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CURRENT PROBLEMS IN RESEARCH

Reactions of Grafts against Their Hosts

Transplantation immunity works both ways—hosts destroy grafts and grafts may harm hosts.

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Even the layman is now aware that, with the exception of one or two tissues such as cornea and cartilage to which special dispensations apply, organ or tissue grafts transplanted from the body of one person to that of another do not normally survive in their new host for longer than a week or two. In man, only identical twins are excepted from this generalization. There are now several cases on record where one twin has successfully given a sound kidney to replace the diseased and nonfunctioning kidneys of the other.

This state of affairs is certainly not peculiar to man, for it obtains in all vertebrates so far investigated: frogs, goldfish, chickens, rats, mice, rabbits, squirrels, dogs, and monkeys, to mention only a few animals in the now formidable list. The demise of *homografts,* as grafts of tissues or organs transplanted between genetically unrelated individuals of the same species are known, is certainly not attributable to inadequacies of grafting procedures, such as temporary interruption of blood supply. This has been repeatedly demonstrated by comparing the short duration of homografts with the successful survival of *autografts* of the same tissue —that is, grafts donated by an animal to itself.

In grafting complex and relatively large organs, such as kidneys, it is of course obligatory to couple up the main blood vessels with those of the host at the operation if the grafts are to stand any chance of survival. With small grafts, on the other hand, such as thin sheets or pieces of skin or fragments of ovarian tissue, close approximation with living, freshly exposed and vascularized mesenchymal tissues of the host is all that is necessary. The juxtaposed graft and host tissues soon become knit together through the activity of fibroblasts, and revascularization—another facet of the healing process—is accomplished within a day or two as the consequence of a sort of end-to-end union between some of the small severed vessels of the graft and those of the host tissue.

Because of the ease with which they may be transplanted and their subsequent accessibility for observation and removal for histological study, skin homografts have been studied most extensively. With very few qualifications the conclusions reached from these studies appear to hold good for homografts of other normal tissues and of solid, malignant tissues transplanted to many different sites in the body.

Immunological Nature of Homograft Rejection

One of the most fundamental advances in homograft research was Medawar's unequivocal demonstration in 1944 that skin homograft incompatibility is not innate but is an immunological phenomenon. All homografts enjoy at least a short period of well-being during which they are almost impossible to differentiate from autografts. The duration of this period depends upon the amount of foreign tissue grafted: small homografts usually live longer than large ones, though this dosage effect is weak. The onset of inflammation and swelling in a skin homograft, accompanied at the microscopic level by an infiltration of its dermis by leucocytes (mainly lymphocyte-like cells) from the host, the eventual cessation of its blood supply and its rapid deterioration to a necrotic scab, are all direct or indirect consequences of the host's immunological reaction (the so-called homograft reaction) against the foreign cellular antigens present in the graft. One of the best expressions of the intensity or severity of the host's response is the survival time of the graft.

Individuals that have reacted against solid tissue homografts, or against cellular homografts such as leucocyte or spleen cell suspensions injected intravenously or by other routes, are sensitized so that subsequent homografts from the original donor, or from another of similar antigenic constitution, undergo an accelerated destruction. With skin homografts this summary rejection frequently goes hand in hand with failure to heal in properly. This "second-set" phenomenon at present constitutes the only test applicable to *all* species for determining whether a given type of tissue homograft, cell suspension, or extract prepared therefrom has sensitized a recipient. That there is no cellular or tissue specificity involved in tissue transplantation immunity was suggested by a body of evidence showing that the sensitization evoked in a recipient by one type of tissue or cellular homograft is fully effective against subsequent homografts of other types of tissue from the same donor. More recently, analyses making use of the principle of immunological tolerance (see below) have shown that *all* living tissue cells of the body of a given individual must have identical

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complements of the cellular antigens responsible for transplantation immunity.

