ACTIVE AND PASSIVE IMMUNITY TO TRANSPLANTATION OF FOREIGN BONE MARROW IN LETHALLY IRRADIATED MICE

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WORK in several laboratories has shown beyond doubt that lethally irradiated mice may be induced to recover by the intravenous implantation of haematopoietic cells from normal mice, and that their recovery is due to repopulation of the host by the injected cells and their progeny (Ford, Hamerton, Barnes and Loutit, 1956). Barnes and Loutit (1954) showed that restoration could not be effected by cells of an allogeneic, that is genetically foreign (Gorer, Loutit and Micklem, 1961) mouse against whose antigens the host had previously been immunized. In other words, the state of immunity had not been abolished by the radiation. Three interpretations of this were possible. Either the injected bone marrow cells were destroyed by humoral antibody circulating in the serum of the host; or they were the object of a cellular immune response mounted by sensitized radioresistant cells; or they were affected by both kinds of response. The immune response to solid homografts of skin is mediated primarily by cells (Billingham, Brent and Medawar, 1954; Billingham and Brent, 1956), and it is not clear that humoral antibody, though it may be formed (Amos, Gorer, Mikulska, Billingham and Sparrow, 1954; Micklem and Brown, 1961), plays any part in the rejection of such solid grafts. Against certain tumours (notably lymphomas), on the other hand, humoral antibody is undoubtedly active both in vitro and in vivo (Gorer, 1958).

The experiments reported in this paper were undertaken to extend information on the bone marrow treatment of pre-immunized irradiated mice, and to discover whether destruction of injected bone marrow cells in these mice was due to a cell-mediated or a humoral reaction. While the experiments were in progress, Gorer and Boyse (1959), Santos, Cole and Garver (1959), and very recently Garver and Cole (1961) reported work showing that immunity to injected marrow suspensions could be passively transferred by means of specific antisera. Our results with some, but not all, strain combinations confirm those of the above workers, and also show that the amount of antiserum needed may be very small.

MATERIALS AND METHODS

Stocks

All the mice used in these experiments were bred at Harwell. They belonged to the strains CBA, A, C57BL and C3H, all of which are inbred by strict sib-mating with frequent reselection of lines. All mice were 3–5 months old at the time of irradiation.

Immunization

Mice were immunized against iso-antigens by one of three methods.

Method 1.—Three injections of approximately 10^7 adult spleen cells suspended in saline were given at intervals of a fortnight—the first intravenous, the second intraperitoneal and the third subcutaneous. Recipients were irradiated or bled for serum 10–14 days after the third injection.

Method 2.—Two successive orthotopic grafts of foreign skin were applied, the second 20 days after the first. Serum was collected 36 days after the second graft was completely shed.

Method 3.—Approximately 10^7 adult spleen cells were injected intraperitoneally. Five to 6 days later the recipients were grafted orthotopically with skin from the same donor strain. Serum was collected 14 days after application of the graft.

Some serum pools prepared in CBA mice against A or C57BL iso-antigens by Methods 1 and 3 were titrated for haemagglutinins by the dextran method of Gorer and Mikulska (1954) and found to have titres of 1/16-1/128, the lower titres being produced by Method 1. Sera prepared by Method 2 probably had titres around 1/64 (Micklem and Brown, 1961). These antisera were cytotoxic in the presence of complement to the corresponding lymphoid cells when tested *in vitro* by a modification of the method of Gorer and O'Gorman (1956).

Irradiation

Mice were X-irradiated in boxes of 5 by the standard technique of this laboratory (Corp, 1957). The dose was 1007 rads \pm 3 per cent (250 kV.; 14 ma.; H.V.L. 1·2 mm. Cu.) which is 99 per cent lethal to our CBA, C3H and A mice within 30 days, nearly all the mortality being within 14 days.

Suspensions for intravenous therapy

The method of preparing these has been described by Bridges, Loutit and Micklem (1960).

EXPERIMENTAL RESULTS

Experiment 1

Two hundred and twenty CBA \bigcirc , 80 A \bigcirc , 20 C3H \circlearrowleft , and 27 C57BL \bigcirc mice were used as hosts in this experiment. The aim was to confirm that pre-immunization of mice against allogeneic mouse tissue antigens (in this case spleen) prevented their recovery after irradiation and treatment with bone marrow of the same foreign strain. In addition we tested the capacity of C57BL \circlearrowright antigens to immunize C57BL \circlearrowright hosts against a subsequent injection of C57BL \circlearrowright bone marrow. In most strain combinations, three experimental groups were run concurrently :—

1. Allogeneic bone marrow or bone marrow and spleen \rightarrow irradiated preimmunized hosts.

2. Allogeneic bone marrow \rightarrow irradiated non-immune hosts.

3. Saline only \rightarrow irradiated non-immune hosts.

The third group was omitted when C3H and C57BL hosts were used. Method 1 was used for immunization.

