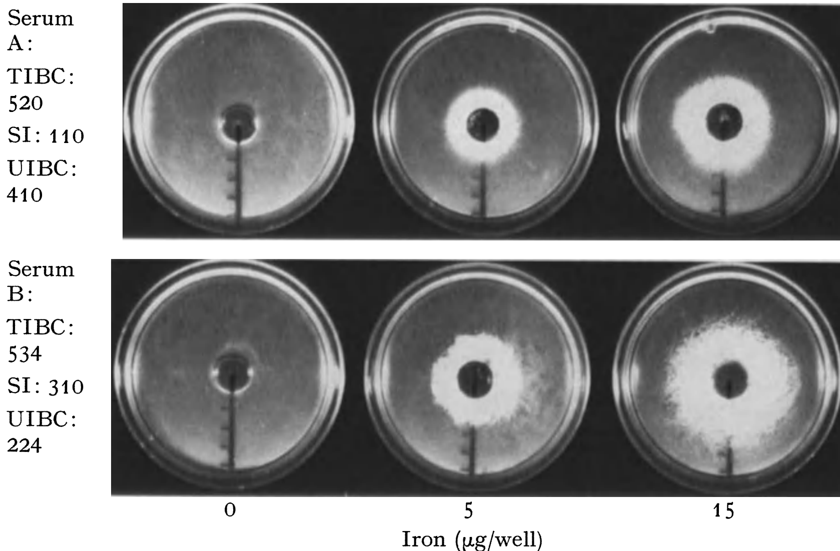


Fig. 3. Bacillary growth on strongly tuberculostatic serum A- and weakly tuberculostatic serum B-agar medium around wells which received 0, 5, and 15 μg of iron. [Reprinted from KOCHAN et al., Infec. Immun., **4**, 130–137 (1971 b)]

cells grown in iron-rich media. To determine whether M is produced in an iron-rich medium, BCG cells were grown on the surfaces of SAUTON (6 μg of iron/ml) and WHITE-SNOW (0.07 μg of iron/ml) media (WHITE and SNOW, 1968). Equal masses of bacilli were extracted with 70% ethanol containing 0.05 % Tween 80 and, after evaporation of alcohol, various dilutions of extracts were tested for serum tuberculostasis-neutralizing quality by the agar-plate diffusion method. Results showed that tubercle bacilli produce M in iron-rich and iron-poor media. Larger amounts of M were found on bacillary cells in iron-poor medium. The demonstration of M on bacilli grown in iron-rich medium suggests that this substance is present on bacillary surface at all times and participates in the transport of the metal into bacillary cells irrespective of the amount of iron present in culture medium (KOCHAN et al., 1971 b).

Probably because of its poor water solubility, M remains for the most part associated with the lipoidal cell wall of tubercle bacillus. This association can be disturbed by Tween 80, a water-dispersible, nonionic, surface-active agent in which M is readily soluble. This pronounced solubility of M in Tween solution enabled us to obtain M not only by extracting bacilli with ethanol

(SNOW, 1970), but also by washing them in Tween 80 solution (KOCHAN et al., 1971 b). We found also that M is present in Tween-containing spent media; 0.05 % of this surfactant in the medium was sufficient to cause a considerable release of M during bacillary growth. If such release of M were to occur in the body of a host under the influence of body surfactants, then this phenomenon would promote bacillary survival; continuous formation and release of M would result in concentrations at which bacilli would obtain iron from Tr-iron complexes and grow unhindered. Recent results (GOLDEN and KOCHAN, unpublished data) indicate that the growth-stimulating effect of surfactants may occur only at very low concentrations; at higher concentrations, all M is released and bacillary growth is stopped because of iron deficiency. The surfactant-induced inhibition of tubercle bacilli can be effectively overcome by M or by high concentrations of iron. Our results also indicate that virulent cells differ from avirulent cells in possessing on their surfaces more lipoidal material which is necessary for the association of M with bacillary cell. The firmer association of M with virulent than with avirulent cells makes the virulent cells more resistant to activity of surfactants and may be the factor determining or contributing to the growth of tubercle bacilli *in vivo*.

III. Effect of Iron on the Fate of Bacteria in Tissues

The role of phagocytic mononuclear cells in the defense of a host against facultative intracellular bacterial parasites has been the most widely studied aspect of host-parasite relationships during the past two decades. Many investigators agree that macrophages of immune hosts possess the ability to limit bacterial multiplication but others are reluctant to accept this conclusion. The cellular antibacterial factors have not as yet been identified. This failure disturbs the proponents of the cellular concept of immunity and cements doubts in the opponents. Usually "cellular immunologists" have limited their research to comparative analyses of the fate of a facultative intracellular parasite in normal and immune hosts, or in infected tissue cultures of normal and immune macrophages. Since the investigations were performed in the presence of antibacterial humoral fluids, sera, or antibiotics, some investigators have understandable reservations about the conclusion that the inhibited bacterial growth is an expression of cellular activity alone. They point out that the increased cellular permeability elicited by the hypersensitive response of immune cells and the increased pinocytotic activity of immune cells in hosts with antibacterial humoral fluids or in antibacterial tissue culture media may favor the accumulation of antibacterial factors in immune rather than in normal macrophages (KOCHAN and SMITH, 1965; RALSTON and ELBERG, 1969a; PATTERSON and YOUMANS, 1970). If so, then the role of phagocytic cells in the defense of the immune host would be limited to the provision of a site and favorable conditions for the expression of antibacterial activity of humoral factors.

It is surprising that immunologists interested in the concept of cellular immunity have made little effort to identify antibacterial factors in cell extracts. Only infrequently have attempts been made to monitor bacterial survival in extracts prepared from normal and immune macrophages. The determination of bacterial fate in cell extracts may offer a method of studying host-intracellular parasite relationships on a molecular rather than a cellular level.

A. Fate of Bacteria in Macrophages

It is not the purpose of this review to discuss the concept of cellular immunity. There are several recently published reviews discussing the role of phagocytes in immunity to facultative intracellular parasites (LURIE, 1964; MACKANESS, 1968; DANNENBERG, 1968; YOUMANS and YOUMANS, 1969; MACKANESS and BLANDEN, 1970). It is necessary, however, to present here some results which show that extracellular factors can influence intracellular parasites because such findings may be pertinent to the understanding of the role of iron in the host-parasite relationship.

Results obtained by following the fate of facultative intracellular bacterial parasites in tissue cultures of macrophages collected from normal and vaccinated animals indicated the existence of cellular immunity. These results showed that the parasites multiplied more slowly in immune than in normal macrophages. Unfortunately, little or no attention was devoted to antibacterial effects on intracellular parasites of serum or antibiotics added to the tissue culture media. This disregard for activities of extracellular factors affecting intracellular bacteria was based upon an assumption that such factors, if present, would exert similar antibacterial effects in immune and in normal phagocytes. That this assumption was erroneous was shown by studies which demonstrated that externally supplied molecules may exert stronger effects on bacteria in immune than in normal macrophages. KOCHAN and SMITH (1965) and PATTERSON and YOUMANS (1970) found that tubercle bacilli grew as rapidly in immune as in normal macrophages; however, in the presence of growth inhibitory human serum or streptomycin, the intracellular mycobacterial growth was more inhibited in immune than in normal phagocytes. Similar findings were reported by RALSTON and ELBERG (1969b), who found that the activity of immune macrophages in the absence of immune serum against *Brucella melitensis* is minimal; only in the presence of immune serum did the restriction of bacterial growth become significant. RALSTON and ELBERG (1969a) suggested that macrophages exert more effect in the presence of growth-inhibitory serum for *Brucella* either because they are able to concentrate antibacterial factors in intracellular loci or because they contribute an additional inhibitory factor. The concentrating activity of phagocytic cells for streptomycin has been reported by BONVENTRE (1968) and by CHANG (1969). It is also possible that extracellular antibacterial factors exert more activity in immune than in normal phagocytes because of increased cellular perme-

ability which is elicited in immune-hypersensitive cells by intracellular parasites or their antigens (KOCHAN and SMITH, 1965).

The importance of the medium in tissue culture studies has been appreciated by WHEELER and HANKS (1965) and HANKS (1966) who found that intracellular growth of tubercle bacilli is not so much influenced by components, metabolism, or immunological properties of host cells as by compounds and conditions provided by the extracellular environment. External supplies of factors such as mycobactin, iron, or glycerol stimulated intracellular growth of bacilli as effectively as though the latter were in test tubes. In the cell-culture systems, each factor alone converted indolent intracellular infections into fulminating ones. These investigators summarized a considerable body of evidence that large molecular compounds, both inhibitors and stimulators, gain access to phagocytic vacuoles and influence intracellular growth.

The passive transfer of immunity from vaccinated to normal animals with cells but not with serum is cited frequently in favor of the cellular concept of resistance to facultative intracellular parasites. This observation, however, offers little support for the cellular hypothesis for the following reasons: (1) According to our findings with antimycobacterial sera, passive transfer of small amounts of hypoferremic and tuberculostatic serum from BCG-vaccinated animals to normal animals fails to induce a state of hypoferremia and tuberculostasis in recipients. Hypoferremia-associated tuberculostasis can only be induced or increased by vaccination and not by passive serum transfer. Therefore, the failure to transfer protection passively with immune serum does not necessarily indicate that humoral antibacterial factors play no role in immunity; (2) it has been well established that delayed hypersensitivity can be transferred with cells (CHASE, 1945) or with cell-materials (KOCHAN and BENDEL, 1966; HILL and NISSEN, 1971) from hypersensitive to normal animals. The state of hypersensitivity may increase pinocytosis or permeability of cells for humoral antibacterial factors. In this case, the acquired resistance to facultative intracellular parasites would not be determined by cells alone, but by the interdependence of humoral and cellular antibacterial properties.

B. Fate of Bacteria in Extracts of Macrophages

Recent reports dealing with the question of cellular immunity suggest that this immunity can be demonstrated most readily in activated macrophages. Such activated cells are collected from heavily infected animals during a limited period during the course of infection (MACKANESS, 1970). Also, it is possible to obtain activated macrophages from vaccinated animals but the donors have to be treated in a special way and the cells have to be collected at a certain time. For instance, GOIHMAN-YAHR et al. (1969) vaccinated guinea pigs with 0.5 mg of heat-killed BCG cells; three weeks later, these animals were injected intravenously with 3.2 mg of bacilli and activated macrophages were collected from peritoneal cavities five days later. It has been observed that the activated state lasts for about 3 weeks after sensitization in macro-

phages and is characterized by increased pinocytotic activity and increased numbers of lysosomes and mitochondria. The increased microbicidal activity of activated macrophages has been associated with increased content of hydrolytic enzymes in lysosomes of immune cells (Cohn and Wiener, 1963; Dannenberg, 1968; Blanden, 1968; Mackaness, 1970). Since the use of activated cells for the demonstration of cellular immunity is strongly emphasized by its proponents, this review will only discuss research in which activated cells were used for the preparation of cell-extracts.

The most important attribute of the activated macrophage is its non-specific microbicidal activity, which may be an expression of increased amounts of lysosomal enzymes acting on molecules present on the surfaces of various bacteria. If so, it should be possible to demonstrate strong antibacterial effects in extracts prepared from activated cells. Kanai and Kondo (1970a) tested antimycobacterial effects of lung tissue extracts. They separated large-molecular complex fractions from lysosomal pellets prepared from normal and activated cells. Upon exposure of tubercle bacilli to these complex fractions they found that the sample obtained from activated lung cells possessed anti-mycobacterial activity whereas the corresponding fraction from normal cells was inactive. However, upon further fractionation of the complex samples, the purified materials exerted similar antimycobacterial activity irrespective of whether they originated from normal or activated cells. These antimycobacterial materials were identified as free fatty acids and peptides. Except for the observation that lysosomal compounds had a strong affinity for the surface of tubercle bacilli, neither this nor the accompanying papers (Kanai and Kondo, 1970b; Kondo et al., 1970) indicated what role lysosomal enzymes play in antimycobacterial activity. Results of work performed by Brown et al. (1969) showed that increased lysosomal activity is associated with vigorous bacterial multiplication rather than with bacterial inhibition. These investigators infected macrophages with *M. tuberculosis* or *Mycobacterium lepraemurium* and observed that cells with low contents of lysosomal enzymes inhibited intracellular growth better than cells with high contents of these enzymes. This study suggested, therefore, that the lysosomal enzymes are not detrimental but are actually beneficial to facultative intracellular parasites as they provide the parasites with low molecular weight nutrients.

Considering the essential iron requirement of the tubercle bacillus, we investigated the availability of this metal for bacillary utilization in cell extracts. Iron is stored in mammalian cells mainly in combinations with ferritin (Harrison, 1964), a well-defined water-soluble crystalline protein. The molecule consists of a protein shell which surrounds ferric hydroxide micelles; the metal accounts for up to about 40–44% of the weight of the ferritin molecule. Whether or not ferritin iron is available for utilization by invading pathogens is not known. It has been observed that the growth of *L. monocytogenes* can be enhanced by inclusion of ferritin in the growth medium (Kingdon and Sword, 1970). On this basis, these investigators suggested that ferritin-iron and possibly other stores of iron in cells become available to the

invading pathogens. Direct evidence of ferritin-iron utilization by intracellular bacterial parasites is not as yet available. In recent experiments we tested the ability of mycobacteria to obtain iron from twice-crystallized horse-spleen ferritin (Pentex, Inc.). Various quantities of ferritin, containing 330 μg of iron per 1 mg of the protein, and iron in the form of ferric ammonium citrate salt, were added to wells made in tuberculostatic serum diluted 1:4 in iron-poor Dubos agar medium. Results showed that ferritin fails to supply tubercle bacilli with iron; to achieve bacillary growth measuring 10 mm in diameter around the wells we had to use more than 1 mg of ferritin (about 400 μg of iron) and only 15 μg of iron as ferric ammonium citrate (unpublished data).

Another iron-binding cellular protein called lactoferrin has been found in leukocytes. This iron-chelating material constitutes a major component of the specific (secondary) granules in the cells (MASSON et al., 1969; BAGGIOLINI et al., 1970). Lactoferrin-iron complexes are much more stable at reduced pH levels than those formed by Tr and iron and therefore lactoferrin can express its iron-chelating activity at acidic pH levels associated with the process of phagocytosis. Since lactoferrin displays bacteriostatic properties in iron-poor media, it may possibly also inhibit intracellular parasites in phagocytic vacuoles into which secondary granules discharge their contents. These considerations suggest that the antibacterial activity of lactoferrin in cells is the same as that of Tr in serum. If so, the activity of lactoferrin should be revealed by the agar-plate diffusion test described above, as was the antimycobacterial activity of Tr.

Attempts were made in our laboratory to detect antimycobacterial activities of ferritin and lactoferrin in materials prepared from normal and activated liver cells of guinea pigs (KOCHAN et al., 1972). Cell-extract, cell-membrane, lysosome-extract, and lysosome-membrane fractions were prepared from liver cells by modifying a method described by WEISSMANN and THOMAS (1962) in the preparation of lysosomal materials for our purpose. The antimycobacterial activities of cell-fractions were determined by the agar-plate diffusion test (KOCHAN et al., 1971 b), in which zones of bacillary inhibition around fraction-containing wells indicate the strength of antibacterial activity. We found that lysosome extracts of normal and activated cells exerted no antimycobacterial activity, cell extracts and lysosome membranes exerted some activity, and cell membranes exerted the strongest activity (Table 5). This comparative study of antituberculous activities of cell fractions showed that fractions prepared from activated cells exerted somewhat more antibacterial effect than fractions of normal cells. In contrast to the neutralization of serum antimycobacterial activity with iron, the metal had no alleviating effect on the antituberculous activities of the cell fractions. Fig. 4 shows that the addition of Tween 80 (polyoxyethylene sorbitan monooleate) to cell extracts potentiated their antimycobacterial activities; the addition of the detergent to lipase solution created a strong antituberculous effect. The antimycobacterial effects exerted by cell fractions, by products of Tween 80-cellular materials or -lipase interactions, and by oleic acid can be eliminated or significantly decreased by

Table 5. Antimycobacterial effects of cellular fractions prepared from normal and activated liver cells

Type of liver cells[a]	Cellular fractions	Widths (mm) of growth-free area in dilutions				
		1:2	1:4	1:8	1:16	1:32
NLC	Cell extract	4	2	0	0	0
ALC	Cell extract	5	4	2	0	0
NLC	Cell membrane	4	3	2	0	0
ALC	Cell membrane	7	5	3	3	1
NLC	Lysosome extract	0	0	0	0	0
ALC	Lysosome extract	0	0	0	0	0
NLC	Lysosome membrane	3	2	1	0	0
ALC	Lysosome membrane	3	2	1	0	0

[a] Normal liver cells (NLC) and activated liver cells (ALC) were used for the preparation of cellular fractions. Concentration of each fraction was corresponding to the original amount of tissue used for its preparation (1 mg of tissue per 5 ml of buffered sucrose); only lysosome extract was used in concentrated form. [Reprinted from KOCHAN et al., Infec. Immun., **6**, 142–148 (1972)].

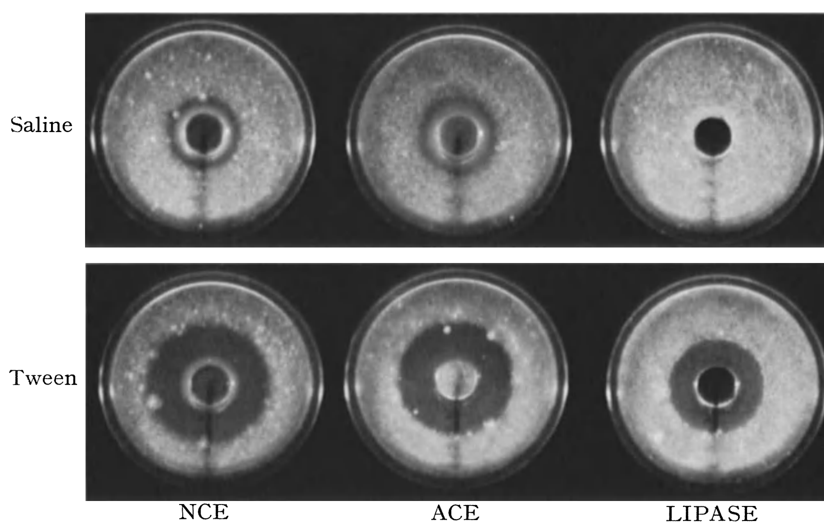


Fig. 4. Growth inhibition around wells which received normal cell-extract (NCE), activated cell-extract (ACE), or 2 mg of lipase solution. Each material was diluted 1:2 either in saline solution itself or saline containing 2% of Tween 80; the diluting fluids themselves had no antimycobacterial activity. Inhibitions in mm: NCE-saline 4, NCE-Tween 11, ACE-saline 5, ACE-Tween 10, lipase-saline 0, lipase-Tween 8. [Reprinted from KOCHAN et al., Infec. Immun., **6**, 142–148 (1972)]

albumin (KOCHAN et al., 1972). This detoxifying activity of albumin as well as other features, such as the stability of the antituberculous products to boiling and the potentiation of the antituberculous effect when the test materials are subjected to longer periods of incubation, suggest that the cell-

associated antimycobacterial effects are exerted by fatty acids. Our recent experiments showed that a close correlation exists between amounts of titratable fatty acids in cell-fractions and the intensity of their antimycobacterial activity. Other investigators have found that fatty acids possess strong antimycobacterial activity (NIEMANS, 1954) and that the production of oleic acid from Tween 80 by tissue lipases may account for the antituberculous activity in mixtures of tissue extracts and Tween 80 (KOTANI et al., 1962). The observation that the main antimycobacterial activity is associated with the washed cell-membrane fraction is of particular interest; it shows that cell membranes possess bound lipases which produce antimycobacterial fatty acids by their action on lipoproteins or phospholipids. Thus, all ingredients necessary for the antituberculous effect are present in the phagosome: a high level of lipase, a high content of phospholipids in phagosomal membranes, and extreme toxicity of fatty acids for tubercle bacilli.

The antimycobacterial effects of cell-materials described are not based on a Tr-iron-mycobactin interplay but on the production of antimycobacterial fatty acids. It is difficult to assess the *in-vivo* importance of this *in-vitro* phenomenon but some circumstantial evidence suggests its potential importance in native and acquired immunity to facultative intracellular parasites. It has been reported that BCG-induced alveolar macrophages display a 2- to 3-fold increase in lipase (COHN and WIENER, 1963). We are finding that serum of "activated" guinea pigs exhibits tuberculostasis which cannot be neutralized by iron and whose intensity is closely correlated with the content of fatty acids (KOCHAN and BERENDT, unpublished data).

IV. Iron-Induced Susceptibility to Bacterial Infections

Since JACKSON and BURROWS (1956) published their original observation on the enhancing effect of iron on the progression of bacterial infection, several groups of investigators have obtained results showing a profound effect of the metal on the host-parasite relationship. It has been reported that the administration of iron promoted the development of diseases in animals infected with *Klebsiella pneumoniae, Pseudomonas aeruginosa* (MARTIN et al., 1963), *S. typhimurium* (CHANDLEE and FUKUI, 1965), *L. monocytogenes* (SWORD, 1966), *P. septica* and *E. coli* (BULLEN and ROGERS, 1969). Not only iron salts but also compounds containing iron have been found to increase the susceptibility of animals to bacterial infections. SWORD (1966) found that mice injected with hemin or erythrocyte-destroying antiserum became more susceptible to *Listeria* infection than untreated animals and the degree of induced susceptibility was equal to that in mice treated with ferric ammonium sulfate. Similarly, haematin hydrochloride, lysed red blood cells, or crystalline haemoglobin treatment greatly enhanced the virulence of *E. coli* in normal guinea pigs (BULLEN et al., 1968a). These results not only demonstrated a promoting effect of iron on bacterial infection, but also suggested that the activity of the metal could not

be attributed to its neutralizing effect on the suggested antibacterial activity of iron-free Tr (LANDAU et al., 1964). In compounds containing iron the metal is not free to saturate Tr and their promoting effects on bacterial infections must therefore be attributed to the availability of iron for bacterial utilization.

The availability of iron for bacterial utilization has been found to increase susceptibility to bacterial infection not only in normal but also in immune animals. The protective effects of antisera against *Cl. welchii* and *P. septica* were abolished with iron-containing compounds (BULLEN et al., 1967; BULLEN et al., 1968b). Since iron compounds stimulate bacterial growth in normal or immune mice and abolish serum bacteriostasis, BULLEN et al. (1971) suggested that the mechanism responsible for antibacterial effects *in vivo* and *in vitro* is the same and can be neutralized with sufficient iron. A similar suggestion has been made by KOCHAN et al. (1969) who observed that the strength of iron-neutralizable serum tuberculostasis in untreated, LPS- or tuberculous cell wall-treated, and vaccinated animals correlated closely with known susceptibilities of such animals to infection with tubercle bacilli.

Results obtained in several laboratories suggest that a close correlation exists between the hyperferremic state and the rapid development of disease in an animal infected with any one of several bacterial parasites. Since hyperferremia seems to be advantageous to the parasite, hypoferremia should benefit the host. This has been found to be the case; when hypoferremia was induced experimentally with endotoxins, the treated animals became more resistant to bacterial infections than untreated animals (CHANDLEE and FUKUI, 1965; PURIFOY and LANKFORD, 1965; PURIFOY et al., 1966). SWORD (1966) used desferrioxamine B methane sulfonate, a specific iron-chelating agent, to deplete iron in mice before and during infection with *Listeria*; this treatment was beneficial to the challenged animals because it induced a hypoferremia which is incompatible with bacterial growth. The host can be benefited in its struggle against infection with some bacterial parasites by the administration of iron-free Tr; MARTIN et al. (1963) found that the increase in levels of unsaturated Tr in blood of rats and mice was beneficial to animals challenged with *K. pneumoniae* and *P. aeruginosa*. Like the hypoferremic state, higher levels of the iron-chelating protein make iron less available for bacterial utilization. Thus, any treatment that lowers the level of free iron in fluids of the host may increase its resistance to many pathogenic microorganisms.

It seems there is ample evidence to support the view that iron significantly influences host-parasite relationships. The availability of iron in hyperferremia favors the growth of many parasites and is therefore associated with susceptibility to bacterial infections and with a rapid progression of disease. The state of hypoferremia is beneficial to the host; parasites fail to obtain sufficient quantities of growth-essential iron and therefore fail to multiply, or multiply with considerable difficulty, producing chronic disease. These conclusions are supported by observations of iron-neutralizing effects on serum antibacterial activities and reconstitution of these activities with iron-binding materials such as Tr, desferrioxamine, ferritin, and others.

Another explanation for the infection-promoting effect of iron can be based upon the detrimental activity of the metal on the host's defense mechanisms. Recently, WAKE et al. (1972) found that attenuated plaque bacilli produce a fatal infection in mice treated with chondroitin-sulfate colloidaliron (Blutal); infected animals treated with iron-free sodium chondroitin sulfate remained healthy. These investigators observed that the administration of Blutal suppressed the development of immunity and suggested that the "virulence-enhancing" effect of iron can be attributed to its interference with the host defense mechanism and not to its growth-stimulatory effect for the bacilli.

There is little evidence to suggest that the disease-promoting effect of iron is attributable to the neutralization of host defense mechanisms. BURROWS (1962) reported that the injection of iron into animals had no effect on the mobilization of phagocytic cells or on their ingestive activities; also iron-treatment did not affect the production of antibodies or increase the toxicity of dead organisms. Similar findings were reported by BULLEN et al. (1967) who observed that iron compounds interfered neither with phagocytosis nor with the fixation of complement by antigen-antibody complexes; KOCHAN and GOLDEN (unpublished results) observed no detrimental effect of iron on phagocytosis of tubercle bacilli by macrophages maintained in tissue cultures. Also, there is other evidence that casts doubt on the hypothesis that exogenous iron impairs defense mechanisms of the host. BURROWS (1963) observed that iron injected even in large quantities into animals infected with avirulent strain of *P. pestis* failed to promote development of disease whereas much smaller iron concentrations enhanced pathogenicity of a virulent strain. If the activity of iron were to neutralize the resistance of the host, iron-treated animals might be expected to succumb to infection with avirulent strains.

Two preliminary experiments in my laboratory showed that treatment of normal and immune mice with iron significantly favored the multiplication of a virulent but not of an attenuated strain of tubercle bacillus. The intraperitoneal injections of ferric ammonium citrate equivalent to 100 μg of iron twice daily promoted more prolific bacillary growth in spleens of iron-treated than in untreated animals. Two and three weeks after infection with the $H_{37}Rv$ strain, spleens of iron-treated immune mice contained about five times more bacilli than spleens of saline-treated immune mice, about the same number as spleens of saline-treated normal mice, and about three times less bacilli than spleens of iron-treated normal mice. If substantiated by further experiments, these findings indicate that iron treatment promotes bacillary multiplication in normal and immune animals; it does not completely neutralize the acquired immunity because if it did the bacillary numbers in iron-treated immune and normal animals would be the same. These results support the findings of BURROWS and suggest that the availability of utilizable iron for bacterial growth, and not the resistance-neutralizing property of the metal, is of crucial importance to the survival of parasites in host fluids and tissues.

V. Summary and Conclusions

Native and acquired resistance of a host to bacterial infection, especially to facultative intracellular parasites, can often be attributed to limitations in the availability of growth-essential iron. Since this metal is bound to Tr in plasma and to ferritin in cells, the infectious bacteria have to produce materials which can remove iron from the proteins and supply it to the pathogens. Some bacteria, such as *M. paratuberculosis*, have lost the ability to produce iron-providing substances and can therefore grow only when such substances are added to iron-containing media.

The presence of a natural iron-chelating agent on the bacterial cell infecting the body of an animal does not guarantee that the cell will be supplied with a sufficient amount of iron. For example, M present on the surface of the tubercle bacillus can help the parasite to obtain the required iron only when Tr in the animal plasma is almost fully saturated with iron or when the blood is enriched with added M, which removes iron from Tr and supplies the metal to the bacilli. This crucial role of M in the survival of the tubercle bacillus has led to the development of a mechanism in bacterial cells which responds to iron starvation by producing increased quantities of M. The quantity of M and its affinity for the bacillary cell seem to determine the survival of tubercle bacilli in tissues of the host. Results obtained in our laboratory (KOCHAN et al., 1971; GOLDEN and KOCHAN, unpublished data) have shown that virulent cells possess not only more M but also M that is much more firmly bound to the bacterial surface of virulent than of avirulent cells. Avirulent bacilli exposed to surfactants such as Tween 80 or lecithin lose M much more rapidly than virulent cells; the depletion of M from the surface of bacilli stops their multiplication. The persistance of M on virulent tubercle bacilli may explain their ability to survive in the body of a host in which they are more able to retain M than avirulent cells in spite of the activities of various surfactants. Thus, M not only ensures survival of tubercle bacilli by providing the parasites with growth-essential iron, but also, because of the firmness of its association with the bacillary cell, may act as a virulence factor.

Our knowledge of the protective responses of the host to invasion by non-toxigenic bacteria, especially by members of the facultative intracellular parasite group, is rather limited. Many investigators have reported that animals respond to bacterial infection, vaccination, or treatment with certain bacterial products by the development of hypoferremia. This limitation in the availability of utilizable iron in the blood of resistant hosts tends to prevent the spread of the disease by submitting the parasite to iron starvation. It has been reported that infectious hypoferremia results from the shift of iron from blood to phagocytic cells of the reticuloendothelial system (VANNOTTI, 1957). It is possible that this shift in serum iron stimulates the production of increased amounts of ferritin in the macrophages as does the administration of large amounts of various iron compounds or rapid breakdown of haemoglobin (HARRISON, 1964). Ferritin is commonly found within lysosomes and phago-

somes and electron microscopic studies have shown that it surrounds phagocytized bacteria within phagosomes of mouse spleen macrophages (ARMSTRONG and SWORD, 1966). It has been shown in our laboratory that ferritin binds iron with surprising avidity and can keep it from bacterial utilization. The possibility exists, therefore, that ferritin limits bacterial multiplication within phagocytic cells. According to this reasoning, then, the shift of iron from blood to phagocytic cells constitutes an important defense mechanism: it determines the development of antibacterial hypoferremia in blood and induces formation of ferritin which binds iron and limits bacterial multiplication in phagocytic cells.

It is questionable whether the host can respond to bacterial invasion by an increase in the level of Tr, even though such an increase would limit iron availability for the growth of bacteria. In our experience the serum iron falls in vaccinated animals or animals treated with bacterial products, while a constant level of Tr is maintained. It has been reported, however, that patients with hypogammaglobulinemia have high levels of iron-free Tr; the increase in this iron-chelating protein may represent a compensatory defense mechanism (MARTIN and JANDL, 1960). One of the most effective defense mechanisms a host could develop against "iron-dependent" parasites would be the production of antibodies to bacterial chelators providing iron. Our attempts to produce such an antibody to the hapten-like M, or to detect it in sera of vaccinated animals, have been unsuccessful. It has been suggested by MIDDLEBROOK (1970) that chemical coupling of the haptenic lipoidal material present in the methanol extract of mycobacterial cells to a protein carrier may induce formation of protective antibodies in vaccinated animals. It is possible that the existence of such antibodies at a very low concentration in immune animals accounts for failures of their detection by serological or passive serum protection techniques. Therefore, attempts should be made to stimulate production of high and detectable levels of antibodies to M with techniques which would endow the molecule with potent immunogenic activity.

Our recent studies indicate that phagocytic cells possess a defense mechanism against bacterial parasites that is based on the production of bactericidal substances. This antibacterial activity is associated with cell membranes and not with the enzymatic content of lysosomes. The antibacterial products seem to be fatty acids which have been shown to exert significant toxic effects against various bacterial parasites. The increased amounts of ferritin and increased production of fatty acids in immune cells may well determine the increased resistance of a host to facultative intracellular parasites.

The results presented in this review indicate that the defense of a host against certain bacterial parasites is primarily determined by the ability of the host to limit the availability of iron which is essential for bacterial growth. Since the host plays an active part in the depletion of utilizable iron not only in serum but possibly also in phagocytic cells, this phenomenon is closely associated with the mechanism of acquired immunity. In contrast to humoral and cellular immunities, such immunity, based on limitations in trace element

nutrition, could be called nutritional immunity. It is quite obvious that there is no sharp distinction between native and acquired nutritional immunity. Since many bacteria depend on the availability of identical essential ions (CLIFTON, 1957), resistance based on trace element nutrition is not specific; *i.e.*, one parasite can induce nutritional immunity against another parasite as long as both parasites require the same essential ion. The relationship between the host and tubercle bacilli, based on interactions among Tr, ferritin, iron and M, offers strong support for the proposal that the study of nutritional require-ments of parasites in host tissues may uncover new ways for the development of effective immunity to many microbial parasites.

Acknowledgements. I acknowledge with thanks my indebtedness to EUGENE D. WEINBERG for his contributions to the concepts discussed in this article. I also thank CAROLE A. GOLDEN for her help in most of my experimental studies during the past seven years.

Support for this work has been provided by research grant GB-24114 from the National Science Foundation and by funds provided by the Committee on Faculty Research, Miami University.

References

ARMSTRONG, B. A., SWORD, C. P.: Electron microscopy of *Listeria monocytogenes*-infected mouse spleen. J. Bact. **91**, 1346–1355 (1966).

BAGGIOLINI, M., DE DUVE, E., MASSON, P. L., HEREMANS, J. F.: Association of lactoferrin with specific granules in rabbit heterophile leukocytes. J. exp. Med. **131**, 559–570 (1970).

BAKER, P. J., WILSON, J. B.: Hypoferremia in mice and its application to the bioassay of endotoxin. J. Bact. **90**, 903–910 (1965).

BICKEL, H., GÄUMANN, E., KELLER-SCHIERLEIN, W., PRELOG, V., VISCHER, E., WETTSTEIN, A., ZÄHNER, H.: Über eisenhaltige Wachstumsfaktoren die Sideramine, und ihre Antagonisten, die eisenhaltigen Antibiotica Sideromycine. Experientia (Basel) **16**, 129–133 (1960).

BLANDEN, R. V.: Modification of macrophage function. J. retic. Soc. **5**, 179–202 (1968).

BOISSEVAIN, C. H.: Growth promotion of the tubercle bacillus by serum albumen. Proc. Soc. exp. Biol. (N.Y.) **44**, 110–112 (1940).

BONVENTRE, P. F.: Uptake of dihydrostreptomycin by macrophages in cultures. J. retic. Soc. **5**, 592–597 (1968).

BORNSIDE, G. H., MERRITT, C. B., WEIL, A. C.: Reversal by ferric iron of serum inhibition of respiration and growth of *Bacillus subtilis*. J. Bact. **87**, 1443–1451 (1964).

BROWN, C. A., DRAPER, P., HART, P. D.: Mycobacteria and lysosomes: a paradox. Nature (Lond.) **221**, 658–660 (1969).

BULLEN, J. J., CUSHNIE, G. H., ROGERS, H. J.: The abolition of the protective effect of *Clostridium welchii* type A antiserum by ferric iron. Immunology **12**, 303–312 (1967).

BULLEN, J. J., LEIGH, L. G., ROGERS, H. J.: The effect of iron compounds on the virulence of *Escherichia coli* for guinea pigs. Immunology **15**, 581–588 (1968a).

BULLEN, J. J., ROGERS, H. J.: Bacterial iron metabolism and immunity to *Pasteurella septica* and *Escherichia coli*. Nature (Lond.) **224**, 380–382 (1969).

BULLEN, J. J., ROGERS, H. J., LEWIN, J. E.: The bacteriostatic effect of serum on *Pasteurella septica* and its abolition by iron compounds. Immunology **20**, 391–406 (1971).

BULLEN, J. J., WILSON, A. B., CUSHNIE, G. H., ROGERS, H. J.: The abolition of the protective effect of *Pasteurella septica* antiserum by iron compounds. Immunology **14**, 889–898 (1968b).

BURNHAM, B. F.: Investigation on the action of the iron-containing growth factors, sideramines, and iron-containing antibiotics, sideromycins. J. gen. Microbiol. **32**, 117–121 (1963).

BURROWS, T. W.: Genetics of virulence in bacteria. Brit. med. Bull. **18**, 69–75 (1962).
BURROWS, T. W.: Virulence of *Pasteurella pestis* and immunity to plague. Curr. Top. Microbiol. Immunol. **37**, 59–113 (1963).
CANETTI, G.: The tubercle bacillus in the pulmonary lesion of man. New York: Springer Publishing Company, Inc. 1955.
CHANDLEE, G. C., FUKUI, G. M.: The role of iron in endotoxin induced non-specific protection. Bact. Proc. p. 45 (1965).
CHANG, Y. T.: Suppressive activity of streptomycin on the growth of *Mycobacterium lepraemurium* in macrophage cultures. Appl. Microbiol. **17**, 750–754 (1969).
CHASE, M. W.: The cellular transfer of cutaneous hypersensitivity to tuberculin. Proc. Soc. exp. Biol. (N.Y.) **59**, 134–142 (1945).
CLIFTON, C. E.: Introduction to bacterial physiology. New York-Toronto-London: McGraw-Hill Book Company, Inc. 1957.
COHN, Z. A., WIENER, E.: Particulate hydrolases of macrophages. I. Comparitive enzymology, isolation and properties. J. exp. Med. **118**, 991–1008 (1963).
COLLINS, F. M.: Cross-protection against *Salmonella enteritidis* infection in mice. J. Bact. **95**, 1343–1349 (1968).
COSTELLO, R., IZUMI, T.: Measurement of resistance to experimental tuberculosis in albino mice. J. exp. Med. **133**, 362–375 (1971).
COURMONT, P., GARDERE, H.: Le pouvoir bactericide du serum et des liquides pleuraux sur la bacille de Koch. J. Med. François **92**, 164–187 (1933).
COURMONT, P., GARDERE, H.: Importance clinique de la recherche du pouvoir bactericide du serum des tuberculeux. Rev. Tuberc. **3**, 312–315 (1937).
DANNENBERG, A. M., JR.: Cellular hypersensitivity and cellular immunity in the pathogenesis of tuberculosis: specificity, systemic and local nature, and associated macrophage enzymes. Bact. Rev. **32**, 85–102 (1968).
DAVIES, R.: The influence of normal blood and certain of its constituents on the growth and viability of the tubercle bacillus. Tubercle (Edinb.) **20**, 1–49 (1938).
DEMAIN, A. L., HENDLIN, D.: "Iron-transport" compounds as growth stimulators for *Microbacterium* sp. J. gen. Microbiol. **21**, 72–79 (1959).
EFFORT, R. J.: The passive transfer of immunity to tuberculosis. Ph. D. dissertation, Stanford University, 1954.
EMMART, E. W., SEIBERT, F. B.: The effect of tuberculous and sensitized sera and serum fractions on the development of tubercles in the chorio-allantoic membrane of the chick. J. Immunol. **50**, 143–160 (1945).
FRANCIS, J., MACTURK, H. M., MADINAVEITIA, J., SNOW, G. A.: Mycobactin, a growth factor for *Mycobacterium johnei*. I. Isolation from *Mycobacterium phlei*. Biochem. J. **55**, 596–607 (1953).
FRANCIS, J., MADINAVEITIA, J., MACTURK, H. M., SNOW, G. A.: Isolation from acid-fast bacteria of a growth-factor for *Mycobacterium johnei* and a precursor of phthiocol. Nature (Lond.) **163**, 365 (1949).
GOIHMAN-YAHR, M., RAFFEL, S., FERRARESI, R. W.: Dalayed hypersensitivity in relation to suppression of growth of *Listeria monocytogenes* by guinea pig macrophages. J. Bact. **100**, 635–640 (1969).
HANKS, J. H.: Host-dependent microbes. Bact. Rev. **30**, 114–135 (1966).
HARRISON, P. M.: Ferritin and haemosiderin. In: Iron metabolism. Berlin-Göttingen-Heidelberg: Springer 1964.
HILL, W. C., NISSEN, B.: The antigen receptor in delayed-type hypersensitivity. II. Passive sensitization of guinea pigs. J. Immunol. **106**, 421–426 (1971).
IZUMI, T., COSTELLO, R.: Temporal development of resistance to pulmonary tuberculosis in swiss albino mice. J. exp. Med. **133**, 376–388 (1971).
JACKSON, S., BURROWS, T. W.: The virulence-enhancing effect of iron on non-pigmented mutants of virulent strains of *Pasteurella pestis*. Brit. J. exp. Path. **37**, 577–583 (1956).
JACKSON, S., MORRIS, B. C.: Enhancement of growth of *Pasteurella pestis* and other bacteria in serum by the addition of iron. Brit. J. exp. Path. **42**, 363–368 (1961).
KALLOS, P., NATHAN, E.: Über die Einwirkung menschlicher Sera auf humane Tuberkulosebazillen in der Kirchnerschen Tiefenkultur. Z. Immun.-Forsch. **76**, 343–354 (1932).
KANAI, K., KONDO, E.: A suggested role of the lysosomal membrane as a part of the defense mechanism against tuberculous infection. Jap. J. med. Sci. Biol. **23**, 295–302 (1970a).

Kanai, K., Kondo, E.: Separation and properties of "in vivo grown tubercle bacilli" associated with the lysosomal membrane. Jap. J. med. Sci. Biol. **23**, 303–314 (1970b).

Kingdon, G. C., Sword, C. P.: Effects of *Listeria monocytogenes* hemolysin on phagocytic cells and lysosomes. Infec. Immun. **1**, 356–362 (1970).

Kirchner, O.: Besitzen Tuberkulose- und Nichttuberkulosesera ein bakterizides Vermögen gegenüber dem Tuberkelbazillus? Z. Immun.-Forsch. **74**, 56–77 (1932).

Kochan, I.: Mechanism of acquired immunity in tuberculosis. Ph. D. dissertation, Stanford University, 1958.

Kochan, I.: Mechanism of tuberculostasis in mammalian serum. I. Role of transferrin in human serum tuberculostasis. J. infect. Dis. **119**, 11–18 (1969).

Kochan, I., Bendel, W. L., Jr.: Passive transfer of tuberculin hypersensitivity in guinea pigs. J. Allergy **37**, 284–294 (1966).

Kochan, K., Cahall, D. L., Golden, C. A.: Employment of tuberculostasis in serum-agar medium for the study of production and activity of mycobactin. Infec. Immun. **4**, 130–137 (1971b).

Kochan, I., Golden, C. A., Bukovic, J. A.: Mechanism of tuberculostasis in mammalian serum. II. Induction of serum tuberculostasis in guinea pigs. J. Bact. **100**, 64–70 (1969).

Kochan, I., Ishak, K., Said, M., Stotts, J.: Study on the tuberculostatic factor of mammalian serum. Amer. Rev. resp. Dis. **88**, 818–826 (1963b).

Kochan, I., Patton, C., Ishak, I.: Tuberculostatic activity of normal human sera. J. Immunol. **90**, 711–719 (1963a).

Kochan, I., Pellis, N. R., Golden, C. A.: Mechanism of tuberculostasis in mammalian serum. III. Neutralization of serum tuberculostasis by mycobactin. Infec. Immun. **3**, 553–558 (1971a).

Kochan, I., Pellis, N. R., Pfohl, D. G.: Effects of normal and activated cell-fractions on the growth of tubercle bacilli. Infec. Immun. **6**, 142–148 (1972).

Kochan, I., Raffel, S.: A property of immune sera inhibitory for the growth of the tubercle bacillus. J. Immunol. **84**, 374–383 (1960).

Kochan, I., Smith, L.: Antimycobacterial activity of tuberculostatic factor on intracellular bacilli. J. Immunol. **94**, 220–227 (1965).

Kondo, E., Kanai, K., Nishimura, K., Tsumita, T.: Analysis of host-originated lipids associating with "in vivo grown tubercle bacilli". Jap. J. med. Sci. Biol. **23**, 315–326 (1970).

Kotani, S., Kutaura, T., Inui, S., Hashimoto, S., Chimori, M.: Antimycobacterial activity of extracts of mononuclear leukocytes from peritoneal exudates of guinea pigs. Biken J. **5**, 133–154 (1962).

Landau, J. W., Dabrowa, N., Newcomer, M. D., Rowe, J. R.: The relationship of serum transferrin and iron to the rapid formation of germ tubes by *Candida albicans*. J. invest. Derm. **43**, 473–482 (1964).

Lankford, C. E., Kustoff, T. Y., Sergeant, T. P.: Chelating agents in growth initiation of *Bacillus globigii*. J. Bact. **74**, 737–748 (1957).

Laurell, C. B.: Metal-binding plasma proteins and cation transport. In: F. W. Putnam (ed.), The plasma proteins, vol. I. New York: Academic Press 1960.

Lurie, M. B.: On the mechanism of immunity in tuberculosis. The host-parasite relationship under the conditions of a localized agar focus of infection and the generalization of the disease in normal and immunized rabbits. J. exp. Med. **63**, 923–946 (1936).

Lurie, M. B.: Studies on the mechanism of immunity in tuberculosis: The role of extracellular factors and local immunity in the fixation and inhibition of growth of tubercle bacilli. J. exp. Med. **69**, 555–577 (1939).

Lurie, M. B.: Studies on the mechanism of immunity in tuberculosis: The fate of tubercle bacilli ingested by mononuclear phagocytes derived from normal and immunized animals. J. exp. Med. **75**, 247–268 (1942).

Lurie, M. B.: Resistance to tuberculosis: Experimental studies in native and acquired defensive mechanisms. Cambridge, Mass.: Harvard University Press 1964.

Mackaness, G. B.: The immunology of antituberculous immunity. Amer. Rev. resp. Dis. **97**, 337–344 (1968).

Mackaness, G. B.: The mechanism of macrophage activation. In: Infectious agents and host reactions. Philadelphia-London-Toronto: W. B. Saunders Company 1970.

Mackaness, G. B., Blanden, R. F.: Cellular immunity. In: Infectious agents and host reactions. Philadelphia-London-Toronto: W. B. Saunders Company 1970.

MARKS, J.: A growth factor for *Mycobacterium tuberculosis*. J. Path. Bact. **66**, 151–156 (1953).
MARKS, J.: The *Mycobacterium tuberculosis* growth factor. J. Path. Bact. **67**, 254–256 (1954).
MARTIN, C. M., JANDL, J. H.: Inhibition of virus multiplication by transferrin. In: Metal binding in medicine. Philadelphia: J. B. Lippincott. 1960.
MARTIN, C. M., JANDL, J. H., FINLAND, M.: Enhancement of acute bacterial infections in rats and mice by iron and their inhibition by human transferrin. J. infect. Dis. **112**, 158–163 (1963).
MASSON, P. L., HEREMANS, J. F., SCHOONE, E.: Lactoferrin, an iron-binding protein in neutrophilic leukocytes. J. exp. Med. **130**, 643–656 (1969).
MEISSNER, G.: Bakterizide und entwicklungshemmende Wirkung des Blutes auf Tuberkelbazillen. Zbl. Bact. **106**, 210–236 (1928).
MIDDLEBROOK, G.: Tuberculosis: Retrospects and prospects. In: Infectious agents and host reactions. Philadelphia-London-Toronto: W. B. Saunders Company. 1970.
MORRISON, N. E., ANTOINE, A. D., DEWBREY, E. E.: Synthetic metal chelators which replace the natural growth-factor requirements of *Arthrobacter terregens*. J. Bact. **89**, 1630 (1965).
MYRVIK, Q., WEISER, R. S.: A tuberculostatic serum substance possessing lysozyme-like properties. Amer. Rev. Tuberc. **64**, 669–674 (1951).
NEILANDS, J. B.: Some aspects of microbial iron metabolism. Bact. Rev. **21**, 101–111 (1957).
NIEMANS, C.: Influence of trace amounts of fatty acids on the growth of microorganisms. Bact. Rev. **18**, 147–163 (1954).
OSHIMA, S., FUGITA, Y., TAKEOKA, A., NAKAJIMA, N., TSUJI, S.: Chemical analysis of the role of humoral factors in native and acquired resistance to tuberculosis. Amer. Rev. Tuberc. **78**, 884–898 (1958).
PAGEL, W.: The effect of blood serum on the growth of tubercle bacilli in the depth of liquid media. J. Path. Bact. **50**, 111–119 (1940).
PATTERSON, R. J., YOUMANS, G. P.: Multiplication of *Mycobacterium tuberculosis* within normal and "immune" mouse macrophages cultivated with and without streptomycin. Infect. Immun. **1**, 30–40 (1970).
PEKAREK, R. S., BOSTIAN, K. A., BARTELLONY, P. J., CALIA, F. M., BEISEL, W. R.: The effects of *Francisella tularensis* infection on iron metabolism in man. Amer. J. med. Sci. **258**, 14–25 (1969).
POLLACK, J. R., AMES, B. N., NEILANDS, J. B.: Iron transport in *Salmonella typhimurium*: Mutants blocked in the biosynthesis of enterobactin. J. Bact. **104**, 635–639 (1970).
POLLACK, J. R., NEILANDS, J. B.: Enterobactin, and iron transport compound from *Salmonella typhimurium*. Biochem. biophys. Res. Commun. **38**, 989–992 (1970).
PURIFOY, D. J., LANKFORD, C. E.: Studies of host-parasite competition for iron. Tex. Rep. Biol. Med. **23**, 637 (1965).
PURIFOY, D. J., WILKINS, T. D., LANKFORD, C. E.: Enhancement of growth and infectivity of *Salmonella typhimurium* by iron and iron (III)-chelates. Bact. Proc. p. 55 (1966).
RAFFEL, S.: The nature of acquired immunity in tuberculosis. Specific and nonspecific factors (cellular and humoral). Bull. int. Un. Tuberc. **30**, 52–59 (1960).
RAFFEL, S.: Immunity, 2nd ed. New York: Appleton-Century-Crofts Inc. 1961.
RALSTON, D. J., ELBERG, S. S.: Serum-mediated immune cellular responses to *Brucella melitensis*. IV. Infection of macrophages under anaerobic conditions. J. Bact. **97**, 471–480 (1969a).
RALSTON, D. J., ELBERG, S. S.: Serum-mediated immune cellular responses to *Brucella melitensis* REVI. II. Restriction of *Brucella* by immune sera and macrophages. J. retic. Soc. **6**, 109–139 (1969b).
ROGERS, H. J.: Bacteriostatic effects of horse sera and serum fractions on *Clostridium welchii* Type A, and the abolition of bacteriostasis by iron salts. Immunology **12**, 285–301 (1967).
ROGERS, H. J., BULLEN, J. J., CUSHNIE, G. H.: Iron compounds and resistance to infection. Further experiments with *Clostridium welchii* Type A in vivo and in vitro. Immunology **19**, 521–538 (1970).
SCHADE, A. L.: The microbiological activity of siderophilin. In: Protides of the biological fluids. New York: Elsevier Publishing Co. 1961.

Schade, A. L.: Significance of serum iron for the growth, biological characteristics, and metabolism of *Staphylococcus aureus*. Biochem. Z. **338**, 140–148 (1963).

Schade, A. L., Caroline, L.: An iron-binding component in human blood plasma. Science **104**, 340–341 (1946).

Seidman, P., Chan, E. C. S.: Growth of *Arthrobacter citreus* in a chemically defined medium and its requirement for chelating agents. J. gen. Microbiol. **60**, 417–420 (1970).

Snow, G. A.: Mycobactins: Iron-chelating growth factors from mycobacteria. Bact. Rev. **34**, 99–125 (1970).

Stephenson, M.: Bacterial metabolism, 3rd ed. London: Longmans, Green 1949.

Sword, C. P.: Mechanisms of pathogenesis in *Listeria monocytogenes* infection. I. Influence of iron. J. Bact. **92**, 536–542 (1966).

Tsuji, S., Ito, K., Oshima, S.: The role of humoral factors in native and acquired resistance to tuberculosis. Amer. Rev. Tuberc. **76**, 90–102 (1957).

Tsuji, S., Oshima, S., Takeoka, A.: The ring method. A method for analyzing the effect of serum on the growth of tubercle bacilli *in vitro*. Amer. Rev. Tuberc. **77**, 524–528 (1958).

Twort, F. W., Ingram, G. L. Y.: A method for isolating and cultivating the *Mycobacterium enteritidis chronicae pseudotuberculosis bovis*, Jöhne, and some experiments on the preparation of a diagnostic vaccine for pseudo-tuberculous enteritis of bovines. Proc. roy. Soc. B **84**, 517–530 (1912).

Vannotti, A.: The role of the reticuloendothelial system in iron metabolism. In: Physiology of the reticuloendothelial system. Oxford: Blackwell Scientific Publications 1957.

Wake, A., Morita, H., Yamamoto, M.: The effect of an iron drug (Blutal) on host response in experimental plague infection. Jap. J. med. Sci. Biol. **25**, 75–84 (1972).

Wang, C. C., Newton, A.: Iron transport in *Escherichia coli*: Roles of energy-dependent uptake and 2,3-dihydroxybenzoylserine. J. Bact. **98**, 1142–1150 (1969).

Weinberg, E. D.: Roles of metallic ions in host-parasite interactions. Bact. Rev. **30**, 136–151 (1966).

Weinberg, E. D.: Roles of iron in host-parasite interactions. J. infect. Dis. **124**, 401–410 (1971).

Weissmann, G., Thomas, L.: Studies on lysosomes. I. The effects of endotoxin, endotoxin tolerance and cortisone on the release of acid hydrolases from a granular fraction of rabbit liver. J. exp. Med. **116**, 433–450 (1962).

Wheeler, W. C., Hanks, J. H.: Utilization of external growth factors by intracellular microbes: *Mycobacterium paratuberculosis* and wood pigeon mycobacteria. J. Bact. **89**, 889–896 (1965).

White, A. J., Snow, G. A.: Methods for the separation and identification of mycobactins from various species of mycobacteria. Biochem. J. **108**, 593–597 (1968).

Youmans, G. P., Youmans, A. S.: Recent studies on acquired immunity in tuberculosis. Curr. Top. Microbiol. Immunol. **48**, 129–178 (1969).

Zitrin, C. M., Wasz-Höckert, O.: Preliminary experiments on passive transfer of protective humoral antibodies in tuberculosis. Amer. Rev. Tuberc. **76**, 256–262 (1957).

Antibodies to Receptors Recognizing Histocompatibility Antigens[1]

H. RAMSEIER[2]

Table of Contents

1. An Account of a System Evaluating Antigenic Recognition in vivo and in vitro and its Immunological Basis 31
2. Nonspecific Blocking of the Recognition Process 38
3. Specific Inhibition of Antigenic Recognition 42
3.1. Antisera to Transplantation Antigens 42
3.2. Antisera to Receptors for Transplantation Antigens 45
4. Demonstration and Basis of Anti-Receptor Antibody Specificity 55
4.1. Specificity of Cell-Induced Anti-RS Antisera 55
4.2. Specificity of Alloantiserum-Induced Anti-RS Antisera 58
5. An Account of two Investigations on Specific Blocking of Antigenic Recognition 59
5.1. Enhancement and Recognition of Tumor-Specific Transplantation Antigens 59
5.2. Transplantation Tolerance 64
6. Perspectives 70
References 75

1. An Account of a System Evaluating Antigenic Recognition in vivo and in vitro and its Immunological Basis

Almost every experiment done in the field of immunology is in one way or another related to antigenic recognition. In this respect, transplantation immunology is no exception. Success or failure of grafts of organs or lymphoid cells is ultimately dictated by mechanisms of antigenic recognition. While, for example, events like rejection of skin grafts or the outcome of graft-versus-host reactions are processes relatively remote, in time, from the actual recognition step, there are in vivo reactions which need shorter time intervals between recognition and immune manifestation.

The immune and the normal lymphocyte transfer reactions (ILTR and NLTR) are well-known examples of this. The basis of these reactions is that if viable suspensions of lymphoid cells of allogeneic origin, sensitized in the case of ILTR or normal in that of NLTR, are inoculated into the skins of normal hosts, cutaneous inflammatory reactions of the delayed type develop. Depending on whether sensitized or normal lymphoid cells have been used to elicit

[1] Experiments by the author were supported in part by a grant from the Swiss National Foundation for Scientific Research and by a grant from the Stiftung Julius Müller zur Unterstützung der Krebsforschung.

[2] Basel Institute for Immunology, 487 Grenzacherstrasse, 4058 Basel, Switzerland, and the Division of Experimental Microbiology, University of Zürich, Zürich, Switzerland.

these reactions, they arise more quickly or more slowly. Cutaneous inflammatory reactions of this nature have been studied in the species guinea pig (BRENT and MEDAWAR, 1967), rabbit (DVORAK et al., 1963), man (AMOS et al., 1965; GRAY and RUSSELL, 1965) and chicken (WARNER, 1964), and extensively also in the Syrian hamster (RAMSEIER and BILLINGHAM, 1966). Studies with animals of the latter species were especially revealing since three lines of inbred animals were available. In histoincompatible strain combinations, ILTR and NLTR could easily be induced and resembled each other, but differed chronologically and in terms of intensity. For this reason NLTR were studied more closely since it was felt that lymphoid cells from normal animals would provide a more informative tool to investigate cell-antigen interactions. Employing parental strain and Fl hybrid animals, studies on the immunogenetic basis of NLTR revealed that they were graft-versus-host reactions (RAMSEIER and BILLINGHAM, 1966; RAMSEIER, 1969a). On the basis of numerous lines of evidence (RAMSEIER, 1969a) it has become quite apparent that cutaneous reactions of the ILTR and NLTR type might express the ability of injected cells to recognize alien host transplantation antigens. Since it was of interest to find the source of these host antigens (and thus the hosts' contribution to these reactions), hamsters were lethally irradiated (1500 r) before immunocompetent cells of allogeneic origin were injected intracutaneously. Irradiation caused a massive and rapid drop of the peripheral blood lymphocyte count and protracted but equally severe granulocytopenia after initial granulocytosis. In the skins of irradiated hosts, very feeble or no ILTR or NLTR could be observed (RAMSEIER and BILLINGHAM, 1966; RAMSEIER and STREILEIN, 1965), indicating that the main source of host antigens was with circulating small lymphocytes. Their destruction by irradiation must have been the reason for the failure of irradiated hosts to display cutaneous reactions.

The likelihood of this interpretation was documented by results of experiments in which allogeneic donor strain node cells were mixed in vitro with node cells syngeneic to the irradiated host and were then injected intracutaneously (RAMSEIER and STREILEIN, 1965). Skin reactions resembling ILTR or NLTR were now obtained in irradiated hamster hosts, presumably because of a local replacement of cells killed by irradiation and needed as targets for allogeneic donor strain node cells. Violent skin reactions were routinely obtained whenever node cells from hamsters sensitized by skin graft rejection were mixed with similar numbers of node cells from animals representing graft antigens (parental or Fl), but also when node cells of two histoincompatible strains of hamsters (parental+parental or parental+Fl hybrid) were mixed and inoculated. Thus, sensitized two-way and one-way reactions as well as normal two-way and one-way reactions could be elicited.

Since little reactivity was recorded in irradiated hamsters after inoculation of unmixed lymphoid hamster cells, hosts were tested for their responsiveness to unmixed lymphoid cells from other species, such as rats, mice, and guinea pigs. Injections of these cells indeed failed to incite reactivity. Cutaneous

reactions of intensities comparable to those obtained with mixed hamster node cells were, on the other hand, observed when mixtures of allogeneic node cells from rats, from mice or from guinea pigs were injected (RAMSEIER and STREILEIN, 1965; RAMSEIER, 1969a). Sensitized as well as normal two-way and one-way reactions could be elicited with cells from these species.

The availability of inbred strains of rats and mice led to an investigation into the immunogenetic basis of these reactions. When rat cells were used it became clear that thymic lymphocytes, while serving well as a source of transplantation antigens, i.e. as target cells, failed to function as responding cells. Results obtained with node cells from normal inbred mice permitted conclusions about interactions of cells from strains differing at the strong H-2 locus or at multiple non-H-2 loci. These resulted in more intense skin reactions than did mixtures of cells from congenic strains differing with respect to one or two non-H-2 loci only. Even differences with respect to H-1+H-3, to H-1 or to H-Y (C57BL/6) resulted in minor, but perceptible reactivities (RAMSEIER and STREILEIN, 1965).

The skin of hamsters has thus proved to be an ideal milieu for the study of interactions of histoincompatible cells. All aspects of this interaction bore the characteristics of a recognition process. The observation that node cells from inbred strain A animals (be it hamster, rat, mouse or guinea pig) mixed and injected together with similar cells from (A×B)Fl hybrid animals (from the same species) resulted in the development of delayed cutaneous reactions is an indication that A cells recognized on (A×B)Fl cells histocompatibility antigen B. While they are compressed with respect to the time scale, these reactions are but a reflection of what takes place in transplantation reactions. Thus the interaction of an immunologically important part of an animal, its lymphoid cells, appeared to give as much information as do experimental models involving the whole animal.

Skin reactivity in the irradiated hamster was further investigated. To determine the type of cells infiltrating skin lesions, those provoked by the injection of incompatible mouse node cells were excised and stained imprints prepared. Two types of cells were found in 24 h-old lesions: small mononuclear cells, forming a minority, and polymorphonuclear (PMN) cells, constituting the majority (up to 95%) (RAMSEIER, 1969a, b). Mononuclear cells were most probably those of the mixed-cell inoculum, since at the time of injection (28 h after irradiation) the blood of hamsters contained negligible numbers of lymphocytes (RAMSEIER and STREILEIN, 1965). The large number of PMN cells, however, must have come from the host.

Clearly, then, PMN cells accumulating at the site of injection of histoincompatible cells must have been responsible for the development of macroscopically visible lesions and most probably not the injected cells themselves. Their interaction, however, might have resulted in the formation of a *mediator* with granulotactic activity.

If such a mechanism were operative, then it might be possible to mimic it under in vitro conditions. Thus, experiments were set up with the aim to

find a mediator for PMN cells in the supernatant of cultured histoincompatible cells. Ultimately, the following procedure was adopted for what is now called the PAR assay (product of antigenic recognition). Accounts of the test procedure containing technical details are given elsewhere (RAMSEIER, 1969b, c). Suspensions of viable lymphoid cells (from nodes or, in most instances, from spleens) were prepared in Hanks' BSS (or medium 199 in early experiments), containing penicillin and streptomycin, but no serum. Suspensions were adjusted to contain 2×10^7/ml cells. In mixtures of incompatible cells (experimental mixtures), 0.5 ml of the cell suspension from one and 0.5 ml of that from the other partner of the mixed culture were added to Falcon tissue culture dishes (60×15 mm) containing 4 ml of Hanks' BSS, again without serum but with antibiotics. One-way mixed cultures thus consisted of 10^7 A lymphoid cells and 10^7 $(A\times B)$Fl lymphoid cells. Control cultures were set up similarly, except that to 4 ml of Hanks' BSS 1 ml (2×10^7) of A lymphoid cells or 1 ml (2×10^7) of $(A\times B)$Fl lymphoid cells were added. At times, additional controls consisted of 5 ml of Hanks' BSS. Mixed and unmixed cultures were incubated at 37°C in a moist atmosphere of 95% air to 5% CO_2 for periods of time permitting maximal responses. The optimal time of incubation was found to vary depending on the strain combination used but was generally in the range of 4 to 10 h (RAMSEIER, 1971a). During cultivation, a product of antigenic recognition (PAR) was formed in dishes containing mixed but not in those containing unmixed cells (or only Hanks' BSS). To demonstrate the leucotactic activity of this mediator, culture supernatants were carefully harvested, centrifuged to remove accidentally harvested cells, and were lyophilized. Samples were reconstituted with 0.06 M phosphate buffer, pH 7.9, in one-fifth the original volume. Concentrated supernatants showed a precipitate which contained the leucotactic activity. After reconstitution the samples were incubated for approximately 1 h at 4°C, and the precipitate was centrifuged and washed once in phosphate buffer. The precipitate was finally resuspended in one-tenth of the original volume. Samples were well mixed and were injected intracutaneously in doses of 0.1 ml into fields marked on the close-clipped backs of hamsters. In early experiments irradiated hamsters were used (RAMSEIER, 1967). Each hamster served as host for 16–18 skin reactions. A given number of doses from any particular sample was inoculated in as many hamsters as were needed for an experiment, usually 6–8. The various experimental and control samples were distributed as randomly as possible into each hamster grid.

When concentrated cell-free culture supernatants of mixed and unmixed mouse, rat or hamster node cells, and also of concentrated medium, were injected into the skins of irradiated hamsters cutaneous reactions developed. They were of about the same intensity as those obtained in similar hosts after the injection of mixed and unmixed node cell suspensions. Again, supernatants of histoincompatible cell mixtures provoked violent reactions, whereas those of controls, including medium, were of weak intensity (RAMSEIER, 1969a). Reactions could be elicited regularly by means of supernatants from cultures

composed of sensitized node cells cocultivated with allogeneic node cells of the sensitizing type, and also from cultures made up of normal node cells taken from genetically disparate donors of the same species.

These findings indicated that culture supernatants contained a mediator with leucotactic activity. Confirmation of this was provided by imprints of skin lesions prepared 24 h after inoculation of culture supernatants revealing predominantly PMN cells (RAMSEIER, 1969a, b).

In view of the fact that the blood of irradiated hamsters contained almost exclusively PMN cells, predominance of this cell type in skin lesions was not surprising. Blood counts of normal, unirradiated hamsters, on the other hand, showed about 70% mononuclear cells and only about 30% PMN cells (RAMSEIER, 1969a). To find out whether or not the mediator was leucotactic in general or specifically granulotactic, culture fluids were injected into the integument of *normal* hamsters. There they provoked similar skin lesions and determinations of cell types found in imprints of 24 h lesions revealed that more than 96% of infiltrating cells were PMN's (RAMSEIER, 1969a, b).

For this reason normal outbred agouti hamsters of local breed were tried as hosts. They proved well suited and were from then on used routinely to demonstrate PAR.

The marked infiltration of an almost pure population of PMN cells into lesions elicited by PAR and the hamster skins' extreme thinness offered an unique opportunity for the quantitative measurement of antigenic recognition by estimating numbers of accumulated PMN cells. This was done by subjecting excised and fragmented 24h- old skin reactions to a short course (3–9 min) of trypsinization, a procedure resulting in the release of PMN cells which could then be counted conventionally under phase contrast (RAMSEIER, 1967, 1969a, b, c). Figures of PMN cells obtained by this procedure from experimental samples were corrected by subtracting those determined in controls. Whenever control values were equal to or slightly higher than experimental values, this is indicated by zero in the tables presented.

Once the assay system for quantitative estimations of antigenic recognition had been developed it was of importance to first demonstrate whether results obtained with it would correlate to skin reaction intensities and, foremost, to well established facts of immunogenetics.

A close correlation between skin reactivity and number of PMN cells counted from these reactions was observed, as shown in Table 1. MHA hamsters, employed 28 h after 1500 r, served as hosts for injections of concentrated supernatants of cultures of node cells taken from animals of histoincompatible strains of mice, hamsters and rats (Table 1). In these early experiments culture medium 199 was used and the cells were cultured for 48 h (which later turned out to be much too long). Scoring (RAMSEIER and BILLINGHAM, 1966) and trypsinization of skin reactions were performed 24 h after inoculations. Numbers of PMN cells determined were low, chiefly because an unsatisfactory lyophilizer had to be used. Despite these restrictions, it is important to note that reaction intensities and PMN counts corresponded. The experimental

Table 1. Correlation between degrees of histoincompatibility, skin reaction intensities and PMN cell counts (Adapted from RAMSEIER, 1967)

Species	Mixed node cell cultures or unmixed control cultures	Antigenic disparity	24 h skin reactions	
			scores	PAR[a]
Rat	DA+Lewis	Ag-B, 1,4	+++	2.74±0.26
	DA+(LewisxDA)Fl	Ag-B, 1,4	+++	2.15±0.28
	DA	none	±	0.37±0.09
	Lewis	none	±	0.50±0.07
	(LewisxDA)Fl	none	±	0.25±0.05
	Medium 199	—	±	0.28±0.02
Mouse	CBA+C57BL/6	H-2+non-H-2	+++	2.72±0.13
	CBA+C3H	non-H-2	++	1.32±0.11
	C57BL/6♀+C57BL/6 ♂	Y	+	0.76±0.08
	CBA	none	±	0.30±0.14
	C3H	none	±	0.20±0.07
	C57BL/6 ♀	none	±	0.28±0.04
	C57BL/6♂	none	±	0.23±0.02
Hamster	CB+MHA	strong	+++	2.00±0.15
	CB+LSH	strong	+++	1.50±0.14
	MHA+LSH	trivial	±	0.38±0.10
	CB	none	±	0.30±0.07
	MHA	none	±	0.20±0.04
	LSH	none	±	0.26±0.04

[a] $\times 10^6$ PMN cells/skin reaction (±SE)

results in Table 1 are uncorrected and values obtained for control reactions have been included to document their order of magnitude.

