PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Observations and Experiments on the Susceptibility and Immunity of Rats towards Jensen's Rat Sarcoma.

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[Plates 1 and 2.]

CONTENTS.

Part I.—Conditions of Susceptibility and Immunity.

1. *The Condition of Susceptibility.*

The Jensen's rat sarcoma used in this investigation was kindly provided by the Imperial Cancer Research Fund, and has been in continual propagation in this laboratory during the past five years.

The inoculation of normal rats with this sarcoma results in the large majority of cases in a growing tumour. The behaviour of the tumour has been investigated in detail.

Four types of growth are clearly distinguishable; they are :—

(1) Cases in which the rate of growth of the tumour is uniform; these are referred to as "Progressive" tumours.

(2) Cases in which the rate of growth gradually diminishes as the tumour increases in size; these are referred to as "Retarded" tumours.

(3) Cases in which, after attaining a certain size, the tumour oscillates between narrow limits for long periods of time (often for months); these are referred to as "Oscillating" tumours.

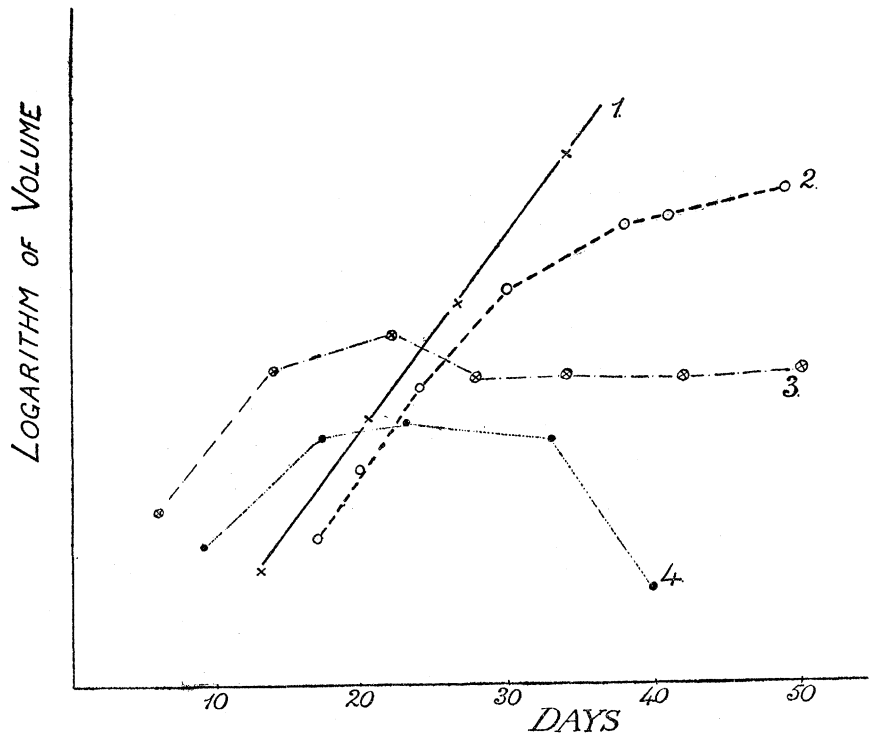

Fig. 1.

(4) Cases in which the tumour spontaneously disappears; these are referred to as "Disappearing" tumours.

The test as to whether a tumour belongs to type 1 or 2 is to make frequent measurements of the superficial area of the tumour. If these areas are raised to the power 3/2 and their logarithms when plotted are found to lie on a straight line, then the rate of growth of the tumour has been uniform; a typical case may be seen in (1), fig. 1. No. 2 illustrates the gradual diminution in the rate of growth which serves to distinguish type 2 from type 1. Nos. 3 and 4 illustrate the oscillating and disappearing types respectively.

The probability of success on re-inoculating animals which are bearing or have borne tumours of any of the types specified may be gauged from the data in Table I.

Table I.

Character of tumour.	No. of rats.	Result of re-inoculation.	Subsequent behaviour of tumour.
Progressive ...	18	Graft took	Progressed.
	2	,, ,,	Retarded.
	2	,, ,,	Recurred after operation.
	1	Graft took but regressed	Recurred after operation, then regressed.
	1	Graft did not take	Retarded.
	1	,, ,, ,,	Recurred after operation, then regressed.
	2	,, ,, ,,	Did not recur after operation.
Retarded	1	Graft took	Progressed.
	1	Graft took but disappeared	Retarded.
	1	Graft did not take	Progressed, then retarded.
	3	,, ,, ,,	Oscillatory.
	2	,, ,, ,,	Retarded.
	3	,, ,, ,,	Disappeared.
	1	,, ,, ,,	Recurred but regressed.
	1	,, ,, ,,	Did not recur after operation.
Oscillating ...	1	Graft did not take	Oscillated for six weeks, then progressed.
	1	,, ,, ,,	Oscillated.
	4	,, ,, ,,	Gradually disappeared.
	1	Graft took	Oscillated.
	1	,, ,,	No recurrence after operation.
Disappearing	1	Graft took	Subsequently grew.
	8	Graft did not take	Disappeared.
	5	,, ,, ,,	Did not recur after operation.
	1	Graft took	Subsequently disappeared.
	21	Graft did not take	,, ,,

Judging from the behaviour of this strain of sarcoma in about 2000 inoculations during the last four years, it is estimated that about 70 per cent. of inoculations yield growing tumours of types 1 and 2, about 5 per cent. of an oscillating nature, and 25 per cent. which spontaneously disappear. These

latter often attain to considerable size; tumours measuring as much as 6 sq. cm. have subsequently been observed to disappear.

2. *The Condition of Immunity.*

(a) *General.*—Rats are rarely found to be immune to a first inoculation of the sarcoma cells. The animals in which the resulting tumours spontaneously regress are almost invariably resistant to a second inoculation; when tested two or three months later, they are generally in a similar state. Our studies upon the immune condition have been largely made upon rats which at one time have borne tumours, and their resistant nature proved by the failure of a second inoculation.

The wide variations observed in the behaviour of the inoculated sarcoma cells are not due to differences in the inoculated material. A progressively growing tumour may be removed from an animal, made into a uniform emulsion and inoculated into a batch of, say, 20 normal rats of about 100 grm. weight each. It may be fairly confidently stated that the subsequent tumours will furnish examples of all four types of growth which have been described; this being so, it is rational to attribute the varying fate of the sarcoma cells to the defensive mechanism which the rats are able to bring to bear against these cells. This defensive mechanism is one which may eventually overpower the growing sarcoma cells, causing their complete disappearance (*vide* type 4); such animals are immune to subsequent inoculation, and the sarcoma cells of tumours in the course of disappearing are rarely transplantable with success (*vide* fig. 2). The oscillating tumours indicate a condition in which the growth of the cells is just balanced by the defensive mechanism; animals bearing such tumours are generally found to be immune to a subsequent inoculation. The transplantation of oscillating tumours gives a moderate number of growing tumours (*vide* fig. 3).

With still smaller degrees of defensive power on the part of the animal there appear retarded tumours, and, lastly, progressive tumours. In these last named, the defensive mechanism is not always entirely absent, for re-inoculation of the animals does not invariably result in growing tumours. Recourse is naturally had to tumours of type 1 or 2 for the continued propagation of the tumour.

(b) *Production of Immunity.*—If sarcoma cells are exposed to the β-rays from radium under suitable conditions of exposure (1), it is found that when the irradiated cells are inoculated into normal rats they do not develop into tumours. If the degree of irradiation is not too prolonged, the animals are frequently found to be immune to a fresh inoculation of normal sarcoma cells.

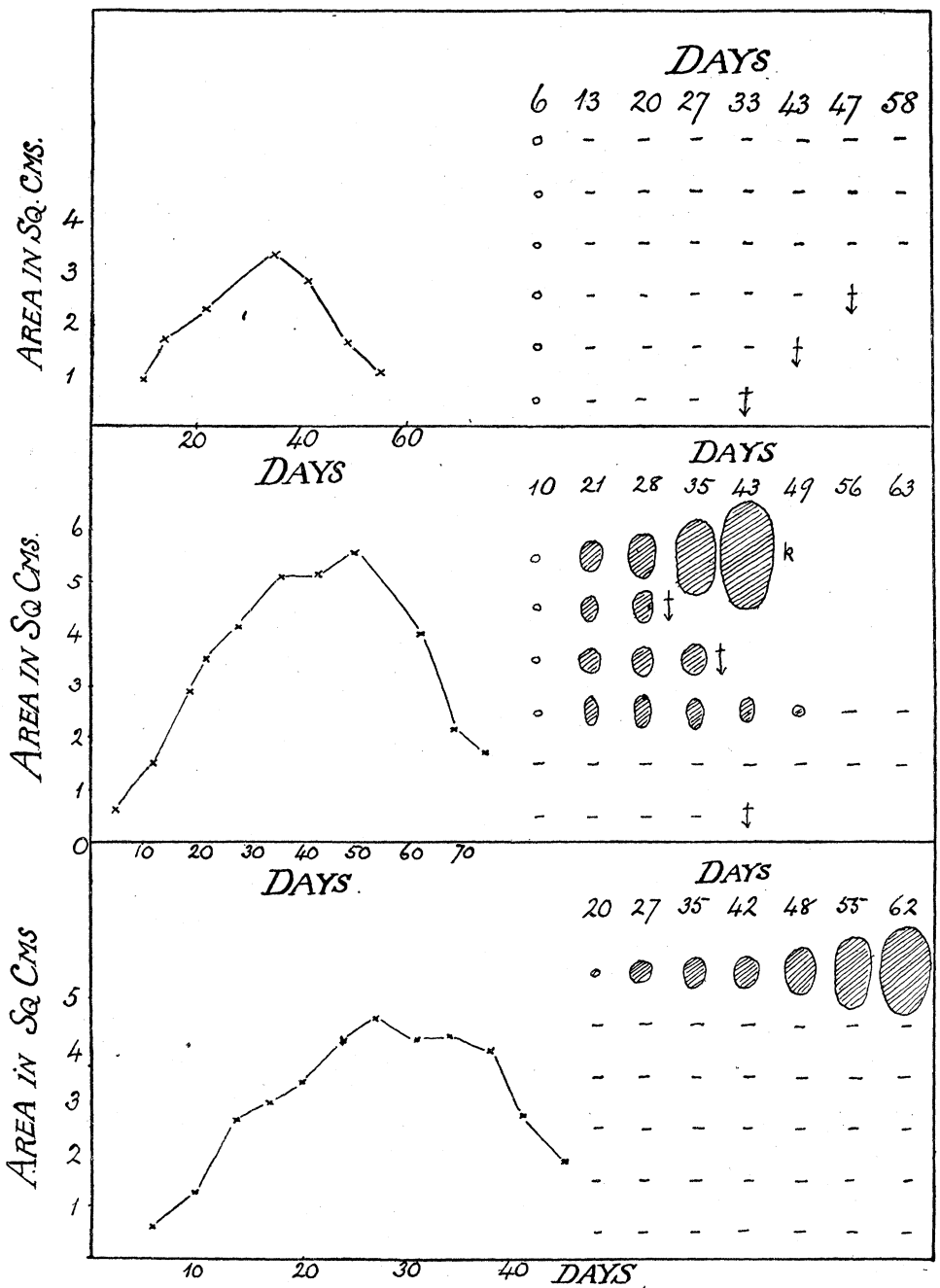

FIG. 2.—The plotted line shows the tumour growth up to the day of inoculation. The result of the inoculation is seen in the charts to the right.

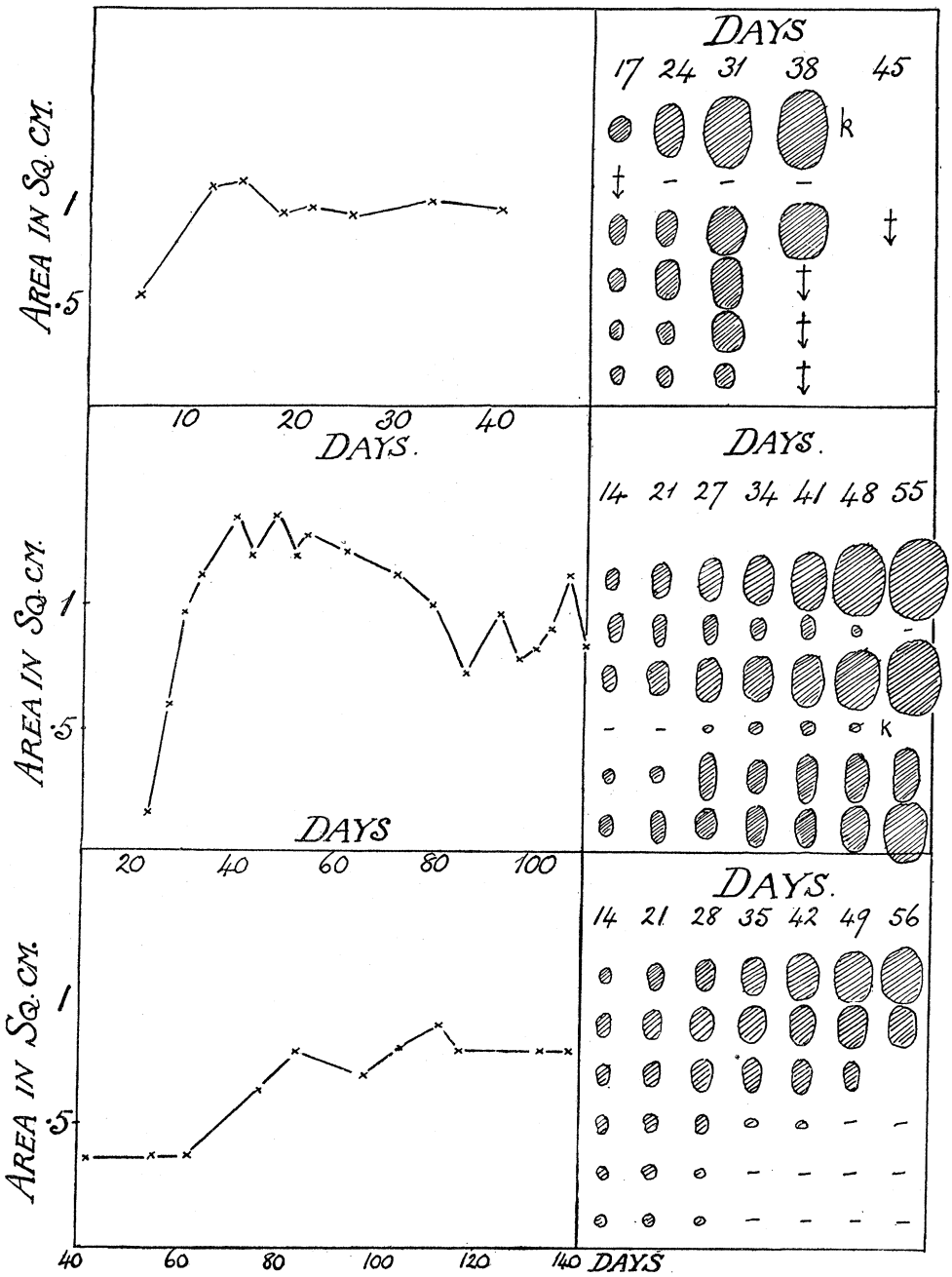

FIG. 3.—The plotted line shows the tumour growth up to the day of inoculation. The result of the inoculation is seen in the charts to the right.