Table I shows the survival times seen in the four strain-combinations tested. These results show that except in the $C3H \rightarrow CBA$ strain combination all mice in Group 1 died within 14 days of irradiation—*i.e.* within the normal period of death from acute radiation-induced aplastic anaemia. Gross autopsy showed no difference between Group 1 and Group 3, in which mortality was 100 per cent within 2 weeks. All mice had small lymph-nodes and spleen, minute thymus, and pale liver and kidneys. The bone marrow was haemorrhagic and gastro-intestinal haemorrhage was common, though not universal. Histological examination of 14 animals in Group 1 confirmed the basic lesion as aplasia of the bone

			Number of		Number dead in		Number dead in		Survivors
\mathbf{Hosts}	Donor material		mice		$\ll 14 \mathrm{days}$		15-30 days	3	$> 30\mathrm{days}$
CBA immune to A .	A bone marrow		25		25		0		0
CBA immune to A .	A bone marrow and spleen	·	25	·	25	•	0	•	0
Normal CBA	A bone marrow		25	•	2		1	•	22
Normal CBA	Saline only	·	25	·	25	·	0	•	0
A immune to CBA .	CBA bone marrow		25		25		0		0
A immune to CBA .	CBA bone marrow and spleen	·	25	·	25	·	0	•	0
Normal A	~~···		25		1		0		24
Normal A	Saline only	•	5	•	5	•	0	•	0
CBA immune to C3H .	C3H bone marrow		25		18		4		3
CBA immune to C3H .	C3H bone marrow and spleen	·	25	·	25	·	0	•	0
Normal CBA			25		1		3		21
Normal CBA	Saline only	·	25	·	25	•	0	•	0
C3H immune to CBA .			10				0	•	0
Normal C3H	CBA bone marrow	·	10	•	0	•	0	•	10
C57BL ♀ immune to C57BL ♂	C57BL 5 bone marrow	•	13	•	9	•	0	•	4
37 1 0	C57BL \mathcal{J} bone marrow	•	14	•	2	•	0	•	12

 TABLE I.—Experiment 1: Mortality of Normal and Actively Iso-immunized Mice after Lethal Whole-body X-irradiation and Treatment with Foreign Haematopoietic Tissue

marrow. A large proportion of Group 1 died also in the C3H \rightarrow CBA and C57BL $3 \rightarrow$ C57BL 2 combinations.

In Group 2 deaths within 14 days were the exception : almost all animals were successfully restored by the injected bone marrow and died later at times which varied with the strain combination. Such late deaths are now generally considered to be initiated by an immune reaction by the grafted tissue against the host, although in the CBA \rightarrow A and CBA \rightarrow C3H strain combinations such a reaction, if it takes place, is feeble. (An account of secondary mortality in weakly and strongly reacting strain combinations is in preparation).

These results confirmed those of Barnes and Loutit (1954). Repopulation and restoration of irradiated mice with foreign bone marrow was indeed prevented when the hosts had been immunized against the appropriate iso-antigens. The death of a majority of immunized mice in the $C3H \rightarrow CBA$ and $C57BL \textcircled{O} \rightarrow C57BL \textcircled{Q}$ combinations showed that the effect was not solely dependent on differences at the H-2 histocompatibility locus, and that even the antigens associated with the Y-chromosome were able to immunize C57 \bigcirc hosts effectively.

Experiment 2

This experiment was designed to show whether repopulation could be prevented by the injection of a fairly large amount (0.4 ml.) of immune serum 1–2hr. before lethal irradiation. Fifteen CBA 3^r mice were injected intravenously before irradiation with 0.4 ml. of pooled serum from CBA mice immunized by Method 1 against A-strain spleen cells. Fifteen control mice received 0.4 ml. of pooled normal CBA serum. All were intravenously injected $\frac{1}{2}$ -4 hr. after irradiation with 2-5 × 10⁶ nucleated cells from the bone marrow of normal A donors. Mortality is shown in Table II. The animals which received immune serum all died within 14 days from aplasia of the bone marrow, while the controls died between 30 and 120 days after irradiation from "secondary disease".

