'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS

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THE experiments to be described in this article provide a solution—at present only a 'laboratory' solution—of the problem of how to make tissue homografts immunologically acceptable to hosts which would normally react against them. The principle underlying the experiments may be expressed in the following terms: that mammals and birds never develop, or develop to only a limited degree, the power to react immunologically against foreign homologous tissue cells to which they have been exposed sufficiently early in fœtal life. If, for example, a fœtal mouse of one inbred strain (say, *CBA*) is inoculated *in utero* with a suspension of living cells from an adult mouse of another strain (say, *A*), then, when it grows up, the *CBA* mouse will be found to be partly or completely tolerant of skin grafts transplanted from any mouse belonging to the strain of the original donor.

This phenomenon is the exact inverse of 'actively acquired immunity', and we therefore propose to describe it as 'actively acquired tolerance'. The distinction between the two phenomena may be made evident in the following way. If a normal adult *CBA* mouse is inoculated with living cells or grafted with skin from an *A*-line donor, the grafted tissue is destroyed within twelve days (see below). The effect of this first presentation of foreign tissue in adult life is to confer 'immunity', that is, to increase the host's resistance to grafts which may be transplanted on some later occasion from the same donor or from some other member of the donor's strain. But if the first presentation of foreign cells takes place in fœtal life, it has just the opposite effect: resistance to a graft transplanted on some later occasion, so far from being heightened, is abolished or at least reduced. Over some period of its early life, therefore, the pattern of the host's response to foreign tissue cells is turned completely upside down. In mice, it will be seen, this inversion takes place in the neighbourhood of birth, for there is a certain 'null' period thereabouts when the inoculation of foreign tissue confers neither tolerance nor heightened resistance—when, in fact, a 'test graft' transplanted in adult life to ascertain the host's degree of immunity is found to survive for the same length of time as if the host had received no treatment at all.

Earlier Work

The literature of experimental embryology is rich in evidence that embryos are fully tolerant of grafts of foreign tissues. It is less well known (though no less firmly established) that embryonic cells transplanted into embryos of different genetic constitutions may survive into adult life, although their hosts would almost certainly have rejected them if transplantation had been delayed until after birth. The transplantation of embryonic melanoblasts[1] provides the most conspicuous evidence of this phenomenon—not because melanoblasts are peculiar in their immunological properties, but simply because their genetic origins are at once betrayed by the pigmentation of the cells into which they ultimately develop. Unfortunately, experiments with embryonic melanoblasts, having been done with quite different purposes in mind, do not make it possible to decide whether survival into adult life is due to an antigenic adaptation of embryonic cells which have been obliged to complete their development in genetically foreign soil, or whether it is due to a suppression or 'paralysis'[2] of the host's immunological response.

An exactly comparable phenomenon has been described by Owen[3], who found that the majority of dizygotic cattle twins are born with, and long retain, red blood cells of dizygotic origin: each calf contains a proportion of red cells belonging genetically to itself, mixed with red cells belonging to the zygote lineage of its twin. There is no reason to doubt that this is because the cattle twins, being synchorial, exchange blood in fœtal life through the anastomoses of their placental vessels. (This is not a peculiarity of cattle, for a human twin with red cells of dizygotic origin has lately been described[4].) Inasmuch as the provenance of the red cells was revealed by their reactions with specific agglutinins, it is most unlikely that the survival of foreign erythrocyte-forming cells into adult life was made possible by any kind of antigenic adaptation. Moreover, we have found that the majority of cattle twins at birth and for long after are fully tolerant of grafts of each other's skin[5]. Being freshly transplanted, these grafts can have had no opportunity to 'adapt' themselves antigenically to foreign hosts, but they survived nevertheless.

The experiments of Cannon and Longmire[6] have a direct bearing on the phenomenon of actively acquired tolerance. About 5–10 per cent of skin grafts exchanged between pairs of newly hatched chicks of different breeds are tolerated and survive into adult life; but the percentage of successes falls rapidly as the age at which the chicks are operated increases, and reaches zero by the end of the second week. These results will be referred to later.

Experiments with Mice

A single experiment will be described in moderate detail: the recipients were mice of *CBA* strain, the donors of *A* strain. The data for transplantations between normal mice of these strains are as follows. The median survival time of *A*-line skin grafts transplanted to normal *CBA* adults (regardless of differences of sex, or of age within the interval 6 weeks–6 months) is $11{\cdot}0 \pm 0{\cdot}3$ days[7]. In reacting against such a graft, the host enters a state of heightened resistance; a second graft transplanted up to sixty days after the transplantation of the first survives for less than six days, and immunity is still strong, though it has weakened perceptibly, after four months. Heightened resistance may be passively transferred to a normal *CBA* adult by the intraperitoneal implantation of pieces of lymph node excised from a *CBA* adult which has been actively immunized against *A*-line skin[8].

