THE ANTIBODY RESPONSE OF RATS DEPLETED OF LYMPHOCYTES BY CHRONIC DRAINAGE FROM THE THORACIC DUCT

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Experimental procedures which deplete lymphoid tissue of small lymphocytes have provided circumstantial evidence that small lymphocytes play a part in primary immune responses. Thus, the primary antibody response of animals can be depressed or abolished by agents which damage lymphoid tissue *in vivo* (1–6) or by neonatal thymectomy which prevents its normal development (7). Although the striking histological abnormality in such animals is a severe depletion of small lymphocytes it cannot be concluded that this is the only abnormality. The view that the immunological deficiency is due solely to a lack of small lymphocytes would be greatly strengthened if the unresponsiveness could be corrected by injections of small lymphocytes from normal animals.

Adult rats can be depleted of small lymphocytes by the chronic drainage of cells from a thoracic duct fistula, and they will still give a normal secondary antibody response (8). In contrast, the present experiments have shown that the primary antibody response is severely depressed in such rats but that it can be restored by injecting small lymphocytes from other rats of the same highly inbred strain.

Methods

The general plan of the experiments was as follows. Lymph from the thoracic duct of unanesthetized rats was allowed to drain away from a fistula for 5 days. The animals were then released from their restraining cages and their fistulae closed. The primary immune response of such animals to tetanus toxoid and sheep erythrocytes was compared with the response of normal rats and with the response of lymphocyte-depleted rats which had received injections of thoracic duct cells from normal, non-immunized members of the same highly inbred strain.

Rats.—Adult male and female rats of highly inbred albino and hooded strains were used in most of the experiments. Members of a randomly bred colony were used in one experiment to study the effect of thoracic duct drainage on the weight of lymphoid tissue. At the beginning of each experiment, the animals were 10 to 16 weeks of age and weighed 180 to 240 gm.

Operative Procedures.—The thoracic duct was cannulated in its short intra-abdominal course by the method of Bollman, Cain, and Grindlay (9). During the prolonged collection of lymph the rats were kept in restraining cages (10) where they received a continuous intravenous infusion of Krebs-Ringer solution containing heparin and streptomycin. The details of

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this infusion and the general management of the restrained rats have been described by Gowans and Knight (11). Unless otherwise stated, lymph was allowed to drain away from the thoracic duct for 5 days before the rats were released from restraint. They were then anesthetized while the thoracic duct cannula was either gently pulled out or cut short and pushed under the skin. These procedures will be referred to as "closure of the fistula" although they resulted in a transient chylous ascites. However, within 1 week the lymph drainage from the intestine was reestablished: after a fatty meal, the intestinal lymphatics could be traced into a leash of vessels which followed the aorta into the thorax.

Sham-operated animals were subjected to the same operative trauma as the lymphocyte-depleted rats with the exception that the thoracic duct was not cut or ligated; a cannula was sutured in place beside it. The animals were then confined for 5 days in restraining cages while they received the same postoperative treatment as the lymphocyte-depleted rats.

Injection of Thoracic Duct Cells.—A number of lymphocyte-depleted rats were injected intravenously with thoracic duct cells from normal, non-immunized rats. Lymph from the donor animals was collected at room temperature for periods up to 12 hours in sterile flasks containing 5.0 ml of Krebs-Ringer solution to which 1.0 mg of heparin and 0.5 mg of streptomycin were added. The lymph was centrifuged at 100 *g* and the pellet of cells was resuspended in 2.0 ml of tissue culture medium "199" (12) for injection. In a number of experiments thoracic duct cells were cultured *in vitro* for 24 hours before they were injected into lymphocyte-depleted recipients. This procedure exploits the fact that large lymphocytes die more quickly than small lymphocytes during a period of incubation *in vitro*; it yields inocula containing almost exclusively small lymphocytes (13). In the account which follows, the cells in lymph with a diameter $> 8 \mu$ in smears will be referred to as "large" lymphocytes; no morphological subdivision within this group will be attempted. Suspensions of cells were injected into the tail veins 24 or 48 hours before the administration of antigen and in no case did they cause distress.

Immunization.—(*a*) Sheep erythrocytes (Wellcome Research Laboratories, Beckenham, England): the erythrocytes from whole sheep blood in Alsever's solution were washed three times in 0.85 per cent sodium chloride and made up to a 1 per cent suspension for injection. The rats were lightly anesthetized with ether, and 1.0 ml of the suspension containing approximately 10^8 erythrocytes was injected into the lateral tail vein. In some experiments sheep erythrocytes were infused intravenously over a prolonged period. For this purpose, the rats were kept in restraining cages while the cells were infused into the femoral vein at 4 ml per hour by means of a roller pump. (*b*) Tetanus toxoid (Wellcome Research Laboratories): 10 Lf of fluid tetanus toxoid in 1.0 ml was injected intraperitoneally into anesthetized rats. Three weeks later 10 Lf of the same preparation was injected intravenously into the lateral tail vein.

Measurement of Antibodies.—Samples of tail blood from anesthetized rats were allowed to stand at room temperature for approximately 3 hours before the sera were separated by centrifugation. The sera were inactivated at 56°C for 30 minutes and titrated immediately or stored at −25°C.

The antibody response to tetanus toxoid was measured by the tanned red cell hemagglutination technique of Fulthorpe (14, 15). Serial twofold dilutions of serum were made in 0.9' ml of borate buffer, pH 8.2, beginning at a dilution of 1.20; 0.1 ml of a 1.25 per cent suspension of tetanus toxoid-sensitized sheep erythrocytes was added to each tube. The tubes were shaken after the addition of the cells and left overnight at room temperature. The titrations were read the following morning and the end point was selected as the last tube which showed a smooth carpet of cells with a faint ring of unagglutinated cells at the periphery.