Genetic Determination of Transplantation Antigens

The most valuable tools for the analysis of homograft incompatibility, and those which revealed at an early date that it was genetically determined, are the many different inbred or *isogenic* (genetically uniform) strains of mice. These have been produced by many consecutive generations of brother-sister matings. With one important qualification mentioned below, *isografts* (grafts exchanged between members of the *same* inbred strain) are permanently successful, like grafts exchanged between a pair of identical twins, since they all have exactly the same genes and consequently the same gene-determined cellular transplantation antigens. In recent years, following the pioneer work of C. C. Little, combined genetic and transplantation studies have shown that the antigenic substances responsible for eliciting homograft reactions between *different* isogenic mouse strains are determined by multiple dominant Mendelian genes—known as *histocompatibility* genes—and that there are at least 15 different genetic loci involved, though only three of these have been subjected to analysis. The most important of this trinity is the so-called H-2 locus, which may be tenanted by not less than 10 alleles or alternative genes. Some of the alleles determine the formation of transplantation antigens so powerful that hosts which differ from their donors simply at this one locus—that is, with respect to a single antigen—may destroy a homograft within less than two weeks.

Provided that the entire complement of genetically determined transplantation antigens possessed by a homograft is also fully represented in the host's own complement, that graft will survive indefinitely like an autograft or an isograft since it fails to confront the host with anything that is "foreign" to it. The F_1 hybrid animals produced by mating parents from two *different* inbred homozygous strains are highly important, as we shall see later. Since each hybrid receives a set of chromosomes from both of its parents, it has all the histocompatibility genes, and therefore the transplantation antigens, present in each parental strain. Consequently, hybrid animals are incapable of reacting against homografts from either of the parents. It will be noted, however, that a parental strain graft on an F_1 hybrid host itself lacks those antigens of the host which the latter derived from the other parent. For this reason, F_1 hybrid grafts are acceptable to neither parental strain (see Fig. 1).

In addition to the three histocompatibility loci referred to above, there is one other, known to be present in both mice and rats, which seems to provide a transplantation immunity system of exquisite simplicity. Males differ genetically from females in that they possess a Y chromosome which the females lack, and the data at present available strongly suggest that a weak transplantation antigen is determined by a locus present on that chromosome. Males will accept isografts from male or female donors, but females normally reject isografts from male donors. In the C57BL mouse strain the median expectation of survival of male skin isografts on females is about 27 days. It has been shown that this factor is present in and determines exactly the same antigen in all male mice so far tested, irrespective of their strain, though for some reason not all females are capable of responding to it.

Merely to say that homograft incompatibility is a genetically determined immunological phenomenon is not enough. To conclude this very brief introductory account of the immunology of homograft rejection it is necessary to say a little about the type of immunological response involved and the nature of the postulated cellular transplantation antigens which provoke it.

Cellular Nature of Transplantation Immunity

The body's immunological defense mechanism usually responds to invasion by foreign microorganisms or to inoculation with foreign antigens such as bovine serum albumen or sheep red blood cells (which immunologists frequently employ) by the production of specific antibodies—protein molecules which are elaborated in the lymphoid tissues and are present in the globulin fraction of the serum. Directly or indirectly these products of the immunological reflex lead to the inactivation and elimination of the particular agent which evoked their formation. However, despite the fact that serum antibodies are formed in response to tissue

