FURTHER ATTEMPTS TO TRANSFER TRANSPLANTATION IMMUNITY BY MEANS OF SERUM

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IT has been established beyond question that serum antibodies, demonstrable by haemagglutination (Gorer, 1947) or by nephelometric methods (Bollag, 1956), are formed in response to the grafting of homologous tissues ; but there is strong evidence that, at least as far as serum haemagglutinins are concerned, these are not the operative agents of transplantation immunity (Mitchison and Dube, 1955 ; Kaliss, 1957). Attempts to transfer a state of heightened resistance against homografts of skin, using serum from specifically immunised mice, have so far been uniformly unsuccessful. Even regimens of daily or two-daily intraperitoneal inoculations of relatively large dosages of " immune " serum have failed to shorten the survival time of skin homografts in mice (Billingham, Brent and Medawar, 1954). The recent experiments of Voisin and Maurer (1956), while confirming that immunity may be transferred with cells, have given equivocal results with homologous " immune " serum.

The purpose of this paper is to report on an extension of our earlier work in mice. Not only has an attempt been made to evoke a more vigorous and generalised immunological response from the serum donors, but special attention has been paid to the possibilities : (1) that the dosages of serum used in previous experiments might have been too high ; (2) that it might be advantageous to begin treatment of the secondary hosts several days before transplanting to them their test-grafts ; and (3) that the serum might be more effective if administered intravenously rather than intraperitoneally.

MATERIALS AND METHODS

The design of the present experiments was exactly as before (Billingham, Brent and Medawar, 1954). After immunisation of mice (primary hosts) of one inbred strain with tissues from animals of a donor strain, the serum was prepared from the pooled blood of the primary hosts—obtained by cardiac puncture—and then injected into normal adult mice (secondary hosts). Primary and secondary hosts were always members of the same inbred strain. The capacity of the serum to confer heightened resistance (*i.e.*, immunity) on the secondary hosts was assessed in terms of the reaction of the recipients against skin homografts from the donor strain. Curtailment of the grafts' survival times compared with those of grafts on normal untreated animals, combined with certain other diagnostic features, would have shown that immunity had been transferred by the serum.

The mice used in these experiments belonged to the sublines of the Strong A and CBA strains, for which accurate transplantation data have already been obtained (see Billingham, Brent, Medawar and Sparrow, 1954). The primary hosts were invariably immunised three times, at three-weekly intervals, in order to exact a maximal response. Each immunisation entailed the intraperitoneal and subcutaneous injection of living spleen and kidney cells

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from the donor strain, the subcutaneous injections being carried out in widely separated areas in the hope that this would activate most of the lymph nodes. The fact that the regional lymph nodes are known to be primarily responsible for the destruction of homografts (Mitchison, 1954, 1955*a*; Billingham, Brent and Medawar, 1954) appears to justify such an approach. In addition, the first immunisation included the transplantation of a skin graft from the donor strain. In other words, the greatest possible care was taken to bring about the hyperimmunisation of the serum donors, which were bled on the 8th day after the final immunisation. In order to obviate any possible deleterious effects of storage, fresh serum was used almost invariably. This entailed careful planning of the immunisations so that groups of primary hosts became available for bleeding on the appropriate days. On the very few occasions on which stored serum was used it had been preserved at 4° for periods never exceeding 48 hr.

RESULTS

The results are summarised in the Table. None of the experiments indicates

TABLE.—*Attempts to Transfer Immunity directed against Skin Homografts by means of Serum*

Strain combination.	Experiment.	Dose of serum per day of administration (ml.).	Total dose (ml.).	Days of administration.*	No. of secondary hosts.	Appraisal of test grafts.
A → CBA	1	0·25	0·25	0	7	No immunity
	2	0·25	0·5	4, 6	4	,, ,,
	3	0·25	0·75	−1, 0 6	10	,, ,,
	4	0·25	1·25	−2 0, 2, 4, 6	3	,, ,,
	5	0·5	1·5	−2 0, 2	3	,, ,,
	6	0·25	1·5	−4, −2 0, 2, 4, 6	2	,, ,,
CBA → A	7†	0·25	0·25	0	5	,, ,,
	8	0·25	1·0	−2 0, 2‡, 4‡	4	,, ,,

* Day of transplantation = 0.
† Grafts treated with immune serum *in vitro* for 6 hours before transplantation
‡ Injections on these days were made intravenously.

that " immune " serum transferred a state of immunity to secondary hosts. These results, considered together with our previous findings, show that the outcome of the experiments was not affected by (*a*) variation over the range 0·25 ml. to 1·5 ml. in the total amount of serum administered, (*b*) the injection of the immune serum over a period beginning as early as 4 days before the transplantation of the test-grafts and ending as late as the day of graft biopsy, and (*c*) the use of the intravenous as well as the intraperitoneal route of injection. Equally ineffective was the prolonged impregnation of very thin grafts with " immune " serum before transplantation, combined with the intraperitoneal inoculation of the host with serum on the day of grafting (experiment 7).

