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Quantitative Studies on Tissue Transplantation Immunity. IV. Induction of Tolerance in Newborn Mice and Studies on the Phenomenon of Runt Disease

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QUANTITATIVE STUDIES ON TISSUE TRANSPLANTATION IMMUNITY

IV. INDUCTION OF TOLERANCE IN NEWBORN MICE AND STUDIES ON THE PHENOMENON OF RUNT DISEASE

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[Plate 10]

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A simple method for the induction of actively acquired tolerance of homologous tissues in newborn mice is described (§§2·1, 2·2 and 2·3). With some donor/recipient strain combinations a high proportion of mice injected intravenously with homologous tissue cells is rendered tolerant of skin grafts from animals of the donor strain (§§3·1, 3·4 and 3·5), provided that the cells are administered within 24 h of birth. As the age of the recipients increases, so the proportion of tolerant mice falls (§3·2); a 'neutral period' in the life of the recipients lies between the latest age at which they can be rendered tolerant and the earliest age at which they can be rendered immune. The induction of tolerance is less effective when homologous cells are injected intraperitoneally into newborn mice, and (with our strains) wholly ineffective when they are injected subcutaneously (§3·3). Tolerance can be induced with spleen, bone marrow or thymus cells (§3·4), and abolished ('adoptive immunization') with sensitized spleen and thymus cells as well as with lymph node cells (§5·2). Further experiments on the specificity of tolerance are described (§5·1). In newborn rabbits, the intravenous inoculation of homologous spleen or thymus cells results in the induction of tolerance in only a small proportion of animals (§3·6).

The injection of adult homologous lymphoid cells into newborn mice leads to the development of a syndrome described as runt disease because, in its extreme form, it is characterized by gross retardation in development (§3·5). Runt disease is marked by varying degrees of involution of the recipients' lymph nodes, and by pathological changes in the spleen, liver and other organs (§3·7). In its acute form it is fatal, but when more mildly expressed it need not be a serious handicap (§3·5).

Two possible causes of runt disease are considered and subjected to experimental test (§4). The theory that runt disease is infective in origin cannot be upheld (§4·1). On the contrary, it is shown conclusively that runt disease is immunological in origin, and that it is the consequence of an immunological reaction of the homologous cells against the tissues of their hosts (§6·2). For (*a*) the injected homologous cells persist indefinitely in the tissues of their hosts (§3·8); (*b*) although the injection of F_1 hybrid spleen cells into newborn mice of the parental strains is usually quite harmless (§4·3), the injection of parental strain cells into F_1 hybrid recipients is followed by the typical syndrome of runt disease (§4·6); (*c*) mice can be protected from runt disease if they receive, together with the homologous cells, *adult isologous* spleen cells (§4·4); (*d*) the severity and frequency of runt disease are enhanced if sensitized homologous spleen cells are injected—i.e. cells from donors already sensitized by host strain tissues (§4·5); (*e*) acute runt disease can be avoided by the injection of adult tissue cells containing no, or only a very small proportion of, immunologically competent cells (§§4·2, 6·3); and (*f*) the severity and incidence of runt disease are largely determined by antigenic differences between donor and host strains (§6·2). Finally (*g*) it is already known that embryonic cells, though adept in producing tolerance, fail altogether to produce runt disease (§6·3).

Tolerance of homologous tissues can occur in the complete absence of runt disease and cannot therefore depend upon the lymphoid hypoplasia that accompanies it (§6·3). On the other hand, runt disease frequently depends upon the induction of tolerance by the injected cells, which are therefore able to persist and to react against the tissues of the host over a relatively long period of time. With certain donor/recipient strain combinations in which runt disease is particularly violent and rapid in onset, the induction of complete tolerance need not be necessary (§6·3), the young hosts succumbing to the disease before their own defence mechanism has matured sufficiently to destroy the cells that cause it.

With some donor/recipient strain combinations, the susceptibility to runt disease of F_1 hybrid mice injected with spleen cells from mice of the parental strains falls off with increasing age (§4·6), despite the fact that with this experimental design even *adult* recipients may be expected to be 'tolerant' of the injected cells. Similarly, the injection of adult mice made tolerant at birth with large doses of donor strain spleen cells does not bring about an increase in the severity of the disease (§4·7). The production of runt disease in a severe form seems therefore to depend upon the hosts' own lymphoid organs being relatively undeveloped when confronted with foreign lymphoid cells.

It has been shown by Simonsen that the splenomegaly produced in chickens injected *in ovo* or soon after hatching with homologous spleen or blood cells is due to a 'graft-against-host' reaction (§6·2), and the same interpretation has been put upon the 'secondary disease' which often occurs in irradiated mice protected against the effects of radiation by adult homologous spleen or bone

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marrow cells (§6·4). These two phenomena and runt disease are therefore considered to have a common etiology.

The occurrence of runt disease is a sensitive indicator of the presence of immunologically competent cells in the inocula used to induce tolerance. The finding that blood leucocytes and thymus cells can cause runt disease when injected into newborn mice (§4·2) suggests that at least some of the cells among them are capable of immunological reactivity. Bone marrow cells, whilst bringing about some degree of lymphoid hypoplasia, do not cause acute runt disease, and they may therefore be used conveniently for the induction of tolerance with those donor/recipient strain combinations in which the injection of spleen cells is always lethal.

Certain clinical implications are discussed (§6·5). It is suggested that the possibility of 'graft-against-host' reactions and the occurrence of immunologically competent cells in adult blood should be borne in mind in the interpretation of haemolytic disease and in devising treatments of any kind which depend upon the transplantation of lymphoid cells.

1. Introduction

The present paper is a sequel to previously published investigations on the phenomenon of actively acquired tolerance of homologous tissue grafts (Billingham, Brent & Medawar 1953, 1955, 1956*a*). Mice, owing to the existence of a wide variety of inbred strains, are especially suitable both for the analysis of the various consequences of tolerance induction and for studies on the mechanism of tolerance itself. A major technical problem left unsolved by our earlier work was the production of large numbers of tolerant mice for experimental purposes: using a fine mince of homologous tissues (liver, testis, spleen and kidney) the injection of embryos *in utero*, preferably before the 18th day of gestation, appeared to be obligatory. Injection of these preparations into *newborn* mice, either subcutaneously or intraperitoneally, resulted in so small a proportion of tolerant animals as to suggest that at the time of birth most mice had already outlived the 'tolerance-responsive' phase of life.

Taking a lead from work on newly hatched chickens (Billingham *et al.* 1956*a*), in about 40% of which tolerance of skin homografts can be induced provided that homologous blood cells are injected *intravenously* immediately after hatching, we devised a simple technique for the intravenous injection of newborn mice. Compared with other routes of injection, the intravenous administration of cell suspensions may be expected to lead to a more rapid and widespread dispersal of the cells in the recipient's body, and thus make for more effective induction of tolerance. The inoculum used in our preliminary trials was homologous adult blood (Billingham & Brent 1956). The very feeble tolerance obtained in this way was attributed to the inadequate dosage represented by the leucocytes contained in 0·05 ml. of blood, and the investigations therefore turned to the use of cell suspensions of spleen. Quite apart from the ease with which they can be prepared, such suspensions are well known to act as highly effective antigenic stimuli when injected into adult mice. It soon became apparent (Billingham & Brent 1957) that, in some mouse strains at least, this method was both simpler and more effective than the inoculation of embryos, with its high mortality rate through abortion and maternal antagonism.

An unfortunate accompaniment of this new method was the occurrence of a disease which led to the premature death or impaired development of a variable proportion of the subjects, depending upon the particular donor/recipient strain combination used. In our preliminary analysis (Billingham & Brent 1957) this disease, to be referred to as 'runt

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disease' on purely descriptive grounds, was provisionally ascribed to an immunological reaction of the inoculated homologous spleen cells against their antigenically foreign hosts. The concept that homografts might be capable of reacting against their hosts is by no means a new one: it was first put forward by Dempster (1951, 1953) and Simonsen (1953) on the basis of histological studies of homotransplanted kidneys. Provided that homologous tissue grafts are equipped with cells capable of immunological reactivity and that they are, by one means or another, permitted to survive in the host, there should indeed be nothing to prevent them from reacting against the host's tissue antigens. The present study provides experimental support for this concept, the survival of the grafted cells here being assured by the simultaneous induction of immunological tolerance.

It has long been known that transplantation of adult homologous spleen and other tissues to the chorioallantoic membrane of the chick embryo may cause an enlargement of the host's own spleen, sometimes accompanied by pathological changes both in the spleen and in other host organs (Murphy 1916; Danchakoff 1916). More recently it has been observed that the injection of homologous adult blood is frequently fatal in chick embryos, but not in 1-day-old chicks (Brandley, Thorp & Prickett 1949; Billingham *et al.* 1956*a*). Brandley *et al.* observed lesions in the bone marrow, spleen and liver of embryos which had been injected with adult blood, and they suspected that 'they were associated at least in part with homoioplastic tissue reactions'. The correctness of this view has now been conclusively demonstrated by Simonsen (1957; Cock & Simonsen 1958), whose careful studies on the reactions of homologous spleen cells and blood leucocytes against young chicken hosts closely parallel our own work in mice.

The present paper will deal in turn with a detailed study of the induction of tolerance in newborn mice belonging to a wide variety of strains, and with the etiology of the disease which frequently afflicted at least a proportion of mice injected neonatally with homologous tissue cells of certain histological types. In addition, further studies on the phenomenon of tolerance itself will be described.

2. Materials and operative procedures

2·1. *Mice and control data on homograft survival times*

The majority of the mice used belonged to laboratory sublines of the following isogenic strains maintained at University College: Strong *A*, *CBA*, *AU*, *C*57 and *C*3*H*. Some of the work, however, was carried out at the California Institute of Technology on mice belonging to strains *CBA/Jax* and *A/Jax* obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor*; because these two lines are not necessarily isogenic with their British counterparts they will be designated CBA^j and A^j.

The median survival time (m.s.t.) of skin homografts transplanted between normal adult members of some of these strains had previously been determined from histological scoring of biopsy specimens removed at regularly spaced intervals (Billingham, Brent, Medawar & Sparrow 1954). With the remaining strain combinations the m.s.t. was estimated from the outward appearance of skin homografts on panels of 6 to 9 animals. The m.s.t. for all strain combinations used is set out in table 1.

* We are grateful to Dr G. D. Snell for making these mice available to us.

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In order to provide a visible measure of the degree of tolerance conferred upon the mice by neonatal injections of homologous cells, all mice received skin grafts from another member of the original donor strain 6 to 8 weeks after birth. The implications of the recent discovery that *within* most isogenic mouse strains skin grafts transplanted from males to females usually provoke and succumb to a homograft reaction (Eichwald, Silmser & Wheeler 1957; Eichwald, Silmser & Weissman 1958; Krohn 1958) have been taken into account in the design of the experiments to be described. Eichwald (1956) had already drawn attention to the possible importance of such intrastrain incompatibilities in experiments on acquired tolerance of tissue homografts.

TABLE 1. MEDIAN SURVIVAL TIMES OF SKIN HOMOGRAFTS EXCHANGED BETWEEN ADULT MICE OF DIFFERENT INBRED STRAINS

strain combination	m.s.t. ± standard error (days)	strain combination	m.s.t. ± standard error (days)
$A \rightarrow CBA$	11·0 ± 0·3	$A \rightarrow C57$	7
$CBA \rightarrow A$	10·2 ± 0·3	$C57 \rightarrow A$	8
$A \rightarrow AU$	9·0 ± 0·3	$CBA \rightarrow AU$	9
$AU \rightarrow A$	9·1 ± 0·4	$CBA \rightarrow C3H$	13
$C3H \rightarrow A$	10	$C3H \rightarrow CBA$	15
$A \rightarrow C3H$	11		

Whenever the survival time of a test-graft did not differ significantly from the m.s.t. of the control mice (table 1), it was inferred that the mouse had remained in a state of unaltered reactivity despite its neonatal injection; if, on the other hand, the m.s.t. was exceeded by at least 3 days the mouse was classified as displaying some measure of tolerance. Those mice on which the grafts survived in a completely autograft-like condition for a minimum of 50 days (though of course retaining their donor-specific hair coloration) were considered to be 'highly tolerant'. Very few of these highly tolerant mice subsequently succeeded in rejecting their grafts.

A state of *immunity* (rather than tolerance) was revealed by a test-graft when its survival time proved to be considerably shorter than the m.s.t. of the controls—or alternatively, when the graft, biopsied 6 days after transplantation, was found by microscopical examination to suffer from a degree of epithelial destruction which greatly exceeded that of grafts removed from control animals after the same interval (see Billingham, Brent, Medawar & Sparrow 1954).

2·2 *Preparation of cell suspensions from spleen, thymus and bone marrow*

Two or more spleens (depending upon the number of mice to be injected) were removed aseptically from adult mice, cut into a few fragments and pressed gently through a fine-meshed stainless-steel sieve with a flat-ended glass pestle. The expressed pulp was taken up in at least 10 ml. of Ringer-phosphate solution and the coarse suspension sucked fairly gently in and out of a pipette to break up the larger tissue clumps into their component cells. Vigorous handling of these suspensions was deliberately avoided because it is known to damage the cells and to strip the cytoplasm from many of them. Indeed, however carefully spleen cell suspensions are prepared by this method, contamination with free nuclei is inevitable. The various cell dosages given in this paper include a small proportion of free nuclei.

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Because the intravenous injection into baby mice of cytoplasmic debris is usually fatal, it was necessary to remove this by centrifugation of the suspensions at speeds of about 500 *g* for 5 min. The supernatant fluid, which was rich in cytoplasmic particles and other cellular debris, was discarded, and the sediment resuspended in Ringer-phosphate solution so that each spleen equivalent was contained in about 0·75 ml. This suspension was allowed to stand in a narrow tube for 3 to 4 min in order to allow cell aggregates of harmful size to settle out; the supernate was made up almost entirely of single cells suitable for

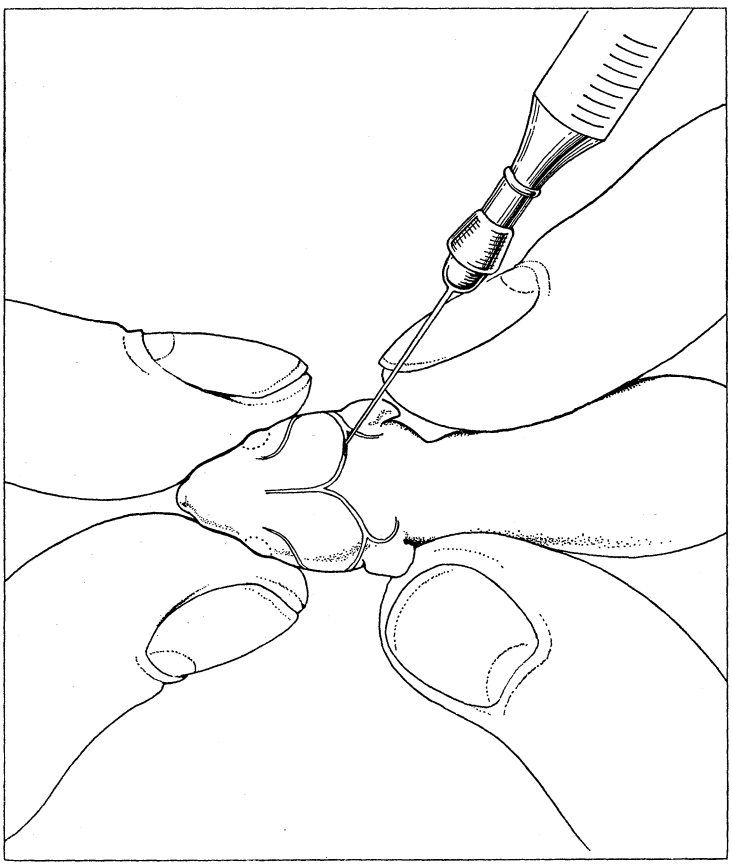

FIGURE 1. Illustrating the intravenous route of injection in newborn mice: injection into the sigmoid sinus (see §2·3).

inoculation by the intravenous route. Accurate cell counts were made of all suspensions and their volumes adjusted accordingly. Experience has shown that an anti-coagulant need not be used with suspensions prepared in this manner.

