ON THE SURVIVAL AND TRANSPLANTABILITY OF ADULT MAMMALIAN TISSUE IN SIMPLE PLASMA.*

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PLATES 22 TO 26.

This communication deals with the characteristics of the growth of adult mammalian tissues after transference to fresh medium. In all cases the medium was simple plasma, and the tissues were therefore not stimulated by the addition of tissue extract.

In 1911 Carrel[1] published the results of his experiments on the continued growth of thyroid tissue in simple plasma. At that time he stated that he had not obtained a third generation of cells. Later he and his assistant Ebeling published several papers[2] in which they describe growth persisting for over a year, but in the majority of these cases the subcultures were made in a medium of plasma and tissue extract so that growth was stimulated.[3] Even in such cases Carrel informs me that he has noticed a tendency for the cultures of adult tissues cultivated in media which contain no tissue extract to die; and in a later paper[4] he states that even with embryonic tissue the growth after several passages in normal plasma alone becomes progressively reduced and often stops altogether.

It is necessary that the characteristics of the growth of unstimulated adult tissue be considered before any conclusions can be made as to the effects of various substances upon the rate of growth. The present experiments are therefore concerned only with the

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[1] Carrel, A., and Burrows, M. T., *Jour. Exper. Med.*, 1911, xiii, 416.

[2] Carrel, *idem*, 1912, xv, 516. Ebeling, A. H., *idem*, 1913, xvii, 273.

[3] Carrel, *idem*, 1913, xvii, 14.

[4] Carrel, *idem*, 1913, xviii, 287.

growth of tissue taken from the adult rabbit in the unaltered plasma of the same animal.

The technique used in the preparation of the primary cultures is that of Carrel,[5] which I have described fully elsewhere.[6] In preparing the subcultures, or transferences, the piece of growing tissue was cut out of the plasmatic medium with a cataract knife and transferred to a bowl of sterile Ringer's solution, where it was washed for a few seconds. A drop of plasma was placed on a fresh sterile cover-slip, the piece of tissue transferred to it, and the plasma spread evenly with a cataract knife. After a few seconds coagulation of the plasma had taken place and the cover-slip was then inverted over a cell slide and sealed in position with molten paraffin. The preparation was then placed in the incubator. Throughout the experiments the most careful aseptic precautions were used, as there is considerable risk of infection. The various steps including the washing in Ringer's fluid were carried out at room temperature, the plasma alone being kept on ice to prevent coagulation. In most cases the whole piece of tissue was transferred, but if growth was well marked it was divided into two, three, or four pieces, each of which was transferred to the new plasmatic medium.

The total number of cultures made in the present investigation was 147. Of these 115 showed growth in the first culture, the remainder probably having died owing to some technical error, as they were mostly isolated specimens in a series of six made from the same tissue and under identical conditions. The characters of the growth of the subsequent subcultures, which varied with the nature of the tissue used, will be descibed below.

Thyroid.—Of this tissue thirteen cultures were made, being taken in groups of six, three, and four from different animals on different dates. Of these thirteen cultures only nine grew, but these when subcultured continued to grow until the third or fourth generation. The specimens from the first animal grew well until the tenth generation, but after this they died suddenly. This was probably in part due to the fact that the plasma of a hare was accidentally

[5] Carrel, *Jour. Exper. Med.*, 1912, xv, 516.

[6] Walton, A. J., *Jour. Path. and Bacteriol.*, 1914 (in press).

used instead of that of a rabbit. Up to the time of death the cultures had lived for a period of forty-two days.

