Jasmin (yellow).—Gave anthocyanin only, more readily by means of zinc and 2N HCl than by electrolysis.

Primrose (yellow).—Easily produced anthocyanin by either method, even in fairly warm HCl with zinc only anthocyanin was produced. The glucoside produced in this case seemed to be more stable to hydrolysis than in the majority of cases.

Lemon Peel.—Extract in HCl, in presence of the peel, reduced with zinc gave only anthocyanin.

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*Variations in the Growth of Adult Mammalian Tissue in Autogenous and Homogenous Plasma.**

By Albert J. Walton, M.S., F.R.C.S., B.Sc.

(Communicated by Prof. W. Bulloch, F.R.S. Received February 18,—Read March 26, 1914.)

(From the Bacteriological Laboratory of the London Hospital.)

[Plates 19 and 20.]

In 1910 Carrel commenced his researches on the growth of tissues outside the body. In 1907 Harrison had succeeded in growing the embryonic tissues of the frog, using coagulable lymph as a medium. In 1910 Harrison and Burrows improved this method and successfully cultivated the tissues of mammalian embryos. Carrel has so modified the technique that the method is now applicable to the study of the growth of all mammalian tissues. He used as a medium the plasma of the animal either in its natural state or modified by the addition of various substances. Since then, he and his collaborators have published a large number of papers, and by their work it has been fully established that tissues of animals, whether embryonic or adult, grow well *in vitro*; that by changing the medium and so removing the catabolic substances life can be greatly prolonged—tissues have been kept alive and growing for periods considerably longer than a year; and that the growth of the tissues can be greatly modified by the addition of various substances to, or otherwise altering the composition of, the plasmatic medium.

* Throughout this paper the term "autogenous" is used to indicate plasma obtained from the same animal as the tissue, "homogenous" to indicate that obtained from another animal of the same species.

In previous communications I have described fully the characters of the growth of adult mammalian tissue in primary and in sub-cultures. Having determined the nature of this growth, it became possible for me to investigate the variation, if any, of the growth in autogenous and homogenous plasma. The results of this investigation are detailed in this paper.

The tissues of adult rabbits were used and the technique of Carrel was carefully adhered to. Four hundred and thirty-three cultures were made, the testicle, thyroid, and kidney being the tissues mainly used. A few experiments were made with tissues of the spleen but owing to the amount of emigration of cells, apart from true growth, which occurs with this tissue it was found difficult to make accurate comparative experiments. These experiments were therefore discontinued.

The details of the experiments on the testicular tissue and the results will be fully described. The other tissues will be considered more briefly as the experiments on them were carried out on the same lines.

Testicle.

One hundred and twenty-two cultures were made with testicular tissue. It was found that the plasma of the rabbit kept in ice would not continue fluid for longer than about an hour. After this time it coagulated and became useless. To overcome this difficulty the plasma was placed in a sterile tube which was corked and sealed with paraffin, and the tube was immediately placed in a mixture of salt and ice contained in a thermos flask. By changing the mixture of salt and ice every two or three days the plasma could be kept frozen hard for an indefinite time. When required for use it was removed from the mixture and at room temperature soon became fluid.

Experiment 1.—Plasma was removed from Rabbit A six days previous to the cultural experiment and kept frozen. At the time of the cultural experiment blood was collected from the carotid artery of Rabbit B and the plasma separated. Cultures of testicle of Rabbit B were made in the usual way in both plasmata. Twelve cultures were made in the homogenous plasma and twelve in the autogenous. Of those in the homogenous, good growth occurred in all, whilst of those in the autogenous plasma only eight grew and growth was less extensive in these.

Experiment 2.—Cultures of testicle were made in homogenous plasma which had been kept frozen for three days and in fresh autogenous plasma. In both the same testicular tissue was planted and the cultures were made at the same time and under identical conditions. The cultures in homogenous plasma again gave 100 per cent. of positive results whilst those in autogenous

plasma gave only 75 per cent. positive. The growth was again more extensive in the homogenous plasma.

Although the above experiments seemed to show that growth was better in homogenous than in autogenous plasma, it was possible that in both cases the autogenous plasma happened to be what one may describe as a bad variety. To solve this question a third experiment was performed three days later than the second experiment. The same two plasmata which were used in the second experiment had been preserved frozen and were used again. They were now both homogenous to the culture tissue and were respectively six and three days old.

