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The Effect of Thymectomy on the Lymphoid Tissues of the Mouse

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THE thymus has been shown to produce a lymphocytosis stimulating factor (LSF) (Metcalf, 1956). On injection into baby mice or thymectomized adult mice, thymic LSF produces a temporary lymphocytosis reaching its maximum point 3–7 days after injection (Metcalf, 1959).

The present experiments were carried out to observe the effect of thymectomy in mice on peripheral lymphocyte levels and lymphoid organs.

The results indicate that thymectomy leads to a fall in peripheral lymphocyte levels and an atrophy of the lymphoid organs. The findings also suggest that the mode of action of thymic LSF may be stimulation of the division of large and medium lymphocytes and their maturation into small lymphocytes.

MATERIALS AND METHODS

Mice

Mice used were males of the inbred strain $C_{57}Bl$ obtained from the Jackson Memorial Laboratory, Bar Harbour, Maine.

The mice were housed in tins, four to a tin, and fed purina chow and drinking water, *ad lib.* The temperature of the animal room was kept at 75° F. (24° C.).

Thymectomy

Thymectomy and sham thymectomy were performed when the mice were between 4–6 weeks of age. The operative technique used was that of Kaplan (1950), the anaesthetic being combined intra-peritoneal nembutal and open ether. Sham-thymectomized mice were submitted to the full operative procedure, including opening the thoracic cavity, but the thymuses were left intact. Operative wounds were closed with black silk.

Post-operatively, the mice were administered oral 'Terramycin' (oxytetracycline) in their drinking water (8 g. per gallon or 4.5 litres) for 2 weeks.

Mortality during the operations ranged between 5–25 per cent and deaths were due, either to acute blood loss or excess anaesthesia in association with collapse of both lungs. No deaths occurred in the post-operative period or in the subsequent weeks of observation.

White-Cell Counts

Absolute and differential white-cell counts were performed on tail blood obtained by pricking the tail vessels with a sharp scalpel blade. Absolute white-cell counts were performed using human white-cell diluting pipettes and haemocytometers. The diluting pipettes were

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filled with undiluted blood to the first division only on the stem of the pipette – the final dilution of the blood in the pipette being, therefore, 1 in 100.

Diluting fluid used was 3 per cent acetic acid with a trace of methylene blue added. Fuchs-Rosenthal counting chambers with a well depth of 0.2 mm. were used throughout these experiments.

Differential white-cell counts were performed on slide preparations stained with Wright's stain. Two hundred cells were counted following a fixed sampling pattern.

Haematocrit Estimations

Haematocrit estimations were performed on tail blood using capillary haematocrit tubes. The tubes were sealed, centrifuged at 3000 rev./min. for 3 minutes and the haematocrit value (PCV) read using a Drummond capillary haematocrit scaler.

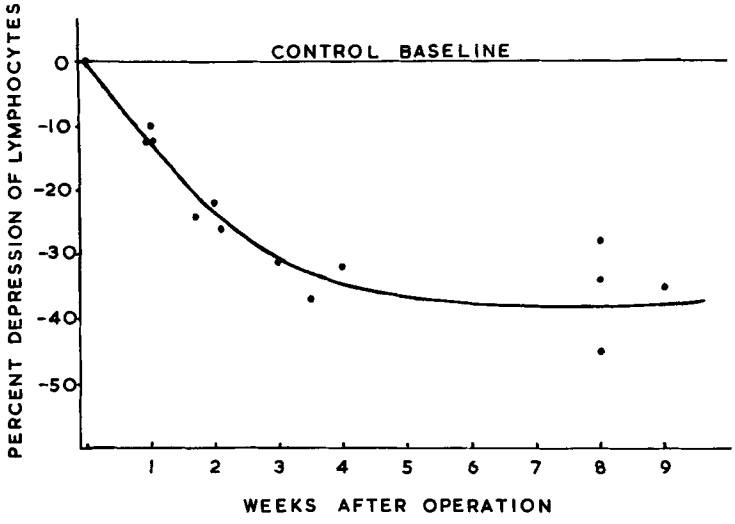

FIG. 1. Effect of thymectomy on the peripheral blood lymphocyte levels of $C_{57}Bl$ mice. Each point is the mean value of four thymectomized mice compared with the mean value for a paired group of sham-thymectomized mice.