As pointed out, any assay for the measurement of antigenic recognition is only of value if it can be shown unequivocally to rest on firm immunological grounds. To study this aspect of the PAR assay, experiments were initiated, in which immunologically competent cells, so-called aggressors, were cultivated with cells carrying recognizable transplantation antigens, so-called target cells. Targets were from three sources. First, advantage was taken of the genetically determined fact that (A×B)Fl hybrid animals cannot react immunologically against transplantation antigens carried by parents A and B. Such Fl hybrids cells can, on the other hand, react against antigens of strains C, D, etc. Thus mixed cultures composed of A and (A×B)Fl or of B and (A×B)Fl lymphoid cells are immunological one-way reactions, because only aggressors A and B can recognize transplantation antigens on (A×B)Fl target cells which carry antigens B and A, respectively. Cultures of C and (A×B)Fl must, in contrast, be characterized as two-way reactions, because cells C will recognize on Fl cells antigens A and B and (A×B)Fl hybrid cells are capable of recognizing on the other partner transplantation antigens C. Second, rat thymocytes, which completely or almost completely lack immunocompetence (BILLINGHAM et al., 1962; BILLINGHAM and SILVERS, 1964) formed another source. Third, lymphoid cells from mice rendered tolerant neonatally, and regarded as in-

Table 2. Formation of PAR and immunogenetic basis of lymphoid cell interactions (Adapted from RAMSEIER, 1969c)

Species	Mixed cell cultures	Reaction type	PAR[a]
Rat	DA node cells+Lewis node cells	2-way	4.69
	DA node cells+(LewisxDA)Fl thymocytes	1-way	1.76
	DA node cells+Lewis thymocytes	1-way	2.72
	Lewis node cells+(LewisxDA)Fl thymocytes	1-way	2.33
	Lewis node cells+DA thymocytes	1-way	1.76
	DA thymocytes+Lewis thymocytes	0-way	0.02
	DA thymocytes+(LewisxDA)Fl node cells	0-way	0.02
	Lewis thymocytes+(LewisxDA)Fl node cells	0-way	0.20
	DA thymocytes+(LewisxDA)Fl thymocytes	0-way	0.30
	Lewis thymocytes+(LewisxDA)Fl thymocytes	0-way	0.30
Mouse	CBA node cells+C57BL/6 node cells	2-way	4.67
	CBA node cells+(CBAxC57BL/6)Fl node cells	1-way	4.00
	CBA node cells+(AxCBA)Fl node cells	1-way	3.58
	CBA node cells+(AxC57BL/6)Fl node cells	2-way	4.13
	A node cells+CBA tolerant of A node cells	1-way	4.61
	C57BL/6 node cells+CBA tolerant of C57BL/6 node cells	1-way	2.97
	CBA tolerant of A node cells+(AxC57BL/6)Fl node cells	2-way	3.02
	CBA tolerant of C57BL/6 node cells+ (AxC57BL/6)Fl node cells	2-way	3.50
	CBA tolerant of A node cells+(AxCBA)Fl node cells	0-way	0.12
	CBA tolerant of C57BL/6 node cells+ (CBAxC57BL/6)Fl node cells	0-way	0.04

[a] $\times 10^6$ PMN cells per skin reaction. Average of 6-38 skin reactions.

competent with respect to those transplantation antigens of which tolerance was induced, were also employed.

With rat or mouse lymphoid cells (from nodes or spleens) cultures were initiated which were composed of aggressors and targets from these various sources. Cultures were grown in Hanks' BSS for 4 h in the case of rat cells and for 7 h in the case of mouse cells. Comparatively low PAR values were recorded because of the low quality lyophilizer.

A summary of the results obtained is shown in Table 2. They demonstrate that PAR formation was intimately correlated with the ability to recognize antigenic foreignness (RAMSEIER, 1969c). Thus, whenever PAR was formed, the cell mixture was composed of aggressor and target cell types, except in immunological two-way reactions in which both partners were at the same time aggressors and targets. Whenever PAR formation was absent, cells with aggressor abilities were lacking in the mixed cell cultures. Highly incompatible, but immunologically incompetent cells, like thymocytes from two strains of rats or thymocytes from one parental strain and Fl hybrid node cells failed to form PAR. Mere histoincompatibility in the absence of cells endowed with the ability to recognize foreignness did not lead to PAR formation. The firm

immunological basis on which PAR elaboration rested was perhaps best illustrated by experiments employing cells from mice made specifically tolerant. Cells from CBA mice tolerant of A and from CBA mice tolerant of C57BL/6 failed to recognize the tolerogen, i.e. the antigens of those cells against which tolerance was induced.

Satisfactory evidence has therefore been obtained for the view that mixed cell cultures will elaborate PAR only if at least one of the partners is competent to interact with cells carrying histocompatibility antigens of recognizable foreignness. From evidence accumulated so far, the ability to recognize is the privilege of viable immunocompetent cells. This ability can be measured either by scoring cutaneous reactions developing in hamsters or, more accurately, by counting PMN cells accumulated in reaction lesions, released by trypsin. Since the latter procedure is much more objective it was employed throughout the work to be discussed.

2. Nonspecific Blocking of the Recognition Process

Among the many types of mixed cell culture listed in Table 2, those of the normal one-way reaction variety furnish most information on the recognition event. When lymphoid cells from normal animals A and from (A×B)Fl hybrids are cocultivated, only A aggressors can recognize because Fl cells are for genetic reasons incapable of perceiving A antigens on aggressors as foreign and thus formed ideal targets. Such mixtures are, therefore, composed of two cell populations with distinctly different properties and, consequently amenable to differentiated treatments aimed at blocking recognition.

Numerous agents could be shown to result in nonspecific blocking of the recognition process (RAMSEIER, 1971b). To test these, aggressor or target spleen cells at concentrations of 2×10^7/ml of Hanks' BSS were treated with the agent for 20 to 30 min at 37°C under tissue culture conditions. Cells were then washed three times in the same medium, counted and readjusted to 2×10^7/ml viable cells and were cultivated with untreated partners. Untreated cells were subjected to the same procedures and at the same cell concentration but agents were absent from Hanks' BSS. Positive controls were cultures in which both partners of cell mixtures were used untreated. All experimental cell cultures consisted of one treated and of one untreated partner. The response, in terms of PAR, of these cultures is given as percentages of positive controls or in terms of absolute values ($\times10^6$ PMN cells per skin reaction).

It was to be assumed that in normal one-way reaction mixtures aggressor cells A recognized transplantation antigens B of (A×B)Fl target cells by virtue of a surface structure, which might well be protein in nature. As such it would be susceptible to hydrolytic enzymes. Mouse and rat aggressor and target cells were, therefore, subjected to treatments with trypsin before cultivation with untreated cell partners. As the results in Table 3 demonstrate, aggressors of both species were prevented from recognizing antigens by very small amounts of trypsin. No such effect was observed when target cells were

Table 3. Effect of trypsin on the recognition of transplantation antigens

Species	Mixed spleen cell cultures		% PAR
	aggressors	targets	
Mouse	C57BL/6 untreated	(A×C57BL/6)Fl untreated	100.00
	C57BL/6+12.5 μg/ml	(A×C57BL/6)Fl untreated	11.74
	C57BL/6+6.25 μg/ml	(A×C57BL/6)Fl untreated	140.57
	C57BL/6 untreated	(A×C57BL/6)Fl+100 μg/ml	106.05
Rat	DA untreated	(Lewis×DA)Fl untreated	100.00
	DA+25 μg/ml	(Lewis×DA)Fl untreated	3.38
	DA+12.5 μg/ml	(Lewis×DA)Fl untreated	69.59
	DA+6.25 μg/ml	(Lewis×DA)Fl untreated	120.94
	DA untreated	(Lewis×DA)Fl+100 μg/ml	120.60

Cultivation was for 7 h. (Adapted from RAMSEIER, 1971b).

treated with comparatively high concentrations of trypsin. The effect of trypsin must be regarded as nonspecific because both rat and mouse cells were equally affected (RAMSEIER, 1971b).

These results hinted at the possibility that the surface structures in question were receptors or recognition structures (RS) for transplantation antigens. It is now well appreciated that receptors are immunoglobulin-like structures at least as far as bone marrow- or bursa-derived (B) cells are concerned (SELL and GELL, 1965; ADA et al., 1970; GREAVES, 1970; LESLEY and DUTTON, 1970; PERNIS et al., 1970; RAFF et al., 1970; TRUFFA-BACHI and WOFSY, 1970; WIGZELL and MÄKELÄ, 1970; RABELLINO et al., 1971; UNANUE, 1971). From various lines of evidence (MILLER et al., 1971; SPRENT and MILLER, 1971; RAMSEIER and LINDENMANN, 1972b) it appears, however, that the cell type involved in the recognition of transplantation antigens might be of thymus-derived (T) cell origin. Of receptors on these cells it is assumed that their frequency is very low (RAFF et al., 1970; RABELLINO et al., 1971). The surprisingly low concentrations of trypsin needed to inactivate these receptors would be consistent with this. Furthermore, trypsin is known to cleave the hinge region of IgM molecules (PAUL et al., 1971) under conditions not too dissimilar to those used here. This might indicate that inhibition of antigenic recognition by trypsin has been brought about by cleavage and, in turn, might suggest that receptors for transplantation antigens are chemically similar to IgM.

Another aspect of these experiments is concerned with the question of resynthesis of receptors presumably inactivated by trypsin. Under in vitro conditions, in a rather poor medium (Hanks'BSS) and during a cultivation time of only 7h, as employed in the PAR assay, resynthesis of receptors could hardly be expected. It might, however, take place under in vivo conditions. Accordingly, C57BL/6 spleen cells were treated as before with concentrations of trypsin ranging from 6.25 to 200 μg/ml and were injected in doses of 10^6 cells intravenously into newborn (A×C57BL/6)Fl mice in a lethal graft-versus-host assay. With one

Table 4. Activity and specificity of an anti-lymphocytic serum on the recognition of mouse, rat and hamster transplantation antigens

Species	Mixed spleen cell cultures		% PAR
	aggressors	targets	
Mouse	CBA untreated	(CBA × C57BL/6)Fl untreated	100.00
	CBA+ALS 1:128–1:2048	(CBA × C57BL/6)Fl untreated	0.00
	CBA+ALS 1:4096	(CBA × C57BL/6)Fl untreated	125.65
	CBA untreated	(CBA × C57BL/6)Fl+ALS 1:32–1:512	82.66–92.04
	C57BL/6 untreated	(CBA × C57BL/6)Fl untreated	100.00
	C57BL/6+ALS 1:1024	(CBA × C57BL/6)Fl untreated	0.00
	C57BL/6 untreated	(CBA × C57BL/6)Fl+ALS 1:32–1:128	77.59–92.52
	C3H untreated	(A × C3H)Fl untreated	100.00
	C3H+ALS 1:1024	(A × C3H)Fl untreated	7.95
	C3H untreated	(A × C3H)Fl+ALS 1:32–1:128	94.40–112.22
	A untreated	(A × C3H)Fl untreated	100.00
	A+ALS 1:1024	(A × C3H)Fl untreated	9.04
	A untreated	(A × C3H)Fl+ALS 1:32–1:128	85.99–96.30
Rat	DA untreated	(BN × DA)Fl untreated	100.00
	DA+ALS 1:1024	(BN × DA)Fl untreated	0.00
	DA untreated	(BN × DA)Fl+ALS 1:128	157.65
	BN untreated	(Lewis × BN)Fl untreated	100.00
	BN+ALS 1:1024	(Lewis × BN)Fl untreated	0.00
	BN untreated	(Lewis × BN)Fl+ALS 1:128	119.57
Hamster	CB untreated	(CB × MHA)Fl untreated	100.00
	CB+ALS 1:1024	(CB × MHA)Fl untreated	0.00
	CB untreated	(CB × MHA)Fl+ALS 1:128	139.01

Cultivation was for 4 h in CBA→C57BL/6 and C57BL/6→CBA as well as for rat and hamster strain combinations. Others were cultivated for 7 h. (Adapted from RAMSEIER, 1971b)

exception, all 38 newborns injected died, as did 13 animals that received untreated parental strain cells (RAMSEIER, 1971b). This provided evidence that the ability of aggressor cells to recognize foreign transplantation antigens had suffered no permanent impairment.

Nonspecific inhibition of the process of antigenic recognition could also be demonstrated with anti-lymphocyte serum. When in mixed spleen cell cultures either aggressor cells or target cells were pretreated before cocultivation with an ALS preparation raised in a New Zealand white rabbit against CBA node cells and showing strong immunosuppressive qualities [as determined by prolonged survival of allogeneic skin grafts (RAMSEIER, 1970a)], the results summarized in Table 4 were obtained. From these it is clear that ALS acted on recognizing cells only and had no effect on target cells. This is consistent with results obtained by LEVEY and MEDAWAR (1967) which showed that ALS-treated CBA spleen cells did not induce splenomegaly in adult (CBA × C57BL)Fl mice, whereas cells treated with normal rabbit serum or not at all

did. Normal New Zealand white rabbit serum lacked activity against both aggressors and targets. The results in Table 4 demonstrate, furthermore, that ALS in the concentrations listed showed a remarkable degree of nonspecificity in its ability to block recognition not only of mouse, but also of rat and Syrian hamster transplantation antigens. The action of ALS as revealed in these experiments is not inconsistent with the idea that one of the fundamental immunosuppressive mechanisms of these sera is blocking of antigenic recognition. This activity is, however, neither strain nor even species specific; an observation confirmed in experiments by CASPARY et al. (1971). The extreme nonspecificity of ALS would also explain the success of cross-species grafts (MONACO et al., 1966; LEVEY and MEDAWAR, 1966; LANCE and MEDAWAR, 1968, 1970; BALNER et al., 1969; SHAFFER, 1969) and underlines the impression that ALS induces wholesale immunosuppression (BALNER and DERSJANT, 1969; DEODHAR and CRILE, 1969; MANDEL and DE COSSE, 1969; STANBRIDGE and PERKINS, 1969; WOODS, 1969; LAW, 1970).

Inhibition of antigenic recognition of a somewhat restricted nonspecificity was obtained employing rabbit anti-PAR antisera. These were raised in New Zealand white rabbits by three injections (of which the first two were with Freund's complete adjuvant) of PAR produced by culturing allogeneic mixtures of untreated rat, mouse and hamster node cells obtained from normal animals. By the same procedure rabbit sera to rat control culture fluids (cultivation of syngeneic DA or (Lewis × DA)Fl node cells only) were raised for control purposes. These proved to be devoid of activity (RAMSEIER, 1971b). An antiserum raised against PAR formed by the interaction of DA and (Lewis × DA)Fl node cells, however, inhibited antigenic recognition, presumably again by virtue of its ability to block receptors on DA aggressor spleen cells. This activity could be demonstrated up to a serum dilution of 1:8192. The serum lacked anti-target [(Lewis × DA)Fl] cell activity (RAMSEIER, 1971b). When, on the other hand, the serum was tested for its anti-aggressor cell activity, using an inhibitory working dilution of 1:2048, on spleen cells obtained from various rat, mouse and hamster strains, results as summarized in Table 5 were obtained. The xenogeneic anti-rat PAR serum (anti-[DA + (Lewis × DA)Fl]PAR) appeared to have blocked receptors for various rat transplantation antigens on all types of aggressor cells tested. Thus, no strain specificity was apparent. The same serum, however, showed no ability to block receptors on mouse aggressor cells. When used to treat similar cells from Syrian hamsters, a curious and as yet unexplained cross-reactivity could be observed. As demonstrated by the activity of rabbit anti-PAR sera to inactivate PAR (rather than to interfere with receptors on aggressor cells, as above), this cross-reactivity turned out to be mutual (RAMSEIER, 1971b). On the whole, however, anti-rat, mouse and hamster PAR antisera, while lacking strain specificity, were at least species specific.

In summary, these results suggest that the more sophisticated the agent with which blocking of antigenic recognition was attempted, the more specific inhibition was. The nature of the agents used to intervene with the recognition

Table 5. Activity and specificity of a rabbit anti-[DA+(Lewis×DA)Fl] PAR serum (1:2048) on the recognition of rat, mouse and hamster transplantation antigens

Species	Mixed spleen cell cultures		% PAR
	aggressors	untreated targets	
Rat	DA untreated	(Lewis×DA)Fl	100.00
	DA+anti-rat PAR	(Lewis×DA)Fl	0.00
	DA untreated	(BN×DA)Fl	100.00
	DA+anti-rat PAR	(BN×DA)Fl	7.63
	Lewis untreated	(Lewis×DA)Fl	100.00
	Lewis+anti-rat PAR	(Lewis×DA)Fl	0.00
	Lewis untreated	(Lewis×BN)Fl	100.00
	Lewis+anti-rat PAR	(Lewis×BN)Fl	0.00
	BN untreated	(BN×DA)Fl	100.00
	BN+anti-rat PAR	(BN×DA)Fl	0.00
	BN untreated	(Lewis×BN)Fl	100.00
	BN+anti-rat PAR	(Lewis×BN)Fl	1.84
Mouse	C57BL/6 untreated	(CBA×C57BL/6)Fl	100.00
	C57BL/6+anti-rat PAR	(CBA×C57BL/6)Fl	112.70
	CBA untreated	(CBA×C57BL/6)Fl	100.00
	CBA+anti-rat PAR	(CBA×C57BL/6)Fl	113.70
	A untreated	(A×C3H)Fl	100.00
	A+anti-rat PAR	(A×C3H)Fl	86.07
	DBA/2 untreated	(C57BL/6×DBA/2)Fl	100.00
	DBA/2+anti-rat PAR	(C57BL/6×DBA/2)Fl	100.47
Hamster	CB untreated	(CB×LSH)Fl	100.00
	CB+anti-rat PAR	(CB×LSH)Fl	63.47
	LSH untreated	(CB×LSH)Fl	100.00
	LSH+anti-rat PAR	(CB×LSH)Fl	52.94

Cultivation was for 4 h for DA→BN, C57BL/6→CBA, CBA→C57BL/6 and for DBA/2→C57BL/6 as well as for hamster strain combinations. Other cultures were for 7 h at 37°C. (Adapted from RAMSEIER, 1971b)

process made specific blocking unlikely. To achieve this goal, more specific agents were required. For this, two possibilities offered themselves: specific blocking of transplantation antigens on target cells and specific blocking of receptors on aggressor cells. The latter goal could be achieved by the application of anti-recognition structure (anti-RS) sera and the former by the employment of alloantisera.

3. Specific Inhibition of Antigenic Recognition

3.1. Antisera to Transplantation Antigens

Inbred animals grafted with allogeneic skin are known not only to develop cellular immunity to the antigens present in the graft but also to form alloantibodies to these same antigens (AMOS et al., 1954; PALM, 1962, 1964; MISHELL et al., 1963; SNELL and STIMPFLING, 1966; HILDEMANN, 1967; RAMSEIER and

Table 6. Blocking of recognition of rat transplantation antigens by alloantisera

Mixed spleen cell cultures		%PAR
aggressors	targets	
DA untreated	(Lewis × DA)Fl untreated	100.00
DA untreated	(Lewis × DA)Fl + DA anti-Lewis 1:32–1:512	0.00
DA untreated	(Lewis × DA)Fl + DA anti-Lewis 1:1024	93.46
DA + DA anti-Lewis 1:32	(Lewis × DA)Fl untreated	99.22
DA + Lewis anti-DA 1:32	(Lewis × DA)Fl untreated	83.18
DA untreated	(Lewis × DA)Fl + Lewis anti-DA 1:32	100.00
Lewis untreated	(Lewis × DA)Fl untreated	100.00
Lewis untreated	(Lewis × DA)Fl + Lewis anti-DA 1:32–1:128	0.00
Lewis untreated	(Lewis × DA)Fl + Lewis anti-DA 1:256	87.81
Lewis + Lewis anti-DA 1:32	(Lewis × DA)Fl untreated	115.75
Lewis + DA anti-Lewis 1:32	(Lewis × DA)Fl untreated	98.36
Lewis untreated	(Lewis × DA)Fl + DA anti-Lewis 1:32	93.46

Cultivation was for 7 h. (Adapted from RAMSEIER and LINDENMANN, 1971 a)

PALM, 1967). Alloantibodies of the proper specificity should be well suited to block the process of antigenic recognition because of their putative ability to coat on Fl hybrid target cells those antigens aggressors normally recognize. On the other hand, alloantibodies should have no effect on the receptors of aggressor cells. To test these premises, rat, mouse and hamster spleen cells to be used as aggressors or as targets were pretreated, as described above, with alloantisera before being cultivated with untreated spleen cells (RAMSEIER and LINDENMANN, 1971 a). All alloantisera employed were posttransplantation sera harvested three weeks postoperatively in the case of strong histoincompatibilities or 35 days after grafting in H-Y difference. Sera were heat-inactivated and sterilized by filtration.

Characteristic examples on the specificity of intervention with antigenic recognition by means of alloantisera are given in Tables 6–8 for rat, mouse and hamster cell systems, respectively. The results of these experiments provided good evidence for the anti-target cell activity of alloantisera. Though titers differed, these sera blocked antigenic recognition in every instance and it is reasonable to assume that this had been achieved by coating of antigens. This mechanism must be of high specificity, for treatment of hybrid cells, say, of (Lewis × DA)Fl origin which have both Lewis and DA transplantation antigens with serum DA anti-Lewis blocked on these cells Lewis but not DA antigens and vice versa (Table 6). This specificity was also observed in the mouse system studied (Table 7). Antigenic structures on Fl cell surfaces must thus be well separated entities. When alloantisera were used to treat aggressor cells they proved to be harmless. In this respect it is of interest to note that treatment of, for instance, DA aggressors with a concentration of serum Lewis anti-DA which, as shown by its effect on (Lewis × DA)Fl targets, can be

Table 7. Blocking of recognition of mouse transplantation antigens by alloantisera

Mixed spleen cell cultures		% PAR
aggressors	targets	
A untreated	(A×CBA)Fl untreated	100.00
A untreated	(A×CBA)Fl+A anti-CBA 1:256–1:4096	0.00
A untreated	(A×CBA)Fl +A anti-CBA 1:8192	107.12
A+A anti-CBA 1:32	(A×CBA)Fl untreated	101.74
A+CBA anti-A 1:32	(A×CBA)Fl untreated	112.64
A untreated	(A×CBA)Fl+CBA anti-A 1:256	107.42
CBA untreated	(A×CBA)Fl untreated	100.00
CBA untreated	(A×CBA)Fl+CBA anti-A 1:256	0.00
CBA untreated	(A×CBA)Fl+CBA anti-A 1:512	90.23
CBA+CBA anti-A 1:32	(A×CBA)Fl untreated	122.15
CBA+A anti-CBA 1:32	(A×CBA)Fl untreated	116.61
CBA untreated	(A×CBA)Fl+A anti-CBA 1:256	107.65

Cultivation was for 7 h. (Adapted from Ramseier and Lindenmann, 1971a)

Table 8. Blocking of recognition of hamster transplantation antigens by alloantisera

Mixed spleen cell cultures		%PAR
aggressors	targets	
CB untreated	(CB×LSH)Fl untreated	100.00
CB untreated	(CB×LSH)Fl+CB anti-LSH 1:128, 1:256	0.00
CB untreated	(CB×LSH)Fl+CB anti-LSH 1:512	141.31
CB+CB anti-LSH 1:32	(CB×LSH)Fl untreated	109.26
MHA untreated	(CB×MHA)Fl untreated	100.00
MHA untreated	(CB×MHA)Fl+MHA anti-CB 1:128, 1:256	0.00
MHA untreated	(CB×MHA)Fl+MHA anti-CB 1:512	116.90
MHA+MHA anti-CB 1:32	(CB×MHA)Fl untreated	116.90

Cultivation was for 4 h. (Adapted from Ramseier and Lindenmann, 1971a).

assumed to have blocked all DA antigens, failed to block receptors for Lewis transplantation antigens. This finding also held for the inverse rat strain combination (Table 6) and for the two mouse strains studies (Table 7). It indicated that receptors and structures representing transplantation antigens must be arranged on the cell surface in such a manner that they cannot be blocked nonspecifically.

It has been pointed out earlier that the PAR assay is capable of measuring comparatively weak histocompatibility antigens, such as those controlled by the Y locus (Table 1). As a demonstration of the remarkable sensitivity of the PAR assay system, the results obtained in an attempt to block the Y antigen on C57BL/6 male spleen cells are set out in Table 9. The anti-Y serum employed was raised in C57BL/6 female mice by the rejection of one C57BL/6 male skin graft. When used to treat C57BL/6 female aggressor spleen cells (which lack antigen Y), this serum failed to exert activity, but was amazingly active against C57BL/6 male spleen cells (Ramseier and Lindenmann, 1971a).

Table. 9. Blocking of recognition of H-Y mouse transplantation antigens by alloantiserum

Mixed spleen cell cultures		%PAR
aggressors	targets	
C57BL/6 ♀ untreated	C57BL/6 ♂ untreated	100.00
C57BL/6 ♀ untreated	C57BL/6 ♂+anti-Y 1:16–1:8192	0.00
C57BL/6 ♀ untreated	C57BL/6 ♂+anti-Y 1:16384	104.09
C57BL/6 ♀+anti-Y 1:32–1:128	C57BL/6 ♂ untreated	94.70–101.67

Cultivation was for 10 h. (Adapted from RAMSEIER and LINDENMANN, 1971 a).

Taken together, results on interventions with processes of antigenic recognition by means of alloantisera revealed remarkable degrees of specificity. Alloantisera were not only capable of blocking on Fl hybrid target cells only one of the two antigens present (while leaving the others to be fully expressed) but were completely inactive with respect to the recognizing abilities of aggressor cells. In all systems studied [including the blocking of non-H-2 determined transplantation antigens (RAMSEIER and LINDENMANN, 1971 a)], an anti-receptor activity of posttransplantation sera could not be found. Furthermore, the sensitivity of the PAR assay permitted demonstration of alloantisera activities not possible by other procedures, such as those against non-H-2 or H-Y determined mouse transplantation antigens or against hamster histocompatibility antigens (Table 8) (RAMSEIER and LINDENMANN, 1971 a).

Thus far evidence has been presented for highly specific inhibitions of the process of antigenic recognition via blocking of transplantation antigens of target cells. It will now be documented that recognition of transplantation antigens can be blocked with equally high specificity by the employment of antisera against aggressor cell receptors specific for given transplantation antigens.

3.2. Antisera to Receptors for Transplantation Antigens

Well known facts of transplantation immunogenetics and serology permitted postulation and, in conjunction with the PAR assay, testing of anti-receptor (anti-RS) antisera. Among others, facts on immunogenetics state that, if an (A×B)Fl hybrid animal is injected with parental strain lymphoid cells, it develops signs of graft-versus-host disease. This disease can be lethal if Fl hosts are either newborn or rendered immunological cripples by various means such as irradiation, thymectomy, treatment with drugs etc. (BILLINGHAM, 1968). Whenever Fl hosts are immunologically fit, graft-versus-host reactions take a more benign course, frequently characterized by enlargement of lymphoid organs. These reactions are known to be initiated by the reactivity of the injected parental strain lymphoid cells against transplantation antigens the Fl host has inherited from the other parent. Because of their foreignness, these antigens stimulate parental strain donor cells to proliferate (FOX, 1962) and to mount an immune attack against the host. Since for genetic reasons

cells from both parents (A and B) of (A × B)Fl hybrids are accepted permanently by these hosts and can consequently continue their immune attack indefinitely, there is no obvious reason for this reaction to stop in immunologically fit adult Fl hybrids (BILLINGHAM, 1968). In contrast to immunologically deficient hosts, this is, however, the situation normally observed. One possible reason for this sudden stop is the marked proliferation of host cells, which has been noted as splenomegaly or lymph node enlargement (SIMONSEN, 1962; FORD et al., 1970). Therefore, as normal adult Fl hybrid hosts are capable of bringing donor cell invasion to a halt, they must have an agent to do this. For the following reasons, a likely candidate for this agent is anti-RS antibody.

The primary attack of, for instance, A strain lymphoid cells injected into (A × B)Fl hosts and directed against transplantation antigens B must almost certainly be due to the function of recognition structures (RS) of A cells for antigens B. It can thus be postulated that A immunocompetent cells possess RS(B). However such A cells also possess receptors for antigens C, for induction of graft-versus-host reaction is equally possible after A lymphoid cells have been injected into (A × C)Fl hybrid hosts. Obviously, populations of A immunocompetent cells have RS for many different transplantation antigens, *except for their own*. Under normal conditions, A lymphoid cells injected into A animals are not expected to react immunologically. These same reflections also hold, of course, for the other parent, B, of (A × B)Fl hybrids. Again, lymphoid cells from animals B will have receptors for a variety of transplantation antigens; to escape self-destruction they must, however, lack RS(B). We might thus postulate that populations of immunocompetent cells have receptors for all transplantation antigens of the species to which they belong, but none for their own antigens. As on many other occasions in Science, this is not new. It is but a rephrasing of Paul Ehrlich's horror autotoxicus, stating that self-recognition normally is excluded.

As pointed out, the secondary event in graft-versus-host reactions is characterized by a marked proliferation of Fl hybrid host lymphoid cells. (A × B)Fl lymphoid cells too, have receptors, for they are perfectly capable of reacting against transplantation antigens C, D, E, etc., in response of which they might, for example, form PAR as attested by the results in Table 2. Since, however, (A × B)Fl hybrids have inherited *codominantly* transplantation antigens from parents A and B, their lymphoid cells must lack RS(A) and RS(B) for the same reasons which prohibited cells from animals A and B from possessing RS(A) and RS(B), respectively. Very likely, then, the hosts' response cannot be directed against any foreign transplantation antigens. But since there is a host response, it must be directed against something. One possibility is that it is directed against the only foreign matter introduced, namely the receptor structure.

It is thus not unlikely that upon introducing A lymphoid cells carrying RS(B) into (A × B)Fl hosts lacking RS(B), hosts respond to RS(B) by the formation of anti-RS(B) antibodies. Similar anti-RS antibodies, but of a

different specificity, are to be expected if B lymphoid cells are injected into adult (A × B)Fl hosts. The anti-RS antiserum formed will be anti-RS(A). Such anti-RS antibodies can be postulated to be exclusively directed against corresponding receptors since contamination of the immune response by a reaction against transplantation antigens or other receptors is not to be expected. On the other hand, the injection of parental strain immunocompetent (and thus potentially antibody-forming) cells into histocompatible Fl hosts where they are confronted with transplantation antigens of the other parent, could conceivably lead to the formation of alloantibodies. Thus inoculation of A lymphoid cells into (A × B)Fl hosts might induce formation of A anti-B and that of B cells formation of B anti-A alloantibodies. However, demonstration of these antibodies might be difficult as the Fl environment affords their immediate neutralization (RAMSEIER and LINDENMANN, 1969, 1971 b, c).

From a theoretical point of view it should, therefore, be possible to induce anti-RS antibodies and, at the same time, alloantibodies simply by injecting parental strain node or spleen cells into adult Fl hybrid animals of the proper genetic constitution.

A simple fact of serology is that an antiserum to any antigen unites with that antigen to form complexes. This implies that antisera have immunoglobulin molecules with combining sites for the antigen in question. An alloantiserum A anti-B (for instance, a posttransplantation serum) possesses, therefore, molecules endowed with the property of recognizing antigen B. The antigen-combining site of immunoglobulin molecules of serum A anti-B might thus be conveniently renamed RS(B). If this straightforward assumption is correct, it should again be possible to provoke formation of anti-RS(B) antiserum if RS(B) as alloantiserum A anti-B were to be injected into animals lacking this activity, such as (A × B)Fl hosts. These hosts do not have cellular RS(B), as pointed out above, nor do they have or are they ever expected to form humoral RS(B), i.e. A anti-B antibodies. Fl hybrid animals, furthermore, offer themselves as ideal hosts because possible complications arising from allotypic differences expressed on immunoglobulin molecules are naturally avoided (RAMSEIER and LINDENMANN, 1971 d, 1972 a).

To test these simple premises, antisera to cell-bound RS and to RS in alloantiserum were prepared. To induce the former, moderate doses of parental strain spleen or node cells were injected intravenously or intraperitoneally into adult Fl hybrids. Early on, sera were raised by injecting 10^6 cells weekly for 6 weeks, and hosts were bled 2 weeks after the last cell dose. This protocol was devised to avoid complications of a graft-versus-host nature and because appearance of a serum activity was only considered likely after repeated injections. However, no outward signs of graft-versus-host activity followed one single dose of 6×10^6 parental strain node cells and sera, obtained two weeks afterwards, proved as active as those harvested following the step-wise protocol. It was later found that a single dose of 10^6 cells would induce as good anti-RS antibody titers as other regimens. It became thus standard to inject this dose and to bleed animals two weeks later.

Whenever attempted, cell-induced anti-RS sera were obtained. To date 52 mouse anti-RS, 17 rat anti-RS and 2 hamster anti-RS antisera have been prepared and tested. Depending on the immunocompetence of injected parental strain cells, some of these sera were active and some were inactive. Active sera were obtained whenever parental strain node or spleen cells were injected; inactive sera were harvested after injections of immunoincompetent cells, such as rat or mouse thymus lymphocytes, lymphoid cells from mice rendered immunologically tolerant, epidermal cells or heat-killed immunocompetent cells. Antisera were prepared against RS for transplantation antigens determined by loci considered as strong, such as H-2 and Ag-B, as moderately strong, like multiple non-H-2 or as relatively weak, such as H-Y.

Some of these anti-RS sera have been prepared repeatedly but at different times in the course of this investigation. When tested for their inhibitory titers, variations encompassed one and rarely two steps of two-fold dilutions. Similar variations were observed when a given serum was tested repeatedly. All cell-induced anti-RS antisera were routinely heat-inactivated and sterilized by Millipore-filtration.

Antisera to alloantibody-RS were also induced following different protocols. To date, 8 anti-alloantisera were raised in rats by injecting twice (at an interval of two weeks) equal volumes of an alloantiserum and Freund's complete adjuvant intradermally into adult Fl hybrid hosts, each receiving 10 doses of 0.1 ml. Animals were bled two weeks later. The alloantisera used as antigens were posttransplantation sera of different specificity obtained three weeks after only one full-thickness skin graft. Alloantisera as well as anti-alloantisera were heat-inactivated and sterilized by filtration (RAMSEIER and LINDENMANN, 1971 d). Titers of these sera varied by one step of a two-fold dilution; a variation also encountered when one and the same serum was tested on different occasions. Control sera to these anti-alloantisera were prepared by exactly the same procedure, except that heat-inactivated and filtered normal rat serum was injected instead of alloantiserum.

Recently, a number of rat anti-alloantisera were prepared without Freund's complete adjuvant by injecting 1 ml of alloantiserum intradermally on only one occasion. Animals were bled two weeks later. Anti-RS titers were comparable to those obtained when adjuvant and repeated injections were employed.

Anti-RS antisera resulting from both immunization procedures had to be named somehow, an undertaking that unfortunately led to a rather cumbersome terminology. For cell-induced anti-RS sera the following designation was adopted: the injection of A lymphoid cells carrying RS(B) into (A×B)Fl hosts resulted in an anti-RS(B) activity which will be called (A×B)Fl anti-A RS(B), whereby (A×B)Fl is the anti-RS former and A the cell population against whose RS(B) the antiserum is directed. Since cell-induced anti-RS antisera can also be provoked by procedures not discussed in this review, the genetic origin of anti-RS producers must not necessarily be Fl. It is for this reason that simplifications in terminology are not easily accomplished. Nevertheless, matters might be eased somewhat by neglecting the designation of the

animal strain in which anti-RS has been induced. Similar complications were encountered in creating a precise terminology for alloantiserum-induced anti-RS sera. The injection of alloantiserum A anti-B, which is RS(B) into (A×B)Fl hosts resulted in an anti-RS(B) activity which should properly be called (A×B)Fl anti-(A anti-B). Again (A×B)Fl marks the strain to which anti-RS-forming animals belonged and (A anti-B) the serum possessing the antigen-binding capacity for antigen B [= RS(B)]. Omitting the designation of the producer strain again eases terminology.

Examples of the action of anti-RS antisera produced in Fl hybrid hosts either by the injection of RS-bearing cells or by that of RS-carrying allo-antisera on the process of antigenic recognition are set out in Tables 10, 11 and 13.

The data in Table 10 demonstrate that cell-induced anti-RS antisera could be obtained in the three species tested. Anti-RS titers observed varied from one strain combination to the next but were, nevertheless, much higher than was anticipated. This, however, should not be taken to indicate that these antisera were of extraordinary potency but rather seems to be an expression of the great sensitivity of the PAR assay.

As pointed out before, injection of parental strain lymphoid cells into adult Fl hybrid animals not only leads to the formation of anti-receptor antibodies, but also to the production of alloantibodies. Examples of the extent of the alloantibody activity present in Fl hybrid sera as compared to the anti-RS activity are given in Table 11 for the three species indicated. Despite the fact that the Fl hybrid environment in which such alloantibodies were formed should effect their neutralization, these activities were found in all Fl sera prepared and tested. Since some neutralization by host tissues must have taken place, conceivably involving antibodies of high affinity, it is probable that low affinity alloantibodies were detected.

Cell mixtures of all three species were not inhibited by normal Fl serum (RAMSEIER and LINDENMANN, 1969). As the data for PAR in Table 12 show, neither normal Fl rat serum nor inactive rat sera had any effect on aggressor or target cells.

Two examples of the action of rat anti-RS sera each produced by two injections of alloantisera mixed with equal volumes of Freund's complete adjuvant and one example of a similar rat anti-RS antiserum produced by only one inoculation of alloantiserum without adjuvant are given in Table 13. It will be noted that high titers of anti-RS activities were obtained in all cases. Indeed, better anti-RS activities attended the use of alloantiserum-RS as antigen as compared to cell-bound RS. As a comparison of rat anti-RS (Lewis) antisera in Tables 10 and 13 will reveal, the alloantiserum-induced activity was 8 times more potent. Though only few examples have been given here, the higher activity of alloantiserum-induced antisera has been a general experience for all rat sera prepared. Preliminary experiments with similar mouse anti-RS sera indicated that this might also pertain to this species (RAMSEIER, unpublished).

Table 10. Activity of antisera directed against node cell-bound RS

Species	RS-bearing cells and hosts	Anti-RS specificity	Mixed spleen cell cultures		% PAR
			aggressors	untreated targets	
Rat	DA→(Lewis × DA)Fl	(Lewis × DA)Fl anti-DA RS(Lewis)	DA untreated	(Lewis × DA)Fl	100.00
			DA+anti-RS 1:2048	(Lewis × DA)Fl	7.65
			DA+anti-RS 1:4096	(Lewis × DA)Fl	81.63
	Lewis→(Lewis × DA)Fl	(Lewis × DA)Fl anti-Lewis RS(DA)	Lewis untreated	(Lewis × DA)Fl	100.00
			Lewis+anti-RS 1:512	(Lewis × DA)Fl	0.58
			Lewis+anti-RS 1:1024	(Lewis × DA)Fl	128.61
Mouse	C57BL/6→(A × C57BL/6)Fl	(A × C57BL/6)Fl anti-C57BL/6 RS(A)	C57BL/6 untreated	(A × C57BL/6)Fl	100.00
			C57BL/6+anti-RS 1:2048	(A × C57BL/6)Fl	4.99
			C57BL/6+anti-RS 1:4096	(A × C57BL/6)Fl	102.64
	A→(A × C57BL/6)Fl	(A × C57BL/6)Fl anti-A RS(C57BL/6)	A untreated	(A × C57BL/6)Fl	100.00
			A+anti-RS 1:1024	(A × C57BL/6)Fl	0.00
			A+anti-RS 1:2048	(A × C57BL/6)Fl	131.16
Hamster	CB→(CB × LSH)Fl	(CB × LSH)Fl anti-CB RS(LSH)	CB untreated	(CB × LSH)Fl	100.00
			CB+anti-RS 1:512	(CB × LSH)Fl	1.56
			CB+anti-RS 1:1024	(CB × LSH)Fl	84.60
	MHA→(CB × MHA)Fl	(CB × MHA)Fl anti-MHA RS(CB)	MHA untreated	(CB × MHA)Fl	100.00
			MHA+anti-RS 1:1024	(CB × MHA)Fl	0.00
			MHA+anti-RS 1:2048	(CB × MHA)Fl	99.86

Cultivation was for 7 h.

Table 11. Anti-RS and alloantibody activities of lymphoid cell-induced Fl hybrid sera

Species	Induction of Fl hybrid serum	Activities	Mixed spleen cell cultures: aggressors	Mixed spleen cell cultures: targets	% PAR
Rat	Lewis→(Lewis × DA)Fl		Lewis untreated	(Lewis × DA)Fl untreated	100.00
		anti-Lewis RS(DA)	Lewis + serum 1:256–1:1024	(Lewis × DA)Fl untreated	0.00
			Lewis + serum 1:2048	(Lewis × DA)Fl untreated	100.00
		Lewis anti-DA	Lewis untreated	(Lewis × DA)Fl + serum 1:32–1:256	0.00
			Lewis untreated	(Lewis × DA)Fl + serum 1:512	102.32
Mouse	CBA→(A × CBA)Fl		CBA untreated	(A × CBA)Fl untreated	100.00
		anti-CBA RS(A)	CBA + serum 1:256–1:1024	(A × CBA)Fl untreated	0.00
			CBA + serum 1:2048	(A × CBA)Fl untreated	96.30
		CBA anti-A	CBA untreated	(A × CBA)Fl + serum 1:16–1:64	0.00–3.19
			CBA untreated	(A × CBA)Fl + serum 1:128	100.51
Hamster	MHA→(CB × MHA)Fl		MHA untreated	(CB × MHA)Fl untreated	100.00
		anti-MHA RS(CB)	MHA + serum 1:256–1:1024	(CB × MHA)Fl untreated	0.00–6.83
			MHA + serum 1:2048	(CB × MHA)Fl untreated	99.86
		MHA anti-CB	MHA untreated	(CB × MHA)Fl + serum 1:32–1:256	0.55–5.16
			MHA untreated	(CB × MHA)Fl + serum 1:512	147.56

Rat cell were cultivated for 7 h, others for 4 h.

Table 12. Lack of activity of normal rat Fl serum and of inactive rat sera on antigenic recognition

Serum	Mixed spleen cell cultures		% PAR
	aggressors	targets	
	DA or Lewis untreated	(Lewis × DA)Fl untreated	100.00
Normal (Lewis × DA)Fl serum	DA + serum 1:8–1:512	(Lewis × DA)Fl untreated	113.65–132.23
	DA untreated	(Lewis × DA)Fl + serum 1:8–1:256	120.99–159.94
10^6 (Lewis × DA)Fl spleen cells →(Lewis × DA)Fl rats	DA + serum 1:32	(Lewis × DA)Fl untreated	92.98
	DA untreated	(Lewis × DA)Fl + serum 1:32	104.82
10^6 Lewis spleen cells →Lewis rats	Lewis + serum 1:32	(Lewis × DA)Fl untreated	146.67
	Lewis untreated	(Lewis × DA)Fl + serum 1:32	121.64
	DA + serum 1:32	(Lewis × DA)Fl untreated	110.97
	DA untreated	(Lewis × DA)Fl + serum 1:32	115.75

Cultivation was for 7 h.

Table 13. Activity of antisera directed against rat alloantiserum-RS

Serum-RS	Anti-RS	Mixed spleen cell cultures		% PAR
→hosts	specificity	aggressors	targets	
Lewis anti-BN(=RS(BN)) +FCA→(Lewis×BN)Fl	(Lewis×BN)Fl anti-(Lewis anti-BN) =anti-RS(BN)	Lewis untreated	(Lewis×BN)Fl untreated	100.00
		Lewis+serum 1:32–1:8192	(Lewis×BN)Fl untreated	0.00
		Lewis+serum 1:16384	(Lewis×BN)Fl untreated	122.30
		Lewis untreated	(Lewis×BN)Fl+serum 1:16–1:64	119.33–123.79
BN anti-Lewis (=RS(Lewis)) +FCA→(Lewis×BN)Fl	(Lewis×BN)Fl anti (BN anti-Lewis) =anti-RS(Lewis)	BN untreated	(Lewis×BN)Fl untreated	100.00
		BN+serum 1:32–1:8192	(Lewis×BN)Fl untreated	0.00
		BN+serum 1:16384	(Lewis×BN)Fl untreated	91.91
		BN untreated	(Lewis×BN)Fl +serum 1:16–1:64	88.61–98.35
DA anti-Lewis(=RS(Lewis)) →(Lewis×DA)Fl	(Lewis×DA)Fl anti-(DA anti-Lewis) =anti-RS(Lewis)	DA untreated	(Lewis×DA)Fl untreated	100.00
		DA+serum 1:32–1:16384	(Lewis×DA)Fl untreated	0.00
		DA+serum 1:32768	(Lewis×DA)Fl untreated	104.38
		DA untreated	(Lewis×DA)Fl+serum 1:16–1:128	93.05–104.38

Cultivation was for 7 h for DA→Lewis and for 4 h for others. FCA=Freund's complete adjuvant.

Table 14. Lack of activity of inactive rat sera on antigenic recognition

Serum	Mixed spleen cell cultures		% PAR
	aggressors	targets	
	DA or BN untreated	(BN×DA)F1 untreated	100.00
DA NS+FCA→(BN×DA)F1 rats	DA+serum 1:32–1:256	(BN×DA)F1 untreated	81.05–95.62
	DA untreated	(BN×DA)F1+serum 1:32	88.14
	BN untreated	(BN×DA)F1+serum 1:32	99.87
BN NS+FCA→(BN×DA)F1 rats	BN+serum 1:32–1:256	(BN×DA)F1 untreated	96.98–110.77
	BN untreated	(BN×DA)F1+serum 1:32	105.32
	DA untreated	(BN×DA)F1+serum 1:32	102.44

Cultivation was for 4 h. NS=normal rat serum; FCA=Freund's complete adjuvant.

It was considered not unlikely that in the course of induction of anti-RS sera by means of alloantisera, not all of the antigen would be used up or bound to host tissues. However, PAR values of Table 13 indicated that alloantiserum activities which might have been passively transferred through F1 hybrid animals could not be detected. This, on the other hand, does not exclude their presence, for when employed in high concentrations(1:8, 1:16), alloantibodies often display a prozone effect and thus escape detection.

In correspondence with the lack of activity shown by "mock" cell-induced F1 sera (Table 12), similar inactive sera, prepared by injecting normal rat serum together with adjuvant into F1 hybrid rats (Table 14), were totally devoid of anti-aggressor (anti-RS) or anti-target (alloantibody) activities (see also RAMSEIER and LINDENMANN, 1971d).

It has thus become quite clear that the process of recognition of transplantation antigens can be inhibited by antibodies to antigen receptors of recognizing cells. Even though cell-induced F1 sera, and possibly also alloantiserum-induced F1 sera, contain, besides anti-RS, also some alloantibody activity, these two activities in one and the same serum address themselves to different immunological entities. As exemplified in Table 11 these entities are RS or transplantation antigens. This already affords a clear separation. Another means of demonstrating coexistence of two different activities present in cell-induced F1 antisera has been selective absorption. The results of these studies have recently been reviewed elsewhere (RAMSEIER and LINDENMANN, 1972b). It

might, for the present discussion, suffice to state that the alloantibody component of such sera can be removed by absorption with cells carrying the proper antigens without harming the anti-receptor activity of the same serum, and indeed without changing the titer. Conversely, the anti-RS activity can be neutralized equally well in these sera with lymphoid cells possessing the fitting receptors without inflicting damage to the alloantibody activity.

4. Demonstration and Basis of Anti-Receptor Antibody Specificity

A question of great significance is whether these anti-RS sera were capable of blocking antigenic recognition specifically. If this were to be answered in the affirmative, it would ultimately mean that anti-RS antisera contained antibody molecules directed against receptors for single transplantation antigenic specificities. Consequently, anti-RS sera directed against numerous antigenic specificities, such as those discussed so far, might be populations of as many species of anti-RS molecules as there are transplantation antigens. For obvious reasons, the final analysis of this problem can only be solved conclusively by employing congenic strains of animals differing at one single antigenic specificity. Such studies are in progress. They have so far revealed that when appropriate congenic strains of mice were used, PAR formation in response to a single H-2 antigenic specificity (antigen 32) was demonstrable. Furthermore, antibodies could be induced to receptors for antigen 32 by injecting both RS-bearing cells and alloantiserum into suitable Fl hybrids (Ramseier, unpublished). However, even when noncongenic inbred strains of animals are employed, specificity should reveal itself by a lack of cross-reactivity if combinations of animals were to be chosen which fail to recognize identical antigenic determinants.

4.1. Specificity of Cell-Induced Anti-RS Antisera

If anti-RS antisera were to act specifically in the sense alluded to above, i.e. one receptor specificity for each antigenic specificity, then an antiserum provoked against receptors of lymphoid cells from mouse strain A for CBA antigens should block on A cells only RS(CBA). Consequently, treatment of A lymphoid cells with serum anti-A RS(CBA) [provoked in (A×CBA)Fl hosts] should result in failure of mixed spleen cell cultures composed of A and (A×CBA)Fl to produce PAR. The very same population of A lymphoid cells, also treated with serum anti-A RS(CBA), should, however, be capable of recognizing transplantation antigens C57BL/6 on (A×C57BL/6)Fl target cells.

Experimental data relevant to this argument are set out in Table 15. The results of three different recognition systems demonstrate that, whereas aggressor cells were prevented from recognizing transplantation antigens by treatment with the proper anti-RS antiserum, this serum failed to block receptors on cells of the same population for unrelated transplantation antigens (Ramseier and Lindenmann, 1971 b, c).

Table 15. Specificity of cell-induced mouse anti-RS antisera

Mixed spleen cell cultures		%PAR
aggressors	untreated targets	
A untreated	(A×CBA)Fl	100.00
A untreated	(A×C57BL/6)Fl	100.00
A+anti-A RS(CBA)	(A×CBA)Fl	2.27
A+anti-A RS(CBA)	(A×C57BL/6)Fl	84.96
A+anti-A RS(C57BL/6)	(A×C57BL/6)Fl	0.53
A+anti-A RS(C57BL/6)	(A×CBA)Fl	77.78
C57BL/6 untreated	(A×C57BL/6)Fl	100.00
C57BL/6 untreated	(CBA×C57BL/6)Fl	100.00
C57BL/6+anti-C57BL/6 RS(A)	(A×C57BL/6)Fl	8.69
C57BL/6+anti-C57BL/6 RS(A)	(CBA×C57BL/6)Fl	95.39
C57BL/6+anti-C57BL/6 RS(CBA)	(CBA×C57BL/6)Fl	17.32
C57BL/6+anti-C57BL/6 RS(CBA)	(A×C57BL/6)Fl	89.85
CBA untreated	(A×CBA)Fl	100.00
CBA untreated	(CBA×C57BL/6)Fl	100.00
CBA+anti-CBA RS(A)	(A×CBA)Fl	1.25
CBA+anti-CBA RS(A)	(CBA×C57BL/6)Fl	126.90
CBA+anti-CBA RS(C57BL/6)	(CBA×C57BL/6)Fl	0.00
CBA+anti-CBA RS(C57BL/6)	(A×CBA)Fl	106.89

Cultivation was for 7 h. All sera were used in dilution 1:512. (Adapted from RAMSEIER and LINDENMANN, 1971b).

The basis of this exclusive specificity might be found in the antigenic specificities recognized by the aggressors employed. For the first system of Table 15 (A→CBA and A→C57BL/6) one has to assume that A cells recognized on (A×CBA)Fl targets H-2 transplantation antigens of CBA origin, i.e. antigen 32. Treatment of A aggressors prior to cocultivation with (A×CBA)Fl targets with anti-A RS(CBA) = anti-RS(32) blocked the recognition process. Since antiserum anti-A RS(CBA) is only anti-RS(32), treated aggressors were capable of recognizing C57BL/6 antigens, because in this case A cells recognized H-2 antigens of C57BL/6 origin, i.e. antigens 2, 22, 33, 39 and 46. Clearly, then, even if in a given population of A aggressors receptors for antigen 32 were blocked, this did not involve receptors for antigens 2, 22, 33, 39 and 46. These were left free to interact with the antigens in question and PAR could be formed. Conversely, blocking of receptors on A cells for these latter H-2 antigens, as accomplished by treatment with antiserum anti-A RS(C57BL/6) blocked recognition of antigens 2, 22, 33, 39 and 46 but not that of antigen 32. Again, one might assume that recognition of the CBA antigen by A cells treated with serum anti-A RS(C57BL/6) was successful because RS(32) was not blocked by a serum anti-RS(2, 22, 33, 39, 46).

Similar models can be constructed for the additional two systems given in Table 15. It will be noted that cross-reactivity cannot possibly take place in any of these three strain combinations tested reciprocally. However, there are

Table 16. Total and partial cross-reactivity of cell-induced mouse anti-RS and alloantiserum

Reaction No.	Mixed spleen cell cultures aggressors	targets	%PAR
1	CBA untreated	(A × CBA)Fl untreated	100.00
2	CBA untreated	(CBA × DBA/2)Fl untreated	100.00
3	CBA + anti-CBA RS(A)	(A × CBA)Fl untreated	2.22
4	CBA + anti-CBA RS(DBA/2)	(A × CBA)Fl untreated	0.00
5	CBA + anti-CBA RS(DBA/2)	(CBA × DBA/2)Fl untreated	0.58
6	CBA + anti-CBA RS(A)	(CBA × DBA/2)Fl untreated	65.30
7	CBA untreated	(A × CBA)Fl + CBA anti-A	1.66
8	CBA untreated	(A × CBA)Fl + CBA anti-DBA/2	0.00
9	CBA untreated	(CBA × DBA/2)Fl + CBA anti-DBA/2	0.00
10	CBA untreated	(CBA × DBA/2)Fl + CBA anti-A	66.47

Cultivation was for 4 h. All sera were used in dilution 1:32.

mouse strain combinations in which cross-reactivity can be demonstrated. Table 16 contains data of two anti-CBA RS sera of which one can be substituted for the other in one strain combination but only partly so in the other.

An anti-CBA RS(DBA/2) serum [provoked in (CBA × DBA/2)Fl mice by the injection of 10^6 CBA spleen cells] blocked on CBA aggressors receptors for DBA/2 antigens as evidenced by the lack of PAR formation (reaction 5). The anti-RS titer of this serum was 1:512. From an analysis of H-2 antigenic specificities involved, it might be assumed that this inhibition was due to the blocking of CBA RS(4, 6, 10, 12, 13, 14, 27, 28, 29, 31, 34, 35, 36, 40, 41, 42, 43, 45, 46). Another antiserum, anti-CBA RS(A) [produced in (A × CBA)Fl mice by the inoculation of 10^6 CBA spleen cells] was shown to inhibit normal function of CBA receptors for A antigens (reaction 3). The anti-RS titer of this serum was 1:1024. CBA spleen cells will recognize on A target cells H-2 antigenic specificities 4, 6, 10, 12, 13, 14, 27, 28, 29, 35, 36, 41, 42, 43 and 45. It will now be noted that all antigenic specificities CBA aggressors were able to recognize on DBA/2 target cells were also recognized by these same cells on A targets. Consequently, serum anti-CBA RS(DBA/2) should not only prevent CBA aggressors from recognizing DBA/2 but also A transplantation antigens. The results of reactions 5 and 4 in Table 16 furnish evidence. This cross-reactivity should, however, not hold in the reverse situation, for CBA aggressors recognized on DBA/2 targets H-2 antigens 31, 34, 40, 41 and 46, which are not recognized by these same cells on A targets. An anti-CBA RS(A) serum might, therefore, not be able to substitute completely for an anti-CBA RS(DBA/2) serum. However since serum anti-CBA RS(A) was directed against receptors for many antigenic determinants common to targets A and DBA/2, partial cross-reactivity might result. When this was tested, the results (reaction 6 of Table 16) fulfilled the expectation.

In preparing these Fl sera, not only anti-RS but also alloantibody activities were obtained. The injection of 10^6 CBA spleen cells into (CBA×DBA/2)Fl mice provoked formation of CBA anti-DBA/2 alloantibodies. When tested in the PAR assay an anti-target cell activity of 1:128 was found. Similarly, inoculation of (A×CBA)Fl mice with 10^6 CBA spleen cells resulted in activity CBA anti-A (titer of 1:64). The alloantibody components of the two Fl sera were found to follow exactly the same rules as the anti-RS components. When used to treat (A×CBA)Fl and (CBA×DBA/2)Fl target cells, serum CBA anti-A and serum CBA anti-DBA/2 blocked recognition processes (reactions 7 and 9, Table 16). Serum CBA anti-DBA/2, however, could also be employed in the blocking of A antigens (reaction 8) because its activity was directed against all antigenic specificities CBA aggressors can recognize on A target cells. Substitution was only partial when serum CBA anti-A was used to treat (CBA×DBA/2)Fl target cells (reaction 10), because this serum failed to coat DBA/2 H-2 antigens 31, 34, 40 and 46. These were recognized by untreated CBA aggressors and the interaction with these 4 antigenic specificities resulted in the formation of as much as 66% of PAR. This figure compares very favorably with that of 65% PAR in the partial anti-RS inhibition and seems to indicate that these 4 antigens (or possibly only antigens 31 and 34) are comparatively strong. The recognition of all other antigens involved accounted for only one-third of PAR formed.

Criticism may, however, be raised against these interpretations because antigens determined by non-H-2 loci have so far been neglected. This objection could be invalidated by experiments employing congenic strains of mice. Using animals of strains B10.A, B10 (C57BL/10) and hybrids of (B10.A×B10.D2)Fl and (B10×B10.D2)Fl it was shown that anti-RS and alloantibody components of Fl sera displayed similar total or partial cross-reactivities as was found for the noncongenic mouse strains presented in Table 16 (RAMSEIER and LINDENMANN, 1972b).

4.2. Specificity of Alloantiserum-Induced Anti-RS Antisera

The specificity of anti-RS antisera induced in Fl hosts by the injection of alloantiserum (together with Freund's complete adjuvant) has, so far, only been studied in rats. Although numerous inbred strains of rats are now available, information on antigenic specificities for the strains employed is not yet available. Therefore, results obtained do not offer insight into fine aspects of the process of antigenic recognition, although it is known that among the several specificities present in these strains (PALM 1962, 1964; ELKINS and PALM, 1966; RAMSEIER and PALM, 1967) some are unique for some strains, whereas others are shared by two or more strains (PALM, 1971). Since the rat strains employed were of strong histoincompatibility, inhibition results should nevertheless reveal as exquisite a specificity, provided alloantiserum-induced anti-RS sera were to behave like cell-induced anti-RS sera.

Table 17. Specificity of alloantiserum-induced anti-RS antisera

Mixed spleen cell cultures		%PAR
aggressors	untreated targets	
Lewis untreated	(Lewis × BN)Fl	100.00
Lewis untreated	(Lewis × DA)Fl	100.00
Lewis + anti-RS(BN) 1:4000	(Lewis × BN)Fl	0.00
Lewis + anti-RS(BN) 1:32	(Lewis × DA)Fl	119.25
Lewis + anti-RS(DA) 1:2000	(Lewis × DA)Fl	0.90
Lewis + anti-RS(DA) 1:32	(Lewis × BN)Fl	144.86

Cultivation was for 4 h (Lewis → BN) or for 7 h (Lewis → DA);
Anti-RS(BN) = (Lewis × BN)Fl anti-(Lewis anti-BN);
Anti-RS(DA) = (Lewis × DA)Fl anti-(Lewis anti-DA).

A typical example of this specificity is given in Table 17 for Lewis aggressors confronted with BN or DA transplantation antigens offered as (Lewis × BN)Fl or (Lewis × DA)Fl spleen cells, respectively. Treatment of Lewis spleen cells with serum (Lewis × DA)Fl anti-(Lewis anti-BN) = anti-RS(BN) blocked on these cells receptors for BN transplantation antigens. Lewis cells so treated, however, remained perfectly capable of recognizing DA antigens, presumably again because receptors for DA antigens were not affected by the anti-RS(BN) serum employed. On the other hand, recognition of DA antigens could be blocked by treating Lewis aggressors with a serum (Lewis × DA)Fl anti-(Lewis anti-DA) = anti-RS(DA), but Lewis cells treated with much higher than the inhibitory serum concentration were capable of recognizing BN antigens. These results confirm earlier data on the specificity of anti-alloantisera (Ramseier and Lindenmann, 1971d) and form a valid argument for the view that these sera are as specific as are cell-induced anti-RS sera.

5. An Account of two Investigations on Specific Blocking of Antigenic Recognition

The availability of a test system by which demonstration of specific blocking of antigenic recognition by either anti-target cell or by anti-aggressor cell sera was possible, prompted an investigation into two aspects of transplantation immunity: the action of so-called enhancing antisera and the problem of transplantation tolerance.

5.1. Enhancement and Recognition of Tumor-Specific Transplantation Antigens

It is a well established fact that autochthonous tumor cells possess, in addition to the normal complement of antigens, new antigenic determinants which are foreign to the very individual that harbors the tumor (Old and Boyse, 1964; Hellström and Möller, 1965; Klein, 1966). Since it is known

that lymphoid cells of the tumor-bearing host can recognize and react against these antigens in a fashion similar to that to transplantation antigens, they have been referred to as tumor-specific transplantation antigens (TSTA). Numerous lines of evidence are now available indicating the potential of lymphoid cells of tumor-bearing animals to react immunologically against autochthonous, or in the case of inbred animals, syngeneic tumors (HELLSTRÖM and HELLSTRÖM, 1970). This beneficial immune reaction is, however, strongly counteracted by so-called enhancing antibodies. While it has been known for some time that the presence of such sera enhances tumor growth (KALISS, 1958), it was not clear whether their action was anti-lymphoid cell aggressor or anti-tumor cell target. Recent elegant experiments by the HELLSTRÖM'S have now indicated that the blocking activity of enhancing sera is directed against the new TSTA of malignant cells (HELLSTRÖM and HELLSTRÖM, 1970).

Experiments of a preliminary character using the PAR assay system appear to confirm this. A number of CBA mice were injected with methylcholanthrene to induce tumors in all 4 extremities. One of numerous mice developing tumors on two extremities after about 3 months was killed, the two tumors (HR and VL) were excised and cut into small fragments which were injected subcutaneously (interscapular) into virgin CBA mice. Tumors took and were from then on propagated when fully developed, i.e. at intervals of 2–3 weeks, into new CBA mice by trypsinizing fragmented sarcoma and injecting 5×10^6 tumor cells subcutaneously. In vivo propagation was successful for sarcoma HR (stopped after 18 passages) but only partially so for tumor VL (lost after 10 passages). The targets used in the PAR test were freshly trypsinized tumor cells and, occasionally, tumor fibroblasts grown in tissue cultures. For mixed cultures (of which PAR-containing supernatant was taken and processed as described) 10^7 aggressor node or spleen cells were cocultured with 2 to 4×10^6 freshly trypsinized and washed tumor cells or with approximately 10^6 washed fibroblasts under conditions otherwise identical to those mentioned earlier for the cocultivation of lymphoid cells.

A first series of experiments was designed to test whether spleen cells from normal and from tumor-bearing CBA mice would recognize freshly trypsinized or cultured CBA tumor cells as foreign and to what extent this recognition would differ, quantitatively, from that of H-2 different transplantation antigens determined in the same experiments. The results are summarized in Table 18. They revealed that lymphoid cells from normal CBA and C57BL/6 mice recognized both CBA sarcoma (HR and VL) to a degree that was not too different from that resulting from an interaction with allogeneic antigens. Variations in the amount of PAR determined were to be expected, because culture time was fixed at 7 h, a time which might have been optimal for some but suboptimal for other combinations. No attempts were made in these experiments to determine optimal cultivation periods. In contrast to tumor tissue lymphoid cells from normal CBA mice cultivated with syngeneic embryo or kidney fibroblasts were not able to form PAR, whereas similar cells from C57BL/6 mice were.

Table 18. Recognition of CBA TSTA and of H-2 mouse transplantation antigens by normal CBA and C57BL/6 lymphoid cells and by lymphoid cells from tumor-bearing CBA mice

Mixed cell cultures		PAR ± SE ($\times 10^6$ PMN cells/ skin reaction)
aggressors (node + spleen cells from)	targets	
Normal CBA / normal C57BL/6	(CBA × C57BL/6)F1 node + spleen cells	5.59 ± 0.18 / 5.43 ± 0.33
Normal CBA / normal C57BL/6	CBA HR tumor fibroblasts	3.83 ± 0.24 / 1.63 ± 0.08
Normal CBA / normal C57BL/6	CBA VL tumor fibroblasts	4.29 ± 0.32 / 3.64 ± 0.27
Normal CBA / normal C57BL/6	CBA HR tumor trypsinized	2.78 ± 0.29 / 3.94 ± 0.61
Normal CBA / normal C57BL/6	CBA VL tumor trypsinized	6.66 ± 0.12 / 6.48 ± 0.34
Normal CBA / normal C57BL/6	CBA embryo fibroblasts	0.12 ± 0.06 / 4.39 ± 0.42
Normal CBA / normal C57BL/6	CBA kidney fibroblasts	0.06 ± 0.05 / 2.65 ± 0.22
HR tumor-bearing CBA	(CBA × C57BL/6)F1 node + spleen cells	6.17 ± 0.25
HR tumor-bearing CBA	CBA HR tumor trypsinized	2.87 ± 0.20
VL tumor-bearing CBA	(CBA × C57BL/6)F1 node + spleen cells	5.24 ± 0.25
VL tumor-bearing CBA	CBA VL tumor trypsinized	6.54 ± 0.29

Cultivation was for 7 h.

Lymphoid cells from CBA mice bearing both types of tumors were capable not only of recognizing allogeneic transplantation antigens (C57BL/6) but also of recognizing TSTA on syngeneic tumor cells.

It is thus obvious that the success of tumor growth is not due to lack of antigenic recognition on the part of the hosts' lymphoid cells, a condition for an immune attack, but to an agent blocking the recognition phase. As pointed out, enhancing serum is believed to be this inhibitory agent. While this is no longer debated, the question of where exactly enhancing sera act has been, until recently, an open one. Since the PAR assay can easily distinguish between agents acting on aggressors or targets, the problem of the site of action of these sera was studied.

Eight CBA mice bearing large HR tumors (3rd in vivo passage) were exsanguinated. The serum was heat-inactivated, sterilized by Millipore-filtration and was later used to treat aggressor or target spleen cells as indicated in Tables 19–21.

It was first necessary to demonstrate that a serum with putative enhancing activity would not interfere with the recognition of allogeneic antigens. The data in Table 19 show that a serum from HR tumor-bearing CBA mice displayed neither anti-aggressor nor anti-target cell activity. In this respect, the lack of anti-target cell activity is particularly relevant for this showed that the serum contained no alloantibodies. The action of this serum was then investigated in cultures composed of aggressor spleen cells from normal CBA micewhich were cocultivated with freshly trypsinized CBA HR target tumor

Table 19. Lack of activity of an enhancing serum on the recognition of allogeneic antigens

Mixed spleen cell cultures		% PAR
CBA agressors	(CBA × C57BL/6)Fl targets	
Untreated	untreated	100.00
+ serum 1:32	untreated	91.97
+ serum 1:64	untreated	119.34
+ serum 1:128	untreated	100.36
+ serum 1:256	untreated	136.86
Untreated	+ serum 1:32	117.15
Untreated	+ serum 1:64	104.20
Untreated	+ serum 1:128	129.20
Untreated	+ serum 1:256	119.34

Cultivation was for 4 h.

Table 20. Effect of enhancing serum on the recognition of CBA TSTA by syngeneic CBA spleen cells

Mixed cell cultures		% PAR
CBA aggressor spleen cells	CBA target tumor cells	
Untreated	untreated	100.00
+ serum 1:32	untreated	107.67
+ serum 1:64	untreated	83.63
+ serum 1:128	untreated	112.54
+ serum 1:256	untreated	102.06
Untreated	+ serum 1:32	0.00
Untreated	+ serum 1:64	1.77
Untreated	+ serum 1:128	0.00
Untreated	+ serum 1:256	0.00
Untreated	+ serum 1:512	103.83

Cultivation was for 7 h. Aggressor spleen cells were from normal CBA mice; targets were from the 17th in vivo passage of CBA tumor HR.

cells, representing TSTA. The results of these experiments (Table 20) indicated that the serum had no effect on aggressors but blocked what must have been TSTA up to a dilution of 1:256 on tumor cells.

In a third type of experiment, a "semi-autochthonous" recognition system was attempted by employing as aggressors spleen cells from 5 CBA mice bearing CBA HR sarcoma. These spleen cells were cultivated with freshly trypsinized CBA HR tumor cells. Tumor cells were from two of these 5 mice. For this reason, because pools of cells were used, and because the enhancing serum employed was from a different group of CBA mice, these experiments were not truly autochthonous. The results obtained (Table 21) were identical to those of the syngeneic recognition system (Table 20).

Table 21. Effect of enhancing serum on the recognition of CBA TSTA by spleen cells from tumor-bearing CBA mice in a "semi-autochthonous" recognition system

Mixed cell cultures		% PAR
Aggressors (spleen cells from tumor-bearing CBA's)	CBA target tumor cells	
Untreated	untreated	100.00
+ serum 1:32	untreated	118.92
+ serum 1:64	untreated	88.16
+ serum 1:128	untreated	86.79
+ serum 1:256	untreated	82.45
Untreated	+ serum 1:32	0.00
Untreated	+ serum 1:64	6.13
Untreated	+ serum 1:128	0.00
Untreated	+ serum 1:256	0.00
Untreated	- serum 1:512	66.49

Cultivation was for 7 h. Aggressors and targets were from CBA mice of the 17th in vivo passage of CBA tumor HR.

Table 22. Site of action of enhancing serum in autochthonous recognition of TSTA

Mixed cell cultures		% PAR
aggressor spleen+node cells	target tumor cells	
Cells from mouse 1 untreated	tumor from mouse 1 untreated	100.00
Cells from mouse 1+serum 1:32	tumor from mouse 1 untreated	104.17
Cells from mouse 1 untreated	tumor from mouse 1+serum 1:256	17.46
Cells from mouse 1 untreated	tumor from mouse 1+serum 1:512	115.67
Cells from mouse 2 untreated	tumor from mouse 2 untreated	100.00
Cells from mouse 2+serum 1:32	tumor from mouse 2 untreated	142.96
Cells from mouse 2 untreated	tumor from mouse 2+serum 1:256	2.25
Cells from mouse 2 untreated	tumor from mouse 2+serum 1:512	0.00

Cultivation was for 7 h. Aggressor lymphoid cells, serum and target tumor cells were from the 18th in vivo passage of CBA tumor HR.

In a last series of experiments truly autochthonous recognition was achieved in so-called "one-mouse experiments". For this two CBA mice bearing HR sarcoma were chosen. Each mouse was first exsanguinated to obtain enhancing serum (which was heat-inactivated), the tumor was excised, trypsinized and washed and used as a source of target cells, and finally a suspension of node and spleen cells from this individual was prepared and employed as aggressor cells. The results obtained (Table 22), though quite clear-cut, suffer from the limited number of serum dilutions which, for technical reasons, could be tested. The results show correspondence to the syngeneic and "semi-autochthonous" recognition systems and indicate that the serum of a tumor-

bearing antimal most likely prevents reaction of lymphoid cells against TSTA by shielding these latter antigens.

