# Further Studies on the Effects of the Removal of the Chief Lymphoid Organs*

KEIYA FUKUTANI

*Department of Anatomy, Yamaguchi Medical Shool, Ube*

*(Director: Prof. Bunsuke Osogoe)*

(Received May 30, 1959)

In view of the fact that the lymphatic tissue is widely dispersed over the whole body, the operative removal of this tissue is very difficult. It is for this reason that only few experiments of this type have hitherto been reported in the literature. So far as the writer is aware, *Sanders* and *Florey* (1940) are the first who succeeded in removing a large part of the organized lymphoid tissues in rats and rabbits without stopping the growth of the operated animals. Later, *Andreasen* and *Gottlieb* (1947), *Andreasen* et al. (1948), *Karasawa* (1954a) and *Maeda* (1955) reported similar experiments on guinea-pigs, rabbits or rats. The experiments to date have demonstrated that extensive extirpation of the lymphoid tissue produces a marked lymphopenia persisting for several weeks or more and a compensatory hyperplasia of the remaining lymphoid tissue which is accompanied by a new formation of lymphoid cell aggregations in the liver and bone marrow. However, it should be noticed here that the observations of the previous workers are confined to the early effects of the ablation of lymphoid tissue within a month or two, or within at most 3 months after operation. Moreover, as regards the compensatory mechanism for marked reduction in the amount of lymphoid tissue, quantitative information is scanty.

Keeping these in mind, the author has intended in the present study to gain additional information concerning the ablation of lymphoid tissue by extending the observation period as long as to 6–10 months after operation, using rats as experimental animals. In this study it was also attempted to use chemical method of DNA determination for quantitative evaluation of the compensatory mechanism that might be expected to occur in various organs in response to a marked reduction in the amount of lymphoid tissue after operation.

## MATERIAL AND METHODS

The animals employed were young mature albino rats of a subline of the *Wistar*

* Aided by a grant to Professor *Bunsuke Osogoe* from the Ministry of Education (Grant-in-Aid for Fundamental Scientific Research, Cooperative)

strain, weighing around 200 g. All rats were reared on a standard laboratory diet* and water ad libitum, supplemented once a week with cabbage or other vegetables.

As a preliminary study, the weights of the lymphoid organs were measured in 11 normal animals. The results are listed in Table 1. It was found that total lymph nodes of young adult male rats amounted to about 0.5 per cent of the body weight. The weight of the thymus and spleen were found to be about 0.15 per cent and 0.5 per cent of the body weight, respectively.

Table 1. Weight (g) of lymph nodes and lymphoid organs in 11 normal adult male rats weighing around 200 g.

| Group | Weight in gram | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |
| Lymph nodes | | | | | | | | | | | | |
| Mesenteric | 0.29 | 0.38 | 0.43 | 0.29 | 0.23 | 0.28 | 0.24 | 0.27 | 0.28 | 0.34 | 0.28 | 0.300 |
| Caecal | 0.06 | 0.05 | 0.03 | 0.05 | 0.05 | 0.06 | 0.06 | 0.05 | 0.06 | 0.06 | 0.03 | 0.050 |
| Portal | 0.02 | 0.04 | 0.04 | 0.01 | 0.02 | 0.02 | 0.02 | 0.03 | 0.01 | 0.01 | 0.02 | 0.021 |
| Renal | 0.03 | 0.05 | 0.02 | 0.03 | 0.03 | 0.03 | 0.05 | 0.03 | 0.04 | 0.05 | 0.02 | 0.034 |
| Para-aortic | 0.06 | 0.05 | 0.06 | 0.07 | 0.05 | 0.08 | 0.05 | 0.06 | 0.05 | 0.08 | 0.04 | 0.059 |
| Postgastric | | 0.02 | | 0.02 | | 0.01 | 0.01 | | 0.02 | 0.02 | 0.01 | 0.010 |
| Illiac | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.011 |
| Sacral | 0.04 | 0.03 | 0.03 | 0.04 | 0.03 | 0.03 | 0.02 | 0.04 | 0.03 | 0.02 | 0.02 | 0.030 |
| Mediastinal | 0.06 | 0.04 | 0.05 | 0.04 | 0.03 | 0.03 | 0.04 | 0.03 | 0.03 | 0.04 | 0.04 | 0.039 |
| Superficial cervical | 0.20 | 0.18 | 0.17 | 0.20 | 0.14 | 0.20 | 0.15 | 0.20 | 0.16 | 0.19 | 0.20 | 0.180 |
| Deep cervical | 0.08 | 0.08 | 0.06 | 0.07 | 0.09 | 0.05 | 0.07 | 0.05 | 0.09 | 0.07 | 0.06 | 0.070 |
| Posterior axilary | 0.63 | 0.10 | 0.10 | 0.08 | 0.15 | 0.10 | 0.09 | 0.10 | 0.08 | 0.08 | 0.07 | 0.089 |
| Internal axillary | 0.03 | 0.05 | 0.06 | 0.06 | 0.07 | 0.06 | 0.04 | 0.04 | 0.04 | 0.06 | 0.04 | 0.050 |
| Inguinal | 0.04 | 0.03 | 0.02 | 0.03 | 0.02 | 0.03 | 0.03 | 0.03 | 0.04 | 0.02 | 0.05 | 0.030 |
| Popliteal | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.013 |
| Total lymph nodes | 0.96 | 1.12 | 1.09 | 1.02 | 0.93 | 1.00 | 0.91 | 0.95 | 0.96 | 1.07 | 0.91 | 0.986 |
| Lymphoid organs | | | | | | | | | | | | |
| Thymus | 0.31 | 0.38 | 0.26 | 0.30 | 0.27 | 0.47 | 0.37 | 0.33 | 0.25 | 0.25 | 0.30 | 0.308 |
| Spleen | 1.05 | 1.58 | 0.75 | 0.85 | 0.79 | 1.10 | 0.60 | 1.02 | 0.85 | 1.11 | 0.90 | 0.963 |
| Total lymphoid organs | 1.36 | 1.86 | 1.01 | 1.15 | 1.06 | 1.57 | 0.97 | 1.35 | 1.10 | 1.36 | 0.20 | 0.271 |

The terminology is the same as used in the papers of *Sanders* and *Florey* (1940) and of *Monden* (1958).

In a series of 8 rats, having an initial weight of around 200 g (range 192–230 g), all lymph nodes except portal, mediastinal, superficial and deep cervical nodes as well as the thymus and the spleen were removed (*subtotal extirpation group*). Operations

* The diet consisted chiefly of unpolished rice, pressed barley and dried small sardines, with a small amount of cod liver oil and minerals ($CaCO_3$+NaCl).

were conducted under ether anesthesia in three stages with an interval of 7 days. In stage I the thymus was removed, in stage II the spleen and the mesenteric lymph nodes as well as several small lymph nodes in the peritoneal cavity (coecal, portal, renal, para-aortic, postgastric, iliac and sacral nodes) were extirpated, and in stage III the lymph nodes lying beneath the skin (i.e., superficial and deep cervical, axillary, inguinal, and popliteal lymph nodes). The total weight of the removed lymph nodes amounted to about 85 per cent or more of that of the entire lymph nodes (cf. Table 2). For thymectomy the technique described by *Segaloff* (1949) was used. The operation produced bilateral pneumothorax, but the collapsed lung rapidly recovered by the procedure recommended by *Andreasen* and *Gottlieb* (1947), that is, by quickly compressing the resilient wall of the thorax and allowing to expand again after suturing.