FIG. 4 (Normal Spleen).

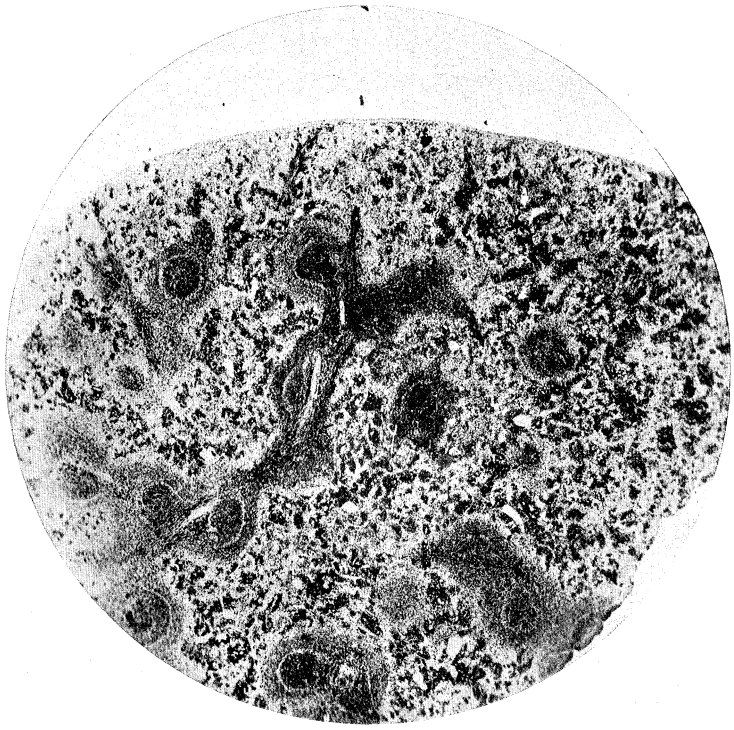

FIG. 5 (Immune Spleen).

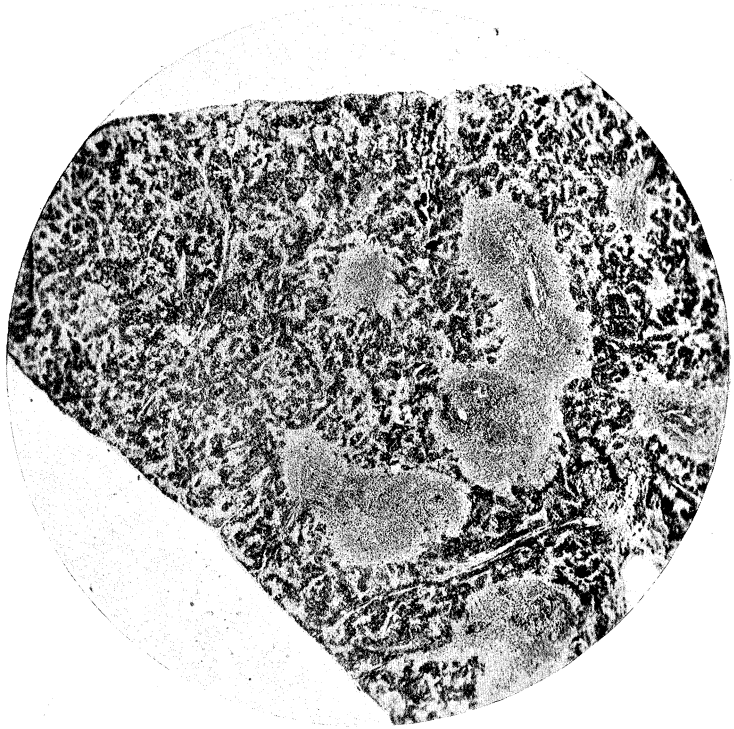

FIG. 6 ("Massive" Spleen).

Rats may be made immune in a similar manner if the sarcoma cells are previously exposed to radium emanation in a concentration of about 0·5 millicurie per c.c., the exposure lasting about one hour (*loc. cit.*). The amount of irradiated material inoculated has usually been 0·5 c.c. in the right and left axillæ; after two or three weeks the immunity of the animals is tested by inoculation.

(c) *Condition of Tissues.*—A microscopic examination of the spleen shows, in a very large number of cases, a striking difference between the normal and the immune animal. This contrast is provided by a very large increase in the total number of lymphocytes and plasma cells in the spleen of immune animals compared with that usually obtaining in the normals. (This increase was found to be accentuated in the immune rats after they had received a large inoculation, *i.e.*, 0·3 or 0·4 c.c.) Other observers have shown a similar increase in these cells throughout the connective tissues (2).

Figs. 4, 5, 6 (Plates 1 and 2) illustrate the contrast referred to in the three types of spleen.

This contrast in the appearance of the spleen seemed to warrant a more extended investigation. The attempt was made to see whether this massing of the lymphocytes and plasma cells was directly associated with the development of the immune condition.

In the first place, an examination of the spleen was made in a large number of animals which were in different conditions as regards their toleration to the growth of sarcoma cells. They were classified as Normal (N), *i.e.*, rats practically certain to bear tumours if inoculated; Immune (I), *i.e.*, rats proved to be resistant to inoculation; "Massive" (M), *i.e.*, immune rats which had been inoculated with $6 \times 0{\cdot}05$ c.c. sarcoma emulsion with negative results; Progressive (P), *i.e.*, animals which were supporting the growth of a progressively growing tumour; and Disappearing (D), *i.e.*, rats in which tumours were spontaneously disappearing.

By giving numerical values, from 0 to 12, to the lymphocyte and plasma-cell content of the spleen, it was possible to classify these various types, and to show that an increased content was not necessarily associated with the immune condition, nor did an immune animal invariably exhibit a high content of these particular cells in its spleen, although this was the usual condition.

To eliminate all personal bias in the matter, the whole collection of slides (numbering about 300) of the sections of the various spleens was examined without any knowledge on the part of the observer of the particular class to which any slide belonged. Numerical values of the cell content were

written on the slides, which were then tabulated. The results are shown graphically in fig. 7.

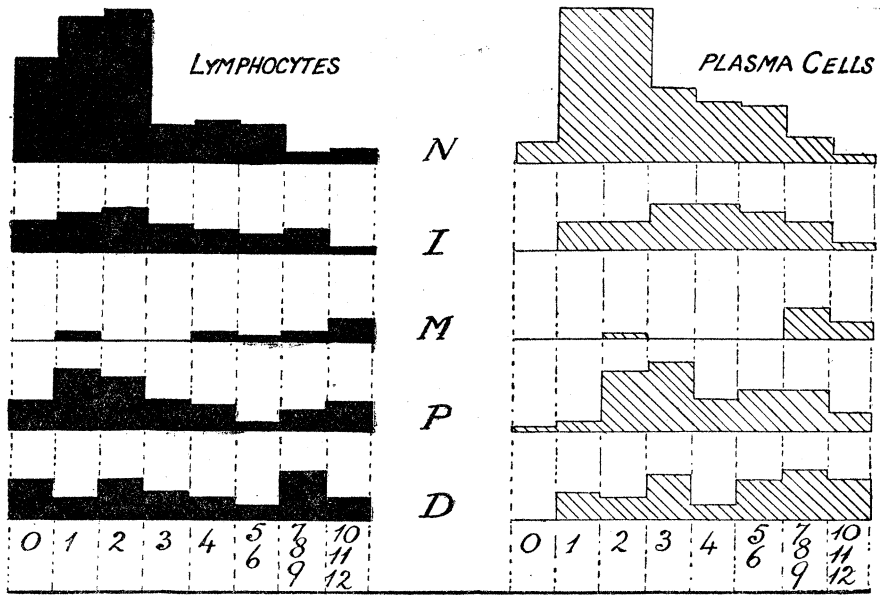

FIG. 7.

The finding of an occasional normal animal, or one bearing a progressively growing tumour with a very high lymphocyte and plasma-cell content, does not, however, rule out the possibility that in such cases these cells may be playing some part in what may still be called immunity. Reference to Table I shows that a small number of animals, exhibiting all the outward signs of susceptibility, simultaneously exhibit an immunity to the graft, for they resist re-inoculation.

The question remained, therefore, as to whether these few animals corresponded with the small proportion of their class, giving a high lymphocyte and plasma-cell count. This was put to the test direct in the following way: A batch of normal rats was taken, and a small portion of their spleens removed; they were then given an inoculation into the right axilla.

The animals grew tumours, of which frequent records were kept; when at least four measurements upon each rat had been made, selection was made of those which had progressive tumours (Type 1); a further portion of their spleens was then removed, and their lymphocyte and plasma-cell content again determined.

It was found that the majority, 19 out of 22, of the rats growing

progressive tumours were positive to a fresh inoculation, and that in some of these cases a considerable increase in their leucocyte and plasma-cell content occurred; in other animals which were resistant to a re-inoculation, although bearing progressive tumours, there was no appreciable increase in the lymphocyte and plasma-cell content directly associated with the appearance of the immune condition.

The outstanding features of the investigation are:—

(1) The lymphocyte and plasma-cell content of normal rats varies within wide limits, but the number having a low content is very much larger than the number having a high content [*vide* diagram (N)].

(2) The immune animal is just about as likely to have a low as a high content (I); "Massive" immunes (*vide* fig. 7) are almost always packed with cells of the kind considered (M).

(3) There is about an equal chance in animals which bear either a progressive or a disappearing tumour, having a high or low cell content (P), (D).

Any observed increase in the lymphocyte and plasma-cell content is not due to intolerance of the spleen to repeated interference with it—this was shown by separate tests upon normal rats. Neither can the variations be induced by inoculating dead cells into the animals; this was tested by inoculating boiled sarcoma cells with two subsequent examinations of the spleen content—no appreciable variation of the lymphocyte and plasma-cell content occurred.

Tumours in Living Rat Tissues before Inoculation.—Microscopical examination [*vide* p. 20 (*d*)] shows that the sarcoma cells can no longer be found after five or six days in the immune rat. If, however, the graft be removed at shorter intervals and re-inoculated into normal rats, the sarcoma cells are still viable in a fair percentage of cases, after having been in an immune animal for as long a period as three days (*vide* Table II).

Protocol.—0·05 c.c. of minced tumour was placed in the axillæ and spleens

Table II.

No. of days the tumour was left in the normal and immune rats.	The growth of the tumour after having been in the axillæ of immune rats.	The growth of the tumour after having been in the axillæ of normal rats.	The growth of the tumour after having been in the spleen of immune rats.	The growth of the tumour after having been in the spleen of normal rats.
1	8/12	8/12	8/12	6/12
2	—	—	5/9	8/9
3	6/20	17/21	10/18	18/21
6	0/11	90–100 per cent.	0/11	90–100 per cent.

of normal and immune rats. After varying lengths of time this was removed; small pieces were then taken from the margin (avoiding as far as possible the necrotic centre), and placed in the axillæ of normal rats. The denominators of the fractions give the number of rats inoculated, the numerators the number of rats in which the inoculation resulted in measurable tumours.

Result.—The tumour was not killed nor its subsequent growth affected by exposure for three days to the living subcutaneous or splenic tissues of immune rats. Exposure for six days to the tissues of immune rats prevented the tumour subsequently growing.

3. *The Effect of Experimental Alteration of Susceptible and Immune Animals upon Subsequent Inoculation.*

(a) *The Influence of Splenectomy upon Tumour Growths.*—The study of the modes of growth of the sarcoma, and the observance of the immunity thereto, led to a search being made for the factors which control this latter condition.

Attention was directed to the spleen, and a preliminary investigation was made by extirpating this organ in normal and immune rats and seeing whether any change was induced to the subsequent fate of sarcoma when inoculated into such animals.

Normal and immune rats were splenectomised and then inoculated with 0·05 c.c. of sarcoma at periods ranging from 0 to 28 days after the operation. The data in Table III show that the splenectomy of normal rats does not affect the subsequent growth of the tumour in them; it shows also that in immune rats the act of splenectomy does not abolish the immunity to a subsequent graft. Brancati (3) and Apolant (4) have shown that with rat and mouse carcinoma, splenectomy favours growth in normal animals.

(b) *The Effect of a Large Inoculation.*—Whatever view may be held as to

Table III.