Hemolysin titrations were performed in plastic agglutination trays and a standard rabbit hemolytic serum was included with each group of seven test sera. Serial twofold dilutions of serum were made in veronal buffer, pH 7.2 (Oxo Ltd., London, England), beginning at a dilution of 1:2.5. Sheep erythrocytes were washed three times in 0.85 per cent sodium chloride,

once in veronal buffer, and then resuspended in buffer to make a 1 per cent suspension. One volume of sheep erythrocytes was added to two volumes of diluted serum and the suspension was incubated at 37°C for 30 minutes. Preserved guinea pig serum (Wellcome Research Laboratories) was diluted with seven parts of distilled water immediately before use. One volume of this solution (corresponding to a 1:10 dilution of guinea pig serum in saline) was added to the suspension of sheep erythrocytes which was incubated at 37°C for a further 30 minutes. The cells were resuspended after 15 and 30 minutes and the end point was selected as the last dilution of serum which showed complete hemolysis after 30 minutes' incubation. The immune response was expressed as a peak titer and a "mean total titer." The mean total titer was a measure of the total amount of antibody formed during the first 3 weeks; it was the mean of the hemolysin titers, 4, 6, 8, 10, 14, and 21 days after the injection of sheep erythrocytes. Some rats had a low titer of hemolysin in their serum before any injection of sheep erythrocytes; these rats were excluded from the experiments.

TABLE I

Mean Output of Lymphocytes from the Thoracic Duct of 15 Inbred Rats on Successive Days after Cannulation

The rats were males and females, 10 to 16 weeks of age and 180 to 240 gm body weight

Days after cannulation	Daily output of lymphocytess ($\times 10^9$)	
	Mean and SD	Range
1	1028 ± 197	768 – 1349
2	659 ± 148	341 – 888
3	378 ± 90	245 – 511
4	263 ± 49	170 – 331
5	200 ± 38	125 – 281
Total	2564 ± 390	1685 – 3163

RESULTS

The Effect of Lymph Drainage on Lymphoid Tissue.—The output of lymphocytes from the thoracic duct fell progressively during the 5 day period of lymph drainage (16, 17) and in 15 rats a mean of about 2.5×10^9 cells was collected from the fistula (Table I). The depletion of lymphocytes which followed the drainage of lymph was reflected in a reduction in the size of all the lymph nodes. Table II records the weights of lymph nodes from lymphocyte-depleted rats in which cell-free lymph was continuously reinfused into the femoral vein during the period of drainage. The nodes weighed much less than those from normal or sham-operated rats of the same age and weight. This decrease in weight occurred both in the mesenteric lymph node and in lymph nodes whose efferent lymphatics did not drain into the fistula. Small lymphocytes normally circulate from blood to lymph through all the lymph nodes of the animal (11, 19): drainage from the thoracic duct would therefore be expected to cause a general fall in their weight. The thymus was smaller in both the sham-operated and lymphocyte-depleted animals. An examination of many animals revealed a considerable variation in the size of the spleen after chronic drainage

of lymph from the thoracic duct; both of the lymphocyte-depleted rats recorded in Table II had spleens that were heavier than normal.

The main histological change in the lymph nodes of lymphocyte-depleted rats was a gross depletion of the cortical small lymphocytes; the medullary areas were normal. The periarteriolar lymphocyte sheaths of the spleen were reduced to small cuffs of cells. The reticulum structure of the spleen was normal and there was no obvious reduction in the capacity of cells in the sinuses of the red pulp and in the perifollicular zone to take up colloidal carbon or colloidal iron after an intravenous injection.

Chronic drainage of lymph from a thoracic duct fistula resulted in a marked

TABLE II

Wet Weight of Lymphoid Tissue from Normal, Sham-Operated, and Lymphocyte-Depleted Rats Belonging to a Randomly Bred Albino Colony

They were 12 to 14 weeks of age and 215 to 235 gm body weight.

No. of rats	Treatment	Thymus	Spleen	Mesenteric node	Other nodes*	Lymphocytes from fistula
		mg	*mg*	*mg*	*mg*	*mg*
8‡	None	252 ± 27	721 ± 150	256 ± 23	586 ± 59	—
2	Sham-operated	170, 187	908, 1132	230, 231	492, 557	—
2	Lymphocyte-depleted	115, 125	905, 1030	110, 123	286, 355	326§

* Mesocolic, portal, postgastric, renal, paraortic, iliac, sacral, posterior abdominal wall, inguinal, popliteal, superficial and deep mediastinal, superficial and deep cervical, posterior and internal axillary lymph nodes (nomenclature from Sanders and Florey, 18).

‡ Mean and standard deviation.

§ Packed cells from lymph collected during 5 days' drainage from thoracic duct of 1 rat.

lymphopenia. As shown in Fig. 1 the number of small lymphocytes in the peripheral blood returned slowly to normal after the fistula was closed.

The Normal Response to Tetanus Toxoid.—A single intraperitoneal injection of tetanus toxoid into a normal rat gave no response that could be measured by the tanned red cell hemagglutination technique. The response to a second intravenous injection of toxoid was therefore taken as evidence of sensitization by the first injection. Twelve inbred rats were challenged with an intravenous injection of tetanus toxoid 3 weeks after a sensitizing injection. As shown in Fig. 2 antibody was first detected between the 2nd and 4th day after the second injection and rose to a maximum on the 6th day after which there was a gradual fall.

