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ON

TRANSPLANTATION

Edited by

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and

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PREFACE

THE Ciba Foundation held a symposium on transplantation of tissues in 1953 and there have been many conferences elsewhere in recent years, notably in Paris in 1957 and in Liège in 1959. This subject's "take" is so vigorous that fresh material for a further international conference in London in 1961 would clearly soon be abundant, and Professor Medawar had already arranged this with the Director before his Award of the Nobel Prize set the seal on this project.

The Foundation itself suffered transplantation on this occasion; our own building being closed for reconstruction, we were fortunate to be accepted as a graft by the noble and ancient Society of Apothecaries, through the courtesy of its Master, Mr. A. M. A. Moore, and members of the Court. We are deeply indebted to them, and to the Society's staff. The graft was temporary, but we hope that the comparatively infant Foundation may prove that it is for ever tolerant to any call made upon it by our generous hosts of the Society.

The enthusiastic contributors to this volume, and its editors, hope that its publication will provoke a proliferative response in readers concerned with this complex and rapidly developing subject.

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OPENING REMARKS

P. B. MEDAWAR

EVEN though it makes a sad beginning to the Conference, our first thought on this occasion must be of the name that was missing when the Director read his roll call. Although Peter Gorer's discoveries and ideas will pervade all our discussions, now and for many years to come, we shall miss him grievously as a colleague and as a friend. Gorer had for many years been the world's leading authority on the serology and serological genetics of homograft reactions. He began his work in 1932, at University College, under J. B. S. Haldane; and there, using first a human serum and then a rabbit immune serum, he demonstrated isoantigenic variation in the red cells of mice. Very soon after he was able to demonstrate immune isoantibodies in mice, and in his classical papers from the Lister Institute in 1937 and 1938 he gave us all but conclusive proof of the immunological character of the reaction against homografts of tumours. His work attracted little attention, at all events in England; the war came, and his thoughts turned to other things; and it was not until after the war, in collaboration with his brilliant colleagues at Bar Harbor, that he began that detailed serological and genetical analysis of tissue transplantation in mice that underpins the entire theory of tissue transplantation. The more recent developments of his own and his pupils' work are very familiar to you: the demonstrations of passive immunity toward leukotic tumours, and of the vulnerability of lymphoid and myeloid cells to the action of humoral antibodies; the demonstration and titration of cytotoxins; the analysis of the mysterious "X-factor"; the work that has raised the possibility of a synergic co-operation between humoral antibodies and sensitized lymphoid cells. But

apart from these and other particular discoveries, we shall miss him above all for his general comprehension and grasp of all the problems of transplantation. With the Director's warm approval, let us dedicate our present proceedings to the memory of Peter Gorer.

One of Gorer's last works was to try to overhaul the terminology of transplantation research. We who study transplantation cannot acquit ourselves of the charge of making our ideas known to each other in a terminology that is etymologically ludicrous and inconsistent with certain older immunological usages. At the very least I suggest that we should follow Gorer (Table I) in substituting "allogeneic" for "homologous", and in replacing "isologous" by "isogenic" or, much better, by "syngeneic". Incidentally "enhancement" is another offender. It is a word to which Nathan Kaliss has given an exact meaning: "enhancement" is an abrogation of the homograft reaction mediated through the action of specific humoral antibodies. Where humoral antibodies are not known to be involved, should we not use a non-committal word like "promotion"—Flexner's word, dating from 1907—instead?

At the last Ciba Foundation conference on transplantation, eight years ago, I began with a general review of transplantation theory of which the keynote was our incomprehension or ignorance of much that we should understand or know. No such review is called for today. We have in any event some reason to be satisfied with progress that has been made since we last met here. The serological analysis and genetic dissection of the H-2 locus I have already referred to. Here now, in no particular order, are some of the other accomplishments of the past eight years of research on homografts: the demonstration that, in many inbred strains of mice, male grafts are unacceptable to females—one of the most surprising single facts uncovered by the study of transplantation; the serological interpretation of "enhancement"; the discovery and analysis of "graft-versus-host" reactions, a discovery which

PROPOSED REVISIONS OF "TRANSPLANTESE"

