On the Action of Radium Rays upon the Cells of Jensen's Rat Sarcoma.

By S. Russ, D.Sc.,* and Helen Chambers, M.D.

(Communicated by H. G. Plimmer, F.R.S. Received March 14,—
Read May 29, 1913.)

[Plates 12 and 13.]

The experiments of B. H. Wedd and one of us† have shown that if freshly excised portions of mouse carcinoma are exposed to X-rays or the β-rays from a few milligrammes of radium for a comparatively brief period (*circa* 1 hour), the irradiated material will not grow on subsequent transplantation. This line of experimental work has here been extended to Jensen's rat sarcoma, the initial material for which was kindly provided by the Imperial Cancer Research Fund. From several points of view this tumour provides excellent material for the investigations in question. Inoculations are successful in practically 100 per cent., for out of 125 inoculations into normal rats, 124 gave growing tumours; the rate of growth is rapid, tumours measuring 2×2 cm. frequently being obtained in 15 days after the inoculation of 0·1 c.c. of tumour emulsion. Spontaneous absorption of the tumours, however, is not uncommon; five disappearances have occurred in 53 rats, all of which were under observation for a minimum period of 20 days.

The Inhibitory Effect of Irradiation by β-Rays.

Thin slices of rat sarcoma from a rapidly growing tumour were exposed between sterile sheets of mica to the β-rays from a source of radium, having an intensity of 1·58 mgrm. per square centimetre. Small pieces of the irradiated material were then inoculated into the right axillæ of a number of normal rats, into the left axillæ of which small pieces of non-irradiated tumour were also inoculated.

The amount of tumour tissue which can be irradiated in this way is necessarily small and the inoculations were, therefore, made with a hypodermic needle and stilet, the pieces of tumour tissue being as nearly as possible of the same size.

This procedure was adopted for the same tumour material for three different periods of irradiation, *i.e.* 30 minutes, 1½ hours, and 3 hours,

* Part of this work was done during the tenure of a Beit Memorial Fellowship.

† 'Journ. Path. and Bact.,' 1912, vol. 17.

six animals being inoculated for each series. They were examined at frequent intervals, and fig. 1 gives in outline one half the actual sizes of the tumours which formed.

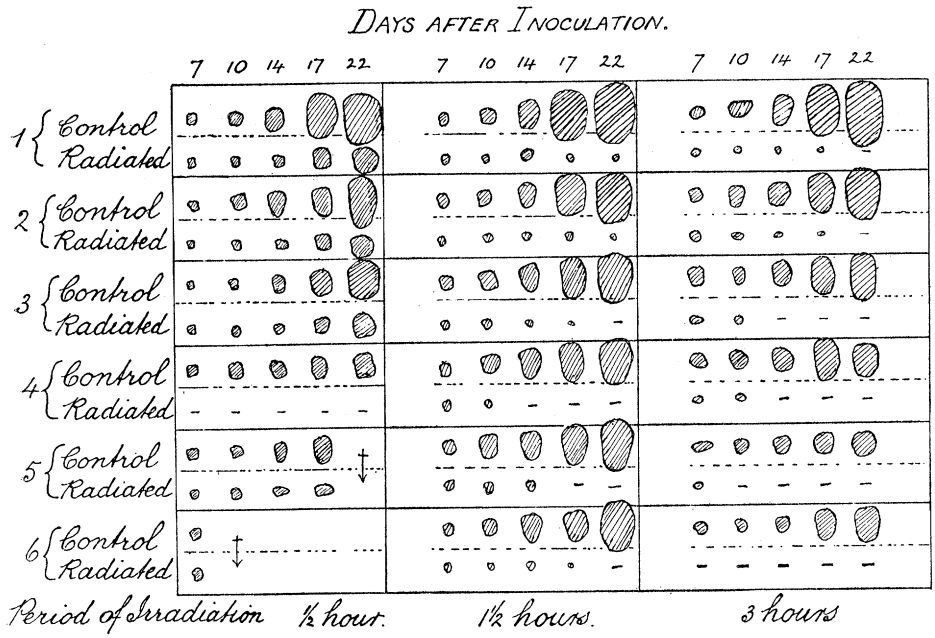


Fig. 1.

It will be seen that as a result of irradiation for 30 minutes there is a slower growth of the irradiated than of the untreated tumour.

When the period of irradiation is increased to 90 minutes, the inoculated material, although apparently increasing in size for some days, was in all cases eventually absorbed in the animal which was simultaneously supporting the growth of the control tumour. Extension of the period of irradiation to three hours ensures the progressive and complete absorption of the tumour cells. There is a close similarity in the action of the β-rays in retarding or preventing the growth of rat sarcoma tissue to the results recorded for mouse carcinoma by Wedd and Russ in the paper to which reference has been made.

The Action of Radium Emanation on Tumour Tissue.

Preliminary experiments were made by mincing a tumour with a Haaland mincer and adding to it sufficient normal saline for the mixture to flow into a small glass bulb. Radium emanation was then supplied in a concentration of about 0·5 millicurie per cubic centimetre. After 32 minutes 0·1 c.c. of

the irradiated emulsion was inoculated into each of six rats, 0·1 c.c. of a control portion of the emulsion being inoculated at the same time into the opposite axillæ of the same animals. No tumours developed from the irradiated material, but in each animal a rapidly growing tumour formed from the control emulsion.