Suspensions of thymocytes were prepared by a similar technique. In order to obtain suspensions of bone marrow cells, the femurs were removed from young adults and stripped as far as possible of all adherent muscle and connective tissue. The ends were cut off with scissors and the marrow expelled with a jet of Ringer-phosphate solution applied through a no. 26 gauge needle mounted on a syringe. Disintegration of the expelled marrow was brought about by sucking it in and out of a pipette. As with spleen cells and thymocytes, the marrow cells were washed and counted before use.

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2·3. *Injection of newborn mice*

Cell suspensions were injected into newborn mice either through the orbital branch of the anterior facial vein (as illustrated by Billingham & Brent, 1956, figure 4, plate 10) or alternatively, through the sigmoid sinus as shown in figure 1. The firmness with which this latter vessel is united to the dura and its straight course immediately beneath the skull make it a very satisfactory alternative to the anterior facial vein. The use of a no. 30 gauge hypodermic needle (diameter 0·3 mm, bore 0·2 mm) is obligatory. Experience has shown that newborn mice are not harmed by the injection of up to 75 mm^3 of suspensions containing as many as 16 million cells. Occasionally respiratory movements may cease after injection, but recovery can usually be brought about by the application of intermittent pressure to the abdomen, or by rhythmically flexing the body, to ventilate the lungs. Our overall mortality attributable to this operative procedure has been well under 5 %.

Whenever possible the baby mice chosen for injection were taken from mothers which had already raised a previous litter. First litters were found to be highly unsatisfactory.

3. Results

3·1. *Induction of tolerance in newborn* A *strain mice with adult* CBA *spleen cells*

The first series of experiments, which form a basis for much of the work to be described in later sections, was designed to investigate the capacity of intravenously injected adult *CBA* strain spleen cells to induce tolerance when injected into newborn *A* strain hosts. The selection of this particular strain combination, though accidental, was extremely fortunate for reasons which will become apparent later (see §3·5). All the members of a total of eleven *A* strain litters, whose ages ranged from 2 to 24 h, were injected intravenously with dosages of *CBA* spleen cells varying from 4 to 10 million, and the survivors were subsequently challenged with *CBA* test grafts. The proportion of injected animals which died immediately as a consequence of injection, or soon after through maternal neglect, was less than 10 %.

The results of these experiments, summarized in table 2, show that some degree of tolerance was induced in 95 % of mice injected within 24 h of birth, the majority being highly tolerant. Despite variation in the dosage of *CBA* spleen cells the results were very uniform within the limits of 4 to 10 million. Although the relationship between cell dosage and tolerance response has not been investigated systematically, experiments with other strain combinations have shown that a dosage of 2 million cells is still highly effective, and the results given in §4·2 indicate that even 1 million cells still constitute a powerful tolerance-conferring stimulus. However, although 250 000 *CBA* spleen cells administered to *adult A* strain mice will sensitize the recipients fairly strongly against *CBA* skin grafts transplanted 3 to 10 days later, the same number will be almost completely ineffective in conferring tolerance on newborn *A* strain mice: only three out of thirteen mice gave evidence of being feebly tolerant, the survival times of the *CBA* grafts on these animals being 15, 15 and 16 days. This suggests that although the cell dosage/tolerance response curve for a newborn mouse is probably very flat-topped, like the cell dosage/sensitization

response curve for an adult mouse (Billingham, Brent & Mitchison 1957), the effective tolerance-conferring dosage of cells is considerably higher than that required for sensitization.

TABLE 2. INDUCTION OF TOLERANCE IN NEWBORN *A* STRAIN MICE BY INOCULATION WITH 4 MILLION TO 10 MILLION ADULT *CBA* SPLEEN CELLS BY DIFFERENT ROUTES

route	age of recipients	no. grafted	no. tolerant	no. highly tolerant
	12 h	39	37 (95 %)	31 (80 %)
	24 h	14	14 (100 %)	11 (78 %)
	48 h	16	9 (56 %)	5 (31 %)
intravenous	4 days	15	2 (13 %)	1 (7 %)
	7 days	18	0*	—
	10 days	14	0*	—
	15 days	16	0*	—
intraperitoneal	12 h	29	19 (65 %)	7 (24 %)
	24 h	15	10 (67 %)	3 (20 %)
subcutaneous	12 h	23	0	—

* In the majority of the mice injected later than the fourth day after birth the pattern of breakdown of their test grafts indicated feeble sensitization.

3·2. *Time relationships: the 'neutral' period*

The data in table 2 (see also figure 2) show that, as the age of the baby mice increased beyond the first 24 h, the proportion of mice in which tolerance was induced fell sharply. Forty-eight hours after birth as many as 46 % of the injected mice had already entered the 'neutral' period—i.e. the period in which the injection of homologous cells does not significantly alter the recipients' response to skin grafts transplanted 6 to 8 weeks later. By the 4th day this proportion had risen to 87 %. The injection of cells into 7-day-old mice left the animals in a feeble but just measurable state of sensitization, for their test grafts

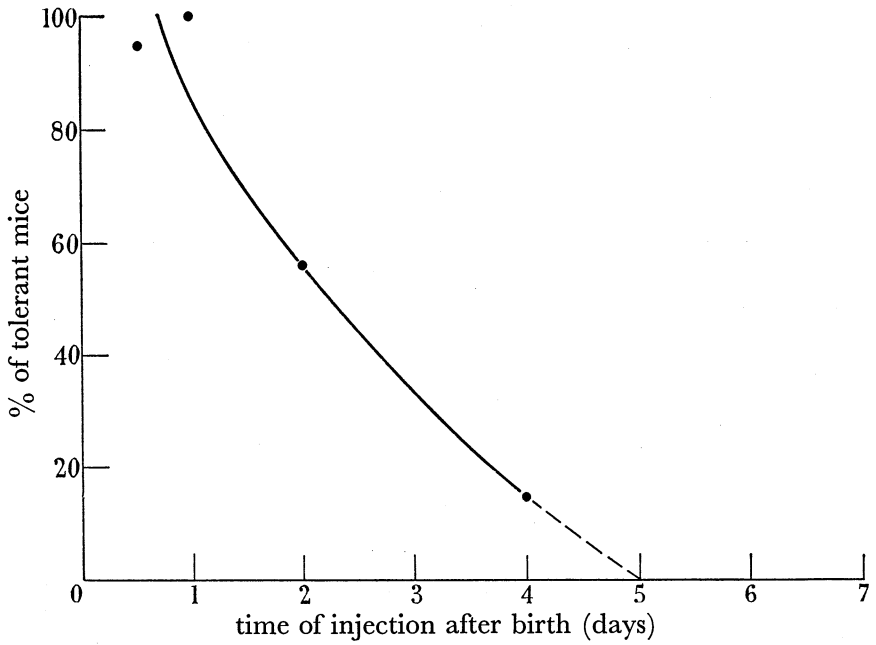

FIGURE 2. Illustrating the incidence of tolerance among *A* strain mice injected with *CBA* cells within the first week of life (see §3·2). Injection on the 7th day resulted in a weak immunity.

broke down rather more quickly than would be expected from the control data. Surprisingly enough the degree of sensitization was only just perceptibly stronger when cells were injected into 15-day-old mice. Both the 7- and 15-day groups of mice had to be injected intraperitoneally — intravenous inoculation having become extremely difficult owing to the increasing thickness, and therefore opacity, of the skin.

These findings show that, at least so far as *CBA* antigens are concerned, the tolerance-responsive phase of *A* strain baby mice extends well into the second day of life. The comparatively feeble levels of immunity revealed 4 to 6 weeks later in the mice which were injected either on the 7th or the 15th day after birth might be due to either (*a*) a decline in the immunity (the immunity elicited in adult mice by relatively large dosages of homologous spleen cells declines much more rapidly than the immunity produced by skin grafts—see Billingham, Brent, Brown & Medawar 1959), or (*b*) the immaturity of the immunological response mechanism at the time of injection.

3·3. *The efficacy of other routes of injection*

A number of newborn *A* strain litters were injected either intraperitoneally or subcutaneously (on both sides of the body) with a total of 5 to 10 million adult *CBA* spleen cells—a dosage of proven efficacy if administered directly into the blood stream. Although 65 % of the mice injected intraperitoneally later displayed some degree of tolerance, only about 20 % could be classified as highly tolerant. The subcutaneous route turned out to be *wholly* ineffective, not a single mouse out of twenty-three having had tolerance conferred upon it by the neonatal injection of homologous cells. The grafts on all non-tolerant mice had survival times which did not differ materially from the m.s.t. of the controls. The difference in effect between the two routes of inoculation is almost certainly due to the fact that the intraperitoneal route allows the injected cells more rapid access to the lymphoid tissues of the hosts. It is evident that in the strain combination used here the intravenous route of injection is by far the most satisfactory.

3·4. *Induction of tolerance with different types of tissue cells*

In the experiments described so far, and in many to be described in later sections, spleen cells have been employed simply because of the ease with which they can be prepared in large numbers and in a suitable form for intravenous injection. The average number of cells which can be obtained from one adult spleen by the method described above is about 100 million. In the work now to be described the ability of *CBA* bone marrow cells, thymocytes and intact skin grafts to induce tolerance in newborn *A* strain mice was investigated.

As shown by table 3, bone marrow cells were quite as effective as spleen cells in conferring tolerance (figure 5, plate 10). For reasons which are not known thymocytes were demonstrably inferior, both in the total number of mice with some degree of tolerance and the proportion of highly tolerant animals.

The efficacy of skin homografts was tested by grafting eight newborn *A* strain mice (less than 20 h after birth) with very thin *CBA* grafts, about 3·5 mm square, which had been prepared by stripping the skin from the dorsum of the ear cartilage (see Billingham & Medawar 1951). The grafts were transplanted to appropriately small beds prepared on

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the side of the chest, the skin overlying the exceedingly delicate panniculus carnosus having been carefully snipped away with scissors. The dressings—small rectangles of rice paper held in place by a strip of thin surgical tape wound round the thorax—were removed after 4 days. Degenerative changes were observed in every graft from the 16th or 17th day, and breakdown was completed by the 21st day. When 80 days old, the mice were again grafted with normal *CBA* body skin: the prompt rejection of these second grafts in all animals left no doubt that the recipients had been sensitized by their earlier grafting.

TABLE 3. INDUCTION OF TOLERANCE IN NEWBORN *A* STRAIN MICE BY INTRAVENOUS INJECTION OF 5 TO 10 MILLION ADULT *CBA* THYMOCYTES OR BONE MARROW CELLS WITHIN 24 H OF BIRTH

type of cell injected	no. of mice grafted	no. tolerant	no. highly tolerant
thymocytes	18	11 (61 %)	5 (28 %)
bone marrow	13	12 (92 %)	10 (77 %)

From the data available it is not possible to decide whether or not the survival times (about 20 days) of skin homografts transplanted to newborn mice reveal the existence of a feeble tolerance. Taking into consideration a possible delay of about 2 days before the grafts become fully vascularized and therefore able to exert an antigenic effect on the hosts, as well as the likelihood that a skin graft is too regional in its effect to be a satisfactory tolerance-conferring stimulus, it seems reasonable to attribute the relatively long survival times to the immunological immaturity of the hosts. The fact that these animals were undoubtedly more strongly sensitized than mice injected with *CBA* spleen cells 7 or 15 days after birth (see §3·2) suggests that, even in baby mice, the immunity elicited by orthotopically grafted skin lasts longer than that elicited by cells injected intraperitoneally.

3·5. *Induction of tolerance by neonatal inoculation with adult homologous spleen cells in other strain combinations*

In view of the great facility with which tolerance could be induced in *A* strain mice by injecting them intravenously at birth with *CBA* spleen cells, the method was applied to thirteen other donor/recipient strain combinations. The results are summarized in table 4.

There was an enormous variation in the tolerance-responsiveness of the hosts from one donor/recipient strain combination to another. For example, when baby *CBA* mice were injected with *A* strain cells, the proportion of highly tolerant animals was only about half that obtained when the hosts belonged to the *A* strain and the donors were *CBA*'s. The results obtained with strains *A* and *C3H* were also asymmetrical. With the two strain combinations $A \rightarrow C57$ and $CBA \rightarrow AU$ the proportion of mice in which tolerance was conferred was strikingly low. The origin of this variability is probably twofold: first, the state of immunological maturity of the mice at the time of birth (upon which their tolerance-response must depend) probably varies from strain to strain; and secondly, the magnitude of antigenic disparity between donor and recipient, an incomplete measure of which is the difference between their *H* series of multiple alleles (see Gorer 1956; Snell 1953, 1957). As this disparity increases, as Hašek (1956) and Billingham & Brent (1956) have argued, it becomes increasingly difficult to confer tolerance on newborn animals or embryos. This

second factor is probably the more important; the first certainly cannot be invoked to explain the results of experiments in which mice of the *same* recipient strain were injected with cells of different donor strains. Our findings therefore suggest very strongly that the termination of the tolerance-responsive phase in the life of a mouse (relative to its birth) depends to a large extent upon the antigenic properties of the injected homologous cells.