The Effect of Lymphocyte Depletion on the Primary Immune Response to Tetanus Toxoid.—Fig. 2 shows the response of 5 inbred rats which received a first injection of tetanus toxoid immediately after closure of the fistula. When these animals were challenged with tetanus toxoid 3 weeks later, they failed to show

an antibody response that could be measured by the tanned red cell hemagglutination technique. No antitoxin could be detected in the sera of these rats by an *in vivo* assay in mice 6 days after the second injection of tetanus toxoid; the inability to detect antibody by this method indicated that there was less than 0.01 units of tetanus antitoxin per ml of serum.

The Effect of Lymphocyte Depletion on the Secondary Immune Response to Tetanus Toxoid.—The effect of lymphocyte depletion on the secondary immune

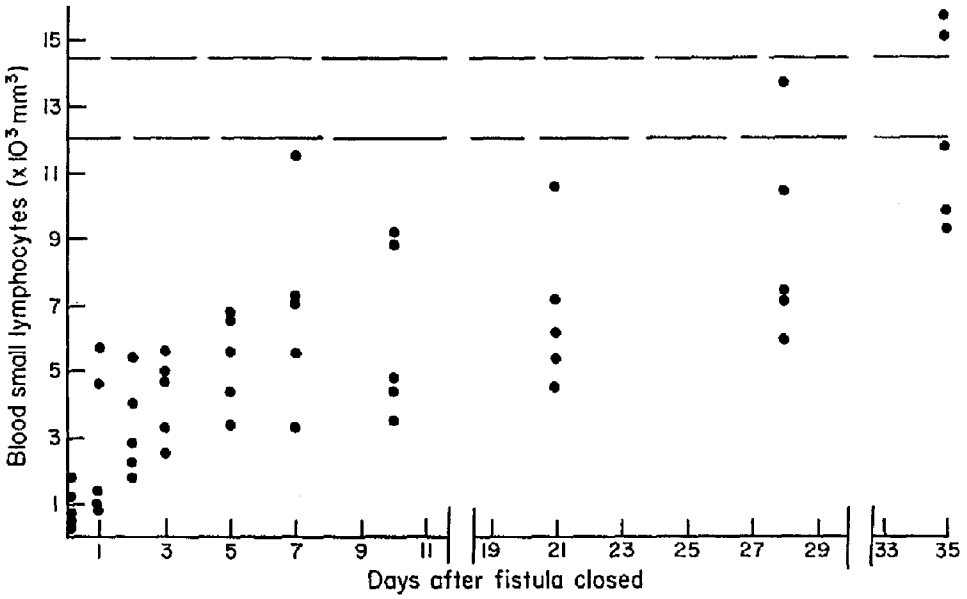

FIG. 1. Concentration of small lymphocytes in the peripheral blood of 5 lymphocyte-depleted rats at various times after the closure of a thoracic duct fistula. The interrupted horizontal lines show the range of values in 10 normal rats of the same inbred strain. At the time of cannulation the rats were 10 to 16 weeks of age and 180 to 230 gm body weight.

response was studied by injecting 2 inbred rats with a second injection of tetanus toxoid immediately after the closure of the thoracic duct fistula, the sensitizing injection having been given 3 weeks earlier. These animals showed a brisk antibody response (Fig. 2).

The Effect of Lymphocyte Depletion on the Primary Immune Response to Sheep Erythrocytes.—The hemolysin response to a single intravenous injection of 10^8 sheep erythrocytes became progressively smaller when the interval between cannulation and closure of the fistula was increased. Fig. 3 shows that the response was slightly depressed after drainage for 2 days while it was virtually abolished in 9 out of 10 rats after drainage for 5 days. However, when the same dose of sheep red cells was infused intravenously over a period of 12 hours (as opposed to being given as a single rapid injection) a slight antibody response

was observed in rats which had been drained for 5 days. The response was greater when the dose of antigen was increased. The response of normal rats was also greater when large doses of sheep red cells were infused. These results are recorded in Table III.

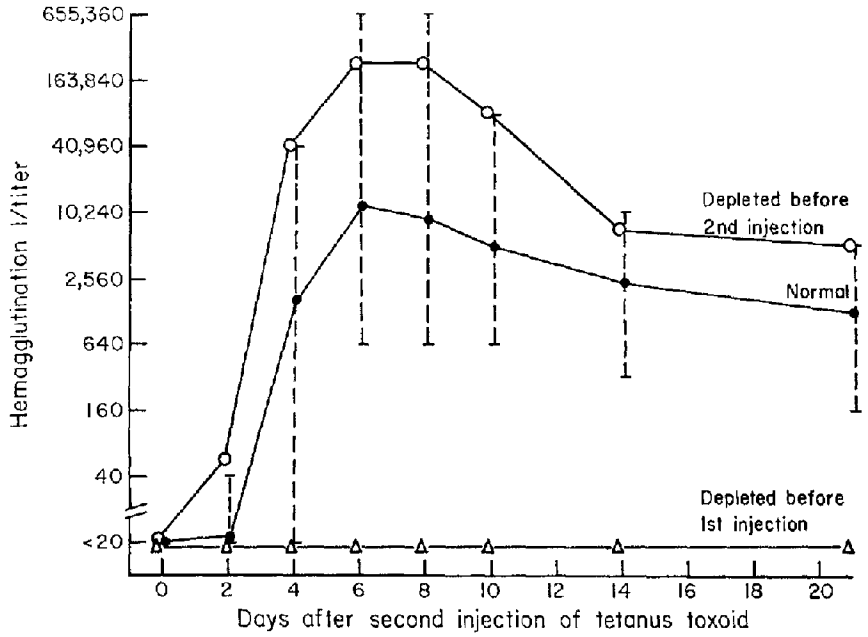

FIG. 2. Response to tetanus toxoid in normal and lymphocyte-depleted rats. Each point is the mean hemagglutination titer at various times after the second of two doses of 10 Lf of fluid toxoid given 3 weeks apart; first dose intraperitoneally; second dose intravenously. ●——●: the mean response of 12 normal rats. The interrupted vertical lines represent the range of titers in this group. △——△: the mean response of 5 rats in which the first dose of toxoid was given immediately after closure of the fistula. ○——○: the mean response of 2 rats in which the second dose of toxoid was given immediately after the fistula was closed; the first dose was given 3 weeks before the thoracic duct was cannulated. The primary immune response but not the secondary immune response was affected by thoracic duct drainage.

