

## STUDIES ON LYMPHOID ACTIVITY.

### VI. IMMUNITY TO TRANSPLANTED CANCER INDUCED BY INJECTION OF OLIVE OIL.

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PLATES 34 TO 36.

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It has been frequently suggested that the lymphoid cells are in some way concerned with the absorption and digestion of fats and lipoids. Recently a number of investigators have reported on the cellular changes following injections of these substances. Ramond<sup>1</sup> found that olive oil injected intraperitoneally is gradually absorbed by white cells of the lymphoid variety, and Clark<sup>2</sup> noted that the subcutaneous injection of olive oil exerts what he considers to be a chemotactic influence on the lymphatic endothelium and lymphocytes. Bergel<sup>3</sup> confirmed and extended these observations by finding that the cellular exudate after an intrapleural or intraperitoneal injection in animals of fatty oil or oil emulsion of lecithin is almost entirely made up of the lymphoid type of cell.

It is well known that the local reaction following an injection of homologous living tissue in mice consists mainly of a lymphoid cell outpouring<sup>4</sup> similar to that described above. Murphy and Nakahara<sup>5</sup> observed that this local reaction is accompanied by evidences of increased proliferative activity among the lymphoblastic cells of the spleen and lymph nodes. It may be stated in passing that mice thus injected with homologous tissue become highly resistant to

<sup>1</sup> Ramond, M. F., *Compt. rend. Soc. biol.*, 1904, lvi, pt. 2, 95.

<sup>2</sup> Clark, E. R., and Clark, E. L., *Am. J. Anat.*, 1917, xxi, 421.

<sup>3</sup> Bergel, S., *Berl. klin. Woch.*, 1919, lvi, 915; *Z. exp. Path. u. Therap.*, 1920, xxi, 216; *Ergebn. inn. Med.*, 1921, xx, 36.

<sup>4</sup> Da Fano, C., *Z. Immunitätsforsch., Orig.*, 1910, v, 1.

<sup>5</sup> Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, xxxi, 1.

transplanted cancer.<sup>6</sup> A like stimulation of the proliferative activity of the lymphoid cells may be induced by certain physical agents,<sup>7</sup> with resultant increased resistance to cancer transplants.<sup>8</sup>

In view of these observations it was regarded as of interest to determine whether or not the local reaction to oil is accompanied by a general lymphoid stimulation and, if so, the effect on the resistance to cancer inoculation in mice.

#### *General Lymphoid Response to Injections of Olive Oil.*

Commercial olive oil,<sup>9</sup> described as the first expression, was used in the following experiments. The injections were made intraperitoneally, followed by a histological study of the general condition of the lymphoid organs, with special attention to the number of mitotic figures present as this had been shown to be a fair index of the degree of stimulation.<sup>10</sup>

*Experiment 1.*—Twenty-five normal white mice of about the same age and size were divided into five groups of five mice each. The mice of Group A received an intraperitoneal injection of 0.1 cc. of the oil, Group B received 0.2 cc., Group C, 0.3 cc., Group D, 0.5 cc., and Group E, 0.7 cc. One animal from each group was killed for histological study 24 hours, 48 hours, 3 days, 4 days, and 5 days after the injection.

*Group A.*—Mice of this group received each 0.1 cc. of the oil. No unusual features were found in lymphoid organs of any of the mice, excepting one that was killed 4 days after the injection, and the germ centers of the lymphoid tissue of this mouse showed a marked increase of mitotic figures.

*Group B.*—Mice of this group received each 0.2 cc. of the oil. The mouse killed 24 hours after the injection showed no unusual condition, but four others killed at 48 hours to 5 days did show, particularly in germ centers (Fig. 1), a definite increase in mitosis.

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<sup>6</sup> Bashford, E. F., *Brit. Med. J.*, 1906, ii, 209.

<sup>7</sup> Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1. Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17. Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

<sup>8</sup> Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 25. Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 429.

<sup>9</sup> It was estimated that this product contained about 30 per cent of cottonseed oil. This was determined by the density of the brown color following the addition of nitric acid, using as a standard known dilutions of the cottonseed oil.

<sup>10</sup> Nakahara, W., and Murphy, Jas. B., *Anat. Rec.*, 1921, xxii, 107.