Lymphoid Organ Investigations

At various times after operation, groups of thymectomized and sham-thymectomized mice were killed with ether. The mice were weighed, then autopsied. Liver, thymus and spleen weights were determined by weighing the organs wet, using a torsion balance. Inguinal lymph node weights were determined by dissecting the nodes free of fat and weighing both nodes together.

The lymphoid tissues were then fixed in Zenker's formalin, blocked in paraffin, sectioned at 5 μ, and stained lightly with haematoxylin and eosin.

Mice were inspected at post mortem for completeness of thymectomy. Thymectomy was complete in all mice reported in this series.

None of the mice showed evidence of intercurrent infections and no mice were found with any neoplastic disease – in particular lymphatic leukaemia.

Two mice, one thymectomized one sham-thymectomized, were found at autopsy to have inguinal lymph node enlargement and splenomegaly. Histologically this was found to be due

to generalized sinus reticuloendotheliosis, a disease occurring sporadically in our colony. These mice were excluded from the series.

No mice were found with infected operation sites and no skin lesions, traumatic or infective, were present in the two groups.

Statistical Analysis

Analysis of the results for statistically significant differences was made using the Student 't' series method.

RESULTS

Effect of Thymectomy on Peripheral Lymphocyte Levels

White-cell counts were performed on paired groups of four thymectomized and four sham-thymectomized mice at weekly intervals following operation. Following thymectomy, a slow progressive fall in circulating lymphocyte levels was found relative to levels in sham-operated mice (Fig. 1). This fall became maximal about 4 weeks after operation, reaching levels of 30–40 per cent below those of the sham-operated control mice. This depression in circulating lymphocyte levels was found to persist for the duration of the observation period, 4 months. The lymphocytes in the peripheral blood of thymectomized mice were normal in shape and size, both large and small lymphocytes being present in the same proportions as in normal adult mouse blood.

No significant alteration occurred in polymorph levels, although in some groups of thymectomized mice, elevated levels were found up to 50 per cent above those of the control groups.

Monocyte and eosinophil levels appeared unaffected by thymectomy.

No alteration in haematocrit values was found at any stage post-operatively in thymectomized mice, nor were any nucleated red cells found in the peripheral blood. Reticulocyte counts were not performed.

Effect of Thymectomy of Lymphoid Organ Weights

The effect of thymectomy on lymphoid organ weights was assessed by killing paired groups of thymectomized and control mice and weighing the various organs in the wet state. Mice were killed and examined at either 6 weeks, or 3 months after operation. Whole body weight and liver weight were used as an index of general body growth and nutrition. The lymphoid organs weighed were the spleen and both subcutaneous inguinal nodes. The thymuses of control mice were also weighed.

Results from the paired groups of mice have been pooled as shown in Tables I and II.

As may be seen from these tables, thymectomy did not significantly affect body growth or the weight of a non-lymphopoietic organ, the liver, during the 3-month post-operative period covered by these investigations.

The thymuses in the control mice were average weight for mice of this age, strain and sex. Since both thymectomized and sham-thymectomized groups of mice were housed similarly, had the same diet, and were subjected to the same environmental stimuli, the normal thymus weights of the control mice suggest that the mice were not exposed to any unusual lymphocytolytic stresses.

The weights of the inguinal lymph nodes were consistently lower in the thymectomized animals than in the controls, at both 6 weeks and 3 months post-operation. Thymectomy

was followed by an approximate 25 per cent fall in weights of these organs. This difference was statistically significant at a level of $P<0.01$.

Spleen weights showed no significant decrease in thymectomized mice 6 weeks after operation, but were 25 per cent below those of control mice 3 months after operation. This latter difference was statistically significant at a level of $P<0.001$. Considerable variation

TABLE I

EFFECT OF THYMECTOMY 6 WEEKS PREVIOUSLY ON LYMPHOID ORGAN WEIGHTS

	Number of mice	*Mean body weight g.*	*Mean spleen weight mg.*	*Mean inguinal lymph nodes weight mg.*	*Mean thymus weight mg.*
Sham thymectomy	33	24.7±2.5*	90±24	13±2.4	62±10
Thymectomy	26	25.2±2.5	83±22	9±2.3	...
Statistical significance of difference	...	NS	NS	P<0.001	...