The tissues of this animal showed after the first transference a marked increase of growth, so that whereas in the first culture growth was present only after an interval of two days, in the second and third subcultures there was usually well marked growth after twenty-four hours. Branching, irregular cells were seen projecting from the edge of the tissue (figure 2), and, if any of the old plasma had been transferred with the tissue, the cells soon passed beyond this into the new medium (figures 1 and 2). Here they rapidly grew and formed an irregular network of cells (figure 3). After a further interval of twenty-four hours the network had become sufficiently dense to form a mass of new tissue resembling the original mass that had been transferred (figure 4). If this mass showed marked increase the tissue was divided before transference. In this way one piece of tissue from this animal was subdivided several times, so that after an interval of twenty-one days there were at the fifth subculture nine pieces of tissue each of the same size as the original piece. At this time growth was such that at the end of the second or third day the ring of new growth was equal in width to the diameter of the original piece of tissue. If a piece of rapidly growing tissue was divided into two it was noticed that the growth was delayed on the cut edge, apparently from the trauma, so that when the cells from the uncut edges were forming a halo of branching cells spreading out into the plasma there were only a few cells projecting from the cut edge. These cells, however, grew rapidly and after the second or third day nearly equalled in width the cells growing from the uncut edge. In the case of the other animals, where growth of the thyroid was not prolonged beyond the third or fourth generation, the rapidity of growth decreased markedly after the first transference. In the first subculture growth was more rapid than in the primary culture, as in the case described above. With the second subculture growth occurred more slowly, being no more rapid than in the case of the first culture, or perhaps even less rapid. At the third transference the cells grew slowly, and if a culture was left for five or six days without transference they became rounded instead of branching, and granular in appearance. In a

short time vacuolation of the plasma occurred and granular remains of cells were found floating free in the liquid of the vacuole. If, however, transference was made on the second day a few cells of short life might occasionally be seen.

The difference in the rate and extent of the growth of the tissue of the first and subsequent animals was possibly due to difference in the age of the rabbits used. As the animals were purchased and not bred in the laboratory, it was impossible to estimate their age accurately, but it was noticed that the first animal used in this series was small and apparently young. This question will, however, be considered in a later communication.

Spleen.—Of this tissue sixty-eight cultures were made from twelve different animals, being taken in ten groups of six each, two groups of three and one of two. In all cases growth was present in the first culture, the growing cells being mainly round and wandering cells. Transference was made at periods varying from three to six days; if left later than this the percentage of growths was definitely decreased. After the first transference thirty-four only showed signs of growth, that is, 50 per cent. Of these all showed marked increase in the rate of growth and a very definite change in the nature of the cells. Thus after twenty-four hours a large number of round cells were seen, but in addition to these a few spindle cells were seen projecting from the edge of the tissue. These increased so that by the third or fourth day the tissue was surrounded by a ring of radiating cells as wide as the piece of tissue (figure 5). The cells appear to be of the connective tissue type. The pieces of tissue were again transferred on the second or third day, and in five the growth was so extensive that the piece of tissue was divided into two parts. Of the forty-three resulting cultures of the third generation eighteen alone showed growth. In these the growth was almost wholly of long radiating cells of spindle shape and apparently connective tissue. The growth, however, was not nearly so extensive as in the second generation, and in several, at the end of two or three days, only a few radiating cells were seen passing out into the new medium. In many of these cells globules resembling fat in appearance were seen, and after a short time the cells began to degenerate. The protoplasm became irregular and ill defined, so

that on staining it was not sharply differentiated from the surrounding plasma. The nuclei stained poorly and were also not sharply defined. In the fourth generation only four pieces of tissue showed evidence of growth, and in all cases this was very slight. A few growing cells could be seen on the first or even on the second day, but after this they rapidly disappeared, being converted into a granular mass.

Testicle.—Of this tissue eighteen cultures were made, being taken in groups of six from three different animals. Growth occurred in fourteen in the first culture. The growth was well marked and showed the usual characters of testicular growth. On the third day growth was sufficiently far advanced for subculture. In the second generation the cells grew well and as usual more rapidly and extensively than in the first culture. The cells were of the spindle type, but more closely resembled those of the first culture than in the case of the spleen. Although these cells are spindle cells and are possibly connective tissue, yet they contain more protoplasm and are less elongated than the cells occurring in subcultures of the spleen, so that it is generally possible even in the third and fourth generations to distinguish one tissue from another by the appearance and arrangement of the cells, although in no case is there any reproduction of the normal histological picture of that viscus. For the third culture growth had become so marked that seven pieces were divided into two, resulting in sixteen cultures of the third generation. Of these only eight grew, but they showed extensive and rapid outgrowth of cells (figures 6 to 9). The onset of growth was, however, not so rapid as in the second generation, there being only a small halo of cells after forty-eight hours. After this growth was rapid, and in a further twenty-four hours long projecting masses of cells were seen passing into the surrounding plasma. By the fourth day the cells formed a ring wider than the original piece of tissue (figure 9). Four of the specimens were killed and stained for photographs, and the other four were subcultured. Only one of the latter showed growth, and that to a slight extent, so that after two days a few cells were seen projecting from the edge. These had slightly increased on the third day, but on the fourth the outlines were ill defined and the protoplasm was granular,

while a brown granular deposit had commenced to form in the plasma around the cells.