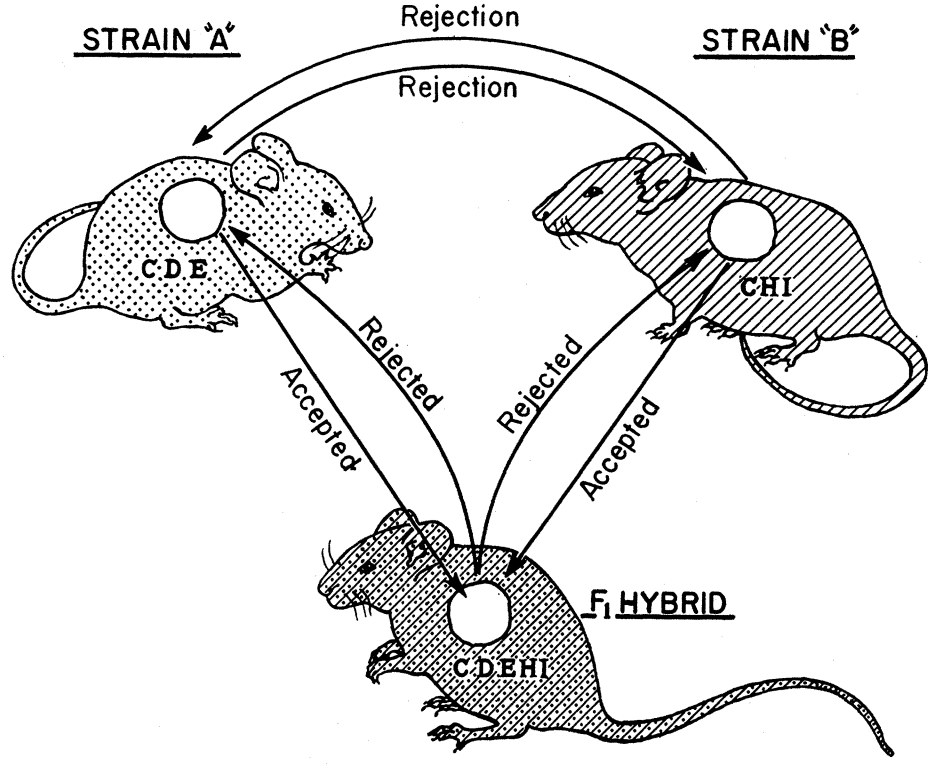

Fig. 1. Fates of skin homografts exchanged between mice of two different inbred strains and their F_1 hybrid. The hypothetical transplantation antigens of these parental strain mice are indicated by the letters *CDE* and *CHI*. Since it receives all the genes present in both parents, the antigenic constitution of the hybrid animal will be *CDEHI*. Only those homografts are accepted which have no antigens that are not also represented in the host.

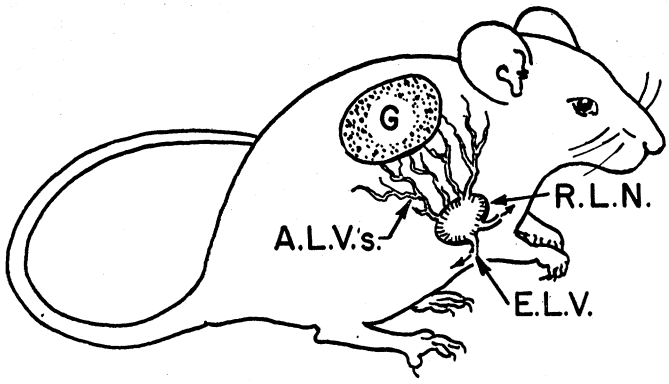

Fig. 2. Mouse bearing a skin homograft, *G*, from which antigenic material escapes and reaches the draining or regional lymph node (R.L.N.) via the afferent lymphatic vessels (A.L.V.). The antigens stimulate the production of immunologically activated cells which leave the node via the efferent vessels (E.L.V.) and so reach the blood stream. They are carried to the homograft, infiltrate its substance, and bring about its destruction.

homografts—they can be revealed by their ability to agglutinate the donor's red blood cells—there is no convincing evidence that these antibodies are in any way responsible for the destruction of homografts of skin or other solid tissues. (They may, however, be the agents of destruction of cellular homografts of lymphocytic cells and of certain types of leucotic and ascites tumors.) For example, serum prepared from the blood of animals which have rejected homografts will not bring about the accelerated rejection of appropriate test tumor or skin homografts if it is transferred to other "virgin" mice of the serum donor's own strain.

As N. A. Mitchison was first to demonstrate, transplantation immunity can be transferred from one animal to another only by means of lymphoid cells prepared from the lymph nodes or the spleen of a sensitized animal. As studies with more familiar types of antigens have established, these organs and the scattered and less clearly defined foci of lymphoid tissue which are present in most other organs and tissues of the body contain those cells which can manufacture antibodies—that is, *immunologically competent* cells, which I shall refer to hereafter noncommittally as lymphoid cells. All the evidence at present available is consistent with the view that the homograft reaction is called into being by the passage of antigens from the graft via the draining lymphatic vessels to the regional lymph nodes where they stimulate the production of immunologically activated cells which resemble the lymphocytes of the blood. These activated cells then leave their site of origin, and after passage through the blood stream infiltrate the homografts for whose destruction they are ultimately responsible (Fig. 2). Some authorities believe that these cells are the vehicles for fixed antibodies which are bound to their surfaces and that these are the antibodies responsible for destroying the homografts. However, it must be emphasized that the actual mechanism whereby these "sensitized" cells bring about graft destruction still awaits experimental elucidation. Other types of *cell-mediated*, as opposed to circulating-antibody-mediated, immunological responses are known: they include the tuberculin reaction and allergies to certain chemicals.

