Nagoya J. med. Sci. 30: 351-364, 1967

ROLE OF THE THYMUS IN THYMECTOMIZED MICE

TATSURO WATANABE

1st Department of Internal Medicine, Nagoya University School of Medicine
(Director: Prof. Susumu Hibino)

SUMMARY

Thymectomy was done on the newborn mice in less than 24 hours after birth, and the following results were obtained.

1) So-called "wasting syndrome" was noted in 60% of thymectomized C_3H mice, and in 50% of thymectomized $C_{57}BL$ mice.

2) A decrease in numbers of the lymphocyte was recognized not only in the peripheral blood but also in the lymphoid tissues of the thymectomized mice.

3) Transplantation of mastocytoma was possible, when thymectomy had been performed in advance, even among the mice of different histocompatibility.

4) When Primol 355 TA vaccine was injected into to extremities of the thymectomized mice and its booster was given subsequently, marked infiltration of the plasma cells was observed in the superficial lymph nodes and the spleen.

5) No significant change in the serum proteins was recognized between the control group and the thymectomized mice.

6) Agglutinin titer against TA vaccine in the thymectomized mice was almost equal to or even higher than that of the control group.

7) No significant difference of the transplantability of mastocytoma was noted between the thymectomized mice and the mice pre-treated with thyectomy in association with the injection of Freund's adjuvant and TA vaccine.

8) Mastocytoma was transplanted into the mice thymectomized either surgically or by X-ray irradiation. Transplantation of mastocytoma was impossible even in the mice pre-treated with thymectomy and selective X-ray irradiation.

I. INTRODUCTION

Very recently it has been found that the thymus plays an important role on the immune mechanism in the living body.

This recent advance has been derived from 2 different investigations. One investigation carried out by Gross[1], Furth[2], and others, recognized that the development of leukemia was inhibited by neonatal thymectomy in the mice which were predisposed to leukemia. Thus, the significance of the thymus on leukemogenesis was recognized. The other investigation, done by Good and Miller[1-7], involved thymectomy performed within 24 hours after birth resulting in significant decrease in the immune reactions in comparison to those of the

渡 辺 達 郎
Received for publication July 18, 1967.

conventional thymectomy.

Good and Miller concluded that the number of lymphocytes decreased not only in the peripheral blood but also in all the lymphatic tissues including even the Payer's plaques in the intestinal tract, and that the animals showed a decreased immunological functions: For example, skin transplantation was successfully performed among different mice showing no histocompatibility, and sometimes even the heterograft between rate and mice was not rejected, moreover, the production of the neutralizing antibody against T_2-bacteriophage was said to be inhibited.

Although the histopathological studies in this field have been relatively scanty, the experiment by Waksman[8] is worthy of note. According to Waksman, lymphocytes of the lymphoid tissues decreased markedly in rats thymectomized early, bot no significant quantitative change was noted in the germinal centers, as well as plasma cells and the cells belonging to the reticuloendothelial system. Our histological date to be presented in the following agree with those of Waksman, but some disagreements are noted as to the results reported by Good and Miller.

It is well recognized that the lymphoid tissues play an important role in immune mechanism, and there exist many different cell groups having immunological competence in the lymphoid tissues. It is of dire interest to note the phenomenon that the production of the circulating antibodies decresed although the plasma cells and the cells in the germinal center-germinoblast (Lennert) —related closely to bacterial antibodies or— globulin remain intact after thymectomy.

In addition, the serum protein pattern is normal in the thymectomized mice, showing no significant change in- and- globulins. This fact can be easily understood because the plasma cells and the germinoblasts are preserved. However, the ability to produce circulating antibodies is reduced, as pointed out by Miller. This discrepancy has prompted our present investigation.

II. EXPERIMENTAL METHODS AND ANIMALS

For the present experiments, mice were used mainly. Thymectomy was done in the neonates within 24 hours after birth. On thymectomy, the anterior portions of both the first and the second ribs were removed together with the sterum, and both lobes of the thymus were aspirated with an aspirator. The skin was closed with an acryl adhesive. Postoperatively, "Naramycin" was applied in order to protect the animals from cannibalism. Fatality due to the surgical procedure and cannibalism was about 30% for the entire experiments.

The strains of mice used included SM, C_{57}BL, C_3H, A/Jax, BALB/c, and LAF_1, and the same strain was used for each experiment. The SM and the

C_3H strains were supplied from Nagoya University. The $C_{57}BL$ and the A/Jax strains from National Institute of Genetics, BALB/c from Takeda Hikari farm, and LAF_1 strain from Cumberland View Animal Farms, Tennessee (U.S.A.).