An experimental series was undertaken on similar lines in order to determine the dosage of irradiation necessary to prevent the growth of the sarcoma tissue. Tumour emulsion was exposed to 0·275 millicurie per cubic centimetre, samples being withdrawn after 15, 30, and 60 minutes; 0·1 c.c. of each sample was inoculated into each of six rats, 0·1 c.c. of the original emulsion being inoculated into an equal number of other rats, making 24 in all; the average weight of the rats in these four series was 39, 34, 42, and 43 grm. respectively. Measurements of the tumours resulting were made by means of callipers at frequent intervals.

In fig. 2 are recorded the sum of the superficial areas of the growths in

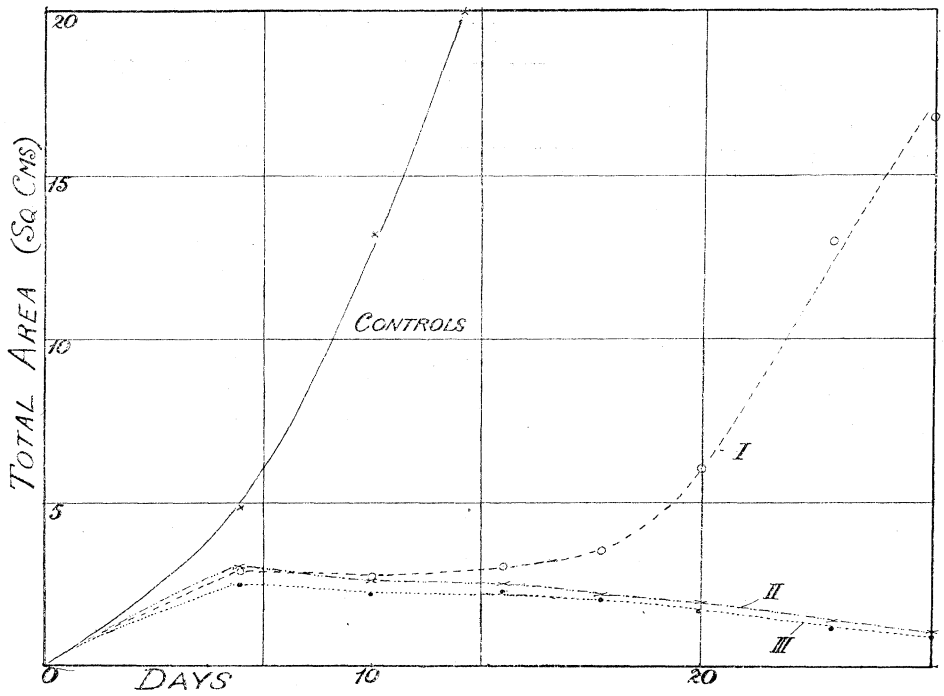


FIG. 2.

the six rats of each series till 27 days subsequent to the inoculations. The tumours in the control animals showed a vigorous growth; in Series I, *i.e.* after irradiation for 15 minutes, a phase of apparent inactivity lasting

about eight days was followed by an almost equally rapid growth. For the more prolonged periods 30 and 60 minutes, *i.e.* Series II and III respectively, the initial reaction, resulting in easily measureable nodules, showed gradual signs of absorption, until after 27 days there was every indication that complete disappearance of the nodules would result. One rat of Series II was killed at this stage, the small nodule was found to consist almost entirely of sarcoma cells (*vide* Plate 12, microphotograph 1).

Continued observations, however, showed that in three of the rats of Series II, nodules remained palpable for a prolonged period and eventually they developed into tumours. In Series III this occurred in one rat and resulted in a fairly rapidly growing tumour. The gradual decline in size of the initial nodules and the subsequent growth of the tumours are depicted for each of these four animals in fig. 3. The full-line curve marked III

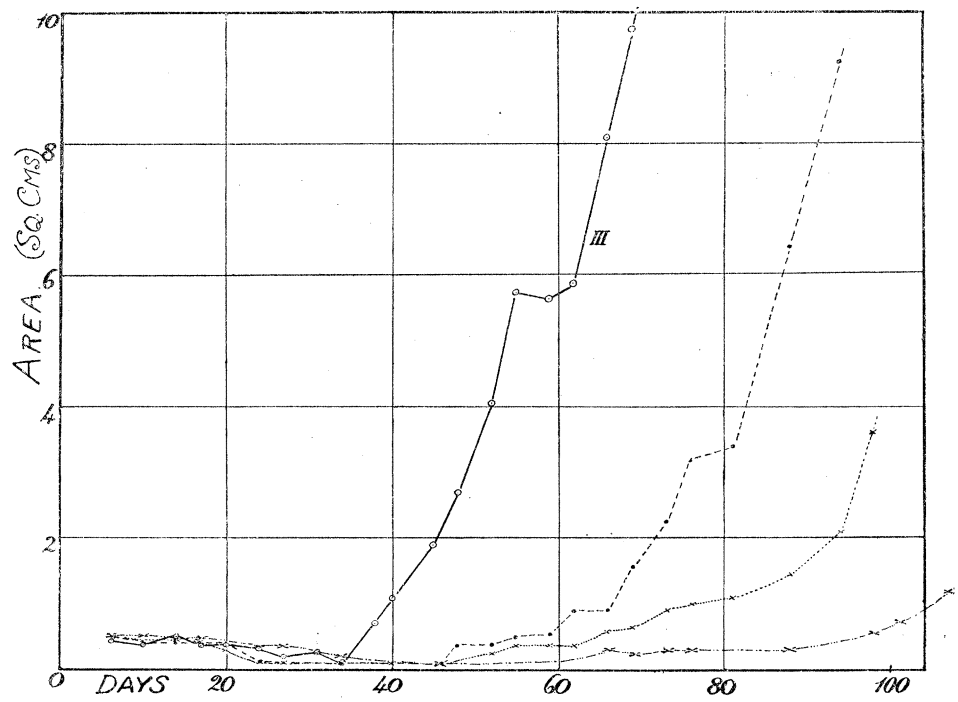


FIG. 3.