*Group C.*—Mice of this group received each 0.3 cc. of oil. An appreciable but less marked increase in the number of mitotic figures was present 48 hours to 5 days after the injection.

*Group D.*—Mice of this group each received 0.5 cc. of oil. Judged by the number of mitotic figures, proliferative activity was retarded in the 24 and 48 hour specimens and about normal in the remaining animals.

*Group E.*—Mice of this group each received 0.7 cc. of the oil. In mice killed at the 24 and 48 hour periods, the lymphoid organs, especially the spleens, were much reduced in size. These small lymphoid organs showed an almost complete suppression of mitosis, reduction in the amount of lymphoid tissue, and, in the splenic pulp, vacuoles of various sizes. At later periods, while the size of lymphoid organs was still small, the rate of mitosis was approximately normal.

The preceding experiment indicates that the most pronounced reaction in the lymphoid organs followed the intraperitoneal injection of a dose of 0.2 cc. of the oil.

*Experiment 2.*—Twelve normal white mice were injected intraperitoneally with 0.2 cc. of olive oil as in Group B of the preceding experiment. The mice were killed in groups of three each at 48 hour, and 4, 7, and 10 day intervals.

Of the three mice killed 48 hours after the injection, one showed unmistakable increase in the number of mitotic figures in the lymphoid germ centers, another a less striking but distinct increase, and a third mouse no appreciable increase.

At 4 and 7 day periods, all the mice showed a greatly increased number of mitotic figures. In one mouse killed at the 4 day period the mitosis was especially exaggerated, and was present not only in, but outside the germ centers.

At the 10 day period, mitotic figures were still abundant in two mice and there were a few in the third.

As a control to the above experiment, a number of normal mice were injected intraperitoneally with 0.2 cc. of liquid petrolatum without leading to an increase of the mitosis.

*Other Organs.*—The livers of a few of the mice injected with oil had many intracellular vacuoles suggestive of fatty inclusions. Also occasionally there were found a marked dilatation of the capillaries and sinus-like spaces of the suprarenal gland and an increase in the number of mitotic figures in the cortical cells (lymphocytes) of the thymus. These findings were of irregular occurrence and, therefore, should not be classed as typical changes induced by the oil injection. No special alterations were noted in the thyroid glands, kidneys, or bone marrow.

*Cytology of the Peritoneal Exudate.*—Smears were taken of peritoneal fluid at autopsy in Experiments 1 and 2. As already pointed out by Bergel,<sup>3</sup> all the smears showed numerous cells of lymphoid group, including typical large and small lymphocytes, so called transitional cells, plasma cells, large cells resembling macrophages, and true endothelial cells. Morphologically, there is a complete series of intergradations from the typical small lymphocyte to the large macrophage-like cell, suggesting that they all belong to a single biologic group, a point which has been emphasized by Bergel.<sup>3</sup> At an early stage (24 hours after the oil injection), there is a considerable number of polymorphonuclear cells, particularly neutrophils and eosinophils, in addition to lymphoid cells, but the number of these granular cells soon falls off. At the 48 hour period, the lymphoid reaction is apparently at its height, while at this time the polymorphonuclear reaction has about subsided (Fig. 2). The local reaction occurs regardless of the amount of oil injected and lasts as long as the oil remains in the peritoneal cavity. When 0.2 cc. of olive oil was injected the reaction tended to subside within 10 days, or hand in hand with the gradual absorption of the oil.

The above experiments indicate that lymphoid tissue as a whole responds definitely to an intraperitoneal injection of olive oil, which, if given in the optimum quantity, brings about a marked stimulation of the proliferative activity of this tissue. Studies on the peritoneal exudates, moreover, confirmed the results reported by Ramond,<sup>1</sup> Clark,<sup>2</sup> and Bergel,<sup>3</sup> regarding the local cellular manifestations about the injected fatty and lipoidal substances.

The relation between the local lymphoid reaction and the stimulation of the germ centers cannot be determined directly. However, in view of the lipolytic function of lymphoid cells,<sup>11</sup> it does not seem improbable that the local lymphoid response to the injected oil is an expression of the attempt of the body to dispose of the injected material. If so, it is conceivable that an optimum grade of activity on the part of lymphoid cells may lead to the general lymphoid stimulation.