5.2. Transplantation Tolerance

One possibility of elucidating the mechanisms by which tolerance of transplantation antigens is induced and maintained is afforded by the system of tolerance abolition. This is achieved by the adoptive transfer of normal lymphoid cells syngeneic to the tolerant animal or by that of syngeneic cells sensitized to the tolerated transplantation antigens (BILLINGHAM et al., 1956, 1963; GOWANS et al., 1963). Experiments on tolerance abolition have led to the concept of "central failure of the immune system", a term indicating that tolerance affects central immune mechanisms which can be regenerated to normal function by the transfer of nontolerant cells. However, this only circumscribes the fact that once it is induced, tolerance is maintained over extended periods of time, but does not give insight into the mechanisms by which tolerance is functioning. Since it can be assumed that new stem cells are constantly being formed in tolerant animals, the critical question appears to be: by what mechanism are novel cells dealt with or, in other words, what mechanism or agent signals virgin cells to remain neutral to the tolerogen and not to become responsive to it? There is indeed ample opportunity for these cells to become reactive, for animals rendered tolerant by the neonatal injection of histoincompatible lymphoid cells are cellular chimeras (BILLINGHAM and SILVERS, 1971). That this stimulus does not lead to responsiveness and, therefore, to spontaneous loss of tolerance, suggests that some regulatory agent is involved.

Recently, HELLSTRÖM et al. (1971) demonstrated in the serum of mice tolerant of transplantation antigens a regulatory factor, claimed to prevent normal reactivity of lymphoid cells of tolerant mice against tolerated antigens. Lymphoid cells of animals A tolerant of B could be shown to react immunologically in an in vitro cytotoxic test against target fibroblasts of strain B, but failed to do so in the presence of serum from animals A tolerant of B. Serum from normal A and B mice lacked blocking factor, and normal A and B lymphoid cells failed to show activity in presence or absence of normal or tolerant serum. The blocking activity of tolerant serum was specific, for serum from animals A tolerant of B could not be substituted by serum from mice B tolerant of A. Lymphoid cells and serum were from mice CBA tolerant of A and from A mice tolerant of CBA. Tolerance induction was by neonatal injections of 5×10^7 (A×CBA)Fl spleen cells and mice were used when tolerant of 1–2 months of age.

As to the nature of this serum factor, no firm commitment is made. It was, however, considered unlikely that it represented soluble transplantation antigens capable of binding to otherwise reactive cells. There is no information as to whether this activity addressed itself to responding lymphoid cells or to the target tissue. On ground of analogy to other systems it is, however, assumed

that this blocking component is enhancing antibody (HELLSTRÖM and HELLSTRÖM, 1970; ALLISON, 1971). As such it would interfere with antigenic recognition by lymphoid cells of tolerant animals by coating tolerated antigens. It is, finally, not quite clear why a blocking serum activity could be removed from cells by simple washing. For serum activities inhibiting antigenic recognition, the experience has been that they stick firmly to their antigens.

The problem of maintenance of transplantation tolerance was approached by employing the experimental tools provided by previous experiments. These have shown that the PAR assay system is capable of detecting and distinguishing serum activities directed against target cells (the enhancing serum activity) from those against cellular receptors (the anti-RS activity).

The question of whether or not tolerance of transplantation antigens was maintained by a serum factor was tested by the effect adoptive transfer of syngeneic lymphoid cells had on test grafts and on activities detectable in the serum. The basic idea was that the state of tolerance might result from an equilibrium delicately maintained by some regulatory agent. The system could be pushed out of balance by confronting tolerant animals with a greater number than usual of responding cells. It was considered likely that, in contrast to the findings of HELLSTRÖM et al. (1971), mice tolerant of transplantation antigens do not reveal this agent in their serum, but might do this after they have been forced to produce more of it when challenged with syngeneic lymphoid cells.

For this study (RAMSEIER, 1972) 30 CBA mice tolerant of A were available. All animals were injected within 24 h of birth with 10^7 (A$\times$CBA)Fl spleen cells intravenously and were challenged, when about 2 months of age, with full-thickness A strain skin grafts. Individuals used bore healthy A test grafts with luxurious fur. Grafts were of rather large size, ranging between 8×12 mm and 20×27 mm. Tolerant animals are referred to as steady-state and of long standing because, after tolerance induction and test grafting, mice had been left untreated for 327 to 616 days.

To decide whether a serum factor was instrumental in maintenance of tolerance, steady-state tolerant mice were bled in groups of 10, 6, 6, and 8 and serum pools were prepared. These sera were tested for an anti-receptor activity and for an anti-transplantation antigen activity. If anti-receptor activity was the responsible regulator, the activity to be expected was against receptors for A antigens, i.e. anti-CBA RS(A). If, on the other hand, shielding of tolerated transplantation antigens was the mechanism by which tolerance was secured, an enhancing serum component of specificity CBA anti-A might have been found. When tested, none of these activities were revealed at dilutions of 1:16, 1:32 or higher.

This finding is not in accordance to that of HELLSTRÖM et al. (1971) but perhaps reflects the outcome of a demand and supply system. Possibly, if steady-state tolerant mice formed a regulatory serum factor it was produced in low amounts because the demand was equally low. This would be compatible with the view that successful maintenance of tolerance involved production

Table 23. Challenge of steady-state CBA mice tolerant of A

Group	No. CBA mice tolerant of A	Days toler.	Challenge inoculum (Lymphoid cells from)
A	7	449–546	200×10^6 CBA spleen
	6		$+\ 20\times10^6$ CBA anti-A spleen
	6		$+100\times10^6$ CBA anti-A node+spleen
B	6	532	20×10^6 CBA anti-A node+spleen
	6		$+150\times10^6$ CBA anti-A node+spleen
C	6	440–506	100×10^6 CBA anti-A node
D	5	488–531	20×10^6 CBA node
	3		not immunized
	all 8		$+100\times10^6$ CBA anti-A node

of just enough inhibitory substance to prevent new stem cells from becoming reactive.

An excess of this inhibitor might be evoked by confronting tolerant mice with an increased demand, i.e. in the case of CBA mice tolerant of A with more responsive CBA cells. This was attempted in thrusts of increasing severity with the aim of breaking tolerance by adoptive transfer of syngeneic cells. The data of these experiments are summarized in Table 23.

In a first test, 7 CBA mice tolerant of A (group A of Table 23) were each given intraperitoneally 2×10^8 spleen cells from normal CBA mice. Quite unexpectedly this did not lead to abolition of tolerance within the observation period of 40 days, although test grafts of 2 mice passed through minor rejection crises. Testing of the serum pool of these mice after this onslought revealed an anti-CBA RS(A) titer of 1:1024 and a CBA anti-A titer of 1:64. Forty days after the first challenge of normal CBA cells, 6 of these tolerant mice were rechallenged with 2×10^7 spleen cells from CBA mice sensitized to A transplantation antigens by skin grafting. No graft rejection occurred. Another 30 days later these same mice were challenged a third time with 10^8 CBA anti-A node and spleen cells each, a procedure that again failed in breaking tolerance. Both challenges with sensitized cells resulted in changes of serum activities: the anti-RS titer fell considerably whereas the alloantibody titer of the same sera rose markedly.

Employing a second group of mice (group B of Table 23) 6 steady-state tolerant mice were challenged each with 2×10^7 sensitized spleen and node cells. Within the observation period of 33 days, 2 mice failed to reject the A test grafts, 2 mice rejected parts of the grafts (resulting in 25% and 50% reduction in graft size) and 2 test grafts showed profound rejection crises.

of long standing with syngeneic lymphoid cells

Rejection of A grafts and observation (days)	Bled days after chall.	Serum activities	
		anti-CBA RS(A)	CBA anti-A
none, few crisis (40)	39	1:1024	1:64
none (30)	29	1:128	1:256
none (3:63, 3:101)	32	1:32	1:1024
2:none } (33)	32	1:1024	1:64
2:partly } (33)	32	1:256	1:256
2:crisis+healing } (33)	32	1:32	1:64
none (68)	61	1:32	1:512
4:days 7–10	14	<1:16	1:256
2:not complete (14)	14	1:64	1:256
none	14	1:1024	1:16
none	—	—	—
5 immunized:none (38)	14	1:256	1:512
3 not immunized:days 8,9	14	<1:16	1:512

When bled separately, the serum pool of mice that failed to reject showed anti-RS and alloantibody titers similar to the serum of group A. Serum pools of the 4 animals in which rejection was incomplete or involved only parts of the large A test grafts displayed comparatively lower anti-RS titers and similar or higher alloantibody titers than mice of group A. When on day 33 these 6 mice were rechallenged with 150×10^6 sensitized CBA spleen and node cells, no graft rejection was observed during a period of 68 days. The serum pool of these mice revealed a low (1:32) anti-RS titer but a substantial (1:512) alloantibody titer.

Tolerance could be abolished in a third group of steady-state tolerant mice when the first challenge was with 10^8 sensitized cells. Group C (Table 23) entailed 6 tolerant mice of which 4 showed complete and 2 incomplete (about 25 %) rejection of A test grafts. Two serum pools were prepared. That from mice which had rejected completely did not display anti-receptor antibodies whereas that from animals which rejected incompletely showed a titer of 1:64. Both sera had, however, identical alloantibody titers (1:256).

From these experiments it appeared likely that anti-receptor antibodies were probably more closely associated with maintenance of transplantation tolerance than were alloantibodies. To test this, the effect of "immunization" of tolerant mice with moderate doses of normal syngeneic cells on the outcome of challenge with a dose of sensitized cells successful in abolishing tolerance was investigated. If anti-receptor antibodies were responsible for maintenance of tolerance, then it was considered possible that this immunization would provoke formation of enough anti-RS antibodies to protect tolerant mice from the tolerance-breaking effect of sensitized cells.

A group of 8 CBA mice tolerant of A was employed (group D in Table 23).

Five of these mice were injected each with 2×10^7 CBA node cells and 3 mice were left untreated. Two weeks later a serum pool from the immunized subgroup was prepared and tested and displayed 1:1024 anti-CBA RS(A) antibodies and only 1:16 CBA anti-A alloantibodies. The serum of the nonimmunized tolerant mice was not tested because a pool of preimmunization serum from all 8 mice lacked both activities. Eighteen days after immunization, all 8 CBA mice tolerant of A were challenged each with 10^8 CBA anti-A node cells. None of the 5 immunized tolerant mice rejected their A test grafts but all 3 nonimmunized animals lost grafts on days 8 and 9. Pools of sera from the 2 subgroups were prepared and tested for anti-RS antibodies and for alloantibodies. Irrespective of whether tolerance was broken or not, the sera showed identical titers for alloantibody CBA anti-A. However, a clear difference revealed itself with respect to anti-CBA RS(A) antibodies. None (less than 1:16) could be found in the serum of mice that had lost tolerance, but substantial activity (1:256) was found in the serum of immunized mice.

It has been shown (Table 2) that spleen cells from CBA mice tolerant of A failed to recognize A transplantation antigens on (A×CBA)F1 target spleen cells. To test the recognition potential of mice that successfully defended tolerance, presumably as a consequence of immunization, individual suspensions of spleen cells from 4 of these mice were prepared and were cultivated with (A×CBA)F1 target cells for 7 h. The response of these cells was compared to that of spleen cells taken from each of 4 mice (from group C of Table 23) in which tolerance was broken due to the adoptive transfer of 10^8 CBA anti-A node cells. None of the spleen cells from immunized mice recognized A transplantation antigens, a result also obtained with spleen cells from 2 steady-state CBA mice tolerant of A for 616 days. On the other hand, the spleens of all 4 mice in which tolerance was broken contained cells capable of recognizing A antigens. The amount of PAR formed was comparable to that resulting from cultivations of spleen cells from normal CBA mice with similar target cells.

These results point to an active role for anti-receptor antibodies in maintenance of transplantation tolerance, but leave that of alloantibodies in a somewhat enigmatic position. That no anti-receptor activity (and also no alloantibody activity) was detectable in the serum of steady-state tolerant mice, is presumably because whatever was formed was immediately used up by spontaneously emerging responsive cells. Introducing into tolerant hosts greater than normal quantities of receptor-bearing cells tended to bring the delicate equilibrium between emergence of responsive cells and neutralization of their receptors out of balance. Hosts now responded with the formation of great amounts of anti-receptor antibodies, the excess of which could be found in the serum.

What cells formed anti-receptor antibodies? As pointed out earlier, only cells lacking the receptors in question can form antibodies (RAMSEIER and LINDENMANN, 1969, 1971b, c, 1972b). For CBA mice tolerant of A this cell population very likely was the (A×CBA)F1 spleen cell inoculum (and its

descendants) which originally conferred tolerance of A. It should be borne in mind that in tolerance induction newborn mice are, in general, injected with 10^7 or more Fl spleen cells, a comparatively high dose of cells which, furthermore, document their continued presence by the fact that tolerant mice are cellular chimeras. Whether or not some of the tolerant animals' own lymphoid cells had permanently lost receptors for A transplantation antigens and would thus have been capable of forming anti-RS(A) antibodies cannot be decided. In steady-state tolerant mice of long standing this might be a possibility. That Fl hybrid spleen cells injected even into nontolerant parental strain mice can form substantial amounts of anti-RS antibodies has recently been shown (RAMSEIER, unpublished) and dispelled doubts about the chimeric cell population's ability to form anti-receptor antibodies.

Once these anti-RS antibodies had been formed, further attempts to break tolerance by sensitized syngeneic cells were futile. The effect of challenge with sensitized cells was a decrease in the anti-RS titer of tolerant hosts. Several reasons appear to account for this. Sensitized cells are cytotoxic (WILSON, 1965; BRUNNER et al., 1966; PERLMANN and HOLM, 1969; GOLSTEIN et al., 1971) and this property directs itself to the Fl population of tolerant chimeras; they are known to possess more receptors than do normal cells (RAMSEIER and LINDENMANN, unpublished) and their humoral product, alloantibody, has been shown to neutralize anti-receptor antibodies (RAMSEIER and LINDENMANN, 1972a, b).

Increase, rather than decrease of activity has been found for alloantibody titers in the sera of tolerant mice challenged with cells already active in forming this activity. This indicated that tolerant hosts had no need for alloantibodies to maintain tolerance. Indeed, an activity pattern revealing direct participation of these antibodies has not become apparent. The role played by alloantibodies is thus far from clear. To relegate these antibodies into a domain of accidental presence affords, however, little comfort. It should be pointed out that the interrelationship of alloantibodies and anti-receptor antibodies is complex and certainly not fully understood. The mutual neutralization of these serum activities (RAMSEIER and LINDENMANN, 1972a) and the possibility of employing alloantibodies as antigens in the induction of anti-receptor antibodies (Table 13) (RAMSEIER and LINDENMANN, 1971d) are examples of this complexity.

The active role of anti-receptor antibodies in maintenance of tolerance appears to be neutralization of receptors for transplantation antigens. This was indicated by challenge experiments but was probably best illustrated by experiments on recognition. Challenge experiments revealed that tolerance could only be broken when tolerant mice lacking enough anti-RS antibodies were confronted with sensitized cells. These most likely killed the potential anti-RS antibody-forming cell population. When, for various reasons, tolerant animals commanded enough anti-receptor antibodies, tolerance could not be broken. Recognition responses indicated that immunized tolerant mice appeared to have neutralized receptors of the sensitized inoculum. Had this not

been the case, recognition due to sensitized cells of the syngeneic challenge inoculum settling in the spleen would have resulted. A response due to sensitized cells was not found. On the other hand, the response of spleen cells from mice in which tolerance of formerly tolerated antigens was broken could not be distinguished from that of normal animals. This suggested that in tolerance-abolished animals recognition was not due to sensitized cells from the challenge inoculum but could be ascribed to cells from the host which had returned to normal reactivity.

On the whole, these experiments led to an interpretation of induction and maintenance of transplantation tolerance which differs from classical views (Billingham et al., 1956; Gowans and McGregor, 1965; Dresser and Mitchison, 1968; Billingham and Silvers, 1971). It also differs from that given by Hellström et al. (1971). Rather than considering transplantation tolerance as a "central failure of the immune system, caused by altering the properties of immunologically competent cells in such a way that a restricted range of reactivity is deleted" (Dresser and Mitchison, 1968; Billingham et al., 1956), the results accumulated favor a "peripheral failure". In contrast to Hellström et al., who advocate a mechanism of "efferent peripheral failure" in the sense that tolerant mice have normally reactive lymphoid cells which are, however, prevented from reacting against tolerated antigens because these are shielded by enhancing antibodies, a mechanism of "afferent" interference with receptors of reactive cells has been revealed here. Normal reactivity of host lymphocytes to tolerated antigens appears to be blocked by antibodies directed against recognition structures.

A mechanism of this sort might also apply to *induction* of transplantation tolerance. Whereas it was never disputed that the Fl hybrid cell population introduced into newborn animals the antigens to be tolerated, a second function of this cell population appears to be formation of anti-receptor antibodies. With these, the few responsive cells existing in newborn animals (Gowland, 1965) are prevented from reacting against the simultaneously introduced transplantation antigens. That not all newborn mice injected with Fl spleen cells become tolerant is the experience of all who have attempted it. While technical factors might play a role in this, it appears also likely that in some animals the tolerance-conferring inoculum was more successful that in others. Early loss of tolerance, also a common experience, could possibly be explained by an escape from the action of anti-receptor antibodies of responding lymphoid cells emerging from a stem cell reservoir.

6. Perspectives

Three major problems beset the central theme of this review. One was whether the test system used, the PAR assay, was to hold what it appeared to promise, the second problem was whether what has been found in vitro would have any relevance to in vivo conditions, and the third problem was

whether recognition resulting in PAR was a phenomenon involving T or B cells.

The credibility of what has been found with the PAR assay would be enhanced significantly if a test system totally independent of it were to reveal similar results. Recently, BINZ and LINDENMANN (1972) attempted this by measuring the uptake of radioactively-labeled rat anti-receptor antibodies by lymphoid cells carrying corresponding receptors. For this, rat anti-RS sera provoked by the injection of alloantisera (raised by rejection of histoincompatible skin grafts) were employed. From these anti-alloantisera (= anti-RS sera) IgG was prepared and iodinated. ^{125}I-anti-RS(A) IgG was found to be fixed by lymphoid cells carrying RS(A) but not by those lacking RS(A). Although nonspecific background binding was present, a 4 to 18-fold difference in uptake documented preferential binding. These studies also confirmed one of the basic ideas on anti-RS antibody formation (RAMSEIER and LINDENMANN, 1969, 1971 b, c, d), namely that (A×B)Fl lymphoid cells are capable of producing anti-RS(A) and anti-RS(B) antibodies because they lack RS(A) and RS(B). Thus, anti-RS(A) antibodies fixed to equal low degrees to A and to (A×B)Fl lymphoid cells [both lacking RS(A)], whereas C cells fixed 5 times greater amounts. On the other hand, an anti-RS(C) antibody was bound to A cells and to (A×B)Fl cells, because both possessed RS(C) but was not taken up by C lymphoid cells, which lacked RS for their own antigens. These and other experiments, showing supporting evidence, leave little doubt that anti-receptor antibodies a) are localized in the IgG fraction of serum proteins, b) can be elicited by alloantibodies representing RS and c) are preferentially bound to cells possessing corresponding RS but not to cells lacking these receptors, although carrying receptors for other transplantation antigens. Furthermore, the analogy of binding results to those obtained by the PAR assay was revealed by concomitant determinations of uptake and PAR titers of the anti-RS sera used.

The second problem about anti-RS antibodies is whether or not these antibodies have any in vivo activity. Already early in this study it was realized that if these antisera inhibited in vivo antigenic recognition, this would be of far-reaching clinical consequences. The major advantage these antisera seemed to offer was *specific* suppression of the immune response to a particular set of antigens, while leaving responses to all other antigens uninhibited (RAMSEIER, 1970b; RAMSEIER and LINDENMANN, 1971 b). It was, at the same time, realized that immunosuppression by anti-RS antibodies might not be easy, particularly since these antisera did not appear to be cytotoxic (DAVIES, 1971). Nothing is known about the binding forces between anti-RS antibodies and RS, and even if this association should turn out to be strong, there is still the possibility that RS-anti-RS complexes might be shed by cells. That such cells are quick in replacing receptors is indicated by experiments of ASHMAN (pers. comm.) and also by the trypsin experiments referred to earlier. These latter experiments (Chapter 2) gave a clear warning, for they demonstrated that an impressive in vitro inhibition failed to show an in vivo correlate. To what

extent the poor medium used throughout these in vitro experiments contributed to the failure of cells to regenerate blocked receptors cannot be decided, but it is possible that the use of more sophisticated media and longer culturing might result in resynthesis of receptors. Perhaps failure to demonstrate anti-RS antibody activity in mixed leukocyte cultures (FISCHER-LINDAHL, pers. comm.; LARNER, pers. comm.) is due to shedding of RS-anti-RS complexes and to rapid replacement of receptors. Thus mixed leukocyte culture reactions appear to be closer to the in vivo conditions than is the PAR assay. Results obtained by the former technique suggest that escape from the inhibitory action of but a few cells might severely counteract suppression.

Despite gloomy prospects, the action of murine anti-RS antibodies on the rejection of H-2 incompatible skin grafts was tested. Antisera were injected in a manner similar to that employed for anti-lymphocyte sera (RAMSEIER, 1970a). The first attempt was encouraging. Skin from A mice grafted on untreated or on CBA hosts treated with normal (A×CBA)Fl serum were rejected normally (11–12 days). Of 7 CBA mice, however, treated with a serum (A×CBA)Fl anti-CBA RS(A) (PAR titer of 1:512) of which 0.2 ml were given subcutaneously on days +1, +3 and +5, only 4 rejected normally. Grafts of the remaining 3 mice did not impress by their healthy appearance, but could also not be considered rejected. A comparison of these grafts for 439 days (when the experiment was terminated) with scars clearly visible on the chests of companion mice which had rejected, on the one hand, and with A skin grafts on CBA mice tolerant of A, on the other, revealed a truly intermediate status. Although presence of A skin could not be denied, only a few long hairs grew on these grafts. Two more similar attempts with this strain combination, involving 6 and 9 CBA hosts (given 0.5 ml anti-RS serum on days +2 and +5 or 0.1 ml on days −1, 0, +1 to +5) were completely unsuccessful. So were also three attempts in which A skin was grafted on a total of 50 C57BL/6 mice. In these tests various injection schedules and also intravenous rather than subcutaneous injections were tried. A last test of this series again entailed grafting of A skin on CBA mice and on CBA mice lightly irradiated (100r) on the day of grafting. The anti-CBA RS(A) serum [provoked in (A×CBA)Fl mice as ascitic fluids] employed had an exceptionally high anti-RS titer of 1:8192. The serum was injected subcutaneously in doses of 0.2 ml either undiluted or diluted 1:10 on days +1, +3 and +5. Normal rejection was observed in all groups, irrespective of whether hosts were irradiated or not and received undiluted or diluted serum, except for a group of 10 irradiated CBA mice which received undiluted anti-RS serum: Six mice rejected normally, in one mouse rejection was obvious by day 155 but in 3 CBA mice the A test grafts were still present on day 266 when the experiment was terminated. The grafts on these mice passed through rejection crises on days 13 to 15, characterized by the formation of thin, serous crusts which, however, disappeared after a few days. At no time could any of these grafts be qualified as excellent; only a few hairs or none at all were visible (RAMSEIER, unpublished).

Having made this experience, it came as no surprise that DAVIES (1971) presented experiments on the activity of anti-receptor antisera to prolong H-2 incompatible skin grafts which, though promising, were only partially successful.

There can be little doubt that attempts at prolonging survival of strongly incompatible skin grafts are highly ambitious undertakings: anti-RS antisera are given passively and, most importantly, they have to neutralize receptors on *all* immunocompetent cells an adult mouse can muster.

That in less ambitious situations an in vivo effect of anti-RS antibodies can be demonstrated has been shown in experiments on tolerance. In tolerant hosts, neutralization of receptors appeared to have been possible whenever comparatively few cells carrying receptors had been introduced. Moreover, due to their chimeric cell population tolerant hosts had their own anti-RS antibody-forming machinery.

An immunological test model which is also more modest than prevention of skin graft rejection and which also, eventually, solicits support from the host, is inhibition of lethal graft-versus-host reactions by anti-RS antibodies. Recently, JOLLER (1972) described the fate of (C57BL/6 × A)Fl mice neonatally injected with 2×10^6 C57BL/6 spleen cells. When parental donor cells were untreated 12.5% of newborns survived. When, on the other hand, C57BL/6 cells were treated before intravenous injection with 1:8 anti-C57BL/6 RS(A) antiserum 56% of newborn hosts survived. Most importantly, when untreated or with anti-C57BL/6 RS(A)-treated C57BL/6 spleen cells were injected into noncorresponding hosts of genetic constitution (CBA × C57BL/6) Fl, survival was low (3.6% for hosts receiving untreated and 7.7% for hosts receiving anti-RS-treated cells). This strongly indicated that the protective effect of anti-RS antisera was specific and that this in vivo specificity was identical to that found in vitro (see Table 15, lines 9 and 10).

Although seemingly straightforward, this system is a highly complex one, for aggressor cells, most probably only temporarily suppressed, find themselves in a rapidly growing host, which also rapidly strives to gain immunocompetence. During this process hosts became capable of forming their own anti-RS antibodies of proper specificity. This enabled them to neutralize newly synthesized donor cell receptors. The issue death or survival thus appears to depend on the outcome of a race between host and donor lymphoid cells.

Another model to document inhibition of receptors for transplantation antigens by anti-RS antisera is blocking of local manifestations of a graft-versus-host reaction, for which the lymph node weight assay of FORD et al. (1970) recommended itself. In this test, parental strain rat spleen cells are injected into the footpads of adult Fl hybrid rats. This leads to an enlargement of the popletial node within 7 days. In work in progress, Dr. S. ZAKARIAN has been able to demonstrate that immunization of (A × B)Fl rat hosts with moderate doses (1–5×10^6) of, say, A spleen cells [carrying RS(B)] or with alloantiserum A anti-B, representing RS(B), markedly reduced the size of nodes when immunized hosts were challenged with 10^7 A spleen cells. At

times nodes from immunized rats could not be distinguished, in weight, from those of Fl rats not challenged at all. However, there was fluctuation in degrees of suppression. This again probably reflects the outcome of a dynamic interaction between blocking and resynthesis of receptors.

On the whole, the experiments of JOLLER and ZAKARIAN suggest that anti-receptor antibodies do act in vivo, provided the system is not overburdened. This, of course, is only a beginning. Rather than utilizing the blocking effect of anti-RS sera directly, it could be used as a vehicle for highly selective targeting of active compounds. It is usually easier to produce punch than to deliver it selectively. As far as selectivity is concerned, it will be difficult to improve on anti-RS antibodies.

A third problem of major importance is the question of whether recognition of antigens resulting in PAR formation is achieved by thymus-dependent (T) or by thymus-independent (B) cells.

Recently, a few lines of evidence favoring T cells as the cell type involved in the recognition process have been reviewed (RAMSEIER and LINDENMANN, 1972b) . These do not, however, establish the T-cell character of the phenomenon byond doubt. The problem is now under further investigation. Results obtained so far (RAMSEIER, unpublished) do not contradict the assumption that recognition is achieved by receptors on T cells. Thus, A strain mouse thymus cells injected into normal or lethally irradiated adult (A × B)Fl hybrid hosts failed to provoke formation of anti-A RS(B) antibodies, whereas similar cells educated by a 5-day sojourn in the spleens of 850 r irradiated (A × B)Fl hosts did so upon injection into normal (A × B)Fl mice. However, such educated A thymus cells treated with anti-θ antiserum and complement when injected into normal (A × B)Fl hosts failed to incite detectable levels of anti-A RS(B) antibodies, presumably because T cells carrying RS(B) were removed from the inoculum.

Should further experiments confirm conclusively that the recognition of transplantation antigens resulting in PAR formation is a property of T cells, rather than of B cells, then the following conclusion might be drawn: since receptors present on these T cells cannot be distinguished antigenically in the experiments reported from recognition structures present in the antigen-combining region of alloantiserum immunoglobulin molecules, produced by B cells, the former might be structurally very similar to the latter. This would imply that the functionally important moiety of the T cell receptor resides in the variable regions of an immunoglobulin molecule.

Acknowledgements. I thank Professor J. LINDENMANN for stimulating discussions and for critical evaluation of this manuscript and Mrs. A. HALLER, Mrs. M. HEIZMANN, Miss R. KELLER, Miss R. LEEMANN and Miss D. PLÜSS for skillful technical assistance.

References

ADA, G. L., BYRT, P., MANDEL, T., WARNER, N.: A specific reaction between antigen labelled with radioactive iodine and lymphocyte-like cells from normal, tolerant and immunized mice or rats. In: Developmental aspects of antibody formation and structure, p. 503, ed. by J. STERZL and I. RIHA. Prague: Academia Publishing House 1970.

ALLISON, A. C.: Cell co-operation in immunity, with special reference to the mode of action of adjuvants. In: Immunopathology VIth internat. symposium, p. 79, ed. by P. A. MIESCHER. Basel: Schwabe 1971.

AMOS, D. B., GORER, P. A., MIKULSKA, B. M., BILLINGHAM, R. E., SPARROW, E. M.: An antibody response to skin homografts in mice. Brit. J. exp. Path. **35**, 203 (1954).

AMOS, D. B., NICKS, P. J., PEACOCKE, N., SIEKER, H. O.: An evaluation of the normal lymphocyte transfer test in man. J. clin. Invest. **44**, 219 (1965).

BALNER, H., DERSJANT, H.: Increased oncogenic effect of methylcholanthrene after treatment with antilymphocyte serum. Nature (Lond.) **224**, 376 (1969).

BALNER, H., DERSJANT, H., VAN BEKKUM, D. W.: Testing of antihuman lymphocyte sera in chimpanzees and lower primates. Transplantation **8**, 281 (1969).

BILLINGHAM, R. SILVERS, W.: The immunobiology of transplantation. New Jersey: Prentice Hall 1971.

BILLINGHAM, R. E.: The biology of graft-versus-host reactions. Harvey Lect. **62**, 21 (1968).

BILLINGHAM, R. E., BRENT, L., MEDAWAR, P. B.: Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. Phil. Trans. B **239**, 357 (1956).

BILLINGHAM, R. E., DEFENDI, V., SILVERS, W. K., STEINMÜLLER, D.: Quantitative studies in the induction of tolerance of skin homografts and on runt disease in neonatal rats. J. nat. Cancer Inst. **28**, 365 (1962).

BILLINGHAM, R. E., SILVERS, W. K.: Some biological differences between thymocytes and lymphoid cells. In: The thymus, p. 41, ed. by V. DEFENDI and D. METCALF. Philadelphia: Wistar Institute Press 1964.

BILLINGHAM, R. E., SILVERS, W. K., WILSON, D. B.: Further studies on adoptive transfer of sensitivity to skin homografts. J. exp. Med. **118**, 397 (1963).

BINZ, H., LINDENMANN, J.: Cellular receptors. Binding of radioactively labelled anti-alloantiserum. J. exp. Med. **136**, 872 (1972).

BRENT, L., MEDAWAR, P. B.: Cellular immunity and the homograft reaction. Brit. med. Bull. **23**, 55 (1967).

BRUNNER, K. T., MAUEL, J., SCHINDLER, R.: *In vitro* studies of cell-bound immunity; clonal assay of the cytotoxic action of sensitized lymphoid cells on allogeneic target cells. Immunology **11**, 499 (1966).

CASPARY, E. A., FIELD, E. J., WOODRUFF, M. F. A.: Nonspecies specificity of anti-lymphocyte serum. Transplantation **11**, 170 (1971).

DAVIES, D. A. L.: Transplantation and tolerance. In: Immunological tolerance to tissue antigens, p. 89, ed. by N. W. NISBET and M. W. ELVES. Oswestry, England: Orthopaedic Hospital 1971.

DEODHAR, S. D., CRILE, G.: Enhancement of metastases by antilymphocyte serum in allogeneic murine tumor system. Cancer Res. **29**, 776 (1969).

DRESSER, D. W., MITCHISON, N. A.: The mechanism of immunological paralysis. Adv. Immunol. **8**, 129 (1968).

DVORAK, H. F., KOSUNEN, T. U., WAKSMAN, B. H.: The "transfer reaction" in the rabbit. I. A histologic study. Lab. Invest. **12**, 58 (1963).

ELKINS, W. L., PALM, J.: Identification of a single strong histocompatibility locus in the rat by normal spleen-cell transfer. Ann. N. Y. Acad. Sci. **129**, 573 (1966).

FORD, W. L., BURR, W., SIMONSEN, M.: A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. Transplantation **10**, 258 (1970).

FOX, M.: Cytological estimation of proliferating donor cells during graft-versus-host disease in Fl hybrid mice injected with parental spleen cells. Immunology **5**, 489 (1962).

GOLSTEIN, P., SVEDMYR, E. A. J., WIGZELL, H.: Cells mediating specific in vitro cytotoxicity. I. Detection of receptor-bearing lymphocytes. J. exp. Med. **134**, 1385 (1971).

GOWANS, J. L., MCGREGOR, D. D.: The immunological activities of lymphocytes. Progr. Allergy **9**, 1 (1965).
GOWANS, J. L., MCGREGOR, D. D., COWEN, D. M.: The role of small lymphocytes in the rejection of homografts of skin. In: The immunologically competent cell: its nature and origin, p. 20, ed. by G. E. W. WOLSTENHOLME and J. KNIGHT. London: Churchill 1963.
GOWLAND, G.: Induction of transplantation tolerance in adult animals. Brit. med. Bull. **21**, 123 (1965).
GRAY, J. G., RUSSELL, P. S.: The lymphocyte transfer test in man. In: Histocompatibility testing, p. 105. Washington, D. C.: National Academy of Sciences—National Research Council 1965.
GREAVES, M. F.: Biological effects of anti-immunoglobulins: evidence for immunoglobulin receptors on "T" and "B" lymphocytes. Transplant. Rev. **5**, 45 (1970).
HELLSTRÖM, I., HELLSTRÖM, K. E., ALLISON, A. C.: Neonatally induced allograft tolerance may be mediated by serum-borne factors. Nature (Lond.) **230**, 49 (1971).
HELLSTRÖM, K. E., HELLSTRÖM, I.: Immunological enhancement as studied by cell culture techniques. Ann. Rev. Microbiol. **24**, 373 (1970).
HELLSTRÖM, K. E., MÖLLER, G.: Immunological and immunogenetic aspects of tumor transplantation. Progr. Allergy **9**, 158 (1965).
HILDEMANN, W. H.: Early antibody production in relation to skin allograft reaction in mice. Transplantation **5**, 1001 (1967).
JOLLER, P.: Inhibition of graft-versus-host reactivity of lymphoid cells by treatment with anti-recognition structure serum. Nature (Lond.) (in press).
KALISS, N.: Immunological enhancement of tumor homografts in mice. Cancer Res. **18**, 992 (1958).
KLEIN, G.: Tumor antigens. Ann. Rev. Microbiol. **20**, 223 (1966).
LANCE, E. M., MEDAWAR, P. B.: Survival of skin heterografts under treatment with antilymphocytic serum. Lancet **1968 I**, 1174.
LANCE, E. M., MEDAWAR, P. B.: Immunosuppressive effects of heterologous antilymphocyte serum in monkeys. Lancet **1970 I**, 167.
LAW, L. W.: Studies of tumor antigens and of tumor-specific immune mechanisms. Transplant. Proc. **2**, 117 (1970).
LESLEY, J., DUTTON, R. W.: Antigen receptor molecules: inhibition by antiserum against kappa light chains. Science **169**, 487 (1970).
LEVEY, R. H., MEDAWAR, P. B.: Nature and mode of action of antilymphocytic antiserum. Proc. nat. Acad. Sci. (Wash.) **56**, 1130 (1966).
LEVEY, R. H., MEDAWAR, P. B.: Further experiments on the action of antilymphocytic antiserum. Proc. nat. Acad. Sci. (Wash.) **58**, 470 (1967).
MANDEL, M. A., DE COSSE, J. J.: The effects of heterologous antithymocyte sera in mice. IV. Alteration of the growth rate of plasma cell tumors. J. Immunol. **103**, 1288 (1969).
MILLER, J. F. A. P., BASTEN, A., SPRENT, J., CHEERS, C.: Interaction between lymphocytes in immune responses. Cell. Immunol. **2**, 469 (1971).
MISHELL, R. I., HERZENBERG, L. A., HERZENBERG, L. A.: Leukocyte agglutination in mice. Detection of H-2 and non-H-2 isoantigens. J. Immunol. **90**, 628 (1963).
MONACO, A. P., WOOD, M. L., GRAY, J. G., RUSSELL, P. S.: Studies on heterologous anti-lymphocyte serum in mice. II. Effect on the immune response. J. Immunol. **96**, 229 (1966).
OLD, L. J., BOYSE, E. A.: Immunology of experimental tumors. Ann. Rev. Med. **15**, 167 (1964).
PALM, J.: Current status of blood groups in rats. Ann. N.Y. Acad. Sci. **97**, 57 (1962).
PALM, J.: Serological detection of histocompatibility antigens in two strains of rats. Transplantation **2**, 603 (1964).
PALM, J.: Immunogenetic analysis of Ag-B histocompatibility antigens in rats. Transplantation **11**, 175 (1971).
PAUL, C., SHIMIZU, A., KÖHLER, H., PUTNAM, F. W.: Structure of the hinge region of the Mu heavy chain of human IgM immunoglobulins. Science **172**, 69 (1971).
PERLMANN, P., HOLM, G.: Cytotoxic effects of lymphoid cells *in vitro*. Adv. Immunol. **11**, 117 (1969).
PERNIS, B., FORNI, L., AMANTE, L.: Immunoglobulin spots on the surface of rabbit lymphocytes. J. exp. Med. **132**, 1001 (1970).

RABELLINO, E., COLON, S., GREY, H. M., UNANUE, E. R.: Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. J. exp. Med. **133**, 156 (1971).
RAFF, M. C., STERNBERG, M., TAYLOR, R. B.: Immunoglobulin determinants on the surface of mouse lymphoid cells. Nature (Lond.) **225**, 553 (1970).
RAMSEIER, H.: Leukotactic factor elaborated by mixtures of genetically dissimilar cells. Science **157**, 554 (1967).
RAMSEIER, H.: Die Transplantationsreaktion als zelluläre Immunität. Basel-New York: Karger 1969a.
RAMSEIER, H.: Recognition of transplantation antigens by immunologically competent cells. In: Cellular recognition, p. 251, ed. by R. T. SMITH and R. A. GOOD. New York: Appleton-Century-Crofts 1969b.
RAMSEIER, H.: Quantitative studies on antigenic recognition. I. Immunological and nonimmunological parameters of the response. J. exp. Med. **130**, 1279 (1969c).
RAMSEIER, H.: Failure of long-established skin homografts on ALS-treated mice to induce transplantation tolerance. Nature (Lond.) **228**, 68 (1970a).
RAMSEIER, H.: Transplantation und Immunität. Bull. schweiz. med. Wiss. **26**, 312 (1970b).
RAMSEIER, H.: Quantitative studies on antigenic recognition. II. Kinetics of the recognition event. Europ. J. Immunol. **1**, 171 (1971a).
RAMSEIER, H.: Quantitative studies on antigenic recognition. III. Nonspecific inhibition of the recognition process. Europ. J. Immunol. **1**, 433 (1971b).
RAMSEIER, H.: Immunization against abolition of transplantation tolerance. Europ. J. Immunol. (in press).
RAMSEIER, H., BILLINGHAM, R. E.: Studies on delayed cutaneous inflammatory reactions elicited by inoculation of homologous cells into hamsters' skins. J. exp. Med. **123**, 629 (1966).
RAMSEIER, H., LINDENMANN, J.: Fl hybrid animals: reactivity against recognition structures of parental strain lymphoid cells. Path. Microbiol. **34**, 379 (1969).
RAMSEIER, H., LINDENMANN, J.: Quantitative studies on antigenic recognition. IV. Specific inhibition of the recognition process by alloantisera. Europ. J. Immunol. **1**, 441 (1971a).
RAMSEIER, H., LINDENMANN, J.: Inhibition of recognition of transplantation antigens by specific antibody. Concept and findings. In: Immunopathology VIth internat. symposium, p. 308, ed. by P. A. MIESCHER. Basel: Schwabe 1971b.
RAMSEIER, H., LINDENMANN, J.: Recognition of transplantation antigens *in vitro*: blocking of recognition structures by specific antibody. Europ. J. Immunol. **1**, 348 (1971c).
RAMSEIER, H., LINDENMANN, J.: Cellular receptors. Effect of anti-alloantiserum on the recognition of transplantation antigens. J. exp. Med. **134**, 1083 (1971d).
RAMSEIER, H., LINDENMANN, J.: Similarity of cellular recognition structures for histocompatibility antigens and of combining sites of corresponding alloantibodies. Europ. J. Immunol. **2**, 109 (1972a).
RAMSEIER, H., LINDENMANN, J.: Aliotypic antibodies. Transplant. Rev. **10**, 57 (1972b).
RAMSEIER, H., PALM, J.: Further studies of histocompatibility loci in rats. Transplantation **5**, 721 (1967).
RAMSEIER, H., STREILEIN, J. W.: Homograft sensitivity reactions in irradiated hamsters. Lancet **1965I**, 622.
SELL, S., GELL, P. G. H.: Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with an antiallotype serum. J. exp. Med. **122**, 423 (1965).
SHAFFER, C. F.: Transfer of immunity against long surviving rat skin xenografts by antilymphocyte serum treated Syrian hamsters. Nature (Lond.) **223**, 1375 (1969).
SIMONSEN, M.: Graft versus host reactions, their natural history and applicability as tools of research. Progr. Allergy **6**, 349 (1962).
SNELL, G. D., STIMPFLING, J. H.: Genetic of tissue transplantation. In: Biology of the laboratory mouse, second edit., p. 457, ed. by E. L. GREEN. New York: McGraw-Hill 1966.
SPRENT, J., MILLER, J. F. A. P.: Activation of thymus cells by histocompatibility antigens. Nature (Lond.) New Biol. **234**, 195 (1971).
STANBRIDGE, E. J., PERKINS, F. T.: Tumour nodule formation as an *in vivo* measure of the suppression of cellular immune response by antilymphocytic serum. Nature (Lond.) **221**, 80 (1969).

TRUFFA-BACHI, P., WOFSY, L.: Specific separation of cells on affinity columns. Proc. nat. Acad. Sci. (Wash.) **66**, 685 (1970).
UNANUE, E. R.: Antigen-binding cells. I. Their identification and role in the immune response. J. Immunol. **107**, 1168 (1971).
WARNER, N. L.: Immunological reactions produced by the local injection of adult fowl leucocytes in chickens. Aust. J. exp. Biol. med. Sci. **42**, 417 (1964).
WIGZELL, H., MÄKELÄ, O.: Separation of normal and immune lymphoid cells by antigen-coated columns. Antigen-binding characteristics of membrane antibodies as analyzed by hapten-protein antigens. J. exp. Med. **132**, 110 (1970).
WILSON, D. B.: Quantitative studies on the behavior of sensitized lymphocytes *in vitro*. I. Relationship of the degree of destruction of homologous target cells to the number of lymphocytes and to the time of contact in culture and consideration of the effects of isoimmune serum. J. exp. Med. **122**, 143 (1965).
WOODS, D. A.: Influence of antilymphocyte serum on DMBA induction of oral carcinoma. Nature (Lond.) **224**, 276 (1969).

Genetics of the F Sex Factor in Enterobacteriaceae

MARK ACHTMAN[1]

With 6 Figures

Table of Contents

I. Introduction . . . 79
A. Nomenclature . . . 80
B. Preliminary Discussion . . . 80
II. Physical Studies on Sex Factor DNA . . . 83
III. Physiological and Genetic Studies on Incompatibility and Replication . . . 86
A. Replication-Control Models . . . 89
B. Replication of Other Plasmids . . . 90
C. Replication-Deficient Mutants . . . 91
IV. Chemical Studies on Conjugational DNA Transfer . . . 93
V. F-Pili . . . 95
A. Role of F-Pili in Male-Specific Phage Infection . . . 96
B. Properties of F-Pili . . . 97
VI. Surface Exclusion . . . 98
VII. Genetic Analysis of Transfer . . . 99
A. Function of *tra* Cistrons . . . 102
B. Other F-Pili Mutants . . . 104
C. Specificity of *tra* Cistrons . . . 104
VIII. Physical Mapping and the Transfer Origin . . . 105
IX. Map Locations of Other Cistrons . . . 108
X. Female-Specific Phage Restriction . . . 109
XI. Still Other Cistrons . . . 110
XII. Relationship between Different Sex Factors . . . 111
XIII. Transfer Regulation . . . 113
A. F-Factor Mutants . . . 113
B. J-Independent Mutants . . . 114
C. Other Forms of Regulation . . . 115
References . . . 116

I. Introduction

Bacterial sex factors and conjugation have supplied the topics for many review articles and the value of yet another one may be questioned. However, the last few years have seen many new developments in this area such that a more informed approach to the problem is now possible. In addition, it seems worthwhile, at this point, to subject what has been done to a critical analysis

[1] Max-Planck-Institut für Biologie, 7400 Tübingen, Correnssstraße 38, West Germany. Present address: Max-Planck-Institut für Molekulare Genetik, Abt. Trautner, 1000 Berlin 33, Ihnestraße 63–73.

and point out several logical predictions and shortcomings. In order to reduce the length of this review to a manageable size, I will largely ignore the large body of information so adequately reviewed in the past except where it is of direct interest to my topic and instead, wherever possible, will refer the interested reader to the appropriate review(s).

A. Nomenclature

The nomenclature proposed by Demerec et al. (1966) and specified, most recently, by Taylor (1970) has gained wide acceptance among bacterial geneticists. Briefly, this nomenclature recommends that each bacterial strain be given a unique designation by a code of one or two letters followed by a number, e.g. M178 or JC3273. The phenotypic properties of these strains may then be referred to by symbols such as Abc^+ or Abc^-, for possession or lack of any one phenotypic property. Each mutation carried by the strain should, once recognized, be assigned a unique designation such as *pmq-n*. Once *pmq-n* has been genetically assigned to a cistron it may then be referred to by *pmqXn*. The cistron itself may be referred to as *pmqX* and the genotype of the strain as pmq^+ or pmq^-.

A similar nomenclature has been proposed for bacterial sex factor mutants (Achtman et al., 1971). According to this system each sex factor mutant (and/or recombinant) should be uniquely designated by a series of four letters followed by a number, e.g. the F*lac* mutant, JCFL18. The genotypic and phenotypic description of the mutant sex factors are then exactly as for bacteria, e.g. JC6630 is a strain carrying JCFL18 and the cells are Tra^- (transfer-deficient) as a result of the JCFL18-encoded transfer mutation *traE18*.

Unfortunately no comprehensive system of nomenclature for sex factors of different origin has yet been proposed. I will use the unpublished system of Adelberg and Low for different F and F′ elements and will refer to resistance transfer factors and colicinogenic factors by the names currently in use. The sex factors mentioned in this article are listed in Table 1.

B. Preliminary Discussion

The following operational definitions are offered for preliminary orientation and will be appropriately referenced and amplified in subsequent sections.

Replication and segregation: The replication and segregation machinery of male cells carrying F1 (or an F prime element) is efficient enough that usually more than 99% of the cells in a culture grown from a single colony still carry F1 (or the F prime element). Occasionally F^- cells are found; these will be referred to as cured cells. Mutations increasing the frequency of cured cells may be episomal or chromosomal and might affect replication, segregation, or both (Novick, 1969).

Conjugational DNA transfer: This is most easily demonstrated in matings between F prime carrying cells and F^- cells where selection may be imposed

Table 1. Designations and a few properties of sex factors often referred to in the text

Sex factor	Selectable properties	Transfer ability	Comments	Reference[a]
F1	none[b]	high	original F-factor	ADELBERG and BURNS (1960)
F2	none[b]	high	original F-prime factor	ADELBERG and BURNS (1960)
F8	Gal$^+$	high	carries *nadA*$^+$, *aroG*$^{+c}$	HIROTA and SNEATH (1961)
F13	Lac$^+$	high	carries *phoA*$^+$, *phoR*$^+$, *tsx*$^+$, *purE*$^+$	HIROTA and SNEATH (1961)
F42	Lac$^+$	high		JACOB and ADELBERG (1959)
F57	His$^+$	high		ACHTMAN et al. (1972)
F450	Gal$^+$	high	carries *bio*$^+$, *att*$^{\lambda}$, *uvrβ*$^+$	FREIFELDER et al. (1971)
R100	Tet^R	repressed		NISHIMURA et al. (1967)
ColI	none[b]	repressed	prototype I-like sex factor	FREDERICQ (1965)

[a] The references given are in all cases to a usable definition of these sex factors which in some cases also coincide with the original definition.
[b] F1, F2 and ColI have no identifiable properties that may be used for the selection of cells that have received them. However, such cells may be identified as chromosomal DNA donors (for F1 or F2) or as producers of colicin I (for ColI). In addition, F2 has homology with part of the *E. coli* chromosome such that *pro*$^+$ is transferred early in conjugation from an (F2)$^+$ donor.
[c] SHARP et al. (1972).

for cells which still carry genetic markers characteristic of the F$^-$ parent but which have also inherited chromosomal genes located on the F prime element. For example F42 transfer to Lac$^-$ Str^R cells may be measured by assaying the number of Lac$^+$ [Str^R][2] cells present after termination of the mating. The transfer efficiency is at least 100% per donor cell in 30 minutes under the appropriate conditions. Transfer-deficient (or Tra$^-$) mutants transfer quantitatively less efficiently, some of them as poorly as $\leqq 10^{-8}$ of the normal efficiency.

Hfr: Hfr cells are genetically defined as cells which transfer bacterial chromosome with a defined sequence in conjugation and which transfer the complete F factor only very late in conjugation after a copy of all the bacterial DNA has been transferred. Because of the incompatibility phenomenon (see below) they are assumed to carry no autonomous F DNA and it is assumed that in Hfr cells replication of F DNA is usually under chromosomal control. On the basis of genetic evidence, CAMPBELL (1962) suggested that chromosomal integration of F1 to give Hfr strains (see Fig. 1) occurred by a single recombinational event between the chromosomal circle and the F circle to give one larger circle. SCAIFE (1967) amplified this model, also on the basis of genetic evidence, to explain F prime formation from Hfr strains by a second

2 Lac$^+$ [Str^R] is an abbreviated form for referring to progeny clones which have maintained the Str^R properties of the recipient cells and have also received *lac*$^+$ alleles from the donor cells.

recombinational event leading to excision. He postulated two types of excision events. One, called type I, gives rise to F prime elements lacking some F DNA and carrying chromosomal DNA formerly located on one side of the integrated F factor. The other, called type II, gives rise to F prime elements containing all the primeval F DNA and also carrying chromosomal DNA formerly located on both sides of the integrated F factor.

Surface exclusion: male cells are poor recipients in conjugation. If we perform parallel matings between an Hfr donor and exponential phase cultures of either an $(F42)^+$ or an F^- recipient, the number of recombinants generated in the former mating is usually approximately 300fold less than in the latter mating. This poor recipient ability, called surface exclusion, is also manifested against F transfer from F^+ cells. Surface exclusion may be removed by physiological means to yield phenocopy Sex^- (surface exclusion deficient) cultures. Surface exclusion is a different phenomenon from incompatibility (see below) although the two have often mistakenly been discussed together.

Incompatibility: cells carrying two closely related sex factors, such as two F prime elements, have only a transitory existence and yield daughter cells carrying only one or the other. The one clear exception is when sex factors are integrated into the chromosome to form a double male strain.

Female-specific phage restriction and male-specific phage sensitivity: a number of bacteriophages give lower efficiencies of plating on male strains than on female strains. With most of these, the difference is only about 100fold but the plaques formed on the male strains are much smaller and more irregular than those formed on female strains. In addition, male strains are usually sensitive to male-specific bacteriophages to which female strains are resistant. Male-specific phage sensitivity is related to the absolute requirement for F-pili in their adsorption and this topic will be dealt with in the section on conjugational DNA transfer.

The above properties are manifested by the F factor and by its related F prime and Hfr derivatives. It is not clear that all other sex factors manifest the same properties. There are at least quantitative differences in replication control, incompatibility, and surface exclusion between F and various R factors. Furthermore, the mechanism of the F type of female-specific phage restriction seems to be unique; in cases where $(R)^+$ cells give a lowered efficiency of plaque formation, this has been attributed to a different mechanism, that of the restriction/modification system (see BOYER, 1971). It is thus clear that information gained with one sex factor cannot be automatically applied to any other. R factors and Col factors also show additional properties. R factors carry dominant alleles for resistance to certain antibiotics and Col factors encode genes for colicin(s) production and immunity against the colicin(s) produced. A discussion of these properties is outside the purview of this article and the interested reader is referred to the review articles by WATANABE (1971), and NOMURA (1967).

Transfer repression: most sex factors other than F promote rather inefficient conjugational DNA transfer (usually about 200 to 500fold poorer

than that of F). This is often true only of established cultures and more efficient transfer is observed with cultures which have only recently received the sex factor. Although the mechanism involved is not yet understood, I will use the convention of NISHIMURA et al. (1967) and refer to this phenomenon as transfer repression and to the agent responsible as the transfer repressor. Since F itself is sensitive to transfer repression by certain R and Col factors, this topic is of relevance to F genetics and will also be discussed in this review.

II. Physical Studies on Sex Factor DNA

A number of different laboratories have now performed measurements of the contour length (electron microscopic) of covalently-closed DNA circles isolated from sex factor-containing cells. These measurements are in very good agreement with each other and lead to an estimate of molecular weight for F1 DNA. However, before discussing these results, I would like to remind the reader that the exact relationship of contour length to molecular weight is still unclear (LANG, 1970; FREIFELDER, 1970) and that the exact molecular weight of λ DNA which has been used as a standard for these measurements is also still under discussion (FREIFELDER, 1970). Thus the molecular weight estimates may be inaccurate by as much as five to ten percent. The reader interested in comparative studies of sex factor and plasmid DNA is referred to the recent review by HELINSKI and CLEWELL (1971) and I will restrict myself primarily to F1 and related F prime elements.

FREIFELDER et al. (1971) have shown convincingly that covalent circles may be isolated from established cultures of $(F450)^+$ cells and that these are not artefacts of the isolation process. If anything, most procedures involved in DNA isolation are likelier to disrupt covalent circles than to generate them. Since covalent circles have also been isolated from $(F42)^+$ cells (FREIFELDER, 1968a), and from mating cultures of $(F1)^+$ and $(F2)^+$ cells (FREIFELDER, 1968b), it is safe to conclude that F factor DNA exists at least occasionally as covalently-closed circular molecules. It is not known what proportion of the intracellular F DNA is in the covalently closed circular state and what proportion is linear but it is possible to make a rough estimate from the data to hand. FREIFELDER (1968a, 1968c) showed that 0.5 to 2.5% of the DNA from $(F42)^+$ cells could be recovered in the form of covalently-closed circles. VAPNEK et al. (1971) showed that 1.7% of the DNA from $(F1)^+$ cells could be isolated in this form. Using the molecular weight estimate of 2.5×10^9 daltons for the non-replicating bacterial chromosome supplied by COOPER and HELMSTETTER (1968) and the molecular weight estimates for F42 and F1 DNA (see below) of 95×10^6 and 62×10^6 daltons, respectively, these values correspond to 0.1 to 0.7 molecules of F42 DNA and 0.7 molecules of F1 DNA per non-replicating chromosome. FRAME and BISHOP (1971) have shown that there are approximately 1.4 F8 DNA molecules per non-replicating chromosome in $(F8)^+$ cells. Thus an appreciable fraction of intracellular F and F prime DNA is in the form of covalently closed circles. Although the biological

role of these circles is not known, FREIFELDER (1968a) has proposed the plausible idea that the covalent circular DNA molecules are molecules temporally (*sic*) trapped between successive replication rounds by the processes of DNA isolation.

KLINE and HELINSKI (1971) have been able to isolate F1 DNA in the form of a supercoiled circle complexed with cellular material. As this may be an intermediate in replication, it is of great interest. The complex can be induced to relax to an open circular form by treatment with proteases, alkali, or sodium dodecyl sulphate, treatments which will not nick covalently closed circles. This implies that proteins and perhaps lipids are present in the complex. The open circular duplex so generated consists of one circular single strand and of one linear single strand. These correspond respectively to the light and heavy strands found upon equilibrium centrifugation in CsCl gradients containing poly (U, G). (See HELINSKI and CLEWELL, 1971 for a discussion of similar relaxation complexes found with other extrachromosomal elements.)

A number of laboratories have measured the contour length of F1 DNA circles and all report a value of approximately 30 μ corresponding to approximately 62×10^6 daltons (KLINE and HELINSKI, 1971; VAPNEK et al., 1971; SHARP et al., 1972; MAAS, W. K., personal communication). The criticism may be leveled against some of these estimates that they were obtained from preparations of covalently-closed circles obtained after ethidium bromide CsCl centrifugation which FREIFELDER (1971) has shown can increase molecular lengths by up to 27%. However the extensive dialysis used by some of these authors and the fact that VAPNEK et al. (1971) did not use ethidium bromide in their DNA isolation procedures argues against this criticism. It is therefore all the more remarkable that earlier molecular weight estimates by FREIFELDER (1968b) based upon the conversion of covalently-closed circles to nicked circles after X-ray irradiation gave molecular weight estimates approximately 30% lower. The new size estimates are large enough to allow space for 100 or more cistrons on F1.

As discussed above (see p. 81 and Fig. 1) a model for F prime formation has been presented by SCAIFE (1967). The model had previously been supported by genetic evidence that at least one F prime element (F13) was of type II since it contained chromosomal DNA from both sides of the integrated F factor in the parental Hfr strain (BRODA et al., 1964; SCAIFE and PEKHOV, 1964). Further support came from genetic evidence that some cured derivatives of Hfr strains still contained homology with F and therefore presumably still contained some integrated F DNA (ADELBERG and BURNS, 1960; RICHTER, 1961). This result is as expected of type I excision events (Fig. 1). These models for F prime formation have been given support from physical experiments by SHARP et al. (1972). These authors performed electron microscopic studies on heteroduplex molecules formed by co-renaturation of denatured F1 and F prime DNA. The contour length measurements of the native DNA molecules lead to molecular weight estimates of 62×10^6 daltons for F1, 78×10^6 daltons for F8, and 114×10^6 daltons for F450. The data presented

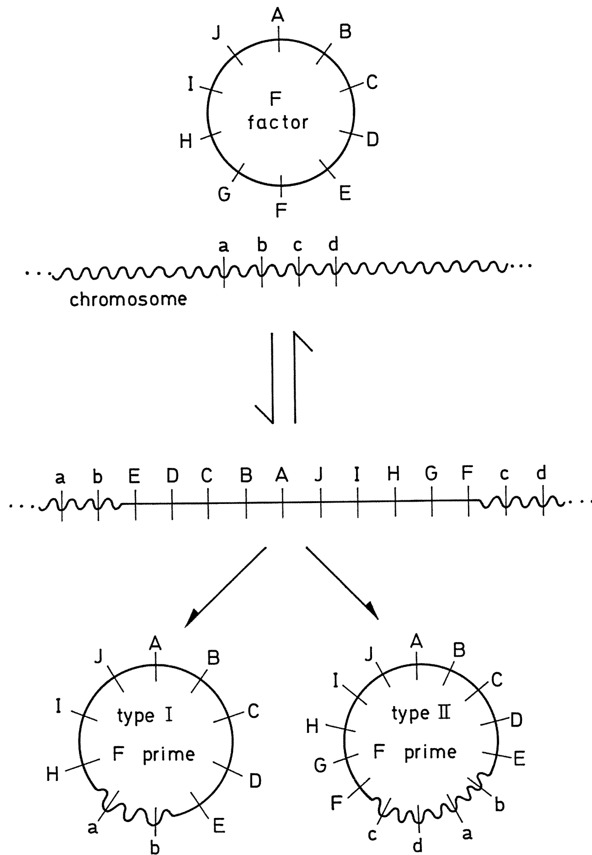


Fig. 1. Model for the formation of Hfr's and F-primes. 10 specific points, A through J, are depicted on F and four specific points, a through d, on the bacterial chromosome. Integration to give the Hfr strain occurs by a single reciprocal recombination between FE and bc. Excision can occur by a single reciprocal recombination event at the same site to yield F and chromosome again or between HG and the chromosome to the left of a to give a type I F-prime or between two sites on the chromosome, one to the left of a and the second to the right of d, to give a type II F-prime. Type I F-primes lack some F DNA while type II F-primes do not. The figure is redrawn from SCAIFE (1967) and this model was first proposed there

may also be used to calculate a molecular weight of approximately 95×10^6 daltons for F42. Furthermore, each of these F prime DNA's could be shown to be lacking some of the F1 DNA and may be considered to be type I F prime elements. The DNA lost is not known to affect any biological functions (see p. 105 for a discussion of physical mapping).

The size of various R factors which can act as sex factors has also been measured by similar techniques and all are roughly similar in size to each other and to F1, approximately $50–70 \times 10^6$ daltons (COHEN and MILLER, 1970; HAAPALA and FALKOW, 1971; NISIOKA et al., 1969, 1970; SILVER and FALKOW, 1970; VAPNEK et al., 1971). The meaning of these size determinations is

somewhat complicated because in some hosts (discussed in more detail on p. 91), some R factors dissociate into at least two smaller replicons (Rownd and Mickel, 1971; Nisioka et al., 1970). One of these replicons is probably the sex factor itself while the other replicon(s) carries markers for antibiotic resistance (Cohen and Miller, 1970a, 1970b; Haapala and Falkow, 1971).

It is of interest to note that the DNA of Col factors ColE1, ColE2, and ColE3, which are not sex factors, but which are transferred very efficiently from sex factor-containing cells, is much smaller than that of F1, on the order of 5×10^6 daltons (Bazaral and Helinski, 1968a) although molecules of dimer and trimer length have been reported (Bazaral and Helinski, 1968b).

III. Physiological and Genetic Studies on Incompatibility and Replication

It was originally observed that it was very difficult to introduce an F prime element stably into an Hfr cell (Scaife and Gross, 1962; Maas and Maas, 1962; Maas, 1963) even under conditions where the F prime element presumably entered the Hfr cells. It was equally difficult to isolate cells carrying two different autonomous F prime elements (Scaife and Gross, 1962; Echols, 1963); instead, the temporary heterozygotes formed seemed to segregate pure clones carrying only one or the other F prime. In F13 × F13 crosses (where one F13 element was *lac*$^-$ and the other *pho*$^-$; Echols, 1963) and F57 × F42 crosses (Achtman, 1969) there seems to be no real preference for which F prime element is segregated to daughter cells. However, F8 is different in that cells carrying F8 and F13 segregate only (F8)$^+$ daughter cells regardless of whether F8 was introduced into an (F13)$^+$ cell or F13 into an (F8)$^+$ cell (Echols, 1963). Perhaps this is due to the F DNA missing in F8 (Sharp et al., 1972; see page 106).

Later work (Dubnau and Maas, 1968) has shown that when an F prime element (F42) is introduced into an Hfr cell, the superinfecting element seems to be prevented from replication and instead is diluted by unilinear inheritance during growth of the culture. The *lac*$^+$ alleles on the F42 element are occasionally stably inherited either through recombination with the chromosome to give homozygotic *lac*$^+$ cells or by chromosomal integration of the F prime element to give a double male strain (Maas and Goldschmidt, 1969). Double male strains, which carry two integrated F factors are quite stable (Clark, 1963). Apparent exceptions to incompatibility where cells seemed to carry both F8 and F42 autonomously or where Hfr cells seemed to carry F42 autonomously (Cuzin and Jacob, 1967b) have been shown to be artefacts and to be due to a high frequency of double male cells in the population (Maas and Goldschmidt, 1969). In fact, it is possible to reduce drastically the number of such pseudo-exceptions to incompatibility by using RecA$^-$ hosts (Dubnau and Maas, 1968).

Another escape from incompatibility in *RecA*$^-$ strains has also been described (Press et al., 1971), namely fusion of two autonomous F prime

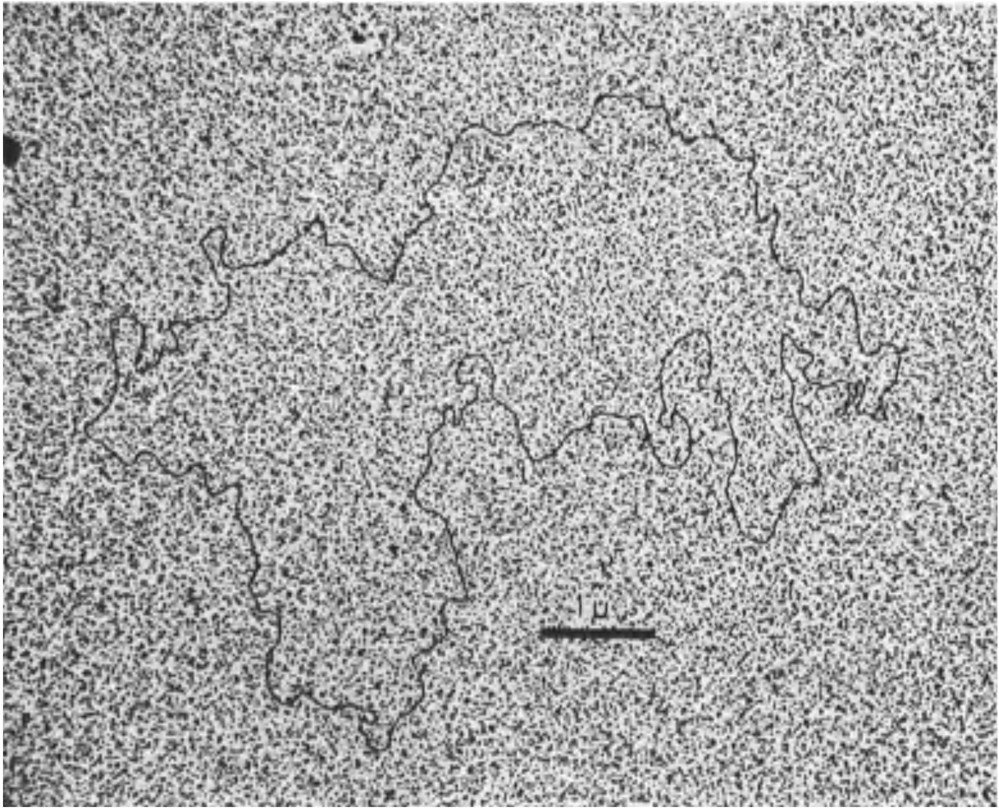


Fig. 2. Electron micrograph of covalently closed circular DNA from an F′*trp*⁺ *arg*⁺ fusion F-prime. The contour length of this F-prime is 39 μ, intermediate between the contour lengths of the two parental F-primes, F′*trp*⁺ (59 μ) and F′*arg*⁺ (31 μ) (MAAS, W. K., personal communication). The photograph is the generous gift of W. K. MAAS

elements into one autonomous F prime element carrying at least some of the chromosomal DNA carried previously by both. In one case an F′ element carrying *trp*⁺ and *att80*⁺ was fused with a different F′ element carrying *metB*⁺ *metF*⁺ *ppc*⁺ *argE*⁺ *argC*⁺ *argB*⁺ and *argH*⁺. The contour length of DNA circles of the F′*trp* element is 59 μ and that of the F′*arg*⁺ element, 31 μ. The contour length of four F′*trp*⁺ *arg*⁺ elements was 39 μ, 39 μ, 63 μ, and 69 μ (MAAS, personal communication). An electron micrograph of DNA from one of the 39 μ fusion F′ elements is shown in Fig. 2.

The incompatibility phenomenon is largely responsible for the present primitive state of F genetics since any complementation analysis depends on being able to use at least partially diploid cells carrying two copies of the cistrons under analysis. The double male carrying two integrated sex factors, does not supply the answer to this problem because such cells are technically difficult to select and because it is uncertain whether both sex factors are expressed at the same time (CLARK, A. J., personal communication). The same

objection applies to fusion F′ elements since it is not obvious without much complicated work whether these carry all the F cistrons of both F factors. This problem of incompatibility is not as severe for R factors where a low frequency of intrachromosomal integration and an apparently lessened efficiency of incompatibility has allowed the maintenance of cells carrying both R factors under conditions of constant selection for the presence of both (HOAR, 1970). However, here as well, the criticism may be levied that the state of expression of the various R factor encoded cistrons in the artificially maintained R/R heterozygote cells is not known.

It would simplify F genetics considerably if incompatibility mutants could be isolated, allowing free coexistence of two autonomous F elements in the same cell. This has not yet been accomplished. However, PALCHOUDHURY and IYER (1971) have reported stable maintenance of two F prime elements in a chromosomal temperature-sensitive replication mutant of *E. coli*. This exciting finding has not yet been subjected to the stringent test devised by MAAS and GOLDSCHMIDT (1969), i.e. the demonstration that the two F elements are compatible in a RecA⁻ derivative of the same strain. Therefore, it is still possible that the apparent compatibility seen by PALCHOUDHURY and IYER (1971) is due to chromosomal integration.

A different approach has been used in an attempt to isolate mutants of F that are incompatibility-deficient. MAAS and GOLDSCHMIDT (1969) have reported the isolation of a RecA⁻ Hfr mutant which allows the establishment of an autonomous F42 element. The integrated F factor in this mutant had apparently lost its transfer and replication functions (MAAS, W. K., personal communication) and it seems likely that the mutant carries a large deletion, deleting most of its F DNA. Since, in general, deletion mutations are less frequent than single point mutations in any one cistron but more frequent than point mutations in two separate cistrons and since such Hfr deletion mutants are the most commonly isolated type of incompatibility mutants (MAAS, W. K., personal communication), it seems likely that F carries at least two cistrons whose gene products are involved in incompatibility. A different type of Hfr mutant has also been isolated (DE VRIES, J. and MAAS, W. K., personal communication) which does not seem to be mutated in its transfer or replication cistrons since transfer- and replication-proficient F primes can be selected from the mutant. Genetic analysis indicated that the incompatibility mutation is linked to the integrated F factor in the mutant Hfr strain. However this does not prove that the incompatibility mutation is on the F factor rather than on the chromosome. It has not yet been possible to demonstrate any impairment of incompatibility with F prime elements derived from this mutant. Despite these reservations it seems likely that the incompatibility mutations in this mutant and in the other type described above are located on the F DNA and therefore that F carries some incompatibility cistrons.