* ± Standard deviation.

TABLE II

EFFECT OF THYMECTOMY 3 MONTHS PREVIOUSLY ON LYMPHOID ORGAN WEIGHTS

	Number of mice	*Mean body weight g.*	*Mean liver weight, g.*	*Mean spleen weight mg.*	*Mean inguinal lymph nodes weight mg.*	*Mean thymus weight mg.*
Sham thymectomy	15	26.6±1.8*	1.63±0.17	101±19	11±2.8	54±14
Thymectomy	13	25.6±1.9	1.51±0.17	75±18	8±2.5	...
Statistical significance of difference	...	NS	NS	P<0.001	P<0.01	...

* ± Standard deviation.

occurred within the various paired groups of animals with regard to splenic size. Histologically this was found to be accompanied by, and probably due to, variations in the degree of infiltration of the spleen by foci of myelopoiesis and erythropoiesis. Since this type of tissue constitutes a major fraction of the weight of the spleen, variations in its amount possiby obscured smaller changes in the weights of lymphoid tissues in this organ, in the thymectomized groups, 6 weeks after operation.

Histology of Lymphoid Tissue following Thymectomy

The changes to be described were seen in thymectomized mice at both 6 weeks and 3 months after-operation. The changes at 3 months were somewhat more pronounced than at 6 weeks and the quantitative data reported was obtained from the group of animals examined 3 months after operation.

On superficial inspection, the lymphoid tissues of thymectomized mice did not differ markedly from control mice. Lymphoid follicles were present in both the spleen and lymph nodes. Germinal centres were also present in the follicles of both organs. However, on closer

inspection, certain quantitative changes were observed. In lymphoid tissues of thymectomized animals, the lymphoid follicles were less tightly packed with lymphoid cells and the germinal centres were less prominent than in control tissues. Whilst these changes were not dramatic, they were consistent and an attempt was made to consolidate these impressions by applying semi-quantitative methods to the comparative study of these lymphoid tissues.

The results obtained from the study of splenic lymphoid tissue will be described first, to show both the methods used and the type of results obtained.