Another series of 12 rats were divided into four groups; in the first group (3 rats) the mesenteric lymph nodes (pancreas Aselli) were extirpated (*partial lymphadenectomy group*), in the second (3 rats) the thymus (*thymectomy group*), in the third (3 rats) the spleen (*splenectomy group*), and the fourth group (4 rats) was subjected to the same 3-stage operative procedure as that described for the *subtotal extirpation group*, except that the lymph nodes and lymphoid organs were exposed, but not removed. The last group is designated as the *sham-operation group.*

In every animal which survived the operation, blood examination and measurement of body weight were carrid out at an interval of 1 month. After losing weight for a day or two, the animals began to grow again and continued to do so at least for 10 months.

The animals of all groups were autopsied 10 months after operation, except 4 animals of the subtotal extirpation group which were killed at the end of the 6th month.

Immediately after the animals had been killed by cervical dislocation and bleeding, the organs to be examined (liver, lung, *Peyer*'s patches, spleen and kidney) were removed and weighed. A portion of each of the excised organs was used for determination of DNA-P and another portion for histological examination after fixing in *Zenker*-formol. The remaining lymph nodes were also removed and weighed, but measurement of DNA-P content was not done in these organs. They were only examined histologically.

For measurements of DNA-P, the *Schneider* method and the diphenylamine test were used. The detailed description of this technique has been made in an earlier paper by *Monden* (1958).

Table 2. Weights (g) of the remaining lymph nodes and lymphoid organs in different group 10 months after operations.

| Groups / Organs / Rat No. | Subtotal extirpation | | | | Partial-lymph adenectomy | | | Thymectomy | | | Splenctomy | | | Sham-operation (Control) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 7 | 8 | 10 | 15 | 16 | 19 | 20 | 22 | 23 | 26 | 30 | 31 | 42 | 43 | 45 | 46 |
| Lymph nodes | | | | | | | | | | | | | | | | | |
| Mesenteric nodes | | | | | — | — | — | 0. 32 | 0. 29 | 0. 43 | 0. 20 | 0. 24 | 0. 28 | 0. 35 | 0. 30 | 0. 29 | 0. 28 |
| Caecal | | | | | 0. 05 | 0. 03 | 0. 06 | 0. 06 | 0. 04 | 0. 10 | 0. 06 | 0. 06 | 0. 04 | 0. 06 | 0. 05 | 0. 04 | 0. 04 |
| Portal | 0. 01 | 0. 01 | 0. 01 | 0. 02 | 0. 03 | 0. 03 | 0. 03 | 0. 03 | 0. 03 | 0. 05 | 0. 01 | 0. 02 | 0. 01 | 0. 04 | 0. 04 | 0. 03 | 0. 03 |
| Renal | | | | | 0. 02 | 0. 02 | 0. 01 | 0. 03 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 02 | 0. 03 | 0. 01 | 0. 02 | 0. 04 |
| Para-aortic | | | | | 0. 08 | 0. 03 | 0. 04 | 0. 05 | 0. 06 | 0. 06 | 0. 04 | 0. 06 | 0. 05 | 0. 06 | 0. 07 | 0. 06 | 0. 08 |
| Postgastric | | | | | 0. 01 | | 0. 02 | 0. 01 | | | 0. 01 | 0. 01 | | 0. 01 | | 0. 01 | |
| Iliac | | | | | 0. 01 | 0. 01 | 0. 03 | 0. 01 | 0. 01 | 0. 02 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 03 | 0. 03 |
| Sacral | | | | | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 01 | 0. 03 | 0. 03 |
| Mediastinal | 0. 06 | 0. 07 | 0. 09 | 0. 08 | 0. 05 | 0. 05 | 0. 06 | 0. 07 | 0. 09 | 0. 08 | 0. 05 | 0. 04 | 0. 06 | 0. 10 | 0. 05 | 0. 07 | 0. 06 |
| Superficial cervical | 0. 02 | 0. 06 | 0. 07 | 0. 07 | 0. 20 | 0. 20 | 0. 27 | 0. 29 | 0. 31 | 0. 38 | 0. 25 | 0. 24 | 0. 27 | 0. 26 | 0. 25 | 0. 28 | 0. 23 |
| Deep cervical | 0. 02 | | | | 0. 08 | 0. 08 | 0. 04 | 0. 09 | 0. 08 | 0. 10 | 0. 10 | 0. 08 | 0. 07 | 0. 10 | 0. 06 | 0. 07 | 0. 06 |
| Posterior axillary | | | | | 0. 07 | 0. 06 | 0. 10 | 0. 10 | 0. 17 | 0. 13 | 0. 09 | 0. 07 | 0. 10 | 0. 09 | 0. 09 | 0. 08 | 0. 07 |
| Internal axillary | | | | | 0. 09 | 0. 06 | 0. 09 | 0. 12 | 0. 14 | 0. 13 | 0. 06 | 0. 10 | 0. 09 | 0. 09 | 0. 09 | 0. 08 | 0. 07 |
| Inguinal | | | | | 0. 05 | 0. 07 | 0. 05 | 0. 04 | 0. 07 | 0. 03 | 0. 05 | 0. 03 | 0. 06 | 0. 05 | 0. 05 | 0. 05 | 0. 06 |
| Popliteal | | | | | 0. 01 | 0. 01 | 0. 01 | 0. 02 | 0. 02 | 0. 02 | 0. 02 | 0. 01 | 0. 01 | 0. 02 | 0. 01 | 0. 02 | 0. 02 |
| Total lymph nodes | 0. 11 | 0. 14 | 0. 17 | 0. 17 | | | | 1. 25 | 1. 33 | 1. 54 | 0. 97 | 0. 99 | 1. 08 | 1. 28 | 1. 09 | 1. 16 | 1. 10 |
| Lymphoid organs | | | | | | | | | | | | | | | | | |
| Thymus | | | | | 0. 06 | 0. 08 | 0. 10 | — | — | — | 0. 08 | 0. 10 | 0. 06 | 0. 10 | 0. 08 | 0. 10 | 0. 08 |
| Spleen | | | | | 0. 96 | 0. 89 | 0. 64 | 0. 79 | 0. 74 | 0. 80 | — | — | — | 0. 82 | 0. 70 | 0. 95 | 0. 75 |
| Total lymphoid | | | | | 1. 02 | 0. 97 | 0. 74 | | | | | | | 0. 92 | 0. 78 | 1. 05 | 0. 83 |