Incompatibility between sex factors has been used as a classification device on the basis that any incompatible sex factors must be closely related (NOVICK,

1969). Thus F elements and the Col factors ColV2 and ColV3 are incompatible and fall into one incompatibility group (MACFARREN and CLOWES, 1967; KAHN and HELINSKI, 1964). However, these three sex factors can be arranged hierarchically in that cells transiently carrying both ColV2 and an F element retain ColV2 preferentially while cells transiently carrying both ColV3 and an F element retain the F element preferentially (MACFARREN and CLOWES, 1967).

A. Replication-Control Models

The molecular mechanism of incompatibility is not known. However, the two major models presented for control of replication have also included mechanisms which account for incompatibility. Both depend on the concept of replicons (JACOB and BRENNER, 1963) which may be defined as autonomous and physically discrete genetic elements with some self control of replication. Thus the bacterial chromosome and the various sex factors are all examples of replicons. In the membrane-attachment model (JACOB et al., 1963), replication is controlled by there being only a limited number of membrane attachment sites to which the replicons must be attached for replication to occur. A segregation unit consists of a region of the membrane with one attachment site for each compatible replicon and upon division of the segregation unit, the attachment sites and the replicons both divide. Incompatibility is explained on the basis that two incompatible replicons both require the same attachment site for division and therefore cannot both replicate at the same time. Thus, one of them will always pass to only one daughter cell and so be diluted out of the population. It is not clear why in an Hfr cell the integrated F element should be under the replication control of the chromosome but still manifest incompatibility and this apparent paradox is probably the strongest argument against invoking an attachment site model to explain incompatibility. In other words, even though attachment sites may be necessary for replication, it seems unlikely that a limited number of attachment sites can explain all the various manifestations of incompatibility.

The second replication-control model is by PRITCHARD et al. (1969), who pointed out that a mechanism is necessary in order to specify when each new replication round should begin. They postulated the existence of an inhibitor of the initiation of replication which is active at one concentration but becomes inactive at a twofold lower concentration and allows a new replication round to begin. The inhibitor is encoded by a gene which is transcribed immediately after the initiation of replication, such that each initiation event produces enough inhibitor to inhibit any subsequent initiation events until the inhibitor is once more diluted twofold by cell growth. If the ratio of F primes to chromosome reached two to one, twice as much F replication inhibitor would be made and two mass doublings (during which the F elements segregate) would be needed to dilute the F replication inhibitor enough to allow a new F replication round. Thus incompatibility would be explained on a random segregation of sex factors with no bias towards the preexisting one. This model

is not at odds with a type of membrane attachment site model since membrane attachment sites could still be involved in segregation of replicated elements.

Replication of F prime elements may be arrested either by the use of temperature-sensitive mutant F primes (CUZIN and JACOB, 1967d) or by the use of acridine dyes such as acridine orange (HIROTA, 1960). The mechanism of the replication arrest by these two means is not clear and in fact it is even possible that some of the temperature-sensitive mutant F primes are defective in segregation rather than replication (NOVICK, 1969). However, both these techniques have now been used to show that when replication is arrested, the pre-existent F prime DNA molecules segregate strictly together with the chromosomal DNA with which they were associated and not with any chromosomal DNA synthesized thereafter (CUZIN and JACOB, 1967a; HOHN and KORN, 1969). This is strong evidence for an association of biological significance between autonomous replicons and may most easily be interpreted by the membrane site attachment model. However, these results do not necessarily imply that there is as strong an association between normally replicating F elements and chromosome since what is true of F DNA whose replication has been blocked may not apply to normally replicating DNA. In fact, it may be relevant to point out here that if the attachment site model is correct then it must still be formulated so as to allow relatively open competition for the newly-formed attachment sites between any two F prime elements. This conclusion derives from the observation that up to 20% of heterozygote cells carrying F57 and F42 can segregate, at the first division, pure clones containing only the superinfecting F prime element (ACHTMAN, 1969). The desired experiment to resolve this question is to pulse label chromosome and F element in some manner and then determine whether the pulse-labeled elements separate in subsequent replication acts.

Evidence is beginning to accumulate concerning the timing of F replication in the cell cycle. The fact that there seem to be about two copies of F DNA per replicating chromosome (FRAME and BISHOP, 1971) can be interpreted to mean that F may initiate replication about the same time as the chromosome but finish its replication round earlier because of its much smaller size. However, ZEUTHEN and PATO (1971) have shown that F42 replicates about one-half to two-thirds of the way through the cell-division cycle regardless of growth rate and its replication is therefore sometimes initiated at times different from those at which that of the chromosome is initiated. This in turn implies that chromosome and F-factor replication are initiated by different types of "signals" and argues against the validity of the cell mass-linked negative control model of PRITCHARD et al. (1969) described above.

B. Replication of Other Plasmids

ColE1 DNA synthesis continues during amino acid starvation conditions which inhibit chromosomal and F DNA synthesis (BAZARAL and HELINSKI, 1970) suggesting that its replication is controlled differently from that of F.

It also seems likely that the replication control of F is different from that of at least some R factors as deduced from their behaviour in *Proteus mirabilis*. This conclusion is only tentative as I know of no comparative studies between F and R factors. It has been reported that *P. mirabilis* cells carrying an F*lac* deletion mutant of F13 or carrying a ColV element in the same incompatibility group as F contain approximately one sex factor DNA molecule per chromosome (FALKOW and CITARELLA, 1965; HICKSON et al., 1967), as is the case in *E. coli*. The situation with R factors is much more complicated (see ROWND et al., 1971, for a general model of R replication). Often the R factor decomposes into at least two replicons (ROWND and MICKEL, 1971; NISIOKA et al., 1970) one of which is the sex factor (COHEN and MILLER, 1970a, 1970b; HAAPALA and FALKOW, 1971). Thus we should really only compare the behaviour of the sex factor replicon itself with that of F. This has only been determined in one case that I know of where it was estimated that there were three to five copies of the sex factor per chromosome (HAAPALA and FALKOW, 1971). The complete undissociated R factor is present in numbers of approximately ten copies per chromosome during exponential phase (ROWND et al., 1966). These ten replicate by a "relaxed control" mechanism where the molecule chosen for any one replication round is chosen at random from all the existing molecules although there is a defined number of rounds of replication per cell-division cycle (ROWND, 1969). The control is relaxed even more during growth into stationary phase such that the number of R DNA molecules per chromosome reaches approximately 20 but regains its former level once more, upon restoration of the cells to exponential growth (KASAMATSU and ROWND, 1970).

The situation with other plasmids may be different still. NOVICK and BRODSKY (1972) have shown that after transduction, *Staphylococcus* penicillinase plasmids commence replication before their stable establishment and segregation. This situation is exaggerated even more upon transduction of a plasmid into a cell containing an incompatible plasmid. Replication begins within 3 generations but cells stably carrying the superinfecting plasmid are not segregated till several generations later. On quantitative grounds, NOVICK and BRODSKY (1972) have been able to exclude explanations based upon the membrane attachment site model, or any other model that links replication and segregation as an explanation for incompatibility.

C. Replication-Deficient Mutants

CUZIN and JACOB (1967d) have reported the isolation of six temperature-sensitive mutants of F42 which are cured with variable efficiencies upon incubation of the host cells at 42°C (Table 2) but which are relatively stable in cells grown at 30°C. As NOVICK (1969) has pointed out, it is not clear whether these mutants affect replication, segregation, or both, since mutations in either process will result in elevated curing. However, these six mutants have generally been accepted at face value as temperature-sensitive replication-

Table 2. Properties of temperature-sensitive F42 mutants[a]

Mutant[b]	$(F')^+$ after 4 generations[c]	Transfer efficiency[d]
F_{ts106}*lac*	80%	$\leqslant 10^{-2}$%
F_{ts115}*lac*	70%	22%
F_{ts114}*lac*	45%	44%
F_{ts108}*lac*	20%	11%
F_{ts62}*lac*	5%	1%
F_{ts113}*lac*	5%	1%

[a] From data reported by CUZIN and JACOB (1967d).
[b] The mutants are designated as originally described. They are all derived from F42.
[c] Percentage Lac^+ clones in the population after growth for four generations at 42°C as as percentage of the percentage of Lac^+ clones in the inoculum. A mutant totally incapable of replication should yield a theoretical value of 6% under these conditions.
[d] Transfer efficiency in 60 min matings at 37° as corrected for the average transfer efficiency of $(F42)^+$ cells (90%) under the same conditions.

deficient mutants. As shown in Table 2, two of the mutants show no signs of replication/segregation at 42°C (F_{ts62}*lac* and F_{ts113}*lac*); both of these also show a marked transfer inefficiency. The other mutants, except one, do show some residual replication/segregation at the nonpermissive temperature and also transfer more efficiently. As described on p. 94, F has two forms of replication, vegetative and transfer replication. Possibly some enzymes are common to both types of replication and the phenotypes in Table 2 might be due to mutations affecting both replication and transfer simultaneously. However, they might also be due to the fact that even a non-leaky replication-deficient mutant would always show some replication *via* transfer. Selection for clean mutants would then select the rare mutants with separate mutations in both replication and in transfer. This latter possibility seems most likely for mutants such as F_{ts62}*lac* since CUZIN (discussion after CUZIN et al., 1967) has shown that the transfer-deficiency of this mutant is not temperature-sensitive and WILLETTS and ACHTMAN (1972) have shown that F_{ts62}*lac* carries a *traG*⁻ mutation (see below).

The primary conclusion from these arguments is that replication/segregation should always be measured under conditions where transfer replication cannot occur. In fact, it is even possible that F_{ts106}*lac* does not carry any replication mutation but is only a transfer mutant. When Tra^- mutants carrying mutations in any of the *tra* cistrons are grown at 42°C they sometimes give elevated numbers of F^- segregants although they are completely stable at 37°C (ACHTMAN and FINNEGAN, unpublished results). This is confirmed by the recent report that some F prime carrying strains undergo extensive curing upon growth at 42°C under conditions of low cell concentrations where DNA transfer is a rare event (STADLER and ADELBERG, 1972). Most likely either replication or segregation of F is normally not as efficient at 42°C as at lower temperatures.

IV. Chemical Studies on Conjugational DNA Transfer

An immense amount of work has been devoted to elucidating the mechanism and properties of DNA transfer, most of which was performed in attempts to resolve whether DNA transfer can proceed in the absence of DNA replication. The answer to this question is still unclear and I refer any interested readers to the reviews by CURTISS (1969) and BRINTON (1971) for a summary of this evidence. I will not discuss it at all here and in this section I will deal primarily with the nature of the DNA which is transferred.

The experiments of GROSS and CARO (1965, 1966) and of PTASHNE (1965) demonstrated that only one strand of DNA preexistent before the beginning of the mating was transferred from the donor to the recipient cells. These authors assumed at the time that the other strand of the duplex was synthesized in the donor cell during the transfer event. It now seems clear instead that *only* that pre-existent strand is transferred and that it is then converted to the duplex form in the recipient while the other strand remains in the donor where it too is converted to the duplex form. This was first indicated by the finding that the DNA isolated from minicells which had been used as recipients in bacterial matings was largely single-stranded (COHEN et al., 1968a, 1968b) as if single-stranded DNA were being transferred which could then not be restored to duplex form in the minicells. Similar conclusions may be drawn from experiments which showed that only one labeled strand of λ phage is physically recoverable from recipients after mating with labeled, λ lysogenic donors (RUPP and IHLER, 1968; IHLER and RUPP, 1969; OHKI and TOMIZAWA, 1968) and from results which showed that only one strand is genetically recoverable after mating (BONHOEFFER and VIELMETTER, 1968; VIELMETTER et al., 1968). Furthermore it has been demonstrated that the end of the λ strand transferred first is always the $5'$ end (OHKI and TOMIZAWA, 1968; RUPP and IHLER, 1968) regardless of the orientation of the F factor. Similar evidence for single-stranded DNA transfer was provided by studies measuring transfer of F DNA itself (VAPNEK and RUPP, 1970).

An alternative possible explanation of the above results involving selective destruction of one strand after transfer of a duplex has been largely ruled out by experiments showing that donor cells labeled before mating in both DNA strands, do actually retain one of the labeled strands during conjugation (VAPNEK and RUPP, 1970). Thus one old strand is transferred and one remains behind. This still does not convincingly prove that the DNA transferred is singlestranded as it is still possible that the DNA is replicated in the donor during the transfer act and then immediately degraded back to the single strand form as it enters the recipient. No evidence exists to support this niggling objection and I will assume the validity of the likelier possibility, that of single-stranded DNA transfer. A similar mechanism presumably involving single-strand transfer is also manifested by R factors (VAPNEK et al., 1971).

The end results of a mating between an F prime cell and an F^- cell is that the F prime has replicated once, leaving one of the daughter replicas in the donor cell and the other in the recipient cell. Thus, in a sense, sex factors have

two types of replication, one similar to that of the bacterial chromosome (and described in section III) and the other mediated by conjugational DNA transfer. It seems likely on consideration of what is known about the properties of the two replication systems that at least some of the enzymes involved are specific for one or the other type of replication, although at the moment none of the enzymes are known. The idea of specificity is borne out by the fact that mating proceeds normally at high temperature in strains carrying a chromosomal temperature-sensitive replication mutation although vegetative replication may be assumed to be inhibited (MARINUS and ADELBERG, 1970). In addition, such mutants, when used as recipients in matings, allow the transferred single strand to be converted to the duplex form and also to be circularized to a covalently-closed circle (VAPNEK and RUPP, 1971).

It is not clear how much DNA is transferred during conjugation, even when the only DNA transferred is that of the sex factor itself. MATSUBARA (1968) measured the length of F DNA shortly after its transfer into recipient cells. The DNA sedimented with an s value corresponding to a molecular weight of 100×10^6 daltons for F1 and 160×10^8 daltons for F42. These numbers are approximately twice as large as the size estimates determined with covalently-closed circles (see p. 84). Similarly, OHKI and TOMIZAWA (1968) found that the DNA transferred in a mating between donors carrying an F *gal* $(\lambda)^+$ (in which λ is integrated into the sex factor) sedimented as a broad peak, part of which was equivalent to the molecular weight of 18 λ DNA molecules and part equivalent to 12 λ DNA molecules. They were able to calculate by hybridization studies that the monomer length of their F *gal*$(\lambda)^+$ was approximately 6 λ DNA equivalents and thus concluded that dimers and trimers were being observed. When these recipient cells carrying dimer and trimer length molecules were incubated for one hour after the mating, the labeled DNA isolatable was all roughly monomer length. Thus it is possible that more than a unit length of DNA may be transferred during mating, as also suggested by the genetic results of FULTON (1965). This is consistent with the possibility that conjugational DNA transfer proceeds according to the rolling circle model (GILBERT and DRESSLER, 1968). However, other interpretations of the data are also possible (W. D. RUPP, personal communication) and it is not entirely excluded that the size estimates were performed on covalently closed- and on nicked-monomer length circles, which sediment rapidly enough that they might be mistaken for dimers and trimers. Thus more work must be done before we can resolve the question of how much DNA is transferred.

Another question that is currently receiving some attention is that of what happens to the transferred DNA. SHULL et al. (1971) have reported that F DNA transferred from F^+ or Hfr cells to F^- minicells may be recovered attached to the membrane of the minicells. This preliminary observation fits with the much more detailed analysis performed by FALKOW et al. (1971) on the fate of replicating R factor DNA after transfer. (This may of course differ from the fate of F DNA since F and R factors can replicate differently; see p. 91.)

These authors found that the R factor DNA was bound as unit-length linear duplex DNA to the recipient cell membrane and was later found free in the cytoplasm, first as nicked circles and then as covalently-closed circles. These results fit with the postulate by FREIFELDER (1968a) that F exists as a covalently-closed circular molecule between replication rounds. No evidence was found for greater than unit length molecules.

V. F-Pili

The molecular basis of conjugation is not understood; it is clear, however, that F-pili must somehow be involved. This conclusion derives directly from the observation by OU and ANDERSON (1970) that DNA may be conjugationally transferred in the absence of cell wall to cell wall contact between donor and recipient cells as observed in the light microscope. Similarly, BRINTON (1965) had reported that he could see mating pairs by light microscopy which seemed to be attached by an invisible thread (F-pili are too thin to be seen in the light microscope). Since the mating process is unaffected by the presence of high concentrations of DNase (BRINTON, 1965) these observations imply that DNA is transferred via some extracellular appendage and the only appendage known to be obligatory is the F-pilus (BRINTON et al., 1964). Furthermore, when F-pili are removed by blending, mating ability is lost and is quantitatively recovered with the same kinetics as is F-piliation (BRINTON, 1965). The sensitivity to blending and the kinetics of reappearance of the other known extracellular appendages, type I-pili and flagellae, are different (NOVOTNY et al., 1969). DNA transfer may be inhibited by treatment with male-specific phages (KNOLLE, 1967; NOVOTNY et al., 1968; IPPEN and VALENTINE, 1967) whose adsorption site is exclusively the F-pilus (see below). Thus F-pili are vitally involved in and absolutely necessary for conjugation (for a summary of further evidence see BRINTON, 1971 and CURTISS, 1969).

However, it is also clear that the presence of F-pili as demonstrated by electron microscopic observations is not sufficient for conjugation. This conclusion derives from several different types of observations. For example, although OU and ANDERSON (1970) did observe some DNA transfer in the absence of cell wall to cell wall contact, this was so in only a minority of cases and they did observe cell wall to cell wall contact in most cases where DNA transfer occurred. Furthermore, type I-pili are also required for efficient DNA transfer (MULCZYK and DUGUID, 1966; MEYNELL and LAWN, 1967). BRINTON (1965) in his experiments on the kinetics of reappearance of transfer after blending would not have observed this since his blending treatment did not remove type I-pili (NOVOTNY et al., 1969). Finally, the regeneration of F-pili preceded the regeneration of donor ability when cells were blended and then chilled and washed in saline (BRINTON, 1965; see also CURTISS et al., 1969). In

summary, both type I-pili and cell-wall contact are implicated, in addition to F-pili, for efficient conjugational transfer and no model for conjugational transfer can be based upon the properties of F-pili alone.

Various models have been presented for the role of F-pili. BRINTON (1965) originally suggested that the F-pilus was a tube with an axial hole through which DNA was transported. More recently (BRINTON, 1971), he has specified slightly different models in which he conceives of the F-pilus as two parallel chains which then conduct (DNA moves through the pilus), convey (the two parallel chains are the visible part of a conveyor belt) or carry (F-pilus is continuously physically degraded at the recipient end and newly synthesized at the donor end) the DNA single strand into the recipient cell. In contrast, MARVIN and HOHN (1969) and CURTISS (1969) have separately specified models in which the F-pilus retracts into the donor cell upon the proper stimulus (DNA phage infection in the former model and contact with a recipient cell in the latter model). Thus according to CURTISS' model, the conjugation bridge is not the F-pilus itself but instead F-pili are only involved in bringing cells together preparatory to mating. These pili retraction models seem unlikely since some of the cells in wall-to-wall contact described by OU and ANDERSON (1970) were also observed to later move back to a more distant position (OU, cited by BRINTON, 1971). However as no direct evidence exists yet for any of these models for the role of F-pili, I will let this topic lapse with the sole conclusion that although F-pili are vitally involved in conjugation, it is not known how.

A. Role of F-Pili in Male-Specific Phage Infection

F-pili are also involved in male-specific bacteriophage infection since the RNA bacteriophages adsorb to the sides of the F-pilus (CRAWFORD and GESTELAND, 1964; BRINTON et al., 1964) and the DNA phages adsorb to the F-pilus tip (CARO and SCHNÖS, 1966). Thus, because of their different mode of adsorption, the mechanism by which the nucleic acid of these phages enters the cell may well differ. Furthermore, the RNA bacteriophages may be divided into at least two major groups with f2 representative of one group and $Q\beta$ of the second (OVERBY et al., 1966a and b). Another group of phages related to f2 (WATANABE et al., 1967a and b) and two more groups related to $Q\beta$ (MIYAKE et al., 1969) have also been described. The two major groups (f2 and $Q\beta$ groups) are also slightly related serologically (KRUEGER, 1969). Despite their similar form of adsorption, it is not *a priori* obvious whether or not all the male-specific RNA bacteriophages use the same mechanism for infecting the cell. In fact, genetic studies have indicated differences in the mechanisms of infection of f2-like and of $Q\beta$-like phages (see below).

Some very exciting results have recently been reported on the mechanism of infection of some of these RNA phages. Earlier studies had shown that

when f2 adsorbs to cell-bound F-pili, the f2 RNA becomes sensitive to destruction by RNase before subsequent events resulting in cell penetration occur (VALENTINE and STRAND, 1965), the RNase-sensitive RNA is bound to F-pili, and the empty virus capsid desorbs into the medium (SILVERMAN and VALENTINE, 1969). Recently, PARANCHYCH et al. (1971) have shown that the A or maturation protein of R17 (closely related to f2), as well as the RNA, bind to the F-pili and that the desorbed phage capsid no longer contains its A protein. In the subsequent infection stages, the F-pili are broken leaving the cell proximal portion attached to the cell and the cell distal portion free in the medium (PARANCHYCH et al., 1971). The A-protein attached to F-pili is itself split into two smaller components (KRAHN et al., 1972), and both halves of the split A-protein and the RNA are transmitted into the cell (PARANCHYCH and KRAHN, 1971; KRAHN et al., 1972). Intracellular entry of MS2 A-protein has also been reported (KOZAK and NATHANS, 1971; MS2 is also closely related to f2). Thus cell-bound F-pili are involved in intracellular penetration of protein as well as nucleic acid and perhaps are even responsible for enzymatic splitting of A-protein.

B. Properties of F-Pili

Our knowledge of the physical and chemical properties of F-pili is still too primitive to explain the biological functions described above on a molecular basis. F-pili are proteinaceous extracellular appendages 85 A (BRINTON, 1965, 1971) to 95 A (LAWN, 1966) wide and up to 20 or more microns long. The number per cell is variable with the strain but is usually approximately 1–1.5 per cell (BRINTON et al., 1964; CURTISS et al., 1969) during growth in nutrient medium although it can reach up to 5 per cell under anaerobic conditions. CURTISS et al. (1969) have made an extensive study of the effects of various conditions on F-piliation.

Although terminal vesicles have occasionally been observed in electron micrographs of F-pili (VALENTINE and STRAND, 1965; LAWN, 1966) these have been shown to be degradation artefacts (BRINTON, 1971). In fact, under appropriate degradative conditions, it is possible to split the pili longitudinally into protein chains of about one-third the diameter of the intact rod (BRINTON, 1971). Since an axial line is clearly visible in F-pili (BRINTON, 1965; VALENTINE and STRAND, 1965; LAWN, 1966), BRINTON (1971) has suggested that the F-pilus consists of two parallel protein chains. Pili containing nucleic acid have not been observed or chemically isolated.

BRINTON (1971) has succeeded in the chemical purification of F-pili although his protocol is not yet published. The general procedure is as follows: F-pili found free in the growth medium are concentrated by iso-electric precipitation followed by cycles of spontaneous aggregation and disaggregation. Finally the pili are banded by two cycles of CsCl buoyant density centrifugation (BRINTON, personal communication). The purified pili show one major protein band on SDS acrylamide gels with a molecular weight of 11800

(BRINTON, 1971) and one minor band (about 0.1 % of total F-pili protein) of greater molecular weight (BRINTON, personal communication). The major band is lacking four amino acids but contains one D-glucose and two phosphate residues per monomer in a very stable linkage (BRINTON, 1971). Although very interesting, it seems likely that these purified F-pili differ from the functional F-pili found on cells. This conclusion derives from the fact that although free F-pili adsorb RNA- (VALENTINE and STRAND, 1965) and DNA-bacteriophages (CARO and SCHNÖS, 1966), they do not induce the adsorbed RNA phages to release their A-protein-RNA complex as cell-bound F-pili do (VALENTINE and STRAND, 1965). Furthermore, cell-bound F-pili can even be prevented from adsorbing RNA phages by treatment with phenethyl alcohol while such treatment has no effect on free F-pili (WENDT and MOBACH, 1969). Thus free F-pili may be considered the enzymatically-inactive degradation product of cell-bound F-pili which do not carry out the enzymatic activities leading to intracellular nucleic acid penetration associated with functional F-pili. It would be of great interest to know the chemical structure of purified enzymatically active F-pili.

The specific density of F-pili in CsCl gradients is not settled. BRINTON'S (1971) purified F-pili had a specific density of 1.257 while the partially purified pili described by WENDT et al. (1966) had a specific density of 1.197 and those of BEARD et al. (1972) had a specific density of 1.296. The reason for these discrepancies is unknown.

VI. Surface Exclusion

Very little of significance is known about surface exclusion. Exponential male cells (F^+, F′, or Hfr) are poor recipients in conjugational matings even when incompatibility is not involved (e.g. in matings against an Hfr donor where the formation of chromosomal recombinants is assayed). Surface exclusion is actively promoted by F as shown by the fact that cells with good recipient ability (Sex^- phenocopies) are generated under conditions of lowered protein synthesis such as growth into stationary phase (LEDERBERG et al., 1952) or growth at 27° C (BONHOEFFER, 1966) or amino acid starvation (CURTISS et al., 1969). Surface exclusion has been clearly demonstrated for the Col I sex factor (MEYNELL, 1969) where Sex^- phenocopies can also be generated by amino acid starvation. ACHTMAN et al. (1971) have shown that surface exclusion acts at least partly at the level of mating pair formation since fewer mating pairs are seen in matings between a Sex^+ (surface-exclusion positive) exponential $(F42)^+$ donor culture and a non-F-piliated but Sex^+ recipient culture than are seen in matings between an $(F42)^+$ and an F^- culture. This effect is specific since more mating pairs are once more seen (ACHTMAN, unpublished results) when the recipient culture carries a Sex^- F42 mutant (see below). However, it is not clear whether the inhibition in mating pair formation is strong enough to account quantitatively for all of surface ex-

clusion. Perhaps surface exclusion also acts to inhibit DNA transfer among a few mating pairs which might be formed.

CURTISS et al. (1969) have concluded that the presence of F-pili is not responsible for surface exclusion. This conclusion is supported by the fact that both minicells derived from F^+ cells and transfer-deficient mutants lacking F-pili can still manifest surface exclusion (COHEN et al., 1968b; ACHTMAN et al., 1971; see below). CURTISS et al. (1968) have also suggested that surface exclusion is a coincidental byproduct of some transfer protein present in the cell surface. This suggestion has no evidence favoring (or contradicting) it.

VII. Genetic Analysis of Transfer

Recently, two sets of genetic analyses of conjugational transfer have been performed; they have revealed hitherto unexpected complexities. The aim of both analyses was to yield a minimal estimate of how many cistrons F carrys whose products are necessary for transfer. This would directly give a minimal estimate of how many transfer proteins there are. To do this, Tra^- (transfer-deficient) mutants of F8 and R100-1[3] (OHTSUBO et al., 1970) and of F42 (ACHTMAN et al., 1971, 1972) were isolated after treatment with mutagens such as nitrosoguanidine, ethyl methane sulfonate, and ICR-191. The survivors were screened for transfer-deficiency. Some of the mutants were instead selected from non-mutagenized cultures on the basis of resistance to male-specific phages (OHTSUBO et al., 1970). The Tra^- mutants isolated include mutants with amber, ochre, UGA, and frameshift mutations (ACHTMAN et al., 1971, 1972). Most of the amber mutants are transferred at near wild type levels from a Su_I^+ host but up to 10^8 fold more poorly from a Su^- host (*op. cit.*).

A genetic analysis of the mutations could not be performed by conventional techniques because of incompatibility. OHTSUBO et al. (1970) therefore examined $(F, R)^+$ cells carrying both an F8 Tra^- mutant and an R100-1 Tra^- mutant for complementation as manifested by elevated transfer levels. Such $(F, R)^+$ heterozygotes are stable since F and R belong to different incompatibility groups. The mutants could be assigned to at least six complementation groups called A, B, C, D, E, and F.

ACHTMAN et al. (1972) measured complementation between Tra^- mutants in transient heterozygotes. Amber-suppressible F42 mutants were transferred from a $T6^s$ amber-suppressing host to Sex^- phenocopy cultures of a $T6^R$ non-suppressing host carrying other F42 mutants. Transient heterozygotes carrying two different F42 Tra^- mutants could be generated with high efficiency by these matings, with up to 30% of the recipient cells becoming heterozygous. The $T6^S$ donor cells were then killed by T6 treatment and survivors were measured for complementation by performing a second mating with an F^- strain. Complementation resulted in an elevated level of transfer from the transient heterozygotes. In order to perform this analysis, a semiautomatic

3 R100-1 is a mutant of R100 which does not make the transfer repressor and therefore transfers almost as well as F itself (EGAWA and HIROTA, 1962).

Table 3. Cistronic assignments of *tra⁻* mutations[a]

	Amber	UGA	Frameshift	Non-suppressible	Total
traA	2	—	1	1	4
traB	3	—	3	6	12
traC	7	1	1	10	19
traD	4	1	2	9	16
traE	2	—	2	2	6
traF	1	—	2	1	4
traG	4	—	4	2	10
traH	3	—	—	6	9
traI	—	—	1	3	4
traJ	—	—	—	2	3
traK	—	—	1	—	1
Total	27	2	17	42	88

[a] from data supplied in Achtman et al. (1972) and Willetts and Achtman (1972). The numbers represent the number of mutants only known to carry a single *tra⁻* mutation, whose mutation could clearly be assigned to one of these eleven cistrons. The inclusion of the mutants described by Ohtsubo et al. (1970) is not possible as it is not known what mutations they carry and many carry double mutations in more than one cistron (Willetts and Achtman, 1972).

technique for performing 144 quantitative bacterial matings concurrently was developed (Achtman, 1971). In a separate analysis, Willetts and Achtman (1972) tested many of the same mutants by introducing DNA from one F42 Tra⁻ mutant to cells carrying a second F42 Tra⁻ mutant by P1 transduction. Presumably these crosses, too, were using a form of transient heterozygotes. P1 is incapable of transducing a complete F42 replicon (Willetts and Achtman, 1972) but apparently can transduce enough F42 DNA to allow transcription and translation of the *tra* cistrons.

These two analyses (Achtman et al., 1972; Willetts and Achtman, 1972) yielded complementary results, one defining nine cistrons and the second confirming the existence of eight of these cistrons and defining two more cistrons for a total of eleven. Most of approximately one hundred Tra⁻ mutants carried mutations assignable to one of these eleven cistrons, labelled *traA* through *traK*. The numbers and types of mutants in these cistrons are listed in Table 3. It is obvious since so few mutants carrying *traA*, *traF*, *traI*, *traJ*, or *traK* mutations are available that F may well carry other *tra* cistrons still as yet undefined. Furthermore, all the mutants described above were selected on the basis that they transferred less than 0.1 % as well as the ancestral F prime and other mutants transferring more efficiently than that were not further tested (Ohtsubo et al., 1970; Achtman et al., 1971). It is thus possible that *tra* cistrons may exist whose products aid but are not essential for DNA transfer and mutants in those cistrons would not have been detected. In agreement with this notion is the fact that Ou and Anderson (1970) found that some, but not all, mating pairs came into temporary wall-to-wall contact. Transfer was usually associated with such contact but was observed to proceed in its absence efficiently enough that mutants in this process would not have

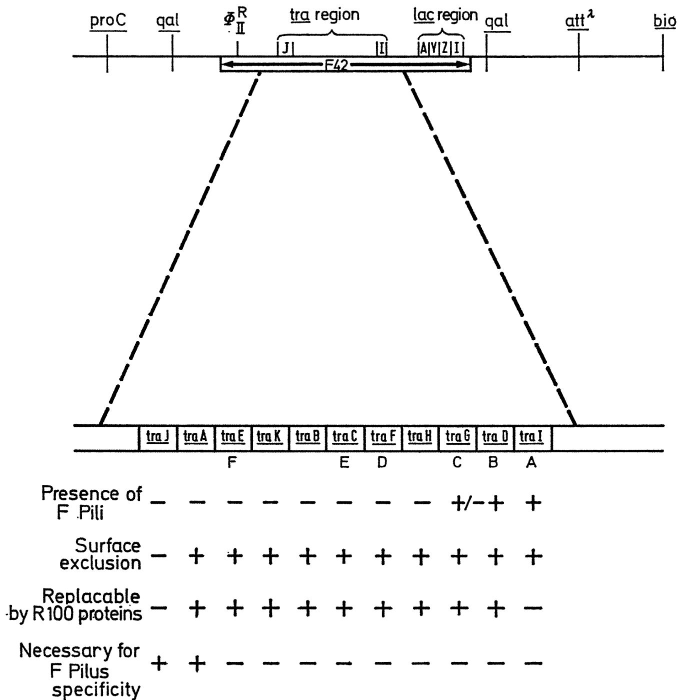


Fig. 3. Order and properties of *tra* cistrons. The top part of the figure shows the *E. coli* chromosome map in the vicinity of the integrated F_{ts114}*lac* element (IPPEN et al., 1971). The lower part of the figure shows an expanded view of the F-part of the chromosome. The distance between any two cistrons is not known. Designations between the two lines in the lower part of the figure are according to ACHTMAN et al. (1972) and WILLETTS and ACHTMAN (1972) while those below the lower line are according to OHTSUBO et al. (1970). The figure is redrawn from data presented by IPPEN-IHLER et al. (1972). The conclusions on F-piliation and surface exclusion come from ACHTMAN et al. (1971, 1972) and WILLETTS and ACHTMAN (1972) while those on plasmid specificity and pilus specificity come from WILLETTS (1971) and WILLETTS and ACHTMAN (1972)

been included among the mutants described here. Thus it seems highly likely that there exist more than the 11 defined *tra* cistrons.

The *tra* cistrons have been ordered in a unique linear sequence by two deletion mapping analyses. OHTSUBO (1970) isolated deletion mutants of F8 generated during P1 · F8 transduction of Rec^- cells. He then performed complementation tests between these and R100-1 Tra^- point mutants which had previously been assigned to complementation groups (OHTSUBO et al., 1970; see above) and was able to order his six *tra* cistrons in a linear array. IPPEN et al. (1971) isolated an Hfr strain in which a temperature-sensitive replication mutant of F42 (Fts_{114}*lac*) is integrated into the *gal* region (near the λ attachment site) of *E. coli*. The strain was then made lysogenic for a temperature-inducible mutant of λ and temperature-resistant mutants were selected. Among these were some deletion mutants in which the deletion ran from λ through to the integrated F and into the *tra* cistrons (IPPEN-IHLER et al., 1972). These deletion

mutants were tested by complementation tests with the F42 Tra⁻ point mutants described above. The tests defined the order of the eleven *tra* cistrons. It should be noted that the order of the cistrons defined by OHTSUBO (1970) is that of *tra* cistrons on an autonomous F8 element while that defined by this analysis (IPPEN-IHLER et al., 1972) is that of *tra* cistrons on an integrated F42 derivative. In any event, the two orders are identical (IPPEN-IHLER et al., 1972) and the order derived from both analyses is shown in Fig. 3.

A. Function of tra Cistrons

Although no concrete conclusions are yet possible on the function of any of the *tra* cistrons, a few isolated pieces of information are available. As shown in Fig. 3, mutations in eight of the eleven cistrons result in the non-production of F-pili. This implies automatically that F-pilus synthesis is a complicated process, certainly more so than is indicated by the chemical composition of purified free F-pili (BRINTON, 1971). It is not known which, if any, of these cistrons codes for the major and minor F-pili proteins isolated by BRINTON (1971; see p. 97). Since *traJ*⁻ mutants lack both F-pili and surface exclusion, BRINTON (1971) has postulated that *traJ* codes for the major F-pilus protein and that monomers of this protein present in the cell membrane and not yet assembled into F-pili are responsible for surface exclusion. This hypothesis, which has no evidence supporting it, is unlikely, as J-independent mutants have been isolated which are able to transfer and promote surface exclusion in the absence of J-protein (see below). Another candidate for the F-pilus structural gene is *traA* which has, in fact, been implicated in specifying F-pilus specificity (see below). However, this does not necessarily mean that it codes for a structural protein. Thus, the answer to which of the defined *tra* cistrons (if any) code for the two known F-pili proteins must await experimental evidence.

Three other cistrons, *traG*, *traD*, and *traI*, are not as obviously associated with F-pilus synthesis and it is likelier that they encode transfer enzymes. All *traI*⁻ mutants and half of the *traG*⁻ mutants[4] make apparently normal F-pili: male-specific bacteriophages can use these sex pili for infection, mating pair formation between the Tra⁻ mutant cells and F⁻ recipients is normal (as measured by the Coulter Counter technique), and seemingly normal F-pili are seen in the electron microscope (ACHTMAN et al., 1971; OHTSUBO et al., 1970). BRINTON (personal communication) has purified F-pili from one *traG*⁻, one *traI*⁻, and one *traD*⁻ mutant. The purified pili from the *traG*⁻ mutant have a different CsCl buoyant density from those of wild type and the pili from the *traI*⁻ mutant have an extra sugar moiety per major F-pili monomer. Although these results must await the analysis of other mutants to confirm that they

[4] It is not known why some *traG*⁻ mutants make F-pili and others do not. Two mutants in fact make F-pili but in lessened numbers (OHTSUBO et al., 1970; ACHTMAN et al., 1971). See ACHTMAN et al. (1972) and IPPEN-IHLER et al. (1972) for a discussion of various possible explanations which are too technical to repeat here.

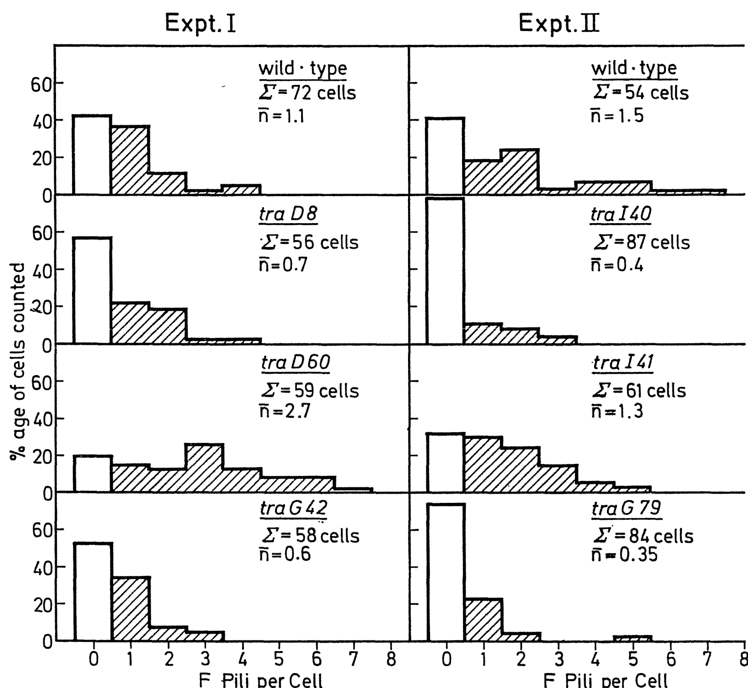


Fig. 4. Pili per cell of various Tra⁻ Mutants. The results obtained here come from two unpublished experiments performed by J. CARNAHAN, C. C. BRINTON, JR. and myself. Exponential broth cultures at 42° C of various F42 mutants were used. F-pili were labeled by allowing adsorption of purified M12 and M13 preparations and the cells were then negatively stained with phosphotungstic acid. Electron microscopic photographs were taken at a magnification of 4 500 × and the negatives were scanned with a magnifying lens. Only pili whose cell proximal end could be seen to be attached to the cell wall were scored and the numbers represent only cells where such a decision was possible. The total number of cells scored and the number average of F-pili/cell are given for each mutant

are cistron specific, they raise the possibility that *traG⁻* and *traI⁻* mutants chemically alter F-pili such that their function in conjugational transfer is adversely affected although superficially normal F-pili are synthesized.

traD⁻ mutants are unique. They make F-pili; in fact, one mutant, JCFL60, makes 2.5 times as many F-pili per cell as the wild type sex factor while a second *traD⁻* mutant, JCFL8, makes normal numbers of F-pili (Fig. 4). However, f2 bacteriophage is unable to infect *traD⁻* cells. The phage particles adsorb but no RNA penetrates the cell (ACHTMAN et al., 1971). Similar mutants, which may also be *traD⁻* mutants but whose transfer ability was never tested, had previously been described (SILVERMAN et al., 1967a and b) and it was found that these mutant cells did not even induce f2 phage to release its RNA. It is very interesting that Qβ, a different type of RNA bacteriophage, and f1, a DNA bacteriophage, infect *traD⁻* mutants with normal efficiency. Thus, Qβ and f2 RNA's have at least partly different mechanisms for intracellular entry. The one *traD⁻* mutant tested (JCFL60) has F-pili which it has not

been possible to distinguish chemically from wild type F-pili (BRINTON, 1971) and since the mutant produces 2.5 times as many pili as the wild type (see above) it has been used as BRINTON'S primary source of F-pili (BRINTON, personal communication). However, *traD*$^-$ mutants are obviously mutated in F-pilus function if not in structure of the major protein and thus chemical analyses of *traD*$^-$ mutant F-pili are not likely to succeed in explaining F-pilus function.

It may be of interest that the two temperature-sensitive Tra$^-$ mutants described (WALKER and PITTARD, 1970; ACHTMAN et al., 1971) both have *traD*$^-$ phenotypic properties at the non-permissive temperature and one of these, JCFL39, was genetically shown to carry a *traD*$^-$ mutation, *traD39* (ACHTMAN et al., 1972).

In summary, complementation analysis of Tra$^-$ mutants has defined eleven *tra* cistrons, at least eight (and possible all eleven) of which are involved in F-pilus synthesis and function. However, it is still possible that *traD*, *traG*, and *traI* code for transfer proteins and are not directly involved in F-pilus synthesis.

B. Other F-Pili Mutants

Mutants have also been described which affect male-specific phage sensitivity but have relatively little effect on DNA transfer. In one case, these resembled *traD*$^-$ mutants in that they were sensitive to $Q\beta$ and fd (related to f1) but resistant to MS2 (related to f2) (MEYNELL and AUFREITER, 1969). Since they were also slightly impaired in transfer they might be leaky *traD*$^-$ mutants. The pili of these mutants were clearly abnormal as they showed an increased number of vesicles (*op. cit.*) which have been shown by BRINTON (1971) to be degradation products. In a second case, BRINTON (personal communication) has also isolated two Tra$^+$ male-specific phage-resistant mutants. These two, however, are resistant to all RNA male-specific phages tested even though they too show the increased vesicle formation. Mutants with similar male-specific phage sensitivities were previously described by SILVERMAN et al. (1968). Purified F-pili from one of BRINTON'S mutants were shown to contain three phosphates per monomer of the major pilus protein (BRINTON, personal communication) whereas the wild type has only two. Most interestingly, these mutants are complementable for male-specific phage sensitivity in crosses against mutants in all but one *tra* cistron: *traA*$^-$ mutants do not complement BRINTON'S male-specific phage resistant mutants and therefore they presumably affect the *traA* protein (BRINTON, personal communication).

C. Specificity of tra Cistrons

WILLETTS (1971) has shown that the *traJ* and *traI* encoded proteins are plasmid-specific. Cells carrying R100-1 and a *traJ*$^-$ or *traI*$^-$ F42 mutant transfer the R100-1 element efficiently but not the mutant F42 element.

Since mutations in both cistrons are recessive in F42×F42 crosses, the easiest interpretation of these results is that F- and R-factors both make plasmid-specific proteins which recognize only the corresponding DNA. Thus R100-1 would also make J- and I-proteins but these would not recognize F DNA and would not promote its efficient transfer.

WILLETTS (1971) was also able to assign pilus specificity to *tra* cistrons. As mentioned above, F-pili and R-pili differ in a number of ways, one of which is the efficiency of plating of male-specific phages. $(R100\text{-}1)^+$ cells give approximately 1 % as many plaques when used as an indicator lawn for M12 (related to f2) infection as do $(F)^+$ cells (NISHIMURA et al., 1967). WILLETTS (1971) found that $(F, R)^+$ cells carrying R100-1 and F42 Tra^- mutants with mutations in any one of most of the *tra* cistrons gave the F-specific level of M12 plating efficiency while $(F, R)^+$ cells carrying R100-1 and either a *traA*⁻ or *traJ*⁻ mutant gave the R-specific level. The easiest interpretation is that *traA* and *traJ* gene products are both involved in the specificity of F-pili. This does not however, imply that they code for either of BRINTON'S pilus protein bands since the relationship of these proteins to F-pilus specificity is not known.

WILLETTS (personal communication) has found that some of the Hfr deletion mutants with deletions extending from the right into *traD* (Fig. 3) are Sex^+ (surface-exclusion proficient) while other deletions extending into *traD* and all longer deletions are Sex^-. Thus, he postulates the existence of a cistron(s) essential for surface exclusion called *traS* which is located between *traD* and *traG*. Point mutants in this hypothetical cistron have not yet been isolated to my knowledge and it cannot be predicted whether $traS^-$ mutants would be affected in transfer efficiency. Mutations in the other *tra* cistrons, except one, do not affect surface exclusion[5]. The exceptional cistron is again *traJ* and all $traJ^-$ mutants are Sex^- (ACHTMAN et al., 1972). The possible role of J-protein will be discussed separately below.

VIII. Physical Mapping and the Transfer Origin

Different Hfr strains isolated after integration of F into the chromosome may transfer chromosomal DNA with different genetic sequences (MATNEY et al., 1964; BRODA, 1967). JACOB and WOLLMAN (1961) proposed that DNA transfer always commences at a particular point (or DNA nucleotide sequence) on F which is called the origin. However, it has not been clear until recently whether, in fact, any F DNA was transferred early in conjugation as predicted by this model. Also it has not been clear whether the intrachromosomal integration event always involved the same point on F (an attachment region) or depended on homology between different chromosomal DNA sequences and different F sequences. Recently, SHARP et al. (1972) have reported physical

5 A few exceptional mutants carrying nonsense mutations in various cistrons are partially Sex^- (WILLETTS, personal communication) but this is probably due to polar effects on the postulated *traS* cistron.

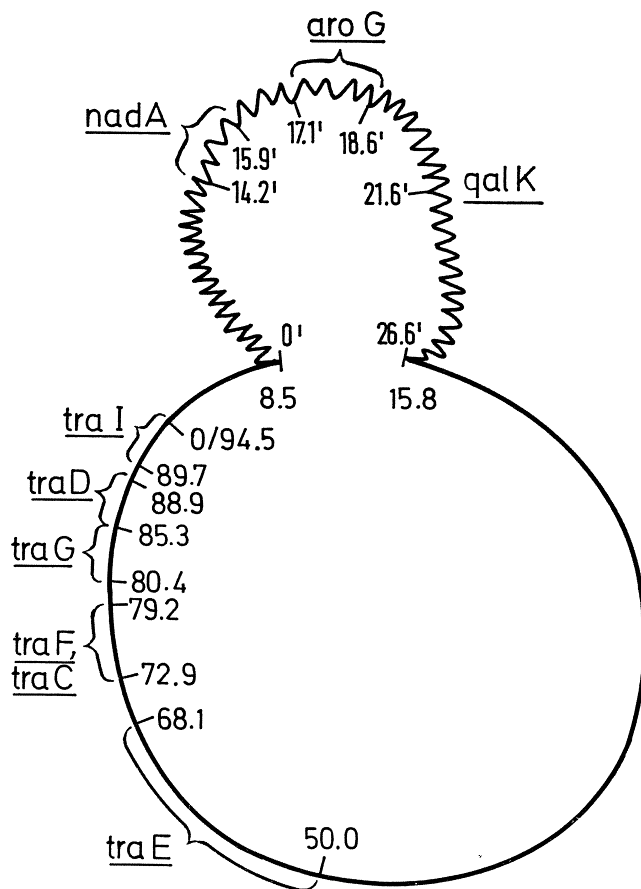


Fig. 5. Physical map of F8. Distances are presented in Kilobases (Kb) as determined from contour length measurements. Position on F DNA is represented by normal numbers and positions on extrachromosomal DNA by primed numbers. The ancestral F1 DNA was 94.5 Kb in length (approximately 30 μ) (SHARP et al., 1972). The position 0 Kb on this map corresponds to one end of the F1 DNA deleted in F42. F8 carries a deletion of F DNA from 8.5 to 15.8 Kb and in its place carries chromosomal DNA of 26.6 Kb length. The *tra* cistrons are labelled according to ACHTMAN et al. (1972) and WILLETTS and ACHTMAN (1972). For the according designation in the OHTSUBO (1970) system, see Fig. 3. The figure is redrawn from SHARP et al. (1972)

mapping of several F prime elements by electron microscopy. By comparing F8 and the Tra⁻ F8 deletion mutants isolated by OHTSUBO (1970) they were able to deduce the physical map of F8 shown in Fig. 5. By comparison of various F prime elements to F and to a particularly useful mutant of F8 which carries an insertion and a duplication, they were able to show that F8 is lacking F DNA from 8.5 to 15.8 Kb (kilobases; see legend to Fig. 5) on the map while F42 and F450 are both lacking F DNA from 0 to 3.0 Kb. The easiest explanation of these results is that the Hfr strains, from which F42 and F450 were isolated, arose by integration of F into different points on the chromosome

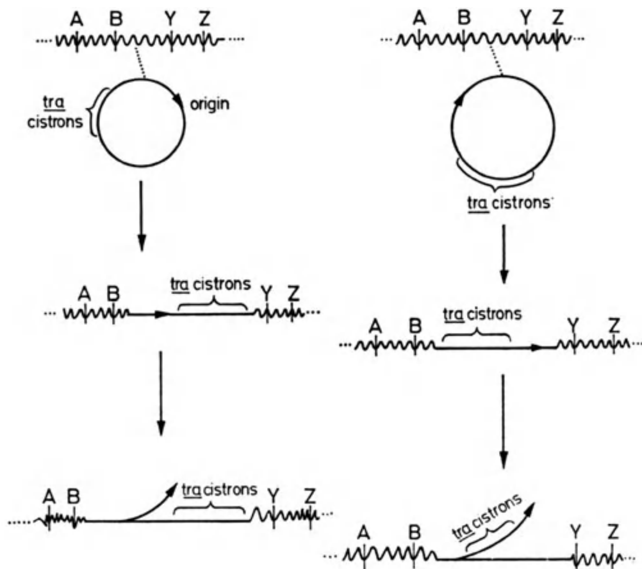


Fig. 6. Integration of F elements. Two types of integration are shown. That in (a) occurs by a single reciprocal recombination event between the chromosome and an area on F between the *tra* cistrons and the origin. That in (b) occurs by a single reciprocal recombination event between the chromosome and a different area on F between the origin and the *tra* cistrons. The Hfr formed in (a) will transfer the *tra* cistrons very late in conjugation while the Hfr formed in (b) will transfer them very early

but using the same point on F. The F prime elements must then have been formed by exactly the same type I (Fig. 1) excision event. F8 however, must have arisen by integration at a different point on F and by a different type I excision event. In a different F element, whose origin is unclear, the region from 0 to 14.5 Kb is deleted. The authors concluded that the DNA of F in the region 0 to 15.8 is a silent region since these F prime elements lacking various parts of this region are Tra+ and replication-proficient. However, as mentioned above (p. 86) F8 does not show the same incompatibility pattern as other F prime elements and instead displaces F13 from transient (F8, F13)+ cells (ECHOLS, 1963). Thus it is equally possible that an incompatibility cistron(s) is located between 8.5 and 15.8 Kb.

The fact that different Hfr strains arose by integration at different points on F implies that if JACOB and WOLLMAN (1961) were correct, then different Hfr strains might transfer different lengths of F DNA early in conjugation. This has been recently proven and it has finally been possible to demonstrate that some Hfr strains do transfer F DNA early in conjugation. BRODA et al. (1972) assayed the ability to complement *tra*$^-$ F42 mutants in transient heterozygotes as a measure of the early transfer of *tra*$^+$ alleles by Hfr donor cells. With a few Hfr strains, all 11 *tra*$^+$ cistrons were transferred early in conjugation. These strains could be assigned to two groups according to their integration site on the chromosome (one transferring *argG*$^+$ early and *thy*$^+$

late in a clockwise fashion and the second transferring *thy*+ early and *his*+ late, also in a clockwise fashion). Hfr strains with F integrated at fourteen other sites on the *E. coli* chromosome did not transfer any *tra*+ alleles early, regardless of whether the direction of transfer was clockwise or anti-clockwise. Thus, at least in some cases, some F DNA is transferred early in conjugation.

Neither the genetic nor the physical location of the origin is known. However, the results of SHARP et al. (1972) show that it is not within 0 to 15.8 Kb. Similarly WILLETTS (personal communication) has shown genetically that the Hfr deletion mutants with deletions running from the right through *traA* but not into *traJ* (see Fig. 3) still possess the origin while longer deletions do not. Thus the origin must lie to the left of *traA* and probably does not lie within the *tra* cistrons. The results of BRODA et al. (1972) described above indicate that in most cases integration occurred somewhere in one large region between the origin and the *tra* cistrons while in two groups of Hfr strains it apparently occurred in a different large region between the *tra* cistrons and the origin as depicted in Fig. 6.

IX. Map Locations of Other Cistrons

No cistrons have yet been mapped other than the *tra* cistrons. However the existence of the deletion mutants allows some statements about where replication and incompatibility cistrons cannot map. All the F8 deletion mutants described by OHTSUBO (1970) are capable of autonomous replication. Since some of these deletions run through the chromosomal DNA region counter clockwise to 68.1 Kb (SHARP et al., 1972), any replication genes carried by F must map between 15.8 and 68.1 Kb. Similarly WILLETTS (personal communication) has shown that the Hfr deletion mutants carrying deletions running from *lac* into *tra* cistrons still manifest incompatibility. Since F42, from which these mutants are derived, lacks F DNA from 0 to 3.0 Kb (SHARP et al., 1972) incompatibility cistrons must map between 3.0 and 68.1 Kb. As mentioned above, one of these may possibly map between 8.5 and 15.8 Kb since F8 lacking this DNA also shows altered incompatibility. Cistrons coding for female-specific phage restriction (see below) must also map in the same general region as the incompatibility and replication cistrons since Hfr deletion mutants deleting the DNA between *lac* and *traJ* still restrict female-specific phages while some longer deletions do not (IPPEN-IHLER et al., 1972).

Unfortunately, none of the techniques described for complementation analysis of *tra*− mutations can be applied to complementation analysis of replication/segregation mutants. F-like R-factors do not complement F replication/segregation mutants (MORRISON and MALAMY, 1970; HIROTA and NISHIMURA, cited in WILLETTS and BRODA, 1969) and complementation for replication or segregation cannot be performed in transient heterozygotes since detection of such complementation depends on selecting stable clones. The most obvious solution would be to use cells forced to carry two F factors

(Cuzin and Jacob, 1965a, 1965b) both of which carry temperature-sensitive replication mutations but this is to be avoided because such populations are very heterogenous due to incompatibility, and often carry the sex factors in the integrated state (Maas and Goldschmidt, 1969). It would be difficult to derive convincing evidence that complementation was being measured from experiments with such cells. These considerations do not mean that a complementation analysis of replication-deficient mutants is impossible; rather they reflect my personal inability to visualize how it could be satisfactorily performed with the present state of the art.

No theoretical problems exist in analysing incompatibility mutants since stable cells carrying two different mutants should readily be isolatable when both carried mutations in the same incompatibility cistron. However, no incompatibility mutants of an F prime element have yet been described despite searches in several laboratories. If such mutants could be isolated, they would be extremely useful for genetic analyses of mutations in other F encoded functions as well as hopefully shedding some light on the incompatibility phenomenon.

X. Female-Specific Phage Restriction

A number of bacteriophages give lower efficiences of plating on male strains (F^+, F′, Hfr) than on F^- strains although this property is strain-dependent (Monner and Boman, 1970; Williams and Meynell, 1971). The phages can readily be separated into two groups. Most of them, such as Φ_{II} (Cuzin, 1965) and T7 (Mäkelä et al., 1964) give immense plaques on F^- bacterial lawns and small, irregular plaques at approximately a 100fold lower frequency on male strains. However, *tau* (Hakura et al., 1964) is different in that it is a temperate phage which gives small plaques on F^- strains and does not plaque at all on male strains. Furthermore, some of the Tra^- mutants of F42 (Achtman et al., 1971, 1972) and of F8 (Ohtsubo et al., 1970) have coincidentally acquired sensitivity to *tau* but not to Φ_{II} or T7. Presumably the F42 mutants carry at least two point mutations, one mapping in a *tra* cistron and the other mapping in a female-specific phage restriction cistron since most mutations in any one *tra* cistron do not affect female-specific phage sensitivity. (The F8 mutants had the properties of deletion mutants extending into the *tra* region.) However, the very existence of these mutants indicates that infection by *tau* is restricted, at least in part, by a mechanism different from that responsible for restriction of phages such as Φ_{II} and T7.

Recent studies may have elucidated some of the mechanisms involved in restriction of the T7/Φ_{II} group of phages. Earlier studies (Cuzin, 1965; Mäkelä et al., 1964; Watanabe and Okada, 1964) demonstrated that these phages adsorb normally to male cells (although the opposite was claimed for Φ_I by Dettori et al., 1961) and Cuzin (1965) reported that Φ_{II} kills male cells without producing plaques. This Φ_{II} killing of male cells has been confirmed by Linial and Malamy (1970) who have also shown that host macromolecular syntheses are inhibited in the male cells within a few minutes after infection.

Furthermore, the phage DNA becomes membrane-associated but seems to be prevented from replication. STUDIER and MAIZEL (1969) had found three sequential classes of proteins are synthesized upon T7 infection of F^- cells. In a further study, MORRISON and MALAMY (1971) have shown that the F-factor may block T7 infection by interfering with the translational control of phage development and resulting in the synthesis of only the first class of proteins. They postulated that F makes two separate inhibitors of T7 translational control, one which prevents synthesis of the second group of proteins once the first group has been made and another which prevents synthesis of the third group of proteins once the second has been made. They have assigned the cistronic designations *pifA* and *pifB* to the theoretical cistrons responsible for these two inhibitors. In apparent agreement with this postulate, they have isolated F mutants (MORRISON and MALAMY, 1971) which allow the second group of proteins to be synthesized but not the third, and double mutants which allow both groups to be synthesized. The former mutants are still somewhat female-specific phage-resistant with intermediate plaque size and intermediate plating efficiency while the double mutants give the same plating efficiency as F^- strains. No genetic analyses of these mutants have yet been published and thus the conclusions about the number of cistrons involved must be considered premature. As there is still controversy on whether T7 is normally under a translational control system, these conclusions on the mechanism of female-specific phage restriction must also be regarded as unproven.

XI. Still Other Cistrons

So far I have described the existence of transfer, surface exclusion, replication/segregation, incompatibility, and female-specific phage restriction cistrons. It is not yet absolutely clear that these are all totally independent. For example, *traS*, the postulated surface exclusion cistron, may also be intimately involved in transfer. Certainly *traJ* is involved in both transfer and surface exclusion. Similarly, to my knowledge, nobody has yet properly tested the temperature-sensitive replication/segregation mutants for incompatibility or female-specific phage restriction. It is obvious that much work remains to be done before a proper genetic analysis of all these cistrons has been accomplished. However, to add to the confusion, I would like to point out the possible existence of still more types of cistrons on F.

F factors are specifically curable by treatment with acridine orange (HIROTA, 1960) and it has been shown that under optimal conditions acridine orange exerts an absolute block on sex factor replication/segregation (HOHN and KORN, 1969). Although it has generally been assumed that this acridine orange sensitivity simply reflects a peculiar attribute of the F replication/segregation system, it is not yet excluded that some other mechanism independent of the normal replication cistrons is involved for which "acridine orange sensitive cistrons" are necessary.

It is also possible that F carries cistrons coding for recombination enzymes. Chromosomal mobilization by an F prime strain is greatly reduced in a *recA*⁻ host (CLOWES and MOODY, 1966; CURTISS, CLARK, cited in WILLETTS and BRODA, 1969). Thus F does not encode a cistron which can replace the chromosomal *recA*. However, WILKINS (1969) has found that $RecB^-$ or $RecC^-$ strains newly infected with an F prime element can show up to 50% of the chromosomal mobilization of a Rec^+ strain. He concluded that this implies that the *recB* and *recC* proteins are not needed for chromosomal mobilization. It seems equally likely that F can substitute its own encoded proteins for the *recB* or *recC* gene products. Furthermore, BRODA and MEACOCK (1971) have shown that the formation of Hfr strains by integration at a non-homologous site proceeds as efficiently in $RecA^-$ as in $RecA^+$ strains. WILLETTS (1971) has demonstrated the occurrence of recombination between F42 and R100-1 in a $RecA^-$ strain. However, this form of evidence cannot be used in support of F encoded recombination enzymes and before such hypotheses can be considered proven, mutants of the sex factors must be isolated which no longer perform these recombination functions. This has not yet been accomplished to my knowledge and alternative explanations are still available for all the examples mustered above, such as host-encoded recombination enzymes different from those encoded by the classical *recA*, *recB*, *recC* cistrons (see review by CLARK, 1971) or recombination enzymes coded perhaps by undiscovered prophages present in the same cell[6]. Thus the question of whether sex factors encode recombination enzymes must still be considered an open one.

XII. Relationship between Different Sex Factors

This topic has been excellently reviewed previously (MEYNELL et al., 1968) and I will only attempt to bring the information up to date. Early attempts at classification of sex factors were based on whether their transfer repressor affected the F factor or not. Those that repressed F-directed transfer were termed fi^+ (WATANABE et al., 1964) or i^+ (EGAWA and HIROTA, 1962) while those not repressing F-directed transfer were termed fi^- or i^-, respectively. This divalent system has the obvious disadvantage of lumping together sex factors which may be totally unrelated except in their inability to repress F transfer as well as those which are simple mutational variants of sex factors which were previously fi^+ or i^+. A much better system has been proposed by LAWN et al. (1967), who found that most sex factors tested could be classified as being F-like or I-like on the basis of whether the sex pili they produced resembled those of F or of Col I with respect to the male-specific phage sensitivity conferred. This system has the advantage of using positive pro-

6 I am indebted to MICHAEL YARMOLINKY for supplying this possible alternative explanation.

perties for identification (production of a specific pilus type) and of being able to accommodate new sex factor groupings. Thus a third type of male-specific bacteriophage, Ike, has recently been described (KHATOON et al., 1972) which defines a third group of sex factors on the basis of sex piliation. This pilus-based classification system also has the advantage that most F-like sex factors are fi^+ or i^+ while most I-like sex factors are fi^- or i^- (MEYNELL and DATTA, 1965, 1966) and the classification thus reflects differences between transfer systems. However, I-like resistance factors *have* been described which repress transfer by F (GRINDLEY and ANDERSON, 1971) and the F-like sex factors, F, ColV2, and ColV3 do not repress their own transfer systems or those of other sex factors. Finally the F-like sex factor ColB2 represses the transfer system of F but not that of an F-like R factor (HAUSMANN and CLOWES, 1971). Thus, F-like and I-like should be reserved for reference only to the male-specific phage sensitivity of the sex pili and cannot be taken to automatically imply anything else.

A different form of sex factor classification comes from incompatibility studies (NOVICK, 1969). On this basis, as mentioned above (p. 89), F, ColV2, and ColV3 form one incompatibility group with some hierarchical preferences. It is also believed that all F-like R factors which are fi^+ form a second incompatibility group although the evidence is still scanty (MITSUHASHI et al., 1962; WATANABE et al., 1964). I-like R factors also probably fall into one incompatibility group but at least three other incompatibility groups exist among fi^- R factors (DATTA and HEDGES, 1971). One of these, called N, corresponds to that group producing sex pili sensitive to the new phage Ike (see above) (*op. cit.*; KHATOON et al., 1972). The male-specific phage sensitivity of members of the two other incompatibility groups is not yet clear. The existence of at least three incompatibility groups among fi^- sex factors may also be inferred from earlier results (BOUANCHAUD and CHABBERT, 1969).

In summary, sex factors have been assigned to groups on at least three principles: whether or not they repress the F transfer system, what sort of sex pili they make, and incompatibility groupings. The latter two seem the most meaningful and so far sex factors have been assigned to three pilus groups and six incompatibility groups. Two of these incompatibility groups, the F group and the F-like R factor group, belong to the same sex pilus group since they specify similar sex pili which allow infection by the same male-specific phages. The two other incompatibility groups tested specify different sex pili. All of these, except the F incompatibility group, encode transfer repressors and those of the F-like sex factors repress F-encoded transfer as well.

It should be noted, however, that although the sex pili specified by F-like R-factors allow infection by F-specific phages, they may allow a lessened efficiency of plating of these phages of up to two orders of magnitude (NISHIMURA et al., 1967). Furthermore, the sex pili encoded by F-like R-factors differ antigenically from those encoded by F (LAWN et al., 1971) and have a different specific density from F-pili (BEARD et al., 1972). Despite this, the

pili subunits are similar enough that cells carrying both F and an F-like R-factor produce phenotypically hybrid pili, carrying the antigenic specificity of both F- and R-pili (LAWN et al., 1971) and with a specific density intermediate between the two (BEARD et al., 1972).

XIII. Transfer Regulation

As mentioned above, many sex factors transfer inefficiently because of negative control exerted *via* a transfer repressor. In (F, R)$^+$ heterozygotes where the R-factor is of the *fi*$^+$, F-like type, the production of F-pili (NISHIMURA et al., 1967) and of surface exclusion (WILLETTS and FINNEGAN, 1970) are repressed but F-specific replication, incompatibility, and female-specific phage-restriction are unaffected (WILLETTS and FINNEGAN, 1970; MORRISON and MALAMY, 1970).

A little is known about the genetics of transfer repression. EGAWA and HIROTA (1962) isolated a mutant of R100, R100-1, which does not repress its own transfer system or that of F (NISHIMURA et al., 1967). It also does not repress surface exclusion (WILLETTS and FINNEGAN, 1970). A general method for isolating de-repressed mutant sex factors has since been published (EDWARDS and MEYNELL, 1968) and a large number of such mutants have been described (*op. cit.*; MEYNELL and DATTA, 1967; OHKI and OZEKI, 1968; HOAR, 1970). These have been shown to fall into two groups on the basis that some mutants are resistant to transfer repression while others do not make the transfer repressor (MEYNELL and COOKE, 1969; FRYDMAN and MEYNELL, 1969; HOAR, 1970). In one case, this was shown by using repression of the F transfer system as an assay for the transfer repressor (MEYNELL and COOKE, 1969) and repression by a ColB factor, which is compatible with F-like R-factors, as an assay for repressor sensitivity. In the second case (HOAR, 1970) advantage was taken of the somewhat relaxed incompatibility between R-factors: clones carrying two R-factors were maintained under constant selection for the presence of both. Recombination between the R-factors was prevented by using a RecA$^-$ host. The mutants were tested in such "hetero-R clones" for sensitivity to repression by a wild-type R-factor and were shown to fall into two groups: repressor-sensitive and repressor-insensitive. Two of the repressor-insensitive mutants were shown by complementation tests to be able to repress the transfer system of other R factors and thus to still be producing a transfer repressor. These results have been interpreted as supporting the model presented by NISHIMURA et al. (1967) that transfer repression is similar to the repression of the *lac* operon. Thus the transfer repressor-insensitive mutants have been referred to as o^c and the mutants not synthesizing a transfer repressor as i^-.

A. F-Factor Mutants

Despite the limited genetic evidence available, it is already clear that the above model will not suffice. Mutants of F have been isolated which are

insensitive to transfer repression (FRYDMAN et al., 1970; FINNEGAN and WILLETTS, 1971). Genetic analysis of four of these mutants showed that they fell into two distinct classes (FINNEGAN and WILLETTS, 1971) as based upon their properties in heterozygous cells transiently carrying R100, F57 and a repressor-insensitive mutant of F42. Two of them were repressed under these conditions and two were not. The former two are only repressed in the presence of both R100 and F57 and not in the presence of R100 alone. This implies that F57 (and presumably all other F-elements) synthesize a diffusible protein which is specific for F DNA and which interacts with the transfer repressor made by R100 to form a functional transfer repressor that only then can act on F. FINNEGAN and WILLETTS (1971) have assigned the cistron designation *traP* to the cistron on F coding for this F-specific protein. Presumably R100 also has a *traP* cistron and synthesizes a P-protein. However the P-protein synthesized by R would be specific for R DNA and could not act on F DNA. FINNEGAN and WILLETTS (1971) have also found that the onset of repression after entry of a repressible F into an $(R100)^{+}$ cell takes a very long time. They have, therefore, suggested that either the synthesis of P-protein by F is slow or the interaction of P-protein with repressor is slow.

The other two F-mutants analyzed by FINNEGAN and WILLETTS (1971) were not repressible even in the presence of F57 and R100 and thus carry cis-dominant mutations which may resemble o^{c} mutations in the *lac* system. FINNEGAN and WILLETTS (1971) have therefore concluded that this cis-dominant class carry mutations in still another cistron, *traO*. Since no genetic tests between the dominant and the recessive mutants were performed, these two cistronic designation of *traO* and *traP* must be considered suspect and premature. It is quite possible to have dominant and recessive mutations in the same cistron and it is not even excluded that these mutations lie in one of the previously-defined *tra* cistrons. However, these results do make it clear that the *lac* operon is not an adequate model for transfer repression.

How does the transfer repressor exert its effect? FINNEGAN and WILLETTS (1971) have suggested that since the effects of transfer repression on transfer and on surface exclusion are mimicked by $traJ^{-}$ mutations, the transfer repressor acts by preventing the *traJ*-encoded J-protein from performing its normal function. Thus it may either inhibit synthesis of J-protein by acting at the transcriptional or translational level, inhibit the J-protein directly, or interact with the J-protein site of action. This only brings up the further question of what J-protein does.