<sup>11</sup> Bergel, S., *Münch. med. Woch.*, 1909, lvi, 64. Fiessinger, N., and Marie, P.-L., *Compt. rend. Soc. biol.*, 1909, lxxvii, pt. 2, 177.

*Cancer Inoculation Experiments.*

The production by injection of olive oil of lymphoid stimulation, essentially similar to the condition of induced potential immunity to transplanted cancers, suggested the possibility of rendering animals resistant by the same method. Experiments were accordingly undertaken to determine this point.

The dose of olive oil was given in a single intraperitoneal injection. Cancer inoculation was made subcutaneously in the left groin, and the rate of growth of tumors was charted thereafter at weekly intervals for 3 weeks. The strain of cancer used was Bashford Adenocarcinoma No. 63, and all the mice were young adults of white variety from the same stock.

*The Degree of Resistance in Relation to the Amount of Olive Oil Injected.*

*Experiment 3.*—In order to determine the optimum dose of olive oil, this substance was injected into mice in different quantities, ranging from 0.1 to 0.7 cc. Cancer inoculation was made in every case 10 days after the injection. The results are summarized in Table I.

TABLE I.  
*Experiment 3.*

Amount of olive oil.	Treated mice.		Controls.	
	Resistance.	No. of mice.	Resistance.	No. of mice.
<i>cc.</i>	<i>per cent</i>		<i>per cent</i>	
0.1	20.5	19	0.0	10
0.2	40.0	18	0.0	19
0.3	25.0	20	5.5	21
0.5	6.1	23	11.1	9
0.7	0.0	9	0.0	9

Since the number of mice in each group is small, slight differences in the percentage of resistance in the several groups should not be considered as significant. However, the fact that emerges is that by injecting 0.2 cc. of olive oil 10 days before giving a cancer inoculation, mice are rendered more resistant to the inoculated cancer than they

normally are. This same point was brought out in three additional experiments, the results of which are shown in Table II.<sup>12</sup>

TABLE II.  
*Experiments 4 to 6.*

Experiment No.	Mice injected with olive oil 10 days before cancer inoculation.		Controls.	
	Resistance.	No. of mice.	Resistance.	No. of mice.
	<i>per cent</i>		<i>per cent</i>	
4	43.7	16	11.1	9
5	52.6	19	10.0	10
6 (Text-fig. 1).	41.0	20	10.5	19

*The Degree of Resistance in Relation to the Time of Cancer Inoculation.*

*Experiment 7.*—In the preceding experiment the cancer inoculations were made 10 days after the oil injection. In order to ascertain the period at which the maximum degree of resistance is manifested, the inoculations in the following experiment were made at various intervals after an injection of 0.2 cc. of the oil. The results are shown in Table III.

TABLE III.  
*Experiment 7.*

Intervals between oil injection and cancer inoculation.	Resistance.	No. of mice.
<i>days</i>	<i>per cent</i>	
5	20.0	10
10	44.4	9
15	30.0	10
25	10.0	10
Control.	0.0	12

In all the former types of induced resistance to transplanted cancer so far studied, there is a period following the treatment during which there is slight, if any, evidence of resistance. This is not only true as

<sup>12</sup> The amount of olive oil should be slightly changed according to the size of the mouse. For a large mouse, weighing over 25 gm., as much as 0.3 cc. can be given.

regards the injection of homologous living tissue,<sup>13</sup> but also equally after exposure to intense dry heat and after small doses of x-rays.<sup>14</sup>

Injected with olive oil 10 days before cancer inoculation				Controls		
1	•	•	•	•	•	•
2	•	•	•	•	•	•
3	•	•	•	•	•	•
4	+	•	•	•	•	•
5	•	•	•	•	•	•
6	•	•	•	•	•	•
7	•	•	•	•	•	•
8	•	•	•	•	•	•
9	+	•	•	•	•	•
10	•	•	•	•	•	•
11	•	•	•	•	•	•
12	+	•	—	•	•	•
13	•	•	—	+	•	•
14	•	+	—	•	•	•
15	+	—	—	•	•	•
16	+	—	—	•	•	•
17	+	—	—	•	•	•
18	+	—	—	•	+	—
19	+	—	—	•	+	—
20	+	—	—	•	+	—
Weeks 1	2	3		1	2	3

TEXT-FIG. 1. Experiment 6. The rate of growth of Bashford Adenocarcinoma No. 63 in mice injected with 0.2 cc. of olive oil 10 days before inoculation, contrasted with the rate of growth in untreated mice.