 TABLE II.—Experiment 2: Mortality of CBA Mice Lethally Irradiated and then Injected Intravenously with A-strain Bone Marrow

	Number of			Number dead in		Number dead in		Survivors		
Hosts		mice		< 14 days		15-30 days		$> 30 ext{ days}$		
Passively immunized before irradiation*		15		15		0		0		
Controls†	•	15	•	0	•	0	•	15		

* Injected intravenously 1-2 hr. before irradiation with 0.4 ml. undiluted CBA anti-A serum.

† Injected intravenously 1–2 hr. before irradiation with 0.4 ml. undiluted normal CBA serum.

This experiment suggested that serum antibodies were alone sufficient to bring about the rejection of grafted marrow; sensitized cells were not necessary.

The next step was to discover how small a quantity of serum could successfully transfer immunity of this kind. A-strain mice were in short supply, so C57BL donors were used in the following experiments.

Experiment 3

One hundred and twenty CBA \circ mice were divided into 4 groups of 30. Half the mice in each group received serum from CBA mice immunized against C57BL spleen cells by Method 1; this was given intravenously $\frac{1}{2}-2$ hr. before irradiation. The other half received normal CBA serum. The experiment was timed so that it was not necessary to store sera for longer than overnight after bleeding of the donors. The maximum period of storage was 18 hr. at 0-4°. All mice were injected intravenously with 2-5 \times 10⁶ nucleated cells from normal C57BL bone marrow $\frac{1}{2}$ -4 hr. after X-irradiation with 1007 rads. The quantity of serum given to each group was as follows :—

- Group 1. 0.4 ml. neat serum.
- Group 2. 0.4 ml. serum diluted 1/10 in saline.
- Group 3. 0.4 ml. serum diluted 1/100 in saline.
- Group 4. 0.4 ml. serum diluted 1/1000 in saline.

The results are shown in Table III.

The minimum effective dose of antiserum was somewhere between 0.004 ml. and 0.0004 ml. It would presumably vary with the number of foreign cells injected, and this was not accurately measured.

In another experiment similar results were obtained with serum from mice immunized with skin grafts (Method 2), although the minimum effective dose was rather larger (0.04 ml.-0.004 ml.).

Experiment 4

The strains C57BL and CBA differ genetically at the important H-2 histocompatibility locus (Snell 1958; Gorer, personal communication). The strains

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	Group			Number of mice		Dilution of serum		Number dead in ≪ 14 days		Number dead in 15–30 days	8	Survivors > 30 days
]	Exptl.			15		Neat		15		0		0
	Control	•	•	15	•	Neat	•	4	•	5	•	6
2	2 Exptl.			15		1/10		15		0		0
	Control	•	•	15	•	1/10	·	3	•	2	·	10
:	B Exptl.			15		1/100		15		0		0
	Control			15		1/100		3		6		6

TABLE III — Experiment 3. Mortality of CBA Mice Injected Intravenously with

* Donors given 3 fortnightly injections of 107 C57BL spleen cells.

0

1/1000

1/1000

C3H and CBA, on the other hand, both have the phenotype H-2^k and give relatively weak immune reactions against each other. Results in Experiment 1 showed that CBA mice immunized against C3H spleen rejected C3H bone marrow administered after lethal irradiation. Could this immunity to weak iso-antigens be passively transferred, as in Experiment 3?

Sixty CBA 3 mice were divided into 2 groups. Thirty were injected intravenously $\frac{1}{2}-2$ hr. before irradiation with pooled antiserum to C3H spleen (prepared by Method 1). The other 30 received pooled normal CBA serum. The volume injected was 0.4 ml. of either neat serum or serum diluted 1/10 in saline. The results are shown in Table IV.

15

15

4 Exptl.

Control .

TABLE IV.—Experiment 4: Mortality of CBA Mice Injected Intravenously with 0.4 ml. of Neat or Diluted CBA Anti-C3H Serum, then Lethally Irradiated and Injected with C3H Bone Marrow

Method of immunizing serum donors		Number of mice	of		Number dead in ≪ 14 days		Number dead in 15–30 days	${ m Survivors}\ > 30~{ m days}$
Method 1* .		15		\mathbf{Neat}	1		1	. 13
Nil (control)		15		\mathbf{Neat}	2		1	. 12
Method 1* .		15		1/10	1		0	. 14
Nil (control)		15		1/10	1		0	. 14
Method 3 [†] .		10		Neat	0		0	. 15
Nil (control)	•	10	•	\mathbf{Neat}	0	•	0	. 15

* Three fortnightly injections of 10⁷ C3H spleen cells.