A completely unexpected finding was the high mortality among the injected mice, summarized in columns 2, 3 and 4 of table 4. Only in four of the fourteen different donor/recipient strain combinations investigated was the total mortality of the subjects less than 10%. With the three strain combinations $C57 \rightarrow A$, $AU \rightarrow A$ and $C57 \rightarrow CBA$ none of the

TABLE 4. ATTEMPTS TO CONFER TOLERANCE ON NEWBORN MICE OF DIFFERENT STRAINS BY THE INTRAVENOUS INJECTION OF 4 MILLION TO 10 MILLION HOMOLOGOUS SPLEEN CELLS WITHIN 24 H OF BIRTH

strain combination	early deaths	late deaths	overall mortality (%)	no. grafted	no. tolerant	no. highly tolerant
$CBA \rightarrow A$	1/58	5/58	10	52	51 (96%)	42 (80%)
$A \rightarrow CBA$	5/28	1/28	21	23	13 (56%)	9 (39%)
$C3H \rightarrow A$	3/22	5/22	36	15	15 (100%)	13 (87%)
$A \rightarrow C3H$	7/27	11/27	67	10	7 (70%)	3 (43%)
$C3H \rightarrow CBA$	—	—	0	5	5 (100%)	5 (100%)
$CBA \rightarrow C3H$	—	—	0	12	12 (100%)	12 (100%)
$A \rightarrow C57$	—	1/17	6	17	1 (6%)	0
$C57 \rightarrow A$	26/26	—	100	0	—	—
$A \rightarrow AU$	3/17	5/17	47	10	6 (60%)	5 (50%)
$AU \rightarrow A$	25/25	—	100	0	—	—
$CBA \rightarrow AU$	2/23	3/23	22	20	5 (25%)	1 (5%)
$C57 \rightarrow AU$	6/9	2/9	89	1	1 (100%)	1 (100%)
$C57 \rightarrow CBA$	13/16	3/16	100	0	—	—
$CBA^j \rightarrow A^j$	20/59	10/59	51	29	29 (100%)	29 (100%)

Note. *Early deaths* include all those mice which died between the 7th and 21st days after injection. Animals which died as an immediate consequence of the neonatal injection, as a consequence of maternal neglect, or during the course of a grafting operation are not included. *Late deaths* refer to mice which died at any time after the 21st day. Some of the mice entered here had actually reached maturity and had been challenged with skin from the original donor strain.

animals survived for long enough to be test grafted, and with the first two all mice died within 7 to 21 days. The outward symptoms of the disease which culminated in these early deaths were constant: the mice grew normally for the first week or more after injection, but then growth ceased and the subsequent loss of weight was nearly always associated with severe diarrhoea. Death usually occurred within a few days of the onset of these symptoms. With the strain combinations $C57 \rightarrow A$ and $AU \rightarrow A$, in which all the injected mice died within less than 3 weeks, uninjected litter-mates grew up quite normally. With other donor/recipient strain combinations there was great variability in the severity of the disease, sometimes even among the members of a single injected litter. Some individuals succumbed quickly to what appeared to be an acute attack of early onset, whereas in others the disease took on a chronic form, the victims lingering on as grossly undersized and backward runts for several months until they eventually died (figure 9, plate 10). The fur of these runts was usually very sparse and staring, and regenerated only slowly after clipping. Their weights rarely exceeded 17 g, and it was by no means unusual to find mice which, 80 days after birth, had attained a weight of only 7 g. Because of their small size

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many runts could not be test-grafted, and some of the larger ones failed to survive the normally trivial hazard of skin grafting. However, all runts which *did* survive the skin grafting operation proved to be completely tolerant for as long as they lived.

It must be emphasized that, although some of them suffered from a slight and temporary retardation in their rate of growth, the great majority of the animals which survived and were found to be tolerant were, to judge from their outward appearance and weight, normal mice (figure 6, plate 10).

3·6. *Attempts to confer tolerance upon newborn rabbits*

Six litters of newborn rabbits were injected, through one of the external mammary veins, with a suspension of adult homologous spleen cells, thymocytes or a mixture of cells of both types (table 5). To ensure a high degree of genetic dissimilarity the donor was selected from a breed differing from that of the mothers. When the donors were killed in order to obtain the tissue from which cell suspensions were to be prepared, skin grafts were removed from the ears, treated with 15% glycerol in Ringer's solution and stored at −79 °C in the manner described by Billingham & Medawar (1952). Thirty to 35 days later the grafts were thawed and the injected rabbits tested with their donor's grafts. Skin storage circumvented the necessity for surgical splenectomy on the live donors.

TABLE 5. ATTEMPTS TO CONFER TOLERANCE ON NEWBORN RABBITS BY INTRAVENOUS INJECTION OF SPLEEN CELLS AND/OR THYMOCYTES

litter	inoculum	no. of cells injected (millions)	survival times of homografts (days)
1	spleen cells	60	< 12
2	spleen cells	80	< 12, < 12, *20*
3	spleen cells	110	< 12, < 12, < 12, < 12
4	spleen cells	150	< 12, < 12, ⩾ 9, *17*
5	spleen cells and thymocytes	320	< 12, < 12, < 12, < 12, *20*
4	thymocytes	780	< 12, < 12, < 12
6	thymocytes	1000	< 12, < 12, < 12, < 12, < 12, *18*

All injections performed within 24 h of birth, the majority within 12 h. Litter no. 4 was divided, half the animals receiving spleen cells and half thymocytes.

Of the twenty-nine rabbits which survived the immediate hazard of injection, three died suddenly between the 24th and the 30th day after injection. All three had looked quite healthy, but histological examination revealed that their spleens were fibrotic and with a marked deficiency of Malpighian corpuscles. The significance of these deaths will be discussed later (§4).

The survival time of skin homografts exchanged between adult rabbits deliberately selected for maximal genetic dissimilarity is known not to exceed 12 days; any grafts which in these experiments survived for longer periods were therefore held to indicate a degree of tolerance in their hosts. Of the twenty-six animals test-grafted, evidence of very weak tolerance was obtained in four (one other animal died 9 days after grafting and its grafts were at that time in such good condition as to suggest that it, too, would have been at least feebly tolerant had it survived). The present findings show, therefore, that the majority of newborn rabbits have passed beyond the tolerance-responsive phase so far as distantly related homologous tissue antigens are concerned.

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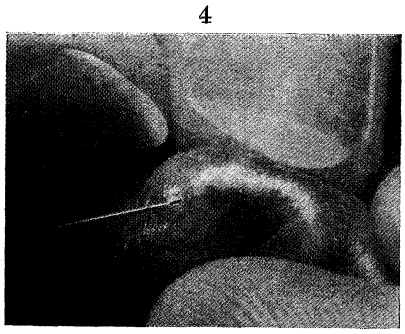

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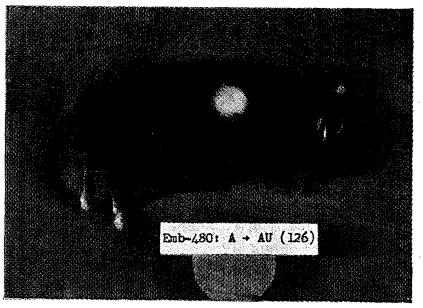

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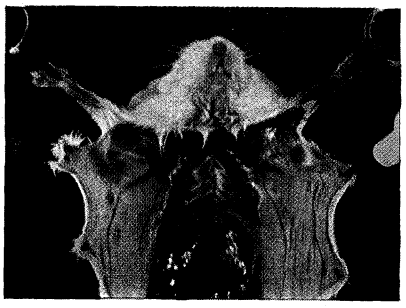

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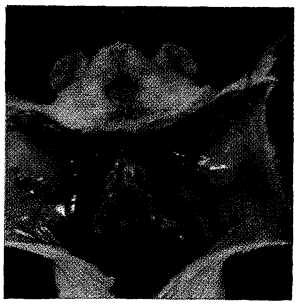

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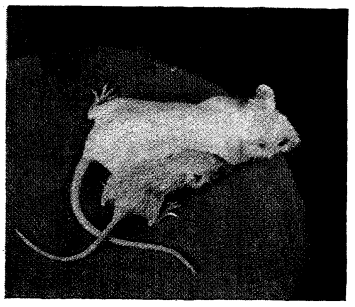

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FIGURE 4. The intravenous injection of a newborn mouse with homologous tissue cells: the 30-gauge needle is about to enter the orbital branch of the anterior facial vein (§2·3; cf. figure 1).

FIGURE 5. A *C*57 skin homograft 160 days after transplantation to an adult *A* strain mouse which had been intravenously injected (within 20 h of birth) with 5 million bone marrow cells from an adult donor (§3·4). The use of bone marrow for tolerance induction avoided the acute and fatal form of runt disease which, with this strain combination, was invariably caused by the injection of adult spleen cells (§3·5).

FIGURE 6. An *A* skin homograft 126 days after transplantation to an *AU* mouse which had been injected intravenously (within 20 h of birth) with 4 million spleen cells from an adult donor. With this strain combination about half of the cell recipients died of runt disease, but—like this mouse—50 % of the survivors were highly tolerant (§3·5).

FIGURE 7. An *A* strain mouse which had been injected neonatally with 6 million *C*57/*CBA* hybrid spleen cells. The mouse died 38 days later (weighing 9 g), and dissection revealed the almost total absence of its lymph nodes—a feature which characterized acute runt disease (§3·7). Note the complete absence of the axillary lymph nodes (cf. figure 8).

FIGURE 8. A normal *A* strain mouse, 33 days old, dissected to display its four axillary lymph nodes.

FIGURE 9. An *A* strain mouse 52 days after birth, when it had been injected intravenously with 5 million *C*3*H* spleen cells. The mouse, which had been suffering from runt disease ever since the first week after its birth, weighed only 6 g when it was killed and photographed. Subsequent dissection revealed a state of extreme lymphoid hypoplasia (see §§3·5 and 3·7). The animal is here shown together with a normal *A* strain mouse of the same age.

FIGURE 10. Two *A* strain litter mates 17 days after birth. The runt had been injected intravenously, soon after birth, with 1 million blood leucocytes obtained from an adult *C*57 donor, and it died 25 days later from runt disease. The larger mouse (short tail) had been injected with 0·07 ml. of plasma prepared from the blood of the same *C*57 donor, and it grew into a normal mouse (§§4·1, 4·2, 6·1 and 6·2).

(*Facing p.* 450)

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3·7. *Abnormalities of the lymphoid tissues of tolerant mice*

Fully tolerant, healthy-looking mice were killed in order to provide tissues for experiments to be described in §3·8, and careful dissections were carried out. Nearly all mice suffered from one striking anatomical abnormality—the involution of their lymphoid tissues as evidenced by the greatly reduced size or even absence of their axillary, brachial and inguinal nodes. Normally these organs are fairly prominent encapsulated bodies which, with the possible exception of the inguinal nodes, are easily distinguishable in even a very fat mouse (figure 8, plate 10). In the tolerant mice, on the other hand, some lymph nodes were reduced in size, yellowish in colour and hard in texture, whilst others had disappeared altogether (figure 7, plate 10). Those lymph nodes which were more or less normal in size were often extremely flabby and somewhat discoloured, and not infrequently it was found that although a mouse displayed atrophy of its lymphoid tissue in some parts of its body, certain of its nodes were enlarged by a factor of about two. Peyer's patches were often reduced in size and occasionally so small as to be barely recognizable.

The spleens of *A* strain mice which were tolerant of *CBA* skin grafts were normal in weight and healthy to outward appearance. However, with the strain combination $C3H \rightarrow A$ a moderate degree of splenomegaly was encountered, the mean weight of the spleens from eleven animals being 155 mg, compared with 112 mg for an extensive control series.

In all runts examined (only a few of which had lived long enough to be grafted with skin and thus to be proved tolerant) lymphoid hypoplasia was extreme: axillary, brachial and inguinal nodes and Peyer's patches could rarely be identified even with the aid of a dissecting microscope. Their spleens were not usually enlarged and were often very pale in colour. Jaundiced livers, with prominent congested vessels visible on their surfaces, were the rule. Another striking observation, made repeatedly in many of the runts which were dissected immediately after death or killed when actually dying, was the semi-gelatinous, foul-smelling condition of their intestines. This was clearly an ante-mortem condition and may be associated with the chronic diarrhoea from which most of these animals had suffered.

The only tolerant mice which survived without the slightest abnormality of their lymphoid tissues were *C3H* mice injected with *CBA* spleen cells, or *vice versa*. The implications of this finding will be discussed below (§§4·2 and 6·1).

Incompletely tolerant mice were examined soon after the breakdown of their test grafts; here lymphoid abnormalities were both less common and less severe than in fully tolerant animals. They usually took the form of barely perceptible discoloration and reduction in size of a few lymph nodes.

The results of the post-mortem examinations of eighty-eight mice of various donor/recipient strain combinations are summarized in table 6.

Histological examination of the spleens from the majority of the healthy-looking fully tolerant mice of different donor/recipient strain combinations revealed nothing strikingly abnormal. Usually the germinal centres of the lymphoid nodules tended to be rather ill-defined, and often had peripheral accumulations of lymphoid cells. The general trend was towards an increase in the number of lymphoid elements present. Even in spleens

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which were enlarged the histological structure did not deviate significantly from the normal. In a few animals the follicular structure of the lymphoid tissue seemed to have disappeared and in its stead there was a diffuse infiltration of the spleen, and occasionally even of its capsule, by lymphocytes or lymphocyte-like cells.

The kidneys and livers of these animals were usually normal, but occasionally prominent accumulations of lymphocytic cells were encountered in both organs. In the kidney these cells were located in perivascular and subcapsular positions; in the liver they occurred in the periportal spaces, and there were perivascular accumulations ranging from small groups to large masses which appeared to have infiltrated the surrounding hepatic parenchyma. Accumulations of lymphocytes in the liver sinusoids, with accompanying stasis, were also observed.

TABLE 6. INCIDENCE OF ABNORMALITIES OF THE LYMPH NODES AND SPLEENS OF HEALTHY-LOOKING MICE MADE TOLERANT BY INJECTION OF HOMOLOGOUS SPLEEN CELLS AT BIRTH

strain combination	no. examined	no. with involution of nodes	degree of involution	no. with abnormal-looking spleens
(*a*) highly tolerant animals				
CBA → *A*	31	29	moderate	0*
A → *CBA*	5	5	moderate	0
C3H → *A*	12	11	severe	11†
A → *C3H*	1	1	severe	0
C57 → *AU*	1	1	severe	0
A → *AU*	4	4	severe	4‡
CBA → *C3H*	12	0	none	0
C3H → *CBA*	5	0	none	0
(*b*) incompletely tolerant animals				
CBA → *A*	15	6	moderate or feeble	0
A → *CBA*	2	1	feeble	0

* Mean weights of spleens from fourteen animals taken at random = 112 mg (mean wt. of normal *A* strain spleen = 112 mg).
† Mean weight = 155 mg.
‡ Macroscopic evidence of splenomegaly but organs not weighed.
'Highly tolerant' animals were those whose grafts had lived for at least 50 days and were healthy at the time of post-mortem examination. 'Incompletely tolerant' animals were dissected at varying intervals after their test grafts had broken down.