Chemical Nature of the Antigens

The nature of the antigenic substances responsible for transplantation immunity has long been a provocative question, especially since even the most conservative methods of killing cells or tissues completely destroy their ability to sensitize if they are grafted or injected. Recently it has been shown that despite their lability, antigenically active substances can be extracted in aqueous solution from living tissue cells of mice and guinea pigs. Preliminary analysis indicates that their determinant groups are amino acid polysaccharide complexes.

Reaction of Grafts against Homologous Animals

So far we have been exclusively concerned with a survey of what is known about the destructive forces which are brought into action when an animal is confronted with a graft of homologous tissue. The primary purpose of this article, however, is to give an account of a new aspect of transplantation immunology: the situation in which, at least in an immunological sense and as a consequence of various experimental artifices, the roles of host and graft are reversed.

In 1953 Dempster and Simonsen, studying the fate of kidney homografts in dogs, independently observed cytological changes in the reticuloendothelial cells (lymphoid-type cells) present in these organs which they postulated might be indicative of antibody formation against the antigens of the host. This hypothesis was perfectly plausible: being continuously perfused with the foreign (from their point of view) blood of the host, the grafted kidneys were certainly thoroughly exposed to an antigenic stimulus. Unfortunately these homografted organs were destroyed by the conventional homograft reaction long before more convincing evidence could be obtained of their possible counterattack against the hosts.

From what has already been said in the introduction, it follows that for a graft to be able to launch and sustain an immunological attack on its recipient there are at least three prerequisites: first, it must contain mature immunologically competent cells; second, the host must possess important transplantation antigens (histocompatibility genes) which are lacking in the graft, so that the host appears foreign to it; and third, the host must itself be incapable of reacting against the graft, at least for a long enough time for the latter to manifest its immunological capabilities—like some Sinbad forced to endure the insults of an Old Man of the Sea whom he cannot dislodge from his shoulders.

During the last few years several different means have been forthcoming for making animals completely or at least temporarily incapable of rejecting tissue or cellular homografts so that they become graft or cellular chimeras, thus fulfilling one of the essential conditions for the revelation of graft-versus-host reactions.

"Runt Disease" in Immunologically Tolerant Animals

It was long believed that embryos or very young animals are incapable of immunological responses, largely on the basis of such empirical facts as the almost universal hospitality of avian embryos to foreign tissue grafts or

microorganisms and the inability of very young animals to form antibodies. Investigations in which living homologous cells were inoculated into embryonic or newborn mice, chickens, and animals of other species have shown that the hosts may in fact respond to these antigens—by becoming *tolerant* of them. To quote one example: if a newborn A strain mouse is injected intravenously or intraperitoneally with a suspension of spleen cells prepared from a CBA strain donor, then when the A strain mouse grows up it will be found to be incapable of reacting against a skin or other tissue homograft from the CBA donor strain. Furthermore, as immunological tests have shown, the splenic cells which called the tolerant state into being show a remarkable "homing" instinct, for they appear to settle out electively in, and so become foreign residents of, anatomically appropriate tissues of the new host—that is, its various lymph nodes, its thymus, spleen, bone marrow, and almost certainly all the small aggregates of lymphoid tissue which are present in most organs of the body. This state of acquired immunological tolerance is highly specific; it extends only to subsequent tissue homografts having the same antigenic constitution as the initial neonatal inoculum, and it depends upon the inability of the host's lymphoid tissues to become sensitized to the antigens to which they were exposed at an early state of their development.

The actual mechanism of immunological tolerance, like the mechanism of antibody formation, is still in the realm of speculation. The principle of tolerance certainly applies to antigens of many different kinds. Its present significance is that it affords one means of enforcing acceptance of homografts upon a host.

In our initial studies on the induction of tolerance in newborn mice with adult splenic cells, L. Brent and I were particularly lucky in our selection of strains CBA and A. The postoperative mortality of our hosts was low, and most of the tolerant animals remained outwardly normal. However, the lymphoid organs of nearly all these animals manifested at least some degree of hypoplasia, a fact which for a while we were at a loss to explain. With other strain combinations, however, and particularly with those which probably differed from their donors with respect to powerful transplantation antigens determined by the H-2 locus, it was observed that many of the mice stopped growing within a week or two of injection, became emaciated, and usually suffered from diarrhea (Fig. 3). Most of the mice thus affected died within a few days, but some lingered on for two or three months as chronically sick and grossly stunted individuals with abnormally sparse fur (Fig. 4). Apart from impairment of growth, the most striking and constant symptom of "runt" disease, as we christened it, was the widespread involution of lymph nodes and the lymphoid or Malpighian centers in the spleen, though frequently distinctive lesions in the liver were observed.