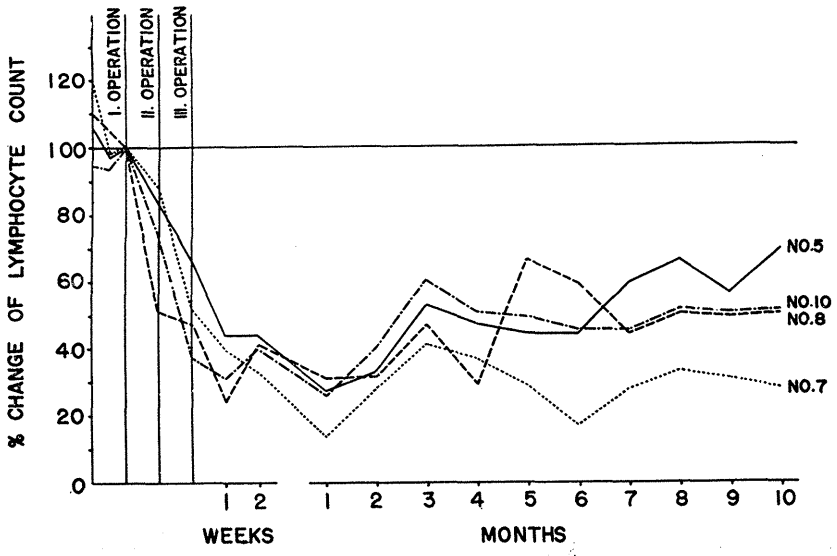


Fig. 1. Changes in percentage of the lymphocyte count after subtotal lymphadenectomy combined with thymectomy and splenectomy.

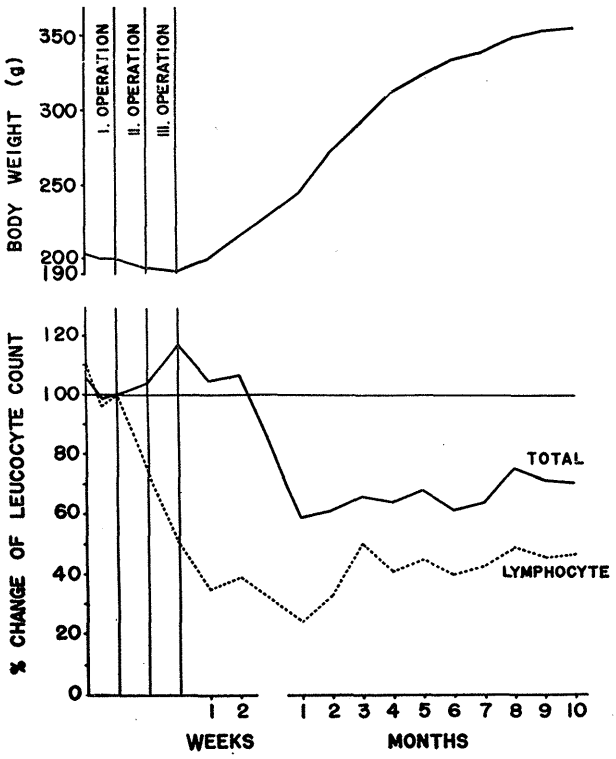


Fig. 2. Changes in percentage of the total white count and lymphocyte count, and the rate of increase of body weight after subtotal lymphadenectomy combined with thymectomy and splenectomy (average 4 rats).

## RESULTS

### 1. *Subtotal extirpation group*

a) *Blood picture* (Figs. 1–3)

Subtotal lymphadenectomy combined with thymectomy and splenectomy caused a pronaunced and persistent lymphopenia. The post-operative lymphocyte counts varied from 24 to 44 per cent (averaging 35 per cent) of the pre-operative values at the end of the first week, 14 to 31 per cent (averaging 24 per cent) at the end of the first month and 28 to 69 per cent (averaging 47 per cent) 10 months after operation. Thus, the normal blood lymphocyte level had not been re-established until 10 months after operation, although there was a slight increase in the number of blood lymphocytes from the 3rd month on (Figs. 1–2).

The total leukocyte level showed a slight elevation before and after the third operation. Thereafter it was also considerably decreased below the pre-operative level. This is chiefly due to the reduction in the number of lymphocytes (fig. 2). A severe post-operative anemia gradually recovered by the third month (fig. 3).

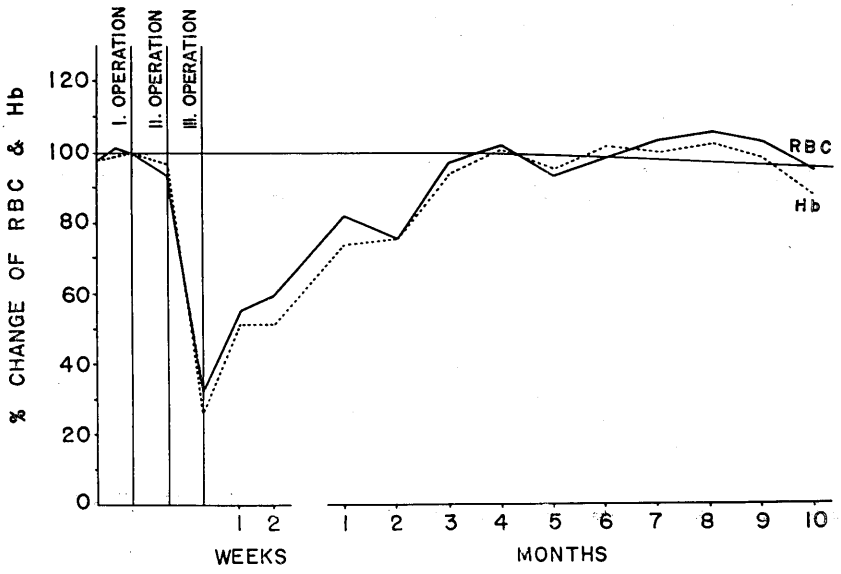


Fig. 3. Changes in percentage of the red cell count and hemoglobin content after subtotal lymphadenectomy combined with thymectomy and splenectomy.

b) *Body weight* (Fig. 2 and Table 3)

Although extensive extirpation of the lymph nodes, thymus and spleen had been performed in this group, the body weight, after showing a slight initial decrease, definitely increased until 10 months after operation, as illustrated in Figure 2. It is

especially noteworthy that the rate of increase of the body weight in this group was significantly greater than that in the *sham-operation group* in which the lymph nodes and lymphoid organs had been exposed, but not removed (Table 3). This seems to indicate that extensive removal of the lymphoid tissue has no decisive influence upon general body growth, so far as the present experimental conditions are concerned.

Table 3. Body weight of rats in different groups 10 months after operations.

| Group | Rat No. | Body weight (g) | |
|---|---|---|---|
| | | Before operation | 10 months after operation |
| Subtotal lymphadenectomy, thymectomy and splenectomy | 5 | 192 | 360 |
| | 7 | 210 | 370 |
| | 8 | 212 | 330 |
| | 10 | 190 | 340 |
| | Average | 201 | 350* |
| Partial lymphadenectomy (removal of mesenteric nodes) | 15 | 198 | 385 |
| | 16 | 190 | 290 |
| | 19 | 199 | 290 |
| | Average | 195 | 322 |
| Thymectomy | 20 | 205 | 290 |
| | 22 | 199 | 290 |
| | 23 | 198 | 380 |
| | Average | 201 | 320 |
| Splenectomy | 26 | 202 | 270 |
| | 30 | 212 | 355 |
| | 31 | 210 | 355 |
| | Average | 208 | 327 |
| Sham-operation | 42 | 200 | 300 |
| | 43 | 204 | 288 |
| | 45 | 198 | 280 |
| | 46 | 210 | 290 |
| | Average | 203 | 290 |

* Significant at 1% level, as compared with the control value in the sham-operation group.

c) *Histological findings in various organs* (Table 4)

The early experiments by *Karasawa* (1954a, b) on rabbits have demonstrated that in response to removal of a large part of lymphoid tissue, there occurs a compensatory hyperplasia of the remaining lymphoid tissue, together with a new formation of lymphoid tissue in the liver and bone marrow. *Sanders* and *Florey* (1940) called attention to a considerable hypertrophy of the peribronchial lymphoid tissue in rats and rabbits after extensive lymphadenectomy. Taking these into consideration, the remaining lymph nodes, *Peyer*'s patches and lymphoid tissues in other organs, such

as liver, lung, kidney and bone marrow were studied in sections.