corresponds to the single occurrence in Series III and the dotted curves to the three rats of Series II. The nodules in the two remaining rats in Series II disappeared after 24 and 81 days. On re-inoculation with 0·1 c.c. of tumour emulsion the first was refractory and the second yielded a growing tumour.

Of the five remaining animals in Series III one died, the growths in two of the rats disappeared in 30 and 38 days, the animals proving refractory to subsequent inoculation. The nodules in the two remaining were palpable for 116 days, when they were excised and found to consist of fibrous tissue.

To attempt to interpret the course of events illustrated in figs. 2 and 3 two alternatives may be considered.

If it be supposed that all of the irradiated cells suffer some damage dependent upon their time of exposure, then the irradiated series might be expected to show a general quantitative sequence, and this appears to be the case. On these lines the tumour cells appear to overcome the effect of their irradiation after a prolonged period in the animal body.

On the other hand if it be supposed that some cells are unaffected by the rays, the delay in the apparent onset of growth would be proportional to the time of exposure, and would depend on the number of cells left undamaged.

Although the observations do not allow of a decision between the alternatives, they show that the irradiated cells increase at a slower rate than do the controls. The areas of the tumours were found by actual measurement, and if these areas are raised to the three halves power, numbers are obtained which are proportional to the volumes of the tumours. On the simplest assumption of continuous cell proliferation and reckoning from the time when growth has certainly started, it is found that the mean life period (T) of the tumour cells in the animals of the different series vary in the following manner :—

Control cells (mean of 6 animals)	T = 3·4 days.
$\frac{1}{4}$ hour irradiated cells (mean of 6 animals) ...	T = 3·9 „
$\frac{1}{2}$ „ „ „ 3 „ ...	T = 8·3 „
1 „ „ (one animal)	T = 5·6 „

Attempted Re-activation of Irradiated Tissue.

The changes produced in the tumour tissue by the irradiation may be internal or external to the cells, or the cell boundaries may be affected.

If a tumour be minced by a Haaland mincer, as has been done in this work and the emulsion be vigorously centrifugalised, about 1 c.c. of fluid may generally be pipetted off from 20 grm. of tissue. If the failure of the tumour to grow after irradiation be due to changes occurring external to the cells, such changes might possibly be counteracted by taking tissue that had been irradiated sufficiently long to prevent its growth and adding to it fluid obtained from non-irradiated tumour tissue in the manner indicated.

This re-activation test has been put into operation three times, the extent of irradiation having been 1 hour 4 mins., 2 hours 35 mins., and 5 hours

40 mins., to a concentration of 0·37, 0·40, and 0·37 millicuries per cubic centimetre respectively. The technique followed was practically the same in each case.

To some of the irradiated tumour emulsion an equal volume of fluid obtained from non-irradiated tumour tissue was added and after allowing the fluid to permeate the irradiated tissue for about 1 hour, 0·1 c.c. of the emulsion was inoculated into a number of normal rats (*i.e.* 6 or 8).

To another portion of the irradiated tumour emulsion an equal volume of normal saline was added, and 0·1 c.c. of the mixture inoculated into (6 or 8) other rats to serve as controls. At the same time 0·1 c.c. of fluid only was injected into a number of rats. No reaction was detected when fluid only was injected. In no one of the cases was the attempt at re-activation successful to the extent of the ultimate production of a growing tumour; indicating that the changes occurring in the tumour tissue as a result of irradiation cannot be counteracted by the action of non-irradiated tumour fluid, and that the irradiation probably causes some change in the cells themselves.

Charts of the animals show that 17 days subsequent to the inoculation of the irradiated emulsion treated with fluid, 16 animals out of 22 showed palpable nodules, compared with 4 out of 21 of the control animals. This result suggests that normal tumour fluid has some action upon the irradiated cells, which delays their absorption by the animal although ineffective in re-activating them.

Histological Examination.

To study the histological changes which occur in the irradiated material after inoculation, three series of rats (36 in all) were inoculated on one side with 0·1 c.c. tumour emulsion, and on the other side with 0·1 c.c. of the same emulsion which had been exposed to a concentration of about 0·45 millicurie per cubic centimetre for periods of 20 minutes (*a*), 80 minutes (*b*), and 24 hours (*c*). These times of exposure ensure that the grafts will (*a*) be slightly delayed in growth, (*b*) just fail to develop into tumours, and (*c*) show no signs of proliferation, respectively. An animal from each series was killed each day for the first week, and then at intervals until the 22nd day after inoculation, the control and irradiated tumours were excised and sections prepared.

Microscopical examinations of the emulsions, after irradiation and before their inoculation into the rats, failed to establish any differences between them and the non-irradiated portions.