Kidney.—Of this tissue thirty-two cultures were made from five different animals, being taken in three groups of six and two groups of eight. Of these only fifteen showed growth in the first culture. As described in a previous communication, the growing cells were almost wholly epithelial in type, forming large plate-like masses of cuboidal cells. Transference was carried out on the third to the fifth day, according to the extent of the growth in the first culture. In the second generation eleven showed growth. This was more rapid than in the first culture and the cells preserved the characteristics of the first culture, being mainly of the epithelial type. The presence of cells of the connective tissue type was, however, rather more marked than in the first culture. There was again a tendency to early vacuolation of the plasma (figure 10). Many of the cells showed evidence of mitosis in the nuclei (figures 11 and 12), but owing to the difficulties of staining in bulk the figures were not as clear as one would desire, although they were more marked in this type of cell than in the spindle-celled connective tissue type. The eleven growing tissues were subcultured, but growth took place only in three, in all of which it was slow and more definitely of the connective tissue type than in the second generation. In no case was growth obtained in the fourth generation.

Liver.—Of this tissue fourteen cultures were made from two animals in groups of eight and six. In nine of these there was active growth. This commenced on the second day and transference was carried out on the third day. In the second generation evidence of commencing growth was seen in three specimens after twenty-four hours. In the other six there was no evidence of growth. Although in the first generation there were a large number of cells which were more or less rounded and which contained a relatively large amount of protoplasm, yet in the second generation the cells were all radiating and of the connective tissue type. This was visible even on the second day (figure 13). The cells grew well and rapidly spread out into the surrounding plasma, so that transference was carried out on the third day. In one case

only in the three specimens of the third generation was any growth observed, and this was only slight. A few radiating cells were seen on the second day, but after a short time these became granular and a brown deposit occurred in the surrounding plasma. The outline of the cells became indefinite and difficult to distinguish from the surrounding plasma, the plasma became vacuolated, the granular deposit increased, and soon all signs of growth ceased.

CONCLUSIONS.

1. Growth of adult mammalian tissue can be prolonged by transference to fresh medium.

2. In a few cases this growth can be continued for ten or eleven generations up to a period of forty days.

3. In the majority of cases growth ceases after three or four generations.

4. After the first transference growth is increased, but in subsequent generations it gradually diminishes and ultimately ceases altogether.

EXPLANATION OF PLATES.

PLATE 22.

Tissue = adult rabbit thyroid; medium = rabbit plasma; culture = fifth subculture.

FIG. 1. Standard. Fixed immediately after coagulation. No growth. 35/1.

FIG. 2. After forty-eight hours. Well marked commencing growth. 35/1.

FIG. 3. After three days. Cells commencing to form a network. 35/1.

FIG. 4. After four days. Well marked network of cells. 35/1.

PLATE 23.

Tissue = adult rabbit spleen; medium = autogenous rabbit plasma; culture = second.

FIG. 5. Fourth day. Marked growth of radiating spindle cells. 35/1.

PLATE 24.

FIG. 6. Tissue = adult rabbit testicle; medium = autogenous rabbit plasma; culture = third. Standard. Fixed one quarter of an hour after coagulation. 35/1.

FIG. 7. After forty-eight hours. 35/1.

FIG. 8. After three days. 35/1.

FIG. 9. After four days. 35/1.

PLATE 25.

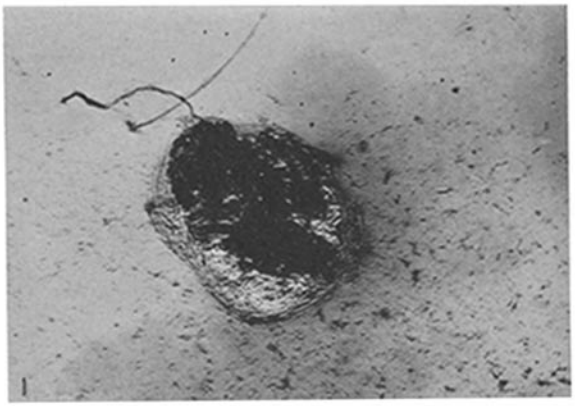
FIG. 10. Tissue = adult rabbit kidney; medium = rabbit plasma; culture = second. After two days. Growth of masses of epithelial cells. Vacuolation of plasma. No growth in vacuole. 35/1.