The chronic drainage of lymph and cells from the thoracic duct had no significant effect on the hemolysin response once antibody formation was initiated. This was demonstrated by injecting 2 rats with sheep erythrocytes immediately after cannulation of the thoracic duct and allowing the lymph to drain away for 5 days. As shown in Fig. 4 these rats gave a normal hemolysin response.

Recovery from the Effects of Lymphocyte Depletion.—To determine the duration of the recovery period which followed the chronic drainage of lymphocytes from the thoracic duct, a single intravenous injection of sheep erythrocytes

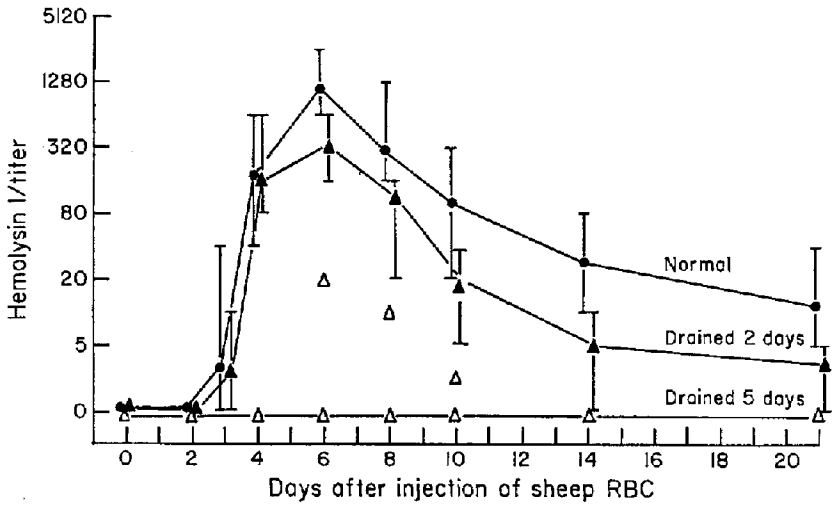

FIG. 3. Hemolysin response to a single intravenous injection of 10^8 sheep erythrocytes in normal and lymphocyte-depleted rats. ●——●: the mean response of 14 normal rats. ▲——▲: the mean response of 4 rats was slightly depressed after 2 days' drainage from a thoracic duct fistula. △——△: the mean response of 10 rats after 5 days' drainage from a thoracic duct fistula; the response was abolished in 9 out of 10 rats and in 1 rat it was greatly depressed. The vertical lines show the range of the hemolysin titers.

TABLE III

Hemolysin Response to a 12 Hour Intravenous Infusion of Sheep Erythrocytes in Normal and Lymphocyte-Depleted Rats

No. of rats	Treatment	Dose of sheep RBC ($\times 10^8$)	1/Peak titer	1/Mean total titer
2	None	1	640, 640	40, 80
2		10	5120, 5120	160, 160
2		250	20480, 80920	1280, 2560
2	Lymphocyte-depleted*	1	20, 20	10, 10
2		10	80, 320	20, 20
4		250	160, 320, 1280, 2560	40, 80, 160, 320

* Lymph drained from thoracic duct fistula for 5 days; infusion of erythrocytes begun immediately after fistula closed.

was given at various times after the closure of the fistula. The peak titer and the mean total titer were used to measure the immune response. Fig. 5 shows that a normal antibody response was elicited approximately 4 weeks after the fistula was closed.

Reversal of Unresponsiveness by the Injection of Thoracic Duct Cells.—It was necessary to determine whether loss of lymphocytes alone was responsible for the immunological unresponsiveness which followed chronic drainage from the thoracic duct or whether other factors such as operative trauma and restraint played a part. An attempt was therefore made to reverse the unresponsive state