Control Grafts.

The day after inoculation the tissue of the graft is almost entirely necrotic, only those sarcoma cells at the edge look in good condition. There is extensive invasion of the graft with leucocytes and much inflammatory œdema of the surrounding tissue. On the second day active proliferation of the sarcoma cells at the periphery is evident, and they soon form an encircling ring of growth around the graft. Sarcoma cells extend outwards into the connective tissue and also invade the central necrotic area. By the 6th and 7th day the mass is completely solid and has the structure of the fully developed tumour (*vide* microphotograph 2).

Irradiation for 24 *Hours.*

On the day after inoculation the graft, as in the control, is almost entirely necrotic. The inflammatory changes and œdema set up in the surrounding tissue are less marked; there is also less invasion with leucocytes, and the sarcoma cells are all apparently degenerate. On the third day they can still be detected, but they show no signs of proliferation. On the fourth day the sarcoma cells have completely disappeared. The graft now consists of granular structureless material, a few leucocytes and nuclei alone being left (*vide* microphotograph 3). There is commencing vascularisation and fibrous tissue formation at the periphery.

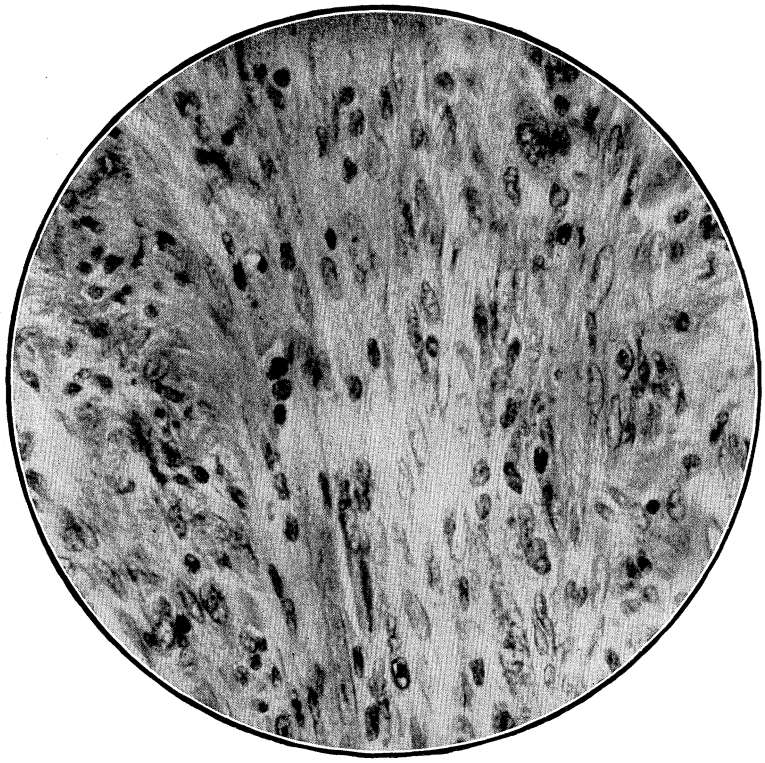
Irradiation for 80 *Minutes.*

On the day after inoculation the reaction of the surrounding tissue to the implanted graft is again less marked than for the controls, and the graft is largely necrotic. Sarcoma cells in good condition can, however, be found at the edge. By the sixth day the graft is almost completely vascularised and many of the sarcoma cells at the edge appear to have proliferated to a slight extent. The condition shows (*vide* microphotograph 4), a distinct contrast with the preceding. At a later stage the graft is largely replaced by fibrous tissue but the sections can be distinguished from those of the 24-hour irradiation series by the presence of a few large sarcoma-like cells embedded in the fibrous tissue.

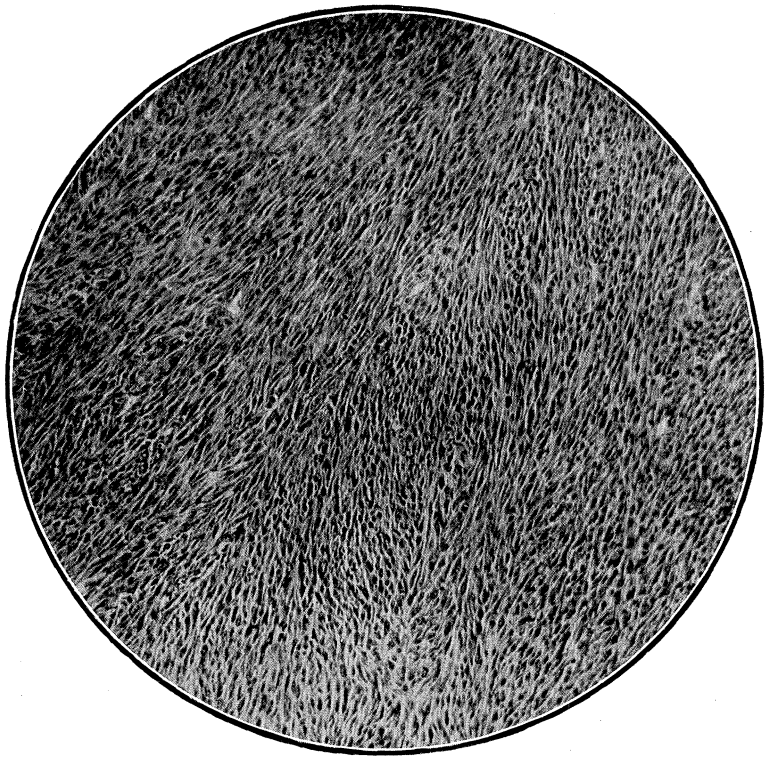
Irradiation for 20 *Minutes.*

Both the experimental and control grafts formed tumours, the former being the smaller.