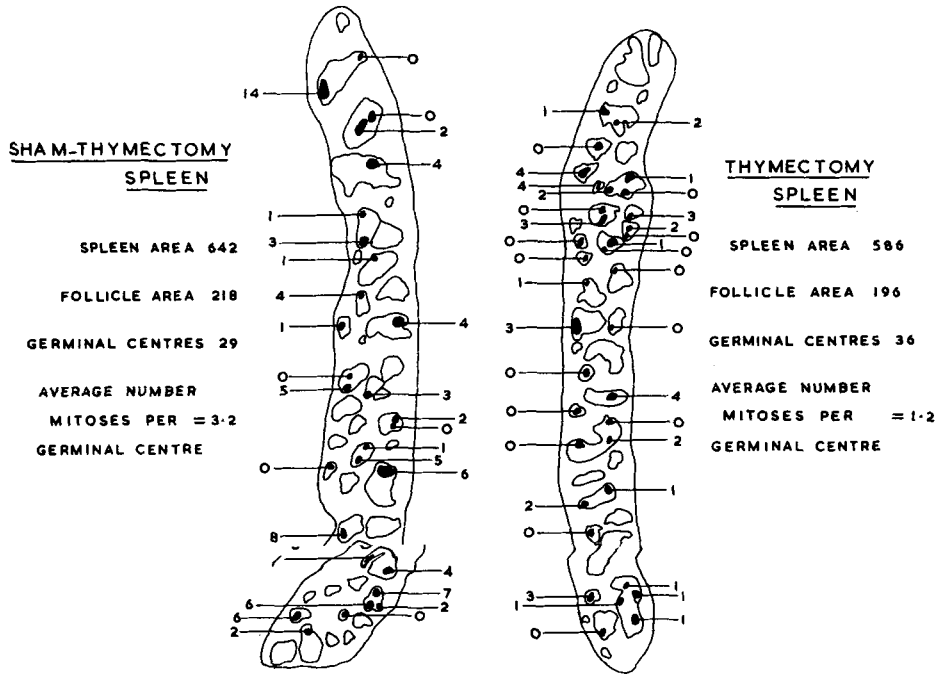

FIG. 2. Tracing of splenic sections, from a thymectomized and a sham-thymectomized mouse showing lymphoid follicles and germinal centres. Number of mitotic figures in each germinal centre is shown.

Longitudinal sections, 5 μ thick, were made through the spleens of 11 thymectomized and 12 sham-thymectomized mice, 3 months after operation. These sections were lightly stained with haematoxylin and eosin. The images of these splenic sections were then projected at × 30 magnification on to drawing paper and the outlines of the spleen, lymphoid follicles and germinal centres traced. These outlines were cut out and weighed on a torsion balance. The weights obtained in mg. were converted to units of area. The results thus give relative areas for the splenic sections and the percentage area occupied by the lymphoid follicles in each spleen. The spleens were then surveyed at × 320 magnification and the mitotic figures in every germinal centre counted and recorded on the drawings. The general appearance of such a spleen survey chart is shown in Fig. 2.

The detailed results obtained in this survey are recorded in Table III. It is obvious that a single longitudinal section through a spleen cannot pass through the centre of every lymphoid follicle encountered. Some lymphoid follicles must be cut tangentially with the result that they will appear smaller than in reality and may appear to lack germinal centres. It is likely, however, that a true size gradient does exist in splenic lymphoid follicles, and that some