In the experiment to be described (Exp. 73), a *CBA* female in the 15–16th day of pregnancy by a

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CBA male was anæsthetized with 'Nembutal', and its body wall exposed by a median ventral incision of the skin. The skin was mobilized but not reflected, and particular care was taken not to damage the mammary vessels. By manipulation of the abdomen with damped gauzes, six fœtuses were brought into view through the body wall. Each was injected intra-embryonically with 0·01 ml. of a suspension of adult tissue cells through a very fine hypodermic needle passing successively through the body wall, uterine wall, and fœtal membranes. (The inoculum itself, consisting of a suspension in Ringer's solution of small organized tissue clumps, isolated cells, and cell debris, had been prepared by the prolonged chopping with scissors of testis, kidney and splenic tissue from an adult male *A*-line mouse.) After injection of the fœtuses, the skin was closed with interrupted sutures.

Five healthy and normal-looking young were born four days later; of the sixth fœtus there was no trace. Eight weeks after their birth, when the lightest weighed 21 gm., each member of the litter was 'challenged' with a skin graft from an adult *A*-line donor. The first inspection of the grafts was carried out eleven days later, that is, at the median survival age of *A*-line skin grafts transplanted to normal *CBA* hosts. The grafts on two of the five mice were in an advanced stage of breakdown; the grafts on the other three (one male and two females) resembled autografts in every respect except their donor-specific albinism. Each of these three grafts became perfectly incorporated into its host's skin and grew a white hair pelt of normal density and stoutness. Fifty days later, one of the three mice received a second *A*-line graft from a new donor, and this graft also settled down without the least symptom of an immunological reaction.

The graft on one of the three animals underwent a long-drawn-out 'spontaneous' involution, beginning somewhat before the 75th day after transplantation and ending with complete breakdown shortly after the 91st day. The other two mice were made the subjects of an experiment[9] designed to show that acquired tolerance is due to a failure of the host's immunological response and not to an antigenic adaptation of the grafted cells. When the two grafts were of 77 and 101 days standing, respectively, and still in immaculate condition, their hosts were inoculated intraperitoneally with chopped fragments of lymph nodes from normal *CBA* mice which had been actively immunized against *A*-line skin. The grafts began to deteriorate 2–3 days later, with signs of vascular congestion and stasis; contracture, hardening and discoloration took place progressively, and the grafts were reduced to dry scabs by the fourteenth day after the nodes had been implanted. It follows that tolerant hosts are fully capable of giving effect to a state of immunity which has been elicited by proxy, and that the tolerated grafts have not lost their ability to respond to it.

The fertility of the mice was entirely unimpaired. Both females repeatedly bore litters of normal size by their male litter mate. When they had grown up, two litters (of six and eight respectively) were challenged with grafts of *A*-line skin. Breakdown of all the grafts was far advanced or complete by the eleventh day after their transplantation.

The results of Exp. 73 demonstrate the great (but not necessarily indefinite) prolongation of the life of homografts made possible by a pre-emptive exposure of their hosts to foreign homologous cells. Beyond this, they demonstrate (*a*) that this prolongation of life is not due to an antigenic transformation of the grafts, nor to a competitive absorption of antibodies by, for example, cells of the fœtal inoculum which had survived into adult life; and (*b*) that acquired tolerance is either not transferred to, or is too weak or too ephemeral to make itself evident in, the offspring.

Two of the five mice of Exp. 73 gave no evidence of increased tolerance of *A*-line cells. We suppose that this was because they were imperfectly injected. Only in one experiment so far has every injected fœtus given rise to a tolerant adult.