<sup>13</sup> Bashford, E. F., Murray, J. A., and Cramer, W., *Proc. Roy. Soc. London, Series B*, 1907, lxxix, 180. Woglom, W. H., *J. Exp. Med.*, 1912, xvi, 629.

<sup>14</sup> Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35. Murphy, Jas. B., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 423.

TABLE IV.  
Experiments 8 to 10.

Experiment No.	Resistance.		
	Group A.*	Group B.	Group C.
8 (Text-fig. 2).	44.4 per cent ( 9 mice).	0.0 per cent ( 9 mice).	0.0 per cent (12 mice).
9	30.0 " " (10 " ).	10.0 " " (10 " ).	0.0 " " (10 " ).
10	50.0 " " ( 8 " ).	10.0 " " (10 " ).	12.5 " " ( 8 " ).

\* Group A was made up of mice injected with the oil 10 days before the cancer inoculation. Group B mice were injected with the oil immediately before the inoculation. Group C comprised the control mice receiving no oil and inoculated with the same tumor.

Injected with olive oil immediately before cancer inoculation			Injected with olive oil 10 days before cancer inoculation			Controls		
1								
2								
3								
4								
5								
6					+	-		
7					+	-		
8					+	-		
9					-	-		
10								
11								
12								
Weeks 1	2	3	1	2	3	1	2	3

TEXT-FIG. 2. Experiment 8. Effect of 0.2 cc. of olive oil on the rate of growth of Bashford Adenocarcinoma No. 63, when administered 10 days and immediately before inoculation.



That a similar state arises after an injection of olive oil is shown by the above experiment. A more complete test of this point is given in Table IV.

*Other Oils.*—Several other oils of different chemical constitution have been tested; namely, cod liver oil, cocoanut oil, sperm oil, and liquid petrolatum (Nujol). Cancer inoculations made 10 days after injection of 0.2 cc. of these several oils induced no appreciable resistance. The tests do not, however, exclude the possibility of a suitable dosage of these oils inducing a result comparable to that given by olive oil.

*Histological Changes Accompanying the Resistance Induced by Olive Oil.*

The following experiments were carried out in order to supply material for a histological study of the nature of the reaction accompanying the resistant state induced by olive oil.

*Experiment 11.*—Ten normal white mice were given an intraperitoneal injection of 0.2 cc. of olive oil each. 10 days later they were inoculated with fragments of a Bashford Adenocarcinoma No. 63, subcutaneously in the left groin. The mice were then killed in pairs 24 hours, 48 hours, 3 days, 4 days, and 5 days after the inoculation and the grafts and the lymphoid organs were removed for histological study.

*Local Cellular Infiltration.*—The occurrence of a characteristic exudate around the cancer grafts in resistant animals has long been known.<sup>15</sup> This local reaction, in which the cells of lymphoid variety take a prominent part, subsides rapidly after the necrosis of the grafts has become complete. On this account the grafts in the present experiment were removed at early periods.

Specimens taken 24 and 48 hours after inoculation showed various types of wandering cells, especially polymorphonuclear leucocytes and fibroblasts, collecting in a great number around the graft. At the 3 day period, however, much of the polymorphonuclear reaction had subsided and there was a marked infiltration of lymphocytes, plasma cells, and fibroblasts (Figs. 3 and 4) closely resembling the local re-

<sup>15</sup> Burgess, A. M., *J. Med. Research*, 1909, xxi, 575. Rous, P., and Murphy, Jas. B., *J. Exp. Med.*, 1912, xv, 270. Tyzzer, E. E., *J. Med. Research*, 1915, xxxii, 201.

action known to occur in cancer-resistant animals. Cell infiltration similar to the latter but in varying amounts was encountered in all the specimens taken at 4 and 5 day periods.