New formation of lymph nodes did not occur at the sites where they had been

Table 4. New formation of lymphocyte aggregations in various organs of rats in different groups 10 months after operations.

| Group | Rat No. | Liver | Bone marrow | Kidney |
|---|---|---|---|---|
| Subtotal lymphadnectomy, thymectomy and splenectomy | 5 | + | − | + |
| | 7 | + | − | ± |
| | 8 | +〜++ | − | ± |
| | 10 | +〜++ | − | + |
| Partial lymphadenectomy (removal of mesenteric nodes) | 15 | − | − | − |
| | 16 | − | − | − |
| | 19 | − | − | − |
| Thymectomy | 20 | ± | − | ± |
| | 22 | − | − | − |
| | 23 | − | − | − |
| Splenectomy | 26 | ± | − | − |
| | 30 | − | − | − |
| | 31 | − | − | ± |
| Sham-operation | 42 | − | − | − |
| | 43 | − | − | − |
| | 45 | − | − | − |
| | 46 | − | − | − |

Fig. 4. Cortex of the superficial lymph node, containing three enlarged germinal centers. Six months after subtotal lymph adenectomy plus thymectomy plus splenectomy. × 100.

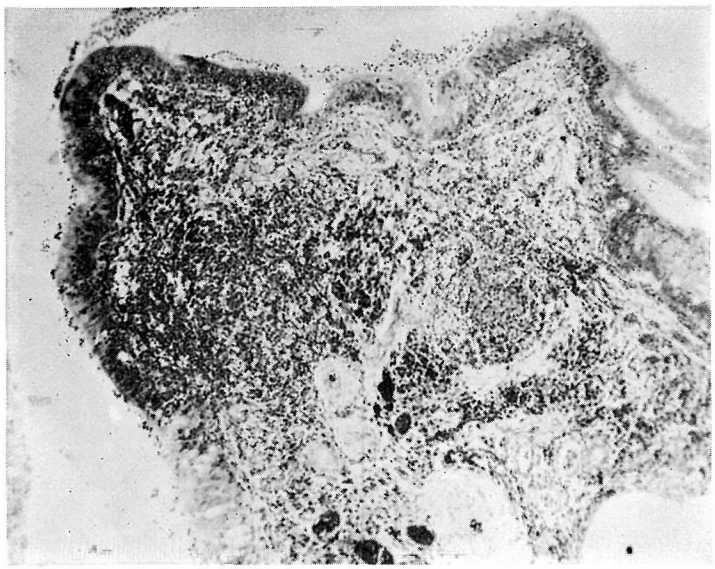

Fig. 5. Peribronchial lymphod tissue of the lung, containing two germinal centers. The months after subtotal lymphadenectomy plus thymectomy plus splenectomy. × 100.

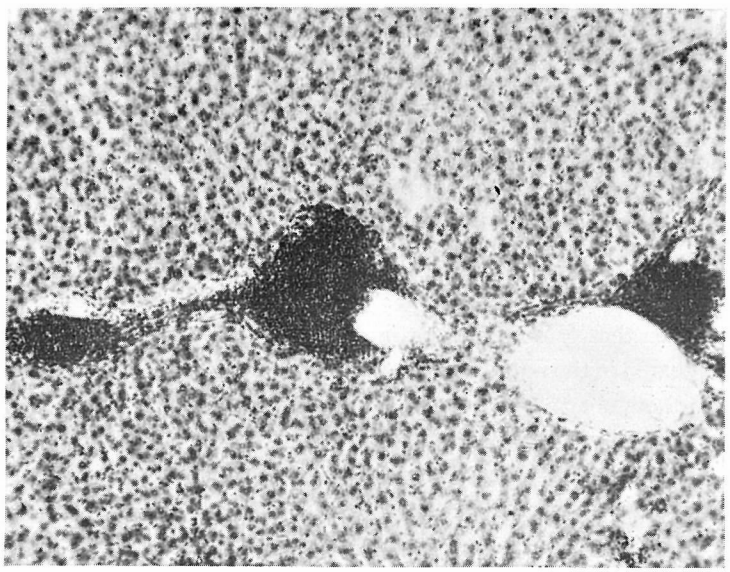

Fig. 6. Lymphocyte aggregations in the periportal spaces of the liver. Six months after subtotal lymphadenectomy plus thymectomy plus splenectomy. Frozen section. × 100.

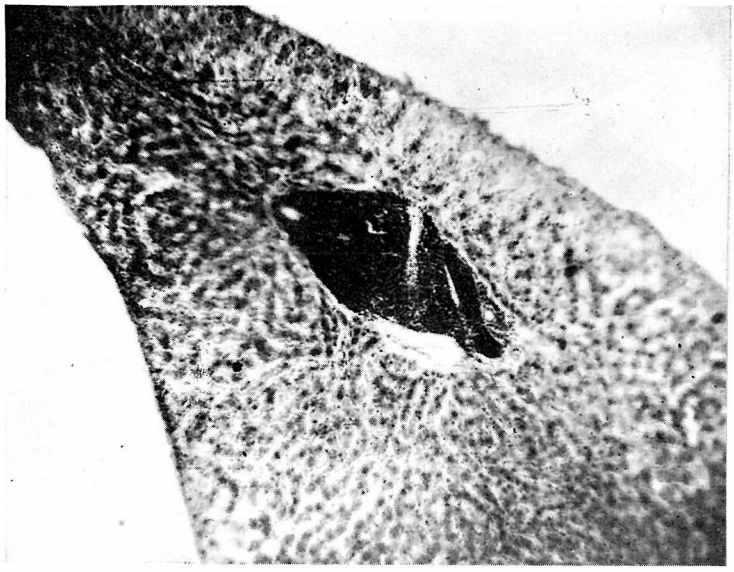

Fig. 7. A large, nodule-like aggregation of lymphocytes in the periportal area of the liver. Ten months after subtotal lymphadenectomy plus thymectomy plus splenectomy. Frozen section. ×100.

removed completely. The remaining lymph nodes were greatly hypertrophied and hyperplasia was prominent in the cortex, the *Flemming*'s secondary nodules (germinal centers), in particular (Fig. 4). A pronounced hyperplasia was also seen in the *Peyer*'s patches, especially in their secondary nodules, as well as in the peribronchial lymphoid tissue of the lung, in which germinal centers were occasionally found (Fig. 5). Such a compensatory hyperplasia of the remaining lymphoid tissues appeared to be more striking at 10 months than at 6 months after operation.