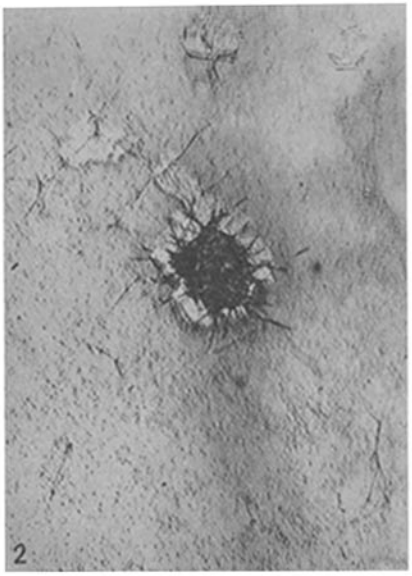
FIG. 11. Same specimen as figure 10. Enlarged to show the appearance of the cells.

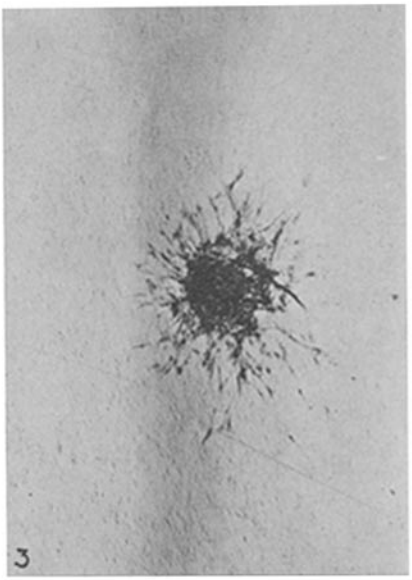
PLATE 26.

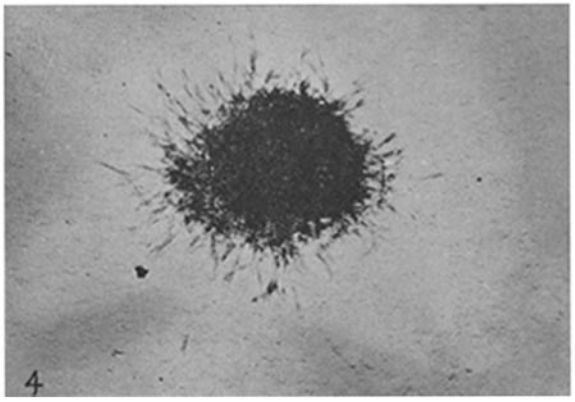
FIG. 12. Tissue = adult rabbit kidney; medium = rabbit plasma; culture = second. Same specimen as figure 10. Enlargement of the growing edge, showing various stages of mitosis in the nuclei.

FIG. 13. Tissue = adult rabbit liver; medium = rabbit plasma; culture = second. After forty-eight hours. Growth of radiating spindle cells.

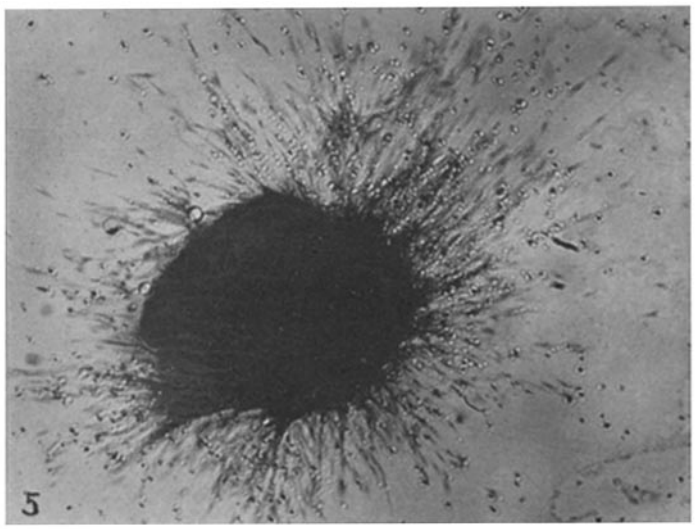








(Walton: Adult Mammalian Tissue in Simple Plasma.)