*Stimulation of Lymphoid Tissue.*—Animals resistant to cancer inoculation tend to develop on inoculation lymphoid hyperplasia.<sup>16,17</sup> Murphy and Nakahara<sup>5,17</sup> have shown that this phenomenon, which is characterized by a marked increase in the number of mitotic figures in germ centers of lymphoid tissue, occurs very soon after cancer grafting in potentially resistant animals.

Spleens and lymph nodes taken as early as 24 hours after cancer inoculation in the above experiment showed that the number of mitotic figures in lymphoid tissue was greater than is seen in normal animals. At the 48 hour and 3 day periods the reaction appears to reach its height and at the latter periods mitotic figures were found in great numbers in the germ centers of the spleen (Fig. 5) and lymph nodes, and often in considerable numbers even in the lymph cord of the node (Fig. 6). It should be stated that one animal each of the 4 and 5 day period failed to show any increase of mitotic figures, an irregularity without significance.

*Blood Lymphocytosis.*—Murphy and his associates have shown that a marked increase in the number of circulating lymphocytes accompanies the state of resistance to transplanted cancer.<sup>18</sup> In order to ascertain whether or not such a lymphoid crisis occurs after cancer inoculation in the mice treated with olive oil, white cell counts were made of a number of such mice.

*Experiment 12 (Text-Fig. 3).*—Nineteen normal white mice were injected intraperitoneally with 0.2 cc. of olive oil and cancer inoculation was made in all of the mice 10 days afterward. Ten of the mice proved to be resistant and nine susceptible to the inoculation.

The average number of lymphocytes per c.mm. of blood in the resistant mice, 1 day before the oil injection, was about 4,800 and of polymorphonuclear leucocytes about 4,700. 3 days after cancer inoculation the lymphocytes increased

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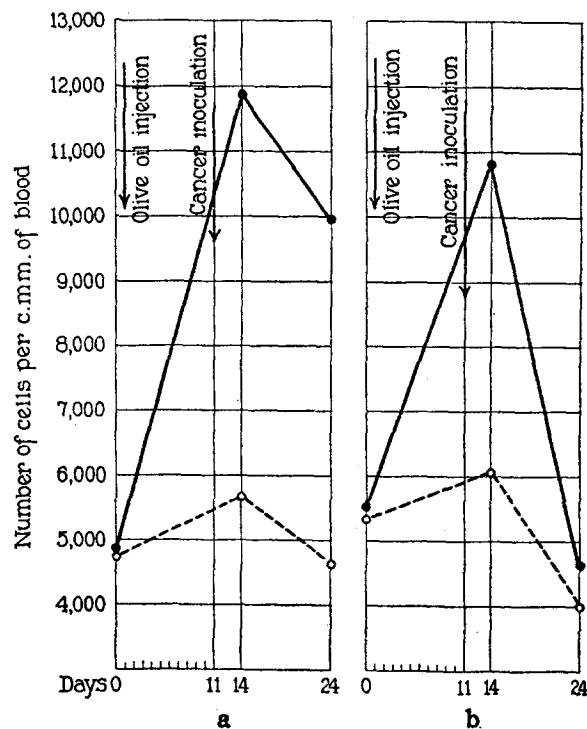
<sup>16</sup> Mottram, J. C., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917-18, xc, 1.

<sup>17</sup> Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 327.

<sup>18</sup> Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204. Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 433.

to about 11,800, while the polymorphonuclear cells were only slightly increased, being about 5,600. 2 weeks after cancer inoculation the lymphocyte count was still high, being about 10,000, while the polymorphonuclear leucocytes had returned to the initial level (about 4,600).

In cancer-susceptible mice, 1 day before the oil injection, the average number of lymphocytes and polymorphonuclear leucocytes was about 5,500 and 5,400



TEXT-FIG. 3. Experiment 12. Composite curves of white cell counts on mice injected with 0.2 cc. of olive oil and inoculated with Bashford Adenocarcinoma No. 63, 10 days later. (a) Composite curves from ten mice proved to be immune; (b) composite curves from nine mice proved to be susceptible. — Lymphocytes. .... Polymorphonuclears.

respectively. The counts were quite high 3 days after the inoculation, the lymphocytes being about 10,800 and the polymorphonuclear leucocytes about 6,000. At the end of the 2nd week after the cancer inoculation, all the cells were much reduced, the lymphocytes being about 4,600 and polymorphonuclear leucocytes about 4,000.