The more important results obtained from the investigation of other litters may be summarized as follows. (1) The conferment of tolerance is not of an all-or-nothing character; every degree is represented, down to that which gives the test-grafts only a few days of grace beyond the median survival time of their controls. (2) The conferment of tolerance is immunologically specific. Thus a *CBA* mouse made tolerant of *A*-line tissue, or vice versa, retains the ability to react with unmodified vigour against skin from a donor belonging to a third strain, *AU*. (Equally clear evidence of the specificity of acquired tolerance is given in the following section.) But it has so far been our experience that the transplantation of (say) *AU* skin to a *CBA* mouse that is tolerant of *A*-line cells will elicit a reaction which, in addition to destroying the *AU* graft, causes an *A*-line graft already in residence to go through a severe immunological crisis. Although we have evidence of a sharing of tissue antigens between strains *A* and *AU*, this phenomenon is difficult to interpret, for the antigens common to strains *A* and *AU* are merely a sub-group of those to which the *CBA* host is manifestly unresponsive. It may therefore turn out that the continued well-being of a tissue homograft upon a tolerant host depends upon the quiescence of the antibody-forming system, and that if this is awakened by tissue antigens other than those of which the host is tolerant, antibodies directed against its, until then, tolerated graft may be formed as well. (3) A wide histological variety of tissue cells is capable of conferring tolerance to homografts of skin. It is by no means obligatory that skin cells, or even epithelial cells, should be among them. (4) We have inoculated ninety-six new-born mice with adult or fœtal tissue cell suspensions to decide whether tolerance can be conferred by exposure to foreign cells at this relatively advanced stage of development. The majority received a single inoculation of cells as soon as possible after birth; a small subgroup of these was injected with 0·05 mgm. cortisone acetate on the same occasion and several more times during the first week of life in an attempt to delay the maturation of the antibody-forming system[6]. The remainder of the new-born mice received repeated injections of foreign tissue cells in increasing quantities over the period of a month from birth. In all, only nine mice (about 10 per cent: cf. the experiments of Cannon and Longmire referred to above) showed an increase of tolerance when tested with a skin graft from the donor strain, and six of these were members of a single litter of eight which had received a single inoculation of cells, without cortisone, immediately after birth. It is of particular interest that when challenged with skin grafts in adult life the great majority of inoculated new-born mice showed neither tolerance nor enhanced resistance. New-born mice are, in general, too old for a tissue inoculum to confer

tolerance, and too young for it to confer immunity; the epoch of birth represents a null period during which the net outcome of exposure to foreign cells is to leave its subjects in a state of unaltered reactivity. (5) Grafts removed from hosts which have tolerated them, and then transplanted to normal mice of the host's strain, survive 2–3 days longer than freshly transplanted homografts of normal skin. Such homografts cannot, however, be compared directly with homografts of normal skin, because a high proportion of the corium of each will probably have been replaced by cells and cellular derivatives of host origin.

Preliminary Experiments with Chickens

Donors and recipients in these experiments were of Rhode Island Red and White Leghorn breeds, respectively. Skin transplanted from two weeks old Rhode Island Red chicks to White Leghorn recipients of the same age, using Cannon and Longmire's methods[6], is completely destroyed within ten days of grafting, to the accompaniment of an inflammatory reaction of conspicuous violence.

The embryonic chick is particularly well suited to experiments which make use of cellular inoculation, because the intravenous route is so easily accessible. Using methods demonstrated to us by Dr. C. Kaplan, whose help has been of the greatest value, we have obtained successful results by transfusing 0·2 ml. unmodified whole blood from an 11–12 day old embryonic Rhode Island Red donor into a chorio-allantoic vein of a White Leghorn embryo of the same age. Fourteen days after hatching, a test-graft of skin was transplanted to the recipient from its original donor. In seven such trials, five grafts showed prolongation of survival; of these, three succumbed within fifty days to the accompaniment of very much subdued inflammatory changes, and two still survive, with normal growth of red feathers, to the present time (125 days).

The success of these experiments has been found to depend on the strict pairing of donors with recipients. The transplantation of skin between our Rhode Island Red chicks when two weeks old showed them to be a highly heterogeneous assembly, in spite of their uniformity of breed characters. It is therefore understandable that chicks made tolerant to grafts from one Rhode Island Red donor will not, in general, accept grafts from another. This strict specificity of acquired tolerance has made it difficult to test the efficacy of grafts transplanted to the chorio-allantois, for an embryonic donor must be killed to provide tissues suitable for grafting. This difficulty has been circumvented by killing a young hatched donor, storing its skin[10] for later use in the test operation, and grafting fragments of a wide variety of its tissues upon the chorio-allantoic membrane of 10–11 day old White Leghorn recipients. Tolerance is conferred less regularly and less completely by this method than by blood transfusion: only three out of seven such experiments have yielded test grafts which survived for longer than twice their normal expectation of life, and those which have done so have given evidence of chronic low-grade inflammatory changes.

Washed blood corpuscles are as effective as whole blood in conferring tolerance; plasma is therefore a dispensable ingredient. If adult blood proves to be capable of conferring tolerance, it should be easy to decide whether red cells or white cells or both are efficacious.