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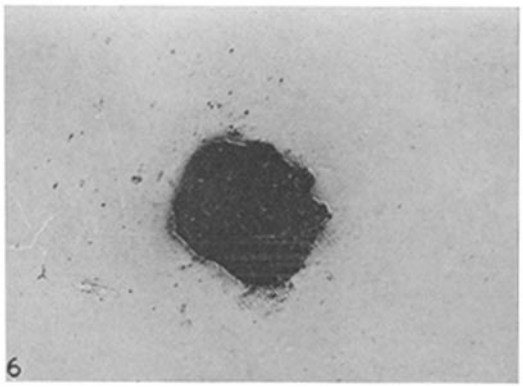

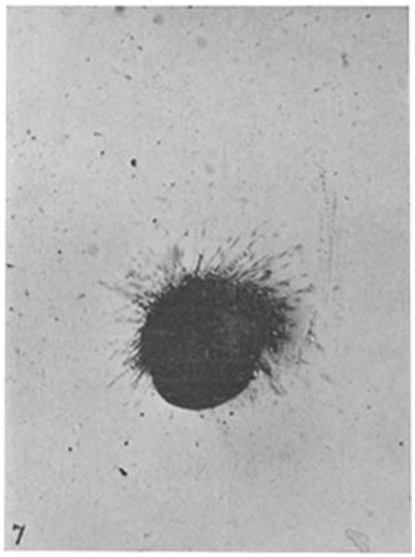

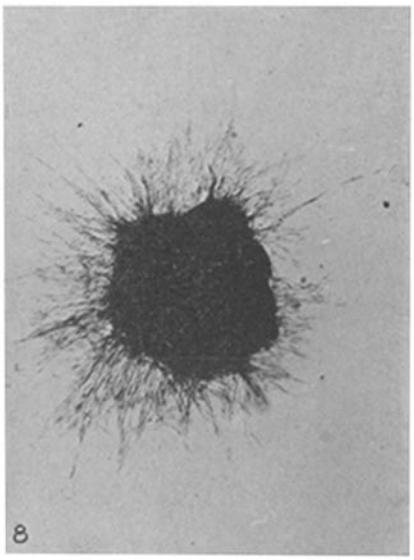

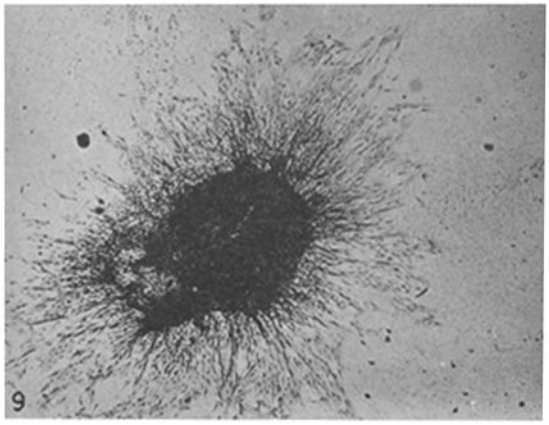