+ Intraperitoneal injection of 107 C3H spleen cells. Grafted 6 days later with C3H skin.

The mortality in experimental and control mice was indistinguishable. Any immunity to C3H antigens transferred in 0.4 ml. of serum was insufficient to prevent repopulation by 2-5 million C3H bone marrow cells. This result was confirmed with another pool of antiserum, prepared by Method 3. In addition, experiments were performed in which C3H bone marrow cells were incubated with antiserum in vitro before being injected into lethally irradiated mice. Again

13

11

there was no evidence that the serum was lethal to the cells; only a small percentage of the cells took up trypan blue or nigrosin after incubation, and all the irradiated mice survived beyond 14 days (Table V).

TABLE V.—Experiment 4: Viability of C3H Bone Marrow Cells after Incubation with CBA Anti-C3H Serum*, Measured by Exclusion of Nigrosin and Capacity to Restore Lethally Irradiated CBA Mice. Controls were Incubated with Normal CBA Serum

Pretreatment of donor bone marrow cells	Number of cells injected per mouse	Percent staining after incubation	Number of mice	${ m Survivors}\ > 30~{ m days}$
l hr. at room temp. in l:l serum: Tyrodes, without added complement Exptl	About 2×10^6 About 2×10^6	. —† .	10	. 10
$\begin{array}{llllllllllllllllllllllllllllllllllll$,		
Exptl	$egin{array}{c} 2\cdot25 imes10^6\ 2\cdot44 imes10^6 \end{array}$. 25 .	10	. 9
Control	$2\cdot44 imes10^6$. 26 .	10	. 10
2 hr. at 37° in 1:2:1 serum : Tyrodes : guinea-pig complement				
Exptl	$2\!\cdot\!46\! imes\!10^6$. 15 .	10	. 10
Control	$egin{array}{c} 2\cdot 46 imes 10^6\ 2\cdot 46 imes 10^6 \end{array}$. 13 .	10	. 9
		_		

* Prepared by Method 3.

† Not counted.

Experiment 5

It seemed almost certain that immunity to bone marrow transplants persisted only so long as the specific antibodies continued in sufficient concentration in the circulation. To confirm this supposition, it was necessary to use a pool of antiserum of known potency, irradiate the serum-injected hosts after various intervals, and inject them with a fixed number of bone marrow cells. The expected duration of passive immunity could be roughly estimated by reference to the reported half-life of mouse γ -globulin—about 2 days according to Dixon, Talmage, Maurer and Deichmiller (1952) and $4\frac{1}{2}$ days according to Humphrey and Fahey (1961).

A pool of immune serum (CBA anti-C57BL) was prepared by Method 3 and stored at -15° in several ampoules until needed. All the serum used in the experiment was thus frozen once only and thawed immediately before use. Its potency was estimated as follows. Various dilutions were injected intravenously into CBA \mathcal{J} mice and 24–26 hr. later the mice were irradiated and injected intravenously with $2 \cdot 0 \times 10^6$ bone marrow cells from 4-month-old C57BL \mathcal{Q} donors. This system differed in two respects from that employed in the previous experiments. Firstly, there was a longer interval (24–28 hr.) between the injections of serum and bone marrow. Secondly, the number of bone marrow cells injected was more closely controlled.

The results of this *in vivo* " titration " are shown in Table VI. The smallest volume of antiserum which was completely effective in preventing repopulation was 0.01 ml. Some effect was seen after the injection of 0.001 ml., but not, paradoxically, of 0.004 ml.