B. J-Independent Mutants

As mentioned on p. 104, $traJ^{-}$ mutants have the same properties as $traI^{-}$ mutants (plasmid specificity), $traA^{-}$ mutants (pilus specificity) and the postulated $traS^{-}$ mutants (surface exclusion). Thus, $traJ^{-}$ mutants are pleiotropic negative mutants. BRINTON (1971) has suggested that the J-protein is the pilus-structural protein, thus adding even more tasks onto an already

over-burdened molecule. An alternative possibility is that J-protein is needed for synthesis or function of the other transfer proteins to occur. For example, J-protein might be a positive control factor regulating one or more *tra* operons. This idea of an operon structure for the *tra* cistrons is supported by the fact that some amber and frameshift *tra*⁻ mutations give poor complementation with other mutations in different cistrons (*traB* with *traC* and *traF*, *traH* with *traG*) and that three other nonsense mutants (whose *tra*⁻ mutations have not been genetically mapped) seem to give no complementation or only poor complementation with mutants mutated in *traK*, *traB*, *traC*, *traF*, *traH* and *traG* (ACHTMAN et al., 1972; WILLETTS and ACHTMAN, 1972). Furthermore, some of these mutants seem to be partially Sex⁻ as if they were polar on *traS* (WILLETTS, personal communication). Reference to Fig. 3 shows that these cistrons are arranged in a block and might be included in an operon. It is not, however, clear whether *traA*, *traS* (postulated), and *traI* are all in the same operon or not, as no effects attributable to polarity have been seen to the left of *traK* or to the right of *traG*. This could be only coincidence or alternatively could indicate that *traA*, *traS* (postulated), and *traI* lie in two or more different operons.

The nature of action of the J-protein has recently been slightly clarified by my isolation of partial Tra⁺ revertants of an amber *traJ*⁻ mutant (Genet. Res., in press). The partial revertants still carry the original amber mutation which is still suppressible and therefore these mutants carry J-independent mutations presumably located outside the *traJ* cistron. The mutants have not yet been analyzed genetically. However, their very existence is strong evidence against the possibility that the J-protein is the pilus-structural protein or any other structural protein and is in complete accord with a positive control role. The mutants have the very interesting property that they transfer at the same level in the presence or absence of R100 transfer repressor, which fits with the idea that the transfer repressor acts on J-protein synthesis or function and therefore does not affect J-independent transfer. It should be mentioned that the normal site of action of the J-protein is probably not altered since the mutants are still sensitive to restoration of J-protein by amber suppression. Of the twelve J-independent mutants isolated, one has regained *traA* activity (pilus specificity) and two have regained both *traA* and *traS* (surface exclusion) activity.

C. Other Forms of Regulation

Even if the J-promoted positive control model were true, it only begins to answer the question of what normally controls transfer. BRINTON (1965) and NOVOTNY et al. (1969) have shown that new F-pili are synthesized within five minutes after shearing of pre-existing F-pili from the cell. On this basis, they have suggested that F-pili are synthesized continuously and then fall off every five minutes. The result is that cells normally only have approximately one F-pilus per cell while free F-pili accumulate in the medium. This

seems quantitatively unlikely since there are usually no more F-pili free in the medium than are attached to cells (BRINTON et al., 1964; LAWN, 1966; NISHIMURA et al., 1967). If F-pili were being synthesized and falling off every few minutes, there should always be a great excess of free F-pili over cell bound F-pili. Instead it seems likelier that the cell is sensitive to loss of its F-pilus and immediately synthesizes a new F-pilus. The loss might be due to a positive act on the part of the cell (see NOVOTNY and LAVIN, 1971) or might be due to natural causes but the implication of this model is that F-pili do not have a predetermined lifetime and that production of new F-pili is normally stimulated by loss of old ones. This model thus implies that there is some form of regulation involved in the synthesis of F-pili.

Similarly, it seems unlikely that cells initiate the DNA transfer process itself until contact with an appropriate recipient cell is made. Again this constitutes a form of regulation. It is conceivable that bursts of J-protein are responsible for F-pilus production and that the bursts are somehow stimulated by the cell's loss of F-pili. But if so, a different regulation mechanism must be involved during the transfer act itself and it seems likely that there is some form of repression of either synthesis or function of transfer enzymes except during the transfer act. These considerations make it clear, that although R-factor transfer repression and its site of action are beginning to be clarified, the whole topic of transfer regulation is still obscure.

Acknowledgment. The author is pleased to have the opportunity to thank his many colleagues who sent unpublished manuscripts and especially pleased to be able to acknowledge helpful criticisms by Dr. FINNEGAN, W. D. RUPP, and W. K. MAAS. The author was supported during the preparation of this review by a Canadian Medical Research Council Fellowship in the laboratory of U. HENNING to both of whom he is very grateful. No serious attempt has been made to review the literature appearing after December, 1971 although a few manuscripts appearing after that date are discussed.

References

ACHTMAN, M.: A genetic study of the F factor. Ph. D. thesis. Univ. of Calif., Berkeley University Microfilms Order No 70-6049. Ann Arbor, Michigan 1969.

ACHTMAN, M.: A semi-automatic technique for conducting many bacterial matings concurrently. Genet. Res. (Camb.) **17**, 261–266 (1971).

ACHTMAN, M., WILLETTS, N., CLARK, A. J.: Beginning a genetic analysis of conjugational transfer determined by the F factor in *E. coli* by isolation and characterization of transfer-deficient mutants. J. Bact. **106**, 529–538 (1971).

ACHTMAN, M., WILLETTS, N., CLARK, A. J.: A conjugational complementation analysis of transfer-deficient mutants of F*lac* in *E. coli*. J. Bact. **110**, 831—842 (1972).

ADELBERG, E. A., BURNS, S. N.: Genetic variation in the sex factor of *E. coli*. J. Bact. **79**, 321–330 (1960).

BAZARAL, M., HELINSKI, D. R.: Circular DNA forms of colicinogenic factors E1, E2, and E3 from *E. coli*. J. molec. Biol. **36**, 185–194 (1968a).

BAZARAL, M., HELINSKI, D. R.: Characterization of multiple circular DNA forms of colicinogenic factor E1 from *P. mirabilis*. Biochemistry **7**, 3513–3519 (1968b).

BAZARAL, M., HELINSKI, D. R.: Replication of a bacterial plasmid and an episome in *E. coli*. Biochemistry **9**, 399–406 (1970).

BEARD, J. P., HOWE, T. G. B., RICHMOND, M. H.: Centrifugation properties of sex pili. J. molec. Biol. **66**, 311—313 (1972).

BONHOEFFER, F.: DNA transfer and DNA synthesis during bacterial conjugation. Z. Vererbungsl. **98**, 141–149 (1966).

BONHOEFFER, F., VIELMETTER, W.: Conjugational DNA transfer in *E. coli*. Cold Spr. Harb. Symp. quant. Biol. **33**, 623–627 (1968).

BOUANCHAUD, D. H., CHABBERT, Y. A.: Stable coexistance of three resistance factors (fi^-) in *S. panama* and *E. coli* K12. J. gen. Microbiol. **58**, 107–113 (1969).

BOYER, H. W.: DNA restriction and modification mechanisms in bacteria. Ann. Rev. Microbiol. **25**, 153—176 (1971).

BRINTON, C. C., JR.: The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in Gram negative bacteria. Trans. N.Y. Acad. Sci. **27**, 1003–1054 (1965).

BRINTON, C. C., JR.: The properties of sex pili, the viral nature of "conjugal" genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. Critical Rev. Microbiol. **1**, 105–160 (1971).

BRINTON, C. C., JR., GEMSKI, P., JR., CARNAHAN, J.: A new type of bacterial pilus genetically controlled by the fertility factor of *E. coli* K12 and its role in chromosome transfer. Proc. nat. Acad. Sci. (Wash.) **52**, 776–783 (1964).

BRODA, P.: The formation of Hfr strains in *E. coli* K12. Genet. Res. (Camb.) **9**, 35–47 (1967).

BRODA, P., BECKWITH, J. R., SCAIFE, J.: The characterization of a new type of F prime factor in *E. coli* K12. Genet. Res. (Camb.) **5**, 489–494 (1964).

BRODA, P., MEACOCK, P.: Isolation and characterization of Hfr strains from a recombination-deficient strain of *E. coli*. Molec. gen. Genet. **113**, 166–173 (1971).

BRODA, P., MEACOCK, P., ACHTMAN, M.: Early transfer of genes determining the transfer function by Hfr strains in *E. coli* K12. Molec. gen. Genet. **116**, 336—347 (1972).

CAMPBELL, A. M.: Episomes. Advanc. Genet. **11**, 101–146 (1962).

CARO, L. G., SCHNÖS, M.: The attachment of the male-specific bacteriophage f1 to sensitive strains of *E. coli*. Proc. nat. Acad. Sci. (Wash.) **56**, 126–132 (1966).

CLARK, A. J.: Genetic analysis of a "Double Male" strain of *E. coli* K12. Genetics **48**, 115–120 (1963).

CLARK, A. J.: Toward a metabolic interpretation of genetic recombination of *E. coli* and its phages. Ann. Rev. Microbiol. **25**, 437–464 (1971).

CLOWES, R. C., MOODY, E. E. M.: Chromosomal transfer from "recombination-deficient" strains of *E. coli* K12. Genetics **53**, 717–726 (1966).

COHEN, A., FISHER, W. D., CURTISS, R., III, ADLER, H. I.: DNA isolated from *E. coli* minicells mated with F^+ cells. Proc. nat. Acad. Sci. (Wash.) **61**, 61–68 (1968a).

COHEN, A., FISHER, W. D., CURTISS, R., III, ADLER, H. I.: The properties of DNA transferred to minicells during conjugation. Cold Spr. Harb. Symp. quant. Biol. **33**, 635–641 (1968b).

COHEN, S. N., MILLER, C. A.: Non-chromosomal antibiotic resistance in bacteria. II. Molecular nature of R-factors isolated from *P. mirabilis* and *E. coli*. J. molec. Biol. **50**, 671–687 (1970a).

COHEN, S. N., MILLER, C. A.: Non-chromosomal antibiotic resistance in bacteria. III. Isolation of the discrete transfer unit of the R-factor R1. Proc. nat. Acad. Sci. (Wash.) **67**, 510–516 (1970b).

COOPER, S., HELMSTETTER, C. E.: Chromosome replication and the division cycle of *E. coli* B/r. J. molec. Biol. **31**, 519—540 (1968).

CRAWFORD, E., GESTELAND, R.: The adsorption of bacteriophage R17. Virology **22**, 165–167 (1964).

CURTISS, R., III: Bacterial conjugation. Ann. Rev. Microbiol. **23**, 69–136 (1969).

CURTISS, R., III, CARO, L. G., ALLISON, D. P., STALLIONS, D. R.: Early stages of conjugation in *E. coli*. J. Bact. **100**, 1091–1104 (1969).

CURTISS, R., III, CHARAMELLA, L. J., STALLIONS, D. R., MAYS, J. A.: Parental functions during conjugation in *E. coli* K12. Bact. Rev. **32**, 320–348 (1968).

CUZIN, F.: Un bactériophage spécifique du type sexuel F^- d' *E. coli* K12. C. R. Acad. Sci. (Paris) **260**, 6482–6485 (1965).

CUZIN, F., BUTTIN, G., JACOB, F.: On the mechanism of genetic transfer during conjugation of *E. coli*. J. cell. Physiol. **70**, Suppl. **1**, 77–88 (1967).

CUZIN, F., JACOB, F.: Analyse génétique fonctionelle de l'épisome sexuel d' *E. coli* K12. C. R. Acad. Sci. (Paris) **260**, 2087–2090 (1965a).

Cuzin, F., Jacob, F.: Existence chez *E. coli* d'une unité génétique de ségrégation formée de différents réplicons. C. R. Acad. Sci. (Paris) **260**, 5411–5414 (1965b).
Cuzin, F., Jacob, F.: Existence chez *E. coli* K12 d'une unité génétique de transmission formée de différents réplicons. Ann. Inst. Pasteur **112**, 529–545 (1967a).
Cuzin, F., Jacob, F.: Association stable de deux épisomes F différents dans un clone d' *E. coli*. Ann. Inst. Pasteur **113**, 145–155 (1967b).
Cuzin, F., Jacob, F.: Mutation de l'épisome F d' *E. coli* K12. I. Mutation défectives. Ann. Inst. Pasteur **112**, 1–9 (1967c).
Cuzin, F., Jacob, F.: Mutations de l'épisome d'*E. coli* K12. II. Mutants a réplication thermosensible. Ann. Inst. Pasteur **112**, 397–418 (1967d).
Datta, N., Hedges, R. W.: Compatibility groups among *fi*⁻ R factors. Nature (Lond.) **234**, 222–223 (1971).
Demerec, M., Adelberg, E. A., Clark, A. J., Hartman, P. E.: A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**, 61–76 (1966).
Dettori, R., Maccacaro, A., Piccinin, G. L.: Sex-specific bacteriophages of *E. coli* K12. G. Microbiol. **9**, 141–150 (1961).
Dubnau, E., Maas, W. K.: Inhibition of replication of an F'lac episome in Hfr cells of *E. coli*. J. Bact. **95**, 531–539 (1968).
Echols, H.: Properties of F' strains of *E. coli* superinfected with F-lactose and F-galactose episomes. J. Bact. **85**, 262–268 (1963).
Edwards, S., Meynell, G. G.: General method for isolating derepressed bacterial sex factors. Nature (Lond.) **219**, 869–870 (1968).
Egawa, R., Hirota, Y.: Inhibition of fertility by multiple drug-resistance factor in *E. coli* K12. Japan. J. Genet. **37**, 66–69 (1962).
Falkow, S., Citarella, R. V.: Molecular homology of F-merogenote DNA. J. molec. Biol. **12**, 138–151 (1965).
Falkow, S., Tompkins, L. S., Silver, R. P., Guerry, P., Leblanc, D. J.: The replication of R-factor DNA in *E. coli* K12 following conjugation. Ann. N.Y. Acad. Sci. **182**, 153–171 (1971).
Finnegan, D. J., Willetts, N. S.: Two classes of F*lac* mutants insensitive to transfer inhibition by an F-like R factor. Molec. gen. Genet. **111**, 256–264 (1971).
Frame, R., Bishop, J. O.: The number of sex-factors per chromosome in *E. coli*. Biochem. J. **121**, 93–103 (1971).
Fredericq, P.: A note on the classification of colicines. Zbl. Bakt., 1. Abt. Orig. **196**, 140–142 (1965).
Freifelder, D.: Studies on *E. coli* sex factors. III. Covalently closed F'Lac DNA molecules. J. molec. Biol. **34**, 31–38 (1968a).
Freifelder, D.: Studies on *E. coli* sex factors. IV. Molecular weights of the DNA of several F' elements. J. molec. Biol. **35**, 95–102 (1968b).
Freifelder, D.: Studies with *E. coli* sex factors. Cold Spr. Harb. Symp. quant. Biol. **33**, 425–434 (1968c).
Freifelder, D.: Molecular weights of coliphages and coliphage DNA. IV. Molecular weights of DNA from bacteriophages T4, T5 and T7 and the general problem of determination of M. J. molec. Biol. **54**, 567–577 (1970).
Freifelder, D.: Electron microscopic study of the ethidium bromide-DNA complex. J. molec. Biol. **60**, 401–403 (1971).
Freifelder, D., Folkmanis, A., Kirschner, I.: Studies on *E. coli* sex factors: Evidence that covalent circles exist within cells and the general problem of isolation of covalent circles. J. Bact. **105**, 722–727 (1971).
Frydman, A., Cooke, M., Meynell, E., Meynell, G. G.: Repressor-insensitive mutants of the F sex factor. J. molec. Biol. **48**, 177–179 (1970).
Frydman, A., Meynell, E.: Interactions between de-repressed F-like R factors and wild type Colicin B factors: superinfection immunity and repressor susceptibility. Genet. Res. (Camb.) **14**, 315–332 (1969).
Fulton, C.: Continous chromosome transfer in *E. coli*. Genetics **52**, 55–74 (1965).
Gilbert, W., Dressler, D.: DNA replication: The rolling circle model. Cold Spr. Harb. Symp. quant. Biol. **33**, 473–484 (1968).
Grindley, J. N., Anderson, E. S.: I-like resistance factors with the *fi*⁺ character. Genet. Res. (Camb.) **17**, 267–271 (1971).

GROSS, J. D., CARO, L.: Genetic transfer in bacterial mating. Science **150**, 1679–1686 (1965).
GROSS, J. D., CARO, L.: DNA transfer in bacterial conjugation. J. molec. Biol. **16**, 269–284 (1966).
HAAPALA, D. K., FALKOW, S.: Physical studies of the drug-resistance transfer factor in *Proteus*. J. Bact. **106**, 294–295 (1971).
HAKURA, A., OTSUJI, N., HIROTA, Y.: A temperature phage specific for female strains of *E. coli* K12. J. gen. Microbiol. **35**, 69–73 (1964).
HAUSMANN, C., CLOWES, R. C.: ColB2-K77, a fertility-repressed F-like sex factor. J. Bact. **107**, 900–906 (1971).
HELINSKI, D. R., CLEWELL, D. B.: Circular DNA. Ann. Rev. Biochem. **40**, 899–942 (1971).
HICKSON, F. T., ROTH, T. F., HELINSKI, D. R.: Circular DNA forms of a bacterial sex factor. Proc. nat. Acad. Sci. (Wash.) **58**, 1731–1738 (1967).
HIROTA, Y.: The effect of acridine dyes on mating type factors in *E. coli*. Proc. nat. Acad. Sci. (Wash.) **46**, 57–64 (1960).
HIROTA, Y., SNEATH, P. H. A.: F' and F mediated transduction in *E. coli* K12. Jap. J. Genet. **36**, 307–318 (1961).
HOAR, D. I.: Fertility regulation in F-like resistance transfer factors. J. Bact. **101**, 916–920 (1970).
HOHN, B., KORN, D.: Cosegregation of a sex factor with the *E. coli* chromosome during curing by acridine orange. J. molec. Biol. **45**, 385–395 (1969).
IHLER, G., RUPP, W. D.: Strand-specific transfer of donor DNA during conjugation in *E. coli*. Proc. nat. Acad. Sci. (Wash.) **63**, 138–143 (1969).
IPPEN, K., SHAPIRO, J. A., BECKWITH, J. R.: Transposition of the *lac* region to the *gal* region of the *E. coli* chromosome: Isolation of *λlac* transducing bacteriophages. J. Bact. **108**, 5–9 (1971).
IPPEN, K. A., VALENTINE, R. C.: The sex hair of *E. coli* as sensory fiber, conjugation tube, or mating arm? Biochem. biophys. Res. Commun. **27**, 674–680 (1967).
IPPEN-IHLER, K., ACHTMAN, M., WILLETTS, N.: A deletion map of the *E. coli* K12 sex factor F: the order of eleven transfer cistrons. J. Bact. **110**, 857—863 (1972).
JACOB, F., ADELBERG, E. A.: Transfert de caractères génétiques par incorporation au facteur sexuel d'*E. coli*. C. R. Acad. Sci. (Paris) **249**, 189–191 (1959).
JACOB, F., BRENNER, S.: Sur la regulation de la synthese du DNA chez les bacteries: l'Hypothese du replicon. C. R. Acad. Sci. (Paris) **256**, 298–300 (1963).
JACOB, F., BRENNER, S., CUZIN, F.: On the regulation of DNA replication in bacteria. Cold Spr. Harb. Symp. quant. Biol. **28**, 329–343 (1963).
JACOB, F., WOLLMAN, E. L.: Sexuality and the genetics of bacteria. New York: Academic Press 1961.
KAHN, P., HELINSKI, D. R.: Relationship between colicinogenic factors E1 and V and an F factor in *E. coli*. J. Bact. **88**, 1573–1579 (1964).
KASAMATSU, H., ROWND, R.: Replication of R-factors in *P. mirabilis*: replication under relaxed control. J. molec. Biol. **51**, 473–489 (1970).
KHATOON, H., IYER, R. V., IYER, V. N.: A new filamentous bacteriophage with sex-factor specificity. Submitted to Virology (1972).
KLINE, B. C., HELINSKI, D. R.: F1 sex factor of *E. coli*: Size and purification in the form of a strand specific relaxation complex of supercoiled DNA and protein. Biochemistry **10**, 4975–4980 (1971).
KNOLLE, P.: Evidence for the identity of the mating-specific site of male cells of *E. coli* with the receptor site of an RNA phage. Biochem. biophys. Res. Commun. **27**, 81–87 (1967).
KOZAK, M., NATHANS, D.: Fate of maturation protein during infection by coliphage MS2. Nature (Lond.) New Biol. **234**, 209–211 (1971).
KRAHN, P. M., O'CALLAGHAN, R. J., PARANCHYCH, W.: Stages in phage R17 infection. VI. Injection of A protein and RNA into the host cell. Virology, **47**, 628—637 (1972).
KRUEGER, R. G.: Serological relatedness of the ribonucleic acid-containing coliphages. J. Virol. **4**, 567–573 (1969).
LANG, D.: Molecular weights of coliphages and coliphage DNA. III. Contour length and molecular weight of DNA from bacteriophages T4, T5 and T7, and from Bovine Papilloma Virus. J. molec. Biol. **54**, 557–565 (1970).

LAWN, A. M.: Morphological features of the pili associated with *E. coli* K12 carrying R factors or the F factor. J. gen. Microbiol. **45**, 377–383 (1966).
LAWN, A. M., MEYNELL, E., COOKE, M.: Mixed infections with bacterial sex factors: Sex pili of pure and mixed phenotype. Ann. Inst. Pasteur **120**, 3–8 (1971).
LAWN, A. M., MEYNELL, E., MEYNELL, G. G., DATTA, N.: Sex pili and the classification of sex factors in the *Enterobacteriaceae*. Nature (Lond.) **216**, 343–346 (1967).
LEDERBERG, J., CAVALLI, L. L., LEDERBERG, E. M.: Sex compatibility in *E. coli*. Genetics **37**, 720–730 (1952).
LINIAL, M., MALAMY, M. H.: Studies with bacteriophage ϕII. Events following infection of male and female derivatives of *E. coli* K12. J. Virol. **5**, 72–78 (1970).
MAAS, R.: Exclusion of an Flac episome by an Hfr gene. Proc. nat. Acad. Sci. (Wash.) **50**, 1051–1055 (1963).
MAAS, R., MAAS, W. K.: Introduction of a gene from *E. coli* B into Hfr and F^- strains of *E. coli* K12. Proc. nat. Acad. Sci. (Wash.) **48**, 1887–1893 (1962).
MAAS, W. K., GOLDSCHMIDT, A. D.: A mutant of *E. coli* permitting replication of two F factors. Proc. nat. Acad. Sci. (Wash.) **62**, 873–880 (1969).
MACFARREN, A. C., CLOWES, R. C.: A comparative study of two F-like colicin factors, ColV2 and ColV3, in *E. coli* K12. J. Bact. **94**, 365–377 (1967).
MÄKELÄ, O., MÄKELÄ, P. H., SOIKKELI, S.: Sex-specificity of the bacteriophage T7. Ann. Med. exp. Fenn. **42**, 188–195 (1964).
MARINUS, M. G., ADELBERG, E. A.: Vegetative replication and transfer replication of deoxyribonucleic acid in temperature-sensitive mutants of *E. coli* K12. J. Bact. **104**, 1266–1272 (1970).
MARVIN, D., HOHN, B.: Filamentous bacterial viruses. Bact. Rev. **33**, 172–209 (1969).
MATNEY, T. S., GOLDSCHMIDT, E. P., ERWIN, N. S., SCROGGS, R. A.: A preliminary map of genomic sites for F-attachment in *E. coli* K12. Biochem. biophys. Res. Commun. **17**, 278–281 (1964).
MATSUBARA, K.: Properties of sex factor and related episomes isolated from purified *E. coli* zygote cells. J. molec. Biol. **38**, 89–108 (1968).
MEYNELL, E., COOKE, M.: Repressor-minus and operator-constitutive de-repressed mutants of F-like R factors: their effect on chromosomal transfer by Hfr C. Genet. Res. (Camb.) **14**, 309–313 (1969).
MEYNELL, E., DATTA, N.: Functional homology of the sex-factor and resistance transfer factors. Nature (Lond.) **207**, 884–885 (1965).
MEYNELL, E., DATTA, N.: The relation of resistance transfer factors to the F-factor (sex factor) of *E. coli* K12. Genet. Res. (Camb.) **7**, 134–140 (1966).
MEYNELL, E., DATTA, N.: Mutant drug resistant factors of high transmissibility. Nature (Lond.) **214**, 885–887 (1967).
MEYNELL, E., MEYNELL, G. G., DATTA, N.: Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. Bact. Rev. **32**, 55–83 (1968).
MEYNELL, G. G.: Exclusion, superinfection immunity and abortive recombinants in $I^+ \times I^+$ bacterial crosses. Genet. Res. (Camb.) **13**, 113–115 (1969).
MEYNELL, G. G., AUFREITER, E.: Selection of mutant bacterial sex factors determining altered sex pili. J. gen. Microbiol. **59**, 429–430 (1969).
MEYNELL, G. G., LAWN, A. M.: Sex pili and common pili in the conjugational transfer of colicin factor Ib by *S. typhimurium*. Genet. Res. (Camb.) **9**, 359–367 (1967).
MITSUHASHI, S., HARADA, K., HASHIMOTO, H., KAMEDA, M., SUZUKI, M.: Combination of two types of transmissible drug-resistance factors in a host bacterium. J. Bact. **84**, 9–16 (1962).
MIYAKE, T., SHIBA, T., SAKURAI, T., WATANABE, I.: Isolation and properties of two new RNA phages SP and FI. Japan. J. Microbiol. **13**, 375–382 (1969).
MONNER, D. A., BOMAN, H. G.: Female strains of *E. coli* K12 as selective hosts for the isolation of female specific mutants of phage ϕII. Biochem. biophys. Res. Commun. **39**, 1017–1020 (1970).
MORRISON, T. G., MALAMY, M. H.: Comparisons of F factors and R factors: Existence of independent regulation groups in F factors. J. Bact. **103**, 81–88 (1970).
MORRISON, T. G., MALAMY, M. H.: T7 translational control mechanisms and their inhibition by F factors. Nature (Lond.) New Biol. **231**, 37–41 (1971).
MULCZYK, M., DUGUID, J. P.: Influence of the state of fimbriation on transmission of the colicinogenic factor ColI between strains of *Sh. flexneri*. J. gen. Microbiol. **45**, 459—477 (1966).

NISHIMURA, Y., ISHIBASHI, M., MEYNELL, E., HIROTA, Y.: Specific piliation directed by a fertility factor and a resistance factor of *E. coli*. J. gen. Microbiol. **49**, 89–98 (1967).
NISIOKA, T., MITANI, M., CLOWES, R. C.: Composite circular forms of R factor deoxyribonucleic acid molecules. J. Bact. **97**, 376–385 (1969).
NISIOKA, T., MITANI, M., CLOWES, R. C.: Molecular recombination between R-factor deoxyribonucleic acid molecules in *E. coli* host cells. J. Bact. **103**, 166–177 (1970).
NOMURA, M.: Colicins and related bacteriocins. Ann. Rev. Microbiol. **21**, 257–284 (1967).
NOVICK, R. P.: Extrachromosomal inheritance in bacteria. Bact. Rev. **33**, 210–263 (1969).
NOVICK, R. P., BRODSKY, R.: Studies on plasmid replication. I. Plasmid incompatibility and establishment in *S. aureus*. Submitted to J. molec. Biol. (1972).
NOVOTNY, C., CARNAHAN, J., BRINTON, C. C., JR.: Mechanical removal of F-pili, type I-pili, and flagella from Hfr and RTF donor cells and the kinetics of their reappearance. J. Bact. **98**, 1294–1306 (1969).
NOVOTNY, C., KNIGHT, W. S., BRINTON, C. C., JR.: Inhibition of bacterial conjugation by ribonucleic acid and deoxyribonucleic acid male-specific bacteriophages. J. Bact. **95**, 314–326 (1968).
NOVOTNY, C. P., LAVIN, K.: Some effects of temperature on the growth of F-pili. J. Bact. **107**, 671–682 (1971).
OHKI, M., OZEKI, H.: Isolation of conjugation-constitutive mutants of Colicin factor Ib. Molec. gen. Genet. **103**, 37–41 (1968).
OHKI, M., TOMIZAWA, J.: Asymmetric transfer of DNA strands in bacterial conjugation. Cold Spr. Harb. Symp. quant. Biol. **33**, 651–658 (1968).
OHTSUBO, E.: Transfer-defective mutants of sex factors in *E. coli*. II. Deletion mutants of an F-prime and deletion mapping of cistrons involved in genetic transfer. Genetics **64**, 189–197 (1970).
OHTSUBO, E., NISHIMURA, Y., HIROTA, Y.: Transfer-defective mutants of sex factors in *E. coli*. I. Defective mutants and complementation analysis. Genetics **64**, 173–188 (1970).
OU, J. T., ANDERSON, T. F.: Role of pili in bacterial conjugation. J. Bact. **102**, 648–654 (1970).
OVERBY, L. R., BARLOW, G. H., DOI, R. H., JACOB, M., SPIEGELMAN, S.: Comparison of two serologically distinct ribonucleic acid bacteriophages. I. Properties of the viral particles. J. Bact. **91**, 442–448 (1966a).
OVERY, L. R., BARLOW, G. H., DOI, R. H., JACOB, M., SPIEGELMAN, S.: Comparison of two serologically distinct ribonucleic acid bacteriophages. II. Properties of the nucleic acids and coat proteins. J. Bact. **92**, 739–745 (1966b).
PALCHOUDHURY, S. R., IYER, V. N.: Compatibility between two F′ factors in an *E. coli* strain bearing a chromosomal mutation affecting DNA synthesis. J. molec. Biol. **57**, 319–333 (1971).
PARANCHYCH, W., AINSWORTH, S. K., DICK, A. J., KRAHN, P. M.: Stages in phage R17 infection. V. Phage eclipse and the role of F-pili. Virology **45**, 615–628 (1971).
PARANCHYCH, W., KRAHN, P. M.: The fate of "A" protein during R17 phage infection. Fed. Proc. **30**, 1264 Abs (1971).
PRESS, R., GLANSDORFF, N., MINER, P., DEVRIES, J., KADNER, R., MAAS, W. K.: Isolation of transducing particles of ϕ80 bacteriophage that carry different regions of the *E. coli* genome. Proc. nat. Acad. Sci. (Wash.) **68**, 795–798 (1971).
PRITCHARD, R. H., BARTH, P. T., COLLINS, J.: Control of DNA synthesis in bacteria. Symp. Soc. gen. Microbiol. **19**, 263–297 (1969).
PTASHNE, M.: Replication and host modification of DNA transferred during bacterial mating. J. molec. Biol. **11**, 829–838 (1965).
RICHTER, A.: Attachment of wild-type F factor to a specific chromosomal region in a variant strain of *E. coli* K12: the phenomenon of episomic alteration. Genet. Res. (Camb.) **2**, 333–345 (1961).
ROWND, R.: Replication of a bacterial episome under relaxed control. J. molec. Biol. **44**, 387–402 (1969).
ROWND, R., KASAMATSU, H., MICKEL, S.: The molecular nature and replication of drug resistance factors of the *Enterobacteriaceae*. Ann. N.Y. Acad. Sci. **182**, 188–206 (1971)
ROWND, R., MICKEL, S.: Dissociation and reassociation of RTF and r-determinants of the R-factor NR1 in *Proteus mirabilis*. Nature (Lond.) New Biol. **234**, 40–43 (1971).
ROWND, R., NAKAYA, R., NAKAMURA, A.: Molecular nature of the drug-resistance factors of the *Enterobacteriaceae*. J. molec. Biol. **17**, 376–393 (1966).

RUPP, W. D., IHLER, G.: Strand selection during bacterial mating. Cold Spr. Harb. Symp. quant. Biol. **33**, 647–650 (1968).

SCAIFE, J.: Episomes. Ann. Rev. Microbiol. **21**, 601–638 (1967).

SCAIFE, J., GROSS, J. D.: Inhibition of multiplication of an F-lac factor in Hfr cells of *E. coli* K12. Biochem. biophys. Res. Commun. **7**, 403–407 (1962).

SCAIFE, J., PEKHOV, A. P.: Deletion of chromosomal markers in association with F prime factor formation in *E. coli* K12. Genet. Res. (Camb.) **5**, 495–498 (1964).

SHARP, P. A., HSU, M., OHTSUBO, E., DAVIDSON, N.: Electron microscope heteroduplex studies of sequence relations among plasmids of *E. coli*. I. Structure of F prime factors. J. molec. Biol., **71**, 471—497 (1972).

SHULL, F. W., FRALICK, J. A., STRATTON, L. P., FISHER, W. D.: Membrane association of conjugally transferred deoxyribonucleic acid in *E. coli* minicells. J. Bact. **106**, 626–633 (1971).

SILVER, R. P., FALKOW, S.: Specific labeling and physical characterization of R-factor deoxyribonucleic acid in *E. coli*. J. Bact. **104**, 331–339 (1970).

SILVERMAN, P. M., MOBACH, H. W., VALENTINE, R. C.: Sex hair (F-pili) mutants of *E. coli*. Biochem. biophys. Res. Commun. **27**, 412–416 (1967a).

SILVERMAN, P. M., ROSENTHAL, S., MOBACH, H., VALENTINE, R. C.: Two new classes of F-pili mutants of *E. coli* resistant to infection by the male specific bacteriophage f2. Virology **36**, 142–146 (1968).

SILVERMAN, P. M., ROSENTHAL, S., VALENTINE, R. C.: Mutant male strains with an altered nucleic acid pump. Biochem. biophys. Res. Commun. **27**, 668–673 (1967b).

SILVERMAN, P. M., VALENTINE, R. C.: The RNA injection step of bacteriophage f2 infection. J. gen. Virol. **4**, 111–124 (1969).

STADLER, J., ADELBERG, E. A.: Temperature dependence of sex-factor maintenance in *E. coli* K-12. J. Bact. **109**, 447–449 (1972).

STUDIER, F. W., MAIZEL, J. V., JR.: T7-directed protein synthesis. Virology **39**, 575–586 (1969).

TAYLOR, A. L.: Current linkage map of *E. coli*. Bact. Rev. **34**, 155–175 (1970).

VALENTINE, R. C., STRAND, M.: Complexes of F-pili and RNA bacteriophage. Science **148**, 511–513 (1965).

VAPNEK, D., LIPMAN, M. B., RUPP, W. D.: Physical properties and mechanism of transfer of R-factors in *E. coli*. J. Bact. **108**, 508–514 (1971).

VAPNEK, D., RUPP, W. D.: Assymetric segregation of the complementary sex-factor DNA strands during conjugation in *E. coli*. J. molec. Biol. **53**, 287–303 (1970).

VAPNEK, D., RUPP, W. D.: Identification of individual sex-factor DNA strands and their replication during conjugation in thermosensitive DNA mutants of *E. coli*. J. molec. Biol. **60**, 413–424 (1971).

VIELMETTER, W., BONHOEFFER, F., SCHÜTTE, A.: Genetic evidence for transfer of a single DNA strand during bacterial conjugation. J. molec. Biol. **37**, 81–86 (1968).

WALKER, E. M., PITTARD, J.: Temperature-sensitive conjugation-defective F factor in *E. coli*. J. Bact. **100**, 319–328 (1970).

WATANABE, I., MIYAKE, T., SAKURAI, T., SHIBA, T., OHNO, T.: Isolation and grouping of RNA phages. Proc. Japan Acad. **43**, 204–209 (1967a).

WATANABE, I., NISHIHARA, T., KANEKO, H., SAKURAI, T., OSAWA, S.: Group characteristics of RNA phages. Proc. Japan Acad. **43**, 210–213 (1967b).

WATANABE, T.: The origin of R factors. Ann. N. Y. Acad. Sci. **182**, 126–140 (1971).

WATANABE, T., NISHIDA, H., OGATA, C., ARAI, T., SATO, S.: Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. VII. Two types of naturally occurring R factors. J. Bact. **88**, 716–726 (1964).

WATANABE, T., OKADA, M.: New type of sex factor-specific bacteriophage of *E. coli*. J. Bact. **87**, 727–736 (1964).

WENDT, L., MOBACH, H. W.: Evidence for two states of F-pili. J. Bact. **97**, 640–646 (1969).

WENDT, L. W., IPPEN, K. A., VALENTINE, R.: General properties of F-pili. Biochem. biophys. Res. Commun. **23**, 375–380 (1966).

WILKINS, B. M.: Chromosome transfer from F-lac^{+} strains of *E. coli* K12 mutant at *recA*, *recB*, or *recC*. J. Bact. **98**, 599–604 (1969).

WILLETTS, N. S.: Plasmid specificity of two proteins required for conjugation in *E. coli* K12. Nature (Lond.) New Biol. **230**, 183–185 (1971).

WILLETTS, N. S., ACHTMAN, M.: A genetic analysis of transfer by the *E. coli* sex factor F, using P1 transductional complementation. J. Bact. **110**, 843—851 (1972).
WILLETTS, N. S., BRODA, P.: The *E. coli* sex factor. Ciba Foundation Symposium on Bacterial Episomes and Plasmids, p. 32–48. Edits: WOLSTENHOLME, G. E. W., O'CONNOR, M. London: J. and A. Churchill Ltd. 1969.
WILLETTS, N. S., FINNEGAN, D. S.: Characteristics of *E. coli* K12 carrying both an F prime and an R factor. Genet. Res. (Camb.) **16**, 113–122 (1970).
WILLIAMS, L., MEYNELL, G. G.: Female-specific phages and F-minus strains of *E. coli* K12. Molec. gen. Genet. **113**, 222–227 (1971).
ZEUTHEN, J., PATO, M. L.: Replication of the F-lac sex factor in the cell cycle of *E. coli*. Molec. gen. Genet. **111**, 242–255 (1971).

Antigenic Competition[1]

MICHAEL J. TAUSSIG[2]

With 6 Figures

Table of Contents

I. Introduction 125
II. Experimental Model Systems of Antigenic Competition 127
A. Protein Antigens: Rabbit Fab and Fc 127
B. Synthetic Polypeptides as Antigens 131
1. Multichain Polypeptides 131
2. Linear Polypeptides 137
C. Heterologous Erythrocytes 140
D. Haptens 143
III. Antigenic Competition and Other Immunological Phenomena 144
A. Tolerance 144
1. Competition in Tolerant Animals 144
2. Can the Process of Tolerance Induction lead to Competition? 146
3. Is there a Competitive Step in Tolerance Induction? 146
B. Genetic Control of Immune Responsiveness 148
C. Cross-Reacting Antigens: Original Antigenic Sin 148
D. Suppression and Enhancement by Antibody 150
E. Cooperation of Antigenic Determinants 154
F. Immune Surveillance 157
IV. Antigenic Competition and Theories of the Immune Response 158
V. The Mechanisms of Antigenic Competition 160
A. Intramolecular Competition 160
B. Intermolecular Competition: Antigen Mixture 162
C. Intermolecular Competition: Sequential Competition 166
VI. Practical Aspects of Competition 168
References 169

I. Introduction

The term antigenic competition dates back as far as 1904 when it was introduced by LEONOR MICHAELIS in connection with the raising of antibodies to serum proteins. In 1902 he had shown that the main result of injection of foreign serum into rabbits was the production of antibodies against globulins, and that in order to obtain anti-albumin it was necessary to inject the relatively purified albumin alone. He concluded that the response to the serum globulin

1 Part of the work described here was carried out while the author was holder of a long term fellowship of the European Molecular Biology Organisation, in Rehovot.

2 Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.

had inhibited the response to serum albumin. MICHAELIS put it thus in the terminology of the time:

"Ein Haptin hindert also in diesem Fall ein zweites, gleichzeitig injiziertes, mit verschiedener haptophorer Gruppe versehenes Haptin an der Entfaltung seiner Antikörper erzeugenden Eigenschaften. Ich möchte dies das Prinzip von der Konkurrenz der Haptine nennen" (MICHAELIS, 1904).

Another very early example is that described by FRIEDBERGER (1904), who found an analogous interference in the response of rabbits to cholera vibrios after immunization with mixtures of cholera vibrios and typhoid bacilli.

In more recent times, however, antigenic competition has not been subjected to the same intense scrutiny as other aspects of the immune response, perhaps because the observations made with competing systems have often seemed random and even contradictory. There is, furthermore, an awareness that some of the present theories of the immune response fail to account easily for antigenic competition, while the phenomenon itself is of such widespread occurrence that it cannot be simply ignored. The purpose of this review, therefore, will be to coordinate some of the current evidence for competition, presenting in particular detail results obtained by means of systems with which the author has been associated. The early literature has been reviewed by ZLATOGOROFF et al. (1929) and ADLER (1959, 1964).

Antigenic competition may be defined as the inhibition of the immune response to one antigen or antigenic determinant which is caused by the administration of another antigen or determinant. It is important to point out the criteria which may be used to distinguish competition from other conditions of poor response, such as genetic low responsiveness, tolerance, etc. In order to at tribute a particular low response to the effects of antigenic competition, it is necessary to show (a) that the potential to respond to the antigen exists and that a response can be elicited under certain conditions; and (b) that the lack of response to that antigen is the result of the presence of another antigenic molecule or determinant. As far as terminology is concerned, the terms "dominant" and "suppressed" have been introduced to refer to the antigens involved in competition, "dominant" referring to the antigen whose presence is inhibitory to another antigen, and "suppressed" to this second antigen. There are cases, however, where a clear-cut distinction between dominant and suppressed antigens cannot be made, for example, where mutual competition inhibits the responses to all the antigens involved.

A further useful distinction is that between different types of antigenic competition. Competition is said to be "intramolecular" if the antigens involved are determinants or antigenic regions located on the same immunogenic molecule. "Intermolecular competition", on the other hand, occurs between the antigens of different molecules, either (a) when these are administered simultaneously as antigen mixtures, or (b) when one antigen is given a certain critical time before the other, an effect which will also be referred to as "sequential competition". There is much evidence to suggest that these three different forms of antigenic competition have distinct underlying mechanisms.

II. Experimental Model Systems of Antigenic Competition

A. Protein Antigens: Rabbit Fab and Fc

The immunogenicity of rabbit IgG and its Fab and Fc fragments in mice provides a particularly interesting example of antigenic competition, demonstrating as it does all three types of competition—intramolecular, intermolecular with antigen mixtures, and intermolecular with antigens given sequentially (TAUSSIG, 1971; TAUSSIG and LACHMANN, 1972). It is a very convenient system for study, since the relevant antigenic groups, Fab and Fc, may be studied as regions within the same molecule, IgG, and may also be readily obtained as separate fragments in a high state of purity. In the results to be described, the assumption is made that Fab and Fc are antigenically non crossreacting, and this is easily verified as far as the specificity of the humoral antibody response is concerned. However, it is known that there is a certain amount of homology in the amino acid sequences of Fab and Fc (SMITH et al., 1971), and one cannot completely rule out the possibility of crossreaction at some level of antigen recognition not manifested in the final antibody specificity.

The main findings with this system were as follows. Rabbit IgG was immunogenic in mice when administered with adjuvant, similar results being obtained with either complete Freund's adjuvant or with alum precipitated antigen and pertussis vaccine. Antibody assays were carried out with suitably coated erythrocytes or modified bacteriophages (TAUSSIG, 1970). When the specificity of the primary antibody response to IgG was examined, most of the antibodies formed over the first 21 days were found to be anti-Fc, with very few anti-Fab antibodies (Fig. 1 a). If the purified Fab fragment itself was administered, however, a much higher and more rapid anti-Fab response was obtained (Fig. 1 b). These results indicated that Fc inhibited the immune response to Fab. Alternative reasons were first sought in features of the IgG molecule unrelated to Fc. Thus Fab determinants on IgG might be hidden or buried and inaccessible to cell-receptors. This seemed unlikely in view of the ability of anti-Fab antibodies raised to the Fab fragment to combine with IgG as completely as with Fab. A second possibility, suggested by work of HENNEY (1969) with human gamma globulin in guinea pigs, was that the early immune response was directed against configurational determinants of the IgG molecule, which would be absent from the isolated fragments, with anti-Fc and anti-Fab appearing only later. However, in the present case of rabbit IgG in mice, all the early antibodies could be absorbed out or inhibited by a mixture of the purified fragments, so that the majority of antigenic determinants must really have been represented on purified Fab and Fc.

The results which further established the ability of Fc to suppress the antibody response to Fab were obtained by the injection of mixtures of the purified fragments. When Fc and Fab in various molar ratios were administered as mixtures in the same adjuvant it was found that the response to Fab was inhibited at a small molar excess of Fc over Fab (3/1) (Table 1). Identical results were obtained with mixtures of Fab and IgG. It should be noted

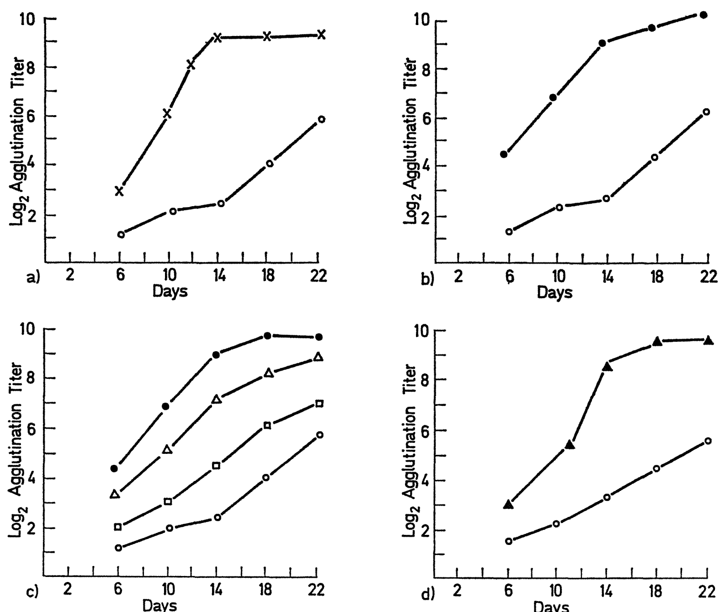


Fig. 1 a—d. Antigenic competition between rabbit Fab and Fc in mice. a Primary anti-rabbit Fc (×——×) and anti-rabbit Fab (○——○) responses in BALB/c mice after inoculation of 50 μg rabbit IgG in FCA. b Primary anti-rabbit Fab responses in BALB/c mice after inoculation of 50 μg rabbit IgG (○——○) in FCA or 35 μg rabbit Fab (●——●) in FCA. c Primary anti-rabbit Fab responses after inoculation of BALB/c mice with 50 μg rabbit IgG in FCA (○——○); 50 μg rabbit IgG in FCA with 25 μg anti-rabbit Fc administered passively (□——□); 50 μg rabbit IgG in FCA with 100 μg anti-rabbit Fc administered passively (△——△); or 35 μg rabbit Fab in FCA (●——●). d Primary anti-rabbit Fab responses after inoculation of 50 μg rabbit IgG in normal BALB/c mice (○——○) or in BALB/c mice tolerant to rabbit Fc (▲——▲)

that with an equimolar ratio of Fab and Fc, a good primary antibody response was obtained to both fragments. This illustrates a general feature of antigenic competition, namely, a marked dependence on the relative amounts of the antigens administered. The conclusion that rabbit Fc inhibited the response to rabbit Fab, and that this was an example of both intramolecular (IgG) and intermolecular (Fc/Fab mixtures) competition was therefore justified.

Fc also successfully inhibited the response to Fab when administered at various times before Fab. The competitive influence of Fc continued for about 7 days. On the other hand it was possible to interchange the roles of dominant and suppressed antigens by administering Fab a short time before Fc, which resulted in significant reduction of the anti-Fc response. The optimal time-period for this sequential competition was 4–6 days. This effect is obviously similar to those found with heterologous erythrocytes (Section II.C), and illustrates another common feature of competition, namely, the dominance of the first of two antigens given in series. It is interesting to note the similar time-course of the suppressive effect obtained for such dissimilar antigenic stimuli as proteins and red cells, indicating perhaps that the time of the

Table 1. The dose-dependence of intermolecular competition

Day	Molar ratio Fc/Fab			
	3/1		1/1	
	Anti-Fc titer	anti-Fab titer	anti-Fc titer	anti-Fab titer
12	6.6 (±0.7)	2.6 (±0.3)	6.3 (±0.5)	6.0 (±0.5)
17	8.6 (±0.7)	4.1 (±0.4)	8.9 (±0.8)	7.8 (±0.5)
22	10.0 (±0.6)	6.9 (±0.6)	11.6 (±0.6)	10.3 (±0.7)

Anti-Fc and anti-Fab titers of groups of BALB/c mice immunized (on day 0) with mixtures of rabbit Fc and Fab in different molar ratios (in complete FREUND's adjuvant). Results as mean $\log_2$ agglutination titers; 10 mice per group. (Data from TAUSSIG, 1971).

peak immune response is not the deciding factor in the optimal time for sequential competition.

A question of obvious interest is how far the recognition of an antigen, and the subsequent response made to it, are important for its dominance in competition. Competition between Fc and Fab was therefore studied under two conditions designed to reduce the immune response to Fc, namely, (a) passive administration of anti-Fc antibody, and (b) the state of tolerance to Fc (TAUSSIG and LACHMANN, 1972). In both these cases, antigenic competition was abolished, and the response to Fab correspondingly enhanced, though only in the case of tolerance to Fc was the anti-Fc response significantly reduced. Intramolecular competition could be abolished by relatively small amounts of passively administered mouse anti-Fc serum (Fig. 1 c); the resulting increase in the anti-Fab response was found to be dependent on the amount of anti-Fc administered. However, in these particular experiments the amount of anti-Fc was not sufficient suppress the response to Fc itself, which continued at a normal level. Thus conditions were obtained under which optimal responses to both Fab and Fc occurred simultaneously. This result has implications for the mechanism of competition, since it shows that the response to Fc cannot take up all the available essential metabolites, etc. and does not simply "crowd out" the response to Fab by occupying all the space in lymphoid issue, as was once envisaged as the explanation of competition. There are various ways in which antibody might interfere with antigenic competition; for example, by improving the localization of antigen in lymphoid follicles, or by interfering directly in the recognition of the dominant antigen by sensitive cells (see Sect. III, D for further discussion). Whatever the explanation, this may constitute an important control mechanism under physiological conditions, as first suggested by BRODY et al. (1967), who have described similar effects in hapten competition (Sect. II, D). Thus, during the primary response to complex antigens, the antibody formed early in the response will be directed towards the dominant antigens on the molecule or particle. The effect of this antibody will tend both to limit the response to the dominant antigens, and

simultaneously to enhance that to other determinants by abolition of competition. Such 'spreading' of the response may be particularly important if, for example, the first-formed antibodies are not effective in removing a pathogen.

The effects of passive anti-Fc on intermolecular competition, with mixtures of Fc and Fab, have been less thoroughly investigated, though here again it was possible be abolish competition with large amounts of anti-Fc (250 μgm per mouse).

The induction of tolerance to Fc was found to have a marked effect on both intramolecular and intermolecular competition. Tolerance was induced by a single injection of 100 μg particle-free Fc. On challenge with either IgG or mixtures of Fc and Fab, there was no evidence of antigenic competition, as shown by the rapid development of high anti-Fab titers. (In the case of challenge with IgG, there was also no evidence of breakage of tolerance to Fc.) These results are clearly analogous to the effects of anti-Fc, with the difference that here the improvement of the anti-Fab response is associated with the lack of response to Fc. The absence of competition in tolerant animals also has important implications for the site of competition, underlining as it does the importance of recognition processes, or of active steps following antigen recognition, in competition. Such steps must be absent in the tolerant animal. One possible site of competition which seems to be ruled out by these observations is the macrophage, since the uptake of labelled antigen by macrophages, as well as their functioning in general, seems to be unaffected by tolerance (HUMPHREY and FRANK, 1967; MITCHISON, 1969a). However it is not a universal finding that tolerance abolishes competition, and different results will be discussed in Sect. III, A.

An interesting facet of the antibody response to IgG, is that the very poor response to Fd determinants also seems to be a result of antigenic competition by Fc, rather than inaccessibility of Fd (TAUSSIG and LACHMANN, 1972). By inhibition of anti-Fab serum with free light chains, it was found that the anti-Fab produced in response to IgG was mainly directed towards light chain determinants. When Fab itself was injected, however, antisera were only marginally inhibited by light chains, and must therefore have contained a high proportion of anti-Fd. A possible explanation is that the Fd determinants in particular were hidden in the IgG molecule, but became more accessible after enzymatic digestion. However, it was found that the abolition of intramolecular competition, whether by passive administration of large amounts of anti-Fc or tolerance to Fc, considerably enhanced anti-Fd production. Thus the poor anti-Fd response to IgG in normal mice is probably a result of competition by Fc, more effective than against the light chain. This raises the possibility that the lack of response to other so-called "immunosilent" determinants on other moleculecules may in fact be a result of unsuspected antigenic competition. Table 2 summarizes the findings on competition between Fab and Fc.

Mention may be made here of other observations of competition with protein antigens. ADLER (1964), for example, has given a detailed description

Table 2. Summary of Antigenic Competition between Rabbit Fc and Fab

1.	Both intramolecular competition (with IgG as immunogen) and intermolecular competition (with mixtures of Fc and Fab) demonstrable. Fc was the dominant and Fab the suppressed antigen. Both anti-light chain and anti-Fd were suppressed by Fc.
2.	Intermolecular competition was dependent on the relative amounts of Fc and Fab in the mixture.
3.	Competition could be abolished by anti-Fc serum without suppressing the response to Fc.
4.	Competition was abolished by tolerance to Fc.

of intermolecular competition between ferritin and bovine gamma globulin. The work of BARR and LLEWELLYN-JONES (1953a, b, 1955), WEIGLE and HIGH (1967) and others will be described in later sections.

B. Synthetic Polypeptides as Antigens

1. Multichain Polypeptides

In many respects the multichain synthetic polypeptides are highly suitable immunogens for the study of antigenic competition. They each possess a relatively restricted number of antigenic determinants, of defined chemical structure and composition, yet there is considerable scope for controlled variation of antigen structure (SELA, 1969). It is possible to follow the antibody responses to separate immunopotent regions located either on the same or on different molecules (MOZES et al., 1969a, b), which is important for the comparative study of intramolecular and intermolecular competition. Furthermore, there is a great deal of background knowledge on the genetic control of the immune response to these antigens, and on the cellular basis of this genetic control (McDEVITT and BENACERRAF, 1969; SHEARER et al., 1971a). The following study of competition between the (Phe, G) and Pro–L regions of synthetic polypeptide antigens illustrates how these factors may be brought to bear on the problem of competition.

The synthetic polypeptides used, one of which is illustrated in Fig. 2, consist of a backbone of poly-L-lysine to which are attached side-chains of either poly-DL-alanine, or poly-L-proline, forming the series denoted as A–L or Pro–L respectively. Short, random sequences of L-tyrosine and L-glutamic acid (T, G), or L-phenylalanine and L-glutamic acid (Phe, G), 4–6 amino acids in length, are attached to the ends of the side-chains. The polypeptides thus formed are denoted as (T, G)-A–L and (Phe, G)-A–L in the A–L series, and as (T, G)-Pro–L and (Phe, G)-Pro–L in the Pro–L series (SELA et al., 1962; FUCHS and SELA, 1964; JATON and SELA, 1968; MOZES et al., 1969a, b). As far as immunological properties are concerned, in the two strains of mice studied (C3H/HeJ and DBA/1), neither (T, G) nor A–L is effectively immunogenic, so that the immunopotent regions under study are (Phe, G) and Pro–L. (Phe, G) may be regarded as essentially haptenic, and both Pro–L and A–L act as 'carriers'.

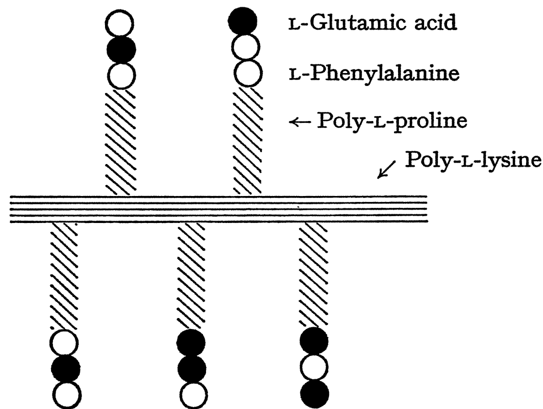


Fig. 2. Schematic diagram of the structure of a multichain synthetic polypeptide (Phe, G)-Pro–L

Both intramolecular and intermolecular competition between (Phe, G) and Pro–L were found to occur; in most cases Pro–L was the dominant antigen and (Phe, G) the suppressed one (TAUSSIG et al., 1972a). Table 3 shows that C3H/HeJ mice responded well to both (Phe, G)-A–L and (T, G)-Pro–L when these molecules were administered separately to different groups of animals, and, as pointed out there was no response to either A–L or (T, G) under these conditions. However, when (Phe, G)-Pro–L was the immunogen, the primary response to (Phe, G) was very poor while that to Pro–L was as good as to (T, G)-Pro–L (Table 3). Once again, the possibility that the (Phe, G) determinants were in some way hidden in the (Phe, G)-Pro–L molecule, was excluded since another strain (DBA/1) was able to respond well to (Phe, G) on Pro–L. Also there is no doubt that Pro–L is well able to act as a carrier for haptenic groups. This, therefore, is another example of intramolecular competition, in which the response to (Phe, G) is inhibited by the response to Pro–L.

Moreover, as with the Fab/Fc system, intermolecular competition was also demonstrated when mixtures of (Phe, G)-A–L and (T, G)-Pro–L were administered. Once again there was a striking dependence on the relative doses of the antigens in the mixture. A molar excess of (T, G)-Pro–L over (Phe, G)-A–L of 5/1 in the immunizing mixture led to competition and suppression of the response to (Phe, G). In contrast, inoculation of mixtures composed of equimolar amounts of the antigens, or an excess of 5/1 of (Phe, G)-A–L, resulted in good responses to both (Phe, G) and Pro–L. Finally, with a large excess of (Phe, G)-A–L of 25/1, the competitive situation was reversed: the response to Pro–L was now inhibited by (Phe, G)-A–L. These effects are detailed in Table 3 (see also Fig. 4a). The interpretation of this dose-dependence of competition is discussed in Sec. V.B.

Intermolecular competition was also found to inhibit priming for a secondary response, and in one case immunological memory (Table 4; see also Fig. 4b, c). C3H/HeJ mice which had received primary inoculation with mixtures of

Table 3. Antigenic competition between the (Phe, G) and Pro—L regions of synthetic polypeptides in C3H/HeJ mice. Data from TAUSSIG et al., 1972)

Competitive situation		Log$_2$ antibody titer	
		anti-(Phe, G)	anti-Pro—L
(i) *Intramolecular*			
Immunogens:			
Primary responses	(Phe, G)-A—L	4.2 ($\pm$0.7)	—
	(T, G)-Pro—L	—	6.7 ($\pm$0.6)
	(Phe, G)-Pro—L	0.9 ($\pm$0.3)	6.9 ($\pm$0.5)
Secondary responses	(Phe, G)-A—L	7.4 ($\pm$0.8)	—
	(T, G)-Pro—L	—	8.3 ($\pm$1.0)
	(Phe, G)-Pro—L	0.6 ($\pm$0.3)	8.6 ($\pm$0.9)
(ii) *Intermolecular*			
(a) Molar ratio (T, G)-Pro—L/(Phe, G)-A—L:			
	5/1	0.4 ($\pm$0.3)	5.7 ($\pm$0.8)
Primary responses	1/1	3.4 ($\pm$0.6)	5.9 ($\pm$0.6)
	1/5	3.9 ($\pm$0.7)	4.2 ($\pm$0.7)
	1/25	4.6 ($\pm$0.7)	0.55 ($\pm$0.3)
(b) Molar ratio (T, G)-Pro—L/A—L:			
Primary responses	1/5	—	4.9 ($\pm$0.5)
	1/25		0.6 ($\pm$0.4)

(Results are expressed as log$_2$ haemagglutination titers, $\pm$S.D., with 10 mice per group.)

Table 4. Intermolecular competition: effect on priming and memory

Primary immunizing mixture (T, G)-Pro—L/ (Phe, G)-A—L (molar ratio)	Secondary immunogen	Anti-(Phe, G)	Anti-Pro—L
5/1	(a) (Phe, G)-A—L	2.5 ($\pm$0.4)	
	(b) (T, G)-Pro—L	—	6.4 ($\pm$0.5)
1/1	(a) (Phe, G)-A—L	1.9 ($\pm$0.3)	—
	(b) (T, G)-Pro—L	—	6.8 ($\pm$0.7)
1/5	(a) (Phe, G)-A—L	5.8 ($\pm$0.6)	—
	(b) (T, G)-Pro—L	—	5.7 ($\pm$0.7)
1/25	(a) (Phe, G)-A—L	8.3 ($\pm$0.7)	—
	(b) (T, G)-Pro—L	—	1.6 ($\pm$0.4)

Groups of 20 C3H/HeJ mice received mixtures of (T, G)-Pro—L and (Phe, G)-A—L in different molar ratios as a primary inoculation in complete FREUND's adjuvant. After 3 weeks, mice from each group were challenged with either (Phe, G)-A—L or (T, G)-Pro—L separately (given in saline). Sera were taken 10 days later and titrated by passive haemagglutination. Results as means of 10, $\pm$S.D.

(T, G)-Pro–L and (Phe, G)-A–L were challenged later with either (T, G)-Pro–L or (Phe, G)-A–L separately. It was found that where competition had caused inhibition of a primary response to one of the antigens, no priming for a secondary response to this suppressed antigen had resulted. This indicates

that competition probably inhibits the very early stages of the response to the suppressed antigen. Moreover, in the group of animals which had received an equimolar mixture of (T, G)-Pro–L and (Phe, G)-A–L in the primary inoculation, immunological memory to (Phe, G)-A–L was not established despite an apparently normal primary response to (Phe, G). Thus, the establishment of memory may be more susceptible to antigenic competition than is the primary response itself.

It has been noted above that an excess of (Phe, G)-A–L could inhibit the primary response to (T, G)-Pro–L by intermolecular competition. An important subsequent finding was that A–L itself was as effective as (Phe, G)-A–L as a competitor against (T, G)-Pro–L, even though A–L was very poorly immunogenic (Table 3). In fact, in the conditions used, i.e. excess of A–L over (T, G)-Pro–L of 25/1, the response to Pro–L was suppressed, but almost no antibody formation against A–L was detected by haemagglutination. It thus appears that immunogenicity, in terms of antibody formation, is not a prerequisite for dominance in intermolecular competition. However, this does not mean that antigen recognition itself is also unnecessary; A–L, for example, is very probably recognized as an antigen since it is able to act as a carrier for haptenic groups. Indeed the carrier aspect of an antigen may be the important feature causing it to compete successfully in intermolecular competition. There are clearly important implications to be drawn for the cellular mechanism of competition, and these will be discussed in Sec. V.B.

The mice used in the above studies, C3H/HeJ, are potentially good responders to both (Phe, G) and Pro–L moieties. DBA/1 mice, on the other hand, while they are also high responders to (Phe, G), are low responders to Pro–L. The genetic control of the ability to respond to Pro–L has been studied in detail (MOZES et al., 1969a, b; SHEARER et al., 1971a, b). A single autosomal, dominant gene, termed Immune Response (Ir)-3 is the controlling locus, and is not linked to the major histocompatibility (H-2) locus, in contrast with the Ir-1 gene which controls the response to (Phe, G), (His, G) and (T, G) (MCDEVITT and BENACERRAF, 1969). The cellular basis of control over the Pro–L response has been studied by limiting dilution analysis, and the inability to respond to Pro–L in DBA/1 mice has thereby been correlated with a reduced number of antigen-sensitive cells available for Pro–L in this strain compared with high responders (MOZES et al., 1970; SHEARER et al., 1971a, b; MOZES and SHEARER, 1971; SHEARER et al., 1972). Of particular importance was the finding that it was the bone marrow-derived (B) and not the thymus-derived (T) cell population that was affected. Unprimed DBA/1 mice had only approximately one fifth as many available B cell precursors as a high responder strain, and the differences in primed animals were much greater (twenty five-fold), whereas the T cell population in DBA/1 was as for high responders. (While the high responder strain studied by these workers was in fact SJL, there are good grounds for the assumption that SJL and C3H/HeJ are identical in respect of the numbers of T and B precursors for Pro–L). A comparison of antigenic competition between C3H/HeJ and DBA/1

Table 5. Antigenic competition between (Phe, G) and Pro—L regions of synthetic polypeptides in DBA/1 mice. Data from TAUSSIG et al., 1972b)

Competitive situation		Log$_2$ antibody titer	
		anti-(Phe, G)	anti-Pro—L
(i) *Intramolecular*			
Immunogens:			
Primary responses	(Phe, G)-A—L	3.9 (±0.3)	—
	(T, G)-Pro—L	—	2.6 (±0.3)
	(Phe, G)-Pro—L	3.4 (±0.4)	2.4 (±0.3)
Secondary responses	(Phe, G)-A—L	6.8 (±0.4)	—
	(T, G)-Pro—L	—	2.4 (±0.2)
	(Phe, G)-Pro—L	6.0 (±0.3)	3.2 (±0.4).
(ii) *Intermolecular*			
Molar ratio (T, G)-Pro—L/(Phe, G)-A—L:			
Primary responses	5/1	0.6 (±0.2)	2.1 (±0.2)
	1/1	4.3 (±0.4	2.3 (±0.4)
	1/5	4.5 (±0.4)	1.9 (±0.3)
	1/25	3.8 (±0.3)	1.4 (±0.4)

Intermolecular competition only is demonstrable. (Results are expressed as log$_2$ haemagglutination titers ±S.D., with 5 mice per group).

strains was therefore made, not only to determine the importance for competition of the level of the response to Pro–L, but also to correlate competition with one or other cell population (TAUSSIG et al., 1972b).

DBA/1 mice were immunized with (Phe, G)-A–L, (T, G)-Pro–L or (Phe, G)-Pro–L, separately, and the results are shown in Table 5. It will be seen that the response to (Phe, G) was as good with (Phe, G)-Pro–L as the immunogen as with (Phe, G)-A–L. This is, of course, in striking contrast to the results described in C3H/HeJ (Table 3), and indicates that intramolecular competition between (Phe, G) and Pro–L does not occur in the low responder strain. Clearly, for intramolecular competition the level of the antibody response to the dominant antigen is a very important factor. On the other hand, when intermolecular competition was examined in DBA/1 mice by inoculation of mixtures of (Phe, G)-A–L and (T, G)-Pro–L in different ratios, no significant difference from C3H/HeJ was found. The relatively small excess of (T, G)-Pro–L over (Phe, G)-A–L of 5/1 caused suppression of the response to (Phe, G), despite the very poor response to Pro–L (Table 3). This result is very similar to the ability of A–L to be dominant in competition without provoking a detectable antibody response. Thus, in intermolecular competition, in contrast with intramolecular competition, the level of the response to dominant antigen is apparently irrelevant to its ability to be successful in competition.

The low response of DBA/1 mice to Pro–L may be "corrected" by the polyribonucleotide, polyadenylic-polyuridylic acid (poly A:U); injection of this after the immunogen restores the response to the level of that of the high responders (MOZES et al., 1971; BRAUN et al., 1971; SHEARER et al., 1971a).

Table 6. Effects of poly A:U on antibody responses to (Phe, G) and Pro—L regions of synthetic polypeptides. (Data from TAUSSIG et al., 1972b)

Immunogen	Without poly A:U		With poly A:U	
	anti-(Phe, G)	anti-Pro—L	anti-(Phe, G)	anti-Pro—L
(Phe, G)-A—L	6.8 (±0.4)	—	7.3 (±0.5)	—
(T, G)-Pro—L	—	2.4 (±0.2)	—	6.4 (±0.4)
(Phe, G)-Pro—L	6.0 (±0.3)	3.2 (±0.4)	2.1 (±0.3)	6.9 (±0.4)

Poly A:U restores both the response to Pro—L and intramolecular competition between Pro—L and (Phe, G). (Results, which are secondary responses, are expressed as $\log_2$ haemagglutination titers ±S.D., with 5 mice per group).

The effect of poly A:U shows a degree of determinant and strain specificity, and in DBA/1 it improves the response to (T, G)-Pro–L without influencing the response to (Phe, G)-A–L. At the cellular level limiting dilution analysis has shown that poly A:U specifically increases the number of B-cell precursors available for (T, G)-Pro–L, while the number of sensitive cells in the T population is unaltered (SHEARER et al., 1971a; MOZES et al., 1971). The influence of poly A:U on the response to (Phe, G)-Pro–L was examined with these points in mind to determine whether the improvement in the response to Pro–L would affect the response to (Phe, G). The results of this experiment were striking and clear-cut, as shown in Table 6. When (Phe, G)-Pro–L was injected into DBA/1 mice, followed after 24 hours by 300 µg poly A:U, the response to Pro–L was enhanced, but that to (Phe, G) was concomitantly depressed. In other words, intramolecular competition between Pro–L and (Phe, G) was restored at the same time as the response to Pro–L was improved. This result was confirmed by the subsequent finding that two other procedures which also correct the low response to Pro–L, namely, injection of macrophage-associated antigen and antigen complexed to methylated BSA, also restored the ability of Pro–L to compete against (Phe, G). The number of antigen-sensitive cells available for Pro–L in the B population was again specifically increased in these cases (SHEARER et al., 1971a).