III. EXPERIMENTAL RESULTS

1) Body weight and the wasting syndroms in the thymectomized mice

Figure 1 and 2 show weight gain in thymectomized mice. Variation in

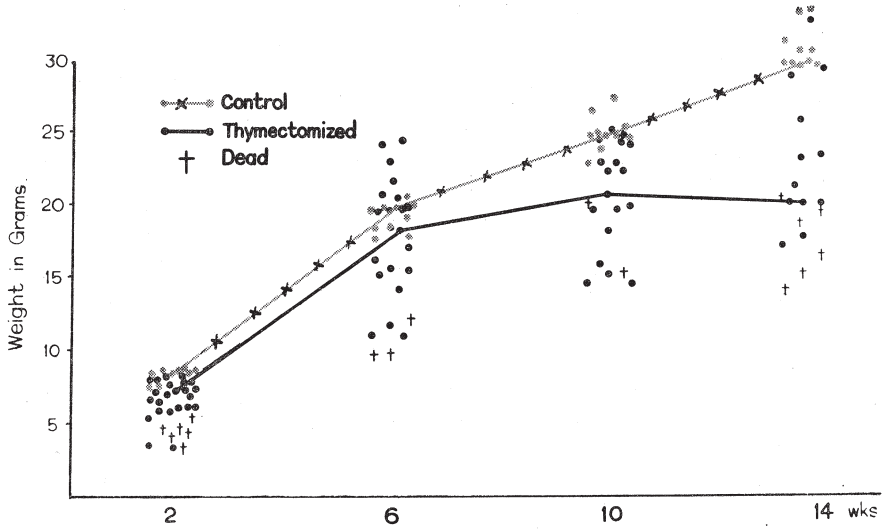


FIG. 1. Effect of neonatal thymectomy on growth and survival of C_3H mice

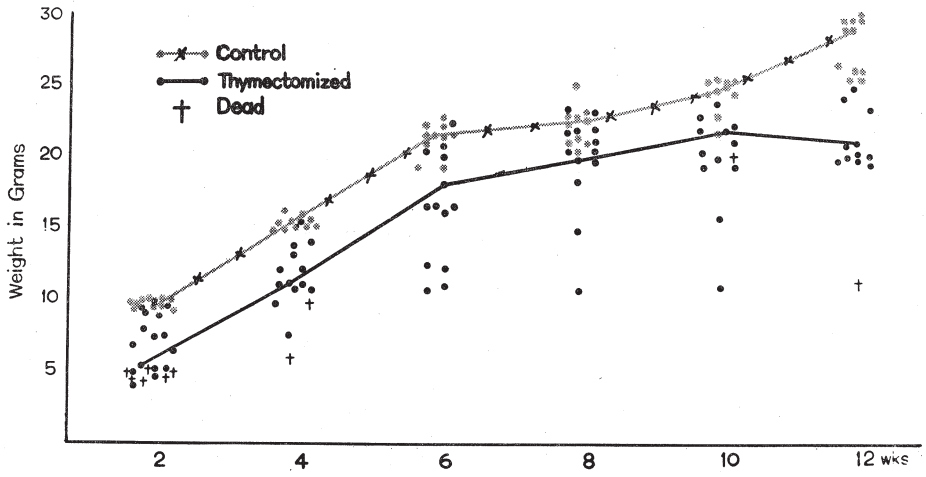


FIG. 2. Effect of neonatal thymectomy on growth and survival of $C_{57}BL$ mice

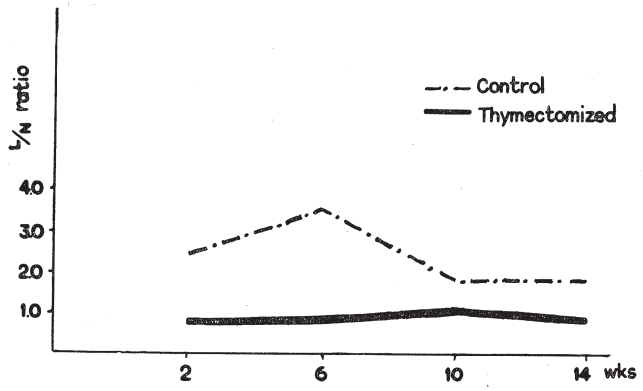
body weight is somewhat different with different strains of mice. Figure 1 is for C_3H strain and Fig. 2 for $C_{57}BL$. The rate of weight gain is poorer for thymectomized mice.

Neonatal thymectomy in mice resulted in wasting syndrome such as coarse haire, anemia, diarrhea and irregular gait, and many of them eventually died (a cross in the figures indicates death). During the follow-up peroid of 14 weeks after birth, death due to wasting syndrome occurred in 60% of C_3H strain mice and in 50% of $C_{57}BL$.

2) Decrease in the number of lymphocytes

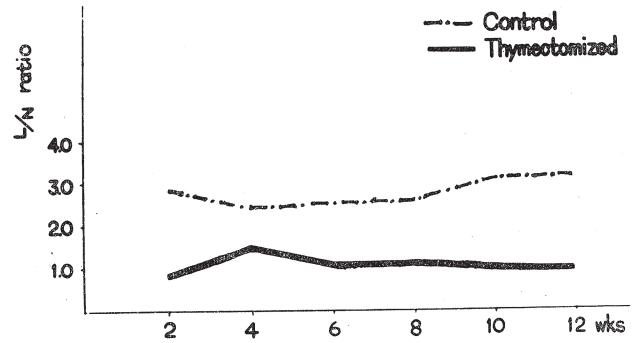
Figures 3 and 4 show the change in the peripheral blood, expressed as the lymphocyte/neutrophil ratio, L/N. As shown in Fig. 3, thymectomy resulted in lymphopenia; in C_3H strain, L/N at the 6th week is almost one, being equal between lymphocyte and neutrophil. In contrast, non-thymectomized mice of the same strain showed L'N to be 3.6. Lymphopenia was still recognized even at the 20th week.

A decrease in the number of lymphocytes was recognized not only in the peripheral blood but also in the lymphoid tissues. Photograph 1 shows the histological change in the spleen of thymectomized mice, while Photograph 2 shows the spleen of non-thymectomized mice. The germinal centers seem to be decreased in number in thymectomized group, but, as seen in Photogarph 3, the germinal centers themselves are not significantly different from those of the control group although the mantle zone shows significant decrease in the



	No. of Mice	L/N ratio 2	6	10	14 wks
Thymectomy within 24 hrs.	29	0.7	0.8	1.2	0.75
Non-thymectomy	10	2.5	3.6	1.7	1.7

FIG. 3. Average L/N ratio of C_3H mice thymectomized at birth and control.



	No. of Mice	L/N ratio 2	4	6	8	10	12 wks
Thymectomy within 24 hrs.	21	0.7	1.4	1.0	1.0	0.9	0.9
Non-thymectomy	10	2.9	2.5	2.6	2.9	3.2	3.2

FIG. 4. Average L/N ratio of C_{57} BL mice thymectomized at birth and control.

number of lymphocytes (Photograph 4).

3) *Transplantation of mast cell tumor (Furth)*

When the thymus is removed early after birth, allogneic tissue transplantation becomes possible. Thus, the importance of the thymus for immunity has been clearly established. Allogeneic tissue transplantation has been also tried for transplantable tumors, mainly breast carcinoma.

We have also studied the role of the thymus in transplantation, using mast cell tumor (Furth), a transplantable tumor in mice.

Mast cell tumor, found by Furth, developed originally in LAF_1 mice which was F_1 hybrid between $C_{57}BL$ and A strains. Transplantation of this tumor is possible in 100% only when LAF_1 mice are used; and not transplantable to C_3H, $C_{57}BL$, A/Jax, and BALB/c mice.

As shown in Table 1, transplantation of the tumor to those different strains of mice is successful if the recipient is thymectomized within 24 hours after birth.

TABLE 1. No. of Mice Taking Mast cell Tumor after Thymectomy SM

Treatment ↓ / Days →	2	5	10	20	30	40	224
Thymectomy within 24 hrs.	12/13 92.3%	22/22 100%	16/23 69.5%	8/10 80%	7/12 58.3%	8/12 66.6%	1/7 14.2%
Sham-thymectomy within 24 hrs.	0/12	0/10	0/7	0/10	0/8	0/11	0/2
Non-thymectomy	1/15 6.6%	0/10	0/22	0/10	0/12	0/16	0/2
Latency wks	2-3	2-3	3-4	1-2	2-3	1-2	1-2

In this experiment, SM strain of mice was used because it was easy to obtain and because it had histocompatibility different from that of LAF_1 mice. As seen in the table, when thymectomized within 24 hours after birth, the tumor was transplantable to SM strain of mice; occasionally, the transplanted tissue was successfully taken by the mice thymectomized even on the 220th day after birth. Thus, permanent tolerance seemed to be possible. The rate of successful transplantation was high for the mice thymectomized early after birth, while it was low when thymectomy was done late after birth. Almost all of our transplantation operations were performed 20 to 30 days after thymectomy, and the rate of successful transplantation of mast cell tumor was about 70%.

As seen in the table, the group receiving sham-thymectomy, in which the thorax was opened surgically but the thymus was not removed, did not take transplanted tissue as was the case with the group not receiving any surgical manipulation. Among the immature mice of SM strain subjected to transplantation operation within 24 hours after birth, transplantation was successful only on one mouse though it was not thymectomized.