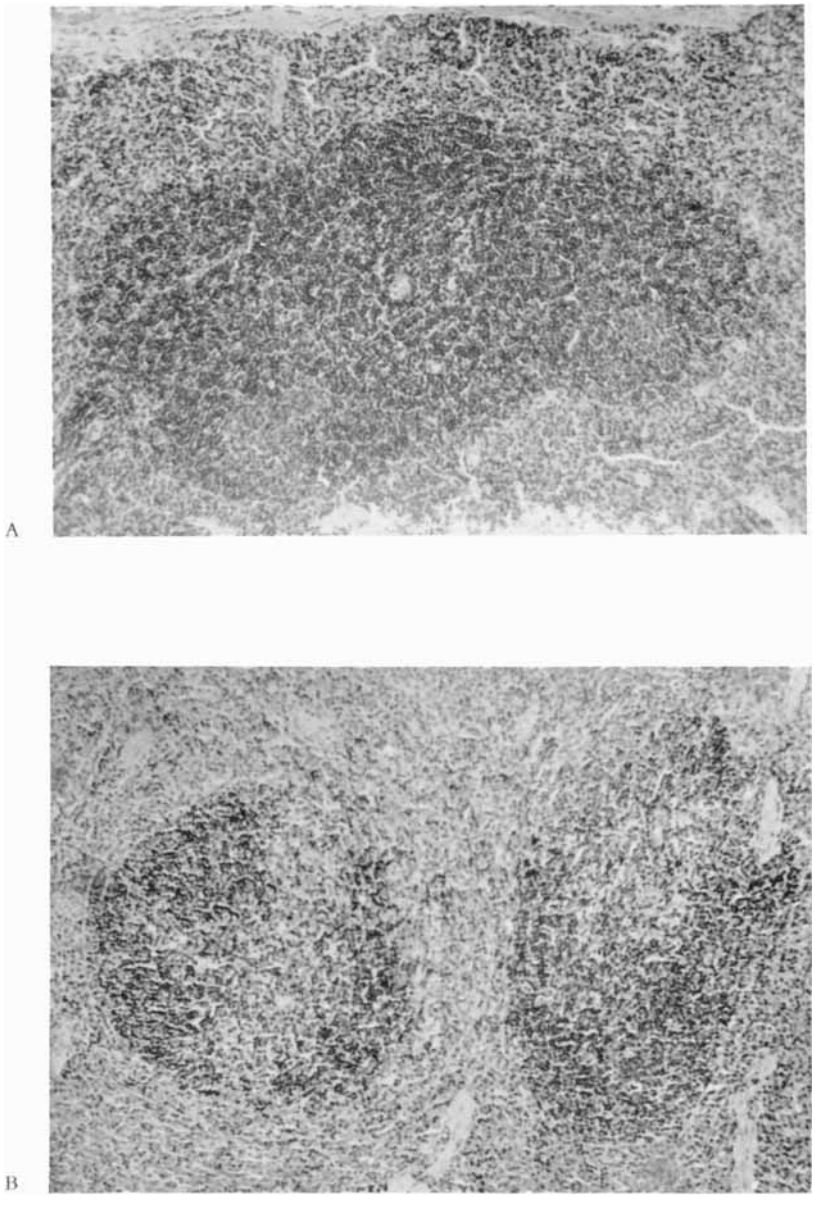

FIG. 3. Splenic lymphoid follicles from a sham-thymectomized (A) and a thymectomized mouse (B). Note the deceased density of packing of lymphocytes in the central area of the follicles in the thymectomized animal. × 150. Haematoxylin and eosin.

TABLE III

EFFECT OF THYMECTOMY ON SPLENIC LYMPHOID FOLLICLES

	Number of spleens examined	*Average 'spleen area'*	*Average % of 'spleen area' occupied by lymphoid follicles*	*Average number of follicles*	*Average % of follicles without germinal centres*	*Average number of germinal centres*	*Average mitotic count per germinal centre*	*Average % germinal centres with*			
								0 mitosis	*1 mitosis*	*2–4 mitoses*	*5+ mitoses*
Sham thymectomy	12	530 ± 160*	26 ± 5	38 ± 7	47 ± 7	24 ± 6	2.6 ± 0.5	18 ± 7	18 ± 5	48 ± 10	16 ± 6
Thymectomy	11	550 ± 150	26 ± 7	39 ± 8	47 ± 15	21 ± 11	1.7 ± 0.6	26 ± 9	31 ± 8	36 ± 7	7 ± 7
Statistical significance of difference	...	NS	NS	NS	NS	NS	$P<0.01$	NS	$P<0.01$	$P<0.01$	$P<0.01$

* ± Standard deviation.

follicles may lack germinal centres. It is reasonable to assume, however, that a section of spleen giving a sample of about 40 lymphoid follicles will reveal approximately the frequency and average size of the lymphoid follicles and the average frequency of germinal centres in such follicles. The relative uniformity of the results obtained appears to confirm these expectations.

In the germinal centres of lymphoid tissue there are usually present a varying number of degenerating cells, and cells phagocytosing such cellular debris. Some of these appearances mimic mitosis, therefore, in counting mitotic cells, only those cells showing clearly identifiable chromosomes were recorded.

From the tables it may be seen that the average 'spleen area' sampled in thymectomized and sham-thymectomized animals was almost identical. Similarly the average number of lymphoid follicles per unit area of splenic tissue and the percentage of the 'spleen area', occupied by these follicles was almost identical in the two groups.

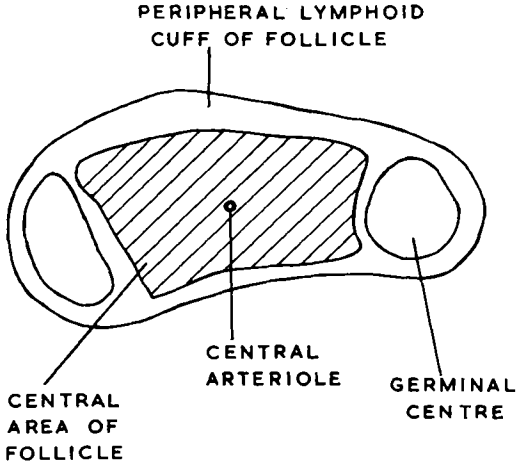

FIG. 4. Schematic representation of splenic lymphoid follicles showing central area. Compare with Fig. 3.