Nouns			*Adjectives*		
Old terms	*Proposed new terms*	*Alternative new terms*	*Old terms*	*Proposed new terms*	*Alternative new terms*
Autograft	Autograft	—	Autologous	Autoplastic autochthonous (acc. to context)	
Isograft	Syngeneic homograft or graft	Isogenic homograft or graft	Isologous	Syngeneic	Isogenic
Homograft (general sense)	Homograft	—	Homologous	Allogeneic	—
Homograft (connoting genetic diversity)	Allogeneic homograft or graft	—			
Heterograft	Xenograft	Heterospecific graft Interspecific graft Heterograft	Heterologous	Xenogeneic	Heterospecific Heterologous

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has led to an astonishing proliferation of research, has provided a valuable new analytical tool, and has established important new principles in the field of general immunology, e.g. of the occurrence of immunologically competent cells in peripheral blood. (Here perhaps I should mention a cognate discovery, that lymphocytes, so far from being ephemeral, are long-lived cells that circulate and recirculate through the lymph nodes.) To continue, the past eight years have witnessed the extraction of sensitizing antigens from tissues and the development of a technique of assay, now being enlarged and reinforced by serological methods; the proof that the allogeneic—"homologous"—bone marrow cells used to repair radiation injury behave as grafts, and that a state having much in common with immunological tolerance may arise when irradiated mice are so treated; the use of transplantation techniques in the analysis of somatic cellular genetics, particularly of the variants that arise in populations of heterozygous tumour cells; the extension of the principles of transplantation immunity far down the ladder of vertebrate evolution, to amphibia and teleosts; the transfer of sensitivity to homografts in human beings by subcellular fractions of blood leucocytes; the revelation of the anomalous position of the golden hamster, and the analysis of why it should be so; the proof that two totally different tissues, skin epithelium and the endocrine component of the ovary, have a qualitatively similar representation of the histocompatibility antigens—with the important theoretical consequence that something between ten and fifteen neutral marker genes are present and at work in tissues which, though descended from the same zygote, have followed altogether separate pathways of differentiation; and the demonstration that in guinea pigs a delayed hypersensitivity reaction accompanies the rejection of homografts, a fact which strengthens the analogy between skin homograft reactions and the cell-mediated immunities. There is much else besides—for example, the slowly growing realization of the great differences between the reactivities associated with "strong" (in mice, with

H-2) and "weak" (non H-2) antigenic differences, accompanied by evidence that these weaker barriers of incompatibility are so much easier to break down than those erected by loci equivalent in strength to H-2. Nor should we forget the discovery of certain isolated phenomena which, though at present unexplained, may be looked back upon as being of crucial importance: the strange outcome of the intravenous injection of dissociated epidermal cells into rabbits; the privileged position of the hamster's cheek pouch; the anomalous behaviour, in guinea pigs, of thyroid homografts which have been allowed to reside for some months in the anterior chamber of the eye; the influence of an ovarian homograft on a later homograft of skin; and so on.

This is an inspiriting record, and one which workers in the field of transplantation research can take pride in. But—let us not forget that we are still quite ignorant of the proximate cause of the death of any homograft; the rôle of serum antibodies in transplantation immunity is still very far from certain; the chemical analysis of transplantation antigens is little more than embryonic; we are ignorant of much of the dynamics or kinetics of transplantation immunity, particularly as it concerns the origin and duration of sensitivity under different conditions; the relationship between tolerance and paralysis has yet to be laid bare; and, above all, we know of no harmless and lastingly effective way to subdue the homograft reaction in adult animals. We still have a very long way to go. In short, I think self-satisfaction and self-reproach should be about evenly balanced in our minds; and in that spirit let us begin this conference.

STUDIES ON TRANSPLANTATION ANTIGENS

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Our work on transplantation antigens during the past two years has consisted of (1) an attempt to correlate the sensitizing power of antigens with their power to excite the formation of humoral antibodies *in vivo* or to inhibit their action *in vitro*; and (2) further investigations of the physical and chemical properties of cellular extracts containing antigenic matter.

The principle underlying the correlation referred to under heading (1) is straightforward. A graft transplanted from (for example) an A-strain donor to a CBA or C3H recipient has two distinguishable effects: it sensitizes its recipient, in the sense that a second graft from an A-strain donor will be destroyed more quickly than its forerunner; and it immunizes its recipient in the more conventional sense of provoking the formation of humoral antibodies. The isoantigens responsible for these two reactions have a common genetic determination, and the temptation is therefore to believe either that they are identical, or—as Snell (1957) has suggested—that they have similar determinant groups and differ only in respect of subsidiary attachments which affect the modality of the immune response. If either of these interpretations is true, then antigenic matter known to sensitize should also absorb (or, in soluble form, should inhibit the action of) the corresponding humoral antibodies.