Experiment 3.—The two frozen plasmata described above were used, one of which was six days and the other three days old. Cultures of testicle were made in these and in fresh autogenous plasma under similar conditions. The results were somewhat similar to those of the last two experiments. Growth was much more extensive in the homogenous than in the autogenous plasma, thus at the end of three days there was but slight growth of the tissue in the autogenous plasma and marked growth in the three-day-old homogenous plasma, 100 per cent. of the pieces growing. In the case of the six-day-old homogenous plasma it was seen that the growth was more extensive than in the autogenous plasma but less than in the three-day-old homogenous plasma, only 70 per cent. of the pieces growing. Sub-cultures were made from each set and it was again seen that after a period of three days growth was much more extensive in the homogenous than in the autogenous plasma. In the homogenous plasma mitotic figures were very abundant and very well marked.

This experiment showed that growth was not better owing to the accidental choice of good homogenous plasmata, for the plasma which in Experiment 2 was autogenous and gave but poor growth when used in Experiment 3, where it was homogenous, gave a good growth in 100 per cent. of the trials. Another very interesting fact became apparent. The homogenous plasma used in Experiment 2 when three days old was successful in 100 per cent. of the trials, but when used in Experiment 3, that is when six days old, gave less growth, and even this appeared in only 70 per cent. of the tissues. These results are shown tabulated on p. 455.

The facts suggested that the variations in growth might be dependent upon the length of time that the plasma had been kept frozen, and further experiments were therefore carried out to elucidate this point. No more sub-cultures were made, however, for it was evident that if the same plasma were used it would not be of the same age and therefore further variants would be introduced.

	Animal A.	Animal B.
Plasma 1	Homog., 3 days old. Good, 100 per cent.	Homog., 6 days old. Medium, 70 per cent.
Plasma 2	Autog., fresh. Slight, 75 per cent.	Homog., 3 days old. Good, 100 per cent.
Plasma 3	—	Autog., fresh. Fair, 60 per cent.

Experiment 4.—Plasmata were removed from two other animals and kept frozen, one eleven and the other eight days before the cultural experiment. Just before this experiment was commenced blood was removed from the lateral ear vein of another animal and the plasma separated. Cultures were then made under identical conditions in the four plasmata, viz.: homogenous eleven days old, homogenous eight days old, homogenous fresh, and autogenous fresh. In the first two groups every piece of tissue died and there was no evidence of growth. The tissues in fresh plasma grew in the usual way and to an equal extent. The growth in the autogenous plasma was perhaps a little more extensive than that in the homogenous plasma (Plate 20, figs. 5 and 6).

These experiments showed that testicle grew better in homogenous plasma that had been kept frozen for three days, but not at all in plasma that had been frozen for more than six to eight days. The question as to whether growth was better in autogenous or homogenous plasma was still undecided. The following experiment was therefore devised to settle this point.

Experiment 5.—Two rabbits were taken. Blood was removed by puncture from the lateral ear vein of each, ten and three days before the cultural experiment, the plasma being separated and frozen. At the time of the experiment blood was removed from the carotid artery of each and the testicle taken out. Thus there were obtained from each animal three plasmata, one which had been frozen for ten days, one for three days, and one fresh, that is six in all. The testicle of each animal was cultivated in all the plasmata, making twelve separate groups. The cultures were fixed at the end of 48 hours and stained so that the early growth-characters might be seen, these being considered more capable of comparison than the later stages when the growth was well advanced.

In the case of the testicle taken from animal A there was no trace of growth in the ten-day-old plasma, whether taken from animal A or animal B. With the three-day-old plasma that from animal A, autogenous, showed well marked growth, but that from animal B, homogenous, showed very slight growth and marked vacuolation of the plasma. With the fresh plasma there was a fair amount of growth in both series, but whereas

that in the autogenous plasma (fig. 4) was considerably less than that in the three-day-old plasma, that in the homogenous was greater than that in the three-day-old homogenous plasma and rather less than that in the fresh autogenous. In the case of the testicle taken from animal B, there was again no trace of growth in the plasma from either animal which had been frozen for ten days (fig. 3), but in the three-day-old plasma there was marked growth in the plasma from animal A, which in this case was homogenous (fig. 2), and little or no growth in the plasma taken from animal B, which in this case was autogenous. With the fresh plasma there was growth in both series, but that in plasma A, homogenous, was more marked (fig. 1) than that in plasma B but was much less than that in the three-day-old homogenous plasma. These results are shown in the following table :—