	No. of rats.	Result of inoculation.
Splenectomised normal rats	26	17 gave progressive tumours. 4 gave disappearing tumours. 5 gave oscillatory tumours.
Splenectomised immune rats	24	All negative.
Control rats not splenectomised	35	22 gave progressive tumours. 10 gave disappearing tumours. 3 gave oscillatory tumours.

the nature of the processes operative in immune animals it seemed advisable to see whether by a large inoculation the immune condition could be overcome. For this purpose 50 immune rats were given six inoculations of 0·05 c.c. of sarcoma cells in the subcutaneous tissues of the flanks and abdomen. In the majority of these small nodules could be felt for 12 to 14 days; in only two cases did they attain sufficient size to be measured.

Twenty immune rats were splenectomised and were then given six or eight inoculations of 0·05 c.c. of tumour. In several cases persistent nodules resulted: in one case a small tumour grew, but subsequently regressed; in another case six large tumours resulted in a single animal.

(c) *Exposure to X-Rays.*—When an animal such as a rat is exposed *in toto* to X-rays, a moderate degree of irradiation is sufficient to produce changes in its blood which can easily be recognised. The white cells are more affected by these rays than are the red cells, and of the white cells the lymphocytes are especially vulnerable; by prolonged irradiation the circulating blood may be temporarily rid of practically all its lymphocytes.

The work which has been described shows that the lymphocytes play some part in the processes by which immunity to the sarcoma cells is maintained in the rat, and it was thought that their relative importance would be shown by the following procedure:

A rat, the immunity of which to sarcoma was proved, was exposed to X-rays for a period sufficient to cause the lymphocytes to drop to a few per cent. of their previous numbers, and an inoculation of sarcoma cells was then made.

The experimental arrangements were as follows:—A rat, proved to be immune, was placed in a small wooden box with a thin lid and exposed to X-rays from a Coolidge tube, the anode of the tube being 27 cm. distant. The radiation selected for this purpose had a wave-length ranging from about 2·6 to $4{\cdot}2 \times 10^{-9}$ cm.

It was produced under the following conditions: the heating current in the Coolidge tube was 4·3 ampères, and the equivalent spark-gap was 6 cm. between spheres 5 cm. in diameter. The quantity of radiation was measured by allowing the beam of X-rays to enter a small gold leaf electroscope placed 225 cm. from the anode. The readings of the instrument were used as a check on the constancy of the radiation from the Coolidge tube, and the instrument was itself checked by the reading produced by the γ-rays from a standard quantity of radium placed at a fixed distance from the electroscope.

There is no generally accepted method by which the quantity of X-radiation may be specified. Some idea, however, of the quantity of radiation

received by the animals in these experiments may be obtained when it is stated that a Sabouraud pastille placed at the centre of the wooden box turned to the standard tint 1 B in $2\frac{1}{2}$ hours ($\frac{1}{3}$ B in 45 minutes, $\frac{4}{5}$ B in 2 hours). This radiation caused the leaf of the electroscope to fall at the rate of about 300 divisions per minute.

Preliminary observation showed that exposing a medium-sized rat for

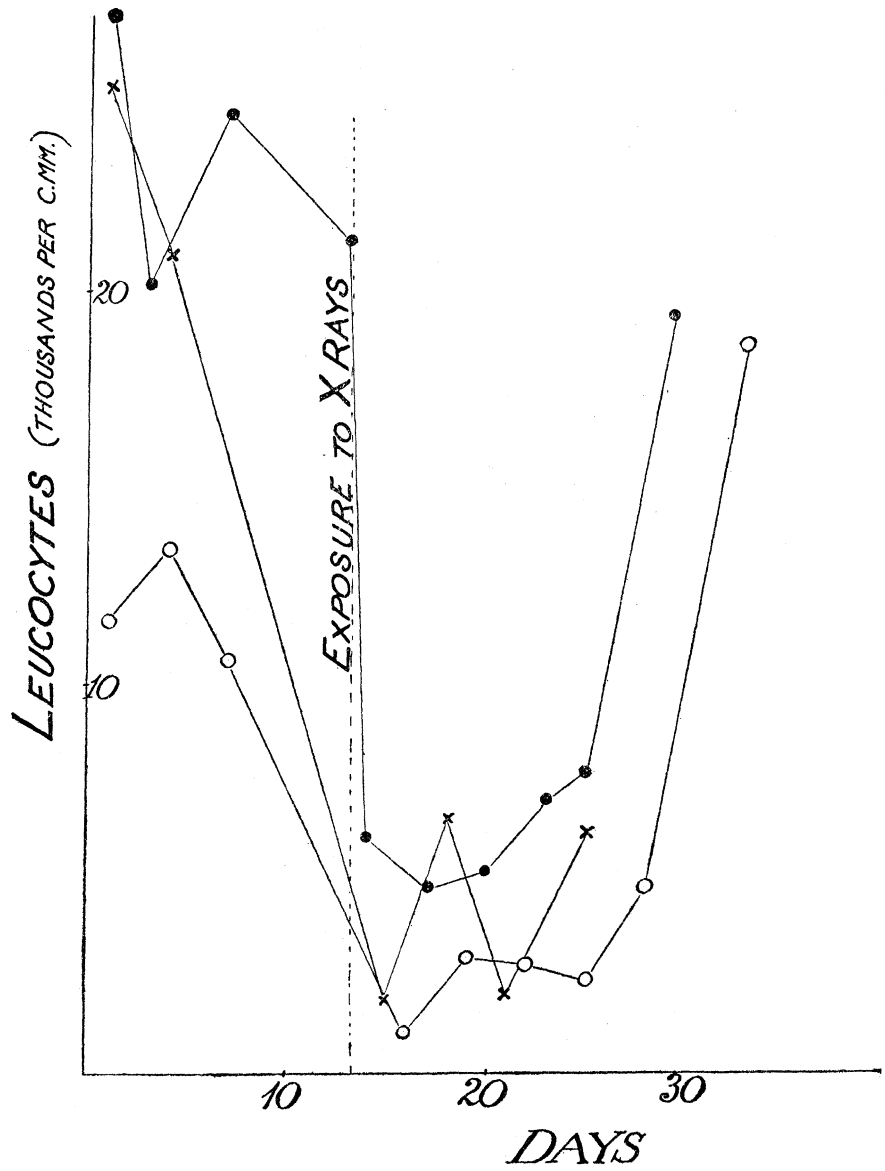

Fig. 8.

½ hour to the radiation under the conditions specified was sufficient to produce well marked changes in its blood; there was, however, a return to a normal content within about 7 days. Prolongation of the exposure to 1 hour leads to a very large reduction in the number of the white cells, so much so that a differential count was hardly practicable. The extent of the destruction of white cells may be gauged from the chart of three rats in fig. 8.

Four rats of proven immunity were irradiated for ¾ hour; the next day they were inoculated with 0·05 c.c. of sarcoma cells, suitable controls being provided. The inoculations into the irradiated animals in no case developed into tumours, but the nodules persisted longer than was the case with ordinary immune rats. Microscopical examination of grafts in the interval 2–6 days after the inoculation showed, however, a distinct contrast to the picture which is typical of a graph of sarcoma cells in an ordinary immune rat (*vide* description, p. 20). The impression was obtained that a little more radiation might lead to a successful growth of sarcoma in the immune animals. Fourteen immune rats were therefore irradiated under the same conditions as before, but for 1½ hours. One or two days after, they were inoculated with sarcoma; the sarcoma cells were found to grow in these animals, which, previous to irradiation, were immune. The chart in fig. 9 shows the gradual development of the tumours. Examination of grafts in these animals showed a condition of growth of the sarcoma which could not be distinguished from that obtaining in normal animals (*vide* figs. 12–14, p. 19).

It will be observed that although growth occurred with the formation of small tumours, there was a great tendency for their subsequent retrogression.

4. *The Effect of Experimental Alteration of the Sarcoma Cell before Inoculation into Susceptible and Immune Animals.*

(a) *The Inoculation of a Mixture of Rat Sarcoma and Rat Spleen.*—The spleens from normal and immune rats were removed and emulsified. A mixture was then made with sarcoma emulsion in varying proportions, which was then inoculated into normal rats.

Illustrations are given of two typical experiments (*vide* figs. 10 and 11). The data in Table IV show the deterrent effect upon growth of the spleen mixtures. Note must be made that when tumour only was inoculated, more sarcoma emulsion was introduced than in the case of the mixtures.

Similar experiments were carried out with liver mixtures; no interference with the growth of the sarcoma was observed.

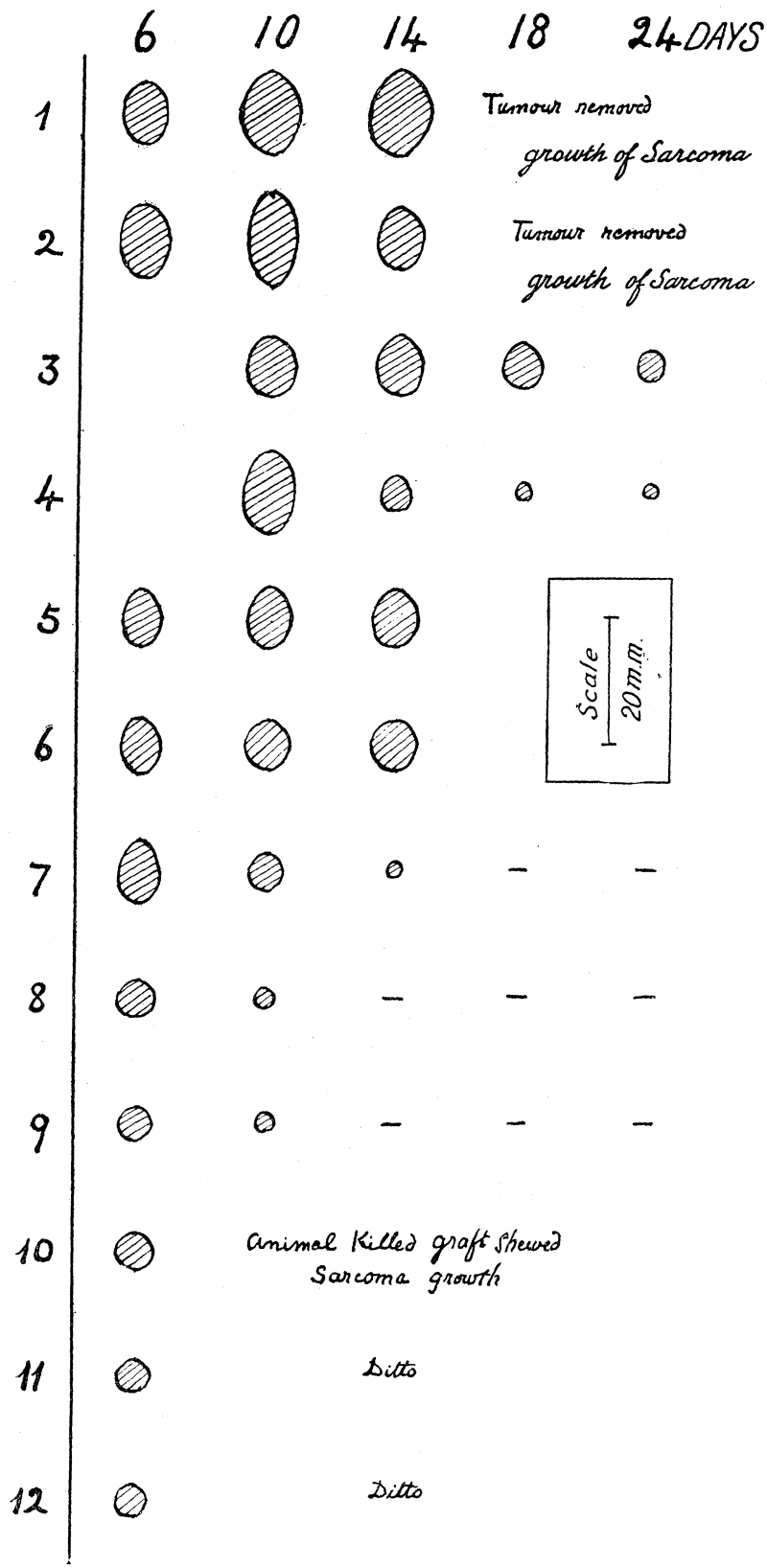
6
10
14
18
24 DAYS
1
Tumour removed
growth of Sarcoma
2
Tumour removed
growth of Sarcoma
3
4
5
6
Scale
20 m.m.
7
—
—
8
—
—
—
9
—
—
—
10
Animal Killed graft Shewed
Sarcoma growth
11
Ditto
12
Ditto

Fig. 9.

Table IV.

	No. of rats inoculated.	Average superficial area at the 14th day.	Average superficial area at the 21st day.
		sq. mm.	sq. mm.
Tumour alone	33	189	270
Tumour with normal rats' spleen ...	20	135	186
Tumour with immune rats' spleen	45	63	117

This deterrent effect of the spleen of immunised rats upon tumour growth is not to be attributed to any immunity which might be eventually produced by the spleen cells. This was shown by inoculating tumour on one side of the rat and spleen on the other; no appreciable effect upon tumour growth was observed.

