THE EFFECT OF SPLENECTOMY ON THE SURVIVAL OF SKIN HOMOGRAFTS IN RABBITS AND ON THE RESPONSE TO CORTISONE.

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FOR many years it has been generally agreed that the spleen is concerned in development of immunity. Removal of the spleen, for example, is said to interfere with the development of an immune response (Hektoen and Curtis, 1915; Hektoen, 1920; Motohashi, 1922; Wolfe, Norton, Springer, Goodman and Herrick, 1950) to an extent which appears largely to depend on the route by which the antigen is administered and the number and size of the doses of antigen that are given (Rowley, 1950*a*, *b*). Immunization of rabbits is accompanied by a proliferation of plasma cells in various organs, including the spleen, which parallels the rise in antibody titre (Bjoerneboe and Gormsen, 1943; Fagraeus, 1947, 1948). Tissue cultures of the spleens from immunized animals have also yielded significant amounts of antibody. Taliaferro (1949) in a summary of the evidence, came to the conclusion that the spleen plays a significant, though not an essential, part in the development of immunity.

Apart from a few exceptional cases, it is a general rule that homografts of skin are finally destroyed by a specific reaction which seems to depend on the development of an immunity response to the grafted tissue. Removal of the spleen might therefore be expected to modify the response to skin homografts if its presence is important in relation to the normal development of immunity. It has also been reported that splenectomy prevents the rise in antifibrinolytic activity of the serum that otherwise follows the injection of either cortisone or ACTH (Ungar and Damgaard, 1951). Ungar believes that cortisone's effect on this system is mediated through the spleen since an extract of spleen, which he has called Splenin A, reproduces the results of giving cortisone, not only in normal animals but in splenectomized animals as well. Ungar, Damgaard and Weinstein (1951) have further observed that, although cortisone and ACTH inhibit the development of an experimental arthritis caused by introducing a specific antigen into the joints of passively sensitized normal guinea-pigs, they fail to do so in splenectomized animals. Splenin A is able to reduce the swelling whether the guinea-pig is splenectomized or not. Billingham, Krohn and Medawar (1951) have shown that cortisone inhibits the development of the immunity response to skin homografts and enables the grafts to survive for 3 or 4 times as long as normal. If this property of cortisone, like the others mentioned by Ungar, were mediated via the spleen and splenins, it would not be evident in splenectomized animals.

Experiments to see whether the absence of the spleen modifies either the normal homograft reaction or its response to treatment with cortisone are reported below. During the course of the work, the opportunity was taken of obtaining further information about the leucocyte count after splenectomy.

MATERIALS AND METHODS.

Animals.—Mature rabbits of both sexes (weighing 2200–3300 g.) from the Department's stock were used. They were caged separately and fed on the pellet diet 18 of Bruce and Parkes (1946). Water was available *ad lib.*

Surgical procedures.—The technique for skin grafting and for assessing the survival time of the homografts was that described by Billingham *et al.* (1951).

Splenectomy was performed aseptically under Nembutal anaesthesia. The spleen was approached through a curved left sub-costal incision, mobilized and removed after the vessels and the pedicle had been cauterized. Any visible accessory splenic tissue was removed at the same time. The completeness of the operation was confirmed at autopsy at the end of the experiment. Remnants of splenic tissue were not found in any of the animals.

A saline suspension of cortisone acetate was used. Either 5 or 10 mg. were administered in a single daily subcutaneous injection, beginning one day before the grafting operation was carried out and continuing for as long as the homografts showed signs of survival.

Quantitative measures of cortisone activity.—The following criteria were employed to assess any differences that might have arisen between the responses of normal and splenectomized rabbits to cortisone.

1. Survival time of the homografts.
2. The rate of healing of the graft bed on the chest and of the sites on the ears which had provided control autografts. Healing was judged by the extent of ingrowth of epithelium from the margins of the wounds, the build-up of granulation tissue and the length of time required for the scabs on the ears to become detached. Contraction of the area of the chest wound was estimated by expressing the area on the twelfth post-operative day as a percentage of the original area at the time of operation.
3. The rate of mitosis (expressed as number of mitoses/mm. of epidermis) in biopsies of autografts on the sixth post-operative day.
4. Changes in the differential white cell count, estimated on samples of blood removed from a small marginal ear vein between 9 a.m. and 10 a.m.
5. Changes in the weight of the liver.
6. Changes in the weight of the adrenal glands.

RESULTS.

Splenectomy was performed 16–66 days before grafting in rabbits that received cortisone and 38–62 days before grafting in those that did not.

The results are stated in the Table. Splenectomy did not alter the normal progress of events after skin grafting in untreated rabbits. Homograft survival time, mitotic activity in autografts, and rate of healing of the wounds were all within normal limits. The Table also shows that the absence of the spleen has not influenced the usual responses to the administration of cortisone. Homograft survival time was prolonged; mitotic activity and wound healing were retarded to at least the same extent as Billingham *et al.* (1951) have already reported. The usual changes in the weights of the liver and the adrenals took place, and also provided no evidence that the effect of cortisone had been modified by the removal of the spleen.