It should, however, be mentioned that the grafts on some of the serum-treated animals displayed outward signs of vascular dilatation and congestion when examined on the 6th post-operative day. This effect was transient and clearly of minor importance to the well-being of the grafts, the survival times of which were not in the least curtailed.

One experiment, not included in the Table, made use of two CBA mice which had previously been made tolerant of A strain tissues (see Billingham, Brent and Medawar, 1956) and which bore A strain skin grafts of 100 days' standing.

Although such animals are themselves unable to react against their grafts, they are nevertheless capable of mediating a state of immunity which may be conferred upon them by the injection of immunologically activated cells. Tolerant mice are therefore ideal subjects for experiments on the passive transfer of immunity with serum. Each mouse received 2·0 ml. of " immune " serum, in divided doses, over a period of 8 days. In one of them the serum was injected intraperitoneally, in the other both intraperitoneally and intravenously. At no stage did the grafts on either mouse reveal the slightest sign of an immunological reaction such as might be expected had the serum contained cytotoxic antibodies.

DISCUSSION

Evidence of the protective action of homologous immune sera has been derived almost exclusively from work on mouse leukaemia. That serum from hyper-immunised donors will protect mice against certain leukaemic cells, provided that cells and serum are first incubated *in vitro*, has been known for some considerable time (Gorer, 1942 ; Mitchison, 1955*b*) ; it has recently been extended to suspensions of Sarcoma 1 cells (Mitchison and Dube, 1955). Gorer and Amos (1956) and Amos (1957) have also shown that it is possible to bring about a true passive transfer of immunity against certain leukaemic tumours, *i.e.*, by the simultaneous injection of the immune serum and the leukaemic cells into the hosts *without* prior incubation. Similar results have now been obtained with the Walker 256 rat carcinoma (Sekla and Barvič, 1956).

As far as the transplantation of *normal* homologous tissues is concerned, the only evidence lending support to the view that protective antibodies are present in " immune " sera—apart from the rather equivocal results of Voisin and Maurer (1956)—lies in the impairment of the proliferative capacity of rabbit skin epithelial cells after their prolonged incubation *in vitro* with the serum from specifically immunised rabbits (Billingham and Sparrow, 1954). However, this effect is too feeble to account convincingly for the violence of the homograft reaction *in vivo*.

The present experiments were designed to test the ability of serum from immunised mice to transfer an immunity measurable by the precocious breakdown of homologous skin grafts, and possible shortcomings of previous attempts on these lines (Billingham, Brent and Medawar, 1954) were carefully avoided. As before, the results have been uniformly negative. It is therefore impossible to escape the conclusion that the sera of these mice did not contain protective antibodies directed against the homologous skin grafts. Indeed, the work of Algire, Weaver and Prehn (1954) and Weaver, Algire and Prehn (1955) has shown that a variety of homologous tissues (including some tumours) remain unharmed when exposed in diffusion chambers to the body fluids of specifically immunised mice, provided that the ingress of host cells is completely prevented. It is also well established that transplantation immunity in respect of both tumour tissue (Mitchison, 1954, 1955*a*) and skin (Billingham, Brent and Medawar, 1954) can be " adoptively " transferred with living cells from the draining lymph nodes and spleens of immunised mice. Our own negative results with serum further support the view that the homograft reaction against normal tissues, like tuberculin sensitivity and other allergic responses of the delayed type, is mediated by cells but not by serum (also see review by Brent, 1957).

It is at present not possible to put forward a satisfactory explanation for the contradictory results obtained with homografts of skin and a variety of solid

tumours on the one hand, and with dissociated leukaemic cells on the other. It is not inconceivable that they may be due to unique properties of the leukaemic tumours (Gorer and Amos, 1956), and the dissociated state of the tumour may well be of crucial importance. Yet Sekla and Barvič's (1956) findings with the Walker rat carcinoma appear to make such an explanation rather less acceptable.

SUMMARY

Further attempts have been made to transfer immunity passively against skin homografts with serum from hyperimmunised mice. Variations in serum dosage, regimen of administration relative to the time of skin grafting, and route of injection have all given uniformly negative results. This finding is in agreement with the thesis that the agent of skin homograft destruction is not present in the serum of immune animals, and that transplantation immunity is transferable with cells only.

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