The tumor which was transplanted to SM mice from LAF_1 mice was retransplanted back to LAF_1 mice. Even when SM mice were used, the tumor was transplanted through many generations when the mice were thymectomized within 24 hours after birth.

Mast cell tumor[9)10)] consists of neoplastic mast cells having similar characteristics with respect to function and morphology as those of normal mast cells. The neoplastic mast cells have many characteristic basophilic granules and contain a large amount of pharmacologically active substances such as heparin, histamine, and serotonin. Thus, on transplantation of the tumor, the cellular changes can be traced morphologically, pharmacologically and biochemically. Taking advantage of this property of the tumor, the tumor transplanted from original LAF_1 to SM mice and further through many generations of SM mice was studied.

Table 2 shows the amount of histamine per one gram of the tumor tissue (γ/g). When SM mice were used as a recipient for 3 generations, no significant difference was noted on the amount of histamine and also on the morphology of the tumor cells. Thus, mast cell tumor can be transplanted even to SM mice through many generations if mice were thymectomized as done in this study. However, it is not too dif-

TABLE 2. Histamine Contentof Mastocytoma in Thymectomized SM Mice

	Histamine γ/g
Original LAF_1	250
1st Generation SM	218
2nd Generation SM	184
3rd Generation SM	225

ficult to assume that the tumor may show some alteration of itself sooner or later, and as to this subject further studies are now in progress.

At any rate, the fact that the mast cell tumor is successfully transplanted to different strains of thymectomized subjects indicates that neonatal thymectomy results in an important immunological defect in allogeneic tumor transplantation. Besides changes in hematological and histopathological picture of the lymphoid tissues in these animals, the transplantability of the tumor may be effectively used in evaluating the effect of thymectomy.

4) Plasma cells in thymectomized mice

As previously mentioned, neonatal thymectomy in the animals results morphologically in reduction of lymphocytes of the lymphoid tissues and also in immunological alteration. Studying the lymphoid tissues in the SM strain mice with mast cell tumor transplanted, lymphocytes were apparently reduced, but not the secondary follicles —germinoblasts— and plasma cells.

The following study was to see the relation between the immunity in a living body and the plasma cell proliferation secondary to thymectomy.

First, an attempt was made to increase the plasma cells in the lymphoid tissues, and the experiments done by Potter *et al.* gave us an important clue for this purpose. Namely, they could produce myeloma experimentally by giving Freund's complete adjuvant or paraffin oil to BALB/c strain mice.

Microphotograph 5 shows the regional lymph node removed one month after injection of 0.5 ml Freund's complete adjuvant (Difco) to the sole of BALA/c strain mice. It shows proliferation of the epithelioid cells including oil droplets, and also abnormal proliferation of plasma cells in the medulla. Photograph 6 shows its touch preparation.

So far, using paraffin oil or Primol 355 (ESSO), we could not produce myeloma but proliferation of plasma cells in the lymph node. For the purpose to proliferate the plasma cells, Freund's adjuvant was more effective than paraffin oil. Proliferation of the plasma cells was produced in the superficial lymph nodes by injecting 0.5 ml of Freund's adjuvant into the extremities of the mice, but not in the spleen. At the same time, the serum protein pattern showed only slight change.

In order to produce proliferation of the plasma cells in the spleen, TA vaccine was injected intravenously, and its booster injection was given into the peritoneal cavity after a certain period of time. TA vaccine for diagnostic use was injected; namely, 0.05 mg of the bacteria was given per 10 g of mouse body weight. As for a booster injection, the same amount of the bacteria was given into the peritoneal cavity 2 weeks later.

The plasma cells usually present around the penicillary arterioles in the spleen. However, after the procedure mentioned above, abnormal proliferation

of the plasma cells was noted in the spleen, and sometimes the white pulp appeared to be filled with the plasma cells.

When the same procedure was followed on the mice thymectomized within 24 hours after birth, proliferation of the plasma cells in the superficial lymph nodes and the spleen was observed clearly in the non-thymectomized control group; sometimes, proliferation was much more pronounced in the thymectomized group. As shown in Photograph 7, abnormal proliferation of the plasma cells not only around the penicillary arterioles but also in the white pulps of the spleen was observed.

Occasionally, in the spleen or the lymph nodes some cells, sometimes looking like Russel cells, were present, having eosinophilic substance and being stained blue with Azan and positive on PAS stain (Photograph 8). Thus, the lymphoid tissues seem to have active protein metabolism.

5) Proliferation of the plasma cells and serum proteins in the thymectomized mice

Tables 3 and 4 show serum protein pattern in the mice with experimentally induced proliferation of the plasma cells. The changes in serum protein pattern were mild in the animals receiving only paraffin oil (Primol 355), somewhat more marked in the group receiving a large amount of Freund's adjuvant into the extremity, and most striking in the group receiving intravenous injection of TA vaccine and booster into the peritoneal cavity. Thus, there was a parallelism noted between the changes in serum protein pattern and the morphological changes of the plasma cells. Table 4 represents the data obtained in thymectomized animals, showing no difference the from data obtained on

TABLE 3. Total Serum Protein and Electrophoretic Pattern

SM-Strain		Case	T.P. (g%)	Serum Pattern (%)			
				Al	α-Gl	β-Gl	γ-Gl
Non-thymectomy		8	6.6 (6.2-7.0)	58 8 (44.6-61.2)	11.9 (8.6-15.0)	21.4 (16.9-25.3)	10.5 (5.8-18.8)
Non-thymectomy	+Primol	8	6.7 (6.4-7.0)	41.8 (35.8-65.6)	15.1 (13.6-20.2)	31.3 (24.6-36.8)	11.8 (8.2-15.7)
	+Adjuvant	8	6.8 (6.2-7.2)	45.9 (43.9-51.7)	13.9 (11.1-16.3)	25.6 (21.7-29.1)	14.6 (10.0-16.9)
	+T.A.B.	10	6.8 (6.0-7.4)	40.7 (36.4-47.2)	18.4 (11.1-24.0)	24.6 (18.9-33.1)	16.4 (10.2-22.9)
	+Adjuvant +T.A.B.	6	7.8 (7.0-8.0)	36.1 (26.8-45.7)	14.5 (9.6-22.0)	32.1 (33.0-33.0)	17.3 (13.2-22.6)
Total		40					

TABLE 4. Total Serum Protein and Electrophoretic Pattern

SM-Strain		Case	T.P. (g%)	Serum Pattern (%)			
				Al	α-Gl	β-Gl	γ-Gl
Thymectomy		6	7.6 (7.2-8.0)	59.9 (58.9-60.7)	15.5 (15.1-15.8)	16.2 (15.2-18.0)	8.6 (8.4-6.0)
Thymectomy	+T.A.B.	10	7.4 (6.2-7.8)	50.2 (41.5-60.8)	20.5 (13.9-27.3)	14.8 (11.5-16.2)	12.3 (9.4-16.0)
	+Adjuvant +T.A.B.	18	7.5 (6.4-8.4)	40.2 (35.0-49.5)	19.4 (10.5-22.8)	23.0 (16.8-27.9)	15.4 (13.9-19.6)
Total		34					

non-thymectomized group.