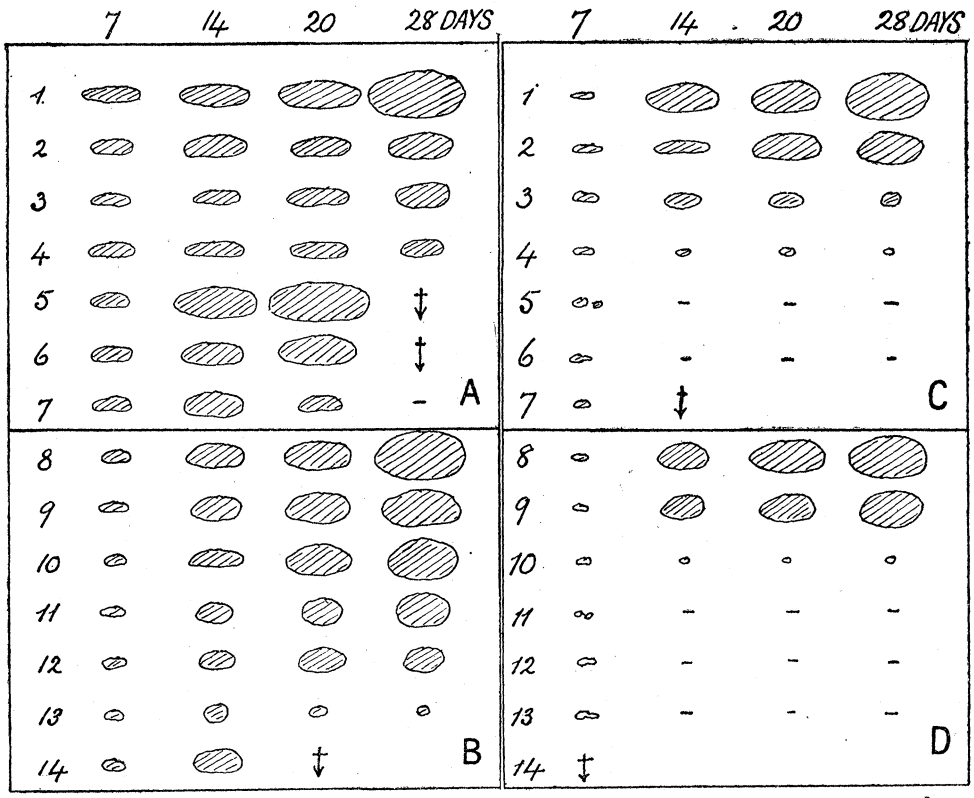

FIG. 10.—A. Tumour only; B. Tumour + Normal spleen; C. and D. Tumour + Immune spleen.

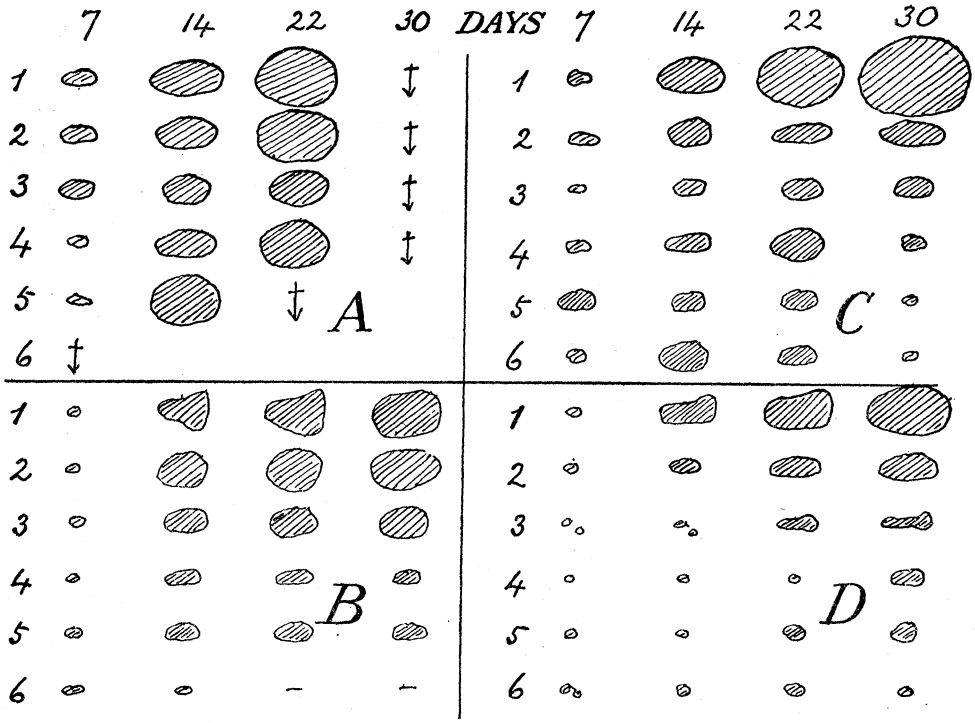

FIG. 11.—A. Tumour only ; B. Tumour + Normal spleen ; C. Tumour + Spleen of tumour-bearing animal ; D. Tumour + Immune spleen.

Tumour Treated with Spleen Extract, Serum, and Plasma of Immune and Normal Rats before Inoculation.

The method of experimentation on these lines was to mix an emulsion of sarcoma cells with either of the above liquids, and after a certain lapse of time to inoculate the emulsion into normal rats.

The spleen extract was prepared by pressing normal and immune spleens in a Buchner's press.

The result of a number of experiments on 102 animals on these lines showed that there was no decided effect upon the subsequent rate of growth of the sarcoma cells.

PART II.—MICROSCOPIC OBSERVATIONS UPON GRAFTS AND TUMOURS.

Side by side with the experiments whose description forms Part I of this paper, microscopic studies have been made of the early stages of growth. These observations upon grafts were carried out in order to determine the reactions of the tissues of the host, and the immediate behaviour of the sarcoma cells, when inoculated under varying experimental conditions.

Material was obtained in the following manner: Rats were inoculated with minced tumour into both axillæ. After varying lengths of time, one graft was removed by operation for histological examination; the other was allowed to remain in order to indicate, by its growth, the degree of susceptibility of the rat. The amount inoculated was 0·05 c.c. The appearances when minute fragments were used were found to be exactly similar to those when 0·05 c.c. was inoculated; in each case the centre of the inoculated mass quickly degenerated, so that, by the second or third day, only cell-débris remained; only the outer surface of the inoculated material for a depth of a few cells remained viable.

Material was fixed in Gilson or Carnoy solution, and stained as a routine with pyronine-methyl green; other methods were also occasionally used, dilute hæmatoxylene (1 in 100 long process), polychrome methylene blue, and azurin 2.

Before describing the microscopic appearances of the grafts, it is necessary to discuss the nomenclature here used with reference to the cells which are found in the connective tissue and reaction tissues of the rat. There can be no confusion about the following cells: fibroblasts, fat cells, mast cells, plasma cells (plasmoidocytes), and polymorphonuclear leucocytes. There remains a polymorphic group of cells, the lymphocytes (polyblasts of Maximow), which have been classified according to their histogenic, hæmatogenic, or endothelial origin, and also according to their characters and functions into adventitial cells, wandering cells, macrophages, clasmatocytes, small amœboid wandering cells, large and small mononuclear cells. Since they appear to grade one into the other as regards both their origin, function, and characters, the term lymphocyte will be here used for the whole group.

1. *The Microscopic Appearances of Subcutaneous Grafts in Susceptible Animals.*

(a) *The Appearance 24 Hours after Inoculation.*—The fatty areolar connective tissue around the inoculated sarcoma emulsion is seen to be distended with a structureless exudate for a distance of from 2 to 4 mm. It is separated from the emulsion by a narrow space or cleft which is often partly filled with collections of polymorphonuclear leucocytes. The groundwork of this reaction tissue consists of oval or round masses (diameter 0·06–0·08 mm.) of hyaline material separated by bands of fine connective tissue fibres. Sparsely scattered over this groundwork, and confined chiefly to the bands between the hyaline masses, are cells of several different kinds (see fig. 12). Close to the emulsion are many polymorphonuclear leucocytes, and immediately outside the crack a few healthy sarcoma cells. At the periphery a small number of mast cells and small fibroblasts are to be seen, as well as fat

globules and cells. Lymphocytes form the rest of the cellular elements, they are more or less evenly distributed throughout the tissue, being a little more numerous close to the graft. Lymphatics, capillaries, and small blood-vessels are scattered through the tissue; in many cases their endothelial cells are seen to be dividing (*vide* fig. 12).

The whole of the inoculated sarcoma emulsion presents degenerative changes, except for a few cells deep just within the cleft, where some healthy sarcoma cells are to be seen. Polymorphonuclear leucocytes are everywhere scattered between the sarcoma cells; the groundwork consists of a structureless granular material. The nuclei of the sarcoma cells are shrunken and their chromatin irregularly distributed. The whole nucleus often stains very deeply, whilst the protoplasm is pale and vacuolated.

(b) *Forty-eight Hours after Inoculation* (*vide* fig. 13).—The reaction tissue now measures 1 to 2 mm. in width, being narrower than in the 24-hour specimen. The groundwork consists of a close network of fibrous material, with only small, narrow hyaline masses in its meshes. Through this groundwork the cells have the following arrangement: Near the emulsion, but outside the cleft, are to be seen many healthy sarcoma cells, some dividing; mixed with them are a few polymorphonuclear leucocytes. Lymphocytes are scattered through the tissue; they are more numerous around the blood-vessels than elsewhere, and more numerous than in the 24-hour specimens.

This appearance of greater numbers may be due to their being more concentrated, owing to the shrinkage in width of the reaction tissue. At the periphery of the reaction tissue, the cellular elements are as in the 24-hour specimen, except that a few plasma-cells are to be seen near the outlying blood-vessels. The blood-vessels are more numerous, and are to be seen close to the sarcoma cells, external to the cleft. The sarcoma cells inside the cleft present more advanced degenerative changes than in the 24-hour specimen; only a few healthy cells are to be seen close to the margin.

(c) *Seventy-two Hours after Inoculation* (*vide* fig. 14).—Immediately outside the cleft, closely packed sarcoma cells now form a band of growth encircling the originally inoculated material, which now consists of structureless cell-débris. Among the sarcoma cells are a small number of lymphocytes, numerous blood-vessels, and lymphatics. Outside this band of growth the reaction tissue is similar to that seen in the 48-hour specimen, except that blood-vessels are more numerous and in places closely packed together, while lymphocytes and plasma-cells are less numerous.

(d) *Four to Seven Days after Inoculation.*—The reaction tissue of the host shows no change. As the sarcoma invades the reaction tissue next to it,

fresh reaction tissue is formed at a distance. In this way growth proceeds, being always separated from the normal tissues of the host by a thin capsule of vascular fibrous connective tissue.

Summary.—The reaction tissue consists first of inflammatory œdema, which subsequently subsides, and allows the tissue elements to become

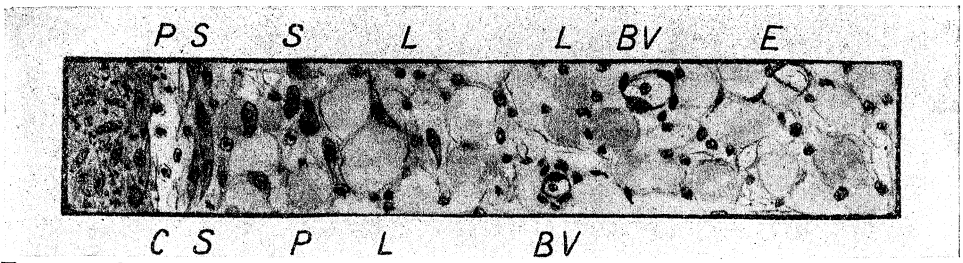

FIG. 12.—Shows a small portion of the margin of the inoculated material, to the left; separated by the "cleft" from a portion of the reaction tissue of the host, to the right; 24 hours after inoculation. S, sarcoma cells outside "cleft"; P, polymorphonuclear leucocytes; BV, blood-vessels; F, fibroblasts; L, lymphocytes; C, cleft.

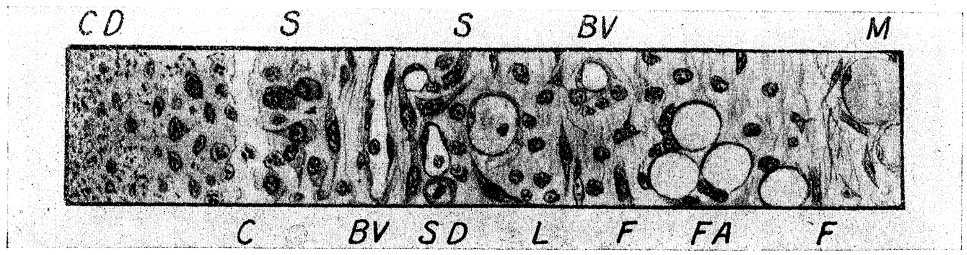

FIG. 13.—Shows the same structures 48 hours after inoculation. F, fibroblast; FA, fat; M, muscle; BV, blood-vessels; L, lymphocyte; S, sarcoma cell; SD, sarcoma cell dividing; CD, cell débris; C, cleft.

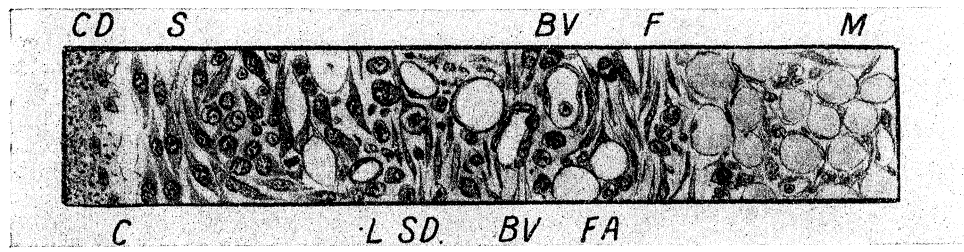

FIG. 14.—Shows same structures 72 hours after inoculation. Letters as above.

more closely packed. After two days the tissue consists of a vascular connective tissue, through which are scattered a few lymphocytes. The sarcoma cells within the cleft soon present degenerative changes, and by the third day only cell-débris remains. At the end of 24 hours, healthy sarcoma cells, some dividing, are seen outside the cleft; on and after the

third day they here form a definite band of growth. As the sarcoma cells extend out into the reaction tissue, so fresh reaction tissue is formed more distantly.

2. *The Microscopic Appearances of Grafts in Immune Animals.*

For this purpose, animals which had had two previous inoculations, with negative result, were used.