The experiments of Hildemann and Medawar (1959) failed to uphold this interpretation. Their failure must be attributed to the inaccessibility of determinant sites in the very crude antigenic

preparations then (1957) in use. At all events, the antigenic preparation of Billingham, Brent and Medawar (1958) can absorb humoral antibodies *in vitro* and excite their formation *in vivo* (Brent, Medawar, and Ruszkiewicz, 1961). The preparation described by Herzenberg and Herzenberg (1961)—probably similar to, but anatomically better defined than our own—and the highly active preparation which Davies will describe shortly (see Davies and Hutchison, 1961) also absorb or inhibit the action of humoral antibodies; both have been subjected to scrupulous tests of specificity of action, and we await with great interest tests of their power to sensitize. Furthermore Lejeune and Kandutsch (this vol. p. 25 and 72) both make use of the specific serological activity of their sensitizing or "enhancing" extracts, and Stetson's analysis (unpublished) of the anatomical distribution of iso-antigens within cells is founded upon their power to inhibit haemagglutination or the action of cytotoxins. The serological activity of tissue extracts with respect to the H-2 system of antigens in mice is therefore established beyond doubt. As our own work has just been published (Brent, Medawar and Ruszkiewicz, 1961) we shall not recapitulate it here.

Instead, we shall discuss three topics under the general heading of studies on the physical and chemical properties of extracts containing sensitizing antigens: (1) problems connected with the solubility or solubilization of antigens; (2) the behaviour of cellular extracts subjected to the action of lipid solvents; and (3) the heat-stability of sensitizing antigens.

In nearly all our experiments (exceptions are noted) we have used A-strain mice as donors of antigenic matter and of grafts, and CBA or C3H mice as their recipients. It is known of CBA mice (Barnes and Krohn, 1957), and can be assumed of C3H mice, that they differ from A-strain mice by antigens segregating at ten or more loci, but the serological and sensitizing activities we describe are dominated by "strong" antigenic differences at the H-2 locus.

Solubility and solubilization of sensitizing antigens

The solubilization of transplantation antigens is important not only as a prerequisite of some forms of physical and chemical analysis, but also because the sensitizing activity of antigens may be expected to vary with the forms in which they are presented to the responding subject.

It will be recollected that the preparation of Billingham and co-workers (1958) begins with the total disruption of A-strain lymphoid cells in distilled water, a process assisted by the exposure of the mechanically prepared homogenate to graded doses of ultrasound. Some old experiments of ours, of which only those with full internal controls are cited in Table I, show that these crude watery extracts contain some antigenic matter in a very finely subdivided form, and that its state of aggregation is profoundly influenced by the presence of electrolytes. If the crude aqueous extract is spun at 27,700 ***g*** (max.) for 30 to 45 min., sensitizing activity is about equally divided between sediment and supernatant fluid (Table I, A). If this supernatant fluid is now again spun for three or four hours at 134,000 to 173,000 ***g***, it is still possible, mainly by the histological analysis of grafts, to discern a trace of sensitizing activity in the second supernatant (Table I, B). However, sensitizing matter behaves quite differently in the presence of electrolyte. If NaCl to a final concentration of 0.15M is added to the supernatant fluid after centrifugation at 27,700 ***g*** (procedure I,A), the first effect is the formation of a heavy precipitate of antigenically inert DNA-protein, and this may be removed by centrifugation at low speeds. After its removal the preparation is again spun at 27,700 ***g*** for 30 min.; most of the activity now appears in the sediment (Table I, C), and experience has shown that the sediment sensitizes more powerfully than the mother liquor from which it was derived. Evidently antigenic fragments tend to aggregate in the presence of electrolyte, and, as colloid theory would lead one to predict, the same consideration

Table I

EFFECT OF ELECTROLYTES ON SOLUBILITY OF ANTIGENIC MATTER PREPARED BY ULTRASONIC DISPERSION OF A-STRAIN LYMPHOID CELLS IN WATER

All experiments A→CBA except 201 and 287 (CBA→A). Sensitivity of recipients measured by degree of epithelial survival in A-strain body skin grafts removed 6 days after transplantation. Dose per mouse expressed in terms of original wet wt. in mg. of lymphoid tissue from which the antigen was extracted. Sec. u/s = time of exposure to ultrasound, in seconds.