6) Proliferation of the plasma cells and TA agglutinin titer in thymectomized mice

Tables 5 and 6 represent the data obtained with TA agglutination test at

TABLE 5. Effect of Neonatal Thymectomy on the Formation of Salmonella Typhi Agglutinin in SM Mice (T.A.B. Vaccinated after 4-7 W Since Birth)

Treatment		Titre of Salmonella Typhi Agglutinin*
Non-thymectomy		9, 9, 8, 8, 8, 7, 7, 6, 6, 5.
	+Adjuvant	9, 8, 8, 8, 7, 6.
Thymectomy		9, 9, 9, 8, 8, 8, 7, 7, 6, 6.
	+Adjuvant	10, 10, 10, 10, 9, 9, 9, 8, 8, 8, 7, 7, 7, 6, 6, 5.

* The titre was expressed as reciprocal of the and point dilution and as powers of the base 2.

TABLE 6. Effect of Neonatal Thymectomy on the Formation of Salmonella Typhi Agglutinin in BALB/c Mice (T.A.B. Vaccinated after 4-7 W Since Birth)

Treatment		Titre of Salmonella Typhi Agglutinin*
Non-thymectomy		8, 7, 7, 7, 6, 6, 6, 6, 6, 5, 5.
	+Adjuvant	8, 8, 7, 7, 6, 6.
Thymectomy		8, 8, 7, 7, 7, 7, 6, 6, 5, 5.
	+Adjuvant	9, 9, 8, 7, 7, 6.

* The titre was expressed as reciprocal of the end point dilution and as powers of the base 2.

the tenth day after the vaccination. The titer was expressed as reciprocal of the end point dilution and as powers of the base 2, Table 5 being for SM strain mice and Table 6 for BALB/c strain mice. The agglutinin titer was not markedly different among the different strains of mice as well as between the thymectomized and non-thymectomized groups. It is interesting to note that the titer seemed to be even higher for the thymectomized group.

7) Proliferation of the plasma cells and tumor transplantation in thymectomized mice

From the previous experiments it is apparent that the circulating antibody at least against TA vaccine is sufficiently produced even in the condition where lymphocytes have been reduced by thmectomy. Table 7 shows the data obtained on transplantation of mast cell tumor after the plasma cells in the

TABLE 7. Transplantability of Tumor-Graft on Treated or Non-treated Thymectomized SM Mice

Treatment	No. of Mice	Tumor Transplantation after Thymectomy at 35-45 days		
Thymectomy within 24 hrs.	24	15/24	62.5%	Depletion of Lymphocyte in Lymphoid Tissue
Thymectomy within 24 hrs.+Compl. Adjuvant	12	8/12	66.6%	Plasmacytosis in Lymph Nodes
Thymectomy within 24 hrs. +T.A. Vaccination	9	3/ 9	33.3%	Increasing of Plasma cells in Spleen
Non-Treatment	16	0/16		

spleen and lymph nodes have been proliferated and the titer of the agglutinin against TA vaccine has been sufficiently elevated. Namely, the rate of successful transplantation on the SM strain mice thymectomized within 24 hours after birth is 62.5%. On the thymectomized mice, to which Freund's adjuvant was injected into the sole, followed by the intravenous TA vaccine and intraperitoneal booster injection, the tumor transplantation rate was almost the same although the lymph nodes and spleen were huge.

Thus, in thymectomized mice, the production of bacterial antibody, at least TA agglutinin, is almost the same as that of the control group, and the antibody titer can also be raised by proliferation of the plasma cells in the lymphoid tissues. From the standpoint of allogeneic tissue transplantation, however, certain immunological defect still exists and it is reasonable to assume that this might be a consequence of neonatal thymectomy.

Thymectomy results in the disappearance of lymphocytes. Therefore, a

defect in transplantation immunity induced by thymectomy does not interfere with the production of the bacterial antibodies carried by the plasma cells. Therefore, the lymphocytes and the plasma cells are independent from each other; and immunologically they seem to be fundamentally different. Our results agree with those of the late Professor Amano[11] that lymphocytes produce cell antibodies while plasma cells produce γ_2 globulin.

8) Selective X-ray irradiation of the thymus

As mentioned previously, wasting syndrome was frequently noted in the thymectomized animals. Wasting syndrome, as pointed out by some investigators, seems to be related to bacterial infection, and the evidences for sepsis were occasionally seen at autopsy. Therefore, thymectomy by irradiation was performed in order to exclude any possibility of infection.

Using a lead plate with a 5 mm hole, irradiation was given to the thymus of C_3H strain mice within 24 hours after birth under the condition of 40 cm distance, 200 kVp, 20 mA and 48 rpm. As shown in Fig. 5, various changes developed with 1,000 r to 2,000 r. The body weight was significantly decreased

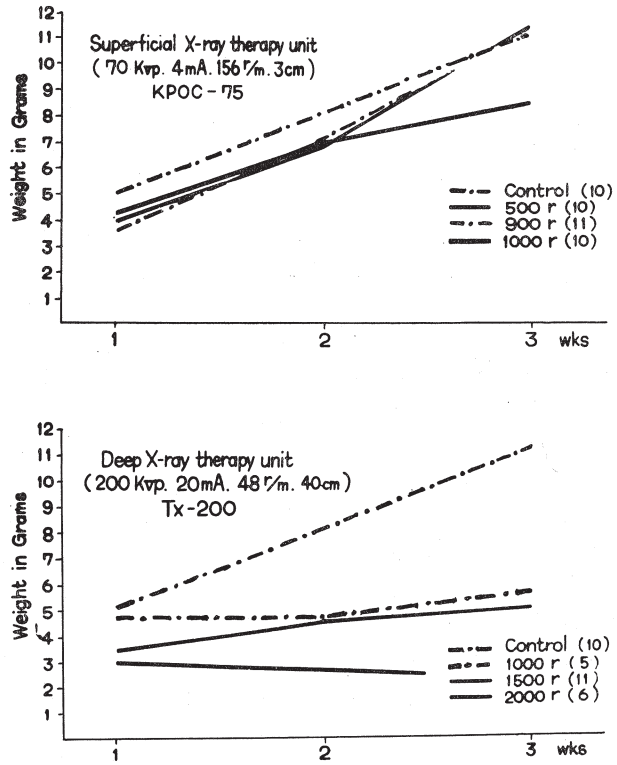
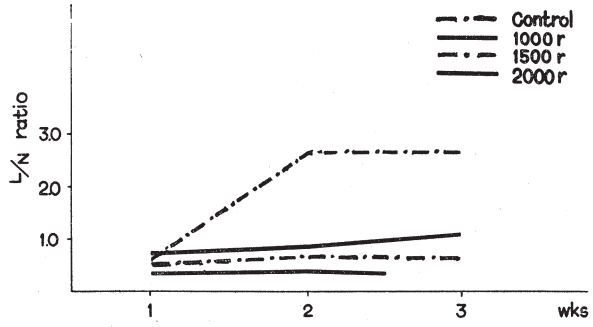


FIG. 5. Effect of superficial and deep X-ray irradiation on growth of neonatal C_3H mice.