These results provide clear indications of the cell types involved in antigenic competition. It appears that the ability of an antigen to compete against others present on the same molecule is related to the number of precursors available for it in the B cell population. Clearly, therefore, intramolecular competition will in many cases be related to the level of the antibody response to the antigens involved, insofar as this is dependent on the number of B cells available for each antigen. On the other hand, intermolecular competition between mixtures of antigens is probably a T-cell effect, and is often unrelated to the level of the antibody response to the antigens involved. Thus, the dominance of (T, G)-Pro–L over (Phe, G)-A–L in antigen mixtures showed no difference between C3H/HeJ high responders to Pro–L and DBA/1 low responders, which correlates with the near identity in the numbers of T cells available for Pro–L in the two strains. This is very similar to the ability of

Table 7. Summary of antigenic competition between (Phe, G) and Pro—L regions of synthetic polypeptides

1.	In C3H/HeJ mice [high responders to both (Phe, G) and Pro—L], both intramolecular competition [(Phe, G)-Pro—L] and intermolecular competition [(Phe, G)-A—L and (T, G)-Pro—L] occured. In general Pro—L was dominant and (Phe, G) suppressed
2.	Intermolecular competition was dependent on the relative amounts of (Phe, G)-A—L and (T, G)-Pro—L in the mixture
3.	A large excess (25/1) of a non-immunogenic molecule, A—L, suppressed the respone to (T, G)-Pro—L
4.	In DBA/1 mice [high responders to (Phe, G), but *low* responders to Pro—L], intramolecular competition was absent, but intermolecular competition was still present
5.	Intramolecular competition restored in DBA/1 by poly A:U

A–L to compete against Pro–L in C3H/HeJ without stimulating antibody production. Both Pro–L in DBA/1 and A–L in C3H/HeJ are molecules for which T cells are available, as shown by limiting dilution analysis and by their ability to act as carriers for haptenic groups in thymus-dependent immune responses, and both can be dominant in intermolecular competition while provoking only a poor antibody response.

These observations are summarized in Table 7 and are discussed further with reference to models for antigenic competition in Sec. V.

2. Linear Polypeptides

Several interesting examples of competition between synthetic linear polypeptides have been described; in many cases the polypeptides are used as haptens on protein carriers. SCHECHTER and SELA (1967), for example, compared the antibody responses in rabbits to poly-L-alanyl, poly-D-alanyl and poly-DL-alanyl proteins. Immunization with poly-L-alanyl or poly-D-alanyl proteins gave rise to non-crossreacting antibodies specific for the stereoisomers. On the other hand, immunization with poly-DL-alanyl proteins led predominantly to production of anti-poly-D-alanine, with very little anti-poly-L-alanine. This was a general phenomenon, occurring also with poly-DL-phenylalanyl and poly-DL-tyrosyl proteins. There was no doubt that poly-DL-alanine, for example, contained sequences of both poly-D- and poly-L-alanine, since these were available for reaction with specific antibody. Also, there was no predominance of D-alanine at the ends of poly-DL-alanine chains, which could have led to preferential production of anti-poly-D-alanine. Clearly, therefore, this is an example of intramolecular competition between sequences composed of D and L amino acids, with the D sequences being the dominant determinants. Very little antibody specific for DL-sequences was produced on immunization with poly-DL-aminoacyl proteins, and it may be that such antibody was also suppressed by the D-sequences.

SCHECHTER (1968) also examined the possibility of intermolecular competition by immunizing with a mixture of poly-L-alanyl-HSA and poly-D-alanyl-HSA. No competition was found, though no attempt was made to vary the relative amounts of the immunogens in the mixture.

The question of whether the intramolecular competition between D- and L-sequences would continue in animals made tolerant to poly-D-alanine was studied by TARRAB et al. (1971). Although there was no increase in anti-poly-L-alanine production in rabbits tolerant of poly-D-alanine and immunized with poly-DL-alanyl proteins, there was a significant increase in the amount of antibodies reacting specifically with DL-sequences. The effects of tolerance on competition are thus rather ambiguous in this case.

An example of intermolecular competition in which the nature of the protein carrier played a particularly critical role was described by SCHECHTER (1968). Poly-DL-phenylalanyl (poly-Phe-) protein conjugates were found to inhibit the antibody response to poly-DL-alanyl (poly-Ala-) proteins, but only when the protein carriers were the same or related molecules. For example, poly-Phe-RSA suppressed the response to poly-Ala-RSA or to poly-Ala-HSA, but not to poly-Ala-RNase. Similarly poly-Phe-RNase was dominant in competition against poly-Ala-RNase, but did not affect the response to poly-Ala-HSA. An important finding was that poly-Phe-RSA was as good a competitor in rabbits made tolerant to it as in normal rabbits. It will be noted that in these experiments the competition only occurred between crossreacting immunogens, even though the crossreaction was at the level of the carrier molecule only. In view of the important role of the recognition of carrier determinants in the response to hapten-carrier conjugates, it is reasonable to interpret these results as competition between crossreacting (or identical) carrier determinants rather than between the polypeptides themselves. One could assume for example, that the affinity of a carrier-recognition site might be slightly higher for poly-Phe conjugates than for poly-Ala conjugates with the same type of carrier, leading to the success of the former in competition. This interpretation assumes a rather high degree of crossreactivity at the carrier level between poly-Phe-RSA and poly-Ala-HSA; the finding that poly-Phe-RSA suppressed the response to both poly-Ala and HSA would, however, support this. The fact that tolerance did not prevent poly-Phe-RSA from competing against poly-Ala-RSA and poly-Ala-HSA could be explained if tolerance to the crossreacting carrier determinants was not complete.

A further case of competition involving poly-DL-alanine may be inferred from the results described by BAUMINGER et al. (1967). The response to poly-DL-alanyl proteins in rabbits was found to be mainly directed towards the haptenic poly-DL-alanine, with a relatively poor response to the protein carrier. However, in rabbits rendered tolerant to poly-DL-alanine by neonatal injection of A–L and subsequently immunized with poly-DL-alanyl proteins, the response to the protein was much enhanced. This may, therefore, be regarded as an example of intramolecular competition between hapten and carrier, in which

the hapten is the dominant determinant, and in which competition is abolished by tolerance to the otherwise dominant poly-DL-alanine. These results are very similar to those of ROELANTS and GOODMAN (1970), (Sec. V.A), and once more affirm the absence of competition in tolerant animals.

Antigenic competition between synthetic polypeptides has also been examined by BEN-EFRAIM and LIACOPOULOS (1967a; 1967b; 1969; 1970). Their results show that in many cases delayed hypersensitivity is as much influenced by competition as is antibody production. In one study (BEN-EFRAIM and LIACOPOULOS, 1967a), the importance of immunogenicity in competition was examined using very weakly immunogenic D-polypeptides. It was found that the delayed response to the random copolymer poly-L-(Tyr, Glu, Lys), which is immunogenic in its own right in guinea pigs, could be inhibited by the very poor immunogen poly-D-(Tyr, Glu, Lys). A high ratio of the antigens was used (60/1 of the D/L polypeptides). Since the D-isomer is known to be slightly immunogenic, their interpretation was that it behaved as an "abortive" antigen; although it was a recognizable immune determinant, it could only rarely induce any but the first stages of a response. Competition would occur at these early stages.

Another interesting example described by the same authors relates antigenic competition to genetic control of the immune response (BEN-EFRAIM et al., 1970). The control of the response to DNP-poly-L-lysine (DNP-PLL) in guinea pigs has been studied in great detail. The random-bred population contains both responder and non-responder animals, and the ability to respond is controlled by a single dominant gene linked to the histocompatibility locus (BENACERRAF et al., 1971). In inbred lines, strain 2 are responders while strain 13 are non-responders. At the cellular level, it appears that the control is exercized at the level of recognition of PLL carrier determinants. Thus, non-responders can be made to respond to DNP-PLL when this is complexed to BSA; in this case, antibody production but not delayed hypersensitivity results. In terms of the cell populations, the defect is presumed to be in the helper cell (T) population, rather than the antibody-forming cell (B) population. The ability of DNP-PLL to compete against poly-(Tyr, Glu, Ala) and poly-(Tyr, Glu) was studied in responder and non-responder animals. It was observed, significantly, that DNP-PLL could indeed compete and was dominant even in non-responder guinea pigs, but that the threshold dose required to show this competition was much higher in non-responders than in responders. This result is important because it is again possible to relate competition to a certain cell population. The implication is that the ability to compete is the result of recognition of DNP-PLL by T cells. Because such recognition is impaired in the non-responders, a much higher dose of DNP-PLL was required to show competition, presumably in order to stimulate the available T cells effectively. This result is very much in keeping with those described for competition by A–L and Pro–L in the absence of good antibody responses to these antigens, where the conclusion was also drawn that intermolecular competition is a result of recognition of antigen by T cells.

C. Heterologous Erythrocytes

When two antigens are injected in sequence with a suitable time-interval between them, it is often found that the response to the antigen given second is suppressed ('sequential competition'). This phenomenon, which is very general, has most often been studied with heterologous red cells from different species used as the competing antigens (ALBRIGHT and MAKINODAN, 1965; RADOVICH and TALMAGE, 1967; EIDINGER et al., 1968; DUKOR and DIETRICH, 1970; HANNA and PETERS, 1970; MÖLLER and SJÖBERG, 1970, 1971; WATERSTON, 1970; GERSHON and KONDO, 1971 a, b), but it occurs equally well between protein antigens (BARR and LLEWELLYN-JONES, 1955; TAUSSIG, 1971) and also when the first stimulus is a graft-versus-host reaction (HOWARD and WOODRUFF, 1961; LAWRENCE and SIMONSEN, 1967; MÖLLER, 1971). It is clearly a form of intermolecular competition, since the competing antigens are of necessity on different molecules or particles, but it differs in many respects, and probably also in causation, from the intermolecular competition which occurs when antigens are administered simultaneously as mixtures. Indeed, antigens which compete vigorously when administered sequentially will very often not compete at all when given together. The precise time which must elapse between administration of the antigens in order to show maximal competition and also the degree of suppression produced, are dependent, however, on two features familiar from other forms of competition, i.e. the immunogenicity and the doses of the antigens involved. In general, the more immunogenic the first antigen and the higher its dose, the more profound the suppressive effect of competition, which will also occur more rapidly. By the same token, the response to a poor immunogen, or to a small dose, is more easily suppressed by sequential competition than that to a good immunogen or a larger dose.

Among the first to examine sequential competition using erythrocytes as antigens were ALBRIGHT and MAKINODAN (1965), and RADOVICH and TALMAGE (1967). Although the basic observations of the two groups were the same, their interpretations were very different. ALBRIGHT and MAKINODAN injected mice first with 2×10^9 sheep RBC, and thereafter with 2×10^8 rat RBC. The antibody response to rat RBC injected within seven days of sheep RBC was significantly depressed, the maximal effect (95 % suppression) occurring when the time interval between injections was 15 hours. This observation was used as evidence for the multipotentiality of antigen-sensitive cells, since it was argued that if these cells were pre-committed to one specificity, non-crossreacting antigens would not interfere with each other. It was further found that the secondary response to rat RBC could not be inhibited by prior injection of sheep RBC. This was taken to mean that memory cells, in contrast with the immature precursors, were relatively differentiated. RADOVICH and TALMAGE (1967) used a similar system to test the suggestion that precursor cells were pluripotential. They showed that sheep RBC and horse RBC would compete with each other, maximal inhibition occurring if a four-day time

interval separated the injection of the antigens. Minimal crossreactivity between the antigens was detected by plaque assay. The authors contended that because competition was maximal when the interval was four days, corresponding to the peak of the response to the first antigen, "it seems unlikely that . . . antigenic competition is in fact competition for anything". A competitive event, it was suggested, would be most likely to occur when the antigens were injected together. Since this was not so it was concluded that competition was probably mediated by humoral, inhibitory factors. Although more recent work also points to a role for inhibitory factors in sequential competition, this particular argument by no means proves the case. In fact, many of the explanations invoking competitive events would predict that the antigen given first in time would be certain to acquire an advantage, by pre-empting multipotent precursor cells, by occupying limited sites of antigen presentation or processing, or by preferentially taking up space in lymphoid tissue, etc. However, RADOVICH and TALMAGE also developed a critical experimental design to probe the question of whether competition was for a multipotent precursor cell. Heavily irradiated (1 000 r) mice received transfers of either 10×10^6 or 50×10^6 normal spleen cells; on the same day as transfer, horse RBC were also injected and sheep RBC were given four days later. It was argued that competition for a multipotent cell should be most noticeable with the lower number of transferred cells. The experimental result showed, on the contrary, that competition was more marked at the higher cell concentration. This was taken as further evidence that competition was the result of the production of a humoral inhibitor by the response to the first antigen, which suppressed the response to the second antigen. Although this experiment indeed tends to rule out the multipotent cell hypothesis, it is worth noting that other interpretations of competition, such as competition for space, metabolites, etc., also predict a greater effect with higher cell numbers.

MÖLLER and SJÖBERG (1970, 1971) have also examined competition between sequential injections of horse RBC and sheep RBC and attempted to prove that competition does not decrease the number of precursors available for the second, suppressed antigen. Spleen cells were removed from mice preimmunized with horse RBC and transferred, together with sheep RBC, into recipient mice irradiated with 350 r. The transferred cells were only marginally less competent than normal cells in their response to sheep RBC. Assuming that there was no host contribution to the response after what was a relatively low level of irradiation, the result shows that there is no competition for immunocompetent cells. A further experiment indicated that the environment within animals preimmunized with one antigen was inhibitory to antibody production by transferred normal cells. Normal spleen cells, or cells primed to sheep RBC, were mixed with sheep RBC and transferred into irradiated (350 r) hosts which had been injected with horse RBC four days prior to irradiation. A very significant suppression of the antibody response of the transferred cells was observed. This result has been confirmed by WATERSTON (1970) and is good evidence for a suppressive milieu in animals undergoing

sequential competition. By analogy with the known ability of T cells to produce humoral effector substances, the authors suggest that T cells responding to the first antigen produce agents, non-specific in their effect, which suppress the proliferation of B cells, hence giving rise to the observation of antigenic competition.

GERSHON and KONDO (1971a) have shown that the presence of T cells is indeed essential to the mechanism of sequential competition. In irradiated mice reconstituted only with bone-marrow cells, a thymus-independent response to e.g. horse RBC was demonstrated. This response, which was mainly IgM, could not be inhibited by the prior injection of non-crossreacting sheep RBC. The addition of T cells both improved the response to horse RBC and also restored the competitive effect of prior injection of sheep RBC. Increasing the number of T cells from 1.5×10^7 to 6×10^7 further improved the competitive effect of sheep RBC without increasing the antibody response to sheep RBC. These observations specifically implicate T cells in sequential competition. Moreover, since the competition was greater when higher numbers of T cells were transferred, it is unlikely that competition was for the commitment of multipotent T cells (following the arguments of RADOVICH and TALMAGE above). Once again, the indication is that T cells, once specifically stimulated by antigen, produce inhibitors which are non-specific in their action.

A rather unusual situation has been described by WATERSTON (1970). Sequential competition was demonstrated *in vivo* between pig and sheep RBC, with much the same results as found by others. However, when an *in-vitro* system of the type described by MISHELL and DUTTON (1967) was used to compare the responsiveness of spleen cells from normal animals with those from animals immunized with pig RBC, the latter were found to be enhanced in their ability to mount a primary response to sheep RBC. The improvement was shown to result from the increased size of a non-adherent cell population, presumably of T cells, since no crossreaction in PFC was found. It is likely, therefore, that the antigens share some 'carrier specificity' not expressed at the serum antibody level. Although the author suggests that the competition *in vivo* resulted from the presence of a minute amount of crossreacting suppressive antibody produced in response to the first antigen, it seems more likely that it is caused by the non-specific inhibitory factors postulated above. These would be diluted out *in vitro*, where instead enhancement would, result from the increased number of T cells recognizing crossreacting carrier determinants.

In summary, sequential competition appears to be the result of the production of non-specific inhibitory factors by T cells in response to the first antigenic stimulus. These factors may well serve the important function of limiting the extent of the immune response, and thus complement the suppressive, regulatory action of antibody itself. Their action is probably to inhibit the proliferation of both T and B cells; they would thus be selflimiting in production, and should only be present while an immune response continues. Any other antigen given during this period is therefore introduced into an

inhibitory environment, with the result that antibody production to it will be relatively suppressed. Sequential competition may thus be seen as a side-effect of the regulation of the immune response. For further discussion, see Sect. V.C.

D. Haptens

Several studies have been described in which conventional haptens were used as the competing determinants (AMKRAUT et al., 1966; BRODY et al., 1967; BRODY and SISKIND, 1969, 1972; SCHWARTZ and LESKOWITZ, 1969; FAUCI and JOHNSON, 1971a, b; TAYLOR and IVERSON, 1971). AMKRAUT et al. (1966) found that competition between the 2, 4 dinitrophenyl (DNP) and arsanilic acid (RAzo) groups only occurred in the intramolecular situation, i.e. with both haptens on the same carrier molecule. The type of carrier used had an important effect on the results. For example, rabbits which were immunized with the haptens coupled to keyhole limpet haemocyanin-$RAzo_{72}DNP_{19}$ KLH-produced anti-DNP, but no anti-RAzo. Animals which received, on the other hand, the same haptens on bovine serum albumin-$RAzo_{24}DNP_{8}$BSA-produced good anti-RAzo as well as anti-DNP titers. Since both conjugates had similar hapten/hapten and hapten/protein ratios, it is possible that the higher immunogenicity of KLH compared with BSA had a role in suppressing the response to RAzo. It was also shown that intramolecular competition was dependent on the ratio of the haptens coupled. Thus, with the conjugate $RAzo_{24}DNP_{25}$BSA, the response to RAzo was suppressed by competition; no such competition occurred with the conjugate $RAzo_{24}DNP_{8}$ BSA. There is a possibility that steric hindrance was a contributing factor in the first case.

BRODY et al. (1967) have also examined competition between DNP and arsanilic acid (RAzo) coupled to rabbit gamma globulin (RGG). Rabbits were immunized separately with either $RAzo_{12}$RGG or DNP_{28}RGG, and the responses compared with those to a mixture of the two conjugates, or to the doubly conjugated molecule $RAzo_{15}DNP_{27}$RGG. Mutual competition was found to occur in both the intermolecular and intramolecular situations, leading to a 60% reduction in anti-DNP and 90% reduction in anti-RAzo produced. Passively administered anti-DNP both suppressed the response to DNP and at the same time reduced the effects of intermolecular competition, i.e., the response to the RAzo group administered simultaneously on a separate carrier molecule was enhanced. Anti-DNP had no effect on intramolecular competition—when DNP and RAzo were present on the same molecule, anti-DNP suppressed the response to DNP but neither suppressed nor enhanced that to RAzo. The same results were obtained with anti-RAzo administered passively. This effect of antibody on antigenic competition is discussed further in Sec. III.D. In a later paper (BRODY and SISKIND, 1969) the affinity of the antibody produced to suppressed antigens during antigenic competition was compared with that formed to the same antigens under optimal immunizing

conditions, and no significant differences were found. This tends to rule out partial tolerance as a cause of competition, since this would be expected to lead to a decrease in average affinity. Nor is there likely to be a restriction in antigen available for sensitive cells in this case, for example as a result of competition during macrophage processing, because an increase in antibody affinity would then be expected. Another interesting feature of competition between these haptens was the requirement that they be injected into the same site, both when given at the same time (Brody and Siskind, 1969) and when administered sequentially with an interval of 1–2 weeks between injections (Brody and Siskind, 1972). If a soluble inhibitor is involved in this case, its local concentration must be critical. The result with sequential competition differs from that between heterologous erythrocytes in mice, where the relative site of injection of the antigens was not important in competition (Eidinger et al., 1968; O'Toole and Davies, 1971).

Schwartz and Leskowitz (1969) described intermolecular competition in the development of delayed hypersensitivity to arsanilic acid. Azobenzene-arsonate-N-acetyl tyrosine (RAzo-tyr), when injected in complete Freund's adjuvant, regularly induced a state of hapten-specific hypersensitivity which could be elicited with RAzo-insulin. When mixed with 100 μg bovine gamma globulin (BGG), sensitization by RAzo-Tyr was inhibited. The induction of tolerance to BGG prevented this competitive effect. Furthermore, intramolecular competition also seemed to occur, since RAzo coupled to poorly immunogenic carriers gave rise to hapten-specific sensitivity, while RAzo on more highly immunogenic carriers failed to do so, suggesting competition between carrier and hapten. Induction of tolerance to the immunogenic carrier protein again restored hapten-specific responsiveness in some cases.

III. Antigenic Competition and Other Immunological Phenomena

A. Tolerance

1. Competition in Tolerant Animals

The question of whether antigenic competition can still occur in animals made tolerant to the antigen which would normally be dominant in competition is an important one. In general, it seems that tolerance to the dominant antigen abolishes its ability to compete in both the intermolecular and the intramolecular situations. Some examples have already been quoted. Thus competition between rabbit Fc and Fab, both within the IgG molecule and with antigen mixtures, did not occur in mice rendered tolerant of Fc (Taussig and Lachmann, 1972, Sec. II.A). Intramolecular competition between poly-γ-D-glutamic acid (PGA) and its carrier, methylated BSA, was abolished when tolerance to PGA was induced (Roelants and Goodman, 1970, Sec. V.A), and a similar example has been described in the work of Bauminger et al. (1967) on competition between poly-DL-alanine and its protein carrier (Sec. II.B.2). Weigle and High (1967) described the inhibition of the antibody response to ovalbumin (OA) as a result of competition by haptenated BSA or

thyroglobulin; once again, tolerance to these dominant antigens prevented competition and improved the response to OA. Similarly, SCHWARTZ and LESKOWITZ (1969) showed that neither intramolecular nor intermolecular competition between the arsenilic acid group and BGG, which results in suppression of delayed hypersensitivity to arsenilic acid, occurred in animals tolerant of the dominant BGG.

The effects of tolerance on three related competitive situations involving rabbit IgG and the multichain synthetic polypeptide (T, G)-Pro–L have recently been studied (TAUSSIG, 1972). The results are shown in Fig. 3. Mice immunized with a mixture (in adjuvant) of (T, G)-Pro–L (1 mg) and rabbit

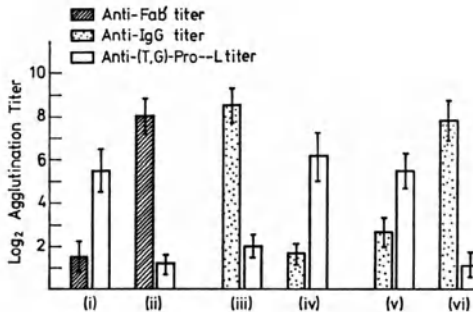


Fig. 3. Absence of Antigenic competition in tolerant animals. Group (i): Anti-rabbit Fab and anti-(T, G)-Pro–L titres 2 weeks after injection of a mixture containing 35 μg rabbit Fab and 1 mg (T, G)-Pro–L in FCA, in normal C3H/HeJ mice. Group (ii): As (i), but in mice tolerant to (T, G)-Pro–L. Group (iii): Anti-rabbit IgG and anti-(T, G)-Pro–L titres 2 weeks after injection of a mixture containing 1 mg rabbit IgG and 1 mg (T, G)-Pro–L, in FCA, in normal C3H/HeJ mice. Group (iv): As (iii), but in mice tolerant to rabbit IgG. Group (v): Antirabbit IgG and anti-(T, G)-Pro–L titres 2 weeks after sequential administration of the antigens [1 mg (T, G)-Pro-L in FCA, followed 4 days later by 1 mg IgG in FCA] in normal C3H/HeJ mice. Group (vi): As (v), but in mice tolerant to (T, G)-Pro–L

Fab' (35 μg) responded well to (T, G)-Pro–L, but the response to Fab' was suppressed by intermolecular competition [Group (i)]. In animals rendered tolerant by a single injection of 2 mg (T, G)-Pro–L in saline, however, this competitive effect did not occur, and the results in the tolerant Group (ii) are thus a reversal of those in Group (i). In competition between (T, G)-Pro–L and rabbit IgG itself, conditions were found where IgG was the dominant and (T, G)-Pro–L the suppressed antigen, namely, injection of a mixture containing equal amounts of each antigen [Group (iii)]. Once again, IgG ceased to be dominant in mice tolerant to it [Group (iv)]. Finally if (T, G)-Pro–L was injected a few days before rabbit IgG, the response to the latter was suppressed in normal mice [Group (v)] but not in mice tolerant to (T, G)-Pro–L [Group (vi)].

As noted in Sec. II.B.2, a different result was obtained by SCHECHTER (1968), who found that poly-L-phenylalanyl-RSA suppressed the antibody response to poly-DL-alanyl-RSA in rabbits tolerant to poly-L-phenylalanyl-

RSA as well as in normal rabbits. Reasons for regarding this as a special case, however, have already been discussed.

It thus appears that, in general, antigenic competition will not take place in an animal which is tolerant to an otherwise dominant antigen. It is probable, therefore, that antigen recognition and the proliferative events following this recognition in a normal animal are an important part of antigenic competition. These results make it unlikely that nonspecific antigen handling, particularly by macrophages, is relevant to the mechanism of competition, especially since this is probably not affected by tolerance (HUMPHREY and FRANK, 1967; MITCHISON, 1969a). Also there is clearly a difference between antigens to which a state of acquired tolerance exists and those which are inherently poor immunogens. Several cases in which the latter can indeed be dominant in competition have been described, so that recognition and the other events necessary for competition must still occur in the case of these antigens, even though antibody production itself may not.

2. Can the Process of Tolerance Induction lead to Competition?

A provocative observation made some time ago by LIACOPOULOS et al. was that the induction of tolerance with very large doses of antigens resulted in a temporary inability to respond to other, unrelated antigens (LIACOPOULOS et al., 1962; LIACOPOULOS and NEVEU, 1964). This threw some doubt on the specificity of tolerance induction and the monopotentiality of antigen-sensitive cells, though the non-specific effect was only be demonstrable for a few days. However, it is now known that during the induction of high-zone tolerance, a passing phase of antibody production may exist (MITCHISON, 1968) and it seems reasonable, therefore, that antigenic competition may occur as a result. There is also a great deal of apparently nonspecific cell proliferation and γ-globulin production during tolerance induced by overloading, and this may have an inhibitory, competitive influence on antibody production of later stimuli. Recently, LIACOPOULOS et al. (1971) have shown that the induction of low-zone tolerance may also inhibit antibody production to unrelated antigens. The most significant finding was that tolerance induced by repeated injection of 0.1 μg BSA was inhibitory to the immune response to OA. However, this effect was only found during the first few days after commencement of the tolerance-inducing schedule; that is, the competitive events occurred long before tolerance could be said to be complete. This may indicate a similarity between some of the early events of both immunity and tolerance induction.

3. Is there a Competitive Step in Tolerance Induction?

A recent study has examined whether antigens which compete for the induction of immunity will also compete for the induction of tolerance (TAUSSIG, 1972). It was hoped that this would be useful not only in defining better the nature of antigenic competition, but would also indicate the essential differ-

Table 8. Induction of tolerance to rabbit Fab in C3H/HeJ mice, under various "competitive" conditions

Dose of Fab (μg)	Antigens Adminstered					
	Fab	Fc [a] ±Fab	Fc [b] ±Fab	IgG [c]	(TG)PL [d] +Fab	TG(PL) [e] +Fab
1	7.5 (0.6)	8.3 (1.1)	8.6 (0.3)	7.1 (0.5)	9.2 (0.8)	8.7 (1.1)
10	2.1 (0.4)	1.5 (0.8)	2.6 (1.0)	2.0 (0.5)	2.7 (0.3)	2.1 (0.9)
25	1.8 (0.7)	0.9 (0.9)	3.0 (0.7)	1.8 (0.7)	1.1 (0.4)	2.0 (1.0)
100	0.9 (0.8)	2.7 (0.5)	2.5 (0.3)	1.7 (0.8)	1.0 (0.3)	2.5 (0.3)

[a] 50 μg Fc.
[b] 500 μg Fc.
[c] Contained amounts of Fab specified in left hand column.
[d] 1 mg (T, G)-Pro—L.
[e] 1 mg (T, G)-Pro—L given 4 days before Fab.

Different amounts of rabbit Fab were administered either mixed with, linked to, or after other antigens, as indicated. All antigens were particle-free and administered in saline. 10 days later, challenge of 35 μg Fab in complete FREUND's adjuvant was given. The results show anti-Fab titers of sera taken 21 days after challenge. (Log_2 haemagglutination titers with S.D. in parentheses, 10 animals per group). Inductions of tolerance to Fc and (T, G)-Pro—L was confirmed in the appropriate cases.

ences between the pathways of immunity and tolerance. The experimental approach was to determine the threshold doses for tolerance induction to purified antigens given either alone or together with other, competing, antigens. The latter were known to dominate in competition for antibody production, but all the antigens were administered in a paralytogenic dose and form. The results indicated that it was not possible to affect the threshold of tolerance by administration of other tolerogens. For example, Table 8 shows that the threshold of induction of tolerance to rabbit Fab' was the same (1–10 μg per mouse) whether it was administered alone, or in the form of IgG, or mixedwith Fc or (T, G)-Pro–L (all as particle-free antigens in saline). Thus, tolerogens apparently do not compete with each other, indicating that there is no competitive step in tolerance induction.

The inability to show competition between tolerogens may mean either that the processes involved in tolerance induction cannot give rise to competition, or alternatively, that they are not susceptible to the influence of competition. The results of LIACOPOULOS et al. (1971) described above have shown that at least the sequential form of competition can be caused even if the first antigen is administered in a tolerance-inducing regimen. One interpretation of the absence of competition between tolerogens is that tolerance induction is a simpler process than immune induction, and that the latter involves additional events which may give rise to or be affected by antigenic competition. A fuller understanding of the cellular processes involved in each, therefore, would probably indicate the sites or mechanism of competition. For example, some of the early events which might be unique to immune induction could include the presentation of antigen to lymphocytes by macrophages (FREI et al., 1965;

DRESSER and MITCHISON, 1968), cooperation between lymphocytes of different origins (MÄKELÄ et al., 1971), and the trapping of antigen by dendritic cells in the follicles of lymph node and spleen (ADA et al., 1964; NOSSAL and ADA, 1971), quite apart from the proliferative events which will occur mainly in immunity. On this basis, antigenic competition might then involve any or all of these, depending on the antigens and the conditions.

B. Genetic Control of Immune Responsiveness

At a time when detailed studies are being made of the genetic control of the ability to respond to complex antigens, or to individual determinants of simple antigens, it is important to note that antigenic competition may give rise to effects superficially similar to those of genetic control. Previous sections have already shown how competition may vary among different strains of imbred mice and guinea pigs. The main point is that a genetically controlled ability to respond to certain dominant antigens on a multideterminant molecule can influence, for better or worse, the response to other determinants on the same molecule. (The same is of course true of antigen mixtures, but these are not usually the object of genetic studies.) It is easy to see that in some circumstances this could give rise to false conclusions. For example, if the ability to respond to the (Phe, G) determinant had been studied in different strains of mice using (Phe, G)-Pro–L as the only immunogen, it would have been found that C3H/HeJ were low responders while DBA/1 were high responders to (Phe, G). In fact, both strains are capable of a high response to (Phe, G), as is shown when (Phe, G)-A–L is used as the immunogen. The genetic difference which does exist between these strains does not control the response to (Phe, G) but the ability to respond to Pro–L, and it is the level of the anti-Pro–L response which in turn influences the response to (Phe, G), being inhibitory in C3H/HeJ but not in DBA/1 (Sec. II.B.1). The criteria which should be applied to distinguish competition from true genetic control have been indicated in the Introduction.

C. Cross-Reacting Antigens: Original Antigenic Sin

The phenomenon of original antigenic sin, which occurs when animals immunized with one antigen are stimulated with a crossreacting antigen, has some interesting features in common with antigenic competition. Examples of antigens used for the study of original antigenic sin include crossreacting influenza viruses (FAZEKAS DE ST. GROTH and WEBSTER, 1966a, b; FAZEKAS DE ST. GROTH, 1967), mutants of β-galactosidase (CELADA, 1971; CELADA et al., 1971), and closely related haptens (EISEN et al., 1967). Extensive studies have been performed by FAZEKAS DE ST. GROTH and WEBSTER (1966a, b) with different strains of influenza viruses in man and experimental animals. The following are representative of their main findings. A particular virus pair studied, SW and FM1, crossreact to a degree of approximately 10–20%; that

is, only 10–20% of the antibodies raised to either one will be cross-absorbable. This crossreacting fraction has a higher affinity for the inducing virus than for the crossreacting virus. It may be assumed that the two viruses possess some determinants which are similar, but not identical, and others which are distinct for each. The original antigenic sin phenomenon is seen when animals are primed with SW, for example, and boosted with FM1. In time of appearance and affinity the antibodies produced are characteristic of those of a secondary response to SW, and accordingly react better with SW than with FM1. Moreover, these antibodies are totally cross-reacting since they can be completely absorbed by either virus, and are more homogeneous than a normal primary or secondary response. The interpretation suggested is that the FM1 boost cross-stimulates a part of the population of cells which was raised in response to SW; a secondary response to the latter therefore occurs at the expense of the expected primary response to FM1. This latter point brings out the analogy between original antigenic sin and intramolecular competition. In the terminology of competition, the cross-reacting determinants are the dominant groups; the response that is suppressed is the normal primary response to the virus used for boosting.

These effects may be seen as the result of a competition for antigen between different cell populations. The population expanded by the first stimulus contains a relatively large number of cells of cross-reacting specificity and high affinity for both viruses. At the time of the second, cross-reacting antigenic stimulus, it therefore captures antigen preferentially, thus effectively preventing the normal primary response. This hypothesis is supported by the finding that when a low dose of SW was used for priming and a high dose of FM1 for boosting, only the early antibody was totally cross-reacting of the original antigenic sin type, while later antibody was of primary anti-FM1 type. Furthermore, when cell populations against both SW and FM1 were raised by priming, original antigenic sin could not be demonstrated on boosting.

Another example of original antigenic sin, perhaps even more closely related to intramolecular competition, has been described by CELADA et al. (1971). The antigens are two forms of β-galactosidase, the wild-type enzyme 'Z' and an inactive mutant 'AMEF'. Antibody raised against Z precipitates both Z and AMEF, and also has the ability to activate the inactive AMEF. Anti-AMEF, on the other hand, although it also precipitates both forms, cannot activate AMEF, but in fact competitively inhibits the activation of AMEF by anti-Z. There is good evidence that the two forms of the enzyme differ in a single amino acid residue, and it is suggested that Z and AMEF differ in a single determinant, the correct conformation of which (as in Z) is essential for enzyme activity. Both anti-Z and anti-AMEF can combine with the determinant as it exists in AMEF, but only anti-Z has the property of changing the enzyme conformation in such a way as to activate AMEF. It is clear that the degree of immunological cross-reaction between Z and AMEF is very much greater than in the case of the influenza viruses, and that the difference is in fact restricted to a single crossreacting determinant. The

advantage of the system is that the activation assay provides a straightforward method for following antibody production to that single determinant.

The main experimental finding was that preimmunization of rabbits and mice with AMEF inhibited the production of activating antibody in response to a later injection of Z. This inhibitory effect could be obtained up to two years after the inoculation of AMEF (in rabbits). The production of precipitating antibody in response to Z, however, was not inhibited by prior exposure to AMEF. It must be remembered that anti-AMEF competitively inhibits activation by anti-Z, so the production of a little activating anti-Z might not be detected. However, the interpretation offered by the authors at the cellular levels is that preimmunization with AMEF raises a population recognizing the critical determinant in its inactive conformation. When Z is given later, this population is cross-stimulated and competes for antigen with naive anti-Z cells. Activating antibody is thereby effectively suppressed, while anti-AMEF is stimulated. This explanation, however, limits itself to the cross-reacting determinant. A more complete hypothesis would be that injection of AMEF raises a large population of memory cells directed against all the determinants which are identical on the two enzymes, together with a smaller population against the critical, cross-reacting determinant. When Z is then injected, it is captured by both these populations, and mainly by the first since it is the larger, with the result that insufficient antigen is available to stimulate a primary response to Z determinants which would lead to activating antibody. This situation is therefore a combination of cross-stimulation and intramolecular competition very similar to the virus pair described above. Once again the inference for intramolecular competition is that since antigen-sensitive cells compete for antigen, the determinants for which the greatest number of immunocompetent cells (or alternatively cells of highest affinity) are available should be dominant in competition, because of their preferential capture of antigen. This is indeed exactly the case in intramolecular competition between the (Phe, G) and Pro–L regions of (Phe, G)-Pro–L (Sec. II.B.1); several other observations are also in direct agreement with this model, which is developed further in Sec. V.A.

D. Suppression and Enhancement by Antibody

Antibody may exert two apparently opposing influences on an immune response, suppression and enhancement, effects which are believed to play an important part in the control of the immune response under physiological conditions. Of several possible mechanisms for antibody-mediated suppression, the most likely seems to be that antibody competes for antigen with cell-bound receptors, thereby blocking cellular recognition. Suppression should therefore be specific for those determinants against which antibody is directed, a prediction which has often been confirmed (reviewed by SCHWARTZ, 1971). The enhancing influence of antibody, on the other hand, may take one of two

forms. In some cases antibody apparently enhances the response to the whole molecule or particle against which it is directed (HENRY and JERNE, 1968; DENNERT et al., 1971). Alternatively the response to some determinants on a molecule may be specifically suppressed by antibody, while the response to others on the same molecule is non-specifically enhanced (PEARLMAN, 1967; McBRIDE and SCHIERMAN, 1970). Two mechanisms may account for antibody-mediated enhancement. The first envisages an improved localization of antigen, implying that antibody may enable antigen to be presented more effectively to antigen-sensitive cells, perhaps by causing trapping of antigen on the surface of macrophages or dendritic cells. This type of enhancement mechanism is most effectively mediated by IgM, although IgG may exert a similar effect. A second cause of enhancement is the abolition of antigenic competition. The preferential production of antibodies towards some determinants on a complex molecule or particle, as in intramolecular competition, is most probably the result of competition for antigen between B-cell populations of different specificities (Sec. V.A). The populations which are largest in number or bear the highest affinity receptors will capture antigen preferentially, with the result that antibody formation may be triggered for only some of the determinants on the molecule. According to this model, antibody against the dominant determinants would impair their recognition by specific B cells to a degree depending on the amount and affinity of the antibody administered, and the probability of interaction of other determinants on the same molecule with antigen-sensitive B cells should thereby be increased. One would therefore expect a loss of competition and concomitantly the enhancement of the response to determinants other than those against which the antibody was specifically directed (see Fig. 5a, b). Suppression of the response to some determinants will therefore be accompanied by enhancement to others. The amount of antibody needed to enhance the response should be less than that required to cause suppression.

These considerations provide an interpretation of many cases of suppression and enhancement by antibody. Examples with protein antigens have been described by PINCUS and NUSSENZWEIG (1969), PINCUS et al. (1971) and TAUSSIG and LACHMANN (1972). As described in Sec. II.A, intramolecular competition between the Fc and Fab regions of rabbit IgG in mice could be prevented by relatively small amounts of mouse anti-rabbit Fc serum (TAUSSIG and LACHMANN, 1972). The result of administration of anti-Fc was, therefore, enhancement of anti-Fab (Fig. 1c). This took place without detectable suppression of the anti-Fc response, i.e., the threshold antibody dose required for enhancement was lower than that for suppression. A similar case was described by PINCUS et al. (1971), in which rabbits were immunized with guinea pig γ_2 immunoglobulin and received rabbit antiguinea pig $F(ab')_2$ serum passively. Their response to guinea pig $F(ab')_2$ determinants was thereby suppressed, but that to Fc determinants was increased. With one particular dose of rabbit anti-$F(ab')_2$, enhancement of anti-Fc was obtained without suppression of anti-$F(ab')_2$ (cf., results of TAUSSIG and LACHMANN). A further significant

point was that $F(ab')_2$ fragments of the rabbit antibody administered were sufficient to bring about suppression, but enhancement required the action of the whole antibody molecule. It is therefore likely that enhancement depends on a combination of improved localization of antigen, which would probably be Fc dependent, and abolition of competitive effects, while suppression only requires blocking of determinants. Another case described by PINCUS et al. (1971) is that of the response of rabbits to human secretory IgA. The response to the "secretory component", a component absent from serum IgA, was enhanced by passive administration of antibodies against serum IgA.

Simultaneous enhancement and suppression have also been found with particulate antigens. PEARLMAN (1967), for example, studied the effect of anti-SRBC antiserum on the response of rabbits immunized with a DNP-SRBC conjugate. The anti-DNP and anti-SRBC responses were followed separately. Depending on the conditions it was possible to suppress anti-SRBC production and enhance the response to DNP; alternatively, both anti-SRBC and anti-DNP could be enhanced; finally, if large amounts of anti-SRBC were administered, both anti-SRBC and anti-DNP could be suppressed. In general, however, suppression only affected the response to those determinants against which the antiserum was directed, whereas enhancement occurred to both heterologous as well as homologous determinants. Moreover, the amount of anti-SRBC required to enhance anti-DNP was less than that required to suppress anti-SRBC production. Once again a reasonable interpretation is that anti-SRBC antibody improves the ability of anti-DNP B cells to combine with the DNP-SRBC conjugate by inhibiting the combination of anti-SRBC B cells with the antigen.

Another interesting situation involving erythrocyte antigens has been described by MCBRIDE and SCHIERMAN (1970). They have studied the response in chickens to chicken red cells (CRBC) bearing the isoantigenic groups A and B. In the GB-1 line of chickens, the B isoantigen acts as a carrier for the A isoantigen, i.e. the birds respond poorly to CRBC bearing group A as the only foreign antigens, but respond well to both A and B when injected with CRBC carrying both groups. They will also respond to CRBC carrying B antigens only. Thus the B antigens are both immunogenic and act as carriers for the hapten-like A antigens. When GB-1 chickens were injected with erythrocytes carrying both A and B (abbreviated as AB cells), the response to B was in general more rapid, and the anti-A response began to rise only after anti-B had reached a peak. One important observation was that the anti-A response to AB carrying cells was enhanced either by passive pre-immunization with anti-B serum, or by injection of the complex of AB cells with anti-B. The anti-B response under these conditions was simultaneously suppressed. Enhancement of anti-A could also be obtained if chickens were hyperimmunized by repeated injection of cells carrying B only, prior to the injection of AB cells. Finally, the cooperative effect of B helping A could not be obtained in birds naturally tolerant of B; nor did passive immunization with anti-B enhance the response to A in these birds.

The following interpretation (which is not that suggested by the authors) would account for these observations. (Note: in this paragraph the designation 'B' refers to the isoantigenic group, rather than to 'bone marrow or Bursa-derived cells'.) Since A is essentially haptenic and B acts as its carrier, injection of AB erythrocytes will lead to the presentation of A to its specific antibody-producing cell precursors by thymus-derived helper cells recognizing group B determinants. In addition to the facilitation of the anti-A response by B, a certain degree of intramolecular competition is assumed to exist between A and B, and in this B acts as the dominant antigen. The action of anti-B antibody is therefore both to suppress the response to B, and at the same time to abolish competition, thereby enhancing further the response to A. This also explains why in normal animals injected with AB cells, the anti-A response does not occur until a certain amount of anti-B has built up in the circulation, since this is required to inhibit the competition between A and B. The effect of preimmunization with B-carrying red cells would be expected to have two antagonistic effects. On the one hand a pool of anti-B antibody would be produced, as well as an increased number of anti-B helper cells, both of which should improve the response to A; on the other hand, the increased population of Bursa-derived cells with anti-B specificity should make the competition between B and A more marked. Since the overall effect is enhancement of anti-A, the former factors are probably decisive. Finally, in B-tolerant chickens the poor response to A is due to the lack of T-helper cells recognizing B, rather than to competitive effects. There is therefore no enhancement of anti-A by passive immunization with anti-B. It should be noted that the explanation given by McBride and Schierman for their results is a different one involving the conversion of haptenic groups into carriers by combination with antibody.

A tacit assumption which has been made throughout this discussion is that suppression by antibody prevents the recognition of antigen by bone-marrow- or Bursa-derived cells (henceforward referred to as B cells) more effectively than it does recognition by helper-T cells. This would be reasonable if T cells possessed higher afinity receptors for antigen than B cells, or had a lower threshold for stimulation. Support for this preferential action of antibody on B cell recognition comes from the results of Kappler et al. (1971). They showed that doses of anti-SRBC serum which suppressed the plaque-forming response to SRBC *in vivo* failed to suppress the helper activity of spleen cells from mice immunized with SRBC when the latter was assayed in an *in-vitro* system. Also the relative insensitivity of cellular immunity to antibody (Uhr and Baumann, 1961), and the finding that rosette-forming (T) cells are less easily inhibited by antiserum than are plaque-forming (B) cells (Greaves et al., 1970), further support this hypothesis. Therefore, since antigenic competition of the sequential type seems to be a result of T-cell stimulation (Sec. II.C), it should be more difficult to inhibit than antibody production. This is indeed what was been found by Gershon and Kondo (1971 b). Injection of SRBC suppressed the response to HRBC given 4 days later, by sequential competi-

tion. Passive immunization with an anti-SRBC serum diluted 1/10 caused suppression of the antibody response to SRBC, but did not affect competition, which continued even in the absence of antibody production to the dominant antigen. Larger amounts of the anti-SRBC serum (1/4 dilution) suppressed both anti-SRBC production and antigenic competition, which would be expected since a large amount of antibody should successfully prevent antigen recognition by both T and B cells.

Another extension of this argument is that if intramolecular competition is a result of competition between B cell populations for antigen, whereas intermolecular competition is rather a side-effect of T-cell stimulation, then in a system in which both forms of competition occur, intramolecular competition should be more sensitive to inhibition by antibody than intermolecular competition. Experiments reported by BRODY et al. (1967) seem to contradict this prediction. As described in Sec. II.D, they demonstrated mutual competition between DNP and arsenilic acid (RAzo) both when the haptens were linked to the same carrier molecule (RAzo-DNP-RGG) and when they were administered on separate carrier molecules (RAzo-RGG and DNP-RGG). When anti-RAzo antiserum was given, the specific antibody response to RAzo was suppressed, but the response to DNP was enhanced only when the haptens were on separate carriers. In this case intermolecular competition was apparently more easily abrogated by antiserum than was the intramolecular form. However, large amounts of antibody were administered, so that in the intramolecular situation steric hindrance by anti-RAzo antibody may also have had a partially inhibitory effect on the recognition of DNP groups on the same molecule, counteracting any enhancement.

Finally, the importance of simultaneous enhancement and suppression by antibody in the control of the antibody response should be recognized. With multideterminant antigens, the first-formed antibody will often be directed only against a certain range of the antigenic groups available, the dominant determinants. This antibody will have the effect of limiting its own production by its specific inhibitory feedback influence. At the same time it will cause the response to be spread to other initially suppressed determinants by the abrogation of antigenic competition and by improved antigen localization. It is easy to visualize situations in the control of infections or in "immune surveillance" where the resulting increase in the diversity of the response will be beneficial.

E. Cooperation of Antigenic Determinants

Cooperation of antigenic determinants present on the same immunogenic molecule is an important event in the induction of an antibody response to many immunogens (RAJEWSKY and ROTTLÄNDER, 1967; RAJEWSKY et al., 1969; COHN et al., 1969; IVERSON, 1970; MCBRIDE and SCHIERMAN, 1970; MITCHISON et al., 1970; LINDENMANN, 1971; MÄKELÄ et al., 1971). The typical cooperation situation is that of a hapten linked to its carrier, and one of the results of cooperation is the carrier-effect, in which the hapten must be coupled

to its original carrier in order to provoke a secondary response (SALVIN and SMITH, 1960; OVARY and BENACERRAF, 1963; MITCHISON, 1967; MITCHISON, 1971 a). In studies designed to demonstrate that cooperation involves the separate recognition of both hapten and carrier, an experimental procedure has been used in which animals receive a hapten-carrier complex at the same time as a second, unrelated protein; later the animals are boosted with the hapten now conjugated to the second protein. The increased response to the hapten in these animals, compared with controls which are not primed with the second carrier protein, has been considered to be a measure of cooperation of carrier and hapten in the secondary response. Such experiments have indeed successfully demonstrated cooperation in rabbits (RAJEWSKY et al., 1969), and guinea pigs (KATZ et al., 1970), but in mice it has been necessary to transfer primed cells into irradiated recipients to demonstrate the effect (MITCHISON, 1971 b). Antigenic competition has been suggested as a possible reason for the failure of this type of experiment in normal mice (MITCHISON, 1971 b) and one can envisage two possible ways in which competition could interfere with the cooperation design. The first is intermolecular competition when mixtures of antigens are given together, or at too close an interval, in the priming stage, since this could inhibit the development of the cell populations to either carrier or hapten. The second possibility is intramolecular competition in the secondary response, in which the response to the heterologous carrier could inhibit rather than help the antibody response to the hapten. This might arise if the sizes, or affinities for antigen, of the cell populations recognizing hapten and carrier were significantly different.

An experiment to test the first of these possibilities has been described by TAUSSIG et al. (1972c). They found that competition arising in the primary response can indeed interfere with later cooperation. The immunogens used were again the synthetic polypeptides (Phe, G)-A–L, (T, G)-Pro–L and (Phe, G)-Pro–L, and for the sake of simplicity (Phe, G) was regarded as a haptenic group and A–L and Pro–L as carriers. A marked carrier effect was found when C3H/HeJ mice were primed with (Phe, G)-A–L and boosted with (Phe, G)-A–L or (Phe, G)-Pro–L; a secondary anti-(Phe, G) response only occurred with the homologous boost. Mice were then primed with mixtures of (Phe, G)-A–L and (T, G)-Pro–L in order to raise populations of anti-hapten (Phe, G) and anti-carrier (Pro–L) cells. A previous section has described how the immunizing ratio of (T, G)-Pro–L/(Phe, G)-A–L is critical to the development of the primary response and immunological memory to (Phe, G) and Pro–L. The results are summarized in Fig. 4, and reference to this shows that the optimal immunizing ratio of (T, G)-Pro–L/(Phe, G)-A–L was 1/5. With other ratios, either the primary response or immunological memory to (Phe, G) was impaired (5/1, 1/1; Fig. 4a, c), or the response to Pro–L was suppressed (1/25; Fig. 4a, b). When mice primed with (T, G)-Pro–L and (Phe, G)-A–L were later boosted with (Phe, G)-Pro–L, a cooperative effect, as judged by anti-(Phe, G) titers, was obtained, but only in the group of animals primed with a 1/5 ratio of the antigens, i.e., where no competition had occurred during

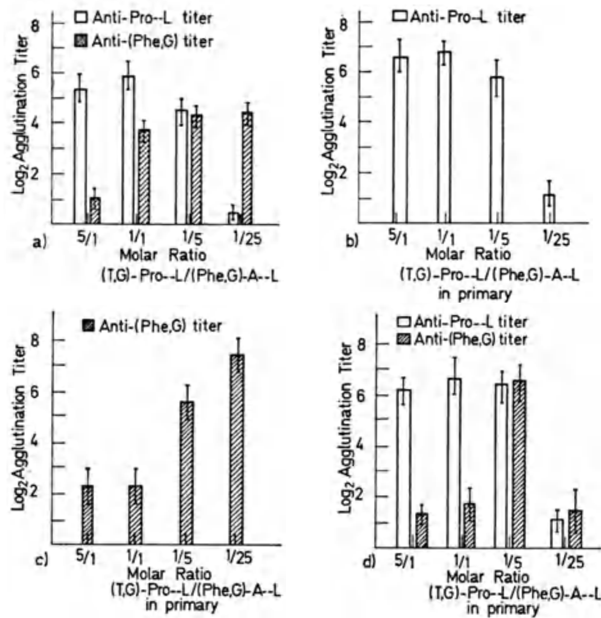


Fig. 4a–d. Antigenic competition and cooperation of antigenic determinants. a Intermolecular competition in the primary response. Primary responses of C3H/HeJ mice after inoculation of mixtures of (T, G)-Pro–L and (Phe, G)-A–L in different molar ratios. b Effect of competition on memory and priming. Secondary anti-Pro–L responses of C3H/HeJ mice after priming with mixtures of (T, G)-Pro–L and (Phe, G)-A–L in different molar ratios and boosting 3 weeks later with (T, G)-Pro–L alone. c Effect of competition on memory and priming. Secondary anti(Phe, G) responses of C3H/HeJ mice after priming with mixtures of (T, G)-Pro–L and (Phe, G)-A–L in different molar ratios and boosting 3 weeks later with (Phe, G)-A–L alone. d Effect of competition on cooperation of antigenic determinants. Anti-(Phe, G) and anti-Pro–L responses of C3H/HeJ mice primed with mixtures of (T, G)-Pro–L and (Phe, G)-A–L in different molar ratios and boosted with (Phe, G)-Pro–L

priming (Fig. 4d). The finding that cooperation could be demonstrated in intact mice without adoptive transfer of cells is in itself significant. These results show that in a system sensitive to antigenic competition, the conditions of priming may be critical since competition may restrict the sizes of cell populations stimulated. Giving the antigens in separate primary injections spaced by about 30 days should overcome this possibility.

The failure of this cooperation design in normal mice in other systems is not, however, so easily explained. For example, Mitchison (1971b) has described experiments in which mice were immunized with NIP-BSA with or a mixture of NIP-OA and BSA, and were then boosted with NIP-BSA. Cooperation could not be demonstrated in these animals, but could be shown to occur if spleen cells from the same animals were transferred into irradiated hosts which were challenged with NIP-BSA. Cell populations adequately primed to both NIP and BSA therefore existed. Leaving aside the contribution of circulating antibody, these results raise the possibility that in the normal

animals intramolecular competition between BSA and NIP occurred (with BSA the dominant antigen) and that such competition had a much less pronounced inhibitory effect with the transferred population in irradiated hosts. The hypothesis that competition is between different cell populations for antigen would in fact predict such a situation. With the relatively lower numbers of sensitive cells in the transferred population compared with the whole animal, intramolecular competition should occur to a much lesser extent.

A related example in which competition interfered with cooperation is that described by TAYLOR and IVERSON (1971). These authors found that skin-painting mice with oxazolone (Ox) enabled it to compete against rather than cooperate with DNP in a secondary response to Ox-DNP-BSA in animals primed with DNP-BGG. The point was made that skin-painting is generally a good means of raising a helper-cell population, as evaluated by cell-transfer experiments. The competition experiments reported, which were carried out without cell transfer, may be taken as further evidence that competition is more effective in intact animals than in irradiated recipients of transferred cells.

Another similar experimental situation relating cooperation and competition is one where animals are prepared for a response to a hapten by prior injection of carrier alone, in the expectation that carrier-primed cells will help the response when the hapten-carrier complex is given later. In some cases the enhanced anti-hapten response is seen (SALVIN and SMITH, 1960; KATZ et al., 1970; MCBRIDE and SCHIERMAN, 1970; LINDENMANN, 1971), but in others there is instead suppression of the primary response to the hapten (DUBERT, 1956; COE and SALVIN, 1964; ASHLEY and OVARY, 1965; RITTENBERG and AMKRAUT, 1966; RAJEWSKY et al., 1969). In these latter cases, intramolecular competition may be responsible, and may be interpreted as successful competition for limiting antigen by a large population of carrier-specific memory cells against the smaller, unprimed population of cells recognizing hapten.

F. Immune Surveillance

A possibility which has received little attention until now is that antigenic competition may have an influence on tumor initiation and development. The central role of the immune system in preventing the appearance of tumors is well established (SMITH and LANDY, 1970), and factors which interfere with the efficiency of recognition of tumor-specific antigens would be expected to improve the chances of tumor growth. Examples which may be attributable to antigenic competition have been described. METCALF (1961), for example, showed that the incidence of reticular tumors was increased in mice subjected to prolonged antigenic stimulation by *Salmonella adelaide* flagellar antigen or BSA. SIEGEL and MORTON (1966) found that injection of BSA in adjuvant into BALB/c mice increased the infectivity of Rauscher leukaemia virus given shortly after. In these instances the immunosuppression caused by the first antigenic stimulus may well have facilitated tumor initiation.

Another possibility is that the immunosuppressive effect of many oncogenic viruses themselves is also related to sequential competition. PETERSON et al. (1963), for example, have described the suppressive effect of Gross passage A virus on the immune response to T2 bacteriophage; DENT et al. (1965) showed that C3H mice injected with the same virus failed to reject skin grafts across a non-H2 barrier. SALAMAN and WEDDERBURN (1966) observed that Friend virus suppressed the response to SRBC if given before, but not after SRBC. In this case, active virus was necessary for the suppression, and a non-oncogenic virus (Riley) had no immunosuppressive effect. Some of these results resemble sequential competition, in which the virus would provide the first antigenic stimulus. On the other hand, if virus-induced immunosuppression is to contribute to oncogenesis, it would probably have to be more effective than the known examples of antigenic competition. By analogy with the postulated release of inhibitors by thymus-derived cells as the cause of sequential competition, it may be suggested that similar inhibitors are released in quantity by virus-infected cells, leading to non-specific immunosuppression. An alternative suggestion is that infection of precursors with virus renders them refractory to antigenic stimulation, so that in effect virus and antigen would compete for the same cells. This is indicated, for example, by the reduction in the number of antigen-sensitive cells in mice injected with Friend virus (FRIEDMAN and CEGLOWSKI, 1968).

IV. Antigenic Competition and Theories of the Immune Response

Antigenic competition has been considered a controversial subject mainly because of the interpretation that antigen-sensitive cells are multipotential in their ability to recognize and respond to antigen. If cells are precommitted, it has been argued, there should be no interference by one antigen in the response to another (e.g. JERNE, 1967; BUSSARD, 1970). The papers by LAWRENCE and SIMONSEN (1967), ALBRIGHT and MAKINODAN (1965), and ALBRIGHT et al. (1970) contain the main points of the argument. LAWRENCE and SIMONSEN (1967) used antigenic competition as part of a general argument, derived initially from a concept of antigenic strength, against clonal selection. Antigenic strength was postulated as being a function of the number of cells available to respond to a particular antigen, with strong antigens being able to engage all available antigen-sensitive cells, and weak antigens being recognized by only a few cells. Supporting evidence for this was that graft-versus-host (GvH) reactivity could be improved by preimmunization for weak but not for strong antigens. Subsequently, it was shown that the number of cells able to respond in a GvH reaction against strong histocompatibility antigens of animals of the same species is very much higher than would be expected from a random distribution of reactivity (SIMONSEN, 1967; NISBET et al., 1969). From this it was supposed that cells engaged in a vigorous immune response to one antigen would show reduced responsiveness to other antigens, that strong antigens should be better competitors than weak, and that the

ability of weak antigens to compete should be improved by preimmunization. These predictions were borne out by the results obtained by LAWRENCE and SIMONSEN. Irradiated F1 hybrids were inoculated with parental lymphoid cells; subsequently SRBC were injected and the PFC response measured. It was found that the response to SRBC injected 7–10 days after parental cells was strongly inhibited, which is interpreted as antigenic competition of the sequential type in which the GvH reaction is the first antigenic stimulus. The response to SRBC given 3 days after parental cells, however, was not suppressed. Weak GvH reactions were much less suppressive than strong ones, but the competitive effect of weak reactions was enhanced by priming. Furthermore, the secondary response to SRBC was also easily inhibited by a GvH response, which suggested that memory cells were also multipotential.

Before interpretation of these experiments is attempted it must be recognized that the GvH reaction is mediated by T cells, and that the antibody response to SRBC is T-dependent. Therefore arguments of multipotency need apply only to T cells and not to B cells (see also TALMAGE et al., 1971). Further, it is possible that the antigen-sensitive cell population does indeed contain a very high percentage of cells recognizing allogeneic histocompatibility antigens while maintaining genetic precommitment of each cell to a single antigen specificity (or at most 2 specificities). This hypothesis was proposed by JERNE (1971) and incorporates the findings of SIMONSEN into the clonal selection theory. Even the experimental findings of LAWRENCE and SIMONSEN are not always compatible with multipotentiality. Thus 3 days should be sufficient time to commit cells to a GvH reaction, yet competition only became manifest after 7–10 days. The inference that memory cells are no more committed than naive cells is also unattractive in view of the specificity of immunological memory. An alternative hypothesis to cellular multipotentiality to account for sequential antigenic competition is that the first response results in the release of inhibitors by T cells which both control the extent of the response in progress and inhibit the initiation of responses to antigens given subsequently. The evidence for this now seems quite strong. MÖLLER (1971), for example, performed experiments similar to those of LAWRENCE and SIMONSEN, inducing GvH reactions by injection of parental cells into untreated F 1 hybrids. The response to SRBC given 7 days later was inhibited. Since the hosts were not irradiated, this inhibition extended to both host and donor cells, a finding which was not due to destruction of host cells. A significant result was that F1 hybrids which were irradiated after being subjected to a GvH reaction would not support anti-SRBC production by transferred, normal parental or F1 cells. It therefore appeared that the environment within the recipient had become inhibitory and would not allow the initiation of other immune responses. This 'inhibitor hypothesis', discussed further in Sec. V.C, not only accounts successfully for most of the observations of sequential competition, including those of LAWRENCE and SIMONSEN, but does so within the widely accepted framework of clonal selection. In these circumstances, antigenic competition seems rather a weak building block for a theory of cellular multipotentiality.

V. The Mechanisms of Antigenic Competition

From the account of antigenic competition given here, one may conclude that the term covers three distinct phenomena which very probably have different mechanisms. They have been termed intramolecular competition, intermolecular competition with antigen mixtures, and intermolecular competition with sequential administration of antigens (sequential competition). It is quite clear that one model will not suffice to account for all the observations, and it appears that three distinct mechanisms exist.

A. Intramolecular Competition

The model proposed to account for the competition between antigens present on the same molecule for the immune response has already been outlined in previous sections. It is an extension of the theory that during the induction of the immune response cells are selected by antigen. This theory and the evidence for it have been described by SISKIND and BENACERRAF (1969), and it accounts successfully for many features of the immune response. In brief, cells are assumed to be genetically precommitted to the production of antibody of one type and specificity; antigen is presumably recognized by cell-bound receptors identical to the antibody secreted by the cell; and a range of cells able to bind a particular antigen will therefore exist, those bearing the highest affinity receptors being the rarest. When antigen is introduced it acts as a selective pressure which becomes greater as its concentration falls. The result is that with respect to every antigenic determinant there will be competition between antigen-sensitive cells, in which those cells bearing receptors of best fit (highest affinity) for that determinant will be progressively more successful as the antigen supply becomes more limiting. This hypothesis of cell-selection by antigen explains the maturation of the immune response (increase in antibody affinity with time), the effects of antigen dose on the amount and affinity of the antibody produced, the low affinity of the antibody found in a state of partial tolerance, and the suppression of antibody production, and selection of higher affinity antibody, by passively administered antibody.

These arguments may be adapted to the case of intramolecular competition if it is borne in mind that an antigen bearing several determinants of different specificities will be recognized by an equivalent number of different populations of specific antigen-sensitive cells. Whenever antigen is limiting in amount, a competition will be set up between cell populations of different specificities for antigen. If some of these populations are of higher cell number than others, or if they have a higher average affinity for antigen, they will capture antigen preferentially. The result will be that antibody production against certain of the determinants on a complex antigen will predominate, at the expense of antibody production against others. The dominance of certain determinants in intramolecular competition is therefore the result of the greater number of cells bearing receptors for those determinants, or their greater affinity, compared with the number and affinity of cells bearing receptors for other

determinants on the same molecule. There may be several reasons why there should be more precursor cells available for some regions of a molecule than for others, for example some immunopotent areas may be antigenically more complex than others, genetic control of the ability to respond will specifically limit the number of precursors available for certain determinants, and prior contact with another, crossreacting antigen may have resulted in immunological memory to some determinants. Thus there is no basic contradiction to the clonal selection theory, which postulates a random distribution of antigen-sensitive cells arising by processes of somatic mutation.

Predictions arising from this hypothesis include the following. (i) Intramolecular competition is a function of antigen dose and is more pronounced at low antigen concentrations than at high. Furthermore, since competition depends on the ratio of antigen-sensitive cells to antigen, it varies with total cell number. This relationship will be demonstrable by cell transfer into irradiated recipients. (ii) Where intramolecular competition occurs, it will be possible to demonstrate differences in the numbers of cells available for the competing determinants, for example, by limiting dilution analysis, or differences in the binding affinities of antibodies raised to the separate determinants. (iii) Where the cell populations recognizing certain determinants have been enlarged by prior immunization, the response to those determinants will in many cases be dominant and suppress the response to others on the same molecule. A secondary response will therefore be more effective in intramolecular competition than a primary response. (iv) Insofar as tolerance and passive antibody administration impair the recognition of or response to specific determinants, they will also prevent the competitive effect of dominant determinants. Passive antibody administration would therefore be expected to have both suppressive and enhancing effects on antibody production (see Sec. III.D.) (v) Intramolecular competition will occur with both thymus-dependent and independent antigens. Many of these predictions have been confirmed by the results described in preceding sections.

It is possible to predict the role of different cell types in intramolecular competition from theoretical considerations. For thymus-dependent antigens let us assume for the sake of simplicity that all determinants are equally capable of cooperating with others on the same molecule, and that, in cooperation, trapping of antigen through recognition of one determinant will present all other determinants equally well to B cells (with the restriction that two different specificities must be present for immunogenicity (RAJEWSKY et al., 1969). If this is correct, then competition between T cells for antigen should not lead to preferential antibody production to any particular determinant. The cell-type involved in intramolecular competition must therefore be the antibody-forming cell precursor or B cell. One possibility, illustrated in Fig. 5 (a) is that competition between B cell populations takes place after antigen has been localized at the surface of T cells, dendritic cells, or macrophages (MITCHISON et al., 1970; NOSSAL and ADA, 1971). The requirement for antigen localization and presentation may in this way contribute to the limitation of

antigen supply. Alternatively, B cells could compete for circulating antigen, being triggered to antibody production by later contact with T cells, as suggested by KRETH and WILLIAMSON (1971). Completely T-independent antigens could then also be expected to show intramolecular competition. These arguments apply to competition for antibody production. Competition for antigen between T cells should, on the other hand, lead to the finding of inhibition of cellular reactions in the same way. Intramolecular competition in delayed hypersensitivity is in fact known to exist.

This hypothesis, that B cells of different specificities compete for antigen, is supported most directly by the experiments with competition between the (Phe, G) and Pro–L regions of (Phe, G)-Pro–L described in Sec. II.B. Pro–L was the dominant region in a high responder strain, C3H/HeJ but not in the low responders DBA/1. The poor response in the latter was shown to be correlated with a reduced number of precursors responsive to Pro–L in the B population (and not in the T population). Furthermore, procedures which corrected the low response in DBA/1, such as administration of poly A:U, were shown both to increase the numbers of B precursors available for Pro–L and to restore the dominance of Pro–L in intramolecular competition. The experiments of ROELANTS and GOODMAN (1970) also directly support the role of antibody-forming cell precursors in competition. Helper cells for poly-γ-D-glutamic acid (PGA) are apparently absent in rabbits, since PGA is non-immunogenic unless complexed with methylated (m)BSA, cannot act as a carrier for haptens, and cannot give rise to delayed hypersensitivity. Nevertheless, PGA was dominant in competition against mBSA when the PGA-mBSA complex was injected. Tolerance to PGA abolished its ability to compete. It is difficult to see how in this case competition could have occurred other than by activity of B cells.

A schematic diagram of the competition envisaged between B cells, and the effects of antibody, is given in Fig. 5a, b. It is interesting—and encouraging—to note that since this hypothesis was formulated, an extremely similar model has appeared (DUTTON, 1972), which, although based on quite different observations, also proposes that B cells compete for localized antigen.

B. Intermolecular Competition: Antigen Mixture

This form of competition is perhaps the most difficult to interpret. Assuming the genetic precommitment of antigen-sensitive cells, antigens which are not linked together should be recognized separately. The existence of intermolecular competition, on the other hand, implies a common event in the recognition or response to different antigens. One way of reconciling these ideas is to invoke an antigen-handling system through which many antigens pass. This has generally been taken to mean macrophage processing of antigen, and some authors have argued that this could be a rate-limiting stage at which direct competition between antigens could occur (BRODY and SISKIND, 1969). However, there is strong evidence against this view, the principal ob-

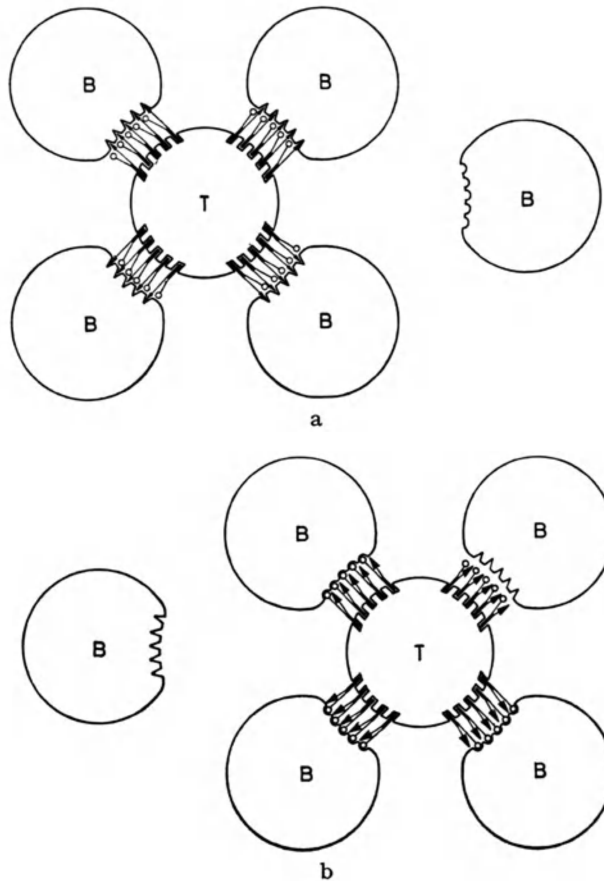


Fig. 5a and b. A model for intramolecular competition. a Competition between bone marrow derived (B) cells of different specificities for antigen. An antigen composed of a carrier ■ bearing two haptenic groups ↑ and ♀ is shown presented at the surface of a thymus-derived (T) cell (though this could also be a macrophage or dendritic cell). The antigen forms a multivalent bridge across to B cells. B cells for the ↑ specificity are present in larger numbers (or bear higher affinity receptors) than those for the ♀ specificity and are therefore more successful in competing for limited antigen. ↑ is thus the dominant determinant and antibody production to ♀ is suppressed. b Abolition of intramolecular competition by antibody against the dominant determinant. Antibody V against the determinant ↑ specifically blocks the combination of that determinant with B cell receptors. B cells bearing receptors for the initially suppressed determinant ♀ are now able to combine with antigen. The result is suppression of antibody formation against ↑ and enhancement of that against ♀

jection being that intermolecular competition is often abolished by tolerance to the dominant antigen, whereas uptake of antigen by macrophages is apparently unaffected by tolerance (Sec. III.A). Another possibility is that the recognition of the dominant antigen results in the release of non-specific inhibitory agents which would act to suppress the response to other antigens. Although this type of explanation adequately accounts for sequential inter-

molecular competition, it is difficult to see why such agents should inhibit the response to one antigen rather than the other when antigens are given simultaneously.