[A] Original crude aqueous preparation before addition of electrolyte: roughly equal subdivision of activity between sediment and supernatant after spinning 30 to 45 min. at 27,700 *g* (max).

Expt.	*Dose*	*Sec. u/s*	*Spin, min.*	*Scores: supernatant*	*Scores: sediment*
217	260	60	45	50, 25, 10, 0	25, 10, 0, 0
287	290	45	30	50, 25, 10, 10, 10	50, 25, 10, 0, 0
319	380	60	30	25, 25, 25, 10, 5	75, 50, 25, 25, 10

[B] Supernatant resulting from procedure [A]: retention of perceptible fraction of sensitizing power by supernatant after further centrifugation at high speeds.

	Sec.		*Spin,*	*Supernatant:*		*Sediment:*	
Expt.	*u/s*	*g (max.)*	*min.*	*Dose*	*Scores*	*Dose*	*Scores*
227	60	134,000	180	320	100, 75, 50	360	50, 50, 0
237	30	173,000	240	450	100, 100, 100, 75	450	10, 10, 10, 0
251	45	173,000	240	360	90, 75, 75, 50	545	100, 75, 25, 5
262	45	173,000	240	430	75, 75, 50, 10	630	0, 0, 0, 0

[C] Supernatant resulting from procedure [A]: after addition of NaCl to 0·15M the greater part of the activity appears in the sediment produced by a further centrifugation at 27,700 *g* for 30 min. Ultrasound: 30 sec. exposure throughout.

Expt.	*Dose*	*Scores: supernatant*	*Scores: sediment*
319	380	50, 50, 25, 25, 5	50, 10, 10, 0, 0
320	430	75, 50, 50, 25, 5	10, 5, 5, 0, 0
334	425	75, 50, 50, 50, 25, 25, 5, 0	25, 0, 0, 0, 0, 0, 0, 0

[D] Partition of activity between sediment and supernatant fluid when $MgCl_2$ or $CaCl_2$ (0·01M final conc.) is added to crude aqueous antigen, followed by centrifugation at ~2500 *g* for 10 min.

201	275	50, 25, 5	50, 0, 0, 0
206	280	100, 75, 75, 50	75, 25, 25, 25, 25, 5, 0, 0
218	270	100, 75, 75, 50, 50, 25, 25, 25	75, 50, 5, 0, 0, 0, 0, 0

applies *a fortiori* when bivalent cations (e.g. $CaCl_2$ or $MgCl_2$ to a final concentration of only 0.01M) are added to the crude aqueous "solution". Under these circumstances most of the antigenic activity appears in the sediment, accompanied by DNA-protein, after centrifugation at low speeds (Table I, D). We can take it that the antigenic matter contained in the original crude aqueous preparation consists of particles of very diverse sizes, and that even in the presence of 0.15M-NaCl some of the material is "soluble" in the sense defined by the above operations. The significance of these facts will be discussed later. The particles could well consist of fragments of cellular membranes, as Dr. Kandutsch has long insisted (Kandutsch and Reinert-Wenck, 1957; and see also Herzenberg and Herzenberg, 1961).

The "crude aqueous preparation" referred to in later experiments consists of an aqueous homogenate to which NaCl (final concentration 0·15M) has been added, and from which DNA-protein and undispersed matter has thereupon been removed by centrifugation at 5000 ***g*** for 10 min. The "antigenic sediment" with which the behaviour of this crude aqueous preparation will be compared is the sediment formed by a further centrifugation at 30,000 ***g*** for one hour. This sediment forms a typically "colloidal" suspension in water or physiological salt solutions, and it flocculates rapidly at pH 5·5 or less. The crude composition of the sediment is summarized in Table II. Its activity is such that, if a discriminating test is used, 0·25 mg. dry weight can be shown to sensitize a mouse; something like 0·5 mg. is needed to remove all detectable antibody from 1·0 ml. of a 1/50 dilution of a homologous antiserum of titre ~ 3200. These results do not encourage us to believe that the material is anything but grossly impure. On compositional grounds no inference can be drawn about the ingredient of the preparation in which activity lies; activity could reside in any one, or in any combination, of carbohydrate, lipid, or protein.

The solubilization of this insoluble sediment raises not one