Thymus and spleen weight on the 20th day after deep X-ray irradiation

	No. of Mice	20 days	
		Thymus (mg)	Spleen (mg)
1000 r Irradiation	5	15.8	43
1500 r Irradiation	11	14.7	33.8
2000 r Irradiation	6	2.1	8.5
Control	10	34.2	56.1
1000 r Superficial X-ray irradiation	10	32.2	48

FIG. 6. Average L/N ratio of C_3H mice at neonatal deep X-ray irradiation.

in comparison to that of the litter mate. With 2,000 r irradiation, all of the mice expired in 2 to 3 weeks. As seen in L/N ratio, lymphocytes decreased markedly, more pronounced than that of the surgically thymectomized group (Fig. 6).

The data shown in the lower part of Fig. 6 indicate the weight of the thymus and the spleen when autopsied on the 20th day after irradiation. They both were decreased in size markedly in comparison to those of the control group. Atrophy of the thymus was especially marked. Photographs 9 and 11 show the histological appearance of the thymus after irradiation, while Photograph 10 show the same of the not irradiated. With irradiation, the thymus shows significantly marked decrease in lymphocytes, and also marked degenerative changes were noted as seen in Photograph 12.

There was no significant histological difference on the spleen between the groups thymectomized surgically and radiologically. However, both procedures resulted in lymphopenia in the peripheral blood.

When mast cell tumor was transplanted to the thymectomized mice with deep irradiation, tumor transplantation was not possible as shown in Table 8.

TABLE 8. Correlation between Thymectomy and Deep X-Ray Irradiation at Birth of C_3H Mice

*Treatment	No. of Mice	Tumor Graft	No. of Mice Dying between				20 days (mg)	
			<1 wks	1-2 wks	2-3 wks	<4 wks	Thymus	Spleen
1,000 r Irradiation at birth	5	0/5	0	2	3		21.6	55.6
1,500 r Irradiation at birth	10	0/10	0	2	8		8.1	14.2
2,000 r Irradiation at birth	13	0/13	0	10	3		1.7	7.5
Thymectomy within 24 hrs.	45	38/45	0	0	4	34		
Non-treatment	16	0/16	0	0	2	14	32.5	73.8
Control	10						34.2	56.1

* Deep X-Ray Therapy Unit.
TX-200, 200 Kvp, 20 mA, 48 r/m, 40 cm.

In contrast, the transplantation was possible in 85% of C_3H strain mice thymectomized surgically.

The fact that tumor transplantation is impossible in the mice thymectomized almost completely with irradiation and with significant lymphopenia seems to be paradoxical on studying the role of lymphocytes in immune mechanism.

IV. CONCLUSION

It is clear that the thymus plays an important role in the immune mechanism of a living body. Suppose that the cellular immunity is closely related to lymphocytes and circulating immunity to plasma cells, the thymus should be more closely related to the production of the cellular antibodies. The purpose of this experiment was to evaluate the above point. From the experimental data obtained, it was concluded that the neonatal surgical thymectomy has a grave effect on tumor transplantation which was carried out as a tool for evaluating cellular immunity, while the procedure does not affect the production of circulating antibodies.

It is known that there are many other cell groups belonging to so-called "immunological competent cell" besides the lymphocytes and the plasma cells. Yet little has been understood about the relation between these cells and the thymus. For this purpose, new immunochemical methods including immunoelectrophoresis and other approach using isotopes should be applied.

Being apparent from the data obtained through X-ray irradiation thymectomy, the characters of the thymus as an endocrinologic organ cannot be neglected in relation to immunity. If the thymus is divided into the cortex

and the medulla, the cortex is quite sensitive to X-ray, while the medulla is quite resistant[12]. This fact is naturally related to LSF (lymphocytosis-stimulating factor, Metcalf)[13], and further to the relationship between immunity and the endocrinological system.

LITERATURE

1) Gross, L., Effect of Thymectomy on Development of Leukemia in C3H Mice Inoculated with Leukemia "Passage" Virus, *Proc. Soc. Exp. Biol. Med.*, **100**, 325, 1959.
2) Levinthal, J. D., Buffett, R. F. and Furth, J., Preventation of Viral Lymphoid Leukemia of Mice by Thymectomy, *Proc. Soc. Exp. Biol. Med.*, **100**, 610, 1959.
3) Miller, J. F. A. P., Immunological Function of the Thymus. *Lancet*, **2**, 748, 1961.
4) Martinez, C., Kersey, J., Papermaster, B. W. and Good, R. A., Skin Homograft Survival in Thymectomized Mice, *Proc. Soc. Exp. Biol. Med.*, **111**, 41, 1962.
5) Martinez, C., Dalmasso, A. P. and Good, R. A., Effect of Thymectomy on Development of Immunological Competence in Mice, *Ann. N. Y. Acad. Sci.*, **113**, 933, 1964.
6) Miller, J. F. A. P., Role of the Thymus in Transplantation Immunity, *Ann. N. Y. Acad. Sci.*, **99**, 340, 1962.
7) Good, R. A., Dalmasso, A. P., Marcinez, C., Archier, O. K. and Papermaster, B. W., Role of Thymus in Development of Immunological Capacity in Rabbits and Mice, *J. Exp. Med.*, **116**, 773, 1962.
8) Waksman, B. H., Arnason, B. G. and Jankovic, B. D., Role of thet hymus in Immune Reactions in Rats. I. II. III., *J. Exp. Med.*, **116**, 159, 1962.
9) Furth, J., Hagen, P. and Hirsch, E., Transplantable Mastocytoma in the Mouse Containing Histamine, Heparin and 5-Hydroxytryptamine, *Proc. Soc. Exp. Biol. Med.*, **95**, 824, 1957.
10) Ono, S., The Mast Cells, *Acta Hemat. Jap.*, **25**, 280, 1962.
11) Amano, S., Antibody producing cells and antibody proteins, *Acta. Hemat. Jap.*, **25**, 840, 1962. (in Japanese)
12) Ghossein, N. A., Azar, H. A. and Williams, J., Local Irradiation of the Thymus, *Amer. J. Path.*, **43**, 369, 1963.
13) Metcalf, D., The Thymic Lymphocytosis Stimulating Factor, *Ann. N. Y. Acad. Sci.*, **73**, 113, 1958.

EXPLANATION OF PHOTOGRAPHS

PHOTO. 1. Spleen at the 8th week after thymectomized 16 hours after birth (SM strain mouse).
PHOTO. 2. The control spleen for comparison to 1.
PHOTO. 3. Germinal center of the mouse shown in 1, showing a decrease in lymphocytes in so-called mantle zone.
PHOTO. 4. Germinal center of the control mouse, showing rich lymphocytes in mantle zone.
PHOTO. 5. Lymph node, an increase in plasma cells in the medulla.
PHOTO. 6. Touch preparation prepared from the same lymph node shown in 5.
PHOTO. 7. Increase in plasma cells in the spleen.
PHOTO. 8. Russel-like cells in the spleen.
PHOTO. 9. The thymus of C3H strain mouse with neonatal thymectomy by X-ray irradiation (2,000 r).
PHOTO. 10. The thymus of the control C3H mouse as shown in 9.
PHOTO. 11. High magnification of the thymus shown in 9. ($\times$400)
PHOTO. 12. Touch preparation prepared from the same lymph node shown in 9.