Some of the spleens of animals which had died of acute runt disease within a week or two of injection showed hyperplasia of the germinal centres of the Malpighian corpuscles and of the reticulum cells of the sinuses, whereas in other spleens there had evidently been a total disappearance of normal follicular structure and a diffuse infiltration of the whole organ with lymphocytes.

The spleens of chronic runts usually showed varying degrees of atrophy of lymphoid elements, ranging from partial to complete loss of follicular structure and a diffuse irregular distribution of cells of the lymphoblast type. The vessels were frequently congested and fibrosis was present to a varying degree. The impression in some cases was that there had been a destruction of lymphoid follicles with a partial replacement of lymphoid cells, the distribution of which was diffuse.

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The livers of chronic runts were usually abnormal, the abnormalities ranging from heavy passive congestions (without evidence of cellular infiltration or necrosis) to varying degrees of necrosis of hepatic cells with or without accompanying lymphocytic infiltration. In extreme cases only a few cords of surviving hepatic cells were left in the perivascular position, the hepatic parenchyma having been replaced by an intensely eosinophilic, sparsely cellular fibrous material suggestive of an amyloid type of degeneration. Bile duct proliferation was common in such cases. The kidneys of these animals were usually normal.

It is unfortunate that neither the lymph nodes nor the bone marrow of these animals were examined histologically.

3·8. *The fate of the tolerance-conferring cells*

There is every reason to believe that, in fully tolerant animals, the neonatally injected homologous cells or their descendants persist indefinitely. The experiments now to be described were based on Mitchison's (1956*a*) design, with the object of determining the ultimate disposition of the foreign cells in their adult tolerant hosts. The method used is illustrated in figure 3. Various organs or tissues were removed from (for example) an adult *A* strain mouse which had been made tolerant by neonatal injection of *CBA* spleen cells.

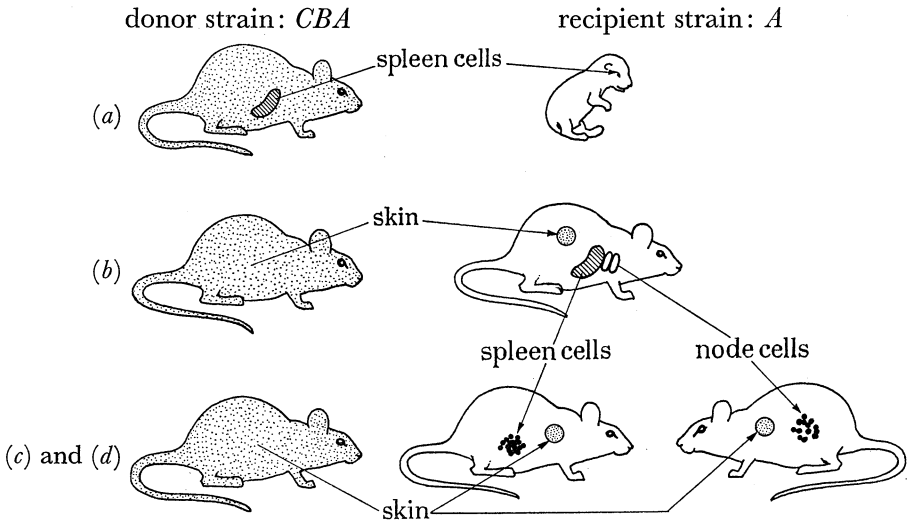

FIGURE 3. Illustrating induction of tolerance, and the test for persistence of donor strain cells in the organs of tolerant animals: (*a*) injection of newborn *A* strain mice with *CBA* spleen cells; (*b*) displaying tolerance by grafting with *CBA* skin; (*c*) removal of host organs, preparation of cell suspensions and their injection into normal *A* strain 'secondary hosts'; (*d*) grafting of the secondary hosts with *CBA* skin (see §3·8).

Cell suspensions were prepared from these tissues and injected intraperitoneally into normal adult *A* strain mice. Four or five days later these *secondary hosts* were tested with skin homografts from *CBA* donors. Accelerated rejection of the grafts indicated the prior sensitization of the secondary hosts with *CBA* antigens and so, by inference, the presence of *CBA* cells in the *A* strain tissues. Some indication of the sensitivity of this test may be derived from the fact that a dosage of less than 250000 *CBA* cells elicits an easily perceptible immune response when injected into normal *A* strain recipients (Billingham *et al.* 1957).

The various tests carried out with tolerant mice of different donor/recipient strain combinations are summarized in table 7. In some of the tests the organs or tissues were removed from single individuals and tested separately by inoculation into one or more secondary hosts. More often, however, organs of the same type were pooled from groups of animals with the same donor/recipient relationship and the resultant composite cell suspensions tested as described above.

TABLE 7. SUMMARY OF TESTS FOR CELLULAR CHIMERISM CARRIED OUT ON ORGANS AND TISSUES OF TOLERANT AND INCOMPLETELY TOLERANT MICE*

type of cell used to induce tolerance	organ or tissue tested for chimerism	no. of tolerant mice tested and strain combination	no. of secondary hosts
(*a*) fully tolerant mice			
spleen cells	spleen	$15 \times CBA \rightarrow A$	7
		$5 \times C3H \rightarrow A$	5
		$1 \times A \rightarrow CBA$	1
	axillary plus brachial nodes	$17 \times CBA \rightarrow A$	6
	thymus	$1 \times CBA \rightarrow A$	1
		$3 \times A \rightarrow AU$	1
		$5 \times C3H \rightarrow A$	2
	bone marrow	$4 \times A \rightarrow AU$	2
		$11 \times C3H \rightarrow A$	4
		$1 \times A \rightarrow CBA$	1
	blood	$4 \times A \rightarrow AU$	4
		$6 \times C3H \rightarrow A$	3
		$1 \times A \rightarrow CBA$	1
	kidneys	$6 \times C3H \rightarrow A$	4
	liver	$5 \times C3H \rightarrow A$	4
thymocytes	spleen	$2 \times CBA \rightarrow A$	3
	nodes	$2 \times CBA \rightarrow A$	1
	thymus	$2 \times CBA \rightarrow A$	1
	marrow	$2 \times CBA \rightarrow A$	1
	blood	$2 \times CBA \rightarrow A$	3
bone marrow	spleen	$2 \times CBA \rightarrow A$	4
	nodes	$2 \times CBA \rightarrow A$	4
(*b*) incompletely tolerant mice			
spleen cells	spleen	$6 \times CBA \rightarrow A$	6
	thymus	$1 \times CBA \rightarrow A$	1
	nodes	$5 \times CBA \rightarrow A$	2

* The fate of test grafts on the secondary hosts indicated chimerism in every tissue tested from each group of fully tolerant mice. No evidence of persistence of foreign cells in any incompletely tolerant mouse.

Every mouse in which tolerance had been induced by the neonatal injection of spleen cells, thymocytes or bone marrow proved to be a cellular chimera. The spleens, lymph nodes, thymuses, bone marrow, kidneys, livers and blood all contained a demonstrable population of foreign cells. Even the minimum quantities of cells obtained from the various organs tested—citrated blood, 0·5 ml.; thymus, whole organ; kidney, whole organ; marrow, two femurs; lymph nodes, combined axillary, brachial and inguinal; spleen, half an organ; liver, 100 mg—contained enough foreign cells to sensitize all secondary hosts. It may be added that 100 mg of liver probably contained relatively few foreign cells since the cells extracted from it elicited only a very low level of immunity.

None of the tissues removed from incompletely tolerant mice (after the destruction of their test grafts) gave evidence of the persistence of foreign cells.

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The possibility that sensitization in these experiments was 'adoptive' in origin (Mitchison 1953, 1954; Billingham, Brent & Medawar 1954), i.e. due to the transfer of sensitized host cells into the secondary hosts, may be dismissed on the following grounds: (1) the tissues were removed from *tolerant* mice, and could not therefore have been in a sensitized state; (2) the dosage of tissues used was too low to account for the high degree of sensitization; (3) tissues which cannot transfer adoptive immunity in mice (e.g. blood and kidney) were nevertheless capable of sensitizing secondary hosts; and (4) tissues from incompletely tolerant mice (i.e. mice which had succeeded in throwing off their skin grafts and which might therefore be *expected* to have been sensitized to a degree) did *not* sensitize the secondary hosts.

With the strain combination *CBA* → *A* it has been shown (see §3·2) that the majority of mice injected with spleen cells on the 4th day after birth manifested neither sensitization nor tolerance towards their subsequent test grafts. This raises the question of the fate of the injected cells. In an attempt to resolve this problem the members of four *A* strain litters (then 4 days old) were each injected intravenously with 10 million *CBA* spleen cells. When they were 40 days old, seventeen out of the twenty-eight surviving mice were challenged with *CBA* skin grafts; the remaining eleven were killed, and the cells from the combined nodes and the spleen of each mouse were injected into a single secondary *A* strain host to test for the persistence of foreign cells. Of the skin-grafted animals only two were tolerant, their grafts having survived for 17 and $>$ 82 days respectively; correspondingly, evidence of cellular chimerism was obtained in only three of the eleven mice tested. None of the animals which were test-grafted and found to be non-tolerant rejected its graft as if it had been previously sensitized. Although this experiment shows clearly that homologous cells introduced into mice during the 'neutral' period are eliminated, it does not provide evidence for the assumption that this elimination was brought about by a typical homograft response. Could it be that in the neutral period the maturing lymphoid tissues, though capable of giving a primary response to an antigen, are nevertheless still too immature to 'memorize' the response? An alternative and, on the face of it, more plausible explanation might be that the response by the young mice against the foreign cells was too weak and dilatory to be detected as much as 5 weeks later; yet the finding that mice injected on the 7th day after birth do reveal a feeble immunity when tested in the same manner is hardly in keeping with this interpretation.

4. The etiology of runt disease: an experimental analysis

On the basis of the evidence so far presented there could be two possible explanations of the disease (§§3·5, 3·7 and 3·8) caused by the inoculation of newborn mice with adult homologous spleen cells—(1) an infective theory: runt disease is caused by the introduction of pathogenic organisms present in or among the spleen cells of the donor strain; or (2) an immunological theory: runt disease is the consequence of an immunological reaction by the inoculated cells against host antigens, the foreign cells having survived because they had induced a state of tolerance in their hosts.

In the sections which follow these two theories will be considered in detail and experiments designed to discriminate between them will be described.

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4·1. *The infection theory*

This explanation of runt disease assumes the transfer into the newborn mice of a pathogen carried within or among the donor spleen cells—a pathogen to which the donor is adapted but which, because it induces immunological tolerance on its own behalf, runs riot in the tissues of the host.

TABLE 8. INOCULATION OF MICE WITH VARIOUS DERIVATIVES FROM ADULT DONORS OF HOMOLOGOUS STRAINS

strain combination	age of hosts	inoculum	route of injection	no. of mice injected	mortality (%)	comments
C57 → A	20 h	spleen cells*	i/v	26	100	all died of acute runt disease
	4 days	spleen cells	i/v	17	0	all healthy mice, none tolerant
	20 h	spleen cells	i/p	16	94	15 died of acute runt disease; survivor highly tolerant
	20 h	spleen cells	s/cut.	19	0	all healthy, normal mice; none tolerant
	20 h	0·07 ml. plasma	i/v	9	0	all healthy, normal mice; none tolerant
	20 h	0·06 ml. whole blood†	i/v	26	15	4 succumbed to acute runt disease; very slight retardation of growth in remainder; none tolerant
	20 h	1 million leucocytes‡	i/v	17	100	all died of acute runt disease
	20 h	thymocytes	i/v	17	59	10 died of acute runt disease; none of survivors tolerant
	20 h	bone marrow cells	i/v	18	5	2 chronic runts; 16/17 survivors tolerant, 11 highly tolerant
AU → A	20 h	spleen cells	i/v	25	100	all died of acute runt disease
	20 h	spleen cells	i/p	5	80	4 died of acute runt disease, survivor not tolerant
	20 h	spleen cells	s/cut.	4	0	all healthy mice
	20 h	bone marrow cells	i/v	16	0	9/16 weakly tolerant
C57 → CBA	20 h	spleen cells	i/v	16	100	all died of acute runt disease
	20 h	bone marrow cells	i/v	3	0	all tolerant; 2 highly tolerant
CBA^j → A^j	20 h	spleen cells	i/v	59	51	30 died of runt disease, all survivors tolerant
	20 h	heated spleen cells (48·5 °C)	i/v	12	0	none tolerant

* The dosage of spleen cells, thymocytes or marrow cells ranged from 5 million to 10 million.
† No. of leucocytes present in 0·06 ml. whole blood ranged from 0·25 million to 0·35 million.
‡ The leucocytes were in the form of buffy coat concentrates.

Direct attempts to transmit the hypothetical pathogen failed. Three A^j mice which had been injected with CBA^j cells and which were dying of the typical runt disease syndrome were killed, and cell suspensions prepared from their spleens and livers were injected into

the ten members of two newborn A^j litters. Since the cells were here injected into isologous hosts their survival need not be questioned on immunological grounds, but all mice remained in perfect health and were found to be normal in every respect when autopsied 3 months later. Furthermore, in the strain combination $C57 \rightarrow A$, in which all injected subjects succumb soon after injection, uninjected mice remained perfectly healthy although they were constantly exposed to the loose faeces of their supposedly infected litter-mates.

As heat treatment (48·5 °C for 20 min) is known to kill mammalian tissue cells, but is unlikely to inactivate most bacteria and viruses, the effect of inoculating heated adult CBA^j-spleen cells into newborn A^j mice was investigated. Whereas the injection of *viable* CBA^j cells caused a mortality of 51 % and complete tolerance in the survivors (see tables 4 and 8), none of the twelve recipients either died or suffered from lymphoid hypoplasia of any kind—and none had become tolerant of CBA^j skin grafts. This experiment suggests that tissue tolerance may be an essential pre-requisite for the occurrence of runt disease (see §6·3).

Further findings, this time obtained with the $C57 \rightarrow A$ strain combination, which tell against the infection theory are that (*a*) the injection of newborn hosts with lightly spun heparinized plasma was entirely innocuous, though blood leucocytes were always lethal (table 8; figure 10, plate 10), and (*b*) the *subcutaneous* injection of spleen cells (which are lethal when injected intravenously) failed to harm the recipients.

These results are difficult to reconcile with an infection theory; when considered in the light of data presented in §§4·2 to 4·6 it will become evident that this theory cannot be upheld.

4·2. *The theory of an immunological reaction by the injected cells against their hosts*

There is a strong *prima facie* case in favour of the theory that runt disease is brought about by an immunological reaction of the injected cells against the tissue antigens of their young hosts: (*a*) the injected cells are immunologically 'competent'; (*b*) they are known to induce tolerance and to persist long enough to do harm; (*c*) the organs overtly affected are those in which the cells have been shown to establish themselves (§3·8): and (*d*) the high degree of variability in the severity of the disease (table 4), which seems to reflect the antigenic disparity between donor and recipient strains (runt disease is not detectable in the most closely related combination, *CBA* and *C3H*).