Another point of interest is the new formation of lymphocyte aggregations in the periportal spaces of the liver (Figs. 6–7). This was first noticed by *Sanders* and *Florey* (1940) in rats and later confirmed by *Karasawa* (1954a,b) in rabbits. It was first demonstrated that 6 months after operation, dense aggregations of lymphocytes frequently appeared in the periportal spaces. Contrary to our expection, however, the degree of lymphocyte aggregations in the liver was not markedly increased at 10 months after operation. It is conceivable, therefore, that the compensatory mechanism of this type would have reached its maximum by 6 months after operation.

In an earlier experiment by *Karasawa* (1954a) on rabbits, a similar new formation of lymphocyte aggregations was also observed in the bone marrow. In contrast, in the present study aggregations of lymphocytes were recognized neither in the bone marrow nor in other organs, except for the kidney in which a slight degree of lymphocyte infiltration was often seen in the peritubular connective tissue (Table 4).

The femoral bone marrow was invariably found to be hyperplastic. Detailed

Table 5. DNA-P content in various organs of rats in different groups 10 months after operations.

| Group | Number of rats examined | Peyer's patches | | | Lung | | | Liver | | | Spleen | | | Kidney | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g |
| Sham-operation | 4 | 0. 177 | 0. 176 | 1. 062 | 2. 140 | 1. 153 | 0. 500 | 10. 27 | 1. 871 | 0. 163 | 0. 587 | 0. 457 | 0. 768 | 1. 910 | 0. 531 | 0. 273 |
| Total lymphadenectomy thymectomy and splenectomy | 4 | 0. 355※ | 0. 482※ | 1. 342※ | 3. 297※ | 2. 396※ | 0. 735※ | 15. 36※ | 3. 651※ | 0. 237※ | —— | —— | —— | 3. 730※ | 1. 013※ | 0. 279 |
| Partial lymphadenectomy (removal of mesenteric lymph nodes) | 3 | 0. 260※ | 0. 350※ | 0. 888※ | 2. 033 | 1. 058 | 0. 537 | 10. 94 | 2. 011 | 0. 201 | 0. 663 | 0. 504 | 0. 761 | 2. 543 | 0. 783 | 0. 326 |
| Thymectomy | 3 | 0. 200 | 0. 191 | 0. 976 | 2. 560 | 1. 209 | 0. 480 | 13. 44 | 2. 871 | 0. 207 | 0. 700 | 0. 536 | 0. 773 | 2. 759* | 0. 615 | 0. 230 |
| Splenectomy | 3 | 0. 223 | 0. 241 | 1. 200 | 2. 890 | 1. 716 | 0. 591 | 13. 08 | 2. 397 | 0. 191 | —— | —— | —— | 2. 530 | 0. 808 | 0. 352 |

※ Significant at 1% level as compared with the control value in the sham-operation group.

* Significant at 5% level.

cytological analysis of bone marrow was not made in the present study, however.

d) *DNA-P content in various organs* (Table 5)

Measurements of DNA-P content in various organs were performed in an attempt to gain quantitative information concerning the compensatory mechanism for a marked reduction in the amount of lymphoid tissue. It was revealed that the DNA-P content was remarkably increased not only in the *Peyer*'s patches but also in the liver, lung and kidney, as compared with the corresponding value in each organ in the sham-operation group (Table 5). In the *Peyer*'s patches, liver and lung, the increase in DNA-P content per organ and per 1 gram of fresh tissue was highly significant ($P<0.01$). This may be partly correlated with the histological findings in these organs, that is, either with hyperplasia or with new formation of lymphoid tissue*. In other words, the observed incease in DNA-P content may be considered to be due to some extent to an increase in the number of lymphocytes in the organs. In contrast, the DNA-P content in the kidney, though considerably increased in the organ as a whole, did not show any noticeable increase when expressed as milli-gram per 1 gram of fresh tissue, as compared with the corresponding values in the control group (sham-operation group). This indicates a marked hypertrophy of this organ without any extensive lymphocyte infiltration.

## 2. *Partial lymphadenectomy group* (Figs. 8 and 12)

In this group only the mesenteric mass of lymph nodes (pancreas Aselli) was removed. Immediately after the removal of this mass, the lymphocyte count sharply dropped nearly to 50 per cent of the pre-operative level and this condition was maintained for 4 months. Thereafter, it gradually returned to normal by the 7th month. The total leukocyte count showed a marked post-operative decrease almost parallel with the fall of the lymphocyte count.

The rate of increase of the body weight after operation was quite steady, showing no difference from that of the control group.

Histological examination of various organs revealed remarkable hyperplasia of the remaining lymphoid tissues (e.g., *Peyer*'s patches), but new formation of lymphocyte aggregations was observed neither in the liver nor in the kidney.

The DNA-P content in various organs (lung, liver, spleen, kidney) was not significantly increased, as compared with the corresponding values in the *sham-operation group*, except in the *Peyer*'s patches.

---

* In this connection, a significant increase in the weight of the liver and lung must also be taken into account.

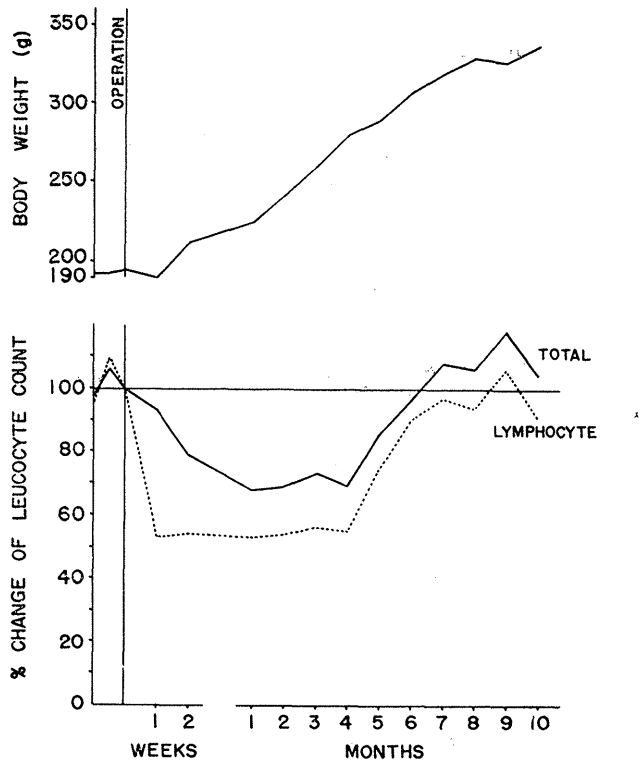


Fig. 8. Changes in percentage of the total white cell count and lymphocyte count, and the rate of increase of body weight after removal of mesenteric lymph nodes (average 3 rats).

### 3. *Thymectomy group* (Figs. 9 and 12)

Thymectomy caused less changes in the leukocyte counts than partial lymphadenectomy (removal of mesenteric nodes). A severe post-operative lymphopenia rapidly recovered within a month or two. The rate of increase of the body weight did not differ from that of the control group.

New formation of lymphocyte aggregations was recognized neither in the liver nor in other organs. Likewise, no significant increase was observed in DNA-P content in these organs. There was, however, a slight degree of hyperplasia of the peribronchial lymphoid tissue in the lung.