Discussion

It is one of the predictions of Burnet and Fenner's[11] theory of immunity that the exposure of animals to antigens before the development of the faculty of immunological response should lead to tolerance rather than to heightened resistance. The homograft immunity system is particularly well suited to the appraisal of such a hypothesis, because the antigens are at once powerful, innocuous and persistent. For this reason, no great weight should be attached to failure of the experiments of Burnet, Stone and Edney[12] to verify it. It must be emphasized, however, that our experiments do not yet bear upon the fundamental problem of whether the production of antibodies represents an *inherited* derangement of protein synthesis, that is, a transformation which can persist through repeated cell divisions after the disappearance of the antigen originally responsible for it. It would be a highly significant fact if a transient exposure to antigen in fœtal life could confer a permanent or very long-lasting tolerance; but in our present experiments there is no reason to doubt that at least some of the cells of the fœtal inoculum survived as long as the tolerant state which they were responsible for creating. At all events, any complete theory of antibody formation must be competent to explain two sets of facts: that although embryos do not make antibodies, they respond to antigens in a manner that prejudices their ability to do so in later life; and that acquired tolerance is highly specific, for antibody-forming cells can be prevented from responding to one antigen without impairing their capacity to respond to any other.

A state of tolerance similar in principle to that which we have described may not necessarily depend upon an exposure to antigens in fœtal life. Phenomena which occur in adult life and which may be cognate with tolerance induced by fœtal inoculation include: (*a*) the highly specific 'immunological paralysis' of mice by the administration of relatively high doses of pneumococcal polysaccharide, made clear by the important experiments of Felton[2]; (*b*) that restraint of certain drug allergies in guinea pigs which, so Chase[13] has shown, may be brought about by the oral administration of the sensitizing chemical—an observation of particular interest, in view of the affinity between transplantation immunity and sensitization reactions of just this type[14]; and (*c*) the enhancement of the growth of tumour homografts by treatment of their prospective hosts with a variety of lyophilized tissue preparations, revealed by the work of Casey, Snell, Kaliss and their colleagues[15]. The relationship between these various phenomena has yet to be determined. We propose to investigate the hypothesis that exposure of the adult antibody-forming system to what may be called a 'tissue hapten' preparation—that is, to tissue ingredients which, although not themselves antigenic, contain the determinant groupings that confer specific activity upon complete antigens—may have the same kind of effect as that produced by an exposure of the immature antibody-forming system of the fœtus to complete antigens.

Actively acquired tolerance may not be a wholly artificial phenomenon. We are inquiring into the possibility that it may occur naturally by the accidental incorporation of maternal cells into a fœtus during normal development.

In dizygotic twin cattle of unlike sex, the confluence of fœtal vessels that leads to red cell

chimærism[3] and tolerance of homografts[6] has long been known to be associated with infertility of the female. In our present experiments, tolerance has been conferred upon female mice by inoculation with male cells without affecting their fertility. The two phenomena are therefore separable, although in cattle they go together and share an anatomical pre-requisite in common; but this is an inference that leaves entirely open the question of whether or not the freemartin state is due to a purely humoral influence of the male fœtus upon its synchorial female twin.

The experiments described in this article will in due course be reported on in full.

Summary

(1) Mice and chickens never develop, or develop to only a limited degree, the power to react immunologically against foreign homologous tissue cells with which they have been inoculated in fœtal life. Animals so treated are tolerant not only of the foreign cells of the original inoculum, but also of skin grafts freshly transplanted in adult life from the original donor or from a donor of the same antigenic constitution.

(2) Acquired tolerance is immunologically specific: mice and chickens made tolerant of homografts from one donor retain the power to react against grafts transplanted from donors of different antigenic constitutions.

(3) Acquired tolerance is due to a specific failure of the host's immunological response. The antigenic properties of a homograft are not altered by residence in a tolerant host, and the host itself retains the power to give effect to a passively acquired immunity directed against a homograft which has until then been tolerated by it.

(4) The fertility of tolerant mice is unimpaired.