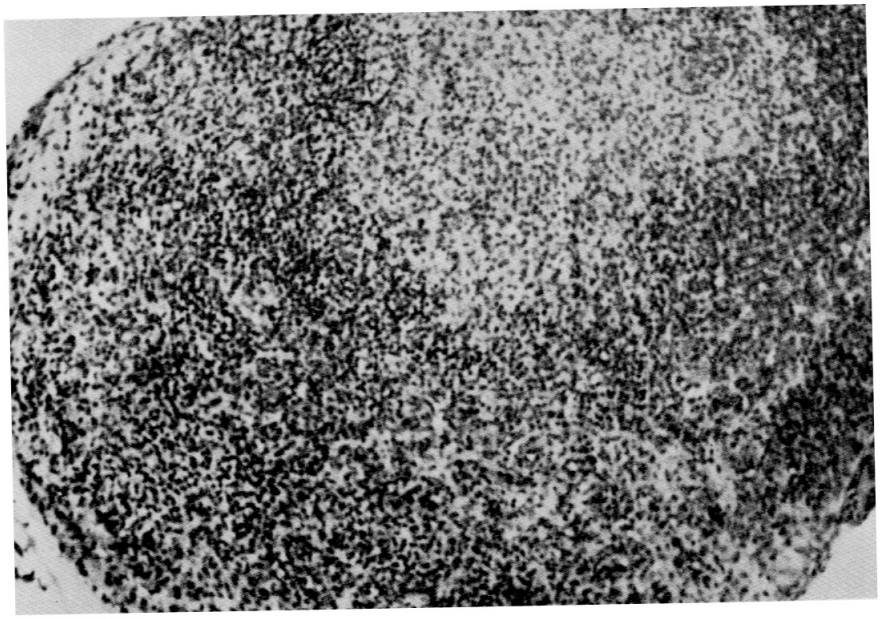


PHOTO. 1

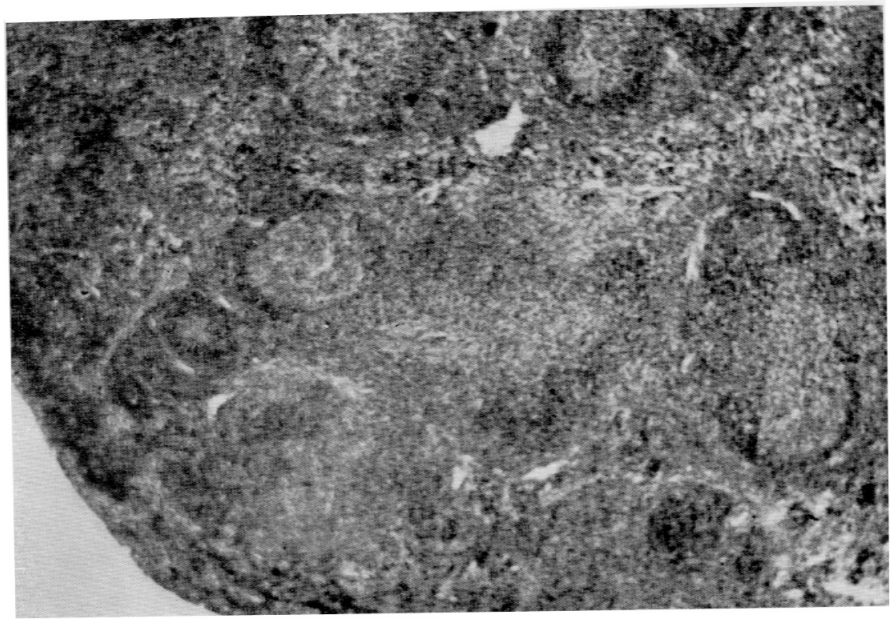


PHOTO. 2

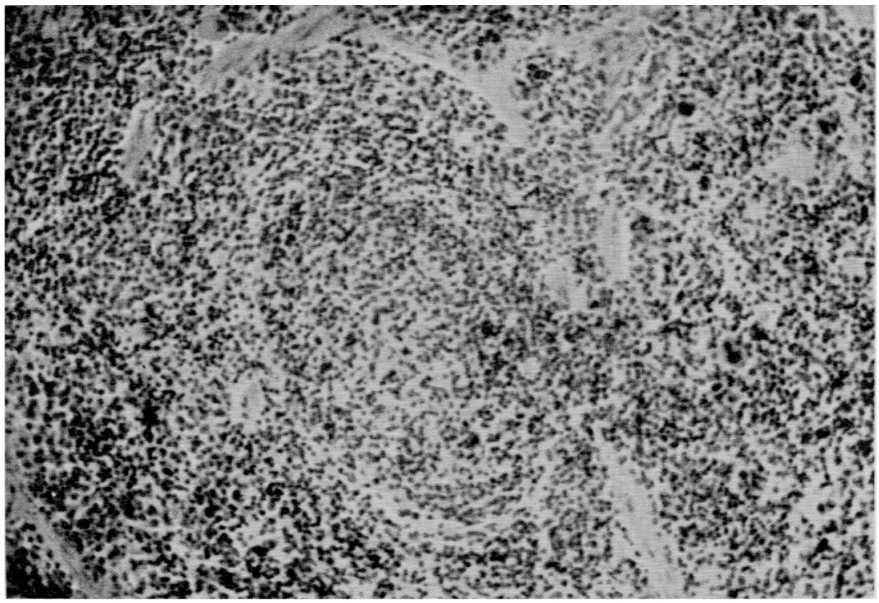


Photo. 3

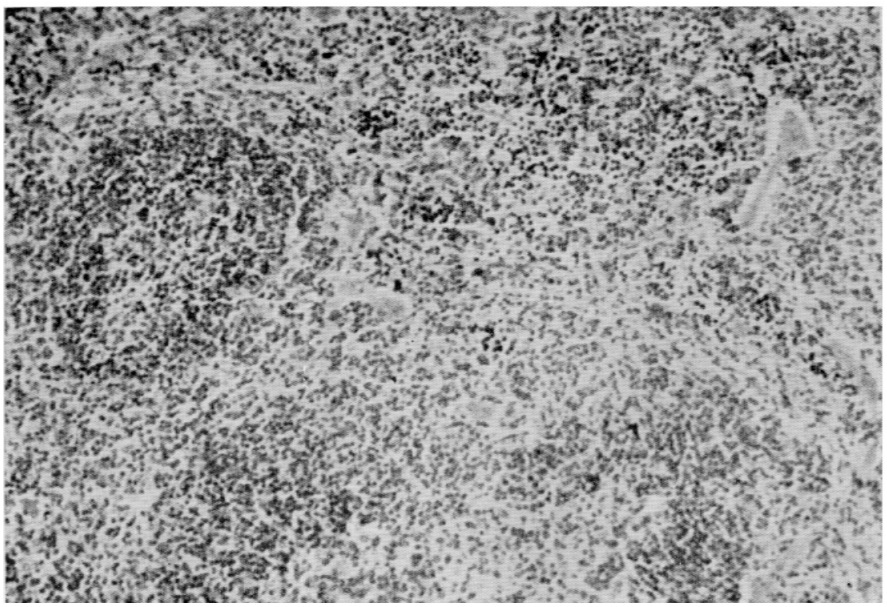


Photo. 4