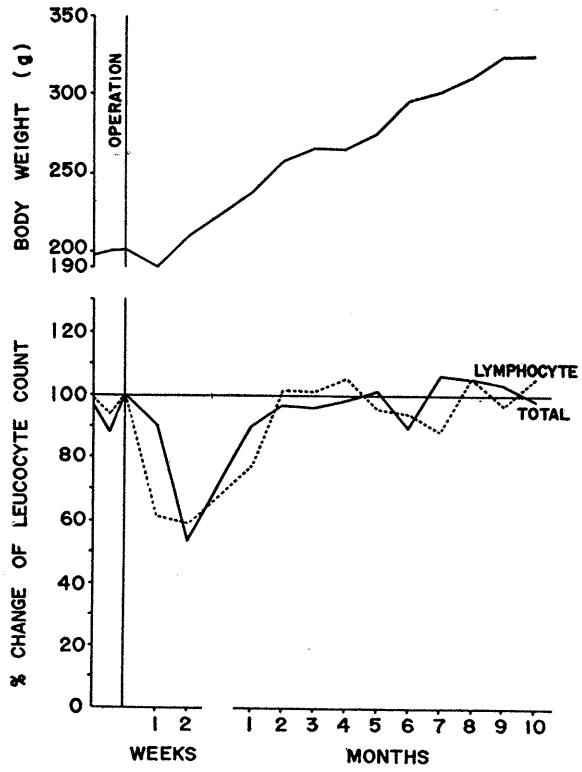


Fig. 9. Changes in percentage of the total white cell count and lymphocyte count, and the rate of increase of body weight after thymectomy (average 3 rats).

4. *Splenectomy group* (Figs. 10 and 12)

In this group the operation caused a slight degree of lymphopenia persisting for two months. It should be noticed here that after splenectomy the total leukocyte count was elevated from 120 to 130 per cent of the pre-operative level. This leukocytosis persisted for about two months and thereafter it gradually declined in degree. Other findings are similar to those in the thymectomy group.

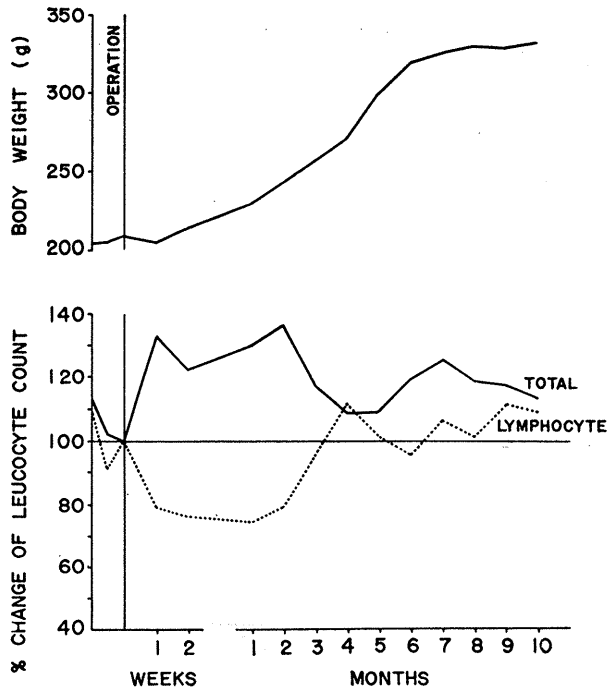


Fig. 10. Changes in percentage of the total white cell count and lymphocyte count, and the rate of increase of body weight after splenectomy (average 3 rats).

5. *Sham-operation group* (Figs. 11 and 12)

In this group, the lymph nodes and lymphoid organs had been exposed, according to the same 3-stage operative procedure as that described for the *subtotal-extirpation group*, but not removed. No remarkable alteration was observed in the leukocyte counts except a slight degree of post-operative leukocytosis.

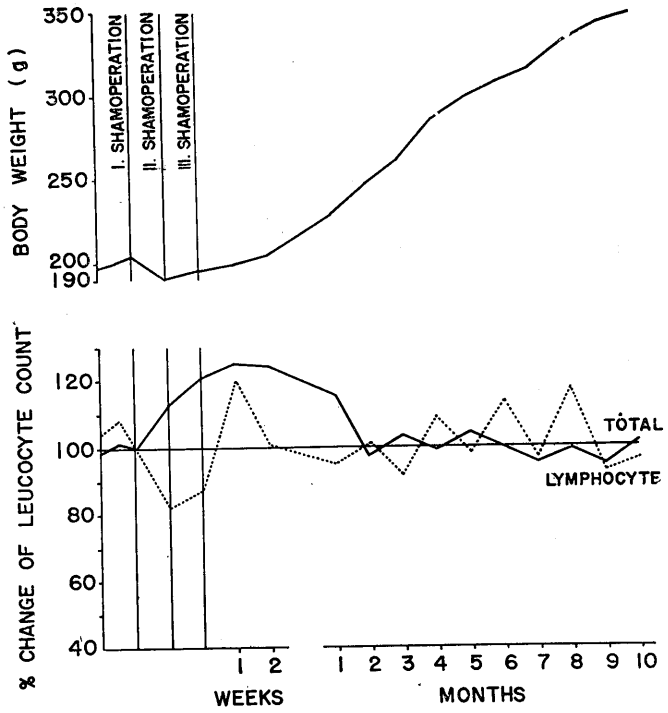


Fig. 11. Changes in percentage of the total white cell count and lymphocyte count, and the rate of increase of body weight after sham-operation (average 4 rats).

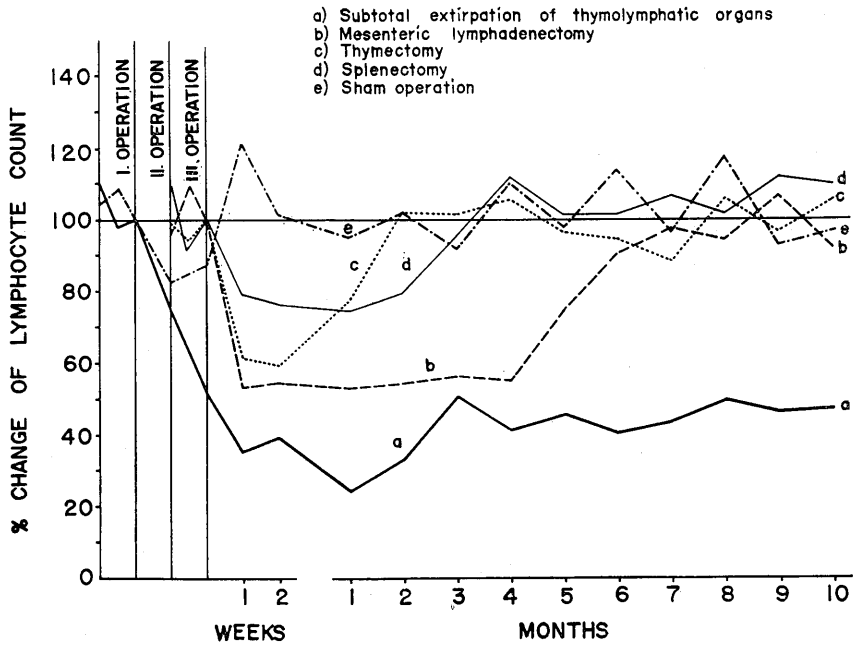


Fig. 12. Summary of the changes of the lymphocyte count in different groups.