For the second part of the experiment, 0.4 ml. of a 1/10 dilution of antiserum (*i.e.* four times the minimum completely effective dose) was injected intravenously

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TABLE VI.—Experiment 5: Mortality of CBA & Mice Injected Intravenously with
0.4 ml. of Various Dilutions of a Single Pool of CBA Anti-C57BL Serum,
then Lethally Irradiated the Following Day and Injected with 2.0×10^6 C57BL
Bone Marrow Cells

Dilution serum inj			Number of mice		$\begin{array}{l} {\rm Number} \\ {\rm dead \ in} \\ \ll 14 \ {\rm days} \end{array}$		Number dead in 15–30 days		${ m Survivors}\ > 30~{ m days}$
1/10	\mathbf{E}^*		10		10		0		0
1/10	C^{\dagger}	•	10	•	0	•	0	·	10
1/40	\mathbf{E}		10		10		0		0
1/40	С	•	10	•	1	•	0	•	9
1/100	Е		10		0		0		10
1/100	С	•	10	•	0	•	2	•	8
1/400	\mathbf{E}		10		4		2		4
1/400	С	•	10	•	0	•	0	•	10
1/1000	\mathbf{E}		10		2		1		7
1/1000	С		10	•	0	•	1	•	9

* Experimental : immune serum injected. † Control : normal serum injected.

into 80 CBA \Im mice, 80 controls receiving a like quantity of normal CBA serum by the same route. Groups of 10 experimental and 10 control mice were irradiated with 1007 rads and injected with $2 \cdot 0 \times 10^6$ C57BL \bigcirc bone marrow cells on the 3rd, 7th, 14th, 21st, 28th, 42nd, 56th and 70th days after the injection of serum. The mortality among these animals is shown in Table VII.

If we take as an index of complete immunity the prevention of repopulation in 90 per cent of the injected mice, then complete immunity was present on the 3rd and 7th days after the injection of antiserum. Immunity was partial on the 14th day, but on the 21st day it was again almost complete—9 out of 10 mice dying by the 15th day after irradiation. A similar paradox was noted above in the results of the first part of the experiment, where 0.001 ml. of antiserum appeared to transfer more immunity than 0.004 ml. On the 28th and subsequent days after the injection of antiserum no detectable immunity remained : mortality in the experimental and control groups was indistinguishable.

DISCUSSION

" Transplantation immunity" by means of antisera

The present experiments parallel in some respects those of Kaliss (1958), Amos and Day (1957), and Gorer and Kaliss (1959). These workers implanted tumour cells in mice which had circulating humoral antibody against the tumour antigens. The antibody was either actively produced by the host in response to immunization with killed tumour preparations, or passively transferred by way of serum from immunized donors. The remarkable differences in the response given by various tumours to various doses of anti-serum have attracted much attention. Some tumours (notably leukoses) are destroyed or inhibited by the action of the specific antiserum; others not merely survive, but grow with increased speed ("enhancement"); yet others may show a dual response, being

TABLE VII.—Experiment 5: Mortality of CBA ♂ Mice Injected Intravenously with 0.4 ml. of a Single Pool of CBA Anti-C57BL Serum (Diluted 1/10); then, After Various Intervals, Lethally Irradiated and Injected with 2.0 × 10⁶ C57BL Bone Marrow Cells

Interval between serum injection and irradiation (days)		Number of mice		÷	Number dead in < 14 days‡		Number dead in 15–30 days‡		${ m Survivors} > 30 { m ~days} { m t}$		
3	\mathbf{E}^{*}		10		10		0		0		
	C^{\dagger}	•	10	•	0	•	1	•	9		
7	Е		10		9		1		0		
	\mathbf{C}	•	10	•	2	•	0	·	7		
14	\mathbf{E}		10		4		1		5		
	\mathbf{C}	·	10	•	0	•	0	·	10		
21	\mathbf{E}		10		7		$2\S$		1		
	\mathbf{C}	•	10	•	1		1	•	8		
28	Е		10		0		3		7		
	\mathbf{C}	•	10	•	0	·	1	•	9		
42	\mathbf{E}		10		2		1		7		
	С	•	10	•	0	·	0	•	10		
56	\mathbf{E}		10		0		1		9		
	С	•	8	•	0	•	0	•	8		
70	\mathbf{E}		10		0		1		9		
	С	•	10		1	•	0	•	9		
		* 17.	· · · ·	ı .							

* Experimental : immune serum injected.

† Control : normal serum injected.

‡ Post-irradiation.

§ Both dead on 15th day.

inhibited by high doses of antiserum and "enhanced" by low doses (Gorer and Kaliss, 1959).

It is possible that normal tissue homografts show a similar range of responses. Billingham, Brent and Medawar (1956) found a small degree of enhancement of skin homografts in mice pretreated with killed tissues. Parkes (1958) found considerable enhancement of orthotopic ovarian homografts in similarly treated rats. In neither case was the enhancing effect passively transferred by injecting serum from a pre-treated animal into a normal host. Kaliss (1958) reported failure to produce enhancement of normal mouse skin homografts in his laboratory.