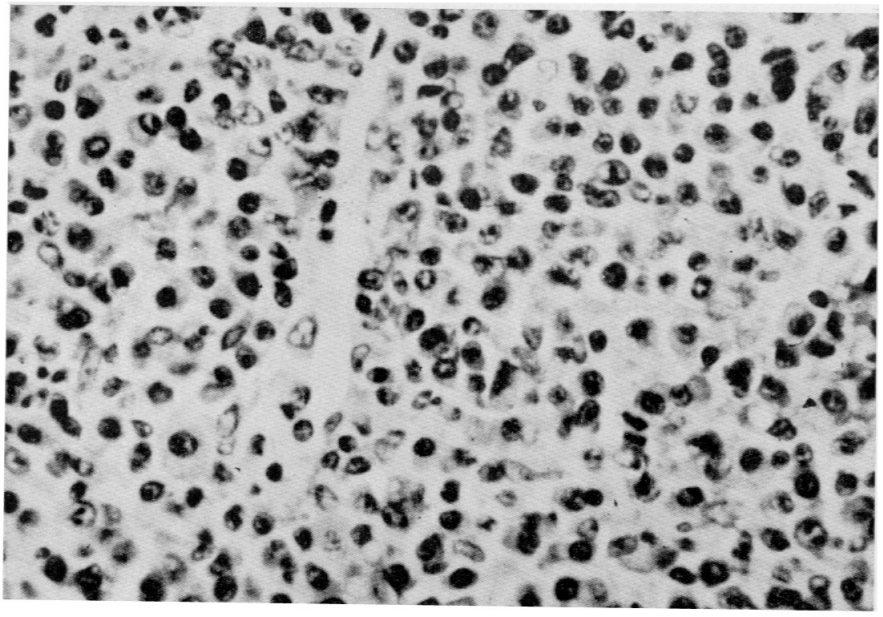


PHOTO. 5

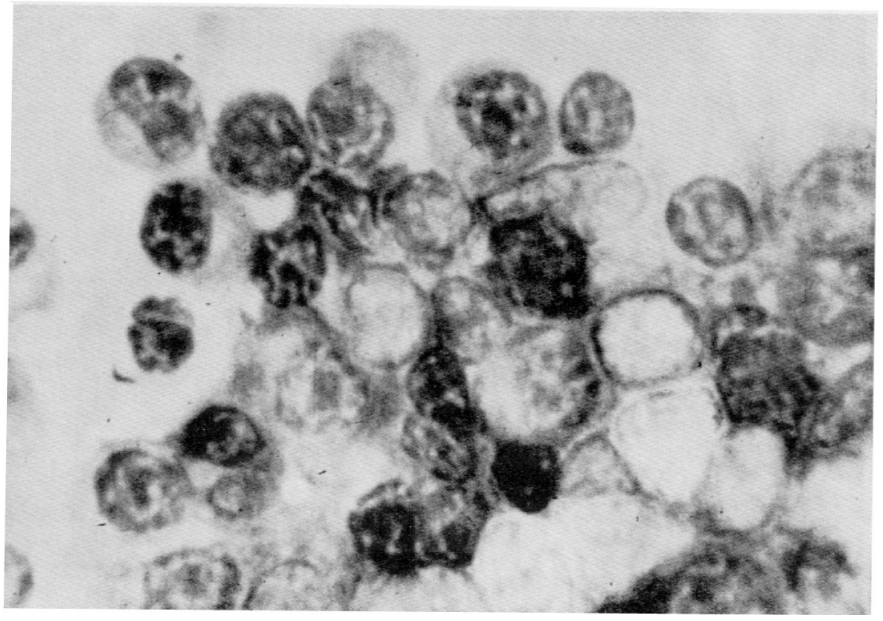


PHOTO. 6

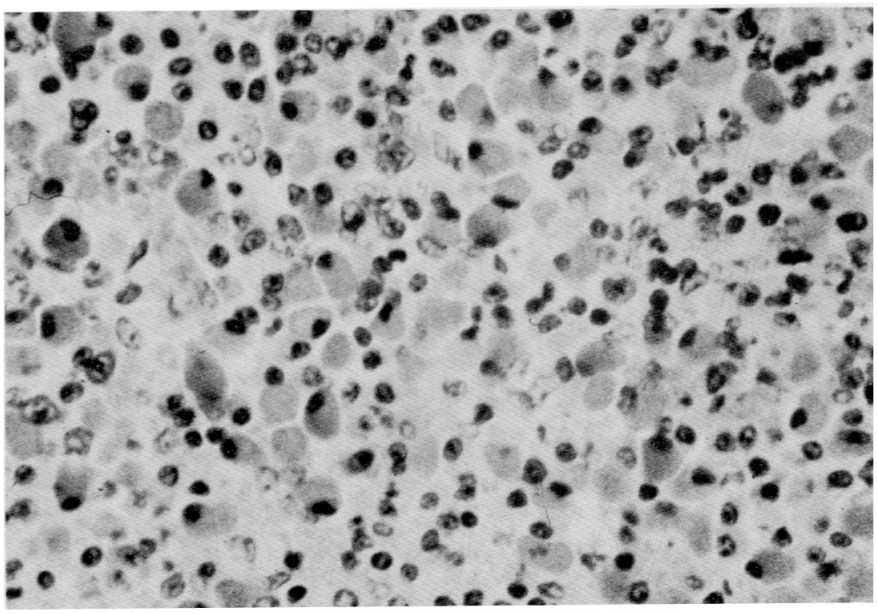


Photo. 7

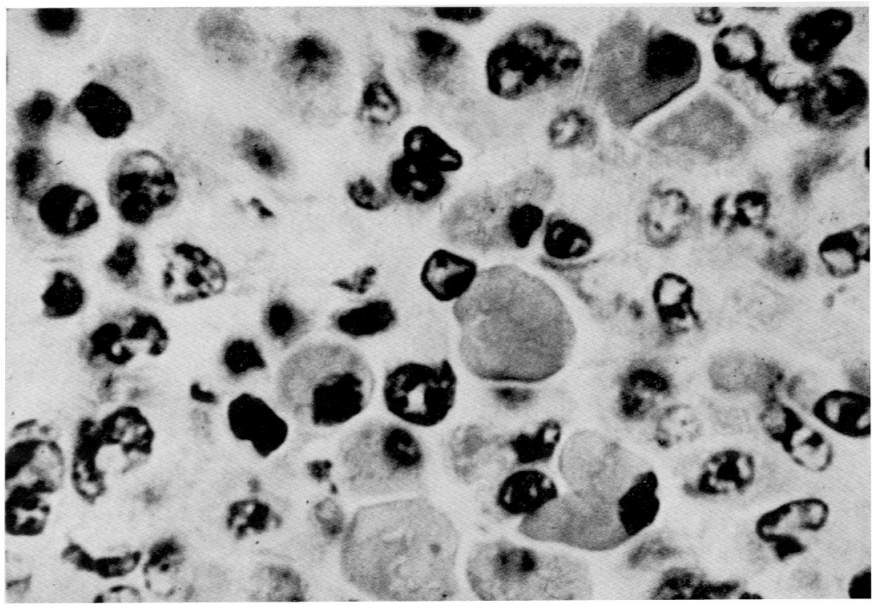