Fig. 3. Three 12-day old "A" strain mice. The two on the left were injected at birth with splenic cells from an adult C57 strain mouse and are now very sick and emaciated. Their uninjected littermate, on the right, is perfectly healthy.

Several different lines of evidence suggested that this disease was the outcome of a reaction on the part of immunologically competent cells, introduced in the neonatal inoculum, against the antigens of the host. Presumably the foreign antigens of the host, confronting these cells in the milieu in which they had settled out (the host's own lymphoid tissues), stimulated them to proliferate and so add weight to the immunological attack. It is almost inconceivable that a dosage of a million splenic cells—all that is necessary to cause fatal runt disease with some strain combinations—is in itself sufficient to do the damage. To cite only a little of the evidence that runt disease is the expression of a graft-versus-host reaction: The severity of the disease is greatest when the donor and host strains are most distantly related genetically, and no symptoms of runt disease are detectable if the donor and recipient strains are very closely related. The severity of the disease is heightened, as one would expect, if splenic cells, instead of being taken from a "virgin" adult donor, are taken from a donor which has previously been sensitized against the cellular antigens of the intended recipient. Finally, and most important, is the fact that runt disease does not appear if splenic cells from an F_1 hybrid donor are injected into newborn mice of one of the parental strains, though the recipients may become tolerant of homografts from the other parental strain.

In attempts to confer tolerance without the intervention of runt disease, leucocyte concentrates prepared from whole blood and suspensions of bone marrow cells were investigated. Contrary to expectation, since the majority of blood leucocytes in normal animals are thought to be expendable end-products incapable of further proliferation, it was found that suspensions of these cells are just as potent as spleen cells in causing runt disease. These results strongly suggest that at least one type of blood leucocyte is immunologically competent and capable of proliferation. Bone marrow cells, on the other hand, although they conferred tolerance very satisfactorily, rarely killed animals even when they were employed with strain combinations which would have invariably been fatal had splenic or lymph node cells been used. This finding is entirely consistent with the fact that marrow, unlike spleen, contains relatively little lymphoid tissue.

Quite independently, a phenomenon essentially similar to runt disease was

discovered in chickens by Simonsen, who had deliberately set out to put his original hypothesis about graft-versus-host reactions on a sound experimental basis. He found that the intravenous inoculation of embryonic chicks with splenic cells from an unrelated adult donor caused a fairly prompt enlargement of the host's spleen and liver, with distinctive pathological lesions in these and other organs, accompanied by an acute hemolytic anemia. The disease usually killed the hosts within a week or two of hatching. Careful histological studies strongly suggested that the affected organs of these animals had undergone varying degrees of replacement of their normal cell populations with proliferating lymphoid cells of donor origin. In the bone marrow, for example, the blood-forming cells were almost entirely replaced by lymphoid cells, and it was presumed that the native cell population had been destroyed immunologically. Simonsen also found that adult whole blood or the leucocytes isolated from it produced splenic enlargement on inoculation into embryos. This spleen-enlarging principle was serially propagated from 3-day old chicks to 18-day embryos and so on, for nine consecutive passages. Here, then, is unequivocal evidence that the inoculated cells must proliferate in their new hosts, presumably in response to the antigenic stimulus. Furthermore, as with the mice, the blood of adult chickens clearly contains immunologically competent cells. The fact that cells from very young donors did not affect their hosts was to be expected, on account of the immaturity of the immunologically competent cells. Indeed, the latter probably became tolerant of their hosts!

It had previously been established by other workers that experimental or natural parabiotic union between avian or mammalian *embryos* permitting a mutual exchange or cross transfusion of blood leads to the production of animals which are cellular chimeras and therefore are tolerant of subsequent grafts of tissues interchanged between them, but are perfectly normal in other respects—that is, there are no symptoms of runt disease.