(a) *The Appearances* 24 *Hours after Inoculation.*—The reaction tissue consists of an inflammatory œdema precisely similar to that seen in susceptible animals. The sarcoma cells also present a similar appearance.

(b) *The Appearances after* 48 *Hours* (*vide* fig. 15).—The groundwork of the reaction tissue is like that seen in susceptible animals; fibroblasts, fat cells, mast cells, blood-vessels, lymphatics, and polymorphonuclear leucocytes have a similar arrangement. In contrast to these similarities, the arrangement of the lymphocytes is strikingly different; they are much more numerous, and are especially abundant just external to the cleft, where they form a solid ring of cells, encircling the inoculated emulsion. At the periphery, plasma cells are to be seen near the blood-vessels. As in susceptible animals, a few sarcoma cells are found outside the cleft; the vast majority, however, present degenerative changes; they are oval or circular in shape, their protoplasm is vacuolated, their nuclei are irregular in shape and contain either no, or very few, fine chromatin granules, and a central large irregular nucleolus. Whereas in the susceptible rat the sarcoma cells external to the cleft are more healthy in appearance than those internal, in the immune rat the reverse is the case.

(c) *The Appearances after* 72 *Hours* (see fig. 16).—The lymphocytes have increased in numbers, so that the solid ring of these cells, just external to the cleft, is somewhat wider than in 48-hour specimens. Towards the outer margin of the reaction tissue, an increased number of fibroblasts are to be seen, and collections of plasma cells around the blood-vessels are numerous. External to the cleft only a few degenerated sarcoma cells are to be found; no dividing sarcoma cells are to be seen. In some cases degenerated sarcoma cells are seen to be embraced by large lymphocytes; in in other cases, nuclei or chromatin fragments are seen in vacuoles in these cells. Degenerated sarcoma cells and chromatin granules contained in vacuoles in large lymphocytes (macrophages), as seen in a graft in an immune animal, but also in all cases where degenerative sarcoma cells are disappearing.

(d) *The Appearances after Four to Six Days.*—The sarcoma cells external to the cleft gradually disappear, so that, by the fifth or sixth day, not a

vestige of them remains. The lymphocytes do not further increase in numbers. Fibroblasts become more numerous, and by the sixth day form a close meshwork around the graft, replacing the lymphocytes, which gradually disappear. The collections of plasma cells at the periphery and near the blood-vessels persist, and may even grow larger. By the sixth day the reaction tissue has the appearance of an early fibrosis.

Summary.—Compared to what occurs in susceptible animals, the reaction in immune animals presents two striking differences, namely, the great accumulation of lymphocytes on the second and third day and the rapid

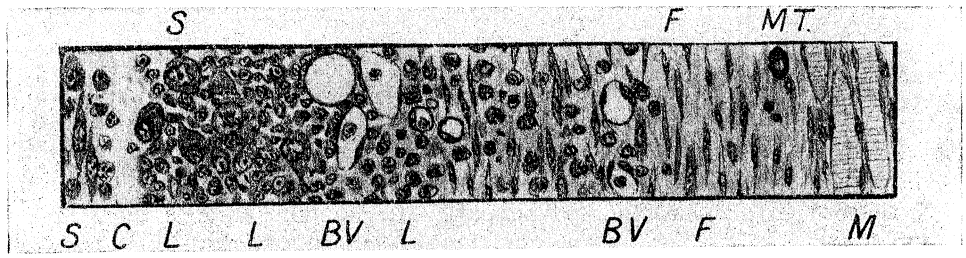

FIG. 15.—Shows the same structures as in fig. 12, but in an immune rat, 48 hours specimen. Mt., mast cell ; M, muscle ; BV blood-vessel ; F, fibroblast ; L, lymphocyte ; S, sarcoma cell ; C, cleft.

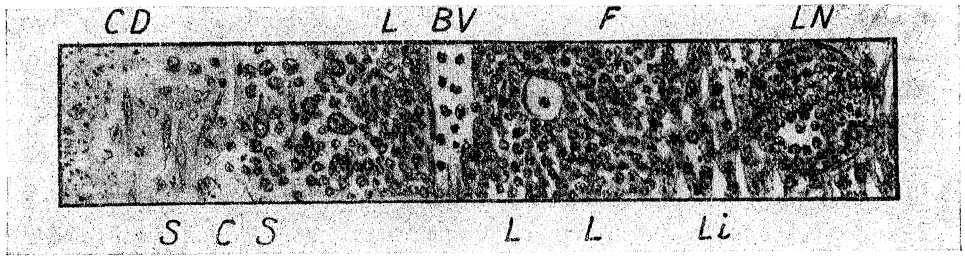

FIG. 16.—Shows the appearances 72 hours after inoculation into the liver of an immune rat. (Subcutaneous inoculation results in a similar appearance.) Li, liver ; LN, lymphoid nodule. Other letters as above.

degeneration and disappearance of the sarcoma cells external to the cleft. The subsequent fibrosis and collection of plasma cells is also not to be seen in susceptible animals. Apart from these differences, the early inflammatory œdema and the later vascular connective tissue appear precisely similar in both kinds of animals.

The Microscopical Appearances when Inoculation is made into Liver and Spleen.—The reaction tissue, both in the case of normal and immune animals, is precisely similar in these organs to that which is produced when inoculation is made into the subcutaneous fatty connective tissue;

24 hours after inoculation the spleen and liver tissues are found to be everywhere separated from the inoculated material by a zone of inflammatory œdema, and subsequently these tissues remain always separated from the sarcoma cells by the reaction tissue.

In immune animals, on and after the third day, collections of lymphocytes and plasma cells are to be seen in the liver substance, up to a distance of 2·5 mm. from the graft (see fig. 16). In normal rat's liver only a few lymphocytes and plasma cells are to be seen. Similar increase is seen in the spleen when inoculation is there made in the case of immune animals.

3. *The Microscopic Appearances of Grafts in Animals which have been Experimentally Altered before Inoculation.*

(a) *Grafts in Animals the Day After the Removal of the Spleen.*—The appearances of grafts in susceptible animals were found to be similar to those seen in unsplenectomised animals. In immune animals there is a delay in the accumulation of the lymphocytes until the fifth or sixth day, and, during this period, the sarcoma cells form a narrow band of growth outside the cleft; subsequently they die out.

(b) *Grafts in Immune Animals which have been given simultaneously Six or Eight other Inoculations.*—As in splenectomised animals, there is a delay in the accumulation of the lymphocytes until the fourth or fifth day, and a similar temporary growth of sarcoma.

(c) *Grafts in Susceptible and Immune Animals which have been Subjected to X-Radiation before Inoculation.*—Experiments have shown (see fig. 9) that the inoculation of immune animals which have been exposed to X-rays for 1½ hours resulted in the growth of measurable tumours, which persisted for about two weeks. A study of the microscopical appearances of grafts under these conditions showed that the reaction tissue and the behaviour of the sarcoma cells, in both susceptible and immune animals, was exactly similar to that which occurs in susceptible animals. A vascular connective tissue is formed by the host, into which the sarcoma cells wander, divide, and form a band of new growth around the central necrotic area. The growth in such animals appears to be just as vigorous as in susceptible animals, as is shown in fig. 17. After about fourteen days the growth becomes surrounded by, and invaded with, lymphocytes, in a manner similar to that which occurs in disappearing tumours (see p. 24). The tumours finally disappear.

Another set of observations was made with animals exposed to X-rays for half the time, namely, ¾ hour. Measurable tumours did not result, but a temporary growth of sarcoma occurred up to the fifth or sixth day,

when accumulation of lymphocytes began, after which the sarcoma cells disappeared.

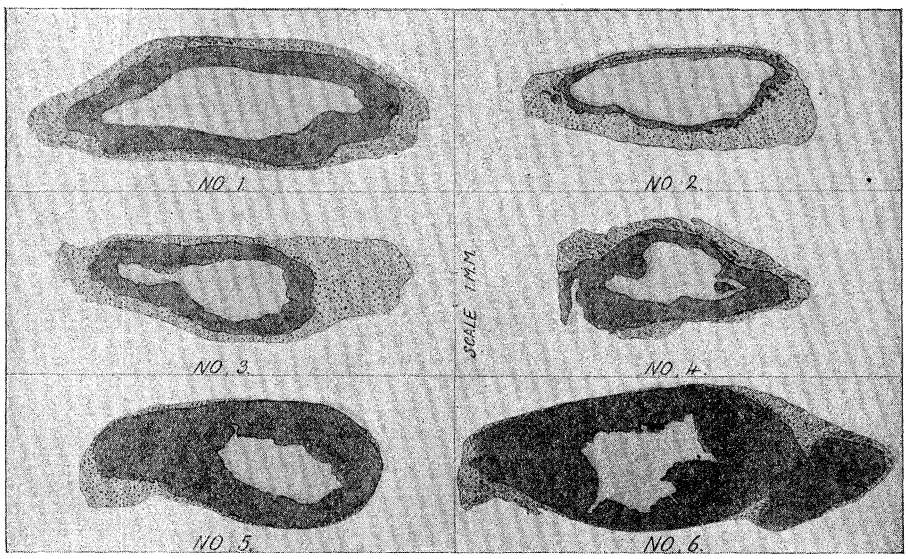

FIG. 17.—Shows tracings made with a projection microscope. Unshaded area—necrotic centre; shaded area—sarcoma growth, except in No. 2, where it represents lymphocytes; dotted area—surrounding connective tissue.

1. 5th day graft in a susceptible animal.
2. 5th day graft in an immune animal.
3. 5th day graft in a susceptible animal exposed to X-rays, 1½ hours.
4. 5th day graft in an immune animal exposed to X-rays, 1½ hours.
5. 10th day graft in a susceptible animal exposed to X-rays, 1½ hours.
6. 12th day graft in an immune animal exposed to X-rays, 1½ hours.

4. *The Microscopic Appearances of Grafts in Animals Inoculated with Sarcoma Cells which have been previously Experimentally Altered.*

Grafts of a Mixture of Sarcoma and Spleen Emulsion Inoculated into Susceptible Animals.—Two series of grafts were studied according as the spleens from susceptible animals or from immune animals were used. Experiments have shown that the inoculation of a mixture of tumour and spleen resulted in delayed growth as compared with the use of tumour alone, and that if the mixture be made with the spleen of immune animals, more interference with growth results than when the spleens from susceptible animals form the mixture (see figs. 10 and 11).

A study of grafts under these conditions shows that very few sarcoma cells are to be seen outside the cleft, until after the fourth or fifth day; during this period, there is some accumulation of lymphocytes in the

surrounding connective tissue. Subsequently the lymphocytes decrease in numbers whilst the sarcoma cells multiply and form a tumour.

Grafts of Boiled Sarcoma in Normal Animals.—At the end of 24 hours the surrounding tissues are hardly at all œdematous, and subsequently there is no reaction tissue comparable to that seen when living sarcoma cells are inoculated; there is no accumulation of lymphocytes, fibroblasts make their appearance early and shut off the inoculated material from the tissues of the host by scar tissue.

Grafts of Irradiated Sarcoma in Normal Animals.—Experiments by Chambers and Russ (*loc. cit.*) have shown that if sarcoma emulsion be exposed to a concentration of 0·45 millicurie per cubic centimetre for periods of (*a*) 20 minutes, (*b*) 80 minutes, and (*c*) 24 hours, on inoculation into normal rats, there result (*a*) grafts which grow at a diminished rate, (*b*) grafts which just fail to grow, and (*c*) grafts which present no sign of proliferation.

In the 24-hour irradiated specimens only a few sarcoma cells are seen outside the cleft, no mitosis occurs, degenerative changes begin early and the sarcoma cells soon die out; a moderate amount of lymphocytic infiltration occurs at an early date. In the 80 minutes irradiated grafts considerable proliferation of the sarcoma cells takes place, but later, degenerative changes supervene which are accompanied by an intense accumulation of lymphocytes; the sarcoma cells eventually die out.

The 20 minutes irradiated specimens present proliferation of sarcoma cells; on about the eleventh day some of these appear degenerated, and at this time some lymphocytic infiltration occurs; subsequently the degenerated sarcoma cells and the lymphocytes disappear, whilst the remaining healthy sarcoma cells continue to proliferate in a normal manner.

5. *The Microscopic Appearances of Disappearing and Oscillating Tumours.*

In the connective tissues surrounding these tumours a great accumulation of lymphocytes is to be seen, which contrasts with the relative absence of these cells in progressive tumours.

Lymphocytes are also seen to be mixed with the marginal sarcoma cells, which present decided degenerative changes. It is only towards the centre of such tumours that healthy sarcoma cells are to be seen, in places where there is no lymphocytic invasion. In the surrounding connective tissue, accumulations of plasma cells occur around the blood-vessels, but only a few of these cells are to be seen mixed with the sarcoma cells. The regression of a tumour proceeds from without inwards, and is accompanied by a gradual invasion of lymphocytes; eventually the tumour is replaced by a fibrous connective tissue, containing lymphocytes and collections of plasma cells.

6. *Comparison of Results.*

The facts which these investigations upon grafts have brought out may be conveniently marshalled under (1) the *rôle* of the lymphocyte, (2) the behaviour of the sarcoma cells, and (3) the reaction tissue of the host.

(1) *The Rôle of the Lymphocyte.*

In susceptible animals they are seen in the reaction tissue in small numbers, making their appearance on the second or third day.

In immune animals the reaction tissue is loaded with these cells from the second and third day until the final disappearance of the sarcoma cells.

Both these characteristic reactions are affected by alteration of the experimental conditions along two distinct lines, namely, by changing the condition of the sarcoma cell before inoculation, or by altering the rat's condition before inoculation.

(a) *Changing the Condition of the Sarcoma Cell.*—If the cell be killed by heat before inoculation, no lymphocytic accumulation occurs in either susceptible or immune animals.