According to this theory the induction of tolerance by the injected cells is the *sine qua non* of runt disease, for otherwise they would be eliminated before they had an opportunity to do harm. Runt disease should not occur if for any reason the cellular inoculum from an adult donor (*a*) represents an insufficient stimulus to confer tolerance, (*b*) consists of cells that are not of an immunologically competent type, and (*c*) consists of cells of an immunologically competent type but introduced either by a route or at a time which does not permit them to induce tolerance. The various experiments designed to test these predictions are summarized in table 8.

Adult *C57* spleen cells injected intravenously into 4-day-old *A* strain mice failed to harm any of them, nor did they confer tolerance. Moreover, the almost uniformly lethal outcome of injecting neonatal *A* strain hosts with adult *C57* spleen cells by the intraperitoneal route, and the complete harmlessness of these cells if inoculated sub-

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cutaneously, closely parallel the relative efficacies of these two different routes in conferring tolerance.

Of twenty-six newborn *A* strain hosts injected intravenously with citrated whole blood (containing 0·25 to 0·3 million leucocytes) from adult *C*57 donors, only four died from acute runt disease, but there was evidence that the growth of sixteen of the survivors was slightly retarded; yet none of them proved to be tolerant when they were test grafted. This finding is in complete accord with expectation: the leucocytes present in 0·06 ml. of blood do not represent an effective tolerance-conferring stimulus. The small proportion of mice which died (four out of twenty-six) is comparable with the small proportion of newborn *A* strain mice which became tolerant after their injection with *CBA* blood (see Billingham & Brent 1956). When leucocyte concentrates prepared from the buffy coat and containing not less than a million leucocytes were employed, acute runt disease was invariably the outcome (see figure 10, plate 10)—a finding which, on the basis of the present hypothesis, suggests that the minimal tolerance-conferring stimulus is less than 1 million cells (but see §6·3). Furthermore, if the graft-versus-host hypothesis is correct, this finding can be accepted as very strong evidence in favour of the concept that among the leucocytes of the blood of adult mice there is at least one cellular ingredient which is immunologically competent (see §6·1).

Since many authorities do not consider that the thymus is capable of immunological responses (see, for example, Harris & Harris 1956; MacLean, Zak, Varco & Good 1957), this suggested itself as a possible alternative source of cells which might confer tolerance without the complication of runt disease. Accordingly, seventeen baby *A* strain mice were injected intravenously with 8 to 10 million *C*57 thymocytes. Ten died of acute runt disease but the remainder grew up normally without showing the slightest symptom of the disease; none was tolerant. This finding suggests that the thymus must contain an immunologically competent cell type. The variability of the results is hard to account for, but it may be recalled that with the donor/recipient strain combination *CBA* → *A* the results were even more variable when thymocytes rather than spleen cells were used to confer tolerance (see §3·4).

Apart from the spleen, blood and thymus, the only other convenient source of cell suspensions is bone marrow. Accordingly, a number of newborn *A* strain litters was injected with bone marrow cells prepared from adult *C*57 donors, whose ages varied from 2 to 4 months. It will be remembered that in this combination the injection of spleen cells was invariably fatal (table 4). The results were exceedingly gratifying, for nearly all the mice survived, and tolerance had been conferred on 94% of them (table 8; figure 5, plate 10). But runt disease had not been *completely* circumvented, for two of the tolerant animals proved to be chronic runts and the remainder all displayed some retardation in development. Nevertheless, the great majority grew into apparently healthy adult mice. When autopsied, the lymph nodes in these mice were found to be highly abnormal: some were barely recognizable, and others were absent altogether. Evidently bone marrow does contain immunologically competent cells, but these appear to be small in number compared with those of the spleen.

Encouraged by these results, we used marrow cells in an attempt to circumvent acute runt disease in two other 'lethal' strain combinations: *C*57 → *CBA* and *AU* → *A*.

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With the first combination only three mice were injected, but all survived and were found to be tolerant; again autopsy revealed a moderate degree of lymphoid hypoplasia. With the *AU* → *A* combination the sixteen mice grew up normally, but only nine gave evidence of being feebly tolerant, and of these only four allowed their test grafts to survive for more than 16 days (table 8). All lymph nodes appeared to be normal when these mice were dissected after the destruction of the grafts. In view of the fact that *AU spleen* cells always killed their *A* strain hosts, the failure of bone marrow cells to induce tolerance suggests that, in this donor/recipient strain combination, full tolerance may not be a necessary pre-requisite for acute runt disease (see §6·3).

4·3. *Inoculation of newborn* A *strain mice with spleen cells from hybrid donors*

According to the 'graft-versus-host' concept, inoculation of new-born *A* strain mice with spleen cells from either *C57/A* or *AU/A* F_1 *hybrid* donors should not cause runt disease because *A* strain antigens are represented in the inoculated cells (which cannot therefore

TABLE 9. INOCULATION OF NEWBORN *A* STRAIN MICE WITH 8 TO 10 MILLION ADULT HYBRID SPLEEN CELLS

strain combination	no. of mice injected	mortality before operation	type of test graft	no. grafted	no. tolerant	survival times of tolerated grafts
C57/A → *A*	15	0	*C57*	15	12*	2 × 17, > 18, > 21, 30, > 31, 33, 25, 4 × > 50
AU/A → *A*	23	0	*AU*	23	8	14, 2 × 20, 23, 30, 2 × 40, 45
AU/CBA → *A*	17	8	(1) *AU*	9	8	20, 2 × 45, 3 × 50, 70, 114
			(2) *CBA*†	9	9	14, 20, 25, 43, 4 × > 91, > 186
C57/CBA → *A*	8	4	(1) *C57/CBA*	4	2	13, 16
			(2) *CBA*‡	3	3	35, 45, 60
C57/CBA → *A*	13	7	*C57/CBA*	6§	4	> 15 but > 20

* Three animals died suddenly 18, 21 and 31 days after grafting: see text.
† Interval between challenging with *AU* skin and *CBA* skin homografts—54 days.
‡ Interval between challenging with *C57/CBA* skin and *CBA* skin homografts—42 days.
§ Two mice did not survive test-grafting (see text).

react against them), but should induce tolerance because the transplantation antigens of the *C57* or *AU* strains are also represented. The experiments described below thus take advantage of the well-known fact that whereas hybrids will not react against parental grafts, mice of the parental strains will do so against hybrid grafts. This principle has also been successfully exploited by Cock & Simonsen (1958) in an analysis of the phenomenon of the splenic enlargement and haemolytic anaemia which occurs after the injection of newly hatched chicks with homologous adult blood.

The results of inoculating newborn *A* strain mice with spleen cells of one or other of the two hybrid types are set out in table 9, rows 1 and 2. In neither case did any of the animals suffer from acute runt disease, and some degree of tolerance was conferred upon some of them. Our results are therefore strikingly similar to those obtained by Cock & Simonsen (1958). Again, it is not easy to account for the low incidence and degree of tolerance conferred in respect of the test grafts from *AU* donors (*AU* cells injected into newborn *A* strain

mice had always proved to be lethal (table 4)), unless it is assumed that tolerance need not be an essential pre-requisite for the occurrence of acute runt disease (see §6·3). More satisfactory in this respect were the experiments involving the injection of *C57/A* hybrid cells: here a very high proportion (80%) of hosts became tolerant of *C57* skin grafts.

An unexpected discovery was made when three of the mice which had received *C57/A* hybrid cells suddenly died—18, 21 and 31 days after skin grafting. Post-mortem examination revealed gross splenomegaly, the organs weighing 886, 536 and 755 mg respectively, and nearly all had conspicuously hypertrophied lymph nodes. Similar though smaller changes were observed in the two highly tolerant animals which were killed 50 days after test-grafting (spleen weights 150 and 293 mg). Because the condition of these spleens at least superficially resembled those of mice suffering from leukemia, cell suspensions were prepared from one of them for inoculation into four normal young *C57/A* hybrid hosts. This did not harm the recipients in any way and did not bring about an enlargement of their spleens or lymph nodes.

When examined histologically it was found that the follicles in most of the enlarged spleens were well differentiated: they showed varying degrees of lymphocytic hyperplasia, and in some of them there were accumulations of lymphocytes in the cords as well as in the primary follicles (see §6·2).

The spleens of all mice injected with *AU/A* hybrid cells appeared to be normal.

It is to be expected that, so far as their response to homologous tissue antigens is concerned, the immunological repertoire of *hybrid* spleen cells is rather more limited than that of spleen cells from either of the parental strains; in other words, the greater the number of antigens represented within a lymphoid cell, the smaller will be its range of response to the tissue antigens of other strains. It was therefore decided to investigate the effect of injecting *AU/CBA* or *C57/CBA* hybrid spleen cells into newborn *A* strain hosts. (It should be borne in mind that spleen cells from the parental strains *AU* and *C57* had always been lethal—see table 4.) This experimental design differed from that described above in that *both* parent strains of the F_1 hybrid spleen donors were homologous to the *A* strain recipients.

Expectation was amply fulfilled: only eight out of seventeen mice injected with *AU/CBA* hybrid cells died, and eight of the nine survivors were found to be at least partially tolerant of *AU* skin homografts (table 9, row 3). Fifty-four days later, by which time all but two of the *AU* grafts had been destroyed, all animals were tested with skin grafts from *CBA* donors. Unexpectedly, all animals displayed some degree of tolerance of these grafts; five out of nine were highly tolerant, and in *all* of them the *CBA* grafts were viable and healthy long after the rejection of the *AU* grafts. The likelihood of hybrid spleen cells surviving in the face of an immunological reaction strong enough to overcome *AU* skin seems rather remote. This puzzling finding will be discussed below in the light of further experimental evidence.

The inoculation of *C57/CBA* hybrid spleen cells gave very similar results, only four out of eight mice dying of acute runt disease (figure 7, plate 10). This time the survivors were first tested with *hybrid* (i.e. *C57/CBA*) skin grafts; two gave evidence of being very feebly tolerant, the grafts surviving for 13 and 16 days respectively. Forty-two days later the four mice were tested with *CBA* skin grafts: one animal unfortunately died several days

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later, but the remaining three proved to be moderately tolerant of their *CBA* grafts (table 9, row 4). Autopsy revealed that these mice suffered from characteristic involutions of their lymph nodes.

The spleen of the animal that died was made the subject of a test for cellular chimerism. Equal portions of a cell suspension prepared from it were injected intraperitoneally into two normal adult *A* strain mice, which were grafted with *CBA* skin 5 days later; both rejected their grafts as if the cell suspensions had sensitized them against *CBA* antigens. This surprising result seemed to suggest that cells carrying *CBA* antigens were present in the spleen of an *A* strain mouse which had already reacted against *C*57/*CBA* hybrid cells. Further evidence was clearly required on this point.

Two more *A* litters were therefore injected with *C*57/*CBA* hybrid spleen cells: seven out of thirteen died of acute runt disease, two did not survive the skin grafting operation, and the remaining four were found to be weakly tolerant of hybrid skin grafts (table 9, row 5). The lymph nodes of the two mice which died displayed the usual lymphoid hypoplasia of runt disease, but their spleens were considerably enlarged (weights 248 and 240 mg respectively). A test for cellular chimerism conducted on one of these spleens, and involving six adult *A* secondary hosts, revealed that it contained cells carrying both *C*57 and *CBA* antigens.

The four survivors of this experiment were autopsied about 100 days after the rejection of their *C*57/*CBA* hybrid test grafts; they also had typical lymph node deficiencies and moderately enlarged spleens (weights 205, 200, 200, and 150 mg respectively). Each spleen was separately tested for the presence of *CBA* antigens as follows: the cells obtained from the whole organ were suspended in 5 ml. Ringer-phosphate, and 1 ml. of the resultant suspension was injected intraperitoneally into three adult *A* mice. Each secondary host therefore received the cells obtainable from about one-fifth spleen. The secondary hosts were test-grafted with *CBA* skin 4 days later. When biopsied 6 days after transplantation, these grafts were found to have the typical appearance of grafts transplanted to strongly pre-immunized animals, i.e. they were poorly vascularized and their epithelial components were totally destroyed. Again it was impossible to escape the unorthodox conclusion that the spleens of these mice contained cells in which *CBA* antigens were represented—despite the fact that the mice had previously reacted against and totally rejected *C*57/*CBA* skin grafts.

Reasons have already been advanced (see §3·8) for believing that the results from chimera tests of this kind cannot be explained in terms of an 'adoptive' transfer of immunity by the host cells. The fact that in this last experiment the chimera tests were carried out as many as 100 days after the destruction of the hybrid grafts adds further to the weight of evidence against such an interpretation (cf. §3·8 on 'incompletely' tolerant mice; also Billingham, Brent & Medawar (1954) on the duration of 'adoptive' immunity).

Two possible interpretations will be considered. The first (suggested to us by Professor P. B. Medawar) is as follows. Hybrid cells, carrying on their surfaces *C*57 and *CBA* antigens, are introduced into newborn mice. Provided that there is some variability among the cells in the extent to which the two sets of antigens are represented (such variability could be either mutational or purely phenotypic), a weak but chronic selection pressure against *C*57 antigens by the *A* strain hosts might result in the elimination of cells

which were predominantly *C57*-like and in the survival and propagation of cells carrying mainly *CBA* antigens on their surfaces. The very low-grade immune responses associated with incomplete tolerance would be ideal for such selective purposes. That a selection pressure against *C57* antigens might indeed exist is hinted at by the fact that the *C57* strain appears to be less closely related than the *CBA* strain to the *A* strain, as shown by (*a*) the shorter survival times of *C57* skin grafts in *A* mice (table 1), (*b*) the ease with which tolerance can be established by *CBA*, as opposed to *C57* antigens, in *A* strain mice (tables 2 and 9, §4·4), and (*c*) the higher incidence of runt disease caused by *C57* cells in *A* hosts (table 4). The hybrid skin grafts would therefore be destroyed because of the incomplete tolerance extended to their *C57* component, without seriously impairing the fuller tolerance established by the *CBA* antigens. Thus the more completely *CBA*-like hybrid cells would persist in the tissues of the hosts. The work of Klein & Klein (1956), Mitchison (1956*b*) and Koprowski, Theis & Love (1956) suggest the existence of a selection phenomenon of this kind, and it has also been invoked to account for the apparent loss of antigenic specificity in transplanted tumours (Gorer 1948; Hauschka 1952; see also Barrett & Deringer 1950, 1952).

A second interpretation of the experimental data given above is that *host* cells may have undergone an antigenic transformation and acquired *CBA*-specific antigens. Both theories will need to be subjected to experimental analysis.

4·4. *Inoculation of newborn* A *strain mice with mixed spleen cells from two different donor strains*

If the 'graft-versus-host' hypothesis is correct one would expect that if, for example, newborn *A* mice were to receive an inoculum of adult *A* strain spleen cells in addition to the normally lethal inoculum of adult *C57* cells, the recipients might be protected against runt disease. The adult isologous cells should provide them with functional machinery with which to eliminate the foreign cells.