There was no difference between the two groups in the percentage of lymphoid follicles showing germinal centres nor was there a significant difference in the number of germinal centres per unit area of splenic tissue.

There were, however, fewer mitotic figures per germinal centre in the thymectomized mice (1.7 per centre) than in the sham-operated mice (2.6 per centre). This difference was, statistically, highly significant with a P value of <0.01. On analysis of the germinal centres in both groups, it was found that there were significantly more follicles with nought to one mitotic figures and fewer follicles with more than two mitotic figures in the thymectomized animals than in the controls.

The figures indicate that the average lymphoid follicle areas were not significantly different in the two groups. However, a closer inspection of the lymphoid follicles revealed definite differences between the two types of follicle, in the tightness of packing of the component lymphocytes. This was most noticeable in the central area of the follicle, particularly around the central arteriole of the follicle (Fig. 3). At the periphery of the follicle, cell packing appeared equal in the two types of follicle. A survey was made of the density of cell packing

in the central area of the follicle, the region of the follicle covered being shown diagrammatically in Fig. 4. Using ×960 magnification, the total number of cells in a fixed field (etched eyepiece) was counted in randomly selected follicles.

The results were as follows:

Type of follicle	*Number of spleens sampled*	*Number of fields counted*	*Mean cell count per field ± SD*
Sham thymectomy	18	110	40±6
Thymectomy	16	100	28±5

There were thus 30 per cent fewer cells in the central area of the follicle in the thymectomized animals, than in the control animals. The cell type predominately present in this area of the follicle is the small lymphocyte. The differential cell counts from the sample areas gave the following mean cell counts per unit area:

	Small lymphocytes	*Medium, large lymphocytes and reticulum cells*
Sham thymectomy	37	3
Thymectomy	22	6

Thus the cell deficit in the follicles in thymectomized animals appeared to be a selective one, involving small lymphocytes. There appeared to be an actual increase in the number of primitive lymphoid cells. This result has a doubtful significance, however, due to the difficulty in typing of cells encountered in the tightly packed tissues of the control follicles.

It was also noted that there were more small lymphocytes in the germinal centres of control mice than in the thymectomized animals. This impression was not tested quantitatively but the finding was fairly consistent throughout the spleens of this series. This may again reflect the paucity of small lymphocytes in lymphoid follicles of thymectomized animals.

In summary, the survey of splenic lymphoid follicles in thymectomized animals showed decreased mitotic activity (−28 per cent) in the germinal centres and a decreased cell content (−30 per cent) of the central areas of the follicles as compared with control animals.

A similar survey was carried out on the lymph nodes from thymectomized and control animals. Here, it proved impossible to orientate the lymph nodes in the paraffin blocks so that a uniform type of cross-section of the node was obtained. Some sections obviously passed tangentially through the cortical follicle zone, whilst others passed mainly through medullary cords. This produced a heterogeneous group of lymph-node sections which could not be handled as individual specimens. There was also extreme variability in the size and shape of the lymphoid follicles.

As a result of these considerations, the survey was limited to an estimation of the total lymph node area, total germinal centre count and mitotic figure count per germinal centre. In all, 26 lymph nodes from thymectomized animals and 24 lymph nodes from sham-thymectomized animals were surveyed. The results are shown in Table IV. Again it was apparent that, as a group, lymph nodes from thymectomized animals contained as many germinal centres per unit area as did control lymph nodes. However, it was again observed that there were fewer mitotic figures per germinal centre (2.8) than in the control germinal centres (4.7). The results from the lymph-node survey thus showed similar changes, following thymectomy, to those seen in the spleen survey. No estimate could be made of the density of cell packing in the lymph nodes due to the extreme variability in architectural patterns encountered. It was again noted, however, that there were consistently more small lymphocytes in the germinal areas of control mice than in those of thymectomized mice.

There was considerable variability in the number of degenerating cells and the amount of cellular debris in the germinal centres in both the spleen and lymph nodes in the two groups. In general, however, there seemed to be more cellular breakdown in the germinal centres of the control mice than in the thymectomized mice. This observation is consistent with the findings of de Bruyn (1948) and of Ringertz and Adamson (1950) that mitotic activity in germinal centres is roughly proportional to the number of pyknotic cells they contain.