If the sarcoma cell be given an exposure to the β- and γ-rays from radium sufficient to prevent any proliferation, some lymphocytes accumulate in the reaction tissue on the second, third, and fourth days.

If the dose of radiation be just sufficient to prevent the growth of a tumour, then in susceptible animals there is a great accumulation of lymphocytes from the fifth day until the final disappearance of the sarcoma cells.

If the dose be sufficient only to delay growth, then an accumulation of lymphocytes occurs about the fifth day, but subsequently they disappear and the growth of sarcoma proceeds as under normal conditions.

If the sarcoma cells be mixed with spleen cells before inoculation, more lymphocytes are found in the reaction tissue than when unmixed sarcoma cells are inoculated into susceptible animals; but they are less numerous than in immune animals.

(b) *Altering the Rat's Condition before Inoculation.*—If rats be splenectomised before inoculation, then in susceptible animals no differences were seen. In immune splenectomised rats there is a decided delay in the onset of lymphocytic accumulation; it is not until the fifth or sixth day that they are present in numbers comparable to that seen in unsplenectomised immune animals on the second or third days.

If immune rats be given six or eight inoculations of 0·05 c.c. instead of two, then a similar delay occurs in the accumulation of the lymphocytes.

If rats before inoculation be given a dose of X-rays sufficient to reduce the number of white cells in the blood by about 90 per cent., then in immune

animals the accumulation of lymphocytes is delayed for as long as two weeks. (A smaller dose of X-rays resulted in less delay.)

The accumulation of lymphocytes which occurs around the sarcoma cells, which by then have grown to a measurable tumour, is precisely similar to what is to be seen when tumours are either disappearing or oscillating.

(2) *The Behaviour of the Sarcoma Cells.*

Before attempting to correlate these facts, it is necessary to consider what has been seen to occur as regards the behaviour of the sarcoma cells under these same experimental conditions. In order that these facts may be displayed side by side, Table V has been prepared; the conditions in disappearing, oscillating, and progressing tumours have been subjoined. Detailed descriptions of the behaviour of the sarcoma cells have already been given, and do not require to be again referred to in the text.

On referring to Table V, it can be seen that, in susceptible animals, lymphocytic accumulation only occurs when injured sarcoma cells are inoculated; and that where uninjured or dead cells are used, very slight lymphocytic accumulation is found. Further, it can be seen that when great accumulation occurs the animal is likely to be subsequently immune.

In immune animals a delay in the accumulation of lymphocytes is seen to be associated with a temporary growth of the sarcoma; but that, later, when accumulation of the lymphocytes takes place, the sarcoma cells die out, and the animals remain unsusceptible.

A similar correlation has been seen to hold in the case of tumours. Animals bearing progressive tumours are usually susceptible, they do not present accumulation of lymphocytes in the surrounding connective tissue; the sarcoma cells are in active division. On the other hand, animals bearing disappearing or oscillating tumours are almost invariably immune, they present great accumulation of lymphocytes; the sarcoma cells are either degenerated or not in active division.

(3) *The Reaction Tissue of the Host.*

Apart from the differences already noted, the local reaction of the tissues of the host appear to be the same in susceptible and immune animals. An inflammatory œdema, lasting for 24 hours, is followed by the laying down of a vascular connective tissue. It is important to note that the formation of blood-vessels in the tissues occurs at the same time, and to the same extent, in immune as in susceptible animals; and that the failure of growth in immune animals cannot therefore be accounted for by the failure of a sufficient vascular supply.

Table V.

		No alteration before inoculation.	The animal altered before inoculation.				The sarcoma cells altered before inoculation.				
			By splenectomy.	By a large inoculation.	By exposure to X-rays for 1½ hours.	By exposure to X-rays for ¾ hour.	Killed by exposure to heat.	By exposure to radium sufficient to prevent the division of cells.	By exposure to radium sufficient to prevent the formation of a tumour.	By exposure to radium sufficient to produce delay of growth of a tumour.	By admixture with spleen cells before inoculation.
In susceptible animals.	The *rôle* of the lymphocyte.	Very few lymphocytes in the reaction tissue.	As in unaltered animals.				Very few lymphocytes in reaction tissue.	Some accumulation of lymphocytes 3rd–4th day.	Great accumulation of lymphocytes on about the 5th day.	Some accumulation 5th–6th day, but subsequently disappear.	Some accumulation of lymphocytes 3rd–4th day.
	The behaviour of the sarcoma cell.	Sarcoma cells multiply and form a tumour.					No growth of sarcoma cells; none outside the "cleft."	No division of sarcoma cells; some outside the cleft.	Some division of sarcoma cells outside the cleft, but subsequently disappear.	Slow division of sarcoma cells and production of a tumour.	Delayed growth of sarcoma, but production of a tumour.
	The subsequent susceptibility of the animal.	The animal may be either susceptible or immune.	Experiments not carried out.				Animal remains susceptible.	Animal remains susceptible.	Animals usually immune.	May be either susceptible or immune.	May be either susceptible or immune.
In immune animals.	The *rôle* of the lymphocyte.	Great accumulation of lymphocytes 2nd or 3rd day.	Accumulation of lymphocytes delayed to 5th or 6th day.	Accumulation delayed to 4th or 5th day.	Accumulation delayed to about 14th day.	Accumulation delayed to 5th or 6th day.	Experiments have not been carried out.				
	The behaviour of the sarcoma cell.	A few sarcoma cells outside the "cleft"; none divide.	Division of sarcoma cells outside cleft up to 5th day; subsequently die out.	Division of sarcoma cells outside cleft up to 4th day; subsequently die out.	Sarcoma cells divide and produce a measurable tumour which subsequently regresses.	Divisions of sarcoma cells outside the cleft up to 5th day; subsequently die out.					
	The subsequent susceptibility of the animal.	Animal remains immune.	Animal remains immune.	Animal remains immune.	Animal remains immune (few experiments only).	Animal remains immune (few experiments only).					

Part III.—General Discussion and Conclusions.

Preparatory to a brief discussion on the trend of our observations, we give below some of the main facts which have been ascertained :—

1. Jensen's rat sarcoma almost invariably grows when inoculated into rats.

2. Once having been inoculated, the rats, in over 90 per cent. of the cases, are immune to a second inoculation. The different phases of growth which the tumours exhibit are intimately bound up with the varying degree of immunity set up by the animal.

3. When a rat is immune to the inoculation of the sarcoma cells, the spleen of the animal generally shows a high content of lymphocytes and plasma cells.

4. Mixture of the spleen with the sarcoma cells before inoculation causes a retardation of growth in the resulting tumour ; this is more marked with the spleen of an immune animal than with that of a normal one.

5. Sarcoma cells may remain as long as three days in an immune rat, and then be successfully re-inoculated ; this period corresponds with the interval required for the accumulation of lymphocytes around the graft.

6. The essential difference in the processes initiated on introducing sarcoma cells into normal and immune rats consists in a marked accumulation of lymphocytes around the graft in the immune rat.

7. By damaging the sarcoma cells by irradiation, the subsequent reaction of the normal animal resembles that of the immune one.

8. By damaging the rat, the accumulation of lymphocytes is delayed and growth of the sarcoma occurs.

9. Rats may be made immune by inoculation of sarcoma cells which have previously been exposed to the β- and γ-rays from radium.

10. By exposure to X-rays, an immune rat may be converted into a tumour-bearing animal.

The majority of normal rats are susceptible; in a few cases only, small nodules follow inoculation. In all such cases, when microscopical examination of the early stages of grafts has been made, some proliferation of the sarcoma cells has been observed. The complete inhibition of proliferation, which occurs in immune animals, has not been observed in normal animals. A complete natural immunity, comparable with acquired immunity, has not been met with.

The microscopical appearances of a graft whose growth is being controlled at an early stage is similar to what is seen in disappearing tumours; what is termed natural immunity appears to be an immunity acquired during the regression of a small nodule.

Further, as will be discussed later, acquired immunity appears to depend on a process similar to that which occurs in disappearing tumours, but in this case the control of growth begins at once, so that no proliferation of the sarcoma cells occurs.

After inoculation, the majority of rats subsequently become immune; in some the immune condition appears soon after inoculation, in which case the tumour resulting from the first inoculation only reaches a small size; if the onset of immunity be further delayed, the tumour may reach a large size before regressing.

The tumour may never regress, but only the rate of growth become less, when the rat is found to be nearly always unsusceptible.

It follows that the onset of unsusceptibility, and the power to inhibit the growth of an established tumour, are closely associated.

It has, however, been seen that an animal bearing a progressively growing tumour, in which, therefore, no measurable inhibition of growth is occurring, may be either susceptible or immune. This might be taken as evidence that the two processes are not identical; on the other hand, a force which is sufficient to kill a few sarcoma cells struggling to establish themselves may have no measurable effect upon a large, well-established tumour. An alternative explanation has been given, on the view that a concomitant immune condition is produced by the re-inoculation itself, which is sufficient to prevent its taking, but insufficient to affect the progressive tumour. As will be seen later, there is considerable evidence that the two conditions—unsusceptibility and the power to inhibit growth of an established tumour—are identical (see p. 30).

Both of the conditions, susceptibility and immunity, are generalised; no matter where inoculation be made, the graft will take or fail to grow, as the case may be.

Immunity depends upon the ability of the animal to prevent the growth of, and to destroy, sarcoma cells when introduced into its body. This condition has been found to be associated with changes in the spleen, in respect of the number of lymphocytes and plasma cells present; nevertheless, it has not been possible to define exactly the relation between these factors. There is, however, other evidence that the spleen plays some part, viz.: (1) When mixtures of spleen and tumour are inoculated, the use of immune spleen causes a greater delay in growth than in the case of normal spleen. (2) If immune animals be splenectomised, the microscopic study of grafts shows that some growth of the sarcoma occurs. (3) If immune animals be splenectomised, and at the same time a large dose of sarcoma be given, in a few cases measurable tumours result. (4) If immune animals

be exposed to X-rays before inoculation, measurable tumours result, and the study of grafts shows that considerable growth of sarcoma occurs; at the same time, profound changes take place in the spleen; lymphocytes, and, to a less extent, plasma cells, are completely destroyed.

Our observations as to the effects of X-rays upon the immune condition are confirmatory of the experiments on similar lines initiated by Murphy and Morton (5), who showed that mice immunised with defribrinated blood could be rendered susceptible to mouse carcinoma by a suitable exposure to X-rays.

Murphy (6) has also shown that the chick embryo, up to the 18th day, will support the growth of rat sarcoma, but that if, during this period, adult chicken spleen or bone marrow be inoculated, then the established rat sarcoma will be destroyed. It was found that this destruction was associated with an accumulation of lymphocytes in the connective tissue around the sarcoma.

Other evidence that the lymphocytes, and less certainly plasma cells, are an important factor in the condition of immunity is, however, forthcoming. The evidence of grafts study is very strong, and it does not appear necessary to repeat this again; the reader is here referred to Part II. (1) The *Rôle* of the Lymphocyte, and also to the observations of da Fano (*loc. cit.*).

Reference may be made again to the fact that sarcoma cells are able to survive in an immune rat for three days, and that this is the period at which the local accumulation of lymphocytes around the inoculated material reaches its maximum. Finally, it may be pointed out that, just as the destruction of sarcoma cells, when introduced into an immune rat, is always associated with a local accumulation of lymphocytes, so when the growth of an established tumour begins to be controlled, there is likewise a great accumulation of lymphocytes in the immediate neighbourhood of the tumour.

It may therefore be concluded that lymphocytes play an important part in the process by means of which an animal is able to destroy sarcoma cells, and that their local presence is necessary for this destruction, and that in their absence locally the sarcoma cells will proliferate.

We are, as yet, entirely ignorant of the mechanism by means of which the lymphocyte is brought to the sarcoma cell. The facts indicate that the lymphocyte does not merely act as a scavenger of sarcoma cells, killed, for instance, by some toxin; for if this were the case, it must be assumed that such actions as splenectomy and X-ray exposure destroy the toxin as well as the lymphocyte, unless the toxin is secreted by the lymphocyte, which is a possibility to be taken into account. The exact time relations between lymphocyte accumulation and sarcoma degeneration have not, as yet, been thoroughly worked out; but from the study of grafts, the degeneration is

roughly concomitant with the local accumulation of the lymphocytes. It is certain that the lymphocytes do not ingest the sarcoma cells until some degeneration has taken place in the latter.

The negative evidence (*vide* p. 16) in the search for a toxin in the circulating fluids indicates that it may be manufactured locally by the lymphocyte. There is also more direct evidence in that where for any reason there is a delay in the local accumulation of lymphocytes, then the sarcoma cells instead of degenerating remain healthy and proliferate even in an immune animal, and it is not until accumulation occurs that growth is controlled. Degenerated sarcoma cells in the absence of lymphocytic infiltration have never been observed, except in the necrotic centre of tumours, where the degenerative changes are different and where lymphocytic infiltration is not seen. It would appear for this reason also that lymphocytic infiltration is not a reaction to dying or dead sarcoma cells, or else it would occur under such conditions.

In favour of there being an association between the production of immunity and the local accumulation of lymphocytes is the fact that in all tumours of the disappearing and oscillating types great accumulation of lymphocytes occurs, and this is associated with unsusceptibility. It would appear therefore that without local accumulation of lymphocytes neither graft destruction, tumour destruction, nor production of immunity can occur. Finally, it may be again mentioned that the condition of immunity to the graft has not in any respect been observed to be due to a failure on the part of the host to supply the necessary connective tissue or blood supply; but all the evidence goes to show that the immunity consists of a positive action on the part of the host.

The above statement refers only to Jensen's rat sarcoma, for in the case of mouse carcinoma Russell (7) has shown that the failure of an inoculation in an immune animal is to be ascribed to a failure on the part of the host to supply the "specific stroma reaction."