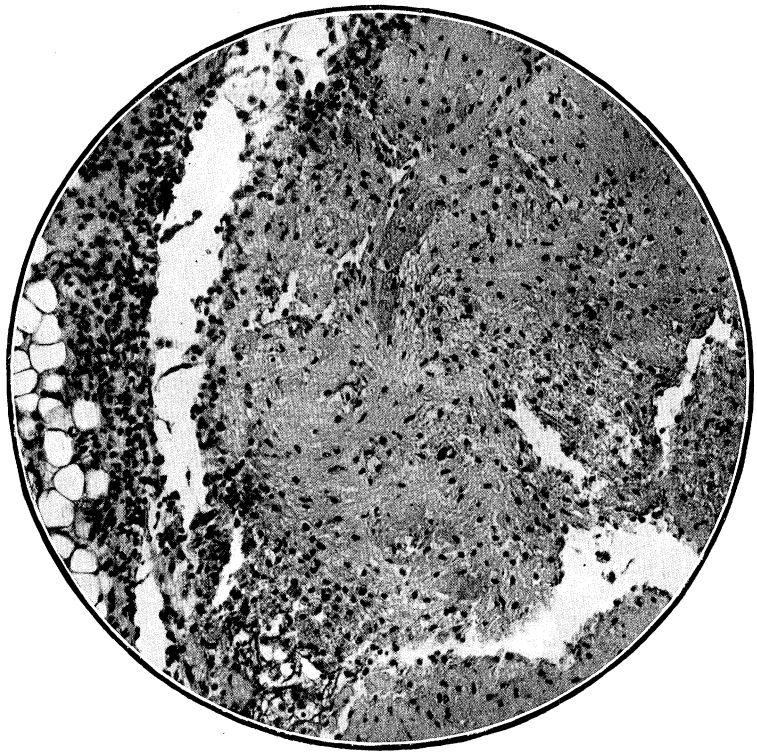
The sections of the experimental grafts are similar to the controls throughout this series, except that in the former the rate of proliferation of the cells is delayed. Moreover 11 days after inoculation the irradiated



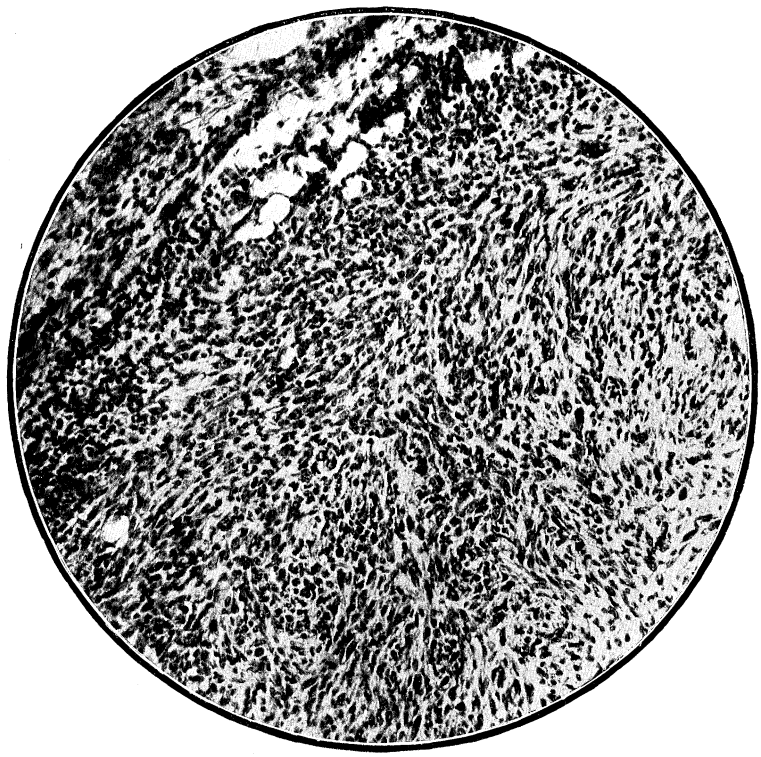
1.



2.



3.



4.

tumour contains numerous very large sarcoma cells, a few of which are multinucleated; they are not found in the actively growing tumours. Cells of this kind have been described by Clunet* and others as occurring in tumours which have been irradiated *in vivo*.

The histological changes indicate that after a long period of irradiation the cells of the growth are killed and are rapidly absorbed. With shorter periods of irradiation, even in cases where no tumour develops, the cells remain at the site of inoculation for a long time, but their capacity for proliferation is diminished. This inability to proliferate is not due, as in immune animals, to failure of the connective tissue to vascularise the graft, but is due to some change in the cells themselves.