In subsequent experiments Simonsen and Cock obtained even more compelling evidence that the splenomegaly and other lesions in the young chicks were the outcome of their cellular homografts reacting against them. Using inbred strains of chickens, Simonsen found that blood from one parental strain caused enlargement of spleens and livers on inoculation into newly hatched F_1 hybrid individuals, whereas adult hybrid blood inoculated into hybrid or parental strain hatchlings was without significant effect, as one would expect *ex hypothesi*. It may be emphasized that in both Billingham and Brent's work and in Simonsen's, care was taken to eliminate any possibility that "runt disease," or its equivalent in chickens, was caused by pathogenic organisms accidentally introduced along with the cells into immature and immunologically defenseless hosts. The disease is unequivocally immunological in origin, and the reacting cells are those of the graft.

It is interesting to recall that the striking splenomegaly that almost invariably follows the transplantation of adult homologous splenic tissue, and not infrequently grafts of other adult tissues, to the chorioallantoic membrane of the embryonic chick has been repeatedly observed for more than 40 years and accounted for by several ingenious hypotheses. Final solution of this problem was not possible until the recent development of the concept of graft-versus-host reactions. The solution depended also on an appreciation of the surprising mobility of apparently fixed lymphoid cells, and of their uncanny power of taking up residence among cells of their own kith and kin and turning upon their new neighbors.

Indeed, with appropriate controls, splenomegaly in the chick embryo furnishes a valid test for the presence of immunologically competent cells in grafts of homologous tissues from adult donors. For example, this test has revealed that adult chickens' skin, carefully perfused to remove all residual blood cells, still retains the spleen-enlarging property—hence must contain immunologically competent cells.

Evidence of the reaction of inocula containing immunologically competent cells against homologous embryonic or very young hosts has now been obtained in other species, including rats, hamsters, and rabbits; preliminary experiments suggest that the phenomenon occurs in salamanders too. The disease may also appear when donor and recipient belong to *different* species, as when the donor is an adult turkey and the recipient a chick embryo. In the reaction to heterografts, as in the normal homograft reaction, the grafted lymphoid cells appear to produce antibodies which may coat the red cells of the host, but there is no evidence that the antibodies are responsible for runt disease. A likely contributory factor to the deaths of the "runts" is probably their gross deficit of lymphoid tissue, which renders them highly susceptible to infection, for example, from pathogens present in their own intestines.

Fig. 4. Two 2-month old "A" strain mice which were injected at birth with C3H strain spleen cells. The smaller animal is suffering from chronic runt disease and weighs only 6 gm. Note the characteristic scruffy condition of its fur. Its brother weighs 22 gm and is perfectly healthy.

Delayed Deaths in Radiation Chimeras

The red blood cell forming and lymphoid tissues are particularly susceptible to ionizing radiations so that, at least in some species, and particularly in the mouse, it is possible to give a dosage of x-irradiation which is sufficient to destroy these two tissues, but does very little damage to the remainder. Of course, this treatment is normally fatal within a few days. However, if the irradiated mice are promptly given an intravenous injection of living cells from the blood-forming tissues of other mice—adult bone marrow or infant spleen—they will recover from the radiation syndrome.

Independent investigations, employing a variety of techniques, have shown that the protection afforded by the inoculated cells depends upon their migration to anatomically appropriate places, where they recolonize the depleted "frameworks" of the marrow spaces and lymphoid tissues and thus restore function. If the inoculated cells come from a genetically similar or isologous donor, the host may expect to live quite happily on its borrowed marrow for a very long time. If, however, donor and recipient belong to different strains the loan may be less satisfactory; deaths of the resultant *homologous chimeras* may occur within a matter of a few weeks or a month or two. Where protection is afforded by cells of *heterologous* origin—for example, rat to mouse—the life expectancy may be even shorter. These "delayed deaths" are attributed to a syndrome variously described as secondary radiation sickness, homologous disease, or wasting disease. The symptoms closely resemble those of runt disease: usually loss of weight, poor condition of the fur, diarrhea, and involution of lymphoid tissue.