TABLE IV

EFFECT OF THYMECTOMY ON INGUINAL LYMPH NODES

	Number of lymph nodes examined	*Average lymph nodes area*	*Average number of germinal centres per lymph node*	*Average number of mitotic figures per germinal centre*
Sham thymectomy	24	58 ± 22*	4.0 ± 2.4	4.7 ± 3.0
Thymectomy	26	57 ± 23	3.6 ± 2.5	2.8 ± 2.1
Statistical significance of difference	...	NS	NS	P = 0.01

* ± Standard deviation.

DISCUSSION

There is fairly general agreement (Rouvière and Valette, 1937; Sanders and Florey, 1940; Turner and Hall, 1943) that excision of lymphoid tissue in animals leads to compensatory increase in weight of the remaining lymph nodes.

At first sight, the thymus, being composed mainly of lymphoid tissue, might be expected to behave in a similar fashion to lymph nodes. Thymectomy might reasonably be expected to lead to compensatory hypertrophy of remaining lymphoid tissues. Indeed, Gyllensten (1953) has reported an increase in lymph-node weights following subtotal thymectomy in immature guinea-pigs.

However, the results of other workers suggest that such may not be the case. Andreasen and Gottlieb (1946) found that if the thymus is removed at the time of excising other lymphoid tissue, no hypertrophy of the remaining lymph nodes occurs. Similarly Nakamoto (1957) has described lymphoid atrophy and peripheral lymphopenia in rabbits following thymectomy.

The investigations on the effect of thymectomy in mice, reported here, confirm that thymectomy leads to a lymphopenia in the peripheral blood, associated with atrophy of the lymphoid tissues.

It should be noted that the changes observed were slow in onset and did not become maximal until at least 4–6 weeks after operation. It is possible that if Gyllensten had extended his observation period on thymectomized guinea-pigs, similar changes would have been observed. Indeed between the 2nd and 4th weeks of his 4-week observation period considerable lymph-node atrophy occurred.

We have reported earlier, that the thymus produces a lymphocytosis stimulating factor (LSF). When thymus extracts are injected into baby mice or thymectomized adult mice, a temporary lymphocytosis is produced. The results of thymectomy, reported here, lend support to the concept that the thymus stimulates lymphopoiesis throughout the body. The results also suggest a possible site of action of thymic LSF in this process.

Thymectomy appears to depress the division rate of the more primitive lymphoid cells (mainly large and medium lymphocytes), at least as judged from germinal centre mitotic activity. Accompanying the decreased mitotic activity of the primitive lymphoid cells, there is also a fall in the small lymphocyte population of the lymphoid follicles. This may be due to increased destruction of small lymphocytes but is more likely to be the result of the normal destruction or utilization of these cells in the body, in the absence of a sufficient production of new small lymphocytes by the germinal centres to replace them.

It is suggested, therefore, that the action of thymic LSF may be to stimulate the division of primitive lymphoid cells and their maturation into small lymphocytes. Thymectomy, by slowing down this process, would lead to a decrease in germinal centre mitotic activity and, as production rate of small lymphocytes continued to be inadequate, a gradual fall in the total population of small lymphocytes both in the lymphoid follicles and the peripheral blood.

It is of interest to note that the lymphoid atrophy and lymphopenia following thymectomy do not increase in intensity indefinitely. After thymectomy, tissue and blood lymphocytes decrease progressively until, at 4–6 weeks after operation, lymphocyte homeostasis appears to achieve a new equilibrium, the animal living thereafter in a stable but relatively lymphopenic state.

SUMMARY

Thymectomy in C_{57}Bl mice is followed by a fall in peripheral lymphocyte levels and atrophy of the lymph nodes and spleen. Histologically, there is a depression of mitotic activity in the germinal centres of the lymphoid follicles, and a differential decrease in the numbers of small lymphocytes in the central areas of these lymphoid follicles.

The results support previous evidence that the thymus produces a lymphocytosis stimulating factor (LSF).

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