	Animal A.		Animal B.	
	Plasma A. Autogenous.	Plasma B. Homogenous.	Plasma A. Homogenous.	Plasma B. Autogenous.
10 days	0	0	0	0
3 days	Very good	Slight	Very good	Slight
Fresh	Good	Fair	Good	Fair

The above experiments showed that, as regards the testicle, growth was not dependent upon any variation in the nature of the cells, for growth was equally good in the series whichever testicle was taken, but it varied directly with the plasmatic medium which was used. The variations in the plasma were not specific to either autogenous or homogenous tissues, for in the experiments given above tissues from both animals grew in the one plasma whether it was autogenous or homogenous, whereas in the other plasma they grew badly in either case. Some plasmata give good growth and others but little, but at present there is not sufficient evidence to show upon what these differences depend.

The fact that growth was always better in plasma that had been frozen for a certain time, whereas, if kept frozen for a longer period, growth entirely ceased, seemed to show that each plasma contains two substances, one of which inhibits growth and the other which stimulates it. By exposure to freezing for two or three days we may suppose the inhibitory substance is destroyed so that growth is increased. After a longer period, about eight days, the stimulating substance is also destroyed and hence there is no growth. Under normal conditions the stimulating substance is in excess

of the inhibitory substance, therefore a certain amount of growth takes place in fresh plasma. In plasmata which are not "good" only a small amount of growth takes place when the plasma has been frozen for three days. This is not so easy to understand; it may be that the stimulating substance is present in a less marked degree, and is therefore all destroyed at an earlier date, so that after the plasma has been frozen for three days there will be little or none present, hence growth will be slight or absent. It was noticed, however, in the cases above where growth was slight that coagulation of the plasma had been incomplete; in some cases, indeed, the plasma had remained quite liquid, so that there was risk of the tissue washing off the slide. It is possible therefore that failure to grow under such circumstances was due to mechanical factors, the plasma failing to form a scaffolding for the growing cells. It is of interest to note that the plasma which failed to coagulate was not serum, for there was no clot present when the frozen material was thawed.

Thyroid.

Of this tissue 167 cultures were made, the experiments being carried out on similar lines to those described for the testicle, but a larger number of cultures were made, so that the plasmata were compared at shorter intervals of time.

Experiment 6.—Homogenous plasma was removed one day previous to the cultural experiment and frozen. Autogenous plasma was removed from the animal at the time of the experiment and cultures of thyroid tissue made in each plasma under identical conditions. Growth was more marked in the homogenous plasma and a greater number of cultures were positive in this.

Experiment 7.—Thyroid tissue was cultivated in eight days' old homogenous plasma and in fresh autogenous plasma. There was no growth in the homogenous plasma, whereas in the fresh autogenous plasma 42 per cent. of the cultures grew and the amount of growth was well marked.

Thus, as in the case of the testicle, growth is better in plasma that has been preserved for one day, but entirely ceases in plasma which has been frozen for eight days.

In the next series the same plasmata were used for several experiments, as in the case of the testicle, so that any given plasma which was autogenous in one experiment became homogenous in the next, and had been kept frozen for periods of time which increased for each successive experiment.

Experiment 8.—Thyroid tissue was cultivated in fresh autogenous plasma and in the plasma used in Experiment 7, which was now five days old.

The homogenous plasma gave 100 per cent. positive results and growth was very well marked in it. Only 13 per cent. grew in the autogenous plasma and growth in it was slight.

Experiment 9.—Thyroid tissue was cultivated—

(1) In plasma taken from animal 7, now nine days old.

(2) In the plasma taken from animal 8, now four days old.

(3) In fresh autogenous plasma.

In the nine-day plasma 60 per cent. of the cultures grew, the growth being fairly extensive.

In the four-day plasma 67 per cent. grew, and the growth was very well marked.

In the fresh autogenous plasma 44 per cent. of the tissues grew, and growth in these was less extensive than in the other groups.

Experiment 10.—Thyroid tissue was cultivated—

(1) In homogenous plasma taken from animal 7, now twelve days old.

(2) In that taken from animal 8, now seven days old.

(3) In that taken from animal 9, now three days old.

(4) In fresh autogenous plasma.