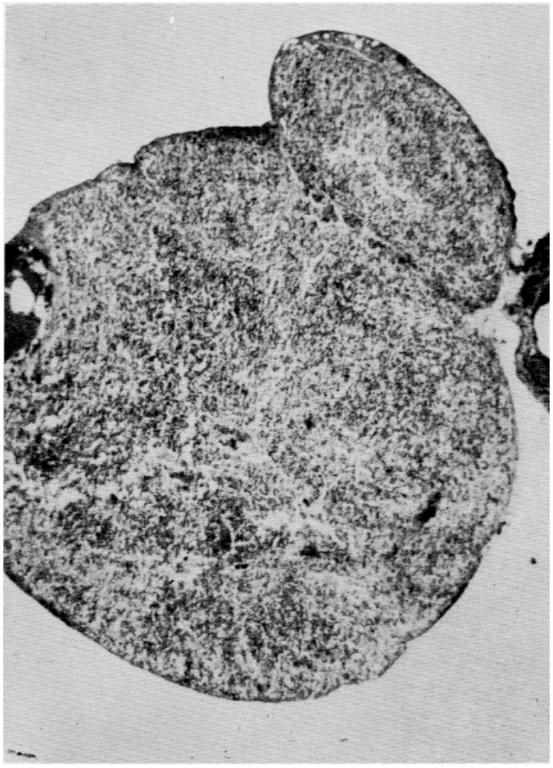
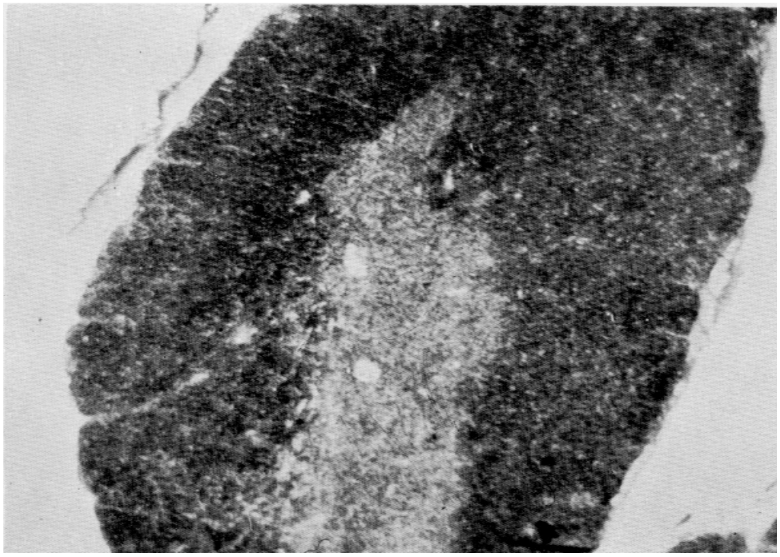


Photo. 8



Рното. 9



Рното. 10

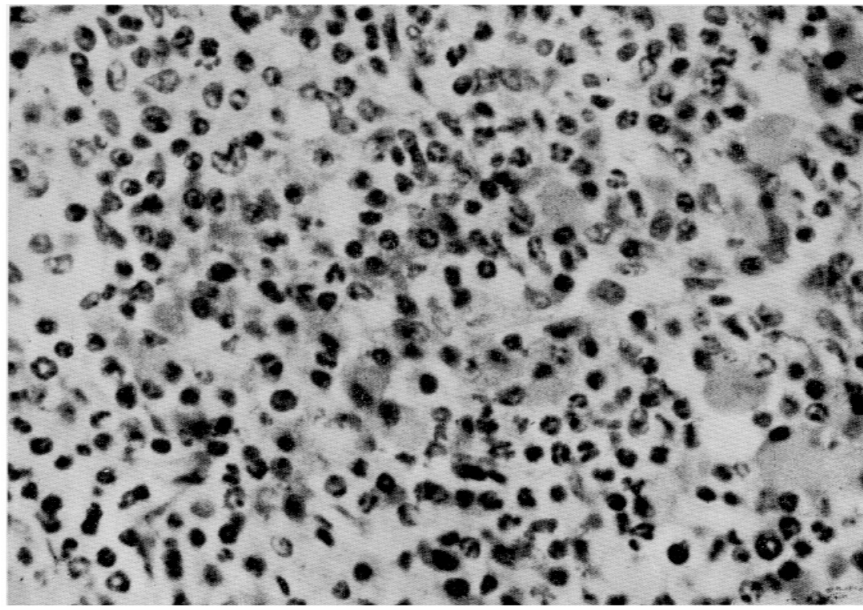


Photo. 11

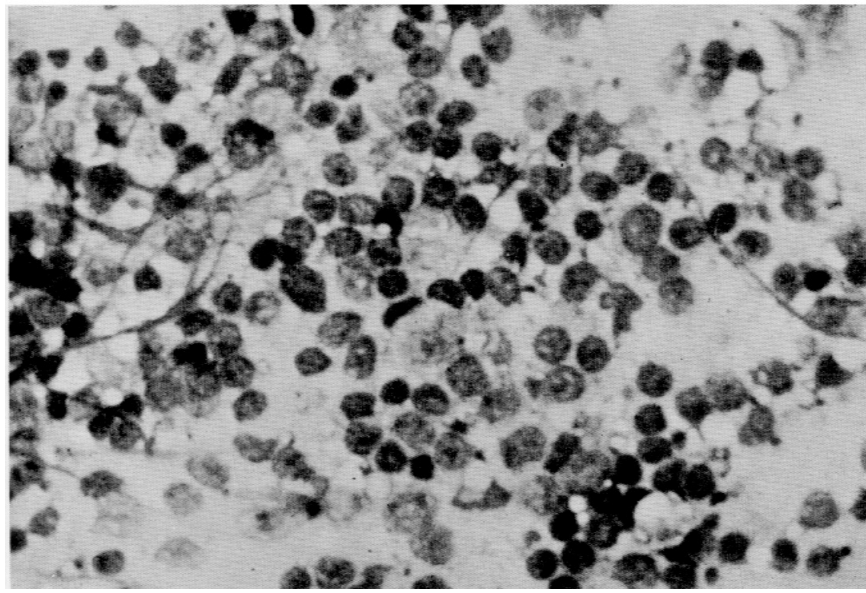


Photo. 12