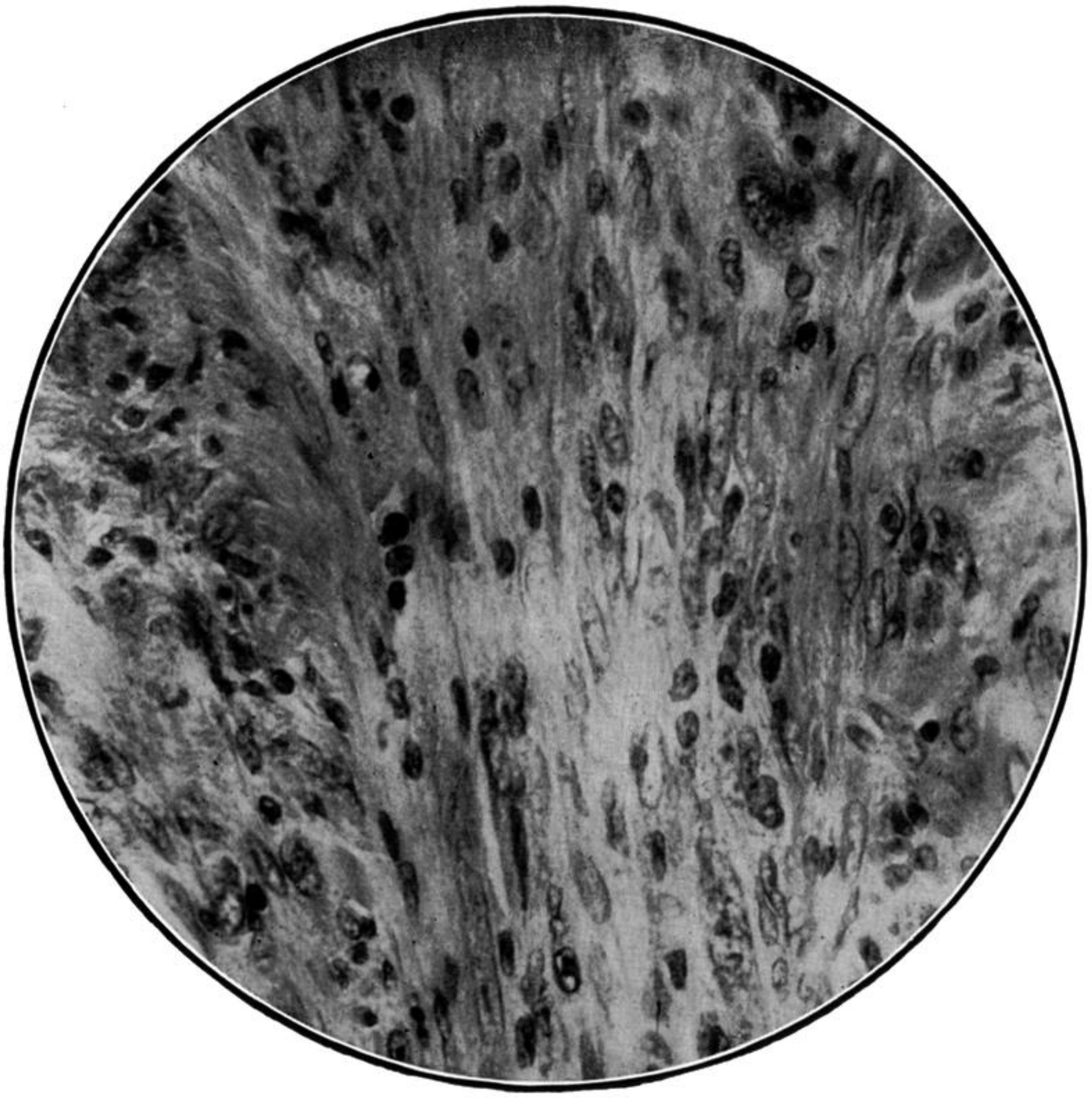
Conclusions.

1. Jensen rat sarcoma when exposed *in vitro* to the β-rays from a source of radium of intensity 1·63 mgrm. per square centimetre for 90 minutes, or to radium emanation of concentration 0·53 millicurie per cubic centimetre for 45 minutes, will not grow upon inoculation into normal rats.