Accordingly, fifteen baby *A* strain mice were injected intravenously with a dosage of 10 million adult spleen cells comprising approximately equal numbers of *C57* and *A* cells. None of the mice died (table 10). However, growth was retarded in the majority and

TABLE 10. INTRAVENOUS INJECTION OF *A* STRAIN MICE WITHIN 20 H OF BIRTH WITH MIXTURES OF ADULT SPLEEN CELLS*

ypes of cell injected	no. of mice injected	mortality through runt disease	type of test-graft	no. grafted	no. tolerant	no. highly tolerant	comments
C57+A	15	0	*C57*	4	0	0	evidence of weak sensitization to test-grafts
C57+CBA	22	15	(i) *C57*	7	0	0	evidence of weak sensitization
			(ii) *CBA*	7	7	7	—
AU+CBA	25	6	(i) *AU*	19	0	0	—
			(ii) *CBA*	19	18	17	—

*Cell dosage per recipient 10 million to 12 million, comprising approximately equal numbers of the two types of cells.

autopsies, carried out when the mice were 80 days old and apparently normal, showed varying and usually moderate degrees of involution of lymph nodes in nine out of fifteen.

Sixty days after their birth four of these mice had been challenged with *C57* skin, and the grafts had been removed for histological appraisal on the 6th post-operative day. This test indicated that, at the time of grafting, the hosts must already have been weakly sensitized against *C57* tissue. The existence of this immunity provides direct evidence for the supposition that protection had been achieved by the destruction of the *C57* cells through the agency of the concomitantly injected adult *A* cells. The abnormalities of the animals' lymph nodes suggest that (*a*) the *C57* cells had caused some tissue damage before they were finally eliminated; and (*b*) regeneration of the damaged organs had not been complete.

We have also studied the situation in which newborn recipients of one strain were injected with cells from two other foreign strains. As table 10 shows, the addition of *CBA* spleen cells to a potentially lethal inoculum of *C57* cells protected about 30 % of *A* strain mice from runt disease. None of the survivors was tolerant of *C57* skin; indeed their reaction towards these test grafts indicated a state of weak sensitization. Yet all proved to be highly tolerant of *CBA* test grafts. When the inoculum consisted of a mixture of *AU* and *CBA* spleen cells the mortality through runt disease was only 24 %. Tolerance of *AU* skin was conferred upon none of the survivors, but again nearly all the survivors were highly tolerant of *CBA* skin. Autopsy revealed that the nodes of most of these animals were deficient in size or number, and there was a history of retardation of growth.

It may therefore be assumed that, in the animals which survived, the *C57* or *AU* cells were destroyed by the *CBA* cells some time after their introduction into the hosts. The fact that the hosts were found to be weakly immune to *C57* or *AU* skin grafts may be attributed to the continued survival of *CBA* cells or their descendants. In the animals which died of acute runt disease the antagonistic cell type may be presumed to have won the upper hand over the *CBA* cells, which were therefore unable to provide protection. That *CBA* cells might have a reasonably good chance of survival at the expense of *C57* or *AU* cells probably follows from their closer antigenic relationship with the tissues of the hosts (see above).

These experiments demonstrate clearly that it may not be easy to render mice tolerant of two or more donor strain tissues at one and the same time.

4·5. *Injection of newborn mice with spleen cells from specifically sensitized donors*

It should follow from the immunological interpretation of runt disease that, if newborn mice were to be inoculated with spleen cell suspensions from donors specifically sensitized against the tissues of their future hosts, then there should be an increase in the severity of the disease. This prediction has been confirmed. It has already been noted (table 4) that if newborn A^j mice were injected with adult CBA^j spleen cells, 51 % succumbed to acute runt disease. A^j mice injected with cells obtained from CBA^j donors which had previously been sensitized to A^j cells showed a much greater mortality (19/20 = 95 %), and death usually occurred somewhat more quickly. The results of this experiment and those of experiments making use of hybrid donors may be considered to constitute, on their own account, proof of the hypothesis that runt disease is brought about by an immunological reaction of the injected cells against the tissue antigens of their hosts.

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4·6. *Inoculation of spleen cells from one parental strain into* F_1 *hybrids*

Apart from injecting newborn mice with adult homologous lymphoid tissue cells there is, as Medawar (1954) has hinted, another way of producing an immunogenetic situation which should enable immunologically competent cells to react against their hosts, but which does not depend upon the induction of tolerance: the injection of F_1 hybrid animals with spleen cells from one or other of the parental strains (cf. §4·3).

TABLE 11. INJECTION OF ADULT HOMOLOGOUS SPLEEN CELLS INTO F_1 HYBRIDS OF DIFFERENT AGES

strain combination	age of mice at injection	no. of cells injected (millions)	no. of mice	no. of early deaths*	no. of late deaths	comments
C57 → C57/A	20 h	10	uninjected: 2	0	0	
			injected: 7	7	—	all died of acute runt disease
	12 days	60	uninjected: 16	0	0	mean wt.† 16 g at 40 days; 20 g at 60 days
			injected: 17	1	4	mean wt. 11 g at 40 days; 17 g at 60 days
	23 days	120	uninjected: 6	0	0	
			injected: 10	0	0	no difference between the injected animals and their controls
AU → AU/A	20 h	8	injected: 6	0	1	all mice suffered from runt disease from 16th day but only 1 died, on 57th day

* Early deaths are those which occurred within the first 3 weeks of life.
† Both sexes almost equally represented in the two groups.

To investigate this possibility a series of *C57/A* hybrid mice of different ages was injected with relatively high dosages of *C57* spleen cells. Some of the mice in each litter were left uninjected as controls (see table 11). As expected, all mice injected soon after birth died from acute runt disease (cf. Cock & Simonsen 1958). All of the seventeen mice injected when 12 days old were retarded in growth as compared with their litter-mate controls; five of these died and were found to have suffered from extreme hypoplasia of their lymph nodes. As the survivors grew older the disparity between their weights and those of the controls declined slowly, but even after 5 months they were perceptibly lighter. Autopsies revealed extreme involution of lymph nodes in the injected animals.

When 23-day-old hybrid mice were injected intraperitoneally with a dosage of 120 million spleen cells, none differed significantly from its control either in weight or appearance.

These findings suggest that, with this particular strain combination, the susceptibility of mice to incompatibility reactions directed against them declines as their age at inoculation increases (see also §4·7). However, the recent experiments of Cole & Ellis (1958) and Trentin (1958) show that, with some inbred strains, even adult F_1 hybrid mice retain their susceptibility to splenic cells of parental origin.

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When six newborn *AU/A* hybrid mice were injected intravenously with *AU* adult spleen cells, symptoms of runt disease were apparent by the 16th day, but all except one of the mice eventually recovered. This result compares rather strangely with the uniformly lethal outcome of inoculating newborn *A* mice with *AU* spleen cells (see table 4). Perhaps it may be taken as a hint that hybrid tissues are somewhat less vulnerable to incompatibility reactions than those of the parental strains.

4·7. *Attempts to cause runt disease in adult tolerant mice with donor strain spleen cells*

An alternative method of testing the susceptibility of *adult* mice to immunological intervention by homologous cells is the injection of large numbers of foreign spleen cells into adult mice which have previously been rendered tolerant of them (cf. §4·6).

Sixteen adult mice fully tolerant of donor strain skin grafts were injected with spleen cells (200 million to 600 million) obtained from animals of the donor strain (table 12). This increase in the number of cells capable of reacting against host antigens did not result in the sickness or death of a single mouse. The six *CBA* mice tolerant of *A* skin grafts, and the six *A* mice tolerant of *CBA* skin grafts, all showed normal increases in weight over observation periods of up to 269 days. Autopsies on two of the mice (*CBA* → *A*) revealed that lymphoid hypoplasia was not significantly different from that encountered in other tolerant mice of the same strain combination which had not been given a supplementary spleen inoculum. Chimera tests of the kind described in §3·8 showed beyond doubt that the spleens of both mice, and the lymph nodes of one, contained *CBA* as well as *A* cells.

TABLE 12. ATTEMPT TO CAUSE RUNT DISEASE IN ADULT TOLERANT MICE BY INOCULATION WITH LARGE DOSAGES OF SPLEEN CELLS FROM DONOR STRAIN

subjects	inoculum per mouse	route of administration	observation period	comments
4 *CBA* mice tolerant of *A* skin grafts	400 million *A* spleen cells	i/v, i/p, s/c	130 days	grafts and hosts unaffected
2 *CBA* mice tolerant of *A* skin grafts	400 million *A* spleen cells*	i/p	220 days	grafts and hosts unaffected
6 *A* strain mice tolerant of *CBA* skin grafts	250 million *CBA* spleen cells	i/v, i/p, s/c	122 days (4 mice) 269 days (2 mice)	5 grafts and hosts unaffected; 1 graft broken down by 76th day
4 *A* strain mice tolerant of *C57* skin grafts†	600 million *C57* spleen cells	i/v, i/p	240 days	grafts and hosts unaffected

* The donors of these spleen cells were adult *A* strain mice which were fully tolerant of *CBA* tissue following their inoculation at birth with *CBA* spleen cells.

† Tolerance had been conferred with *C57* bone marrow cells.

In the case of the spleen tests it might be argued that the powerful immunization of the *CBA* secondary hosts to *A* skin grafts was due to the presence of host blood leucocytes among the spleen cells; it is however unlikely that a similar objection could hold in the case of lymph nodes, in which the contamination with host blood cells must have been relatively slight.

The four *A* mice tolerant of *C57* skin grafts (as a result of neonatal bone marrow injections) are of special interest. Again the injection of a large number of donor strain

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spleen cells failed to kill the animals—despite the fact that *C57* spleen cells injected into *newborn A* mice had always been lethal (table 4). The question of the hosts' age as a factor in susceptibility to runt disease is discussed further in §6·2.

5. Some further studies on the phenomenon of tolerance of homologous tissue cells

The considerable number of highly tolerant animals produced during the course of the work already described provided the material for further experiments.

5·1. *The specificity of tolerance*

In a previous study (Billingham *et al.* 1956*a*) it was shown that *CBA* mice fully tolerant of *A* strain grafts were capable of reacting with normal vigour against skin homografts from a third unrelated strain (*AU*). This work has now been extended to three strains which are all fairly closely related as judged by homograft survival times, i.e. *CBA*, *C3H* and *A*, the former two strains being more closely related to one another than to the *A* strain. The principle of the experiments was simply to test mice of one of these strains, say *A*, which had been made tolerant of skin grafts from a second strain, say *CBA*, for their capacity to react against homografts from the third strain (*C3H*). The results (table 13) show clearly that although tolerance is strain specific, a tolerant mouse can react with *normal* vigour against skin homografts from a second donor strain only if the two donor strains are distantly related. The extent to which a state of tolerance of grafts from one donor strain results in an impairment of a tolerant mouse's ability to reject skin homografts from a second donor strain may be regarded as a measure of the extent to which the two donor strains have important antigens in common.

Table 13. Experiments on specificity of tolerance

strain of tolerant hosts	strain in respect of which tolerant	no. of tolerant mice grafted	strain of second donor	survival times of grafts from second donors	approximate m.s.t. (days)
A	*C3H*	6	*CBA*	13, 17, 23, 25, 30, > 50	24
A	*CBA*	8	*C3H*	14, 16, 20, 20, 23, 24, 2 × > 50	22
C3H	*CBA*	9	*A*	9 × < 12 days	< 12
C3H	*A*	1	*CBA*	25	
CBA	*C3H*	5	*A*	5 × < 12 days	< 12
CBA	*A*	2	*C3H*	30, 45	~37
CBA	*A*	2	*AU*	9, 12 days	~12
A	*CBA*	3	*AU*	3 × 9 days	~ 9

From the findings set out in table 13 (see also table 1) the following conclusions may be drawn concerning the antigenic relationship between strains *A*, *CBA* and *C3H*:

(1) Strain *A* has important antigens which it does not share with the other two strains (see lines 3 and 5 of the table).

(2) Strains *C3H* and *CBA* probably have many important antigens in common (see lines 1 and 2 of the table).

(3) Strain *A* possesses at least some antigens by which strains *CBA* and *C3H* differ from one another (see the very limited data set out in lines 4 and 6).

Since the nodes of nearly all *A* strain mice made tolerant of *CBA* or *C3H* tissues by inoculation with homologous spleen cells at birth show pathological changes, the question arises whether some of the prolongations of survival of test grafts from a second donor strain might not be attributable to an impairment of function of the host's lymphoid tissues. This question is of some importance since it has already been shown that *CBA* mice made tolerant by neonatal inoculation in respect of *C3H* cells, and *C3H* mice likewise made tolerant in respect of *CBA* cells, show no involutionary changes in their nodes and react with normal vigour against *A* strain homografts.

Evidence which is hard to reconcile with this possibility is present in lines 7 and 8 of table 13. It shows that *CBA* mice tolerant of *A* strain tissue and *A* strain mice tolerant of *CBA* tissue both reacted with normal vigour against grafts from *AU* donors; yet these mice manifested the usual involutions of their lymph nodes when subsequently examined. To what extent the foreign cells which are present in the lymphoid tissue of tolerant mice play an active immunological role when the hosts are grafted with skin from a third strain has yet to be determined.

5·2. *The abolition of tolerance with isologous thymocytes or spleen cells*

Previous investigations (Billingham *et al.* 1956*a*) have shown that tolerance can be abolished in either of two ways: (*a*) rapidly, by inoculating tolerant animals with isologous lymph node cells from donors which had been specifically sensitized against the antigens of the original donor strain, or (*b*) slowly, by inoculation with isologous lymph node cells from normal donors.

TABLE 14. ABOLITION OF TOLERANCE BY MEANS OF THYMOCYTES AND SPLEEN CELLS

donor cells	dosage per host	tolerant hosts†	fate of hosts' grafts after inoculation
CBA strain thymocytes from normal donors	$2\frac{1}{2}$ organ equivalents (400 million cells)	2 *CBA* mice tolerant of *A* tissue	completely unaffected, survived +170 days
A strain thymocytes from sensitized* donors	800 million cells	3 *A* mice tolerant of *CBA* tissue	all three broke down within 16 days
A strain spleen cells from sensitized* donors	400 million cells	4 *A* mice tolerant of *CBA* tissue	breakdown of all 4 grafts within 12 days

* Sensitization of donors brought about by grafting with *CBA* skin.
† All hosts were fully tolerant of donor strain skin grafts.

Experiments have now been carried out to determine whether tolerance can be abrogated by inoculation of tolerant mice with isologous thymocytes or spleen cells. As the results set out in table 14 show, tolerance is abolished rapidly by the inoculation of tolerant animals with 400 million to 800 million isologous spleen cells or thymocytes from sensitized donors. It is also apparent that immune spleen cells are rather the more effective in transferring immunity adoptively. There is some suggestion that normal isologous thymocytes are ineffective in abolishing tolerance.