*Experiment 13.*—An experiment similar to the preceding one was carried out with ten mice, five of which were resistant and five susceptible.

In the resistant mice the average number of lymphocytes per c.mm. of blood 1 day before the oil injection was about 4,000; polymorphonuclear leucocytes about 4,400. 2 weeks after the cancer inoculation the lymphocytes were a little over 8,000 while the polymorphonuclear leucocytes had decreased to about 3,400.

In the susceptible mice, 1 day before the oil injection the lymphocytes were about 3,600 and the polymorphonuclear leucocytes about 4,000. 2 weeks after cancer inoculation the lymphocytes were unchanged and the polymorphonuclear leucocytes had perceptibly increased (about 5,700).

The preceding experiments show that a characteristic lymphoid crisis occurs in the blood during the establishment of resistance to cancer grafting induced through olive oil injection. Experiment 12 suggests that even in mice that proved to be susceptible there is apparently an inadequate reaction, which, however, is not of long duration.

It should also be stated that the injection of olive oil alone does not bring about a lymphocytosis. White cell counts were made on ten normal mice injected with 0.2 cc. of olive oil. In certain of the mice there was an increase and in others a decrease in number of circulating lymphocytes during the first 10 days after the oil injection, but the changes were too slight and too irregular to be of importance.

#### DISCUSSION.

The early work of Bashford and his coworkers<sup>6</sup> established the fact that resistance to transplanted cancer in mice could be induced by the inoculation of homologous living tissues. Later Murphy and his associates<sup>14</sup> showed that resistance could be induced by the use of suitable doses of x-rays and intense dry heat. The experiments reported in this paper demonstrate that resistance may be induced by still another means; namely, by the intraperitoneal injection of olive oil. Thus it may be said that resistance to transplanted cancer can be induced by three classes of agents—homologous tissue, a biological agent; x-rays and heat, physical agents; and olive oil, a chemical agent.

There is little direct indication concerning the nature of the common factors responsible for the resistant state induced by these various agents but the manifestations associated with phenomena of resistance are the same regardless of the means used to induce this state. These associated manifestations are, a latent period after the treatment, during which time there is no evidence of resistance,

a local cellular reaction about the inoculated cancer graft, an increase in the number of circulating lymphocytes, and a marked increase in the proliferative activity in the lymphoid organs. The indirect evidence associating the lymphoid cell with the mechanism of resistance to cancer is so strong as to leave little doubt that this cell has an important, if not the most important rôle in bringing about the resistant state.

#### SUMMARY.

The experiments reported in this paper show that it is possible to render mice resistant to transplanted cancer by injections of a suitable quantity of olive oil. In the course of the development of the resistance a definite period of latency is detectable following the oil injection, and the maximum degree of resistance appears at about the 10th day. This state of resistance, as has been determined by histological studies, is preceded by a proliferation of the cells of the lymphoid germ centers and, after the cancer inoculation, is associated with a lymphoid infiltration about the grafts, as well as by a second stimulation of the lymphoid germ centers and an increase in the number of the circulating lymphocytes.

#### EXPLANATION OF PLATES.

##### PLATE 34.

FIG. 1. Germ center of spleen 4 days after an intraperitoneal injection of 0.2 cc. of olive oil. *M*, mitotic figure.

FIG. 2. Peritoneal exudate 48 hours after an intraperitoneal injection of 0.2 cc. of olive oil.

##### PLATE 35.

FIG. 3. 48 hour old cancer grafts and surrounding connective tissue in mouse injected with 0.2 cc. of olive oil 10 days previous to cancer inoculation. Note the extensive cellular infiltration around the graft.

FIG. 4. High power view of an area of infiltration in the above specimen, showing the types of cells participating in the infiltration.

##### PLATE 36.

FIG. 5. Germ center of spleen of mouse injected with 0.2 cc. of olive oil and inoculated with cancer 10 days later. 3 days after cancer inoculation. *M*, mitotic figure.

FIG. 6. Medulla of lymph node of mouse treated similarly to that of Fig. 5. 3 days after cancer inoculation. *M*, mitotic figure.