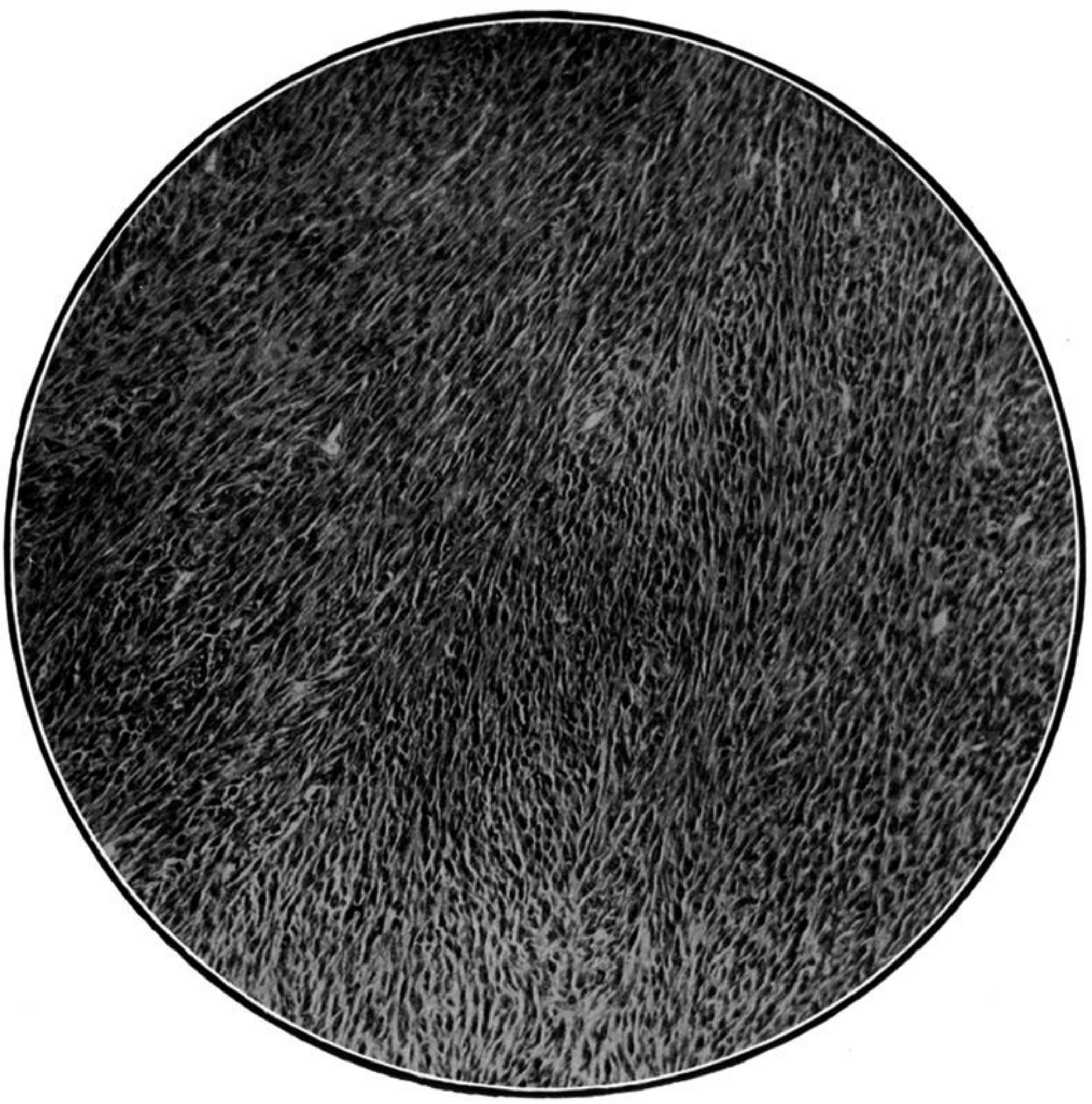
2. The sarcoma cells which have been irradiated may remain in the animal body for more than 60 days before giving evidence of growth.

3. Histological evidence shows that failure of the irradiated sarcoma cells to produce a tumour does not necessarily indicate their destruction at the time of inoculation.

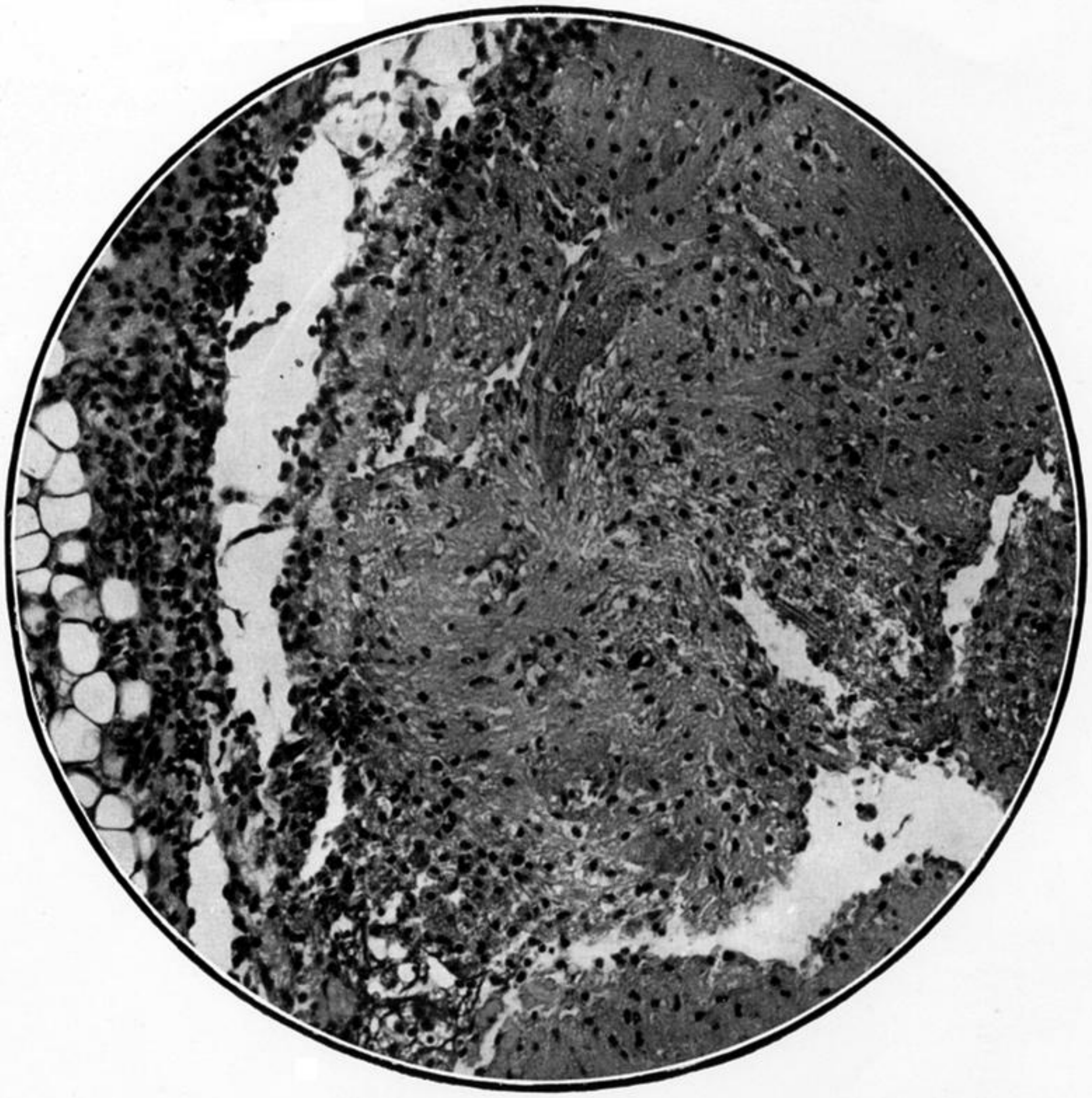
* Clunet, 'Tumeurs Malignes,' 1910.