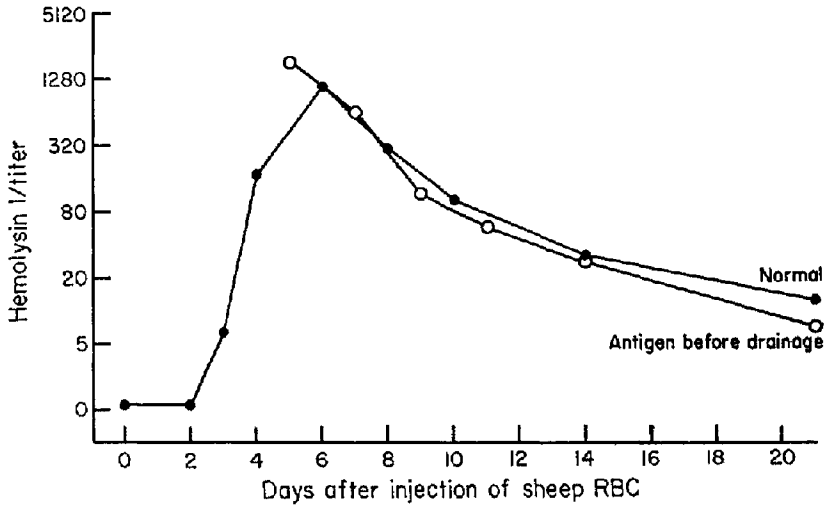

FIG. 4. Hemolysin response to a single intravenous injection of 10^8 sheep erythrocytes in normal rats and in rats injected with sheep erythrocytes immediately before the drainage of lymph from a thoracic duct fistula. ●——●: the mean response of 14 normal rats. ○——○: the mean response of 2 rats injected with sheep erythrocytes immediately after the thoracic duct was cannulated. Lymph was then allowed to drain from the duct for 5 days before the fistula was closed. The response was unaffected when antigen was given before drainage from the thoracic duct had started.

by injecting lymphocyte-depleted rats with thoracic duct cells from normal non-immunized rats of the same highly inbred strain.

In the first experiment sheep erythrocytes alone were injected 7 days after the fistula was closed. During the interval between closure of the fistula and the injection of antigen the rats were housed under normal laboratory conditions. The animals rapidly regained the weight they had lost while under restraint, but they still showed a greatly depressed hemolysin response (Fig. 6). Five inbred rats were injected intravenously with 10^9 thoracic duct cells from normal rats of the same highly inbred strain 6 days after the fistula was closed and 2 rats were injected with 10^9 cells that were first disintegrated by ultrasonic vibration. All the animals were injected intravenously with sheep erythrocytes 24 hours later.

Fig. 6 shows that the antibody response of lymphocyte-depleted rats was restored by living thoracic duct cells but not by disintegrated cells. The interval of 6 days between closure of the fistula and the injection of thoracic duct cells

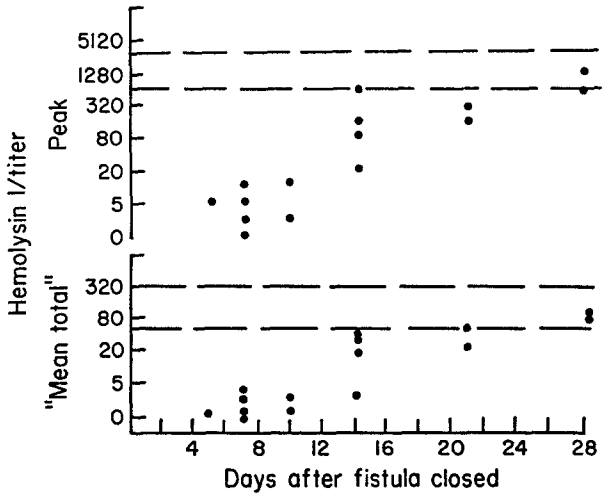

FIG. 5. Hemolysin response to a single intravenous injection of 10^8 sheep erythrocytes in lymphocyte-depleted rats at various times after closure of a thoracic duct fistula. Each point on graph represents the response of a single rat. The interrupted horizontal lines enclose the range of response of 14 normal rats. Lymphocyte-depleted rats showed a normal hemolysin response approximately 4 weeks after the fistula was closed.

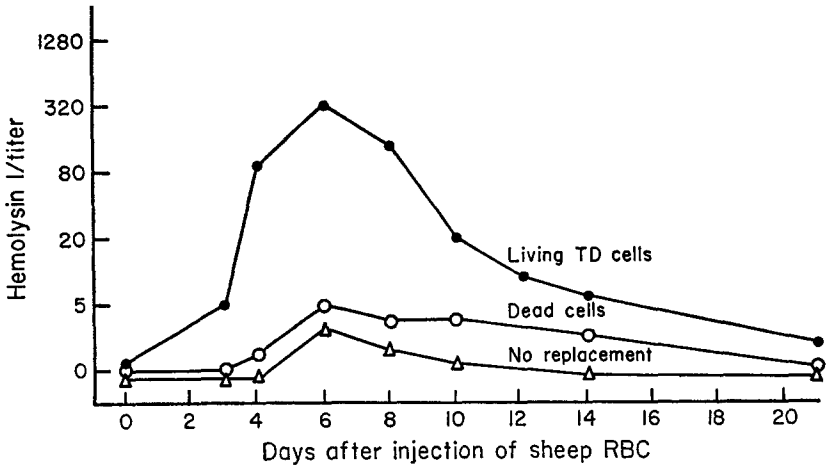

FIG. 6. Hemolysin response to a single intravenous injection of 10^8 sheep erythrocytes in lymphocyte-depleted rats injected intravenously with living and disintegrated thoracic duct cells. Lymphocyte-depleted rats were injected with either 10^9 living thoracic duct cells (●——● mean of 5 rats) or 10^9 disintegrated thoracic duct cells (○——○ mean of 2 rats) 6 days after the fistula was closed and challenged with sheep erythrocytes 24 hours later. △——△: the mean response of 4 rats given sheep erythrocytes alone 7 days after the closure of a thoracic duct fistula. The hemolysin response which was severely depressed in lymphocyte-depleted rats could be restored by living thoracic duct cells but not by disintegrated cells.

allowed the animals to recover from the possible effects of restraint. However, this interval was found not to be necessary; the response of lymphocyte-depleted rats could be restored by an injection of 10^9 lymphocytes immediately after the fistula was closed.