## DISCUSSION

It has been repeatedly shown that extensive extirpation of the lymphoid organs causes a severe and persistent lymphopenia (*Sanders* and *Florey*, 1940; *Andreasen* and *Gottlieb*, 1947; *Karasawa*, 1954a and *Maeda*, 1955). The present observations on rats have confirmed this and added a new finding that the post-operative lymphopenia persisted for a period of 10 months after operation, without stopping the growth of the operated animals.

The fact that the normal blood lymphocyte level had not been re-established until 10 months after operation deserves special attention. From this fact it is firstly conceivable that regeneration or new formation of lymphoid tissue did not take place to a sufficient degree to compensate the removed lymphoid tissue, and secondly, that this condition would have certain influences upon the organism.

Most of the previous investigators agree in that following complete lymphadenectomy, new formation of lymph node does not occur at the site of excision. Although it is reported by some investigators such as *Furuta* (1947) and *Shimizu* (1959) that lymph node regeneration does take place to a slight degree, the regenerated lymph node is usually of mikroscopic size, and hence the amount of the regenerated lymphoid tissue is negligible when contrasted with that of the excised node. It should be emphasized here that, so far as the present mikroscopic observations are concerned, no new formation of lymph node was recognized at the site of excision. For these reasons, lymph node regeneration at the site of excision was not taken into consideration in the present study.

As the second type of compensatory mechanism for an extensive extirpation of the lymphoid organs, hypotrophy or hyperplasia of the remaining lymph nodes and lymphoid tissues must next be considered. A marked increase in weight of the remaining lymph nodes after removal of a large part of the organized lymphoid tissues has clearly been demonstrated by *Turner* and *Hall* (1943). Our results of measurements of DNA-P content in the *Peyer*'s patches and in the lung provide a more reliable quantitative information concerning the compensatory hyperplasia of the remaining lymphoid tissues. In the lung, there was a marked hyperplasia of the peribronchial lymphoid tissue, which seems to be responsible for the observed increase in DNA-P content per organ and per 1 gram of fresh tissue.

New formation of lymphocyte aggregations in the liver may be regarded as the third type of compensatory mechanism. The present observations have revealed a marked increase in DNA-P content per organ and per 1 gram of fresh tissue of the liver, 10 months after subtotal extirpation of the organized lymphoid tissues.

The increase in DNA-P content in the above-mentioned organs means a corresponding increase in the number of cell nucleus, if we assume that the DNA-content per nucleus is constant. This is valid so far as the *Peyer*'s patches and the

peribronchial lymphoid tissue of the lung are concerned. In these organs, an increase in the number of lymphocytes due to hyperplasia is chiefly responsible for the observed increase in DNA-P content per organ. In the liver, on the other hand, not only new formation of lymphocyte aggregations but also an increase in the number of liver cell nuclei must be taken into consideration, because the DNA-P content per organ is significantly increased as compared with the control value. In view of the abundant occurrence of polyploid nuclei in liver cells, it is also conceivable that the observed increase in DNA-P content would partly be due to a relative increase of polyploid nuclei, although substantiating evidence is lacking.

Finally, it should again be emphasized that despite the occurrence of various types of compensatory mechanism mentioned above, the pre-operative blood lymphocyte level was not re-established until 10 months after operation. This indicates that the amount of the lymphoid tissue or the lymphocytopoietic activity is still remained considerably reduced until that time. Of particular interest in this connection is the finding that under this condition the animals showed no abnormality in their general body growth. Since this finding is of primary importance in understanding the physiological functions of the lymphoid tissue, we are planning a more extensive study on the relationship between the amount of lymphoid tissue and general body growth of the animals.

## SUMMARY

1. In a series of the young adult albino rats the chief lymphoid organs (thymus, spleen, mesenteric lymph nodes and other small lymph nodes) were extirpated. The total amount of the removed lymph nodes amounted to about 85 per cent or more of the entire lymph nodes.

2. Such an extensive extirpation of the lymphoid tissue produced a pronounced and persistent lymphopenia. Though the normal blood lymphocyte level was not re-established until 10 months after operation, the operated animals showed no abnormality in their general body growth.

3. There was observed a marked hyperplasia of lymphatic tissue in the remaining lymph nodes, in the *Peyer*'s patches and of the peribronchial lymphoid tissue in the lung at 6 months as well as at 10 months after operation. In addition, there occurred a new formation of lymphocyte aggregations in the periportal spaces of the liver. The regeneration of lymph nodes, however, did not take place at the site of extirpation.

4. The above-mentioned compensatory mechanism for the ablation of lymphoid tissue was substantiated by chemical determinations of DNA-P content in these tissues, which indicated a significant increase in DNA-P content per organ and per 1 gram fresh tissue, not only in the *Peyer*'s patches and lung but also in the liver.

*Acknowledgments*

*I wish to acknowledge the valuable assistance of Drs. Y. Monden and T. Tamashige.*

## REFERENCES

ANDREASEN, E., and O. GOTTLIEB 1947 On the removal of the lymphoid organs and its effect on the blood count. *Acta physiol. scandinav.*, **13**: 35-44.

ANDREASEN, E., J. BING, O. GOTTLIEB, and N. HARBOE 1948 On the possible significance of the lymphoid organs for the production of serum protein in the rat. *Acta physiol. scandinav.*, **15**: 254-263.

FURUTA, W. J. 1947 An experimental study of lymph node regeneration in rabbits. *Am. J. Anat.*, **80**: 437-506.

GYLLENSTEN, L. 1953 Influence of thymus and thyroid on the postnatal growth of the lymphatic tissue in guinea-pig. *Acta anat.*, Suppl. 18=1 ad Vol. **17**: 1-163.

KARASAWA, K. 1954a Experimental studies on the chief source of blood lymphocytes in adult rabbits. *Okajimas Fol. anat. jap.*, **26**: 309-325.

————————. 1954b Formation of lymphocyte aggregations in the periportal spaces of the liver after removal of chief lymphoid organs in adult rabbits. *Okajimas Fol. anat. jap.*, **26**: 371-376.

————————. 1954c The effects of the removal of chief lymphoid organs. *Arch. hist. jap.*, **7**: 55-70. (In Japanese with English summary.)

MAEDA, M. 1955 The effect of the removal of lymphoid tissue upon lymphocytopoiesis. *Collect. Pap. II. Div. Anat. Inst. Kyoto Univ.*, **4**: 259-314. (In Japanese.)

MONDEN, Y. 1959 Quantitative evaluation of total cellular number and cellular density in the thymolymphatic organs of young adult albino rats by means of DNA determination. *Okajimas Fol. anat. jap.*, **32**: 193-206.

SANDERS, A. G., and H. W. FLOREY 1940 The effects of the removal of lymphoid tissue. *Brit. J. exp. Path.*, **21**: 275-287.

SEGALOFF, A. 1949 Thymectomy, in the chapter "Surgery of the Rat" by D. J. Ingle and J. Q. Griffith, Jr. In: *The Rat in the Laboratory Investigation,* edited by E. J. Fariffith, Jr. J. B. Lipincot Comp. Philadelphia, London and Montreal.