In the plasmata from animals 7 and 8 there was no growth at all. In the plasma from animal 9 100 per cent. of the pieces grew and the growth of these was very well marked. In the fresh autogenous plasma 44 per cent. grew. The growth of these was much less marked than that of those grown in the three-day homogenous plasma.

These results are shown in the following table:—

Plasma.	Animal 7.	Animal 8.	Animal 9.	Animal 10.
7	Fresh 42 per cent.	5 days, 100 per cent.	9 days. 60 per cent.	12 days, 0
8	—	Fresh, 13 per cent.	4 days, 67 per cent.	7 days, 0
9	—	—	Fresh, 44 per cent.	3 days, 100 per cent.
10	—	—	—	Fresh, 44 per cent.

It was clear that the increase in the amount of growth which took place when the plasma had been kept frozen for about three days was very marked, thus while autogenous plasma when fresh gave a growth in from 13 per cent. to 40 per cent. of cases, it gave a growth in 100 per cent. of the trials when it had been kept for three days and was homogenous. The fact that some plasmata are good and others bad is also clearly shown by the table. For instance, the plasma of animal 8 is definitely not so good as that of animals

7 and 9. The results obtained in Experiment 9 are specially of interest, for with plasma nine days old growth was obtained. This plasma coagulated well but only gave 60 per cent. of positive results as compared with 100 per cent. obtained with the same plasma when it was only five days old. This would seem to show that the diminution of growth which occurred after the plasma had been kept for a certain period was not entirely due to the lack of power of coagulation, a lack which was considered the possible cause of failure in the case of the testicular tissue. Further experiments were carried out to show whether the increase of growth described above was due to the homogenity of the plasma.

Experiment 11.—Thyroid tissue was cultivated in fresh autogenous and homogenous plasmata. Cultures were also made in plasmata eleven and eight days old respectively. As usual no growth took place in the last two groups. In the fresh autogenous plasma growth occurred in 60 per cent. of the cultures, whilst in the fresh homogenous plasma it was present in 40 per cent. and was rather less marked.

The above results were confirmed by cross experiments carried out in the same way as Experiment 5 was conducted in the case of the testicular tissue.

Experiment 12.—Blood was removed from the lateral ear veins of two rabbits, eight and three days before the experiment. Fresh blood was removed at the time of the experiment and the thyroids were taken out from the two animals at the same time. Cultures were made from each thyroid in all six plasmata. In the eight-day plasmata all four groups showed no growth. The thyroid tissue of animal A showed positive results in 100 per cent. of the trials in the three-day-old autogenous plasma, but no growth at all in the plasma of animal B. With the fresh plasma again there was slight growth in 55 per cent. of the cultures in the autogenous plasma of animal A, and no growth in that of animal B. In the case of the thyroid of animal B there was good growth in 100 per cent. of the trials in the three-day-old plasma of animal A, which in this case was homogenous, and no growth in that of animal B. With the fresh plasma there was fair growth in the plasma of animal A in 64 per cent. of the cultures and none in the plasma of animal B. These results are shown in the table on p. 460.

Thus this experiment confirmed what was found in the case of the testicle, namely, that growth was not dependent upon any quality of the cells or upon the fact that the plasma was homogenous or autogenous, but one plasma was bad so that neither tissue would grow in it, whilst the other was good and gave good results.

	Animal A.		Animal B.	
	Plasma A. Autogenous.	Plasma B. Homogenous.	Plasma A. Autogenous.	Plasma B. Homogenous.
8 days	0	0	0	0
3 days	100 per cent.	0	100 per cent.	0
Fresh	50 „	0	64 „	0

Kidney.

Of this tissue 96 cultures were made. The experiments were all carried out by the method of cross growth, which made all the requisite points clear. One such experiment may be quoted as an example.

Experiment 13.—Plasmata were collected in a manner similar to that used in Experiments 5 and 12. The plasmata were respectively eight days old, three days old, and fresh. In the eight-day-old plasma, as usual, no growth took place. In the case of the three-day-old plasmata the kidney of animal A grew well in the plasma of animal B but not at all in the plasma of animal A. The kidney of animal B also grew well in the plasma of animal B and not at all in the plasma of animal A. With the fresh plasmata growth occurred in the case of both tissues in both plasmata, that in plasma B being rather the better. Growth was in all cases less than that in the three-day-old plasma of animal B.

The results of the kidney cultures supported therefore those obtained with testicle and thyroid.

Summary.