Reversal of Unresponsiveness by Small Lymphocytes.—An attempt was made to determine the cell type in thoracic duct lymph which restored the capacity of lymphocyte-depleted rats to respond to sheep erythrocytes. Advantage was taken of the fact that most of the large lymphocytes die when thoracic duct

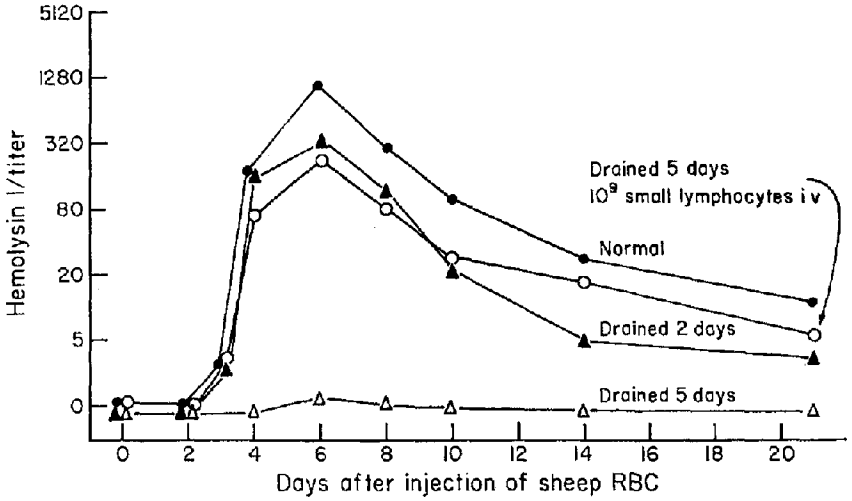

FIG. 7. Hemolysin response to a single intravenous injection of 10^8 sheep erythrocytes in normal rats and in lymphocyte-depleted rats injected intravenously with small lymphocytes (see text). ●——●: the mean response of 14 normal rats. ▲——▲: the mean response of 4 rats after drainage from a thoracic duct fistula for 2 days. △——△: the mean response of 10 rats after 5 days' drainage. ○——○: the mean response of 4 rats depleted of lymphocytes for 5 days and then injected with 10^9 small lymphocytes.

cells are cultivated *in vitro* for 24 hours (13). Differential counts on 2000 cells showed that the donor inocula prepared in this way contained <0.05 to 0.25 per cent large lymphocytes; the remainder of the cells were typical small lymphocytes. Immediately after their fistulae were closed, 4 lymphocyte-depleted rats were injected intravenously with 10^9 incubated thoracic duct cells. Fig. 7 shows that these rats gave a hemolysin response when 10^8 sheep erythrocytes were injected intravenously 2 days later but that this response was lower than the hemolysin response of normal rats. However, Table I showed that approximately 10^9 lymphocytes normally emerge from a thoracic duct fistula between the 2nd and 5th day after cannulation. It can be seen from Fig. 7 that the hemolysin response of lymphocyte-depleted rats injected with 10^9

incubated thoracic duct cells was approximately equal to the hemolysin response of rats after 2 days' drainage from the thoracic duct.

The Influence of "Stress".—Sham operations were performed on 2 rats to determine the effect of operative trauma and restraint on the hemolysin response. Fig. 8 shows that sham-operated animals, immediately after they were released from their restraining cages, gave a normal hemolysin response to an intravenous injection of sheep erythrocytes.

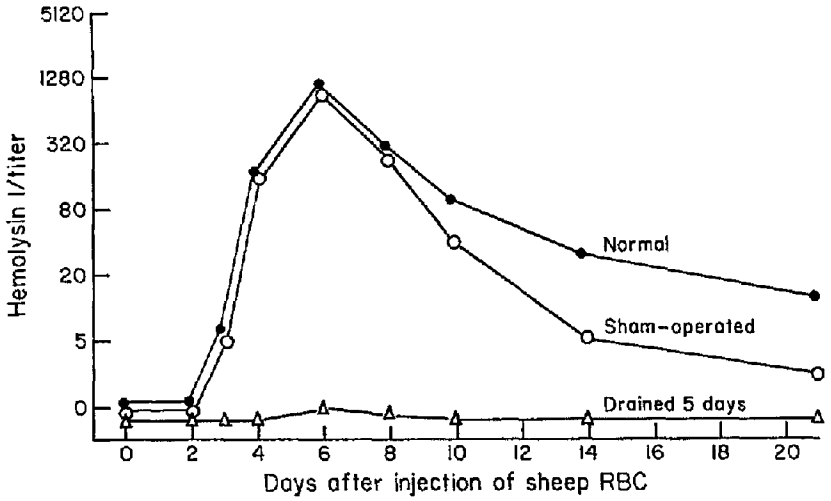

FIG. 8. The effect of sham operation and restraint on the hemolysin response to a single injection of 10^8 sheep erythrocytes. ●——●: the mean response of 14 normal rats. ○——○: the mean response of 2 rats which had been sham-operated and confined in restraining cages for 5 days. △——△: the mean response of 10 lymphocyte-depleted rats. Sham-operated animals showed a normal hemolysin response. The hemolysin response was severely depressed in lymphocyte-depleted rats.

It was unlikely that stress factors mediated by the adrenal cortex contributed to the unresponsive state in lymphocyte-depleted rats because their antibody-forming capacity was still severely impaired 1 week after the fistula was closed. The lymphatic drainage from the small intestine had been reestablished by this time and the animals had regained the weight which they had lost while under restraint. Further, sham-operated animals gave a normal response.