Although all authorities are in agreement that this disease is immunological in origin, some division of opinion still prevails as to whether it is due to a reaction on the part of some of the grafted cells against the host, as Trentin first suggested, or to a gradual recovery of the host's *own* lymphoid cells, through the proliferation of a few survivors, and the immunological destruction or rejection by these host cells of the borrowed hematopoietic or blood cell forming tissue upon which survival of the animal depended. The general conclusion to be drawn from numerous experiments employing the same principles as those used in analyzing runt disease is that the delayed deaths following potentially lethal irradiation are a consequence of the cellular homografts reacting against their hosts. To mention only two of the many important pieces of evidence which support this conclusion: delayed deaths may be avoided by the use of cells from adult F_1 hybrid donors or cells from fetal donors. With lower dosages of irradiation the host is more likely to recover its own immunological defense mechanism so that the reaction of host against the cellular homograft becomes the predominating cause of death. There is, in fact, no reason why attacks from both directions cannot take place concomitantly if lymphoid cells of both donor and recipient types are present. The fact that homologous bone marrow inoculated into newborn mice induces immunological tolerance with only subclinical manifestations of runt disease, whereas homologous marrow inoculated into adult irradiated mice may cause a high proportion of delayed deaths (even with the same donor-recipient combination) is not necessarily paradoxical.

Although in an immunological sense the two situations are comparable—the hosts in both situations being immunologically defenseless—in an anatomical sense there are several important differences. Thus, in the irradiated animal the host's marrow and lymphoid tissues have been destroyed, making plenty of room for the small proportion of immunologically competent ingredients among the donor's marrow cells to establish themselves and build up a population large enough to cause immunological damage; in the newborn animal the limited sites available are already colonized so that there is less opportunity for insurgent cells to develop in sufficient numbers to do serious harm.

"Runt" or "Homologous" Disease in Genetically Tolerant Hosts

There is one immunogenetic situation which should give a graft every opportunity of reacting against its adult host yet require no pretreatment or conditioning of the latter. This obtains when cells from one parental strain are introduced into an F_1 hybrid (see previous section on genetic determination of transplantation antigens).

In some strain combinations, very young hybrid mice inoculated with parental strain cells are highly susceptible to acute runt disease but gradually become resistant; inoculations given more than about two weeks after birth are apparently harmless. Similar results have been obtained with hybrid chickens. In other mouse strain combinations, however, the adult hybrid mice do retain their susceptibility. How is this loss of susceptibility to be explained? It seems reasonable to suppose that the actual dosage of the cellular homograft and the "power" of the antigenic stimulus it receives from the host are important. The fact that sublethal dosages of irradiation increase the vulnerability of adult hybrids hints at another factor: insidious competition of the inoculated cells with the native and established cells for *lebensraum* before they can express their Quisling-like properties. Other things being equal, one might expect adult lymphoid cells to be at some advantage in competing with their homologues in immature hosts, but when recipient as well as donor is adult the situation may well be less favorable for the cells of the latter to gain lodgment unless they have been aided by a little site-clearing by prior sublethal irradiation. Moreover, hybrid cells may, like hybrid animals, possess a "vigor" which exceeds that of the parental strain cells.

One important question still to be resolved concerns the activity or fate of immunologically competent cells in those immunologically tolerant mice, radiation chimeras and F_1 individuals which do not succumb to graft-versus-host reactions. Unquestionably, many of these cells survive for very long periods, if not for the remainder of their host's lives. Do they maintain a chronic though virtually asymptomatic attack on their hosts—like the long-drawn-out course of some autoimmune diseases in which individuals react against some of their own specialized body ingredients?

Parabiosis Intoxication

Surgical parabiosis is, in effect, the grafting of two animals together, and has long formed part of the armamentarium of the endocrinologist. With parabiotic unions between members of the same species, usually rats or mice, a distinctive syndrome, commonly referred to as *parabiosis intoxication*, has long been recognized. Usually one parabiont loses weight and dies within a week or two after union, whereas its

partner appears unaffected—although sometimes both members may die. This disease has not been made the subject of systematic investigation, but there are strong indications that it is the outcome of an immunological incompatibility between the parabionts. For example, littermate rats are less susceptible to it than nonlittermate rats, and inbred mice may be parabiosed with impunity. Also consistent with this interpretation are reports of involution of the lymphoid tissues and spleens of the smaller animals and of a hyperplasia of these tissues in the larger animals. So far as it goes, all the evidence suggests that there is a striking similarity between parabiosis intoxication and homologous or runt disease.