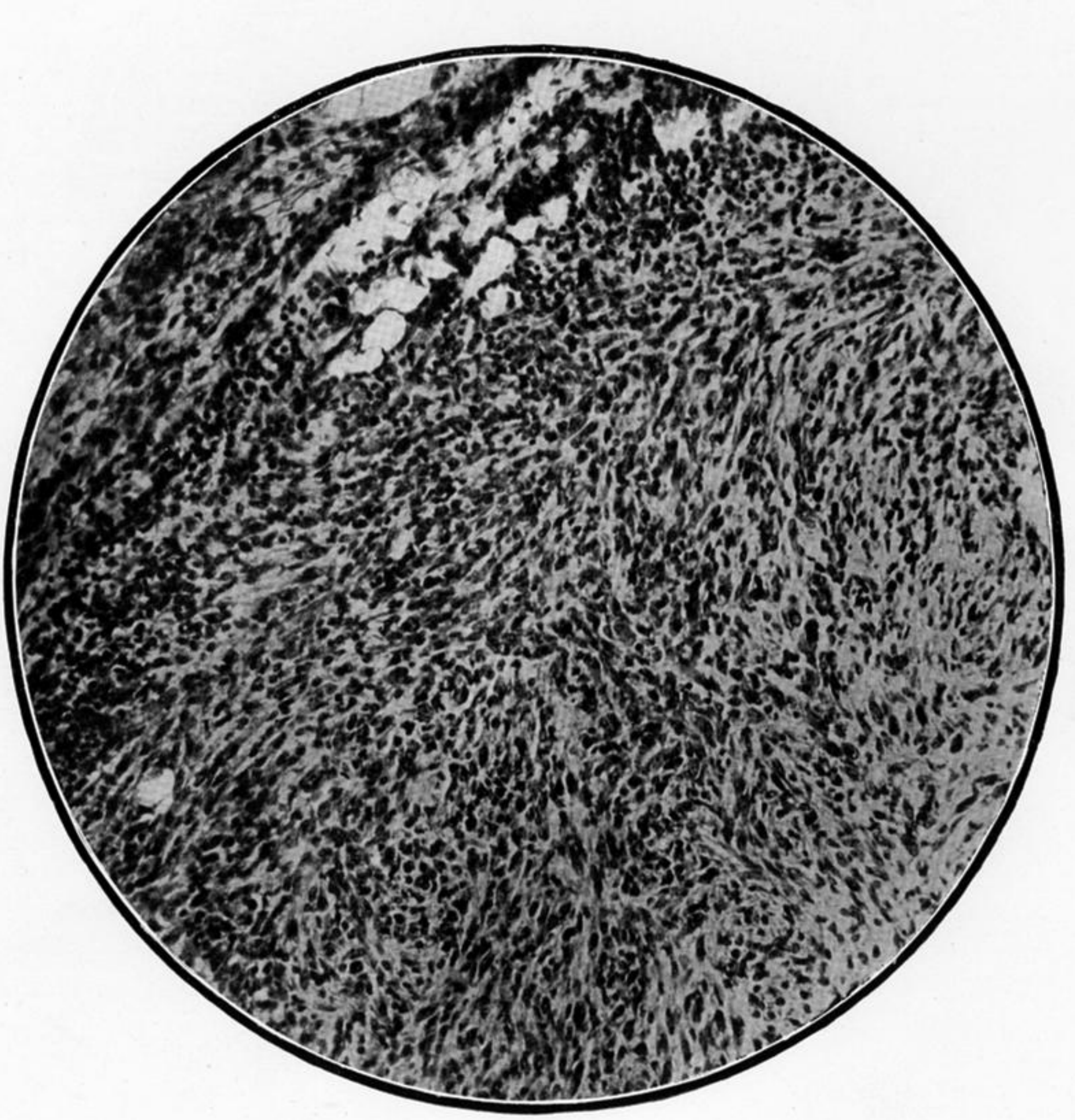
1.



2.



3.



4.