Nevertheless, the influence of stress was studied by removing the left adrenal gland from 2 rats on the day that the thoracic duct was cannulated, the right adrenal gland having been removed 4 weeks before. On the day before cannulation and for the next 5 days, the animals were given 1.0 mg of cortisone acetate (Roussel Laboratories, London, England) per 100 gm body weight intramuscularly and the same dose every 2nd day thereafter. This dose of cortisone was less than the minimum amount which has been shown to reduce the primary hemoly-

sin response to sheep erythrocytes in rats (3). Two other rats were subjected to bilateral adrenalectomy but the thoracic duct was not cannulated; these animals received the same amount of cortisone as the lymphocyte-depleted rats. All the animals were given an intravenous injection of sheep erythrocytes 5 days after complete adrenalectomy.

The results recorded in Fig. 9 show that adrenalectomy alone had no effect on the hemolysin response, but the response was abolished when adrenalectomy was combined with cannulation of the thoracic duct.

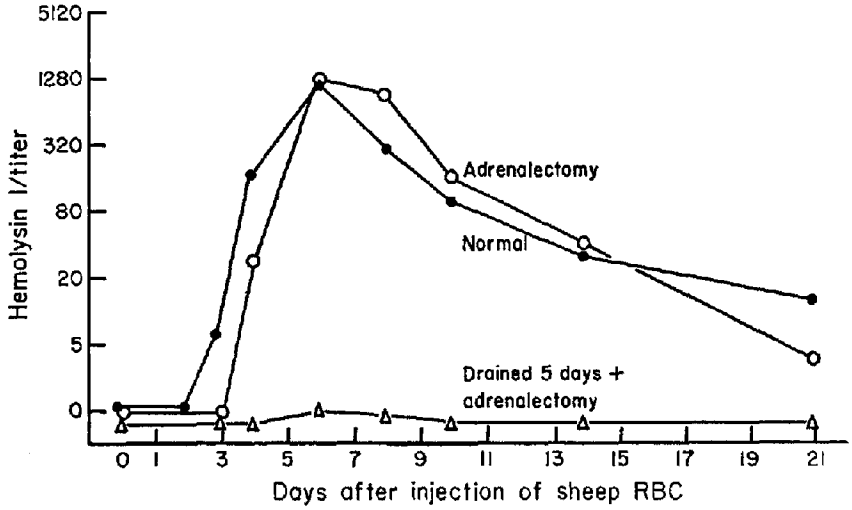

FIG. 9. The effect of bilateral adrenalectomy on the hemolysin response of normal and lymphocyte-depleted rats to a single intravenous injection of 10^8 sheep erythrocytes. ●——●: the mean response of 14 normal rats. ○——○: the mean response of 2 adrenalectomized rats maintained on cortisone (see text). △——△: the mean response of 2 adrenalectomized rats maintained on the same dose of cortisone but in which adrenalectomy was combined with drainage from a thoracic duct fistula for 5 days. The hemolysin response of adrenalectomized rats was normal but it was abolished when adrenalectomy was combined with thoracic duct drainage.

DISCUSSION

During the 5 days which followed cannulation of the thoracic duct in rats approximately 2.5×10^9 cells emerged from the fistula: about 90 per cent were typical small lymphocytes and the remainder were larger lymphocytes. The present investigation showed that the loss of these cells from the fistula severely depressed or abolished the primary immune response of rats to a small dose of either tetanus toxoid or sheep erythrocytes. The unresponsive state was related to the loss of cells because the inability to respond to sheep erythrocytes could be corrected by an injection of thoracic duct lymphocytes from normal, non-immunized rats. Since it was corrected equally well by injecting suspensions of

thoracic duct cells from which large lymphocytes had been virtually eliminated, it is likely that the immunological unresponsiveness was due to a loss of small lymphocytes from the fistula.

It is unlikely that operative trauma or the "stress" of restraint influenced the immunological status of rats after chronic drainage from the thoracic duct. Sham-operated rats treated in the same way as the lymphocyte-depleted animals showed a normal primary immune response when injected with sheep erythrocytes immediately after they were released from restraint. It was also shown that adrenalectomized rats which were maintained with cortisone gave no immune response to sheep erythrocytes when adrenalectomy was combined with thoracic duct drainage. The administration of cortisone could not account for the failure of these rats to respond because adrenalectomized rats maintained on the same dose of cortisone showed a normal hemolysin response.

The appearance of the lymphoid tissue of rats after chronic drainage from the thoracic duct supports the idea that their immunological deficiency is due to a lack of small lymphocytes. The drainage of about 2.25×10^9 small lymphocytes from the fistula caused a reduction in the size of all the lymph nodes, a gross depletion of small lymphocytes in the cortex of the nodes and in the periarteriolar lymphocyte sheaths of the spleen, and a marked lymphopenia. The small lymphocytes which emerge from the thoracic duct of the rat are part of a pool of cells which, in the intact animal, recirculate from the blood to the lymph through all its lymph nodes (11, 19). Thus, drainage from the thoracic duct depletes all the nodes of their small lymphocytes and not merely those which lie in the intestinal lymphatic bed. However, even very prolonged drainage did not remove all the small lymphocytes from lymphoid tissue. Similar changes have been described in the lymphoid tissue of rats thymectomized at birth and the immunological inadequacy of these animals has also been attributed to a lack of small lymphocytes (7, 20).

The lymphocyte-depleted rats were not absolutely unresponsive: they responded if the dose of antigen was increased. Thus very large doses of sheep erythrocytes evoked a hemolysin response after drainage from the thoracic duct and gave a correspondingly greater response in normal rats. A dose-response effect of this kind has also been reported in normal and heavily x-irradiated rabbits after injections of sheep erythrocytes (21). A similar phenomenon was seen in the response of lymphocyte-depleted rats to first-set homografts of skin (22). Here the major factor governing the response is not the size of the graft but the genetic disparity between donor and recipient. Grafts which were exchanged between members of a randomly bred albino colony were normally shed in about 14 days; many have survived for more than 300 days in lymphocyte-depleted rats. On the other hand, grafts from inbred hooded rats were shed in 10 days by randomly bred albino recipients and only survived 1 to 5 days longer in lymphocyte-depleted animals. The significance of these observations is

not clear. It is not yet possible to define the response to "strong" and "weak" antigenic stimuli in cellular terms.