Our own experiments demonstrate that, as with the leukoses of Amos and Day (1957) and Gorer and Kaliss (1959), so with normal bone marrow suspensions, there is destruction or inhibition of the implant in the presence of specific antiserum. It seems probable that this destruction or inhibition takes place almost immediately following intravenous injection of the cells, by a cytotoxic mechanism resembling that which is demonstrable *in vitro* by the method of Gorer and O'Gorman (1956). It is scarcely possible that sensitized host cells played any part in Experiments 2–5, and there is no need to suppose that they did so in Experiment 1. The failure of attempts to prevent growth of C3H cells by means of CBA anti-

C3H serum, despite the active immunity induced against C3H in Experiment 1, may be due simply to failure to produce a potent enough serum. The immunization schedules employed in these experiments certainly result in much lower titres of haemagglutinin than are obtainable by other methods (Brown and Micklem, unpublished). On the other hand this may be one instance in which the intervention of sensitized cells or cell-bound antibody is necessary.

The presence of antiserum results in a state of transplantation immunity to injected bone marrow suspensions. This does not necessarily mean that there is a state of transplantation immunity in a general sense to all tissues carrying the appropriate antigens. Billingham and Brent (1956) have given strong evidence that humoral antibodies alone are ineffective against skin, and the same is probably true of most solid tumour grafts.

Duration of passively transferred iso-immunity

The results of Experiment 5, which was intended to show the duration of passive immunity, are impossible to interpret with certainty owing to their apparent inconsistency. There can be little doubt that the failure of 0.004 ml. of serum to confer immunity to bone marrow injected the following day, while 0.001 ml. of the same serum was partially effective, was due to some imperfection of the test system. One possible variable is the proportion of effective repopulating cells in the bone marrow inoculum. The apparent resurgence of the immunity conferred by 0.04 ml. of serum between the 14th and 21st days after injection may reasonably be ascribed to similar causes. Nevertheless it is difficult to make the results fit with the half-life of antibody quoted by Dixon et al., (1952)-The possibility must be borne in mind that a portion of the injected 1.9 days. antibody may become attached (perhaps temporarily) to cells of the host, like the "cytophilic antibody" of Boyden and Sorkin (1960), and thus escape metabolism at the normal speed. Alternatively the γ -globulin of iso-antibody may be metabolized at a slower rate than that of the antibody measured by Dixon et al. Recent work (Humphrey and Fahey, 1961) shows this to be true of normal γ -globulin, the half-life of which may be as high as $4\frac{1}{2}$ days. A half-life around this figure fits our results better than one of 2 days.

Effect on survival of subliminal levels of antibody

It seemed possible that levels of antibody just insufficient to cause acute death of the irradiated host through failure of repopulation might have some effect on longer term survival. By killing a proportion of the injected cells they might reduce, or delay the onset of, an immune reaction by the graft against the host; or they might act in the opposite way, by stimulating mitosis and so causing such a reaction to develop more rapidly. (The former effect would be analogous to that reported by Kren, Vesely, Frenzl and Stark (1960). They found that the runting syndrome initiated by injecting foreign spleen cells into newborn rats could be inhibited by the previous injection of iso-antiserum to blood of the donor. The erythropoietic elements of the spleen were not destroyed, since the recipients remained blood chimaeras, but runt disease was reduced or prevented.) Close comparison of the time of onset, the duration and the outcome of " secondary disease " in animals with low antibody levels and in controls failed to reveal any differences. Effects such as those postulated, if they existed, were too small to be detected.

SUMMARY

Mice from 3 highly inbred strains were actively immunized against the isoantigens of other inbred strains. In addition C57BL female mice were actively immunized against the iso-antigens, determined by the Y-chromosome, of C57BL They were then given a lethal dose of X-rays followed by an injection males. of bone marrow from donors of the strain against which they had been immunized. In these circumstances the bone marrow therapy, which saved non-immune control mice from acute radiation death, was ineffective.

In two strain combinations, in which there was considerable antigenic disparity between donor and host, immunity could be transferred by injecting serum from immunized donors intravenously. As little as 0.004 ml. of immune serum was effective. It was concluded that serum antibodies were alone able to mediate immunity to transplanted bone marrow.

In a third strain combination, where donor and host had the same antigens determined by the H-2 histocompatibility locus, passive immunity was not demonstrated. In this case, effective immunity may have required the presence of sensitized cells.

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