Two models may be suggested to account for this type of competition. In the first a common presentation mechanism for all antigens is proposed, namely, the surface of reticular dendritic cells of the follicles of lymph node and spleen. The evidence that this does play an important role in antigen localization and presentation *in vivo* has been extensively reviewed (NOSSAL and ADA, 1971). The model, described by TAUSSIG and LACHMANN (1972) assumes that antigen is localized in the first place by a "co-operating antibody" (also termed IgX) which is directed against carrier-specific determinants. The existence of antibody of a special class produced by T cells to mediate cooperation has been discussed by several authors (BRETSCHER and COHN, 1968, 1970; MITCHISON, 1969b; MITCHISON et al., 1970; NOSSAL and ADA, 1971). The present model proposes that initial recognition of antigen by T cells results in the release of IgX, which becomes attached to dendritic cell membranes and thereby presents antigen to specific B cells. If the number of sites for attachment of IgX is limited, a competition for sites will occur between cooperating antibodies directed towards different antigens. An excess of IgX directed against one antigen will therefore competitively inhibit the presentation of other antigens. The production of IgX is assumed to be dependent on antigen dose, in order to account for the dose effects of competition. The model is presented schematically in Fig. 6a.

This scheme was originally proposed to account for all forms of competition, but it is now clear that it could only apply to the intermolecular case. It has a specific advantage, namely, the suggestion that cooperating antibody may also act as an inhibitor of the immune response. In this role it would clearly have the semi-specific properties implied in this type of competition, in that while specifically promoting the response to the antigen which induced it, it would non-specifically inhibit that to other antigens. This scheme however, also has certain disadvantages. It is difficult for example to envisage that sites on dendritic cells for presentation of antigen should be so limited that competition for them could easily occur. Their function as a presentation device would seem unnecessarily restricted if this were the case. In some instances the localization of antigens in follicles during competition has in fact been followed and shown not to be significantly decreased (HANNA and PETERS, 1970; RHODES and LIND, 1971). A variation on this model may therefore be suggested which retains the semispecific properties of IgX as an inhibitor. This proposes that, after its release by T cells, IgX becomes attached, not to the dendritic cell, but directly to the B cell, by way perhaps of receptors for its Fc portion. In this way it would aid the capture and 'focussing' of antigen by B cells specific for the antigen in question, and would temporarily block the IgX site of other B cells (Fig. 6b). Fc receptors for conventional antibody and antigen-antibody complexes have been demonstrated (DUKOR et al., 1970; BASTEN et al., 1972), and it is interesting that PARASKEVAS et al.

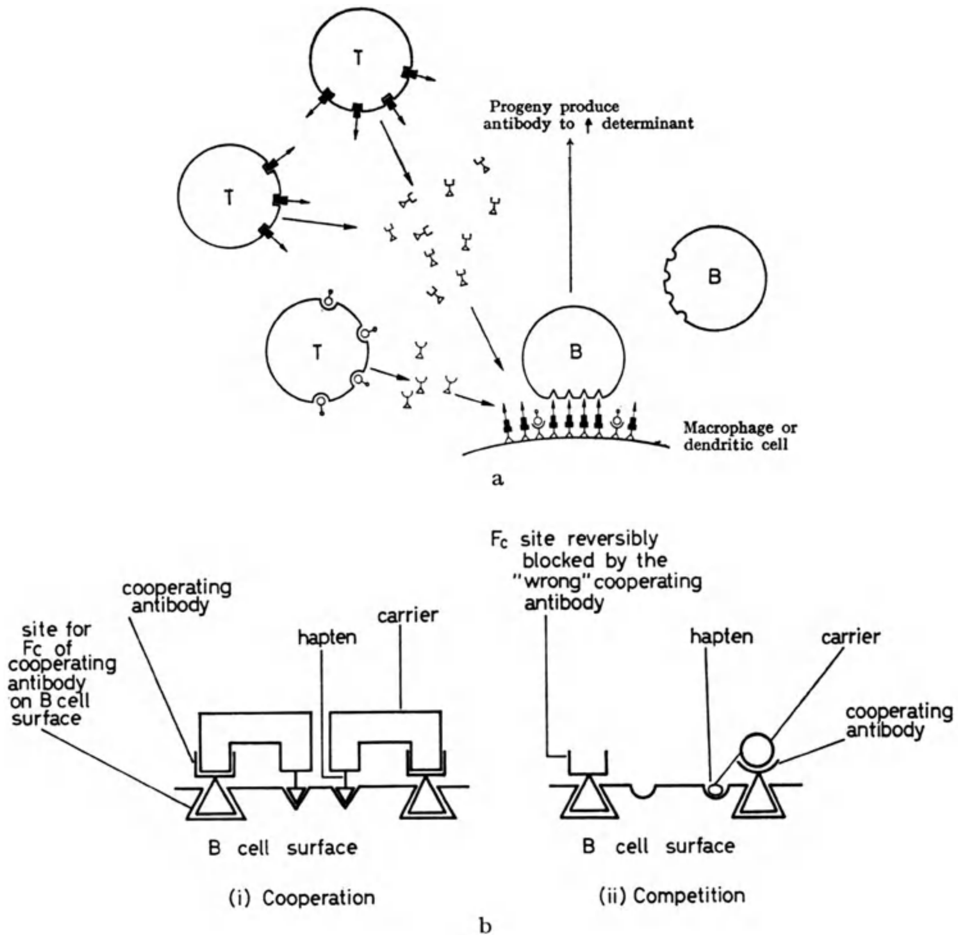


Fig. 6a and b. Models for intermolecular competition. a Intermolecular competition. between two antigens ♠ and ♂ each composed of a carrier ■, ○, bearing a hapten ↑, ♀. Specific thymus-derived (T) cells recognize carrier determinants and release specific "cooperating antibodies" ⅄ and ⅄. These in turn become attached to sites on the surface of macrophages or dendritic cells and thereby present antigen to specific bone marrow-derived (B) cells. If these sites for antigen presentation are limited in number, cooperating antibodies will compete for them as shown. b In this model for intermolecular competition, cooperating antibodies ⅄ and ⅄ (specific for the antigens ⊓ and ♂), after their release by specific T cells, become attached to sites on the B cell surface recognizing their Fc portion. There they trap and "focus" antigen, which must also be recognized by specific B cell receptors, as shown in (i). Competition arises when the two antigens are administered together, and the cooperating antibody for one antigen occupies Fc sites on B cells bearing specific receptors for the other antigen, as shown in (ii)

(1972) have demonstrated Fc receptors on lymphocytes which become inaccessible for a certain period soon after antigen administration.

Either of these models suggesting competition by cooperating antibody, although admittedly speculative in nature, would adequately explain two

of the key characteristics of this type of competition, namely, (i) a dependence on the relative amounts of the antigens involved, and (ii) a lack of dependence on antibody production to the dominant antigen. The dose-dependence would be related to the numbers of antigen sensitive T cells stimulated by different antigen concentrations. The T cell causation of the competition is suggested by the ability of non-immunogenic molecules, such as Pro–L in DBA/1 mice, or A–L, in C3H/HeJ, to take the dominant role in competition. These molecules can act as carriers for haptenic groups, suggesting that they can be recognized by T cells; this T cell recognition would then lead to competition. Both models would also make the prediction that intermolecular competition should not affect the response to T-independent antigens, although such antigens may be capable of stimulating T cells and thus could be dominant themselves in competitive situations. It should be noted that both models would easily accommodate B cell competition, as proposed for intramolecular competition in the preceding section.

C. Intermolecular Competition: Sequential Competition

Many of the effects found when antigens are administered in sequence can be explained by a hypothesis which postulates the production of non-specific inhibitors of the immune response. The main findings this hypothesis has to account for are the following (for references see Sec. II and IV): (i) The antigen administered first in a sequence generally behaves as the dominant antigen, and suppresses the response to other antigens given within approximately the following 10 days. Very often these antigens do not interfere with each other when administered together. (ii) The time during which the competitive effect occurs is not always related to the peak of the immune response to the first antigen. For example, a similar time-course may apply to inhibition by prior injection of heterologous erythrocytes or of protein antigens. (iii) A secondary antigenic stimulus generally has a more marked effect than does a primary. (iv) In general it is not necessary to inject the antigens at the same site. (v) In cell-transfer systems, competition by the dominant antigen increases with the number of the cells transferred, suggesting that it is not not for the commitment of multipotent cells (RADOVICH and TALMAGE, 1967). (vi) The involvement of T cells in sequential competition has been demonstrated (GERSHON and KONDO, 1971 a). Cellular immunity, involving mainly T cells, can interfere with the development of antibody production and cell-mediated responses to unrelated antigens (HOWARD and WOODRUFF, 1961; LAWRENCE and SIMONSEN, 1967; LAPP and MÖLLER, 1969; MÖLLER, 1971). Antibody production can also inhibit cell-mediated responses such as homograft rejection (EIDINGER et al., 1968). (vii) The internal environment of animals receiving antigenic stimulation remains inhibitory even after irradiation (MÖLLER and SJÖBERG, 1970; MÖLLER, 1971). (viii) Sequential competition may be abolished by antibody against the dominant antigen, but the threshold

amount of antibody required to inhibit competition is higher than that to specifically suppress antibody production (GERSHON and KONDO, 1971b). This is the opposite of the finding with intramolecular competition, but is in keeping with a T-cell causation of sequential competition.

The hypothesis for sequential competition is that shortly following the recognition of antigen by T lymphocytes, the stimulated cells release factors which are inhibitory to cell stimulation and division. It is envisaged that these inhibitors serve the important function of regulating the extent of the response by their effect on cell proliferation. This would supplement the feedback control exerted by antibody. Although secreted specifically by T cells, the inhibitors would act on both T and B cells. When a second antigen is injected a certain critical time after a first antigen, it is thus introduced into an already inhibitory milieu, with the result that the suppression observed as sequential competition occurs. Several authors have described inhibitors obtained from normal or immune sera, or culture supernatants including an α-globulin described by MOWBRAY (1963) and COOPERBAND et al. (1968), an inhibitor found by AMBROSE (1969) in stimulated lymph node cultures, and one found in normal and immune mouse serum by VEIT and MICHAEL (1972). Whatever the nature of the inhibitor, the hypothesis explains many of the observations of sequential competition. The effect would be expected to a general one, involving many unrelated antigens, which it does in fact seem to be. The antigens involved in sequential competition would not necessarily compete when administered together, although a certain mutual inhibition might be expected in some cases. A secondary response would be both a more effective competitor and more difficult to inhibit by competition. Furthermore, since the inhibitors would circulate, the relative sites of injection of the antigens would not be an important consideration, which has often been confirmed in sequential competition (EIDINGER et al., 1968; O'TOOLE and DAVIES, 1971) and contrasts with the local nature of the competition with antigen mixtures (BRODY and SISKIND, 1969). It would also be predicted that antigens that do not stimulate T cells would not be able to give rise to sequential competition. On the other hand, the response to T-independent antigens would be inhibited by sequential competition, and the limited results available suggest that this is probably the case (MÖLLER, 1971; KERBEL and EIDINGER, 1971). This prediction also differs from that for competition between antigen mixtures (Sec. V.B).

The assumption has been made here that the inhibitors responsible for sequential competition would be totally non-specific in their effect. In this case the response to the same antigen given for a second time shortly after its first inoculation would be expected to be inhibited. O'TOOLE and DAVIES (1971) have in fact observed this; they showed that intraperitoneal injection of either sheep RBC or horse RBC to mice inhibited the response to sheep RBC given subcutaneously up to 10 days later. However, it is not certain that 'preemption', as the effect was termed, by the homologous antigen is of the same origin as antigenic competition. For example, the preinjection of sheep RBC

had a much more rapid effect than horse RBC, and it seems likely that trapping of specific cells in the spleen as a result of the first injection of sheep RBC also contributed to the suppression.

An alternative theory which might be mentioned here has been put forward by KERBEL and EIDINGER (1971). They noted a correlation between the ability of an antigen to compete, and an increase in spleen size coupled with a reduction in the proportion of cells bearing the theta antigen at the time of maximal competition. It was suggested that the response to the first antigen 'diluted out' T and B cells, interaction of which was essential for the response to the second antigen, and hence caused suppression of the latter. A predicition was made that T-independent antigens would not be affected by competition, and it will be noted that this is the opposite of what is expected in the action of inhibitors. However, the model presented by these authors has several weaknesses, not the least of which is the treatment of the spleen as merely a repository of different interacting cell types, presenting a very misleading oversimplification of the processes involved in immune induction. More important is the fact that the predictions of the model are not borne out in practice. For example, sequential competition is not confined to the site of response to the first antigen, as the model would suggest. Nor is a secondary response, which was found to cause less splenic enlargement than a primary, less able to compete successfully than a primary response, as the authors' data suggest it should be; indeed the opposite has often been shown to be true.

VI. Practical Aspects of Competition

One of the important practical aspects of antigenic competition is its relation to human vaccination. The studies of BARR and LLEWELLYN-JONES (1953a, b, 1955) investigated some of the factors to be taken into account when toxoids and vaccines are administered either in sequence or as mixtures. Their main conclusions, which should be borne in mind in devising immunization schedules for babies and adults, were the following. (i) When several inoculations of different vaccines, toxoids, etc., are to be given in a series, it is important to leave an interval of at least 30 days between injections in order to avoid the possibility of sequential competition. (ii) In the desire to cut down the number of injections in a vaccination programme to a minimum, it is now usual to administer mixtures known as "combined prophylactics". Probably the best known is the triple vaccine (diphtheria and tetanus toxoids with pertussis vaccine). It is obviously necessary to ensure first that such mixtures are 'balanced', so that optimum immunization against all components is achieved, and to avoid the suppression of the response to any component by intermolecular competition. Furthermore, if there is pre-exisiting immunity, it should apply equally to all the components of the mixture, otherwise there is the danger that a secondary response to some components will inhibit the primary response to others. Is should be note that a previous infection may be equivalent in effect to vaccination. Suitable schedules for

multiple vaccinations have been recommended (PARISH and CANNON, 1962). Further discussion of these points can be found in an International Symposium on Combined Vaccines (1967) and in OAKLEY (1968) and COCKBURN (1969).

References

ADA, G. L., NOSSAL, G. J. V., AUSTIN, C. M.: Antigens in immunity. V. The ability of cells in lymphoid follicles to recognise foreigness. Aust. J. exp. Biol. med. Sci. **42**, 331–346 (1964).

ADLER, F. L.: Competition of antigens. In: H. SHAFFER, G. A. LOGRIPPO and M. W. CHASE, eds., Mechanisms of hypersensitivity, p. 539–546. Boston: Little, Brown & Co. 1959.

ADLER, F. L.: Competition of antigens. Progr. Allergy **8**, 41–57 (1964).

ALBRIGHT, J. F., MAKINODAN, T.: Dynamics of expression of competence of antibody-producing cells. In: J. STERZL, ed., Molecular and cellular basis of antibody formation, p. 427–446. New York: Academic Press 1965.

ALBRIGHT, J. F., OMER, T. F., DEITCHMAN, J. W.: Antigenic competition: antigens compete for a cell occurring with limited frequency. Science **167**, 196–198 (1970).

AMBROSE, C. T.: Regulation of the secondary antibody response *in vitro*. Enhancement by actinomycin D and inhibition by a macromolecular product of stimulated lymph node cultures. J. exp. Med. **130**, 1003–1029 (1969).

AMKRAUT, A. A., GARVEY, J. S., CAMPBELL, D. H.: Competition of haptens. J. exp. Med. **124**, 293–306 (1966).

ASHLEY, H., OVARY, Z.: Effect of bovine gamma globulin on subsequent immunisation with dinitrophenylated bovine gamma globulin. Proc. Soc. exp. Biol. (N.Y.) **119**, 311–314 (1965).

BARR, M., LLEWELLYN-JONES, M.: Some factors influencing the response of animals to immunisation with combined prophylactics. Brit. J. exp. Path. **34**, 12–22 (1953a).

BARR, M., LLEWELLYN-JONES, M.: Interference with antitoxic responses in immunisation with combined prophylactics. Brit. J. exp. Path. **34**, 233–240 (1953b).

BARR, M., LLEWELLYN-JONES, M.: Some factors influencing the response to immunisation with single and combined prophylactics. Brit. J. exp. Path. **36**, 147–154 (1955).

BASTEN, A., SPRENT, J., MILLER, J. F. A. P.: Receptor for antibody-antigen complexes used to separate T cells from B cells. Nature (Lond.) New Biol. **235**, 178–180 (1972).

BAUMINGER, S., SCHECHTER, I., SELA, M.: Specificity of immunological tolerance to poly-DL-alanyl proteins. Immunochemistry **4**, 169–181 (1967).

BENACERRAF, B., GREEN, I., BLUESTEIN, H. G., ELMAN, L.: Specific immune responses of guinea pigs. In: AMOS, D. B. (ed.), Progress in immunology, vol. 1, p. 487–494. New York: Academic Press 1971.

BEN-EFRAIM, S., LIACOPOULOS, P.: Inhibition, no effect or enhancement of immune response following injections of mixtures of immunogenic and non-immunogenic synthetic polypeptides. Immunology **12**, 517–524 (1967a).

BEN-EFRAIM, S., LIACOPOULOS, P.: Inhibition of delayed hypersensitivity in guinea pigs after competition between synthetic antigens. Nature (Lond.) **213**, 711–713 (1967b).

BEN-EFRAIM, S., LIACOPOULOS, P.: The competitive effect of DNP-poly-L-lysine in responder and non-responder guinea pigs. Immunology **16**, 573–580 (1969).

BEN-EFRAIM, S., LIACOPOULOS, P., HAREL, S., GILLE, F.: The immune response to DNP-poly-L-lysine in randomly bred guinea pigs and its relation to antigenic competition. In: H. PEETERS (ed.), Protides of the biological fluids, 17th Colloquium, p. 181–183 (1970).

BRAUN, W., ISHIZUKA, M., WINCHURCH, R., WEBB, D.: Cells and signals in immuonlogical non-responsiveness. In: Tolerance to microbial antigens, ed. H. FRIEDMAN, Ann. N.Y. Acad. Sci. **181**, 289–300 (1971).

BRETSCHER, P. A., COHN, M.: Minimal model for the mechanism of antibody induction and paralysis by antigen. Nature (Lond.) **220**, 444–448 (1968).

BRETSCHER, P., COHN, M.: A theory of self-nonself discrimination. Science **169**, 1042–1049 (1970).

BRODY, N., WALKER, J. G., SISKIND, G. W.: Studies on the control of antibody synthesis. Interaction of antigenic competition and suppression of antibody formation by passive antibody on the immune response. J. exp. Med. **126**, 81–91 (1967).
BRODY, N. I., SISKIND, G. W.: Studies on antigenic competition. J. exp. Med. **130**, 821–832 (1969).
BRODY, N. I., SISKIND, G. W.: Studies on antigenic competition. II. Evidence for effect at level of antigen 'processing'. Immunology **22**, 75–86 (1972).
BUSSARD, A. E.: Difficulties of clonal selection. In: J. ŠTERZL and I. RIHA, eds., Developmental aspects of antibody formation and structure, p. 983–989. New York-London: Academic Press 1971.
CELADA, F.: The cellular basis of immunologic memory. Progress Allergy **15**, 223–267 (1971).
CELADA, F., ELLIS, J., BODLUND, K., ROTMAN, B.: Antibody-mediated activation of a defective β-D-glactosidase. II. Immunological relationship between the normal and defective enzyme. J. exp. Med. **134**, 751–764 (1971).
COCKBURN, W. C.: World problems in viral vaccines. In: D. G. EVANS, ed., Immunisation against infectious diseases. Brit. med. Bull. **25**, 121–125 (1969).
COE, J. E., SALVIN, S. B.: The immune response in the presence of delayed hypersensitivity or circulating antibody. J. Immunol. **93**, 495–510 (1964).
COHN, M., NOTANI, G., RICE, S. A.: Characterisation of the antibody to the C-carbohydrate produced by a transplantable mouse plasmacytoma. Immunochemistry **6**, 111–123 (1969).
COOPERBAND, S. R., BONDEVICK, H., SCHMID, K., MANNICK, J. A.: Transformation of human lymphocytes: inhibition by homologous alpha globulin. Science **159**, 1243–1244 (1968).
DENNERT, G., POHLIT, H., RAJEWSKY, K.: Co-operative antibody: a concentrating device. In: MÄKELÄ, O., A. CROSS and T. U. KOSUNEN, eds., Cell interactions and receptor antibodies in immune responses, p. 3–7. London-New York: Academic Press 1971.
DENT, P. B., PETERSON, R. D. A., GOOD, R. A.: A defect in cellular immunity during the incubation period of passage A leukaemia in C3H mice. Proc. exp. Biol. (N.Y.) **119**, 869–871 (1965).
DRESSER, D. W., MITCHISON, N. A.: The mechanism of immunological paralysis. Advanc. Immunol. **8**, 129–181 (1968).
DUBERT, J.-M.: Sur la nature de la reponse secondaire. C. R. Acad. Sci. (Paris) **243**, 1939–1941 (1956).
DUKOR, P., BIANCO, C., NUSSENZWEIG, V.: Tissue localisation of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. Proc. nat. Acad. Sci. (Wash.) **67**, 991–997 (1970).
DUKOR, P., DIETRICH, F. M.: The immune response to heterologous red cells in mice. V. The effect of cyclophosphamide and cortisone on antigenic competition. J. Immunol. **105**, 118–125 (1970).
DUTTON, R. W.: In: J. UHR and M. LANDY, eds., Immunologic intervention, p. 66–70. New York: Academic Press 1971.
EIDINGER, D., KHAN, S. A., MILLAR, K. G.: The effect of antigenic competition on various manifestations of humoral antibody formation and cellular immunity. J. exp. Med. **128**, 1183–1200 (1968).
EISEN, H. N., LITTLE, J. R., STEINER, L. A., SIMMS, E. S., GRAY, W.: Degeneracy in the secondary immune response: stimulation of antibody formation by cross-reacting antigens. Israel J. med. Sci. **5**, 338–351 (1969).
FAUCI, A. S., JOHNSON, J. S.: Suppression of antibody synthesis. I. Evidence for a circulating inhibitor of antibody synthesis demonstrable at the cellular level. J. Immunol. **107**, 1052–1056 (1971a).
FAUCI, A. S., JOHNSON, J. S.: Suppression of antibody synthesis. II. The effect of carrier-specific cells upon haptenic competition. J. Immunol. **107**, 1057–1062 (1971b).
FAZEKAS DE ST. GROTH, S.: Cross-recognition and cross-reactivity. Cold Spr. Harb. Symp. quant. Biol. **32**, 525–536 (1967).
FAZEKAS DE ST. GROTH, S., WEBSTER, R. G.: Disquisitions on original antigenic sin. I. Evidence in man. J. exp. Med. **124**, 331–345 (1966a).
FAZEKAS DE ST. GROTH, S., WEBSTER, R.G.: Disquisitions on original antigenic sin. II. Proof in lower creatures. J. exp. Med. **124**, 347–361 (1966b).

FREI, P. C., BENACERRAF, B., THORBECKE, G. J.: Phagocytosis of antigen, a crucial step in the induction of the primary response. Proc. nat. Acad. Sci. (Wash.) **53**, 20–23 (1965).
FRIEDBERGER, E.: Über die Intensität der Choleramboceptorenbildung beim Kaninchen unter dem Einflusse der Alkoholisierung und der Mischung. Berl. klin. Wschr. **41**, 242 (1904).
FRIEDMAN, H., CEGLOWSKI, W. S.: Cellular basis for the immunosuppressive properties of a leukemogenic virus. Nature (Lond.) **218**, 1232–1234 (1968).
FUCHS, S., SELA, M.: Antigenicity of some new synthetic polypeptides and polypeptidyl gelatins. Biochem. J. **93**, 566–572 (1964).
GERSHON, R. K., KONDO, K.: Antigenic competition between heterologous erythrocytes. I. Thymic dependency. J. Immunol. **106**, 1524–1531 (1971a).
GERSHON, R. K., KONDO, K.: Antigenic competition between heterologous erythrocytes. II. Effect of passive antibody administration. J. Immunol. **106**, 1532–1539 (1971b).
GREAVES, M. F., MOLLER, E., MOLLER, G.: Studies on antigen-binding cells. II. Relationship to antigen-sensitive cells. Cell Immunol. **1**, 386–403 (1970).
HANNA, M. G., PETERS, L. C.: The effect of antigenic competition on both the primary and secondary immune capacity in mice. J. Immunol. **104**, 166–177 (1970).
HENNEY, C. S.: The specificity of the early immune response. I. A comparison of the specificity of delayed hypersensitivity and humoral responses following immunisation with human γG globulin. J. Immunol. **103**, 519–527 (1969).
HENRY, C., JERNE, N. K.: Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. J. exp. Med. **128**, 133–152 (1968).
HOWARD, J. G., WOODRUFF, M. F. A.: Effect of the graft-versus-host reaction on the immunological responsiveness of the mouse. Proc. roy. Soc. B **154**, 532–539 (1961).
HUMPHREY, J. H., FRANK, M. M.: The localisation of non-microbial antigens in the draining lymph nodes of tolerant, normal and primed rabbits. Immunology **13**, 87–100 (1967).
International symposium on combined vaccines. Symp. series immunobiol. standard. Basel-New York: Karger 1967.
IVERSON, G. M.: Ability of CBA mice to produce anti-idiotypic sera to 5563 myeloma protein. Nature (Lond.) **227**, 273–274 (1970).
JATON, J.-C., SELA, M.: Role of optical configuration in the immunogenicity and specificity of synthetic antigens derived from multichain polyproline. J. biol. Chem. **243**, 5616–5626 (1968).
JERNE, N. K.: The problem of antigen competition. In: International symposium on combined vaccines, p. 1–6. Basel-New York: Karger 1967.
JERNE, N. K.: The somatic generation of immune recognition. Europ. J. Immunol. **1**, 1–9 (1971).
KAPPLER, J. W., HOFFMANN, M., DUTTON, R. W.: Regulation of the immune response. I. Differential effect of passively administered antibody on the thymus-derived and bone marrow-derived lymphocytes. J. exp. Med. **134**, 577–587 (1971).
KATZ, D. H., PAUL, W. E., GOIDL, E. A., BENACERRAF, B.: Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody response by carrier preimmunisation. J. exp. Med. **132**, 261–282 (1970).
KERBEL, R. S., EIDINGER, D.: Further studies of antigenic competition. III. A model to account for the phenomenon based on a deficiency of cell-to-cell interaction in immune lymphoid cell populations. J. exp. Med. **133**, 1043–1060 (1971).
KRETH, H. W., WILLIAMSON, A. R.: Cell surveillance model for lymphocyte cooperation. Nature (Lond.) **234**, 454–456 (1971).
LAPP, W. S., MÖLLER, G.: Prolonged survival of H-2 incompatible skin allografts on F_1 animals treated with parental lymphoid cells. Immunology **17**, 339–344 (1969).
LAWRENCE, W., SIMONSEN, M.: The property of 'strength' of histocompatibility antigens and their ability to produce antigenic competition. Transplantation **5**, 1304–1322 (1967).
LIACOPOULOS, P., COUDERC, J., GILLE, M. F.: Competition of antigens during induction of low zone tolerance. Europ. J. Immunol. **1**, 359–363 (1971).
LIACOPOULOS, P., HALPERN, N. B., PERAMENT, F.: Unresponsiveness to unrelated antigens induced by paralysing doses of bovine serum albumin. Nature (Lond.) **195**, 1112–1113 (1962).

LIACOPOULOS, P., NEVEU, T.: Non-specific inhibition of the immediate and delayed types of hypersensitivity during immune paralysis of adult guinea pigs. Immunology **7**, 26–39 (1964).
LINDENMANN, J.: Cross-priming and cross-inhibition by antibody in the influenza virus-host antigen system. Proc. roy. Soc. B **176**, 419–423 (1971).
MÄKELÄ, O., CROSS, A., KOSUNEN, T. U. (eds.): Cell interactions and receptor antibodies in immune responses. London-New York: Academic Press 1971.
McBRIDE, R. A., SCHIERMAN, L. W.: Hapten-carrier relationships of isoantigens. A model for immunological maturation based on the conversion of haptens to carriers by antibody. J. exp. Med. **131**, 377–390 (1970).
McDEVITT, H. O., BENACERRAF, B.: Genetic control of specific immune responses. Advanc. Immunol. **11**, 31–74 (1969).
METCALF, D.: Reticular tumours in mice subjected to prolonged antigenic stimulation. Brit. J. Cancer **15**, 769–779 (1961).
MICHAELIS, L.: Untersuchungen über Eiweißpräzipitine. Dtsch. med. Wschr. **28**, 733–736 (1902).
MICHAELIS, L.: Weitere Untersuchungen über Eiweißpräzipitine. Dtsch. med. Wschr. **30**, 1240–1241 (1904).
MISHELL, R. I., DUTTON, R. W.: Immunisation of dissociated spleen cell cultures from normal mice. J. exp. Med. **126**, 423–442 (1967).
MITCHISON, N. A.: Antigen recognition responsible for the induction *in vitro* of the secondary response. Cold Spr. Harbor Symp. quant. Biol. **32**, 431–439 (1967).
MITCHISON, N. A.: The dosage requirements for immunological paralysis by soluble proteins. Immunology **15**, 509–530 (1968).
MITCHISON, N. A.: The immunogenic capacity of antigen taken up by peritoneal exudate cells. Immunology **16**, 1–14 (1969a).
MITCHISON, N. A.: In: S. LAWRENCE and M. LANDY (eds.), Mediators of cellular immunity, p. 97. New York: Academic Press 1969b.
MITCHISON, N. A.: The carrier effect in the secondary response to haptenprotein conjugates. I. Measurement of the effect with transferred cells and objections to the local environment hypothesis. Europ. J. Immunol. **1**, 10–17 (1971a).
MITCHISON, N. A.: The carrier effect in the secondary response to haptenprotein conjugates. II. Cellular cooperation. Europ. J. Immunol. **1**, 18–26 (1971b).
MITCHISON, N. A., RAJEWSKY, K., TAYLOR, R. B.: Cooperation of antigenic determinants and of cells in the induction of antibodies. In: J. STERZL and M. RIHA, eds., Developmental aspects of antibody formation and structure, p. 547–561. New York: Academic Press 1971.
MÖLLER, G.: Suppressive effect of graft versus host reactions on the immune response to heterologous red cells. Immunology **20**, 597–609 (1971).
MÖLLER, G., SJÖBERG, O.: Effect of antigenic competition on antigensensitive cells and on adoptively transferred immunocompetent cells. Cellular Immunology **1**, 110–121 (1970).
MÖLLER, G., SJÖBERG, O.: Studies on the mechanism of antigenic competition. In: MÄKELÄ, O., A. CROSS and T. U. KOSUNEN (eds.), Cell interactions and receptor antibodies in immune responses, p. 419–432. London and New York: Academic Press 1971.
MOWBRAY, J. F.: Ability of large doses of an $alpha_2$ plasma protein fraction to inhibit production. Immunology **6**, 217–225 (1963).
MOZES, E., McDEVITT, H. O., JATON, J.-C., SELA, M.: The nature of the antigenic determinant in a genetic control of the antibody response. J. exp. Med. **130**, 493–504 (1969a).
MOZES, E., McDEVITT, H. O., JATON, J.-C., SELA, M.: The genetic control of antibody specificity. J. exp. Med. **130**, 1263–1278 (1969b).
MOZES, E., SHEARER, G. M.: Contribution of bone marrow cells and lack of expression of thymocytes in genetic controls of immune responses for two immunopotent regions within poly-(Phe, Glu)-poly-Pro–poly-Lys in inbred mouse strains. J. exp. Med. **134**, 141–161 (1971).
MOZES, E., SHEARER, G. M., SELA, M.: Cellular basis of the genetic control of immune responses to synthetic polypeptides. I. Differences in frequency of splenic precursor cells specific for a synthetic polypeptide derived from multichain polyproline, (T, G)-

Pro–L, in high and low responder inbred mouse strains. J. exp. Med. **132**, 613–622 (1970).
MOZES, E., SHEARER, G. M., SELA, M., BRAUN, W.: Conversion with polynucleotides of a genetically controlled low immune response to a high response in mice immunised with a synthetic polypeptide antigen. In: BEERS, R. F. and W. BRAUN, eds., Biological effects of polynucleotides, p. 197–213. New York: Springer 1971.
NISBET, N. W., SIMONSEN, M., ZALESKI, M.: The frequency of antigensensitive cells in tissue transplantation. J. exp. Med. **129**, 459–467 (1969).
NOSSAL, G. J. V., ADA, G. L.: Antigens, lymphoid cells and the immune response. New York and London: Academic Press 1971.
OAKLEY, C. L.: Prophylaxis of microbial diseases. In: P. G. H. GELL and R. R. A. COOMBS (eds.), Clinical aspects of immunology, 2nd ed. Oxford: Blackwells 1968.
OVARY, Z., BENACERRAF, B.: Immunological specificity of the secondary response with dinitrophenylated proteins. Proc. Soc. exp. Biol. Med. (N.Y.) **114**, 72–75 (1963).
PARASKEVAS, F., ORR, K. B., ANDERSON, E. D., LEE, S.-T., ISRAELS, L. G.: The biologic significance of Fc receptor on mouse B-lymphocytes. J. Immunol. **108**, 1729–1732 (1972).
PARISH, H. J., CANNON, D. A.: Antisera, toxoids, vaccines and tuberculins in prophylaxis and treatment, 6th ed. Edinburgh: E. and S. Livingstone 1962.
PEARLMAN, D. S.: The influence of antibodies on immunologic responses. I. The effect on the response to particulate antigens in the rabbit. J. exp. Med. **126**, 127–148 (1967).
PETERSON, R. D. A., HENDRICKSON, R., GOOD, R. A.: Reduced antibodyforming capacity during the incubation period of passage A leukaemia in C3H mice. Proc. Soc. exp. Biol. (N.Y.) **114**, 517–520 (1963).
PINCUS, C. S., LAMM, M. E., NUSSENZWEIG, V.: Regulation of the immune response: suppressive and enhancing effects of passively administered antibody. J. exp. Med. **133**, 987–1003 (1971).
PINCUS, C. S., NUSSENZWEIG, V.: Passive antibody may simultaneously suppress and stimulate antibody formation against different portions of a protein molecule. Nature (Lond.) **222**, 594–596 (1969).
RADOVICH, J., TALMAGE, D. W.: Antigenic competition: cellular or humoral. Science **158**, 512–514 (1967).
RAJEWSKY, K., ROTTLÄNDER, E.: Tolerance specicity and the immune response to lactic dehydrogenase isoenzymes. Cold Spr. Harb. Symp. quant. Biol. **32**, 547–554 (1967).
RAJEWSKY, K., ROTTLÄNDER, E., PETRE, G., MÜLLER, B.: The immune response to a hybrid protein molecule: specificity of secondary stimulation and of tolerance induction. J. exp. Med. **126**, 581–606 (1967).
RAJEWSKY, K., SCHIRRMACHER, V., NASE, S., JERNE, N. K.: The requirement of more than one antigenic determinant for immunogenicity. J. exp. Med. **129**, 1131–1143 (1969).
RHODES, J. M., LIND, I.: Antigen uptake *in vivo* by peritoneal macrophages from mice exhibiting antigenic competition. Immunology **20**, 839–842 (1971).
RITTENBERG, M. B., AMKRAUT, A.: Immunogenicity of trinitrophenylhemocyanin: production of primary and secondary anti-hapten precipitins. J. Immunol. **97**, 421–430 (1966).
ROELANTS, G. E., GOODMAN, J. W.: Tolerance induction by an apparently non-immunogenic molecule. Nature (Lond.) **227**, 175–176 (1970).
SALAMAN, M. H., WEDDERBURN, N.: The immunodepressive effect of Friend virus. Immunology **10**, 445–458 (1966).
SALVIN, S. B., SMITH, R. F.: Specificity of allergic reactions. II. Azoproteins in the anamnestic response. Proc. Soc. exp. Biol. (N.Y.) **104**, 584–590 (1960).
SCHECHTER, I.: Antigenic competition between polypeptidyl determinants in normal and tolerant rabbits. J. exp. Med. **127**, 237–250 (1968).
SCHECHTER, I., SELA, M.: Preferential formation of antibodies specific toward D-amino acid residues upon immunisation with poly-DL-peptidyl proteins. Biochemistry **6**, 897–905 (1967).
SCHWARTZ, H., LESKOWITZ, S.: Antigenic competition in the development of delayed hypersensitivity to the azobenzenearsonate group. Immunochemistry **6**, 503–606 (1969).
SCHWARTZ, R. S.: Immunoregulation by antibody. In: AMOS, D. B. (ed.), Progr. Immunol. **1**, 1082–1092 (1971).

SELA, M.: Molecular aspects of antigenicity. Science **166**, 1365–1374 (1969).
SELA, M., FUCHS, S., ARNON, R.: Studies on the chemical basis of the antigenicity of proteins. V. Synthesis, characterisation and immunogenicity of some multichain and linear polypeptides containing tyrosine. Biochem. J. **85**, 223–235 (1962).
SHEARER, G. M., MOZES, E., SELA, M.: Cellular basis of the genetic control of immune responses to synthetic polypeptides. II. Frequency of immunocompetent precursors specific for two distinct regions within (Phe, G)-Pro–L, a synthetic polypeptide derived from multichain polyproline, in inbred mouse strains. J. exp. Med. **133**, 216–230 (1971a).
SHEARER, G. M., MOZES, E., SELA, M.: Cellular basis of the genetic control of immune responses. In: AMOS, D. B. (ed.), Progress in immunology, vol. 1, p. 509–529. New York: Academic Press 1971b.
SHEARER, G. M., MOZES, E., SELA, M.: Contribution of different cell types to the genetic control of immune responses as a function of the chemical nature of the polymeric side chains (poly-L-prolyl and poly-DL-alanyl) of synthetic immunogens. J. exp. Med. **135** (1972). In Press.
SIEGEL, B. V., MORTON, J. I.: Depressed antibody response in the mouse infected with Rauscher leukaemia virus. Immunology **10**, 559–562 (1966).
SIMONSEN, M.: The clonal selection hypothesis evaluated by grafted cells reacting against their hosts. Cold Spr. Harb. Symp. quant. Biol. **32**, 517–523 (1967).
SISKIND, G. W., BENACERRAF, B.: Cell selection by antigen in the immune response. Advanc. Immunol. **10**, 1–50 (1969).
SMITH, G. P., HOOD, L., FITCH, W. M.: Antibody diversity. Ann. Rev. Biochem. **40**, 969–1012 (1971).
SMITH, R. T., LANDY, M. (eds.): Immune surveillance. New York-London: Academic Press 1970.
TALMAGE, D. W., RADOVICH, J., HEMMINGSEN, H.: Cell interaction in antibody synthesis. Advanc. Immunol. **12**, 271–282 (1970).
TARRAB, R., SCHECHTER, I., SELA, M.: Antibody response to polyalanyl determinants in tolerant rabbits. Immunochemistry **8**, 1107–1116 (1971).
TAUSSIG, M. J.: Bacteriophage linked assays for antibodies to protein antigens. Immunology **18**, 323–330 (1970).
TAUSSIG, M. J.: Studies on antigenic competition. I. Antigenic competition between the Fc and Fab fragments of rabbit IgG in mice. Immunology **21**, 51–60 (1971).
TAUSSIG, M. J.: Studies on antigenic competition. III. Is there a competitive step in tolerance induction? Europ. J. Immunol. **2**, 118–123 (1972).
TAUSSIG, M. J., LACHMANN, P. J.: Studies on antigenic competition. II. Abolition of antigenic competition by antibody against or tolerance to the dominant antigen: a model for antigenic competition. Immunology **22**, 185–197 (1972).
TAUSSIG, M. J., MOZES, E., SHEARER, G. M., SELA, M.: Studies on the mechanism of antigenic competition. Analysis of competition between synthetic polypeptide antigens in mice. Europ. J. Immunol. (1972a) (in press).
TAUSSIG, M. J., MOZES, E., SHEARER, G. M., SELA, M.: Genetic control of the immune response and antigenic competition. Cell. Immunol. (1972b) (in Press).
TAUSSIG, M. J., MOZES, E., SHEARER, G. M., SELA, M.: Cooperation of antigenic determinants *in vivo:* interference by antigenic competition. Europ. J. Immunol. (1972c) (in Press).
TAYLOR, R. B., IVERSON, G. M.: Hapten competition and the nature of cell-cooperation in the antibody response. Proc. roy. Soc. B **176**, 393–418 (1971).
UHR, J. W., BAUMANN, J. B.: Antibody formation. I. The suppression of antibody formation by passively administered antibody. J. exp. Med. **113**, 935–958 (1961).
VEIT, B. C., MICHAEL, J. G.: Immune response suppression by an inhibitor in normal and immune mouse serum. Nature (Lond.) New Biol. **235**, 238–240 (1972).
WATERSTON, R. H.: Antigen competition: a paradox. Science **170**, 1108–1109 (1970).
WEIGLE, W. O., HIGH, G. J.: The effect of antigenic competition on antibody production to heterologous proteins, termination of immunologic unresponsiveness and induction of autoimmunity. J. Immunol. **99**, 392–398 (1967).
ZLATOGOROFF, S. J., GLUSMAN, M. P., KANDYBA, L. L.: Experimentelle Untersuchungen über gleichzeitige Immunisierung gegen verschiedene Infektionen. Z. Hyg. Infekt.-Kr. **110**, 706 (1929).

Author Index

Page numbers in *italics* refer to the references

Achtman, M. 86, 90, 98, 100, *116*
Achtman, M., Finnegan 92
Achtman, M., Willetts, N., Clark, A, J. 80, 81, 98, 99, 100, 101, 102, 103, 104, 105, 106, 109, 115, *116*
Achtman, M., see Broda, P. 107, 108, *117*
Achtman, M., see Carnahan, J. 103
Achtman, M., see Ippen-Ihler, K. 101, 102, 108, *119*
Achtman, M., see Willetts, N. S. 92, 100, 101, 106, 115, *123*
Ada, G. L., Byrt, P., Mandel, T., Warner, N. 39, *75*
Ada, G. L., Nossal, G. J. V., Austin, C. M. 148, *169*
Ada, G. L., see Nossal, G. J. V. 148, 161, 164, *173*
Adelberg, E. A., Burns, S. N. 81, 84, *116*
Adelberg, E, A., see Demerec, M. 80, *118*
Adelberg, E. A., see Jacob, F. 81, *119*
Adelberg, E. A., see Marinus, M. G. 94, *120*
Adelberg, E. A., see Stadler, J. 92, *122*
Adler, F. L. 126, 130, *169*
Adler, H. I., see Cohen, A. 93, 99, *117*
Ainsworth, S. K., see Paranchych, W. 97, *121*
Albright, J. F., Makinodan, T. 140, 158, *169*
Albright, J. F., Omer, T. F., Deitchman, J. W. 158, *169*
Allison, A. C. 65, *75*
Allison, A. C., see Hellström, I. 64, 65, 70, *76*
Allison, D. P., see Curtis, R., III 95, 97, 98, 99, *117*
Amante, L., see Pernis, B. 39, *76*
Ambrose, C. T. 167, *169*
Ames, B. N., see Pollack, J. R. 11, *29*
Amkraut, A., see Rittenberg, M. B. 157, *173*
Amkraut, A. A., Garvey, J. S., Campbell, D. H. 143, *169*
Amos, D. B., Gorer, P. A., Mikulska, B. M., Billingham, R. E., Sparrow, E. M. 42, *75*
Amos, D. B., Nicks, P. J., Peacocke, N., Sieker, H. O. 32, *75*
Anderson, E. D., see Paraskevas, F. 164, 165, *173*
Anderson, E. S., see Grindley, J. N. 112, *118*
Anderson, T. F., see Ou, J. T. 95, 96, 100, *121*
Antoine, A. D., see Morrison, N. E. 11, *29*
Arai, T., see Watanabe, T. 111, 112, *122*
Armstrong, B. A., Sword, C. P. 25, *26*
Arnon, R., see Sela, M. 131, *174*
Ashley, H., Ovary, Z. 157, *169*
Ashman 71
Aufreiter, E., see Meynell, G. G. 104, *120*
Austin, C. M., see Ada, G. L. 148, *169*

Baggiolini, M., De Duve, C., Masson, P. L., Heremans, J. F. 19, *26*
Baker, P. J., Wilson, J. B. 9, *26*
Balner, H., Dersjant, H. 41, *75*
Balner, H., Dersjant, H., van Bekkum, D. W. 41, *75*
Barlow, G. H., see Overby, L.R. 96, *121*
Barr, M., Llewellyn-Jones, M. 131, 140, 168, *169*
Bartellony, P. J., see Pekarek, R. S. 9, *29*
Barth, P. T., see Pritchard, R. H. 89, 90, *121*
Basten, A., Sprent, J., Miller, J.F.A.P. 164, *169*
Basten, A., see Miller, J. F. A. P. 39, *76*
Baumann, J. B., see Uhr, J. W. 153, *174*
Bauminger, S., Schechter, I., Sela, M. 138, 144, *169*
Bazaral, M., Helinski, D. R. 86, 90, *116*
Beard, J. P., Howe, T. G. B., Richmond, M. H. 98, 112, 113, *116*
Beckwith, J. R., see Broda, P. 84, *117*
Beckwith, J. R., see Ippen, K. A. 101, *119*
Beisel, W. R., see Pekarek, R. S. 9, *29*
Benacerraf, B., Green, I., Bluestein, H. G., Elman, L. 139, *169*
Benacerraf, B., see Frei, P. C. 147, *171*
Benacerraf, B., see Katz, D. H. 155, 157, *171*
Benacerraf, B., see McDevitt, H. O. 131, 134, *172*
Benacerraf, B., see Ovary, Z. 155, *173*
Benacerraf, B., see Siskind, G. W. 160, *174*
Bendel, W. L., Jr., see Kochan, I. 17, *28*
Ben-Efraim, S., Liacopoulos, P. 139, *169*

Ben-Efraim, S., Liacopoulos, P., Harel, S., Gille, F. 139, *169*
Berendt, see Kochan 21
Bianco, C., see Dukor, P. 164, *170*
Bickel, H., Gäumann, E., Keller-Schierlein, W., Prelog, V., Vischer, E., Wettstein, A., Zähner, H. 11, *26*
Billingham, R., Silvers, W. 64, 70, *75*
Billingham, R. E. 45, 46, *75*
Billingham, R. E., Brent, L., Medawar, P. B. 64, 70, *75*
Billingham, R. E., Defendi, V., Silvers, W. K., Steinmüller, D. 36, *75*
Billingham, R. E., Silvers, W. K. 36, *75*
Billingham, R. E., Silvers, W. K., Wilson, D. B. 64, *75*
Billingham, R. E., see Amos, D. B. 42, *75*
Billingham, R. E., see Ramseier, H. 32, 35, *77*
Binz, H., Lindenmann, J. 71, *75*
Bishop, J. O., see Frame, R. 83, 90, *118*
Blanden, R. F., see Mackaness, B. G. 15, *28*
Blanden, R. V. 18, *26*
Bluestein, H. G., see Benacerraf, B. 139, *169*
Bodlund, K., see Celada, F. 148, 149, *170*
Boissevain, C. H. 4, *26*
Boman, H. G., see Monner, D. A. 109, *120*
Bondevick, H., see Cooperband, S. R. 167, *170*
Bonhoeffer, F. 98, *117*
Bonhoeffer, F., Vielmetter, W. 93, *117*
Bonhoeffer, F., see Vielmetter, W. 93, *122*
Bonventre, P. F. 16, *26*
Bornside, G. H., Merritt, C. B., Weil, A. C. 8, *26*
Bostian, K. A., see Pekarek, R. S. 9, *29*
Bouanchaud, D. H., Chabbert, Y. A. 112, *117*
Boyer, H. W. 82, *117*
Boyse, E. A., see Old, L. J. 59, *76*
Braun, W., Ishizuka, M., Winchurch, R., Webb, D. 135, *169*
Braun, W., see Mozes, E. 135, 136, *173*
Brenner, S., see Jacob, F. 89, *119*
Brent, L., Medawar, P. B. 32, *75*
Brent, L., see Billingham, R. E. 64, 70, *75*
Bretscher, P. A., Cohn, M. 164, *169*
Brinton, C. C., Jr. 93, 95, 96, 97, 98, 102, 104, 105, 114, 115, *117*
Brinton, C. C., Jr., Gemski, P., Jr., Carnahan, J. 95, 96, 97, 116, *117*
Brinton, C. C., Jr., see Carnahan, J. 103
Brinton, C. C., Jr., see Novotny, C. 95, 115, *121*
Broda, P. 105, *117*
Broda, P., Beckwith, J. R., Scaife, J. 84, *117*
Broda, P., Meacock, P. 111, *117*
Broda, P., Meacock, P., Achtman, M. 107, 108, *117*
Broda, P., see Willetts, N. S. 108, 111, *123*
Brodsky, R., see Novick, R. P. 91, *121*
Brody, N. I., Siskind, G. W. 143, 144, 162, 167, *170*
Brody, N. I., Walker, J. G., Siskind, G. W. 129, 143, 154, *170*
Brown, C. A., Draper, P., Hart, P. D. 18, *26*
Brunner, K. T., Mauel, J., Schindler, R. 69, *75*
Bukovic, J. A., see Kochan, I. 3, 7, 8, 9, 10, 11, 22, *28*
Bullen, J. J., Cushnie, G. H., Rogers, H. J. 22, 23, *26*
Bullen, J. J., Leigh, L. G., Rogers, H. J. 21, *26*
Bullen, J. J., Rogers, H. J. 8, 21, *26*
Bullen, J. J., Rogers, H. J., Lewin, J. E. 9, 10, 22, *26*
Bullen, J. J., Wilson, A. B., Cushnie, G. H., Rogers, H. J. 22, *26*
Bullen, J. J., see Rogers, H. J. 9, *29*
Burnham, B. F. 11, *26*
Burns, S. N., see Adelberg, E. A. 81, 84, *116*
Burr, W., see Ford, W. L. 46, 73, *75*
Burrows, T. W. 23, *27*
Burrows, T. W., see Jackson, S. 21, *27*
Bussard, A. E. 158, *170*
Buttin, G., see Cuzin, F. 92, *117*
Byrt, P., see Ada, G. L. 39, *75*

Cahall, D. L., see Kochan, I. 3, 12, 13, 14, 15, 19, 24, *28*
Calia, F. M., see Pekarek, R. S. 9, *29*
Campbell, A. M. 81, *117*
Campbell, D. H., see Amkraut, A. A. 143, *169*
Canetti, G. 4, *27*
Cannon, D. A., see Parish, H. J. 169, *173*
Carnahan, J., Brinton, C. C., Jr., Achtman, M. 103
Carnahan, J., see Brinton, C. C., Jr. 95, 96, 97, 116, *117*
Carnahan, J., see Novotny, C. 95, 115, *121*
Caro, L., see Gross, J. D. 93, *119*
Caro, L. G., Schnös, M. 96, 98, *117*
Caro, L. G., see Curtiss, R., III 95, 97, 98, 99, *117*
Caroline, L., see Schade, A. L. 8, *30*
Caspary, E. A., Field, E. J., Woodruff, M. F. A. 41, *75*
Cavalli, L. L., see Lederberg, J. 98, *120*
Ceglowski, W. S., see Friedman, H. 158, *171*
Celada, F. 148, *170*
Celada, F., Ellis, J., Bodlund, K., Rotman, B. 148, 149, *170*

Chabbert, Y. A., see Bouanchaud, D. H. 112, *117*
Chan, E. C. S., see Seidman, P., 11, *30*
Chandlee, G. C., Fukui, G. M. 21, 22, *27*
Chang, Y. T. 15, *27*
Charamella, L. J., see Curtiss, R., III 99, *117*
Chase, M. W. 17, *27*
Cheers, C., see Miller, J. F. A. P. 39, *76*
Chimori, M., see Kotani, S. 21, *28*
Citarella, R. V., see Falkow, S. 91, *118*
Clark, see Curtiss 111
Clark, A. J. 86, 87, 111, *117*
Clark, A. J., see Achtman, M. 80, 81, 98, 99, 100, 101, 102, 103, 104, 105, 106, 109, 115, *116*
Clark, A. J., see Demerec, M. 80, *118*
Clewell, D. B., see Helinski, D. R. 83, 84, *119*
Clifton, C. E. 2, 26, *27*
Clowes, R. C., Moody, E. E. M. 111, *117*
Clowes, R. C., see Hausmann, C. 112, *119*
Clowes, R. C., see Macfarren, A. C. 89, *120*
Clowes, R. C., see Nisioka, T. 85, 86, 91, *121*
Cockburn, W. C. 169, *170*
Coe, J. E., Salvin, S. B. 157, *170*
Cohen, A., Fisher, W. D., Curtiss, R., III, Adler, H. I. 93, 99, *117*
Cohen, S. N., Miller, C. A. 85, 86, 91, *117*
Cohn, M., Notani, G., Rice, S. A. 154, *170*
Cohn, M., see Bretscher, P. A. 164, *169*
Cohn, Z. A., Wiener, E. 18, 21, *27*
Collins, F. M. 2, *27*
Collins, J., see Pritchard, R. H. 89, 90, *121*
Colon, S., see Rabellino, E. 39, *77*
Cooke, M., see Frydman, A. 114, *118*
Cooke, M., see Lawn, A. M. 112, 113, *120*
Cooke, M., see Meynell, E. 113, *120*
Cooper, S., Helmstetter, C. E. 83, *117*
Cooperband, S. R., Bondevick, H., Schmid, K., Mannick, J. A. 167, *170*
Costello, R., Izumi, T. 2, *27*
Costello, R., see Izumi, T. 2, *27*
Couderc, J., see Liacopoulos, P. 146, 147, *171*
Courmont, P., Gardere, H. 3, 4, *27*
Cowen, D. M., see Gowans, J. L. 64, *76*
Crawford, E., Gesteland, R. 96, *117*
Crile, G., see Deodhar, S. D. 41, *75*
Cross, A., see Mäkelä, O. 148, 154, *172*
Curtiss, Clark 111
Curtiss, R., III 93, 95, 96, *117*
Curtis, R., III, Caro, L. G., Allison, D. P., Stallions, D. R. 95, 97, 98, 99, *117*
Curtiss, R., III, Charamella, L. J., Stallions, D. R., Mays, J. A. 99, *117*
Curtiss, R., III, see Cohen, A. 93, 99, *117*
Cushnie, G. H., see Bullen, J. J. 22, 23, *26*
Cushnie, G. H., see Rogers, H. J. 9, *29*
Cuzin, F. 92, 109, *117*
Cuzin, F., Buttin, G., Jacob, F. 92, *117*
Cuzin, F., Jacob, F. 86, 90, 91, 92, 109, *117*, *118*
Cuzin, F., see Jacob, F. 89, *119*

Dabrowa, N., see Landau, J. W. 6, 22, *28*
Dannenberg, A. M., Jr. 15, 18, *27*
Datta, N., Hedges, R. W. 112, *118*
Datta, N., see Lawn, A. M. 111, *120*
Datta, N., see Meynell, E. 111, 112, 113, *120*
Davidson, N., see Sharp, P. A. 81, 84, 86, 105, 106, 108, *122*
Davies, see O'Toole 144, 167
Davies, D. A. L. 71, 73, *75*
Davies, R. 4, *27*
DeCosse, J. J., see Mandel, M. A. 41, *76*
De Duve, C., see Baggiolini, M. 19, *26*
Defendi, V., see Billingham, R. E. 36, *75*
Deitchman, J. W., see Albright, J. F. 158, *169*
Demain, A. L., Hendlin, D. 11, *27*
Demerec, M., Adelberg, E. A. Clark, A. J., Hartman, P. E. 80, *118*
Dennert, G., Pohlit, H., Rajewsky, K. 151, *170*
Dent, P. B., Peterson, R. D. A., Good, R. A. 158, *170*
Deodhar, S. D., Crile, G. 41, *75*
Dersjant, H., see Balner, H. 41, *75*
Dettori, R., Maccacaro, A., Piccinin, G. L. 109, *118*
de Vries, J., Maas, W. K. 88
Devries, J., see Press, R. *86*, *121*
Dewbrey, E. E., see Morrison, N. E. 11, *29*
Dick, A. J., see Paranchych, W. 97, *121*
Dietrich, F. M., see Dukor, P. 140, *170*
Doi, R. H., see Overby, L. R. 96, *121*
Draper, P., see Brown, C. A. 18, *26*
Dresser, D. W., Mitchison, N. A. 70, *75*, 148, *170*
Dressler, D., see Gilbert, W. 94, *118*
Dubert, J.-M. 157, *170*
Dubnau, E., Maas, W. K. 86, *118*
Duguid, J. P., see Mulczyk, M. 95, *120*
Dukor, P., Bianco, C., Nussenzweig, V. 164, *170*
Dukor, P., Dietrich, F. M. 140, *170*
Dutton, R. W. 162, *170*
Dutton, R. W., see Kappler, J. W. 153, *171*
Dutton, R. W., see Lesley, J. 39, *76*

Dutton, R. W., see Mishell, R. I. 142, *172*
Dvorak, H. F., Kosunen, T. U., Waksman, B. H. 32, *75*

Echols, H. 86, 107, *118*
Edwards, S., Meynell, G. G. 113, *118*
Effort, R. J. 4, *27*
Egawa, R., Hirota, Y. 99, 111, 113, *118*
Eidinger, D., Khan, S. A., Millar, K. G. 140, 144, 166, 167, *170*
Eidinger, D., see Kerbel, R. S. 167, 168, *171*
Eisen, H. N., Little, J. R., Steiner, L. A., Simms, E. S., Gray, W. 148, *170*
Elberg, S. S., see Ralston, D. J. 15, 16, *29*
Elkins, W. L., Palm, J 58, *75*
Ellis, J., see Celada, F. 148, 149, *170*
Elman, L., see Benacerraf, B. 139, *169*
Emmart, E. W., Seibert, F. B. 4, *27*
Erwin, N. S., see Matney, T. S. 105, *120*

Falkow, S., Citarella, R. V. 91, *118*
Falkow, S., Tompkins, L. S., Silver, R. P., Guerry, P., Leblanc, D. J. 94, *118*
Falkow, S., see Haapala, D. K. 85, 86, 91, *119*
Falkow, S., see Silver, R. P. 85, *122*
Fauci, A. S., Johnson, J. S. 143, *170*
Fazekas de St. Groth, S. 148, *170*
Fazekas de St. Groth, S., Webster, R. G. 148, *170*
Ferraresi, R. W., see Goihman-Yahr, M. 17, *27*
Field, E. J., see Caspary, E. A. 41, *75*
Finland, M., see Martin, C. M. 21, 22, *29*
Finnegan, see Achtman, M. 92
Finnegan, D. J., Willetts, N. S. 114, *118*
Finnegan, D. S., see Willetts, N. S. 113, *123*
Fischer-Lindahl 72
Fisher, W. D., see Cohen, A. 93, 99, *117*
Fisher, W. D., see Shull, F. W. 94, *122*
Fitch, W. M., see Smith, G. P. 127, *174*
Folkmanis, A., see Freifelder, D. 81, 83, *118*
Ford, W. L., Burr, W., Simonsen, M. 46, 73, *75*
Forni, L., see Pernis, B. 39, *76*
Fox, M. 45, *75*
Fralick, J. A., see Shull, F. W. 94, *122*
Frame, R., Bishop, J. O. 83, 90, *118*
Francis, J., Macturk, H. M., Madinaveitia, J., Snow, G. A. 11, *27*
Francis, J., Madinaveitia, J., Macturk, H. M., Snow, G. A. 11, *27*
Frank, M. M., see Humphrey, J. H. 130, 146, *171*
Fredericq, P. 81, *118*
Frei, P. C., Benacerraf, B., Thorbecke, G. J. 147, *171*
Freifeldeı, D. 83, 84, 95, *118*
Freifelder, D., Folkmanis, A., Kirschner, I. 81, 83, *118*
Friedberger, E. 126, *171*
Friedman, H., Ceglowski, W. S. 158, *171*
Frydman, A., Cooke, M., Meynell, E., Meynell, G. G. 114, *118*
Frydman, A., Meynell, E. 113, *118*
Fuchs, S., Sela, M. 131, *171*
Fuchs, S., see Sela, M. 131, *174*
Fugita, Y., see Oshima, S. 4, *29*
Fukui, G. M., see Chandlee, G. C. 21, 22, *27*
Fulton, C. 94, *118*
Gäumann, E., see Bickel, H. 11, *26*
Gardere, H., see Courmont, P. 3, 4, *27*
Garvey, J. S., see Amkraut, A. A. 143, *169*
Gell, P. G. H., see Sell, S. 39, *77*
Gemski, P., Jr., see Brinton, C. C., Jr. 95, 96, 97, 116, *117*
Gershon, R. K., Kondo, K. 140, 142, 153, 166, 167, *171*
Gesteland, R., see Crawford, E. 96, *117*
Gilbert, W., Dressler, D. 94, *118*
Gille, F., see Ben-Efraim, S. 139, *169*
Gille, M. F., see Liacopoulos, P. 146, 147, *171*
Glansdorff, N., see Press, R. 86, *121*
Glusman, M. P., see Zlatogoroff, S. J. 126, *174*
Goidl, E. A., see Katz, D. H. 155, 157, *171*
Goihman-Yahr, M., Raffel, S., Ferraresi, R. W. 17, *27*
Golden, C. A., Kochan, I. 15, 24
Golden, C. A., see Kochan, I. 3, 7, 8, 9, 10, 11, 12, 13, 14, 15, 19, 22, 23, 24, *28*
Goldschmidt, A. D., see Maas, W. K. 86, 88, 109, *120*
Goldschmidt, E. P., see Matney, T. S. 105, *120*
Golstein, P., Svedmyr, E. A. J., Wigzell, H. 69, *75*
Good, R. A., see Dent, P. B. 158, *170*
Good, R. A., see Peterson, R. D. A. 158, *173*
Goodman, J. W., see Roelands, G. E. 139, 144, 162, *173*
Gorer, P. A., see Amos, D. B. 42, *75*
Gowans, J. L., McGregor, D. D. 70, *76*
Gowans, J. L., McGregor, D. D., Cowen, D. M. 64, *76*
Gowland, G. 70, *76*

Gray, J. G., Russell, P. S. 32, *76*
Gray, J. G., see Monaco, A. P. 41, *76*
Gray, W., see Eisen, H. N. 148, *170*
Greaves, M. F. 39, *76*
Greaves, M. F., Moller, E., Moller, G. 153, *171*
Green, I., see Benacerraf, B. 139, *169*
Grey, H. M., see Rabellino, E. 39, *77*
Grindley, J. N., Anderson, E. S. 112, *118*
Gross, J. D., Caro, L. 93, *119*
Gross, J. D., see Scaife, J. 86, *122*
Guerry, P., see Falkow, S. 94, *118*

Haapala, D. K., Falkow, S. 85, 86, 91, *119*
Hakura, A., Otsuji, N., Hirota, Y. 109, *119*
Halpern, N. B., see Liacopoulos, P. 146, *171*
Hanks, J. H. 17, *27*
Hanks, J. H., see Wheeler, W. C. 17, *30*
Hanna, M. G., Peters, L. C. 140, 164, *171*
Harada, K., see Mitsuhashi, S. 112, *120*
Harel, S., see Ben-Efraim, S. 139, *169*
Harrison, P. M. 18, 24, *27*
Hart, P. D., see Brown, C. A. 18, *26*
Hartman, P. E., see Demerec, M. 80, *118*
Hashimoto, H., see Mitsuhashi, S. 112, *120*
Hashimoto, S., see Kotani, S. 21, *28*
Hausmann, C., Clowes, R. C. 112, *119*
Hedges, R. W., see Datta, N. 112, *118*
Helinski, D. R., Clewell, D. B. 83, 84, *119*
Helinski, D. R., see Bazaral, M. 86, 90, *116*
Helinski, D. R., see Hickson, F. T. 91, *119*
Helinski, D. R., see Kahn, P. *89*, *119*
Helinski, D. R., see Kline, B. C. 84, *119*
Hellström, I., Hellström, K. E., Allison, A. C. 64, 65, 70, *76*
Hellström, I., see Hellström, K. E. 60, 65, *76*
Hellström, K. E., Hellström, I. 60, 65, *76*
Hellström, K. E., Möller, G. 59, *76*
Hellström, K. E., see Hellström, I. 64, 65, 70, *76*
Helmstetter, C. E., see Cooper, S. 83, *117*
Hemmingsen, H., see Talmage, D. W. 159, *174*
Hendlin, D., see Demain, A. L. 11, *27*
Hendrickson, R., see Peterson, R. D. A. 158, *173*
Henney, C. S. 127, *171*
Henry, C., Jerne, N. K. 151, *171*
Heremans, J. F., see Baggiolini, M. 19, *26*
Heremans, J. F., see Masson, P. L. 19, *29*
Herzenberg, L. A., see Mishell, R. I. 42, *76*
Herzenberg, Leonore A., see Mishell, R. I. 42, *76*
Hickson, F. T., Roth, T. F., Helinski, D. R. 91, *119*
High, G. J., see Weigle, W. O. 131, 144, *174*
Hildemann, W. H. 42, *76*
Hill, W. C., Nissen, B. 17, *27*
Hirota, Nishimura 108
Hirota, Y. 90, 110, *119*
Hirota, Y., Sneath, P. H. A. 81, *119*
Hirota, Y., see Egawa, R. 99, 111, 113, *118*
Hirota, Y., see Hakura, A. 109, *119*
Hirota, Y., see Nishimura, Y. 81, 83, 105, 112, 113, 116, *121*
Hirota, Y., see Ohtsubo, E. 99, 100, 101, 102, 109, *121*
Hoar, D. I. 88, 113, *119*
Hoffmann, M., see Kappler, J. W. 153, *171*
Hohn, B., Korn, D. 90, 110, *119*
Hohn, B., see Marvin, D. 96, *120*
Holm, G., see Perlmann, P. 69, *76*
Hood, L., see Smith, G. P. 127, *174*
Howard, J. G., Woodruff, M. F. A. 140, 166, *171*
Howe, T. G. B., see Beard, J. P. 98, 112, 113, *116*
Hsu, M., see Sharp, P. A. 81, 84, 86, 105, 106, 108, *122*
Humphrey, J. H., Frank, M. M. 130, 146, *171*

Ihler, G., Rupp, W. D. 93, *119*
Ihler, G., see Rupp, W. D. 93, *122*
Ingram, G. L. Y., see Twort, F. W. 11, *30*
Inui, S., see Kotani, S. 21, *28*
Ippen, K., Shapiro, J. A., Beckwith, J. R. 101, *119*
Ippen, K. A., Valentine, R. C. 95, *119*
Ippen, K. A., see Wendt, L. W. 98, *122*
Ippen-Ihler, K., Achtman, M., Willetts, N. 101, 102, 108, *119*
Ishak, I., see Kochan, I. 5, *28*
Ishak, K., see Kochan, I. 5, *28*
Ishibashi, M., see Nishimura, Y. 81, 83, 105, 112, 113, 116, *121*
Ishizuka, M., see Braun, W. 135, *169*
Israels, L. G., see Paraskevas, F. 164, 165, *173*
Ito, K., see Tsuji, S. 4, *30*
Iverson, G. M. 154, *171*
Iverson, G. M., see Taylor, R. B. 143, 157, *174*
Iyer, R. V., see Khatoon, H. 112, *119*
Iyer, V. N., see Palchoudhury, S. R. 88, *121*
Izumi, T., Costello, R. 2, *27*
Izumi, T., see Costello, R. 2, *27*

Jackson, S., Burrows, T. W. 21, *27*
Jackson, S., Morris, B. C. 8, *27*

Jacob, F., Adelberg, E. A. 81, *119*
Jacob, F., Brenner, S. 89, *119*
Jacob, F., Brenner, S., Cuzin, F. 89, *119*
Jacob, F., Wollman, E. L. 105, 107, *119*
Jacob, F., see Cuzin, F. 86, 90, 91, 92, 109, *117*, *118*
Jacob, M., see Overby, L. R. 96, *121*
Jandl, J. H., see Martin, C. M. 21, 22, 25, *29*
Jaton, J.-C., Sela, M. 131, *171*
Jaton, J.-C., see Mozes, E. 131, 134, *172*
Jerne, N. K. 158, 159, *171*
Jerne, N. K., see Henry, C. 151, *171*
Jerne, N. K., see Rajewsky, K. 154, 155, 157, 161, *173*
Johnson, J. S., see Fauci, A. S. 143, *170*
Joller, P. 73, 74, *76*