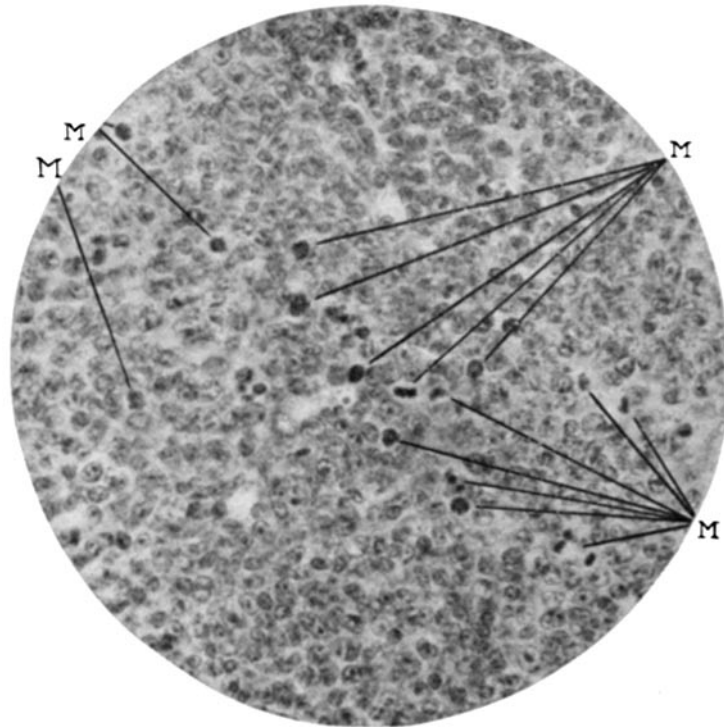


FIG. 1.

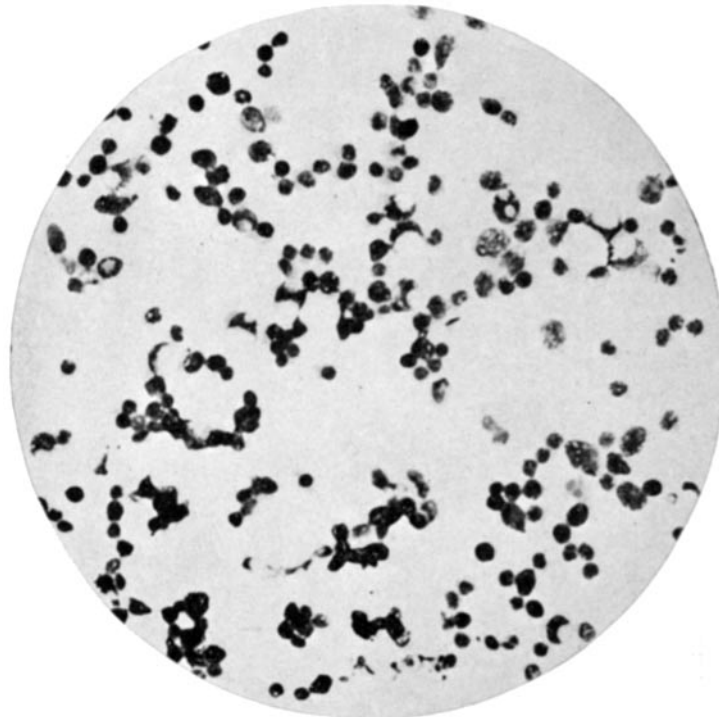


FIG. 2.

(Nakahara: Lymphoid activity. VI.)



FIG. 3.

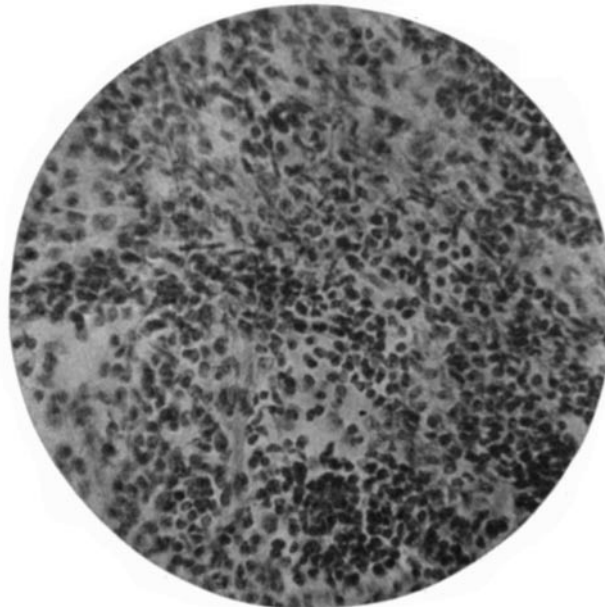


FIG. 4.