The Effect of Splenectomy on the Leucocyte Count.

Observations on 15 rabbits for periods of 10–62 days after operation show that splenectomy is followed by a leucocytosis of about 3000 cells/c.mm., due to a rise in the number of granulocytes which more than compensates for a fall in the number of lymphocytes. The blood picture returns to normal within two weeks of the operation. Mock operations in which the spleen was handled but not removed were followed by a much smaller leucocytosis.

TABLE.—*Summary of Results.*

Animal no.* and body weight (g.) at time of operation and before autopsy.	Survival time of homografts (days).	Mean mitoses/mm. ± st. error 6-day autograft.	Contraction of wound area per cent of original area at 12 days.	Condition of scabs on ears.†			Weight of liver (g.).	Weight of both adrenals (mg.).
				6 days.	9 days.	12 days.		
S.1 2760–2570	9	10·4±0·54	44	++	++++	—	65	484
S.2 2200–2070	7	11·7±0·26	52	+	+++	—	58	623
S.3 2500–2300	7	13·4±0·61	—	++	++++	—	—	—
S.4 3200–3050	6	13·4±0·16	—	—	—	—	—	—
S.5 2480–2350	7	13·4±0·42	47	++	++++	—	68	509
S.6 2900–2450	7	10·0±0·37	51	+	++++	—	65	393
S.7 2740–3100	9	—	42	+	+++	—	93	301
COS.1 (10 mg.) 3020–2380	21	6·3±0·13	73	+	+	+++	135	225
COS.2 (5 mg.) 3080–2720	25	3·5±0·14	73	++	++	+++	166	220
COS.3 (10 mg.) 3300–3080	19	4·5±0·18	62	+	+	+++	280	195
COS.4 (5 mg.) 3090–2650	21	4·6±0·16	66	+	+	++	113	400
COS.5 (10 mg.) 2810–2440	23	3·4±0·36	60	++	++	+++	195	226
COS.6 (10 mg.) 1950–2060	‡	4·1±0·34	—	+	—	—	169	299

* S = splenectomized; COS = splenectomized and treated with cortisone (daily dose in brackets).

† Soft +; hard, dry and discoloured ++; free marginally and peel off +++; resurfaced scabs detached ++++.

‡ Died under anaesthesia on 6th post-operative day.

A profound lymphopenia follows treatment of rabbits with cortisone. The extent of this response was not altered by the absence of the spleen.

DISCUSSION.

These results appear to indicate that the spleen plays no significant part in the development of immunity to homologous skin grafts in rabbits, unless the interval between splenectomy and grafting was long enough for the body to compensate for the loss of whatever contribution the spleen might have made to the immune response. The recent results of Rowley (1950*a*, *b*) indicate, however, that there is no relation between the interval of time between splenectomy and the antigenic stimulus on the one hand, and the extent to which the immunity process is affected on the other. An alternative explanation is based on Rowley's finding that the influence of splenectomy depends on the route of administration of the antigen. Splenectomy inhibited the response to an intravenous injection of antigen, but not the response to subcutaneous or intradermal administration. When skin homografts are applied to a prepared area of granulating tissue the circumstances of absorption of an antigenic stimulus are likely to resemble those of an intradermal injection, in which absorption takes place mainly via the lymphatic system. Similarly, Mitchison (1954) has shown that passive immunity to a tumour can be transferred only by the transplantation of the lymph node draining the area where the tumour was implanted. If local production of anti-

body is predominant under our conditions of skin grafting, then it would not be surprising that removal of the spleen was without effect.

De Langen (1943) has reported that splenectomy in the rabbit is followed by a leucocytosis of 20–50,000 cells/c.mm. due entirely to a rise in the number of granulocytes. Other workers (*e.g.*, Downs, 1948 ; Dury, 1950) also assume that the total white cell count is increased. De Langen suggests, as did Singer, Miller and Dameshek (1941), that by removing a normal inhibitory mechanism, perhaps hormonal in nature, splenectomy permits the release of granulocytes from the bone marrow. Our figures do not suggest that the changes in the count are anything more than the normal concomitants of operative trauma, a conclusion reached earlier by Mole (1925) on rather fragmentary evidence.

Ungar *et al.* (1951) concluded from their experiments that the spleen was a necessary mediator of some of the effects of cortisone. Using a variety of measures for assessing the effectiveness of cortisone, which differ from those employed by Ungar *et al.*, we have been unable to show, in a different species of animal, that the activity of cortisone is in any way curtailed by the absence of the spleen. No useful explanation of this difference can be offered. It would perhaps be interesting to see whether Splenin A can modify the response of a rabbit to skin homografts.

SUMMARY.

Removal of the spleen in rabbits does not interfere with the normal evolution of the immunity reaction by which homografts of skin are destroyed.

The prolongation of the survival time of skin homografts that follows treatment with cortisone is also uninfluenced by splenectomy.

Changes in the leucocyte count after splenectomy are only concomitants of operative trauma.

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