Kadner, R., see Press, R. 86, *121*
Kahn, P., Helinski, D. R. 89, *119*
Kaliss, N. 60, *76*
Kallos, P., Nathan, E. 3, *27*
Kameda, M., see Mitsuhashi, S. 112, *120*
Kanai, K., Kondo, E. 18, *27*, *28*
Kanai, K., see Kondo, E. 18, *28*
Kandyba, L. L., see Zlatogoroff, S. J. 126, *174*
Kaneko, H., see Watanabe, I. 96, *122*
Kappler, J. W., Hoffmann, M., Dutton, R. W. 153, *171*
Kasamatsu, H., Rownd, R. 91, *119*
Kasamatsu, H., see Rownd, R. 91, *121*
Katz, D. H., Paul, W. E., Goidl, E. A., Benacerraf, B. 155, 157, *171*
Keller-Schierlein, W., see Bickel, H. 11, *26*
Kerbel, R. S., Eidinger, D. 167, 168, *171*
Khan, S. A., see Eidinger, D. 140, 144, 166, 167, *170*
Khatoon, H., Iyer, R. V., Iyer, V. N. 112, *119*
Kingdon, G. C., Sword, C. P. 18, *28*
Kirchner, O. 4, *28*
Kirschner, I., see Freifelder, D. 81, 83, *118*
Klein, G. 59, *76*
Kline, B. C., Helinski, D. R. 84, *119*
Knight, W. S., see Novotny, C. 95, *121*
Knolle, P. 95, *119*
Kochan, Berendt 21
Kochan, I. 4, 5, 6, 7, 10, *28*
Kochan, I., Bendel, W. L., Jr. 17, *28*
Kochan, I., Cahall, D. L., Golden, C. A. 3, 12, 13, 14, 15, 19, 24, *28*
Kochan, I., Golden, C. A. 10, 23
Kochan, I., Golden, C. A., Bukovic, J. A. 3, 7, 8, 9, 10, 11, 22, *28*
Kochan, I., Ishak, K., Said, M., Stotts, J. 5, *28*
Kochan, I., Patton, C., Ishak, I. 5, *28*
Kochan, I., Pellis, N. R., Golden, C. A. 10, 12, 24, *28*
Kochan, I., Pellis, N. R., Pfohl, D. G. 19, 20, *28*
Kochan, I., Raffel, S. 4, 7, 8, *28*
Kochan, I., Smith, L. 15, 16, 17, *28*
Kochan, I., see Golden, C. A. 15, 24
Köhler, H., see Paul, C. 39, *76*
Kondo, E., Kanai, K., Nishimura, K., Tsumita, T. 18, *28*
Kondo, E., see Kanai, K. 18, *27*, *28*
Kondo, K., see Gershon, R. K. 140, 142 153, 166, 167, *171*
Korn, D., see Hohn, B. 90, 110, *119*
Kosunen, T. U., see Dvorak, H. F. 32, *75*
Kosunen, T. U., see Mäkelä, O. 148, 154, *172*
Kotani, S., Kutaura, T., Inui, S., Hashimoto, S., Chimori, M. 21, *28*
Kozak, M., Nathans, D. 97, *119*
Krahn, P. M., O'Callaghan, R. J., Paranchych, W. 97, *119*
Krahn, P. M., see Paranchych, W. 97, *121*
Kreth, H. W., Williamson, A. R. 162, *171*
Krueger, R. G. 96, *119*
Kustoff, T. Y., see Lankford, C. E. 11, *28*
Kutaura, T., see Kotani, S. 21, *28*

Lachmann, P. J., see Taussig, M. J. 127, 129, 130, 144, 151, 164, *174*
Lamm, M. E., see Pincus, C. S. 151, 152, *173*
Lance, E. M., Medawar, P. B. 41, *76*
Landau, J. W., Dabrowa, N., Newcomer, M. D., Rowe, J. R. 6, 22, *28*
Landy, M., see Smith, R. T. 157, *174*
Lang, D. 83, *119*
Lankford, C. E., Kustoff, T. Y., Sergeant, T. P. 11, *28*
Lankford, C. E., see Purifoy, D. J. 22, *29*
Lapp, W. S., Möller, G. 166, *171*
Larner 72
Laurell, C. B. 6, *28*
Lavin, K., see Novotny, C. P. 116, *121*
Law, L. W. 41, *76*
Lawn, A. M. 97, 116, *120*
Lawn, A. M., Meynell, E., Cooke, M. 112, 113, *120*
Lawn, A. M., Meynell, E., Meynell, G. G., Datta, N. 111, *120*
Lawn, A. M., see Meynell, G. G. 95, *120*
Lawrence, W., Simonsen, M. 140, 158, 159, 166, *171*
Leblanc, D. J., see Falkow, S. 94, *118*

Lederberg, E. M., see Lederberg, J. 98, *120*
Lederberg, J., Cavalli, L. L., Lederberg, E. M. 98, *120*
Lee, S.-T., see Paraskevas, F. 164, 165, *173*
Leigh, L. G., see Bullen, J. J. 21, *26*
Leskowitz, S., see Schwartz, H. 143, 144, 145, *173*
Lesley, J., Dutton, R. W. 39, *76*
Levey, R. H., Medawar, P. B. 40, 41, *76*
Lewin, J. E., see Bullen, J. J. 9, 10, 22, *26*
Liacopoulos, P., Couderc, J., Gille, M. F. 146, 147, *171*
Liacopoulos, P., Halpern, N. B., Perament, F. 146, *171*
Liacopoulos, P., Neveu, T. 146, *172*
Liacopoulos, P., see Ben-Efraim, S. 139, *169*
Lind, I., see Rhodes, J. M. 164, *173*
Lindenmann, J. 154, 157, *172*
Lindenmann, J., see Binz, H. 71, *75*
Lindenmann, J., see Ramseier, H. 39, 43, 44, 45, 47, 48, 49, 54, 55, 56, 58, 59, 68, 69, 71, 74, *77*
Linial, M., Malamy, M. H. 109, *120*
Lipman, M. B., see Vapnek, D. 83, 84, 85, 93, *122*
Little, J. R., see Eisen, H. N. 148, *170*
Llewellyn-Jones, M., see Barr, M. 131, 140, 168, *169*
Lurie, M. B. 2, 4, 15, *28*

Maas, R. 86, *120*
Maas, R., Maas, W. K. 86, *120*
Maas, W. K. 84, 87, 88
Maas, W. K., Goldschmidt, A. D. 86, 88, 109, *120*
Maas, W. K., see Maas, R. 86, *120*
Maas, W. K., see de Vries, J. 88
Maas, W. K., see Dubnau, E. 86, *118*
Maas, W. K., see Press, R. 86, *121*
Maccacaro, A., see Dettori, R. 109, *118*
Macfarren, A. C., Clowes, R. C. 89, *120*
Mackaness, G. B. 15, 17, 18, *28*
Mackaness, G. B., Blanden, R. F. 15, *28*
Macturk, H. M., see Francis, J. 11, *27*
Madinaveitia, J., see Francis, J. 11, *27*
Mäkelä, O., Cross, A., Kosunen, T. U. 148, 154, *172*
Mäkelä, O., Mäkelä, P. H., Soikkeli, S. 109, *120*
Mäkelä, O., see Wigzell, H. 39, *78*
Mäkelä, P. H., see Mäkelä, O. 109, *120*
Maizel, J. V., Jr., see Studier, F. W. 110, *122*
Makinodan, T., see Albright, J. F. 140, 158, *169*
Malamy, M. H., see Linial, M. 109, *120*
Malamy, M. H., see Morrison, T. G. 108, 110, 113, *120*
Mandel, M. A., DeCosse, J. J. 41, *76*
Mandel, T., see Ada, G. L. 39, *75*
Mannick, J. A., see Cooperband, S. R. 167, *170*
Marinus, M. G., Adelberg, E. A. 94, *120*
Marks, J. 11, *29*
Martin, C. M., Jandl, J. H. 25, *29*
Martin, C. M., Jandl, J. H., Finland, M. 21, 22, *29*
Marvin, D., Hohn, B. 96, *120*
Masson, P. L., Heremans, J. F., Schoone, E. 19, 29
Masson, P. L., see Baggiolini, M. 19, *26*
Matney, T. S., Goldschmidt, E. P., Erwin, N. S., Scroggs, R. A. 105, *120*
Matsubara, K. 94, *120*
Mauel, J., see Brunner, K. T. 69, *75*
Mays, J. A., see Curtiss, R., III 99, *117*
McBride, R. A., Schierman, L. W. 151, 152, 153, 154, 157, *172*
McDevitt, H. O., Benacerraf, B. 131, 134, *172*
McDevitt, H. O., see Mozes, E. 131, 134, *172*
McGregor, D. D., see Gowans, J. L. 64, 70, *76*
Meacock, P., see Broda, P. 107, 108, 111, *117*
Medawar, P. B., see Billingham, R. E. 64, 70, *75*
Medawar, P. B., see Brent, L. 32, *75*
Medawar, P. B., see Lance, E. M. 41, *76*
Medawar, P. B., see Levey, R. H. 40, 41, *76*
Meissner, G. 3, *29*
Merritt, C. B., see Bornside, G. H. 8, *26*
Metcalf, D. 157, *172*
Meynell, E., Cooke, M. 113, *120*
Meynell, E., Datta, N. 112, 113, *120*
Meynell, E., Meynell, G. G., Datta, N. 111, *120*
Meynell, E., see Frydman, A. 113, 114, *118*
Meynell, E., see Lawn, A. M. 111, 112, 113, *120*
Meynell, E., see Nishimura, Y. 81, 83, 105, 112, 113, 116, *121*
Meynell, G. G. 98, *120*
Meynell, G. G., Aufreiter, E. 104, *120*
Meynell, G. G., Lawn, A. M. 95, *120*
Meynell, G. G., see Edwards, S. 113, *118*
Meynell, G. G., see Frydman, A. 114, *118*
Meynell, G. G., see Lawn, A. M. 111, *120*
Meynell, G. G., see Meynell, E. 111, *120*
Meynell, G. G., see Williams, L. 109, *123*
Michael, J. G., see Veit, B. C. 167, *174*
Michaelis, L. 125, 126, *172*
Mickel, S., see Rownd, R. 86, 91, *121*

Middlebrook, G. 2, 25, *29*
Mikulska, B. M., see Amos, D. B. 42, *75*
Millar, K. G., see Eidinger, D. 140, 144, 166, 167, *170*
Miller, C. A., see Cohen, S. N. 85, 86, 91, *117*
Miller, J. F. A. P., Basten, A., Sprent, J., Cheers, C. 39, *76*
Miller, J. F. A. P., see Basten, A. 164, *169*
Miller, J. F. A. P., see Sprent, J. 39, *77*
Miner, P., see Press, R. 86, *121*
Mishell, R. I., Dutton, R. W. 142, *172*
Mishell, R. I., Herzenberg, L. A., Herzenberg, Leonore, A. 42, *76*
Mitani, M., see Nisioka, T. 85, 86, 91, *121*
Mitchison, N. A. 130, 146, 155, 156, 164, *172*
Mitchison, N. A., Rajewsky, K., Taylor, R. B. 154, 161, 164, *172*
Mitchison, N. A., see Dresser, D. W. 70, *75*, 148, *170*
Mitsuhashi, S., Harada, K., Hashimoto, H., Kameda, M., Suzuki, M. 112, *120*
Miyake, T., Shiba, T., Sakurai, T., Watanabe, I. 96, *120*
Miyake, T., see Watanabe, I. 96, *122*
Mobach, H. W., see Silverman, P. M. 103, 104, *122*
Mobach, H. W., see Wendt, L. 98, *122*
Möller, G. 140, 159, 166, 167, *172*
Möller, G., Sjöberg, O. 140, 141, 166, *172*
Möller, G., see Hellström, K. E. 59, *76*
Möller, G., see Lapp, W. S. 166, *171*
Moller, E., see Greaves, M. F. 153, *171*
Moller, G., see Greaves, M. F. 153, *171*
Monaco, A. P., Wood, M. L., Gray, J. G., Russell, P. S. 41, *76*
Monner, D. A., Boman, H. G. 109, *120*
Moody, E. E. M., see Clowes, R. C. 111, *117*
Morita, H., see Wake, A. 23, *30*
Morris, B. C., see Jackson, S. 8, *27*
Morrison, N. E., Antoine, A. D., Dewbrey, E. E. 11, *29*
Morrison, T. G., Malamy, M. H. 108, 110, 113, *120*
Morton, J. I., see Siegel, B. V. 157, *174*
Mowbray, J. F. 167, *172*
Mozes, E., McDevitt, H. O., Jaton, J.-C., Sela, M. 131, 134, *172*
Mozes, E., Shearer, G. M. 134, *172*
Mozes, E., Shearer, G. M., Sela, M. 134, *172*
Mozes, E., Shearer, G. M., Sela, M., Braun, W. 135, 136, *173*
Mozes, E., see Shearer, G. M. 131, 134, 135, 136, *174*
Mozes, E., see Taussig, M. J. 132, 133, 135, 136, 155, *174*
Müller, B., see Rajewsky, K. *173*
Mulczyk, M., Duguid, J. P. 95, *120*
Myrvik, Q., Weiser, R. S. 4, *29*

Nakajima, N., see Oshima, S. 4, *29*
Nakamura, Y., see Rownd, R. 91, *121*
Nakaya, R., see Rownd, R. 91, *121*
Nase, S., see Rajewsky, K. 154, 155, 157, 161, *173*
Nathan, E., see Kallos, P. 3, *27*
Nathans, D., see Kozak, M. 97, *119*
Neilands, J. B. 11, *29*
Neilands, J. B., see Pollack, J. R. 11, *29*
Neveu, T., see Liacopoulos, P. 146, *172*
Newcomer, M. D., see Landau, J. W. 6, 22, *28*
Newton, A., see Wang, C. C. 11, *30*
Nicks, P. J., see Amos, D. B. 32, *75*
Niemans, C. 21, *29*
Nisbeth, N. W., Simonsen, M. Zaleski, M. 158, *173*
Nishida, H., see Watanabe, T. 111, 112, *122*
Nishihara, T., see Watanabe, I. 96, *122*
Nishimura, see Hirota 108
Nishimura, K., see Kondo, E. 18, *28*
Nishimura, Y., Ishibashi, M., Meynell, E., Hirota, Y. 81, 83, 105, 112, 113, 116, *120*
Nishimura, Y., see Ohtsubo, E. 99, 100, 101, 102, 109, *121*
Nisioka, T., Mitani, M., Clowes, R. C. 85, 86, 91, *121*
Nissen, B., see Hill, W. C. 17, *27*
Nomura, M. 82, *121*
Nossal, G. J. V., Ada, G. L. 148, 161, 164, *173*
Nossal, G. J. V., see Ada, G. L. 148, *169*
Notani, G., see Cohn, M. 154, *170*
Novick, R. P. 80, 88, 89, 90, 91, 112, *121*
Novick, R. P., Brodsky, R. 91, *121*
Novotny, C., Carnahan, J., Brinton, C. C., Jr. 95, 115, *121*
Novotny, C., Knight, W. S., Brinton, C. C., Jr. 95, *121*
Novotny, C. P., Lavin, K. 116, *121*
Nussenzweig, V., see Dukor, P. 164, *170*
Nussenzweig, V., see Pincus, C. S. 151, 152, *173*

Oakley, C. L. 169, *173*
O'Callaghan, R. J., see Krahn, P. M. 97, *119*
Ogata, C., see Watanabe, T. 111, 112, *122*
Ohki, M., Ozeki, H. 113, *121*
Ohki, M., Tomizawa, J. 93, 94, *121*
Ohno, T., see Watanabe, I. 96, *122*

Ohtsubo, E. 101, 102, 106, 108, *121*
Ohtsubo, E., Nishimura, Y., Hirota, Y. 99, 100, 101, 102, 109, *121*
Ohtsubo, E., see Sharp, P. A. 81, 84, 86, 105, 106, 108, *122*
Okada, M., see Watanabe, T., 109, *122*
Old, L. J., Boyse, E. A. 59, *76*
Omer, T. F., see Albright, J. F. 158, *169*
Orr, K. B., see Paraskevas, F. 164, 165, *173*
Osawa, S., see Watanabe, I. 96, *122*
Oshima, S., Fugita, Y., Takeoka, A., Nakajima, N., Tsuji, S. 4, *29*
Oshima, S., see Tsuji, S. 4, *30*
O'Toole, Davies 144, 167
Otsuji, N., see Hakura, A. 109, *119*
Ou, J. T. 96
Ou, J. T., Anderson, T. F. 95, 96, 100, *121*
Ovary, Z., Benacerraf, B. 155, *173*
Ovary, Z., see Ashley, H. 157, *169*
Overby, L. R., Barlow, G. H., Doi, R. H., Jacob, M., Spiegelman, S. 96, *121*
Ozeki, H., see Ohki, M. 113, *121*

Pagel, W. 3, *29*
Palchoudhury, S. R., Iyer, V. N. 88, *121*
Palm, J. 42, 58, *76*
Palm, J., see Elkins, W. L. 58, *75*
Palm, J., see Ramseier, H. 42, 43, 58, *77*
Paranchych, W., Ainsworth, S. K., Dick, A. J., Krahn, P. M. 97, *121*
Paranchych, W., Krahn, P. M. 97, *121*
Paranchych, W., see Krahn, P. M. 97, *119*
Paraskevas, F., Orr, K. B., Anderson, E. D., Lee, S.-T., Israels, L. G. 164, 165, *173*
Parish, H. J., Cannon, D. A. 169, *173*
Pato, M. L., see Zeuthen, J. 90, *123*
Patterson, R. J., Youmans, G. P. 15, 16, *29*
Patton, C., see Kochan, I. 5, *28*
Paul, C., Shimizu, A., Köhler, H., Putnam, F. W. 39, *76*
Paul, W. E., see Katz, D. H. 155, 157, *171*
Peacocke, N., see Amos, D. B. 32, *75*
Pearlman, D. S. 151, 152, *173*
Pekarek, R. S., Bostian, K. A., Bartellony, P. J., Calia, F. M., Beisel, W. R. 9, *29*
Pekhov, A. P., see Scaife, J. 84, *122*
Pellis, N. R., see Kochan, I. 10, 12, 19, 20, 24, *28*
Peltre, G., see Rajewsky, K. *173*
Perament, F., see Liacopoulos, P. 146, *171*
Perkins, F. T., see Stanbridge, E. J. 41, *77*
Perlmann, P., Holm, G. 69, *76*
Pernis, B., Forni, L., Amante, L. 39, *76*
Peters, L. C., see Hanna, M. G. 140, 164, *171*
Peterson, R. D. A., Hendrickson, R., Good, R. A. 158, *173*
Peterson, R. D. A., see Dent, P. B. 158, *170*
Pfohl, D. G., see Kochan, I. 19, 20, *28*
Piccinin, G. L., see Dettori, R. 109, *118*
Pincus, C. S., Lamm, M. E., Nussenzweig, V. 151, 152, *173*
Pincus, C. S., Nussenzweig, V. 151, *173*
Pittard, J., see Walker, E. M. 104, *122*
Pohlit, H., see Dennert, G. 151, *170*
Pollack, J. R., Ames, B. N., Neilands, J. B. 11, *29*
Pollack, J. R., Neilands, J. B. 11, *29*
Prelog, V., see Bickel, H. 11, *26*
Press, R., Glansdorff, N., Miner, P., Devries, J., Kadner, R., Mass, W. K. 86, *121*
Pritchard, R. H., Barth, P. T., Collins, J. 89, 90, *121*
Ptashne, M. 93, *121*
Purifoy, D. J., Lankford, C. E. 22, *29*
Purifoy, D. J., Wilkins, T. D., Lankford, C. E. 22, *29*
Putnam, F. W., see Paul, C. 39, *76*

Rabellino, E., Colon, S., Grey, H. M., Unanue, E. R. 39, *77*
Radovich, J., Talmage, D. W. 140, 141, 142, 166, *173*
Radovich, J., see Talmage, D. W. 159, *174*
Raff, M. C., Sternberg, M., Taylor, R. B. 39, *77*
Raffel, S. 3, 4, *29*
Raffel, S., see Goihman-Yahr, M. 17, *27*
Raffel, S., see Kochan, I. 4, 7, 8, *28*
Rajewsky, K., Rottländer, E. 154, *173*
Rajewsky, K., Rottländer, E., Peltre, G., Müller, B. *173*
Rajewsky, K., Schirrmacher, V., Nase, S., Jerne, N. K. 154, 155, 157, 161, *173*
Rajewsky, K., see Dennert, G. 151, *170*
Rajewsky, K., see Mitchison, N. A. 154, 161, 164, *172*
Ralston, D. J., Elberg, S. S. 15, 16, *29*
Ramseier, H. 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 49, 55, 65, 69, 71, 72, 74, *77*
Ramseier, H., Billingham, R. E. 32, 35, *77*
Ramseier, H., Lindenmann, J. 39, 43, 44, 45, 47, 48, 49, 54, 55, 56, 58, 59, 68, 69, 71, 74, *77*
Ramseier, H., Palm, J. 42, 43, 58, *77*
Ramseier, H., Streilein, J. W. 32, 33, *77*

Rhodes, J. M., Lind, I. 164, *173*
Rice, S. A., see Cohn, M. 154, *170*
Richmond, M. H., see Beard, J. P. 98, 112, 113, *116*
Richter, A. 84, *121*
Rittenberg, M. B., Amkraut, A. 157, *173*
Roelants, G. E., Goodman, J. W. 139, 144, 162, *173*
Rogers, H. J. 8, 10, *29*
Rogers, H. J., Bullen, J. J., Cushnie, G. H. 9, *29*
Rogers, H. J., see Bullen, J. J. 8, 9, 10, 21, 22, 23, *26*
Rosenthal, S., see Silverman, P. M. 104, *122*
Roth, T. F., see Hickson, F. T. 91, *119*
Rotman, B., see Celada, F. 148, 149, *170*
Rottländer, E., see Rajewsky, K. 154, *173*
Rowe, J. R., see Landau, J. W. 6, 22, *28*
Rownd, R. 91, *121*
Rownd, R., Kasamatsu, H., Mickel, S. 91, *121*
Rownd, R., Mickel, S. 86, 91, *121*
Rownd, R., Nakaya, R., Nakamura, Y. 91, *121*
Rownd, R., see Kasamatsu, H. 91, *119*
Rupp, W. D. 94
Rupp, W. D., Ihler, G. 93, *122*
Rupp, W. D., see Ihler, G. 93, *119*
Rupp, W. D., see Vapnek, D. 83, 84, 85, 93, 94, *122*
Russell, P. S., see Gray, J. G. 32, *76*
Russell, P. S., see Monaco, A. P. 41, *76*

Said, M., see Kochan, I. 5, *28*
Sakurai, T., see Miyake, T. 96, *120*
Sakurai, T., see Watanabe, I. 96, *122*
Salaman, M. H., Wedderburn, N. 158, *173*
Salvin, S. B., Smith, R. F. 155, 157, *173*
Salvin, S. B., see Coe, J. E. 157, *170*
Sato, S., see Watanabe, T. 111, 112, *122*
Sauton 14
Scaife, J. 81, 84, 85, *122*
Scaife, J., Gross, J. D. 86, *122*
Scaife, J., Pekhov, A. P. 84, *122*
Scaife, J., see Broda, P. 84, *117*
Schade, A. L. 8, *29*, *30*
Schade, A. L., Caroline, L. *8*, *30*
Schechter, I. 138, 145, *173*
Schechter, I., Sela, M. 137, *173*
Schechter, I., see Bauminger, S. 138, 144, *169*
Schechter, I., see Tarrab, R. 138, *174*
Schiermann, L. W., see McBride, R. A. 151, 152, 153, 154, 157, *172*
Schindler, R., see Brunner, K. T. 69, *75*
Schirrmacher, V., see Rajewsky, K. 154, 155, 157, 161, *173*
Schmid, K., see Cooperband, S. R. 167, *170*
Schnös, M., see Caro, L. G. 96, 98, *117*
Schoone, E., see Masson, P. L. 19, *29*
Schütte, A., see Vielmetter, W. 93, *122*
Schwartz, H., Leskowitz, S. 143, 144, 145, *173*
Schwartz, R. S. 150, *173*
Scroggs, R. A., see Matney, T. S. 105, *120*
Seibert, F. B., see Emmart, E. W. 4, *27*
Seidman, P., Chan, E. C. S. 11, *30*
Sela, M. 131, *174*
Sela, M., Fuchs, S., Arnon, R. 131, *174*
Sela, M., see Bauminger, S. 138, 144, *169*
Sela, M., see Fuchs, S. 131, *171*
Sela, M., see Jaton, J.-C. 131, *171*
Sela, M., see Mozes, E. 131, 134, 135, 136, *172*, *173*
Sela, M., see Schechter, I. 137, *173*
Sela, M., see Shearer, G. M. 131, 134, 135, 136, *174*
Sela, M., see Tarrab, R. 138, *174*
Sela, M., see Taussig, M. J. 132, 133, 135, 136, 155, *174*
Sell, S., Gell, P. G. H. 39, *77*
Sergeant, T. P., see Lankford, C. E. 11, *28*
Shaffer, C. F. 41, *77*
Shapiro, J. A., see Ippen, K. A. 101, *119*
Sharp, P. A., Hsu, M., Ohtsubo, E., Davidson, N. 81, 84, 86, 105, 106, 108, *122*
Shearer, G. M., Mozes, E., Sela, M. 131, 134, 135, 136, *174*
Shearer, G. M., see Mozes, E. 134, 135, 136, *172*, *173*
Shearer, G. M., see Taussig, M. J. 132, 133, 135, 136, 155, *174*
Shiba, T., see Miyake, T. 96, *120*
Shiba, T., see Watanabe, I. 96, *122*
Shimizu, A., see Paul, C. 39, *76*
Shull, F. W., Fralick, J. A., Stratton, L. P., Fisher, W. D. 94, *122*
Siegel, B. V., Morton, J. I. 157, *174*
Sieker, H. O., see Amos, D. B. 32, *75*
Silver, R. P., Falkow, S. 85, *122*
Silver, R. P., see Falkow, S. 94, *118*
Silverman, P. M., Mobach, H. W., Valentine, R. C. 103, *122*
Silverman, P. M., Rosenthal, S., Mobach, H., Valentine, R. C. 104, *122*
Silverman, P. M., Rosenthal, S., Valentine, R. C. 103, *122*
Silverman, P. M., Valentine, R. C. 97, *122*
Silvers, W., see Billingham, R. 64, 70, *75*
Silvers, W. K., see Billingham, R. E. 36, *75*

Simms, E. S., see Eisen, H. N. 148, *170*
Simonsen, M. 46, *77*, 158, 159, *174*
Simonsen, M., see Ford, W. L. 46, 73, *75*
Simonsen, M., see Lawrence, W. 140, 158, 159, 166, *171*
Simonsen, M., see Nisbet, N. W. 158, *173*
Siskind, G. W., Benacerraf, B. 160, *174*
Siskind, G. W., see Brody, N. I. 129, 143, 144, 154, 162, 167, *170*
Sjöberg, O., see Möller, G. 140, 141, 166, *172*
Smith, G. P., Hood, L., Fitch, W. M. 127, *174*
Smith, L., see Kochan, I. 15, 16, 17, *28*
Smith, R. F., see Salvin, S. B. 155, 157, *173*
Smith, R. T., Landy, M. 157, *174*
Sneath, P. H. A., see Hirota, Y. 81, *119*
Snell, G. D., Stimpfling, J. H. 42, *77*
Snow, G. A. 11, 14, 15, *30*
Snow, G. A., see Francis, J. 11, *27*
Snow, G. A., see White, A. J. 14, *30*
Soikkeli, S., see Mäkelä, O. 109, *120*
Sparrow, E. M., see Amos, D. B. 42, *75*
Spiegelman, S., see Oberby, L. R. 96, *121*
Sprent, J., Miller, J. F. A. P. 39, *77*
Sprent, J., see Basten, A. 164, *169*
Sprent, J., see Miller, J. F. A. P. 39, *76*
Stadler, J., Adelberg, E. A. 92, *122*
Stallions, D. R., see Curtis, R., III 95, 97, 98, 99, *117*
Stanbridge, E. J., Perkins, F. T. 41, *77*
Steiner, L. A., see Eisen, H. N. 148, *170*
Steinmüller, D., see Billingham, R. E. 36, *75*
Stephenson, M. 2, *30*
Sternberg, M., see Raff, M. C. 39, *77*
Stimpfling, J. H., see Snell, G. D. 42, *77*
Stotts, J., see Kochan, I. 5, *28*
Strand, M., see Valentine, R. C. 97, 98, *122*
Stratton, L. P., see Shull, F. W. 94, *122*
Streilein, J. W., see Ramseier, H. 32, 33, *77*
Studier, F. W., Maizel, J. V., Jr. 110, *122*
Suzuki, M., see Mitsuhashi, S. 112, *120*
Svedmyr, E. A. J., see Golstein, P. 69, *75*
Sword, C. P. 9, 21, 22, *30*
Sword, C. P., see Armstrong, B. A. 25, *26*
Sword, C. P., see Kingdon, G. C. 18, *28*

Takeoka, A., see Oshima, S. 4, *29*
Takeoka, A., see Tsuji, S. 4, *30*
Talmage, D. W., Radovich, J., Hemmingsen, H. 159, *174*
Talmage, D. W., see Radovich, J. 140, 141, 142, 166, *173*
Tarrab, R., Schechter, I., Sela, M. 138, *174*
Taussig, M. J. 127, 129, 140, 145, *146*, *174*
Taussig, M. J., Lachmann, P. J. 127, 129, 130, 144, 151, 164, *174*
Taussig, M. J., Mozes, E., Shearer, G. M., Sela, M. 132, 133, 135, 136, 155, *174*
Taylor, A. L. 80, *122*
Taylor, R. B., Iverson, G. M. 143, 157, *174*
Taylor, R. B., see Mitchison, N. A. 154, 161, 164, *172*
Taylor, R. B., see Raff, M. C. 39, *77*
Thomas, L., see Weissmann, G. 19, *30*
Thorbecke, G. J., see Frei, P. C. 147, *171*
Tomizawa, J., see Ohki, M. 93, 94, *121*
Tompkins, L. S., see Falkow, S. 94, *118*
Truffa-Bachi, P., Wofsy, L. 39, *78*
Tsuji, S., Ito, K., Oshima, S. 4, *30*
Tsuji, S., Oshima, S., Takeoka, A. 4, *30*
Tsuji, S., see Oshima, S. 4, *29*
Tsumita, T., see Kondo, E. 18, *28*
Twort, F. W., Ingram, G. L. Y. 11, *30*

Uhr, J. W., Baumann, J. B. 153, *174*
Unanue, E. R. 39, *78*
Unanue, E. R., see Rabellino, E. 39, *77*

Valentine, R., see Wendt, L. W. 98, *122*
Valentine, R. C., Strand, M. 97, 98, *122*
Valentine, R. C., see Ippen, K. A. 95, *119*
Valentine, R. C., see Silverman, P. M. 97, 103, 104, *122*
van Bekkum, D. W., see Balner, H. 41, *75*
Vannotti, A. 24, *30*
Vapnek, D., Lipman, M. B., Rupp, W. D. 83, 84, 85, 93, *122*
Vapnek, D., Rupp, W. D. 93, 94, *122*
Veit, B. C., Michael, J. G. 167, *174*
Vielmetter, W., Bonhoeffer, F., Schütte, A. 93, *122*
Vielmetter, W., see Bonhoeffer, F. 93, *117*
Vischer, E., see Bickel, H. 11, *26*

Wake, A., Morita, H., Yamamoto, M. 23, *30*
Waksman, B. H., see Dvorak, H. F. 32, *75*
Walker, E. M., Pittard, J. 104, *122*
Walker, J. G., see Brody, N. I. 129, 143, 154, *170*
Wang, C. C., Newton, A. 11, *30*
Warner, N., see Ada, G. L. 39, *75*
Warner, N. L. 32, *78*
Wasz-Höckert, O., see Zitrin, C. M. 4, *30*
Watanabe, I., Miyake, T., Sakurai, T., Shiba, T., Ohno, T. 96, *122*

Watanabe, I., Nishihara, T., Kaneko, H., Sakurai, T., Osawa, S. 96, *122*
Watanabe, I., see Miyake, T. 96, *120*
Watanabe, T. 82, *122*
Watanabe, T., Nishida, H., Ogata, C., Arai, T., Sato, S. 111, 112, *122*
Watanabe, T., Okada, M. 109, *122*
Waterston, R. H. 140, 141, 142, *174*
Webb, D., see Braun, W. 135, *169*
Webster, R. G., see Fazekas de St. Groth, S. 148, *170*
Wedderburn, N., see Salaman, M. H. 158, *173*
Weigle, W. O., High, G. J. 131, 144, *174*
Weil, A. C., see Bornside, G. H. 8, *26*
Weinberg, E. D. 3, 13, *30*
Weiser, R. S., see Myrvik, Q. 4, *29*
Weissmann, G., Thomas, L. 19, *30*
Wendt, L., Mobach, H. W. 98, *122*
Wendt, L. W., Ippen, K. A., Valentine, R. 98, *122*
Wettstein, A., see Bickel, H. 11, *26*
Wheeler, W. C., Hanks, J. H. 17, *30*
White, A. J., Snow, G. A. 14, *30*
Wiener, E., see Cohn, Z. A. 18, 21, *27*
Wigzell, H., Mäkelä, O. 39, *78*
Wigzell, H., see Golstein, P. 69, *75*
Wilkins, B. M. 111, *122*
Wilkins, T. D., see Purifoy, D. J. 22, *29*
Willetts, N., see Achtman, M. 80, 81, 98, 99, 100, 101, 102, 103, 104, 105, 106, 109, 115, *116*
Willetts, N., see Ippen-Ihler, K. 101, 102, 108, *119*
Willetts, N. S. 101, 104, 105, 108, 111, 115, *122*
Willetts, N. S., Achtman, M. 92, 100, 101, 106, 115, *123*
Willetts, N. S., Broda, P. 108, 111, *123*
Willetts, N. S., Finnegan, D. S. 113, *123*
Willetts, N. S., see Finnegan, D. J. 114, *118*
Williams, L., Meynell, G. G. 109, *123*
Williamson, A. R., see Kreth, H. W. 162, *171*
Wilson, A. B., see Bullen, J. J. 22, *26*
Wilson, D. B. 69, *78*
Wilson, D. B., see Billingham, R. E. 64, *75*
Wilson, J. B., see Baker, P. J. 9, *26*
Winchurch, R., see Braun, W. 135, *169*
Wofsy, L., see Truffa-Bachi, P. 39, *78*
Wollman, E. L., see Jacob, F. 105, 107, *119*
Wood, M. L., see Monaco, A. P. 41, *76*
Woodruff, M. F. A., see Caspary, E. A. 41, *75*
Woodruff, M. F. A., see Howard, J. G. 140, 166, *171*
Woods, D. A. 41, *78*

Yamamoto, M., see Wake, A. *23*, *30*
Yarmolinky, M. 111
Youmans, A. S., see Youmans, G. P. 16, *30*
Youmans, G. P., Youmans, A. S. 16, *30*
Youmans, G. P., see Patterson, R. J. 15, 16, *29*

Zähner, H., see Bickel, H. 11, *26*
Zakarian, S. 73, 74
Zaleski, M., see Nisbet, N. W. 158, *173*
Zeuthen, J., Pato, M. L. 90, *123*
Zitrin, C. M., Wasz-Höckert, O. 4, *30*
Zlatogoroff, S. J., Glusman, M. P., Kandyba, L. L. 126, *174*

Subject Index

absorption, selective 54, 55
acridine orange sensitive cistrons 110
agar-plate diffusion test 12, 13, 14, 19
Ag-B locus 36, 48
albumin, detoxifying activity 20
alloantibody, humoral product of sensitized cells 69
—, representing recognition structure (RS) 71
ALS see anti-lymphocytic serum
anautosequestric bacterial species 11
antibacterial factors, cellular 15, 17, 18, 19, 20, 21
— —, extracellular, affecting intracellular bacteria 16
— —, humoral 15, 17
antibodies, antimycobacterial activity of 4
—, cooperating, localization of antigen by 164, 165
—, enhancing 60, 65, 70, 150, 151, 152, 153, 154, 161
— in vaccinated or infected hosts 2
— to mycobactin 10, 25
— to receptors recognizing histocompatibility antigens 31, 49
—, tumor growth enhancing 60
antigen 32 55, 56
—, antibody-mediated, improved localization 151, 152
—, dominant 126
—, suppressed 126
—, trapping by dendritic cells 148, 151
antigenic competition 125
— —, abolition of, enhancement and 151
— — and immune response, similar effects 148
— —, „abortive" immunogenicity and 139
— —, antibody interfering with 129
— —, cell types involved 134, 136, 139, 142
— —, definition of 126
— —, delayed hypersensitivity and 139, 144, 145
— —, dose dependence 128, 129, 132, 133, 164, 166
— —, immunocompetent cells and 141, 150
antigenic competition, intermolecular 126, 162
— —, —, effect of polyribonucleotides 135, 136
— —, —, role of crossreacting carrier mutants 138
— —, —, effect on priming and memory 132, 133, 134
— —, intramolecular 126, 160
— —, —, between hapten and carrier 138, 139, 144
— —, —, effect of carrier 143
— —, —, specific for stereoisomers 137
— —, level of immune response and 135, 136
— —, macrophages and 130, 144, 146
— —, priming of response to suppressed antigen 133, 134
— —, recognition and 129, 130, 134, 139, 146
— —, relation to human vaccination 168
— —, result of high-zone tolerance 146
— —, — of low-zone tolerance 146
— —, sequential 126, 166
— —, —, essential role of T cells 142, 153, 167
— —, —, heterologous erythrocytes and 140, 141, 142, 143
— —, —, immunosuppressive effect of oncogenic viruses 158
— —, —, importance of site of injection of antigens 144
— —, —, inhibitory factors and 141, 167
— —, tolerance to the dominant antigen and 144, 146
— —, tumor development and 157
— cooperation 152, 154, 155, 156, 157
— determinants, „immunosilent" 130
antigenic recognition, cell-bound receptors and 160
— —, competition and 129, 130, 134, 139, 146, 150
— —, cooperation and 155, 156
— —, effect of alloantisera 43, 44, 45, 50, 51, 52, 53, 54, 59
— —, — of anti-lymphocytic serum 40
— —, — of enhancing serum 59, 65
— —, — of trypsin 38, 39

antigenic recgnition, quantitative measurement 35
— —, specific inhibition of 42, 55, 59, 71
— sin, original, phenomenon of 148, 149, 150
antigensensitive cells, competition between 160
— —, precommitment of 162
— —, random distribution of 160
anti-lymphocytic serum 40, 41, 72
— —, immunosuppression and 41
anti-PAR (product of antigenic recognition) serum 41, 42
anti-receptor antibodies 31, 49, 55, 65, 67, 68, 69, 70, 71, 72, 74
— —, IgG fraction and 71
— —, specificity of 55
anti-RS (recognition structure) antibodies, immunosuppression by 71
— — — sera 42, 45, 46, 47, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59, 65, 66, 67, 68, 69, 71, 72, 73, 74
— —, alloantiserum-induced, specificity of 58, 59
— —, cell-induced 48, 58, 59
— —, —, specificity of 55
antisera, enhancing 59, 60, 61, 62, 63
antitoxins 1
autosequestric bacterial species 11

B cells 39, 71, 74, 134, 136, 139, 142, 153, 154, 159, 161, 162, 163, 164, 165, 166, 167, 168
bacteria, digestion by intracellular enzymes 2
—, direct destruction of by specific antibodies and complement 1
—, nontoxigenic 1, 2, 24
bacterial chromosome, molecular weight 83
— conjugation 80, 81, 82, 93, 94, 95, 96, 98
— infection, resistance to 24
— infections, iron-induced susceptibility to 21, 22
— nutrition in infected hosts 3
— sex factors 79ff.
— — —, classification by incompatibility 88, 89
— — —, incompatibility of 81, 82, 86, 87, 88, 89, 91, 98
— — —, nomenclature 80
— — —, relationship between 111, 112
— — —, replication and segregation 80, 89, 90, 91, 92
— — — (Table) 81
— — —, transfer replication 94
— — —, vegetative replication 94
bacteriophages, antibody assays with 127
bacteriostatic activity of lactoferrin 19
— — of transferrin 8
bacteriostasis, alleviation with iron in normal serum 3
—, abolition of in antisera by heme compounds 9
—, alleviation with iron in immune serum 8
— of serum, alleviation of with iron-binding bacterial products 10
BCG 6, 7, 21
— cells 5, 7, 9, 11, 12, 13, 14, 17
— -vaccination 4, 8, 9, 10, 17
β-galactosidase, inactive mutant AMEF, activation by crossreacting antibody 149, 150
beta-globulin fraction with antimycobacterial activity 5
beta-lipoprotein 5
beta proteins, heatlabile 5
beta-2A globulin 5
Blutal 23
body fluids, antimycobacterial effect of 4

carrier-effect 154
cell-antigen interactions 32
coeruloplasmin 5
Cohn fractions, antituberculous factor in 4
Col 80, 81, 82, 83, 89, 91, 111, 112, 113
— DNA, molecular weight 86
— —, replication of during amino acid starvation 90
colicin 82
competition, antigenic see antigenic c.
complement, bacteriostasis and 10
—, tuberculostasis and 4
conjugation see bacterial c.
Coulter Counter technique 102

defense mechanisms, extracellular 3
defenses, natural, of the host 1
dendritic cells 148, 151, 163, 164, 165
Desferal (desferrioxamine B methane sulfate) 7, 22
diseases, chronic 2, 22
—, infectious, iron promoted 21, 22, 23
DNA transfer, conjugational 80, 82, 93, 94, 95, 96, 99, 103, 104, 105
— —, —, inhibition by male-specific phages 95
— —, —, surface exclusion and 99
— —, —, unaffected by DNase 95
Dubos medium 5, 7, 12, 19
endotoxin, hypoferremic effect 9, 10, 22
enhancing serum 59, 60, 61, 62, 63, 65
enterobacteriaceae 79
enterobactin 11
ethidium bromide CsCl gradients 84

exotoxins 1
—, active or passive acquisition of antibodies to 1

F, acridine orange treatment and 110
F (Table) 81
F^- (cured) cells 80, 81, 82
F DNA, attachment region 105
—, cistrons, estimated number of 84
—, contour length 83, 84, 87
—, covalently-closed circles 83, 84, 87, 95
—, excision of 82, 84, 85, 107
—, integration of 81, 84, 85, 105, 106, 107
—, molecular weight, estimation of 83, 84, 85
—, molecular weight, estimation of after transfer 94
—, physical map 106
—, replication-control of 89, 90, 91
—, replication of 80, 81, 82, 84, 86, 89, 90, 113
—, — of, intermediate in 84
—, — of, initiation of 90
—, — of, timing of 90
—, — of, mapping of cistrons involved 108
—, single-stranded, transfer of 93
—, supercoiled circle, complexed with cellular material 84
—, transfer of 93
—, — of and integration site 107, 108
—, transferred to F^- minicells 93, 94
—, transfer replication 92
—, vegetative replication 92
F, double male strains 86, 87
F, heterozygotes, temporary 86
Flac deletion mutant 91
F-pili 82, 95, 111, 112, 113
—, antigenic specificity of 113
—, CsCl bouyant density 98, 102
— mutants, male-specific phage resistant 104
—, number of per cell 97, 103, 104
—, role in conjugational DNA transfer 95, 96, 103
—, — in male-specific phage infection 96, 97, 102
—, specificity 101, 102, 104, 105, 115
—, structural proteins 97, 98, 102, 114, 115
—, synthesis of after shearing 115, 116
—, —, function of tra cistrons 102
F prime (Table) 81
—, fusion of 87, 88
$(F,R)^+$ heterozygotes 99, 105, 113
F replication inhibitor 89
F sex factor, genetics of 79ff.
F, transfer-deficient (Tra^-) mutants 100

fatty acids, antibacterial activity 25
— —, antimycobacterial activity 21
FCA = Freund's complete adjuvant
ferritin 18, 19, 22, 24, 25, 26
flow-curtain electrophoresis 5

graft-versus-host reaction, mediated by T cells 159
— reactions 31, 32, 39, 45, 46, 47, 73, 140, 158, 159
granulotactic activity, mediator with 33, 34, 35
guinea pigs, antimycobacterial activity of liver cells of 19, 20
— —, BCG-vaccinated 8, 9, 17
— —, enhanced virulence of E. coli in 21
— pig serum, bacillary multiplication and 7
— — —, growth-supporting nature of 8
— — —, tuberculostasis exhibited by 21

hamster, hybrid strain (CB×LSH)F1 42, 44, 50
—, — — (CB×MHA)F1 40, 44, 50, 51
—, inbred strain CB 36, 40, 42, 44, 50, 51
—, — — LSH 36, 42
—, — — MHA 35, 36, 44, 50, 51
Hanks's solution 5, 6, 34, 37, 38, 39
heteroduplex DNA molecules 84
Hfr 81, 82
—, incompatibility mutants 88
—, model for the formation of 85, 107
— strains, cured derivatives 84, 110
—, superinfection by F 86
histoincompatible cells, study of interactions of 33, 34, 37
histoincompatibility, H-1 locus 33
—, H-2 locus 33, 36, 48, 55, 56, 57, 58, 60, 72, 73, 134
—, non-H-2 loci 33, 36, 45, 48, 58, 158
—, H-3 locus 33
—, H-Y locus 33, 43, 45, 48
—, Y locus 36, 44
horror autotoxicus (Paul Ehrlich) 46
host antigens, source of 32
HR sarcoma 60, 61, 62, 63
human serum, intracellular mycobacterial growth and 16
hypersensitivity, delayed 17, 139, 144, 145, 162
hypoferremia 8, 9, 10, 17, 22, 24, 25

IgG, antigenic determinants, competition of 127, 128, 129, 130, 131
—, — —, inaccessible 127, 130
— fragments, tolerance to the dominant antigen and competition 144
— —, antigenic competition and 127

IgM, enhancement mechanism and 151
—, similar to receptors for transplantation antigens 39
ILTR = immune lymphocyte transfer reactions 31, 32
immune cells 25
— —, hydrolytic enzymes in lysosomes of 18
— —, hypersensitive response of 15, 17
— —, immunocytotic activity 15
— induction, cellular processes involved 147, 148
— response, control gene in guinea pigs linked to histocompatibility locus 139
— —, cooperating antibody as inhibitor and 164
— —, crossstimulation by crossreacting antigens 149
— —, enhancing effect of antibodies 150, 151, 152, 153, 154, 161
— —, genetic control of 131, 139, 148, 161
— —, Ir-1 locus 134
— —, Ir-3 locus 134
— —, limiting role of T cells 142, 159
— —, maturation of 160
— —, memory cells, differentiated 140
— —, polyribonucleotides and 135, 136
— —, precursor cells, pluripotential 140, 141, 158
— —, regulation of and antigenic competition 143, 148, 158, 159
— —, specific suppression of 71
— —, spreading of 130, 154
— —, suppressing effect of antibodies 150, 161
— —, thymusdependent 137
— sera, acquired antimycobacterial activity of 8
— —, antibacterial effect and iron binding capacities 9, 10
— surveillance 157
— system, central failure of 64, 70
— —, — role in preventing tumors 157
— —, peripheral failure of 70
immunity, cellular, activated macrophages and 17, 18
—, —, concept of 15, 16, 17
—, development suppressed by Blutal 23
—, nutritional 25
—, passive transfer of 17
—, persistence in the vaccinated animal 2
—, role of phagocytes in 16
— to tuberculosis based upon antimycobacterial activity of antibodies 4
—, tuberculous, role of phagocytic cells in 4
inbred strain A animals 33
incompatibility, escape from in RecA$^-$ strains 86, 88
incompatibility groups, classification of sex factors 88, 89, 112
—, models of molecular mechanism of 89
— mutants, search for 109
— of F factors 81, 82, 86, 87, 107
— —, cistrons involved 88, 107
— —, mapping of cistrons involved 108
— of R factors 88, 113
—, ts replication-deficient mutants of E. coli and 88, 91, 92, 109
infection, tuberculous 2
inflammatory reactions of the delayed type 31, 32
I-pili 95, 96, 111, 112
iron, alleviation of bacteriostasis with 3, 8
— -binding materials, secretion of 11
— -chelating bacterial product 10, 12, 13, 14
— -chelating bacterial product, antibodies to 25
— product in leukocytes 19
—, colloidal chondroitinsulfate compound (Blutal) 23
—, defense mechanisms of the host and 23
—, disease-promoting effect 23
—, essential for bacterial growth 2, 3, 10, 15, 22, 24, 25
—, fate of bacteria in tissues and 15
—, ferritin and 18, 24
— metabolism 10
—, minimum requirements for bacterial growth 3
— neutralization of tuberculostasis 6, 9, 19, 21
—, resistance-neutralizing property of 23
—, role in bacterial infections 1, 3, 16, 21, 22, 23, 25
—, — in the survival of serum-exposed tubercle bacilli 4
—, transferrin and 24
— -transferrin, dissociation balance 8
— interplay in tuberculosis 6, 7, 8
—, Vegex "fortified" with 5
irradiation, lymphocyte count after 32

Kirchner medium 5

lactoferrin 19
λ DNA, single-stranded, transfer of from lysogenic donors 93
leucotactic activity, mediator with 33, 34, 35
lipase, antimycobacterial activity and 19, 20, 21
lipopolysaccharide (LPS) 8, 9, 22
liver cells, activated (ALC) 20
— —, normal (NLC) 20
— — of guinea pigs, antimycobacterial activity of cell fractions of 19, 20

lung tissue extracts, antimycobacterial effects 18
lymph node weight assay 73
lymphocytes, cooperation between 148
—, small, circulating 32
—, thymic 33
lysosomes 18, 19, 20, 24, 25

M see mycobactin
macrophages 15, 16, 17, 21, 23, 25, 162, 163, 165
—, activated, increased microbicidal activity 18
—, —, source 17
—, fate of bacteria in extracts of 17
—, multiplication of parasites in 16, 18
—, presentation of antigen to lymphocytes 147, 151
—, tissue culture of 16, 23
mating see bacterial conjugation
media, antibacterial tissue culture 15
—, growth-supporting quality for tubercle bacilli 4
—, suitable for the study of serum tuberculostasis 5, 13
medium 199 34, 35, 36
memory cells 140, 157, 159
methylcholanthrene 60
micelles of ferric hydroxide, ferritin molecule and 18
minicells, F DNA transferred to 93, 94
mononuclear cells 33, 35
mouse, hybrid strain (A × CBA)F1 37, 44, 51, 55, 56, 57, 58, 64, 65, 68, 72
—, — — (A × C57BL/6)F1 37, 39, 50, 55, 56, 73
—, — — (A × C3H)F1 40, 42
—, — — (B10.A × B10.D2)F1 58
—, — — (B10 × B10.D2)F1 58
—, — — (CBA × BL/6)F1 40
—, — — (CBA × C57BL/6)F1 37, 40, 42, 56, 62, 73
—, — — (CBA × DBA/2)F1 57, 58
—, inbred strain A 37, 38, 40, 42, 44, 50, 51, 55, 56, 57, 58, 65, 66, 67, 68, 69, 72
—, — — A3H 36
—, — — B10
—, — — B10.A 58
—, — — BALB/c 128, 129, 157
—, — — C3H 36, 40, 158
—, — — C3H/HdJ 156
—, — — C3H/HeJ 131, 132, 133, 134, 135, 136, 137, 147, 148, 155, 156, 162, 166
—, — — C57BL/6 36, 37, 38, 39, 40, 42, 44, 45, 50, 51, 55, 56, 60, 61, 72, 73
—, — — C57BL/10 58
—, — — CBA 36, 37, 38, 40, 42, 44, 51, 55, 56, 57, 58, 60, 61, 62, 63, 64, 65, 66, 67, 68, 72
mouse, inbred strain DBA/1 131, 132, 134, 135, 136, 137, 148, 162, 166
—, — — DBA/2 42, 57, 58
—, — — SJL 134
mycobacteria, ability to obtain iron from ferritin 19
Mycobacterium lepraemurium 18
— paratuberculosis 11, 24
— phlei 11
— tuberculosis 10, 18
mycobactin (M) 10, 11, 12, 13, 14, 15, 17, 24, 26
—, association with lipoidal cell wall 14, 24
—, solubility in Tween solution 14, 24
—, virulence factor 24

NLTR = normal lymphocyte transfer reactions 31, 32
—, immunogenetic basis of 32
N-Z amine 5

oncogenic viruses, immunosuppressive effect 158
operon 11
oxazolone 157

PAR (product of antigenic recognition) 46, 55, 56, 57, 58, 60, 68, 71, 72, 74
— — — antisera 41
— — — assay 34, 35
parasites, bacterial 1, 2, 10, 25
—, —, ferritin-iron utilization by 19
—, disease-producing 2
—, facultative intracellular 2, 10, 15, 16, 17, 18, 24, 25
—, — —, acquired immunity to 2, 17, 21
—, intracellular, relationships to host 16
—, nontoxigenic 2
—, pathogenic 1, 10
Pasteurella pestis 8, 23
— septica 8, 9, 10, 21, 22
pathogenicity of a virulent strain, iron concentration and 23
—, of nontoxigenic bacteria 1
pathogens, iron dependence 2, 8
—, nutritional capabilities 2
—, potential, and iron-availability 10, 18, 22
penicillinase plasmids of Staphylococcus 91
pertussis vaccine 127
phage infection, male-specific, role of F-pili in 96, 97
— —, —, role of traD$^-$ F-pili in 103
— —, —, role of traG$^-$ F-pili in 102
— —, —, role of maturation (A) protein in 97
— restriction by F-pili mutants 104, 109

phage restriction, female-specific 82, 108, 109, 110, 113
— sensitivity, male-specific 82, 111
phagocytic cells 1, 2, 3, 15, 23, 24, 25
— —, ability of parasites to multiply in 2, 16, 25
— —, concentrating streptomycin 16
— —, role in immunity 16
— —, — in tuberculous immunity 4
— —, tuberculostatic effect in the absence of 4
— vacuoles 2, 17
phagocytosis 2, 19, 23
phagosome 21, 24, 25
pinocytosis 17, 18
plasmid DNA 83
—, replication of 90, 91
PMN see polymorphonuclear cells
polar effects 105, 115
poly (U, G) containing CsCl gradients 84
polymorphonuclear cells (PMN) 33, 34, 35, 36, 37, 38, 61
— —, quantitative measurement of antigenic reaction and 35, 36, 37, 38
polypeptides, synthetic, antigenic competition and 131
—, —, tolerance to the dominant antigen and competition 145
polyribonucleotides, immune response and 135, 136
preimmunization, improved immune response to weak antigens 158, 159
Proskauer-Beck medium 5
proteins, antigenic competition and 127
prozone effect 54
P1 transduction 100, 101

R 82, 83, 88, 113
R, dissociation of 86, 91
R DNA, fate of after transfer 94, 95
—, molecular weight, estimation of 85
—, replication of 91
—, replication of by a relaxed control mechanism 91
—, single-stranded, transfer of 93
R-pili, antigenic specificity of 113
R, transfer-deficient (Tra⁻) mutants of 99
rabbit sera, antibacterial activity 8
rat, hybrid strain (BN × DA)F1 40, 42, 54
—, — — (Lewis × BN)F1 40, 42, 53, 59
—, — — (Lewis × DA)F1 36, 37, 39, 41, 42, 43, 50, 51, 52, 53, 59
—, inbred strain BN 40, 42, 53, 54, 59
—, — — DA 36, 37, 39, 40, 41, 42, 43, 50, 51, 52, 53, 54, 59
—, — — Lewis 36, 37, 42, 43, 49, 50, 51, 52, 53, 59
receptors 39, 46, 55, 59, 65, 68, 69, 70, 71, 72, 73, 74, 127, 150, 151, 160, 164, 165
recognition, antigenic see antigenic r.
—, nonspecific blocking 38
—, specific blocking 55
— structures (RS) 39, 46, 47, 55, 70, 71
—, T cells involved 74, 161, 165, 166
recombination enzymes, coded by F DNA cistrons 111
—, integration and excision of F DNA and 81, 82, 86
replication-control, models of 89
replication-deficient mutants, conjugation at non-permissive conditions 94
— —, incompatibility and 88, 91, 92
replication, initiation of 89, 90
—, membrane-attachment model 89, 90, 91
—, rolling circle model and conjugational DNA transfer 94
— segregation mutants 108
—, segregation unit 89
replicons, concept of 89
RS see recognition structures
resistance, antibiotic 86
—, induction by tuberculous infection 2
—, natural 2
— -neutralizing property of iron 23
— transfer factors 80
restriction-modification system 82
RNA phages, role of maturation (A) protein-RNA complexes 97, 98

Sauton medium 5, 14
sera of tuberculous, antimycobacterial activity in 3
—, tuberculostatic activity by non-antibody inhibitory factors 4
serum, antibacterial activity 10
— globulin, antibacterial 8
— iron (SI) 7, 14
—, ironbinding capacity of 13
— tuberculostasis, antagonistic activity of iron and mycobactin 12
— —, growthpromoting factor and 4
— —, hypoferremia and 10
— —, independence from antibodies or complement 4
— —, iron binding and 6
— —, lysozyme-like substance and 4
— —, serum iron (SI) and 7, 12
— —, suitability of various media for the study of 5
— —, total iron binding capacity (TIBC) and 7
— —, transferrin-iron-mycobactin interplay and 12
SI = serum iron 7, 14
sideramines 11

skin graft, full-thickness 48, 65
— —, allogenic, survival 40
— —, —, cellular immunity and allo-antibodies to 42, 43
— —, rejection of 31, 66, 67, 68, 72, 73
— —, tolerance of 66
— reaction intensities, antigenic recognition and 35, 36, 38
— reactivity 33, 35
streptomycin 16
surface exclusion 82, 98, 99, 101, 102, 105, 113, 114, 115
— — cistron (traS), postulated 105, 110, 114, 115
— —, incompatibility and 82, 98

T cells 39, 71, 74, 134, 136, 137, 139, 142, 153, 154, 159, 161, 162, 163, 164, 165, 166, 167, 168
—, producing suppressors of B cell proliferation 142
thymocytes 36, 37
TIBC = total iron binding capacity 7, 14
tissue fluids, ability of parasites to multiply in 1, 2, 3
tissues, predisposition of to invasion by tubercle bacilli 2
tolerance 36, 37, 38, 59, 64, 65, 66, 67, 68, 69, 70, 126, 160, 163
—, abolition of 64, 66, 67, 70
—, antigenic competition and 126, 129, 130, 131, 138, 139, 144, 145, 146
— induction, absence of antigenic competition 147
—, maintenance of 65, 67, 68, 69
—, partial, antigenic competition and 144
total iron binding capacity (TIBC) 7, 14
toxemia 1
toxigenicity 2
Tr = transferrin
transduction experiments 11
transfer-deficient (Tra^-) mutants 81, 92, 99, 100, 101, 102, 103, 104, 114, 115
— — —, cistrons 100, 101, 102, 104, 110
— — —, cistrons of pilus specificity 101, 102, 104, 105
— — —, phage restriction and 109
transfer efficiency 81, 95, 105
— enzymes 102, 116
— inefficiency in replication-deficient mutants 92
— origin 105, 106, 107, 108
—, polarity of 93, 108
— proteins 99, 104, 105, 114, 115
— regulation 113, 114, 115, 116
— repression 82, 83, 112, 113, 114
— repressor 99, 111, 113, 114, 115
transferrin 5, 6, 7, 8, 9, 10, 11, 12, 19, 22, 24, 25, 26
—, antifungal activity 6
—, bacteriostatic activity 8
—, binding capacity for iron 6
—, iron-binding chelator 6
—, unsaturated, bacteriostasis and 10, 22
—, —, tuberculostasis and 6, 9, 10, 12
transplantation antigens 32, 33, 36, 37, 38, 39, 40, 41, 42, 45, 46, 47, 48, 59, 60, 61, 64, 65, 66, 68, 69, 70, 71, 73, 74
— —, antisera to 42, 45, 55
— —, recognition of, effect of alloantisera 43, 44, 45, 50, 51, 52, 53, 54, 59
— —, —, — of anti-lymphocytic serum 40
— —, —, — of anti-PAR serum 42
— —, —, T cells and 39, 74
— —, tumor-specific, recognition of 59, 60, 61, 62, 63
— reactions 33
— tolerance 59, 64, 65, 67, 68, 70
— —, induction of 70
TSTA = tumor-specific transplantation antigens 60, 61, 62, 63, 64
tubercle bacilli 12, 18, 23
— —, absence of in cell-free tuberculous exudates 4
— —, effect of mammalian serum on the growth of 3, 8
— —, — of ferritin on 19
— —, — of transferrin on 6
— —, growth in macrophages 16
— —, — supported by guinea pig serum 7
— —, inhibitory effect on 4
— —, iron requirement 3, 18
— —, media for 4, 17
— —, predisposition of tissues to invasion by 2
— —, production of mycobactin 14
— —, toxicity of fatty acids for 21
— —, virulent 2
— bacillus, interaction with host 1, 3, 12, 23, 26
— —, mycobactin (M) and 10, 12, 24, 26
— —, strain $H_{37}Rv$ 8, 23
— —, virulent strains, ability to survive in an animal 3
— —, — —, cord-producing 13
tuberculosis, prognosis in 4
tuberculostatic factor, antagonist to 5
— —, transferrin and 5, 6
tuberculostasis, guinea pig serum and 21
—, hypoferremia and 8, 10, 17
— in iron-neutralized serum 6, 9, 22
—, iron-transferrin interplay 6, 8
—, liver cell fractions and 19

—, passive serum transfer and 17
—, unbound iron binding capacity (UIBC) and 13, 14
—, vaccination and 17, 22
tuberculous cell wall (TCW) 8, 9, 22
tumor-specific transplantation antigens (TSTA) 60, 61, 62, 63, 64, 157
Tween 80 14, 15, 19, 20, 21, 24

UIBC = unbound iron binding capacity 13, 14

Vegex 5
virulence, enhancing effect of iron 23
VL sarcoma 60, 61

White-Snow medium 14

Index to Volumes 40—59

Volume 40

Symposium on Chronic Infectious Neuropathic Agents (CHINA) and other Slow Virus Infections. Edited by JACOB A. BRODY, WERNER HENLE, and HILARY KOPROWSKI

Volume 41

M. R. HILLEMAN, Present Knowledge of the Rhinovirus Group of Viruses 1
R. V. WALKER, Plague Toxins-A Critical Review 23
M. PLEMPEL, A. M. WALTER, Biochemische Wirkungsmechanismen antibakteriell wirksamer Antibiotica 43
K. HABEL, Virus-Induced Tumor Antigens 85
M. BOCK, Chemotherapie der Viruserkrankungen 100

Volume 42

Symposium on Insect Viruses, Edited by KARL MARAMOROSCH

Volume 43

E. NORRBY, Biological Significance of Structural Adenovirus Components 1
P. ALBRECHT, Pathogenesis of Neurotropic Arbovirus Infections 44
P. M. BIGGS, Marek's Disease—Current State of Knowledge 92
F. BOREK, Delayed-Type Hypersensitivity to Synthetic Antigens 126
C. JUNGWIRTH, Frühenzyme bei der Vermehrung DNS-haltiger Animalviren . . . 162

Volume 44

B. S. STRAUSS, DNA Repair Mechanisms and Their Relation to Mutation and Recombination 1
P. GRABAR, Embryonic Constituents in Tumors and Antibodies in Cancerous Patients 90
L. GORINI, J. DAVIES, The Effect of Streptomycin on Ribosomal Function 101
K. KIRSCHNER, Allosteric Regulation of Enzyme Activity (An Introduction to the Molecular Basis of and the Experimental Approaches to the Problem) 119

Volume 45

P. B. BLAIR, The Mammary Tumor Virus (MTV) 1
G. GILLISSEN, Sessile Antikörper bei der Tuberkulinallergie 70
P. HAUSEN, Biochemische Vorgänge bei der Vermehrung der Picornaviren, dargestellt am Beispiel des ME-Virus-L-Zell-Systems. 90
J. F. HEREMANS, Immunoglobulin Formation and Function in Different Tissues . . 131

Volume 46

A. L. DEMAIN, J. BIRNBAUM, Alteration of Permeability for the Release of Metabolites from the Microbial Cell 1
D. BIERWOLF, F. FEY, A. GRAFFI, G. PASTERNAK, T. SCHRAMM, Neuere Untersuchungen über murine Leukämieviren 26
J. DRESCHER, Der Hämaggregationstest 96
C. O. DOUDNEY, Ultraviolet Light Effects on the Bacterial Cell 116

Volume 47

W. C. WILCOX, G. H. COHEN, The Poxvirus Antigens 1
R. BREZINA, Advances in Rickettsial Research 20
H. SCHUSTER, Die molekularen Grundlagen der Photoreaktivierung 40
L. HORTA-BARBOSA, D. FUCCILLO, J. L. SEVER, Rubella Virus 69
E. NETER, Endotoxins and the Immune Response 82
OLGA KRIŽANOVÁ, VOJTA RATHOVÁ, Serum Inhibitors of Myxoviruses 125
D. F. H. WALLACH, Cellular Membrane Alterations in Neoplasia: A Review and a Unifying Hypothesis 152

Volume 48

F. FENNER, Conditional Lethal Mutants of Animal Viruses 1
P. C. C. GARNHAM, J. H. HUMPHREY, Problems in Leishmaniasis Related to Immunology 29
J. G. MICHAEL, Natural Antibodies 43
J. ROTTA, Biological Activity of Cellular Components of Group A Streptococci in Vivo 63
Y. OKADA, Factors in Fusion of Cells by HVJ 102
G. P. YOUMANS, ANNE S. YOUMANS, Recent Studies on Acquired Immunity in Tuberculosis 129

Volume 49

B. ROIZMAN, The Herpesviruses — A Biochemical Definition of the Group 1
T. MAKINODAN, T. SADO, D. L. GROVES, G. PRICE, Growth Patterns of Antibody-Forming Cell Populations 80
J. IVÁNYI, J. ČERNÝ, The Significance of the Dose of Antigen in Immunity and Tolerance 114
A. RYTER, Structure and Functions of Mesosomes of Gram Positive Bacteria . . . 151
T. H. JUKES, Recent Problems in the Genetic Code 178

Volume 50

H. G. SCHWEIGER, Cell Biology of Acetabularia 1
S. E. MERGENHAGEN, R. SNYDERMAN, H. GEWURZ, H. S. SHIN, Significance of Complement to the Mechanism of Action of Endotoxin 37
O. J. PLESCIA, The role of the Carrier in Antibody Formation 78
A. GRANOFF, Viruses of Amphibia 107
A. MÁJSKÝ, Antigenicity of Blood Platelets 138
M. SHILO, Morphological and Physiological Aspects of the Interaction of *Bdellovibrio* with Host Bacteria 174

Volume 51

D. C. BISHOP, A. A. GOTTLIEB, Macrophages, RNAs and the Immune Response . . 1
G. WERZ, Cytoplasmic Control of Cell Wall Formation in Acetabularia 27
I. MILER, Specific and Non-Specific Opsonins 63
P. H. DUESBERG, On the Structure of RNA Tumor Viruses 79
J. P. BADER, T. L. STECK, T. KAKEFUDA, The Structure of the RNA of RNA-Containing Tumor Viruses 105
H. HANAFUSA, Virus Production by Rous Sarcoma Cells 114

Volume 52

P. TRAUB, Structure, Function and in vitro Reconstitution of Escherichia coli Ribosomes 1
A. KOHN, P. FUCHS, Communication in Cell Communities 94
M. W. PONS, On the Nature of the Influenza Virus Genome 142

Volume 53

P. Iványi, The Major Histocompatibility Antigens in Various Species 1
S. Alikhanian, Applied Aspects of Microbial Genetics 91
R. J. Erickson, New Ideas and Data on Competence and DNA Entry in Transformation of *Bacillus subtilis* 149

Volume 54

D. Sulitzeanu, Antibody-like Receptors on Immunocompetent Cells 1
T. J. Gill III, Synthetic Polypeptide Metabolism 19
R. Arnon, Antibodies to Enzymes — A Tool in the Study of Antigenic Specificity Determinants 47
H. Küntzel, The Genetic Apparatus of Mitochondria from *Neurospora* and Yeast 94
M. Servin-Massieu, Effects of Freeze-Drying and Sporulation on Microbial Variation 119

Volume 55

Arthropod Cell Cultures and Their Application to the Study of Viruses. Edited by Emilio Weiss

Volume 56

F. Kauffmann, Die moderne Klassifikation und Nomenklatur der Bakterien . . . 1
R. Thomas, Regulation of Gene Expression in Bacteriophage Lambda 13
T. Watanabe, Infectious Drug Resistance in Bacteria 43
G. Balassa, The Genetic Control of Spore Formation in Bacilli 99

Volume 57

V. ter Meulen, M. Katz, and D. Müller, Subacute Sclerosing Panencephalitis . 1
J. Gross, DNA Replication in Bacteria 39
N. H. Ruddle, Approaches to the Quantitative Analysis of Delayed Hypersensitivity 75
R. R. Brubaker, The Genus Yersinia: Biochemistry and Genetics of Virulence . . 111
B. Říhová-Škárová and I. Říha, Host Genotype and Antibody Formation 159

Volume 58

R. C. Boguslaski, R. S. Smith, N. S. Mhatre, Applications of Bound Biopolymers in Enzymology and Immunology 1
M. Majer, Virus Sensitization. 69
E. Benjamini, D. Michaeli, J. D. Young, Antigenic Determinants of Proteins of Defined Sequences 85
M. Levine, Replication and Lysogeny with Phage P22 in *Salmonella Typhimurium* 135
B. A. Phillips, The Morphogenesis of Poliovirus 156

Volume 59

D. W. Kingsbury, Paramyxovirus Replication 1
R. Drzeniek, Viral and Bacterial Neuraminidases 35
R. G. Webster, On the Origin of Pandemic Influenza Viruses 75
T. L. Benjamin, Physiological and Genetic Studies of Polyoma Virus 107
G. Roelants, Antigen Recognition by B and T Lymphocytes 135
E. Mozes, G. M. Shearer, Genetic Control of Immune Responses 167

Current Topics in Microbiology and Immunology

Reprint from Vol. 60

The Role of Iron in Bacterial Infections, with Special Consideration of Host-Tubercle Bacillus Interaction

I. Kochan

Springer-Verlag Berlin · Heidelberg · New York 1973

Reprint from

Current Topics in Microbiology and Immunology

Vol. 60

Antibodies to Receptors Recognizing Histocompatibility Antigens

H. Ramseier

Springer-Verlag Berlin · Heidelberg · New York 1973

Current Topics in Microbiology and Immunology

Reprint from Vol. 60

Genetics of the F Sex Factor in Enterobacteriaceae

M. Achtman

Springer-Verlag Berlin · Heidelberg · New York 1973

Current Topics in Microbiology and Immunology

Reprint from Vol. 60

Antigenic Competition

M. J. Taussig

Springer-Verlag Berlin · Heidelberg · New York 1973