[1] Cf. Rawles, M. E., *Physiol. Rev.*, **28**, 383 (1948).
[2] Felton, L. D., *J. Immunol.*, **61**, 107 (1949).
[3] Owen, R. D., *Science*, **102**, 400 (1945). Owen, R. D., Davis, H. P., and Morgan, R. F., *J. Hered.*, **37**, 291 (1946). Stone, W., Stormont, C., and Irwin, M. R., *J. An. Sci.*, **11**, 744 (1952).
[4] Dunsford, I., Bowley, C. C., Hutchison, A. M., Thompson, J. S., Sanger, R., and Race, R. R., *Brit. Med. J.*, ii, 81 (1953).
[5] Anderson, D., Billingham, R. E., Lampkin, G. H., and Medawar, P. B., *Heredity*, **5**, 379 (1951). Billingham, R. E., Lampkin, G. H., Medawar, P. B., and Williams, H. Ll., *Heredity*, **6**, 201 (1952).
[6] Cannon, J. A., and Longmire, W. P., *Ann. Surg.*, **135**, 60 (1952).
[7] Billingham, R. E., Brent, L., Medawar, P. B., and Sparrow, E. M. (unpublished work).
[8] Billingham, R. E., Brent, L., and Medawar, P. B. (unpublished work). The passive transfer of transplantation immunity was first demonstrated by Mitchison, N. A., *Nature*, **171**, 267 (1953); *Proc. Roy. Soc.*, B (in the press).
[9] The principle underlying this test was formulated by Chase, M. W., Abstr. 49th Gen. Meeting Soc. Amer. Bacteriol., 75 (1949).
[10] Billingham, R. E., and Medawar, P. B., *J. Exp. Biol.*, **29**, 454 (1952).
[11] Burnet, F. M., and Fenner, F., "The Production of Antibodies" (Melbourne, 1949).
[12] Burnet, F. M., Stone, J. D., and Edney, M., *Aust. J. Exp. Biol. Med. Sci.*, **28**, 291 (1950).
[13] Chase, M. W., *Proc. Soc. Exp. Biol. Med.*, N.Y., **61**, 257 (1946).
[14] Mitchison (ref. 8). Medawar, P. B., Colloquia of the Ciba Foundation (in the press).
[15] Summarized and reviewed by Snell, G. D., *Cancer Res.*, **12**, 543 (1952).

TRANSMUTATION OF THE ELEMENTS

TRANSMUTATION of the elements, once the dream of the alchemists, is now being carried out as an everyday operation in the laboratory and on the industrial scale. The present situation and its implications, especially in so far as they concern chemistry, were reviewed in a group of three papers given before Section B (Chemistry) of the British Association at Liverpool on September 8.

In the introductory paper, Dr. R. Spence (Atomic Energy Research Establishment, Harwell) showed how transmutation is occurring continuously due to the radioactive decay of the naturally occurring radioactive elements on one hand and to the absorption of cosmic radiation by the atmosphere on the other. In the case of uranium minerals, in addition to the normal decay series the spontaneous fission of the isotope uranium-235 leads to the production of neutrons, some of which are captured by the uranium-238 isotope, which decays through neptunium-239 to plutonium. Seaborg and collaborators at the University of California have identified extremely small amounts of plutonium (~ 1 part in 10^{14}) in carnotite. Cosmic-ray neutrons react with the nitrogen of the atmosphere to give carbon-14 and to a smaller extent to give hydrogen-3. On the basis of data given by Libby and co-workers at the University of Chicago, it has been calculated that 16 mgm. of radiocarbon are produced in the earth's atmosphere every minute.

The development of particle accelerators and later of the chain-reacting pile has provided us with the means of bringing about transmutations on a comparatively large scale. If the construction of nuclear reactors for the generation of electrical power proceeds as might be expected, it is quite likely that at least one ton of fissile material will be consumed in Britain per annum by 1970. World consumption might conceivably be as much as 100 tons per annum by the end of the century. The long-term future is very much dependent, however, on the successful transmutation of naturally occurring thorium or naturally occurring uranium into the fissile isotopes uranium-233 and plutonium-239, respectively, on an industrial scale. In this way the whole of the natural uranium and thorium supplies could be utilized as nuclear fuel instead of less than 1 per cent of the natural uranium as at present.

Besides the isotopes uranium-233 and plutonium-239, other isotopes of the heavy elements are produced in nuclear reactors, such as neptunium-237, americium-241 and curium-242. These were discussed in more detail by Dr. R. Hurst in the last of the three papers of the symposium. Since fission of 100 tons of nuclear fuel yields about 100 tons of fission products it is important that some effort should be made to utilize this material. The fission products consist of isotopes of elements in the middle of the Periodic System, from zinc to gadolinium. Work is going on both in the United States and in Britain to develop uses for the radiation from fission products such as for the preservation of meat and other foods, for sterilization of pharmaceuticals, for production and treatment of plastics and for bringing about chemical reactions. The fission products also contain the elements technetium and promethium, which do not occur in Nature. It is conceivable that some use will be found for these elements in due course and also for the inert isotopes arising from total decay of the active species.

The second paper, given by Dr. K. Chackett (University of Birmingham), dealt with transmutation induced by accelerated particles. The Bohr