It might be objected that the hemolysin response is not a true primary response because some rats, which were excluded from the experiments, showed a low titer of antibody before they were injected with sheep erythrocytes. Nevertheless, the response to first injections of sheep erythrocytes and tetanus toxoid were similar in that both were abolished by depletion of lymphocytes. The secondary response to tetanus toxoid, on the other hand, was unaffected by this procedure. Rats in which drainage from the thoracic duct was carried out 3 weeks after a first dose of tetanus toxoid and immediately before the second dose gave an antibody response which was, if anything, greater than normal. The secondary response, unlike the primary response, is mediated by cells which cannot be withdrawn from lymphoid tissue by drainage from a thoracic duct fistula. This does not mean that in the intact animal circulating lymphocytes play no part in secondary responses. Mitchell and Gowans (8) found that a "secondary" response to diphtheria toxoid could be obtained in normal rats after a single dose of antigen if this was preceded by an injection of thoracic duct lymphocytes from primarily immunized donors. Clearly some of the donors' lymphocytes (either large or small) had become specifically sensitized during primary immunization.

The present experiments suggest that recirculating small lymphocytes are involved in the primary immune response of rats to small doses of both particulate and soluble antigens. They are probably concerned in an early phase of the response since lymphocyte depletion had no effect when the antigen was given immediately before drainage from the duct. Similar observations have been made when treatment with cortisone (23) or exposure to large doses of ionizing radiation (1) are preceded by a primary antigenic stimulus. Experiments on graft-*versus*-host reactions (13) have shown that small lymphocytes can *initiate* an immunological response, in the sense that they react with antigens in the tissues of the host and set in train the processes which eventually kill it. It would be tempting to suppose that antibody formation may also be initiated by the reaction of small lymphocytes with antigens.

The process of recirculation could itself have an immunological significance. If, as the present work suggests, small lymphocytes play an essential part in primary antibody responses, and they are also a heterogenous population in the sense proposed by Burnet (24), then their recirculation could ensure the selection into the spleen or into any one regional node of those few cells which, for genetic or other reasons, can alone respond to a particular antigen. A similar recruitment into regional lymphoid tissue could occur during the secondary response if primary immunization contributed specifically sensitized small lymphocytes to the total lymphocyte pool (8).

The present experiments have provided no information about the way in

which small lymphocytes mediate primary responses. One difficulty is that antibody is not produced by small lymphocytes but by a line of cells which divides and differentiates to form plasma cells (25, 26). However, it is now clear that the small lymphocyte is not an "end" cell but that it can develop rapidly into a "large pyroninophilic cell" which divides and which resembles morphologically a plasma cell precursor (13). Keuning and his coworkers have shown that such large pyroninophilic cells appear in the white pulp of the spleen (27) and in the cortex of the lymph nodes (28) during the early stages of the primary antibody response in rabbits and have suggested on histological grounds that they arise from small lymphocytes and develop into plasma cells. It would be interesting to know if the dividing "large lymphocytes" which Nossal and Mäkelä (29) have identified in imprints of the regional nodes of rats after primary antigenic stimulation would, in sections, have the morphological characteristics of the large pyroninophilic cell. Although there are strong grounds for supposing that small lymphocytes may be the ultimate precursors of antibody-forming cells it must be emphasized that this has not yet been unequivocally established.

The simplest hypothesis would be as follows. Primary immune responses are initiated by the interaction of small lymphocytes with antigen or possibly with antigen which has been "processed" by reticuloendothelial phagocytes. The extensive studies of Harris (30) could be interpreted in this way. After contact with antigen the small lymphocytes become fixed in lymphoid tissue and no longer circulate between blood and lymph (13); thus, drainage from the thoracic duct immediately after the administration of antigen does not affect the primary response. In the lymphoid tissue the fixed lymphocytes enlarge and give rise to a dividing cell line which perpetuates itself and produces a small number of plasma cells (29). In the secondary response further contact with antigen greatly increases the rate at which the dividing cells produce plasma cells (29, 31). The precise location of the dividing cells within the lymph nodes has not been determined. Aggregates of them may possibly constitute germinal centres which, during secondary responses, either synthesize antibody (32) or generate antibody-forming cells (33).

These speculations rest on the assumption that small lymphocytes participate in primary responses by generating the cells which eventually synthesize antibody. If this assumption is false then the only alternative is that small lymphocytes transfer some antigen-conditioned material to other cell types. There are only the vaguest precedents for such a mechanism.

SUMMARY

The chronic drainage of lymph and cells from a thoracic duct fistula in rats results in a reduction in the weight of all the lymph nodes and of their content of small lymphocytes. The primary immune response to tetanus toxoid or sheep

erythrocytes is severely depressed or abolished in such animals. This unresponsive state is related to the loss of lymphocytes from the thoracic duct fistula and not to stress factors ensuing from the trauma of operation and restraint; it can be reversed by injecting inocula which contain almost exclusively small lymphocytes. In contrast to the severe impairment of the primary immune response in lymphocyte-depleted rats, such animals show a normal response to a second injection of tetanus toxoid. The mechanism by which small lymphocytes mediate the primary immune response is discussed.

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