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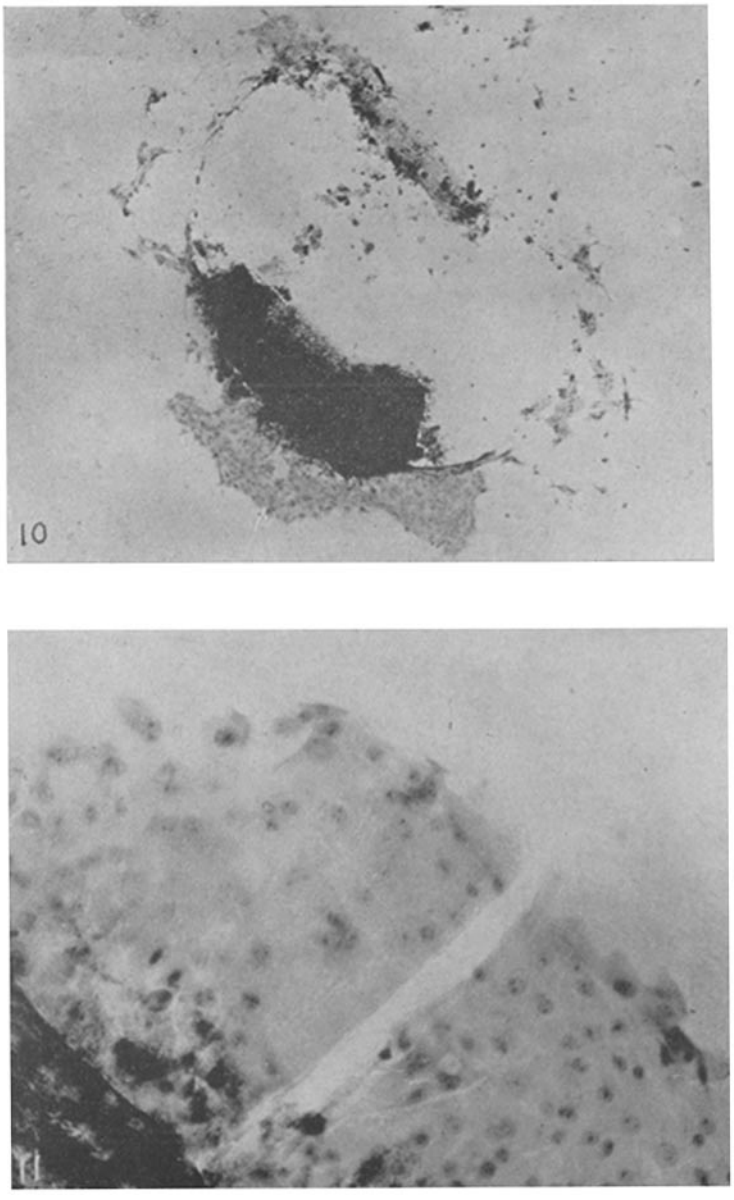

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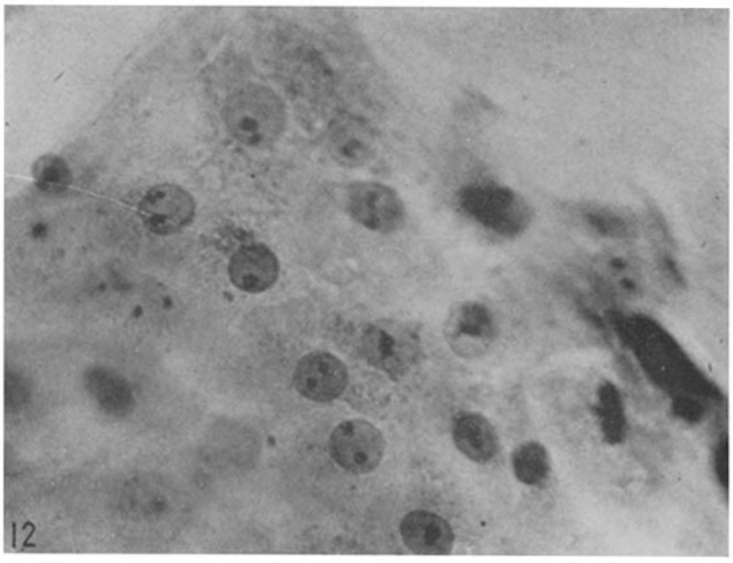

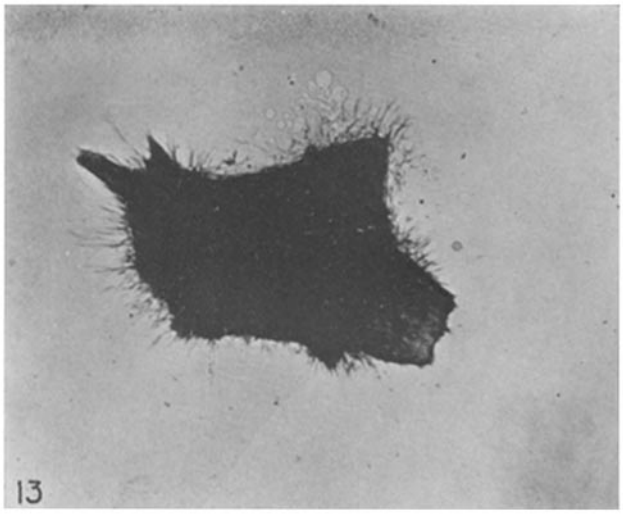

(Walton: Adult Mammalian Tissue in Simple Plasma.)