The Bearing of the Observations upon Malignant Disease in the Human Subject.

Spontaneous disappearance of malignant disease in the human subject is very rare; there is, however, no reason to doubt the clinical observation that occasionally growths, malignant in all their aspects, do spontaneously disappear. Clinical study has also shown that in some cases, although the tumour may not disappear, its growth is not continuously progressive, but that sometimes growth is held in check or may even regress, though finally proving fatal. There is, in fact, ample evidence to show that an active

resistance to the growth of the tumour is in some cases set up in the human subject.

The bearing of our observations upon malignant disease in the human subject is of a dual nature. If the *rôle* which the lymphocyte plays in immunity towards Jensen's rat sarcoma proves to apply also to the human malignant growths, then the lines along which successful treatment may possibly be attained are to some extent indicated. In the first place, it may be possible by stimulating the growth of lymphoid tissues to produce a concentration of these cells at the locality of the growth. This might be accomplished by the inoculation of malignant cells rendered harmless by irradiation or of some other material.

In the second place, reference may be made to the treatment of malignant disease by means of X-rays or the rays from radium (β- and γ-rays). The aim of radiotherapy in malignant disease is to cause the destruction of the malignant cells with a minimum amount of damage to the adjacent normal tissues. Under many conditions of irradiation of the human subject, and especially in the treatment of deep-seated malignant growths, it happens that the normal tissues are subject to a very considerable degree of irradiation.

In view of the fact that the lymphocytes play an important part in the healing of growths of Jensen's rat sarcoma, and because these cells have been shown to be especially vulnerable to these radiations, it follows that particular care should be taken in this treatment of cancer in the human subject to protect the rest of the body from radiation, more especially those parts where lymphocytes occur, namely, the blood, lymph, bone marrow, lymphatic glands, and spleen.

It is known (8) that a small dose of rays may stimulate the production of some of the cellular constituents of the blood. With prolonged irradiation, however, the probability of destroying cells which are capable of resisting the growth of the invading cancer cells suggests the advisability of taking steps to avoid the irradiation of any but the malignant tissues, and, if possible, to restrict the blood supply of all these tissues, malignant or otherwise, through which the radiation penetrates.

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REFERENCES.

1. Chambers and Russ, 'Roy. Soc. Proc.,' B, vol. 86 (1913).
2. Da Fano, 'Imp. Cancer Research Fund, 5th Report,' p. 57.
3. Brancati, 'Tumori,' Anno II, fasc. 1, p. 74.
4. Apolant, 'Zeits. f. Immunitäts Forschung,' Originale 17, p. 219 (1913).
5. Murphy and Morton, 'Journal Exp. Medicine,' vol. 22, p. 204 (1915).
6. Murphy, 'Journal Exp. Medicine,' vol. 19, p. 181 (1914).
7. Russell, 'Imp. Cancer Research Fund, 3rd Report,' p. 341.
8. Aubertin and Beaujard, 'Arch. de Méd. Expérimentale et d'Anatomie Pathologique,' vol. 20, p. 273 (1908).

The Germicidal Action of Ultra-Violet Radiation, and its Correlation with Selective Absorption.

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[Plate 3.]

A new method is here described which enables us to say definitely what portion of the ultra-violet spectrum is especially effective in germicidal action and the wave-length of the radiation at which such action practically ceases. Briefly the method consists of inoculating a gelatine* plate with micro-organisms instead of sensitising it with a silver salt. We find that when a spectrum is formed on this it produces what may be called an image, where germicidal action occurs, and this image may be rendered visible by a process of incubation, which encourages a copious growth of those organisms which have not been affected by the radiation, whereas the affected parts remain practically transparent. Such an exposed and incubated plate can be used as an ordinary negative for producing positive contact prints or, equally well, may be photographed by light reflected and scattered from the bacterial surface.

The study of the action of radiation, visible and otherwise, upon micro-organisms is not a new one. The period 1894–6, associated with the work

* For convenience, an agar plate was used in most of the experiments.

FIG. 4 (Normal Spleen).

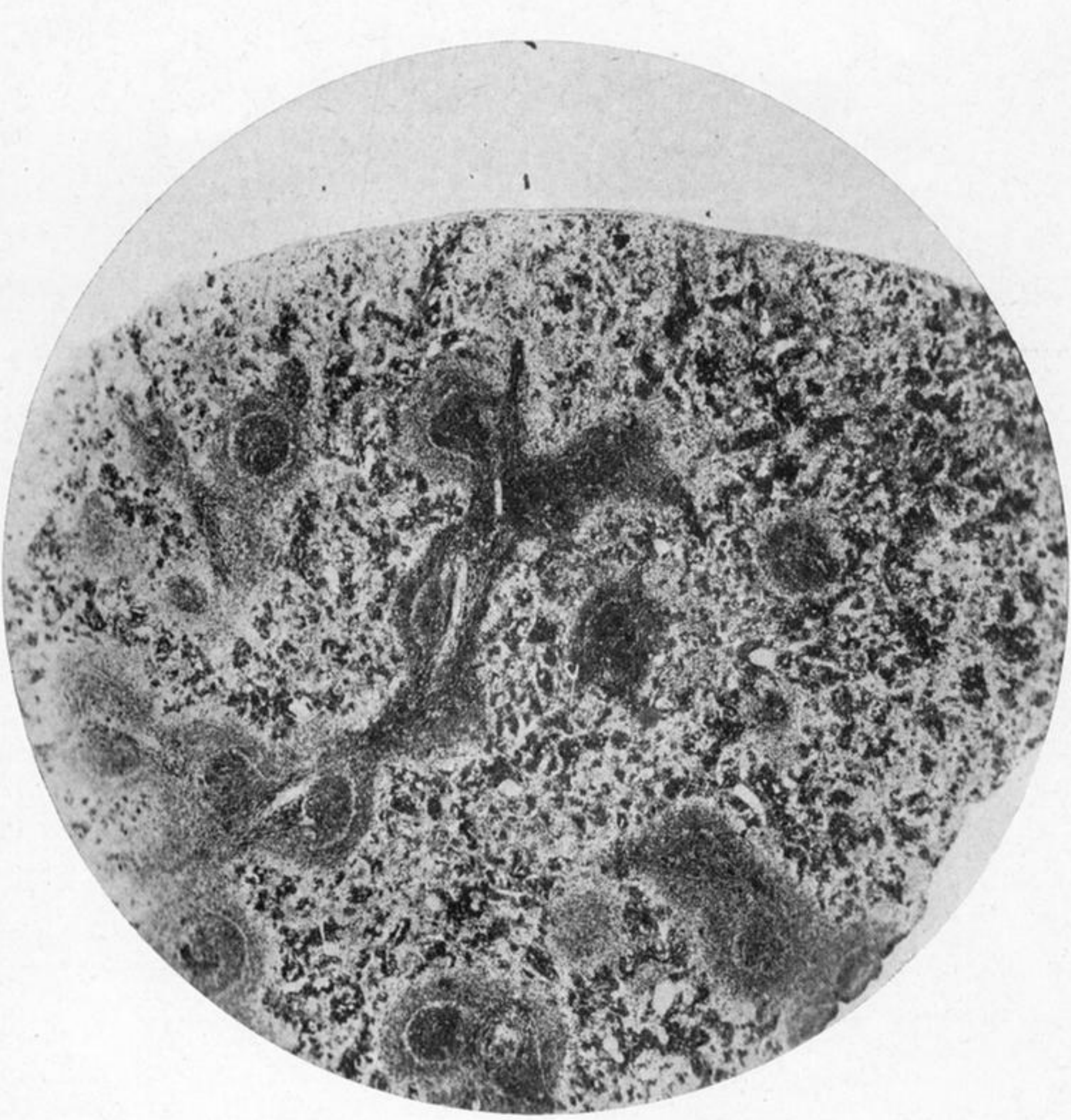

FIG. 5 (Immune Spleen).

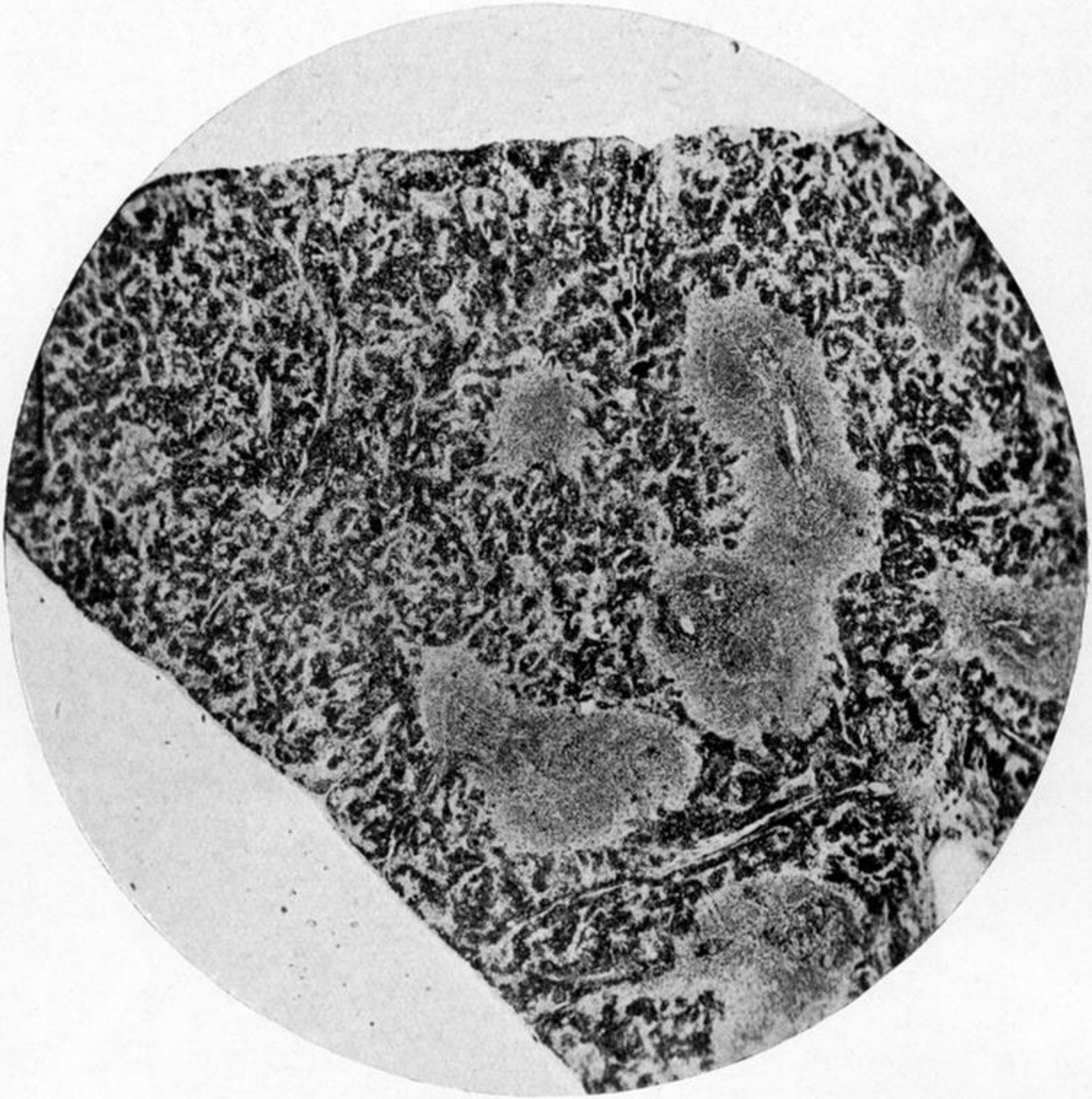

FIG. 6 ("Massive" Spleen).

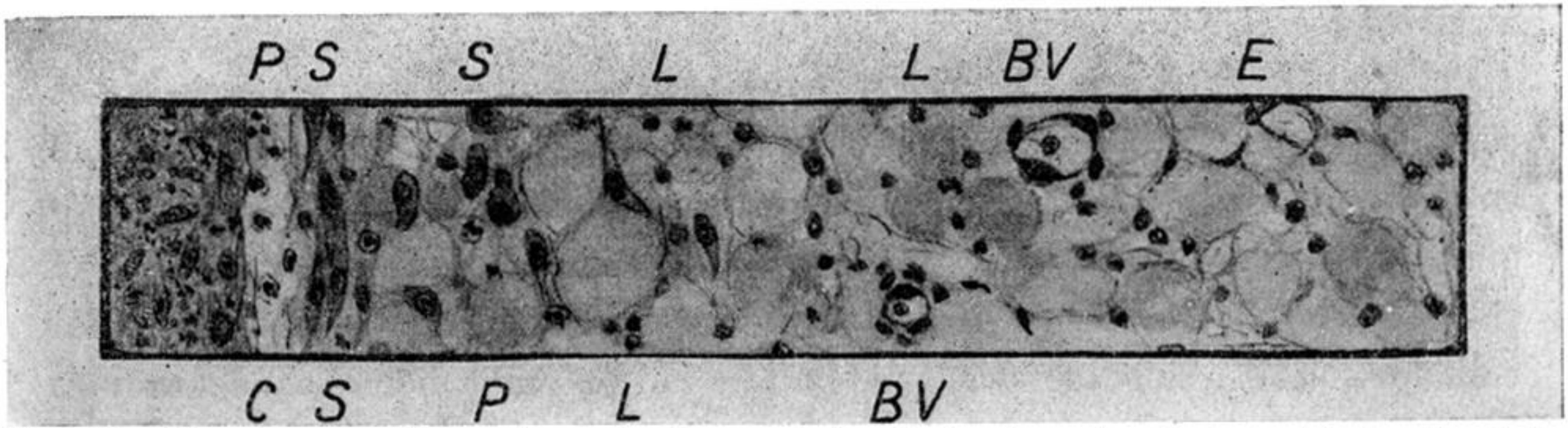

FIG. 12.—Shows a small portion of the margin of the inoculated material, to the left; separated by the "cleft" from a portion of the reaction tissue of the host, to the right; 24 hours after inoculation. S, sarcoma cells outside "cleft"; P, polymorphonuclear leucocytes; BV, blood-vessels; F, fibroblasts; L, lymphocytes; C, cleft.