SHIMIZU, T. 1958 Histological studies on the regeneration of the lymph nodes and lymphatic apparatus. *Collect. Pap. II. Div. Anat. Inst. Kyoto Univ.*, **7**: 1-28. (In Japanese.)

TURNER, M. G., and V. E. HALL 1943 The effect of removal of a large part of the lymphoid system on the weight of the portion remaining in situ. *Anat. Rec.*, **85**: 401-412.

Table 6. DNA-P content of various organs 10 months after sham-operation.

| Rat No. | Liver | | | Lung | | | *Peyer*'s patches | | | Spleen | | | Kidney | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g |
| 42 | 9. 40 | 1. 566 | 0. 166 | 2. 22 | 1. 191 | 0. 535 | 0. 19 | 0. 193 | 1. 098 | 0. 45 | 0. 405 | 0. 810 | 2. 11 | 0. 585 | 0. 277 |
| 43 | 11. 72 | 1. 917 | 0. 163 | 1. 68 | 0. 934 | 0. 556 | 0. 17 | 0. 168 | 0. 990 | 0. 70 | 0. 525 | 0. 768 | 1. 73 | 0. 597 | 0. 302 |
| 45 | 7. 55 | 1. 813 | 0. 159 | 1. 70 | 0. 780 | 0. 459 | 0. 15 | 0. 187 | 1. 293 | 0. 45 | 0. 354 | 0. 784 | 1. 60 | 0. 355 | 0. 222 |
| 46 | 13. 20 | 2. 184 | 0. 165 | 2. 96 | 1. 713 | 0. 450 | 0. 20 | 0. 155 | 0. 968 | 0. 75 | 0. 535 | 0. 714 | 2. 20 | 0. 585 | 0. 292 |
| Mean | 10. 217 | 1. 871 | 0. 163 | 2. 140 | 1. 153 | 0. 500 | 0. 177 | 0. 176 | 1. 062 | 0. 587 | 0. 457 | 0. 768 | 1. 910 | 0. 531 | 0. 273 |

Table 7. DNA-P content of various organs 10 months after total lymphadenectomy, thymectomy and splenectomy.

| Rat No. | Liver | | | Lung | | | *Peyer*'s patches | | | Kidney | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g |
| 5 | 17. 00 | 4. 054 | 0. 238 | 3. 80 | 2. 304 | 0. 604 | 0. 20 | 0. 246 | 1. 230 | 4. 10 | 1. 032 | 0. 252 |
| 7 | 13. 60 | 3. 159 | 0. 231 | 2. 19 | 1. 755 | 0. 801 | 0. 40 | 0. 570 | 1. 425 | 3. 56 | 0. 763 | 0. 215 |
| 8 | 16. 80 | 3. 936 | 0. 234 | 3. 75 | 2. 640 | 0. 704 | 0. 40 | 0. 558 | 1. 395 | 4. 20 | 0. 980 | 0. 234 |
| 10 | 14. 05 | 3. 457 | 0. 246 | 3. 45 | 2. 883 | 0. 835 | 0. 42 | 0. 555 | 1. 321 | 3. 06 | 1. 278 | 0. 417 |
| Mean | 15. 36 | 3. 651 | 0. 237 | 3. 297 | 2. 396 | 0. 735 | 0. 355 | 0. 482 | 1. 342 | 3. 730 | 1. 013 | 0. 279 |

Table 8. DNA-P content of various organs 10 months after thymectomy.

| Rat No. | Liver | | | Lung | | | *Peyer*'s patches | | | Spleen | | | Kidney | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g |
| 20 | 9. 25 | 1. 665 | 0. 180 | 3. 02 | 1. 311 | 0. 436 | 0. 11 | 0. 097 | 0. 826 | 0. 59 | 0. 496 | 0. 840 | 2. 00 | 0. 501 | 0. 251 |
| 22 | 14. 14 | 2. 755 | 0. 195 | 2. 46 | 0. 973 | 0. 396 | 0. 20 | 0. 249 | 1. 245 | 0. 71 | 0. 504 | 0. 772 | 3. 27 | 0. 772 | 0. 235 |
| 23 | 16. 93 | 4. 194 | 0. 247 | 2. 20 | 1. 342 | 0. 610 | 0. 29 | 0. 234 | 0. 704 | 0. 80 | 0. 564 | 0. 707 | 3. 00 | 0. 571 | 0. 191 |
| Mean | 13. 44 | 2. 871 | 0. 207 | 2. 560 | 1. 209 | 0. 480 | 0. 200 | 0. 191 | 0. 976 | 0. 700 | 0. 536 | 0. 773 | 2. 759 | 0. 615 | 0. 230 |

Table 9. DNA-P content of various organs 10 months after partial lymphadenectomy (removed of mesenteric nodes)

| Rat No. | Liver | | | Lung | | | *Peyer*'s pacthes | | | Spleen | | | Kidney | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g |
| 15 | 11. 24 | 2. 070 | 0. 237 | 2. 14 | 1. 422 | 0. 665 | 0. 27 | 0. 303 | 0. 748 | 0. 66 | 0. 357 | 0. 541 | 3. 03 | 0. 640 | 0. 211 |
| 16 | 11. 25 | 1. 788 | 0. 158 | 1. 61 | 0. 996 | 0. 624 | 0. 27 | 0. 386 | 0. 952 | 0. 69 | 0. 592 | 0. 858 | 1. 88 | 0. 963 | 0. 498 |
| 19 | 10. 34 | 2. 178 | 0. 210 | 2. 35 | 0. 758 | 0. 323 | 0. 24 | 0. 360 | 0. 965 | 0. 64 | 0. 564 | 0. 883 | 2. 72 | 0. 744 | 0. 270 |
| Mean | 10. 94 | 2. 011 | 0. 201 | 2. 033 | 1. 058 | 0. 537 | 0. 260 | 0. 350 | 0. 888 | 0. 663 | 0. 504 | 0. 761 | 2. 543 | 0. 783 | 0. 326 |

Table 10. DNA-P content of various organs 10 months after splenectomy.

| Rat No. | Liver | | | Lung | | | *Peyer*'s patches | | | Kidney | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g | weight of organ (g) | mg/ organ | mg/g |
| 26 | 8. 43 | 1. 846 | 0. 221 | 1. 86 | 1. 144 | 0. 607 | 0. 15 | 0. 194 | 1. 290 | 1. 65 | 0. 577 | 0. 441 |
| 30 | 12. 44 | 2. 476 | 0. 198 | 3. 70 | 2. 292 | 0. 620 | 0. 19 | 0. 186 | 1. 266 | 2. 79 | 0. 786 | 0. 280 |
| 31 | 18. 38 | 2. 869 | 0. 156 | 3. 11 | 1. 606 | 0. 546 | 0. 33 | 0. 345 | 1. 045 | 3. 15 | 1. 063 | 0. 337 |
| Mean | 13. 08 | 2. 397 | 0. 191 | 2. 890 | 1. 716 | 0. 591 | 0. 223 | 0. 241 | 1. 200 | 2. 530 | 0. 808 | 0. 352 |