Pregnancy in mammals may also be regarded as a form of parabiosis or homotransplantation. However, the complete separation of the fetal from the maternal blood circulations is generally held to be the most important factor in preserving these animals from immunological extinction. However, in man, hemolytic disease of the newborn is an unpleasant reminder that this barrier is not completely infallible. Hemolytic disease is usually held to be brought about by the accidental entry of "foreign" fetal cells into the mother, where they elicit the formation of antibodies. These then pass back across the placenta into the fetal circulation. However, there is an alternative mechanism which may be worth bearing in mind, now that it has been shown from the experiments on runt disease that adult blood contains immunologically competent cells. Hemolytic disease may be caused by the entry of maternal *leucocytes* into the fetus, where they become established as cellular homografts and so react against the host from *within*. Although there are cogent reasons why this explanation cannot be applicable to all cases of hemolytic disease of the newborn, it would certainly account for the small proportion of recorded cases in which no evidence of sensitization was forthcoming from examination of the mother's blood.

Conclusion

Runt disease in small rodents or chick embryos provides one of the simplest diagnostic tests for immunologic competence of inoculated adult cells. For example, it has shown unequivocally that the thymus contains such cells, a finding paralleled by the fact that homologous disease may follow the inoculation of parental thymocytes into sublethally irradiated F_1 hybrid hosts. Furthermore, experiments currently in progress show that the median survival time of newborn mice inoculated with a constant dosage of cells obtained from different types of lymphoid tissue can be employed as a quantitative measure of their relative immunological efficacy. This test system is already being used in several laboratories to find out which of the types of leucocytes is responsible for the immunological reactivity of peripheral blood. The two principal candidates appear to be the so-called small and large lymphocytes, of which the former is by far the more abundant.

Of clinical interest is the possible use of body "spare parts" if the problem of homograft survival should be solved. Before the widespread utilization of clinical spare parts could be safely undertaken, it would be necessary to evaluate carefully the possible importance of graft-versus-host reactions. Could a kidney homograft, for example, conceivably damage its host by virtue of the small amount of lymphoid tissue it contains? The question is clearly irrelevant in the case of some tissues, such as skin, which lack lymphoid-type cells. The risk of incurring "homologous disease" in man has already been justifiably undertaken. For example, reports have recently appeared in the lay press of the apparently successful treatment of five patients who had accidently received high dosages of irradiation by giving them infusions of adult homologous marrow. Attempts are also being made to employ total body irradiation in the treatment of leukemia, the widespread damage to the patient's blood-forming tissues being made good by infusions of homologous marrow cells. A well-recognized complication here is the fact that functional lymphoid tissue is essential for the individual to protect himself against pathogens. As the experiments already described in this article have shown, one way in which this risk can be eliminated is to employ immature tissues from fetal donors, so that the transplanted lymphoid-type cells will become tolerant of their host.

Bibliography

R. E. Billingham, "Actively acquired tolerance and its role in development," in *A Symposium on the Chemical Basis of Development*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1958), p. 575.

———and L. Brent, "A simple method for inducing tolerance of skin homografts in mice," *Transplantation Bull.* **4**, 67 (1957).

L. Brent, "Tissue transplantation immunity," in *Progress in Allergy* (Karger, Basel, Switzerland, 1958), vol. 5, pp. 271–348.

W. J. Dempster, "Kidney homotransplantation," *Brit. J. Surg.* **40**, 447 (1953).

J. C. Finerty, "Parabiosis in physiological studies," *Physiol. Revs.* **32**, 277 (1952).

P. A. Gorer, "Some recent work on tumor immunity," *Advances in Cancer Research* **4**, 149 (1956).

P. B. Medawar, "The immunology of transplantation," *Harvey Lectures Ser. 52, 1956–57* (1957), p. 144.

———, "The Croonian lecture: the homograft reaction," *Proc. Roy. Soc. (London)* **B149**, 145 (1958).

N. A. Mitchison, "Passive transfer of transplantation immunity," *ibid.* **B142**, 72 (1954).

M. Simonsen, "The impact on the developing embryo and newborn animal of adult homologous cells," *Acta Pathol. Microbiol. Scand.* **40**, 480 (1957).

G. D. Snell, "The homograft reaction," *Ann. Rev. Microbiol.* **11**, 439 (1957).

J. J. Trentin, "Tolerance and homologous disease in irradiated mice protected with homologous bone marrow," *Ann. N.Y. Acad. Sci.* **73**, 799 (1958).