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6. Discussion and conclusions

6·1. *Induction of tolerance in mice*

It is evident from tables 2 to 4 that mice from a wide variety of isogenic strains can be made highly tolerant of skin homografts, provided: (*a*) that the mice are injected with homologous tissue cells immediately or soon after birth; (*b*) that the tolerance-conferring cells are introduced by a systemic route; and (*c*) that, with some strain combinations, appropriate steps are taken to avoid chronic sickness or death attributable to reactions of the inoculated cells against their hosts. These steps include the use of (1) bone marrow or immunologically non-reactive cells in place of spleen cells, (2) foetal in place of adult cells (see below), or (3) cells from an F_1 hybrid donor, one parent of which is a member of the recipient strain (§4·3).

Leaving the problem of deaths among the recipients aside for the moment, it is clear that there is considerable variability among different donor/recipient strain combinations in the proportion of mice rendered tolerant by neonatal injection, a point which has also been noted by other workers (Martinez, Smith, Aust & Good 1958). The reasons for this variability must include differences in the immunological status of newborn mice of various strains, and differences in the degree of genetic dissimilarity (particularly at the *H*-2 locus—see Snell, 1953, 1957; Gorer, 1956) between donor and recipient strains. To illustrate the second (and almost certainly more important) point: the induction of tolerance with donor and recipient strains (*CBA* and *C*3*H*) known to be closely similar at the *H*-2 locus and to react rather feebly towards each other's skin grafts (table 1) was accomplished with ease in all mice injected (table 4). On the other hand, only 39 % of *CBA* mice injected with cells from the *A* strain (which is known to *differ* from the *CBA* strain at the *H*-2 locus, and the grafts of which are rejected more quickly) proved to be highly tolerant. Any general statement concerning the immunological responsiveness of newborn animals of any one species to homologous tissue antigens may therefore be misleading unless the degree of genetic affinity between donor and recipient is taken into account. It may well be, for example, that with rat strains other than those used by Woodruff & Simpson (1955) the tolerance-responsive period does not extend into the second week of post-natal life. In the rabbit it has been our experience that, if donors and recipients are deliberately chosen to be dissimilar, intravenous injection of homologous cells soon after birth will elicit a very low-grade tolerance, and in only 20 % of the injected animals—a result which is rather surprising in view of the fact that the neonatal rabbit can be made tolerant of heterologous serum proteins (Hanan & Oyama 1954; Dixon & Maurer 1955; Cinader & Dubert 1955; Cinader & Pearce 1958).

Acquired tolerance had previously been shown to be strain specific (Billingham *et al.* 1956*a*). Our present rather more extensive data (§6·1) emphasize that this is true only when the two donor strains (i.e. the tolerance-conferring strain and the strain used in the specificity test) are *distantly* related to each other; clearly, a mouse does not become tolerant of antigens to which it has never been exposed in early life. When important antigens are shared by the two donor strains, then tolerance induced by one strain will be extended to skin grafts of the second strain to a degree which reflects the extent of the antigenic overlap. It is possible that the work of Terasaki, Cannon & Longmire (1958) on the specificity of tolerance in chickens is open to a similar interpretation.

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The question of whether a lasting tolerance of homologous tissues depends upon the persistence in the hosts of donor strain cells must for the time being remain unanswered. There can now be no doubt at all that the systemic inoculation of newborn mice with homologous cell suspensions leads to the formation of cellular chimeras, the injected cells surviving and almost certainly proliferating in many host organs (§3·8; Billingham & Brent 1957; see also Martinez, Smith, Aust, Mariani & Good 1958). But it would be premature to conclude that because donor strain cells are present in the tolerant animal the state of tolerance (once induced) could not continue without them. It is true that all attempts to elicit tissue tolerance with non-living preparations which are antigenically active when injected into *adult* mice (Billingham, Brent & Medawar 1956*b*, 1959) have so far failed (Billingham, Brent & Medawar, unpublished). However, the relative weakness of these cell-free antigens, and the physical difficulty of administering them in large amounts and over the length of time required to induce tolerance, suggest caution in the interpretation of these experiments.

6·2. *The causes of runt disease*

It has been shown that, depending on the donor/recipient strain combination, the intravenous injection of homologous adult spleen cells into newborn mice results in the sickness and death of many animals (§3·5). Among the symptoms of this 'runt disease' are greatly retarded growth and development, diarrhoea, varying degrees of hypoplasia of the lymph nodes, and abnormalities in other organs (§3·7). Two theories concerning the cause of runt disease have been subjected to critical experimental tests: (*a*) an infection theory, which envisages the introduction into the newborn hosts of a pathogen present in or among the injected cells, and (*b*) an immunological theory, which attributes runt disease to an immunological response by the adult homologous spleen cells against tissue antigens of the hosts.

The weight of evidence is conclusively in favour of the second interpretation. No experiment designed to test for the presence of an infective organism with the potentiality of causing runt disease has given an affirmative answer (§4·1). On the other hand, the experiments making use of F_1 hybrid cells (§4·3) and F_1 hybrid recipients (§4·6), and those entailing the injection of sensitized cells (§4·5), all show convincingly that the ultimate cause of runt disease must have been an immunological response by the injected cells. Once having induced tolerance in their hosts (§§3·1 to 3·5) they survive, and almost certainly proliferate, in many host organs such as the spleen, lymph nodes, liver and bone marrow, and it is no accident that it is in precisely these organs that the most obvious pathological changes have been observed (§3·7): a local reaction by the foreign cells would indeed be expected to manifest itself in the destruction of nearby tissues.

It is probable that the response of the injected cells against the host takes the form of an orthodox (i.e. cell-mediated) homograft reaction, but the possibility that circulating antibodies also play some role cannot at present be ruled out. The extensive studies of Simonsen (1957) on chicks, and some very limited observations by Billingham & Hildemann (unpublished) on tolerant mice, certainly indicate that homologous cells can form circulating antibodies directed against host cells. Such antibodies might therefore contribute to the general debilitation of the host by causing anaemia.

Although there was a suggestion that most runts were anaemic and suffered from lesions of the liver, this particular aspect of the pathology of runt disease has unfortunately not been studied in sufficient detail to reveal the *immediate* cause of death. The extreme hypoplasia of the lymphoid tissues may well have weakened the immunological defences of the hosts, possibly permitting micro-organisms such as those of the digestive tract to get out of hand.

The data presented in table 4 and elsewhere leave no doubt that the susceptibility of newborn mice to runt disease is largely genetically determined, donor/recipient histocompatibility differences determined by alleles at the *H*-2 locus playing an important part. When antigenic differences between donor and recipient strains were relatively small (as with *CBA* and *C3H* strains), the incidence and severity of the disease were negligible (§3·5). The age of the animals at the time of injection is another factor which may have to be taken into account, for experiments making use of F_1 hybrid recipients showed that, at least in the strain combinations used by us, there was a fall-off in the severity of the disease as the age of the recipients at the time of injection was increased (§§4·6 and 4·7). However, this has been shown not to be true for other strain combinations (Cole & Ellis, 1958; Trentin 1958; also Schwartz, Upton & Congdon 1957).

It is clear, then, that runt disease should be caused only by immunologically reactive cells. With a donor/recipient strain combination such as $C57 \rightarrow A$, in which the injection of spleen cells is always fatal, the ability of cells of different histological types to cause runt disease therefore represents a diagnostic test of their immunological competence. The fact that the injection of bone marrow cells will bring about only a very mild form of runt disease, without mortality, suggests that the crucial cell type is poorly represented in this tissue. On the other hand, the test has fully established the immunological competence, in mice, of at least one type of cell present in the thymus (see also §5·2) and among the blood leucocytes (§4·2). Using splenomegaly as the criterion of immunological reactivity of homologous cells against their embryonic hosts, Simonsen (1957) has demonstrated conclusively that, at least in chickens, the culprit among the blood cells must be capable of rapid multiplication (he was able to passage the agent causing splenic enlargement in nine successive embryonic hosts, using 0·05 ml. blood for each transfer). This would appear to rule out the small lymphocyte which, although it has now been shown to have a much longer life than had been originally supposed (Gowans 1957) and to account for 60 to 80 % of all white cells in the peripheral blood of the mouse (Dunn 1954), is not thought to be capable of mitosis. On the other hand, the work of Terasaki (1959) has shown that the capacity to cause splenomegaly in chick embryos resides entirely in the lymphocyte fraction of adult blood. The sum total of evidence therefore supports Terasaki's contention that it is the large lymphocyte of the blood which possesses the faculty of immunological response.

Our experiments in mice have been closely paralleled by the quite independent investigations of Simonsen (1957) in chickens. Simonsen has found that the injection of adult blood, blood buffy coat cells or splenic cells, either into late embryos or newly hatched chicks, leads to the splenic enlargement and severe anaemia of the hosts, many of which die in the 1st or 2nd week after hatching. These pathological changes (others also occurred in the bone marrow, thymus and liver) occurred only if the cells were injected into the hosts at a time when they would still be expected to elicit immunological tolerance. The

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finding that embryonic cells, or cells from young chicks of up to 8 days old, did not cause splenic enlargement was strong evidence for Simonsen's hypothesis that splenomegaly is due to an immunological reaction initiated by the injected cells against their hosts. This interpretation was fully confirmed by his demonstration that, in the late stages of the disease, the hosts' blood cells were strongly positive when subjected to Coombs's direct test, and by experiments with chickens belonging to inbred lines (Cock & Simonsen 1958): the injection of F_1 blood into newly hatched chicks of one of the parental lines brought about only a very slight degree of splenomegaly—one that could be entirely accounted for by the known residual antigenic diversity among members of the inbred lines.

That splenomegaly in mice may also be symptomatic of a reaction by homologous cells against their hosts is evident from the work of Simonsen as well as from the data presented here. Relevant in this connexion are the experiments of Mitchison (1958), who found that *heterologous* (turkey) spleen cells transplanted to the chorioallantoic membrane of embryos, or blood cells injected directly into the embryonic circulation, are also capable of causing gross splenomegaly in chickens. Further, he was able to show beyond doubt that the chicken host blood cells were coated with turkey antibody, and he too concluded that the enlargement of embryonic spleens in this heterologous situation was brought about by an immunological reaction by the foreign cells against the tissue antigens of their hosts.

In our own study, the greatly enlarged spleens and lymph nodes of some of the *A* strain mice which had been neonatally injected with *C*57/*A* hybrid cells present a particularly intriguing problem. Histological examination revealed that the structure of the spleens was normal except for hyperplasia of the lymphoid centres (§4·3). Similar observations were made on the spleens of a few chronic tolerant runts injected some months after birth with adult F_1 hybrid spleen cells, in an attempt at rehabilitation by providing them with immunologically competent cells which, because they contain the antigens of both donor and recipient strains, should not be able to react against either donor or host cells. At the time of autopsy these animals were also found to be suffering from a high degree of splenomegaly.

What was the cause of the splenic enlargement in these animals? On the face of it, the injected F_1 cells should not have been capable of responding to the antigens of their *A* strain hosts, unless they were for some reason deficient of at least one host antigen. Such an antigenic loss might have occurred as the result of mutation in a few of the cells—a possibility which is not altogether implausible because, in hybrid cells, mutation at a single locus would be sufficient for the expression of an antigenic change. On the other hand, a *host* response against the homologous antigens present in the F_1 cells can be invoked as the cause of splenomegaly only if it is supposed that tolerance induction had not been complete; in fact, the grafts on some of the animals concerned appeared to be in very good condition at the time of autopsy, suggesting that a very high degree of tolerance had been conferred on the recipients. Yet another possibility is that we are dealing with an example of hybrid vigour at a cellular level, the hybrid cells proliferating at a relatively greater rate than the lymphoid cells of the hosts. Finally, it may be mentioned that it is not entirely out of the question that splenomegaly was due to a deficiency of our experimental design,* for in at least one instance a female *A* strain mouse (chromosomes *A X*, *A X*)

* We are grateful to Dr D. R. Newth for suggesting this possibility to us.

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made tolerant by the injection of male hybrid cells (chromosomes *C*57 *Y*, *A X*) was grafted with skin from a female hybrid donor (chromosomes *C*57 *X*, *A X*): splenomegaly might have come about as the result of a very feeble host response against antigens present on the *C*57 *X* chromosome, or by lymphoid cells present in the skin graft at the time of transplantation against the antigens of the *C*57 *Y* chromosome. All these explanations are at present purely speculative, and it is to be hoped that experiments now in progress will help to elucidate this problem.

Another interesting finding was that *A* strain mice injected at birth with *C*57/*CBA* hybrid cells displayed a high degree of tolerance towards *CBA* skin grafts after they had rejected grafts from *C*57/*CBA* donors. This result has been fully discussed above (§4·3).

Finally, it should be added that runt disease of mice has also been encountered by other workers (P. L. Krohn, private communication), and Woodruff & Sparrow (1957) have suggested that the high mortality of rats injected neonatally with homologous spleen cells might be due to a similar phenomenon. Reactions of grafted cells against their hosts have been observed by Egdahl *et al.* (1958) when attempting to induce tolerance of heterologous tissues in rodents, and it is probable (Nakić & Silobrčić 1958) that they are also the cause of a disease which occurs after the parabiosis of adult animals (see Finerty, 1952).

6·3. *The relationship between tolerance and runt disease*

Two questions must be considered under this heading: (*a*) is there any *causal* connexion between tolerance and the lymphoid hypoplasia associated with runt disease; and (*b*) is tolerance a necessary prerequisite for the manifestation of runt disease?

(*a*) There are no grounds for regarding the state of tolerance as a consequence of the hosts' lymphoid hypoplasia. *Foetal* cells are perfectly capable of inducing tolerance without causing this or any other symptom of runt disease, presumably because they become tolerant of the *host* antigens. As examples one may cite the tolerance conferred naturally as the result of anastomosis between the foetal blood circulations of dizygotic cattle twins (Anderson, Billingham, Lampkin & Medawar 1951; Billingham, Lampkin, Medawar & Williams 1952; Billingham & Lampkin 1957) or of man (Dunsford, Bowley, Hutchison, Thompson, Sanger & Race 1953; Booth, Plaut, James, Ikin, Moores, Sanger & Race 1957; Nicholas, Jenkins & Marsh 1957) or, artificially, by the parabiosis of chick embryos, or by the intravenous injection of chick embryos with homologous embryonic blood (Hašek & Hraba 1955; Billingham *et al.* 1956*a*; Simonsen 1957). In none of these have abnormalities of any kind been reported. Particularly relevant in this context are Simonsen's experiments, for they were conducted with the precise object of determining whether or not embryonic homologous cells were capable of being harmful to their hosts. Equally compelling is our demonstration that (1) complete tolerance of *C*57 skin grafts could be conferred upon newborn *A* strain mice without lymphoid hypoplasia of any kind, provided that they were injected with cells from F_1 *C*57/*A* hybrid donors (§4·3; also see Cock & Simonsen 1958), (2) with some strain combinations, tolerance of homologous tissues was never accompanied by abnormalities of the hosts' lymph nodes (§3·5), and (3) the injection of *CBA* bone marrow cells into *A* strain newborn mice, whilst inducing full tolerance, brought about a degree of lymphoid hypoplasia which was negligible compared with that caused by *CBA* spleen cells.

