BIOLOGY

Effect of Lymphocyte Depletion by Thoracic Duct Fistula and Administration of Anti-lymphocytic Serum on the Survival of Skin Homografts in Rats

Since lymphocytes appear to play a crucial part in the homograft reaction it seemed of interest to examine the behaviour of homografts in recipients subjected to chronic lymphocyte depletion by: (*a*) drainage of lymph through a thoracic duct fistula; (*b*) repeated administration of a hetero-specific antilymphocytic serum; (*c*) a combination of these procedures.

The use of a thoracic duct fistula is based on the observation of Gowans, McGregor, Cowen and Ford[1] that rats challenged with tetanus toxoid or sheep erythrocytes after such a fistula has been maintained for 5 days fail to produce antibodies.

The effect of a hetero-specific (rabbit-anti-rat) antilymphocytic serum on the behaviour of homografts of skin and endocrine tissues was investigated some years ago by Woodruff, Woodruff and Forman (cited by Woodruff[2]), but it was not found possible to maintain a lymphocytopenia for more than about 10 days, and no significant effect on homograft survival was observed; the serum used in the experiments recorded here has, however, proved to be much more effective.

Albino rats of an inbred strain were used as skin graft donors, and hooded rats of a second inbred strain as recipients. Fitted split skin grafts (2 cm × 2 cm) were used as described by Woodruff and Simpson[3]. Thoracic duct cannulation was performed as described by Bollman, Cain and Grindlay[4] and Gowans[5]. In rats undergoing lymph depletion the fistula was maintained for 5 days, during which time the total output was 1–2 × 10^9 lymphocytes.

Antilymphocytic serum was prepared initially by giving random bred rabbits three intraperitoneal injections of 2 × 10^8 hooded rat thoracic duct lymphocytes at weekly intervals and bleeding 10 days after the last injection. Immunized rabbits received a 'booster' intraperitoneal injection of 100–200 × 10^6 rat lymphocytes 10 days before subsequent bleeding. The serum was used without prior absorption against rat erythrocytes. It was assayed by measuring its capacity to produce a lymphocytopenia in normal hooded rats, and was accepted as satisfactory only if 2 ml. injected intraperitoneally to a 200-g rat resulted in a fall in the absolute lymphocyte count of the peripheral blood to less than 2,000/mm² in 4 h.

The treatment used and the period of homograft survival (assessed macroscopically) in the various experimental groups are set out in Table 1.

It will be seen that lymph drainage and administration of antilymphocytic serum both resulted in prolonged homograft survival; the maximum effect was obtained when treatment was begun before grafting and continued thereafter.

These results will be reported more fully elsewhere with an appraisal of their biological significance and their possible application in the surgery of replacement.

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Control of Egg Size in Chickens through Dietary Amino-acid Balance

There are conflicting reports regarding the influence of the level of protein and of specific amino-acids in the diet of the hen on egg size. Results from an experiment in this laboratory suggested that the balance, rather than absolute levels, of amino-acids determines the response to different amounts of dietary protein and to amino-acid supplementation of the laying diet. There was also evidence that egg size may be modified quickly by altering dietary amino-acid balance. The following experiment was designed to test the validity of these observations.

Forty-eight White Leghorn laying pullets, 8 months of age, were placed in individual laying cages. The percentage composition of the basal diet (1) fed was: ground wheat 69·09, ground yellow corn 10·0, soy-bean oil meal (44 per cent protein) 7·5, distillers' dried solubles 2·0, iodized salt 0·35, feeding oil (2,250 U.S.P. units vitamin A, 300 I.C.U. vitamin D_3/g) 0·5, vitamin B_{12} premix (20 mg/lb.) 0·06, stabilized animal fat 5·0, bonemeal 1·75, limestone 3·75, manganese sulphate 0·015. The other diets consisted of the basal diet supplemented with amino-acids as follows: (2) 0·25 per cent L-lysine monohydrochloride plus 0·25 per cent DL-methionine (98 per cent pure); (3) 0·255 per cent glycine; (4) 0·25 per cent L-lysine monohydrochloride plus 0·25 per cent DL-methionine plus 0·255 per cent glycine. The basal diet contained 14 per cent protein.

Table 1

Treatment*	Effect of lymphocyte count	No. of animals	Survival of skin grafts (days)	
			Individual values	Mean
Nil	Nil	12	8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 9	8
Lymph drainage from day −5 to day 0	Fell during drainage period to about half initial level; began to rise 1 week later but still below normal by time all grafts destroyed	10	11, 11, 11, 12, 12, 13, 14, 15, 15, 15	13
Antilymphocytic serum 1 ml./day i.p. on days +1 to +14 inclusive	Fell during period of treatment to about quarter initial level, then slowly increased	10	11, 11, 12, 14, 14, 14, 14, 16, 18†, 41	16
Antilymphocytic serum 2 ml./day i.p. on days −7 to −1 inclusive, and 1 ml./day i.p. on days +1 to +14 inclusive	Fell during pre-grafting treatment to about one-fifth initial level; then gradually increased for 13 days after which it fell again	10	18, 18, 22, 27, 29, 30, 30, 32, 32, 46	28
Normal rabbit serum 2 ml./day i.p. on days −7 to −1 inclusive, and 1 ml./day i.p. on days +1 to +14 inclusive	Increased slightly throughout period of treatment	10	8, 8, 8, 8; 8, 8, 8, 8, 8, 8	8
Lymph drainage from day −5 to day 0. Antilymphocytic serum 1 ml./day i.p. on days +1 to +14 inclusive	Fell during period of treatment to about one-tenth original level; then gradually rose and was almost normal 3 weeks after grafting	10	17, 18†, 22†, 26†, 33, 38, 42, 45, 54, 60	35

* The day of skin grafting is reckoned as day 0. † Animal died with healthy graft.