(Nakahara: Lymphoid activity. VI.)

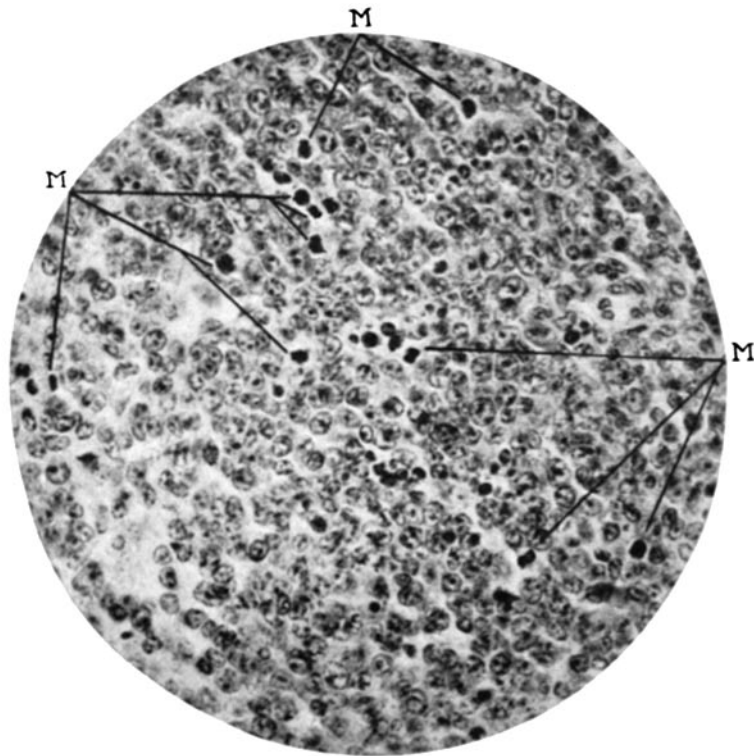


FIG. 5.

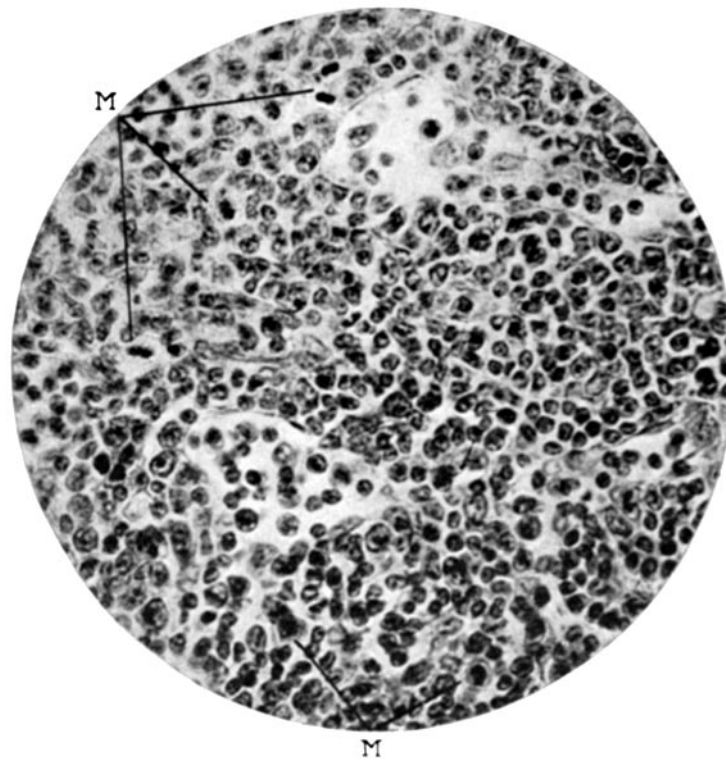


FIG. 6.

(Nakahara: Lymphoid activity. VI.)