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(*b*) With regard to the second question, there can be little doubt that tolerance aids and abets the homologous cells in so far as it enables them to persist long enough to do harm. On the other hand, there are grounds for believing that full tolerance need not *always* be a prerequisite: in strain combinations in which the reaction of the foreign cells is of special violence the hosts might conceivably be killed before their immature but normal defence mechanism had been fully alerted. Evidence in favour of this lies in the demonstration that, whereas *AU* spleen cells injected into *A* strain newborns were invariably fatal (table 4), *AU* bone marrow cells (§4·2) or *AU*/*A* hybrid spleen cells (§4·3)—both of which avoided the mortality due to runt disease—elicited tolerance in only a relatively small proportion of *A* hosts. Unless it is assumed that bone marrow cells and F_1 hybrid spleen cells are less efficient than *AU* spleen cells in inducing tolerance (comparable experiments with the *C*57 strain suggest that such an assumption is ill-founded), it must be concluded that many of the mice dying after the injection of *AU* spleen cells were not at all tolerant at the time of death. Similar results have been obtained by Billingham & Silvers (unpublished) with another strain combination, *C*3*H* and *PRI*.

Nevertheless, there can be little doubt that with many *other* strain combinations, in which runt disease does not follow its most extreme course, a state of tolerance permitting the prolonged survival of the homologous cells is indeed essential for the manifestation of the disease.

6·4. *Runt disease in relation to 'secondary disease' in radiation chimeras*

It is now generally agreed that the protective effect of bone marrow or spleen cells injected into mice soon after high dosage *X*-irradiation is due to the colonization of the depleted host organs by the injected cells (Lindsley, Odell & Tausche 1955; Ford, Hamerton, Barnes & Loutit 1956; Mitchison 1956*a*; Nowell, Cole, Habermeyer & Roan 1956; Vos, Davids, Weyzen & van Bekkum 1956; Makinodan 1956; Ford, Ilbery & Loutit 1957). When homologous (or heterologous) cells are used in order to confer protection, the recipients become cellular chimeras which in some respects resemble the chimeras established by intravenous injection of newborn mice with homologous cells; here the hosts are rendered immunologically non-reactive by the *destruction* of their lymphoid and haematopoietic tissues rather than by the induction of tolerance. It is therefore of great interest that a variable proportion of mice protected with foreign cells have been reported to have died several months later from a disease which has been variously described as 'homologous', 'secondary' or 'wasting' disease. The symptoms of this disease resemble those of runt disease very closely (see also Denko 1956; Congdon & Urso 1957; Barnes, Ford, Ilbery & Loutit 1958).

Two theories have been advanced concerning the cause of death in radiation chimeras. The first turns on the possibility that the hosts' own faculty of response may slowly reappear and, by destroying the widely distributed foreign cells, lead to self-destruction (see Makinodan 1957). The second theory, which was first formulated by Trentin (1956), depends upon the fact that the adult foreign cells which initially protected the mice are capable of normal immunological responses and should therefore be expected to react against the tissue antigens of the hosts. According to this second theory, the causes of runt disease and secondary disease are fundamentally the same.

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There is nothing mutually exclusive in these two theories for, depending partly perhaps on experimental conditions such as the dosage of X-irradiation to which the mice had been exposed or the type of cell used for protection, both host and graft responses may well be involved. That a reaction by the homologous cells against their hosts may often play a predominant role in causing secondary disease can hardly be doubted: secondary disease can be largely avoided if the homologous cells are obtained either from *hybrid* F_1 donors (Trentin 1957; cf. §4·3) or from embryos (Barnes, Ilbery & Loutit 1958; Uphoff 1958; cf. §6·3). In both these cases a reaction by the cells against their hosts is prevented—in the first by the genetic composition of the injected cells, and in the second by their becoming tolerant.

The resemblance between runt disease in tolerant mice and secondary disease in radiation chimeras is further underlined by the finding that the incidence and severity of secondary disease also appear to be largely determined by the genetic disparity between donor and host strains (Uphoff & Law 1958; Barnes, Ford, Ilbery, Koller & Loutit 1957).

It should be pointed out that there is not necessarily any inconsistency in the fact that, whereas adult homologous bone marrow may be fatal in irradiated *adult* hosts, its effect on baby mice is sublethal (§3·4); it may well be that the irradiated adult animal provides a far better opportunity for the relatively small proportion of immunologically competent cells present in bone marrow to proliferate and so to reach a fatally high density (see Owen, Jacob, Moloney & Dunphy 1958).

6·5. *Clinical implications*

At the present time much work is being done with the ultimate object of making homografts of tissues or even whole organs immunologically acceptable to their hosts. The discovery that a graft may, if it contains a significant number of immunologically competent cells, react against the tissues of the host suggests that, at least for some tissues and organs, the clinical homograft problem must now be regarded as composite: not only must the host be made incapable of rejecting the homograft, but the homograft too may have to be prevented from reacting against the tissues of the hosts. Although the second consideration is hardly likely to apply to tissues such as skin, its importance in the transplantation of whole organs such as the kidney has yet to be determined. The use of *foetal* homologous tissues certainly offers one avenue of escape, restricted though it may be. The careful X-irradiation of adult grafts prior to transplantation may be another.

Furthermore, it is evident that any attempts to confer tolerance on newborn human infants by the injection of homologous adult blood leucocytes must now be considered to be fraught with considerable danger, a point which also merits serious consideration when attempting to restore immunological reactivity to hypogammaglobulinemic patients (see Good, Varco, Aust & Zak 1957).

Finally, we wish to draw attention to the possibility that lesions produced in the mammalian foetus as a consequence of maternal iso-immunization need not *necessarily* be produced by humoral antibodies entering the foetus from the maternal circulation; it is not impossible that such lesions are brought about by maternal, immunologically competent blood leucocytes which had for some reason or other gained access to the tissues of the foetus. Hydrops foetalis has some symptoms which resemble those of runt disease (see

Pickles 1949). Our suggestion is open to clinical investigation, for, if true, then one would occasionally expect to find that an infant is born with all the symptoms of iso-immunization but by a mother who has never been sensitized.

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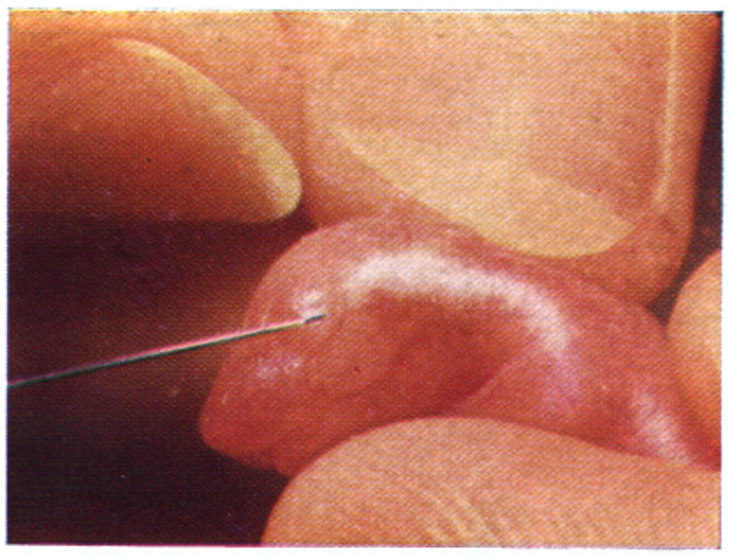

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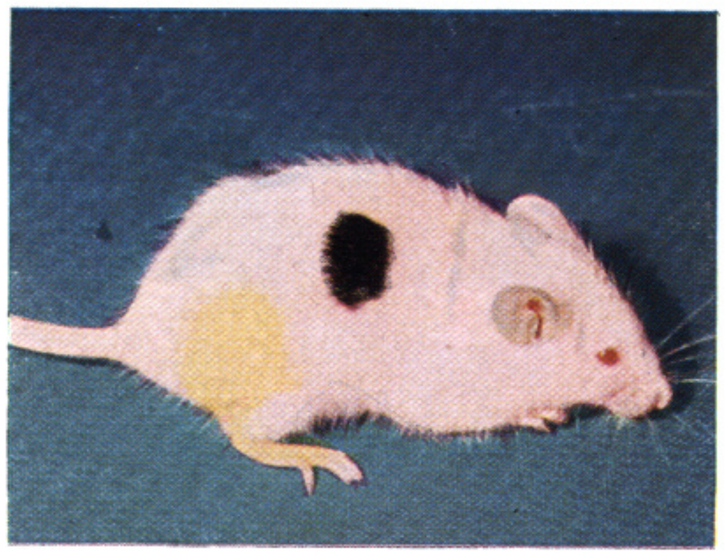

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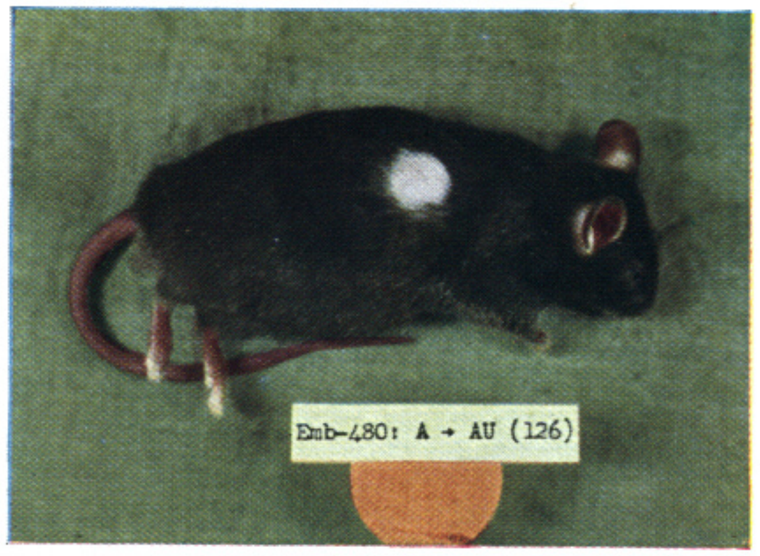

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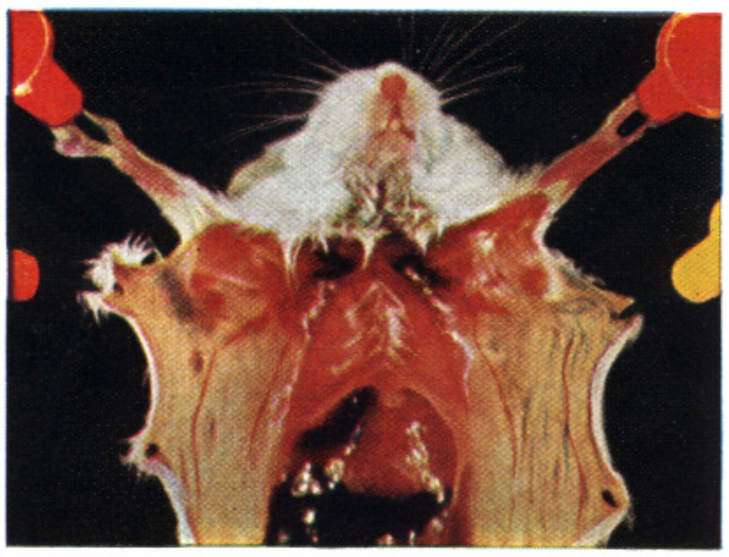

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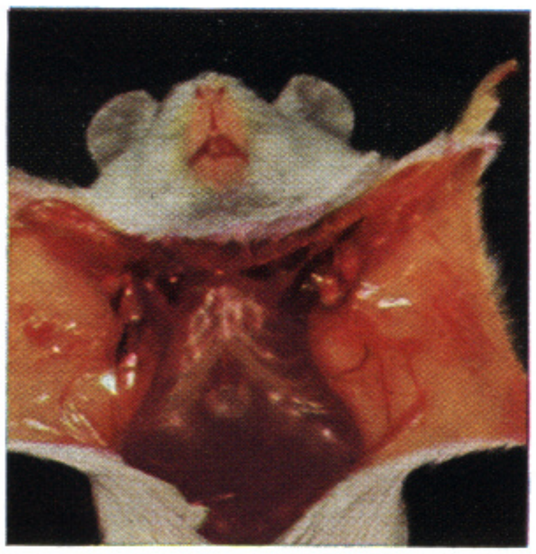

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FIGURE 4. The intravenous injection of a newborn mouse with homologous tissue cells: the 30-gauge needle is about to enter the orbital branch of the anterior facial vein (§2·3; cf. figure 1).

FIGURE 5. A *C*57 skin homograft 160 days after transplantation to an adult *A* strain mouse which had been intravenously injected (within 20 h of birth) with 5 million bone marrow cells from an adult donor (§3·4). The use of bone marrow for tolerance induction avoided the acute and fatal form of runt disease which, with this strain combination, was invariably caused by the injection of adult spleen cells (§3·5).

FIGURE 6. An *A* skin homograft 126 days after transplantation to an *AU* mouse which had been injected intravenously (within 20 h of birth) with 4 million spleen cells from an adult donor. With this strain combination about half of the cell recipients died of runt disease, but—like this mouse—50 % of the survivors were highly tolerant (§3·5).

FIGURE 7. An *A* strain mouse which had been injected neonatally with 6 million *C*57/*CBA* hybrid spleen cells. The mouse died 38 days later (weighing 9 g), and dissection revealed the almost total absence of its lymph nodes—a feature which characterized acute runt disease (§3·7). Note the complete absence of the axillary lymph nodes (cf. figure 8).

FIGURE 8. A normal *A* strain mouse, 33 days old, dissected to display its four axillary lymph nodes.

FIGURE 9. An *A* strain mouse 52 days after birth, when it had been injected intravenously with 5 million *C*3*H* spleen cells. The mouse, which had been suffering from runt disease ever since the first week after its birth, weighed only 6 g when it was killed and photographed. Subsequent dissection revealed a state of extreme lymphoid hypoplasia (see §§3·5 and 3·7). The animal is here shown together with a normal *A* strain mouse of the same age.

FIGURE 10. Two *A* strain litter mates 17 days after birth. The runt had been injected intravenously, soon after birth, with 1 million blood leucocytes obtained from an adult *C*57 donor, and it died 25 days later from runt disease. The larger mouse (short tail) had been injected with 0·07 ml. of plasma prepared from the blood of the same *C*57 donor, and it grew into a normal mouse (§§4·1, 4·2, 6·1 and 6·2).