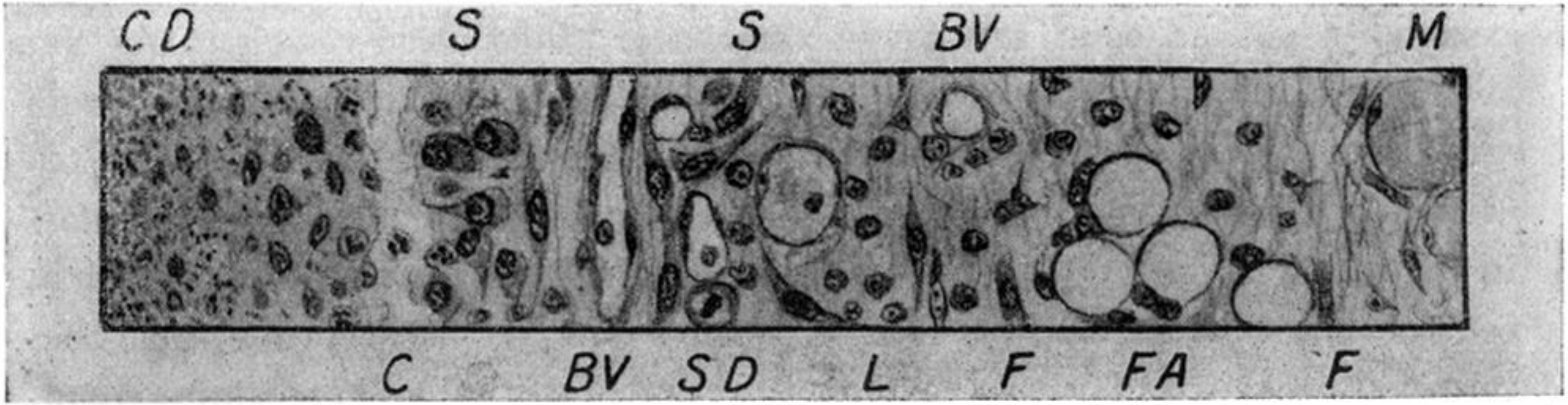

FIG. 13.—Shows the same structures 48 hours after inoculation. F, fibroblast; FA, fat; M, muscle; BV, blood-vessels; L, lymphocyte; S, sarcoma cell; SD, sarcoma cell dividing; CD, cell débris; C, cleft.

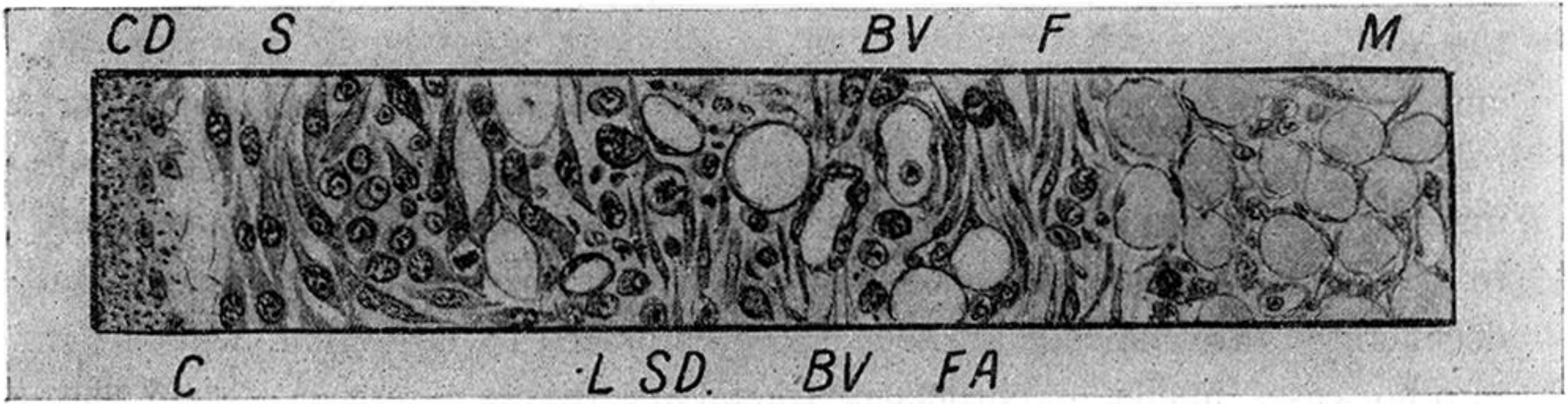

FIG. 14.—Shows same structures 72 hours after inoculation. Letters as above.

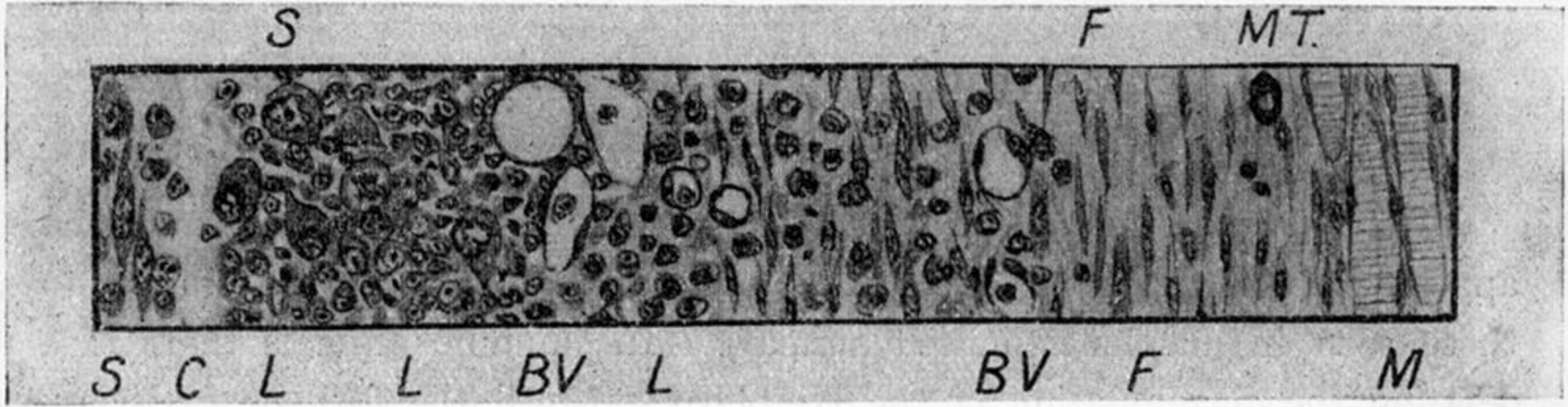

FIG. 15.—Shows the same structures as in fig. 12, but in an immune rat, 48 hours specimen. Mt., mast cell ; M, muscle ; BV blood-vessel ; F, fibroblast ; L, lymphocyte ; S, sarcoma cell ; C, cleft.

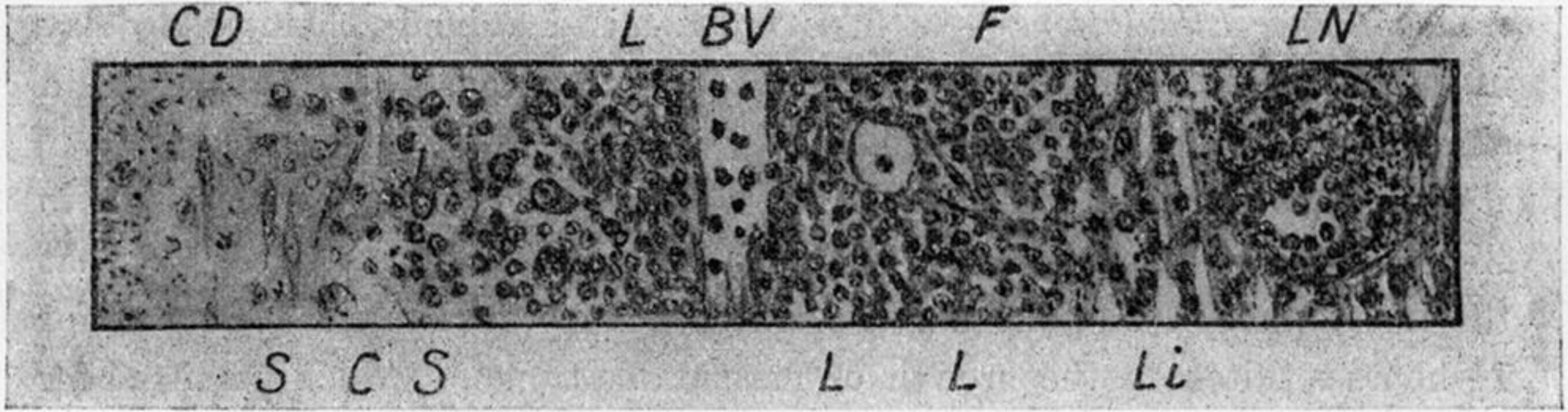

FIG. 16.—Shows the appearances 72 hours after inoculation into the liver of an immune rat. (Subcutaneous inoculation results in a similar appearance.) Li, liver; LN, lymphoid nodule. Other letters as above.

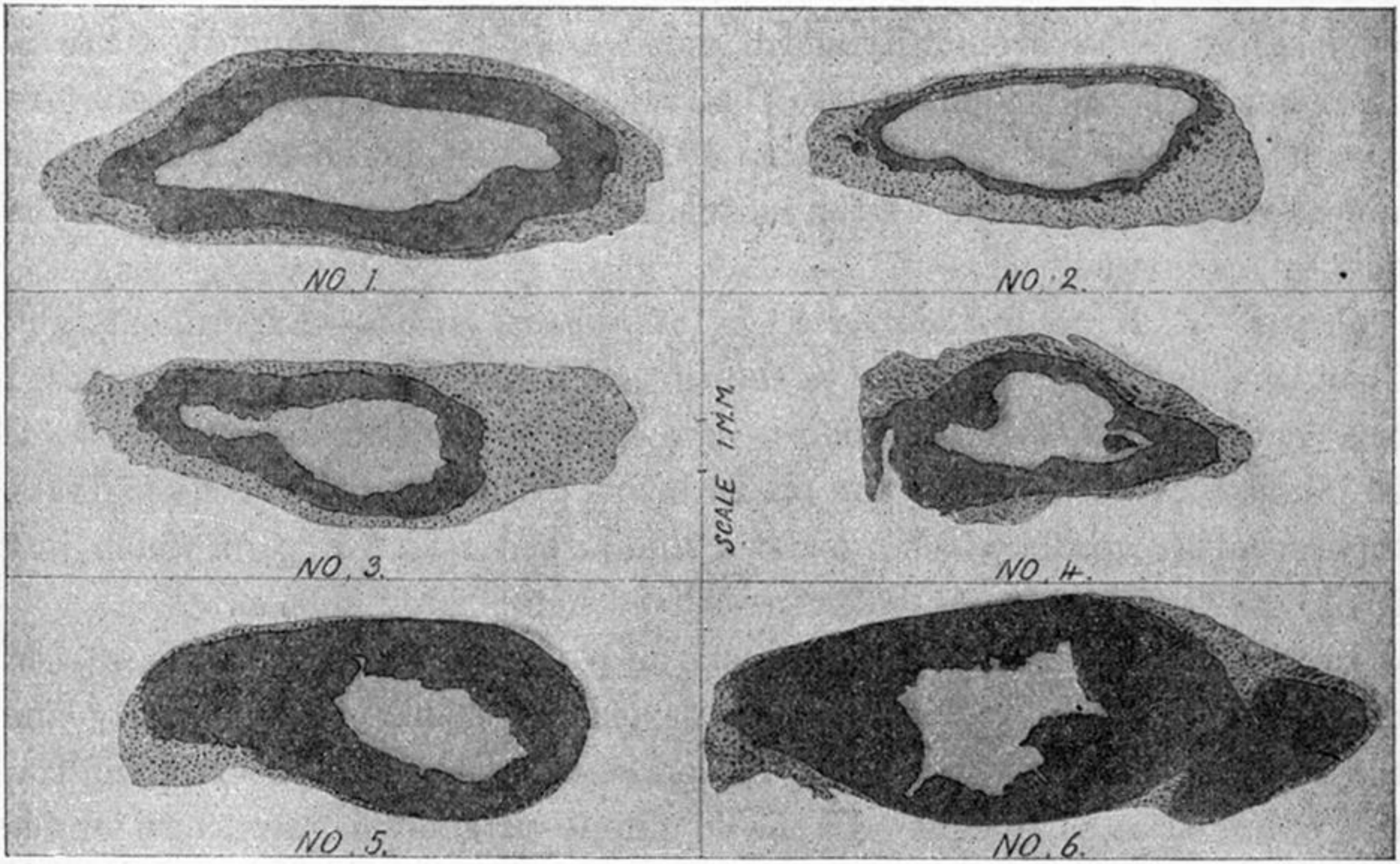

FIG. 17.—Shows tracings made with a projection microscope. Unshaded area—necrotic centre; shaded area—sarcoma growth, except in No. 2, where it represents lymphocytes; dotted area—surrounding connective tissue.

1. 5th day graft in a susceptible animal.
2. 5th day graft in an immune animal.
3. 5th day graft in a susceptible animal exposed to X-rays, 1½ hours.
4. 5th day graft in an immune animal exposed to X-rays, 1½ hours.
5. 10th day graft in a susceptible animal exposed to X-rays, 1½ hours.
6. 12th day graft in an immune animal exposed to X-rays, 1½ hours.