1. The extent of growth of tissues *in vitro* is not dependent upon any quality of the cells themselves.

2. The extent of growth varies directly with the character of the plasma.

3. The variation in the plasma does not depend upon whether it is autogenous or homogenous but upon some cause at present unknown.

4. Fresh plasmata appear to contain substances, inhibitory and stimulating, to the growth of cells, the latter being in excess.

5. The inhibitory substance is lessened, or the stimulating substance is increased, by freezing the plasma for one to three days.

6. The stimulating substance is destroyed after the plasma has been frozen for six to eight days.

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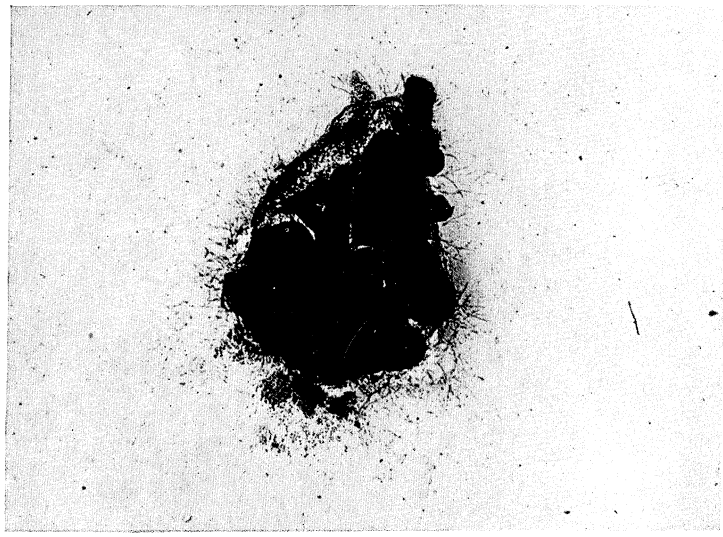

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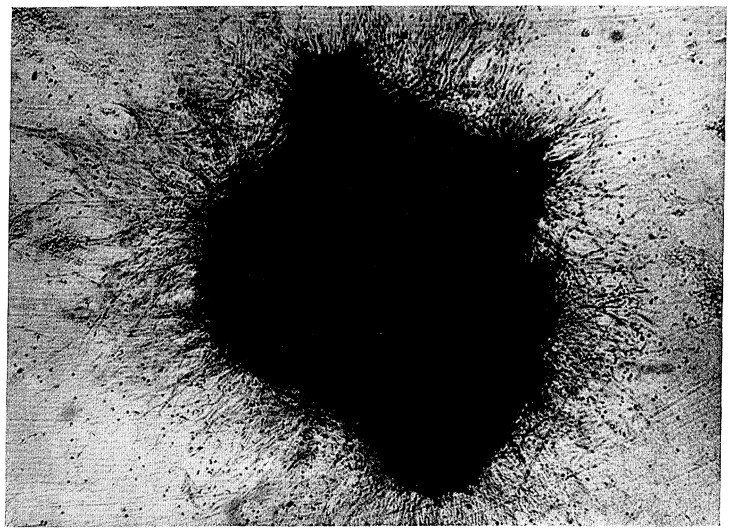

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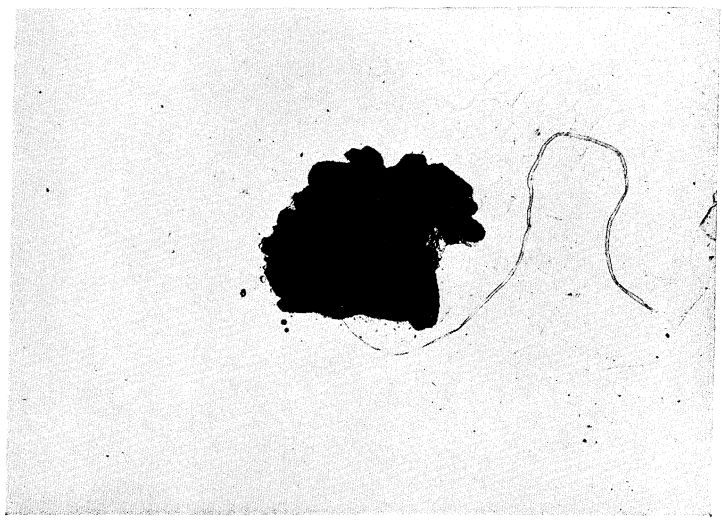

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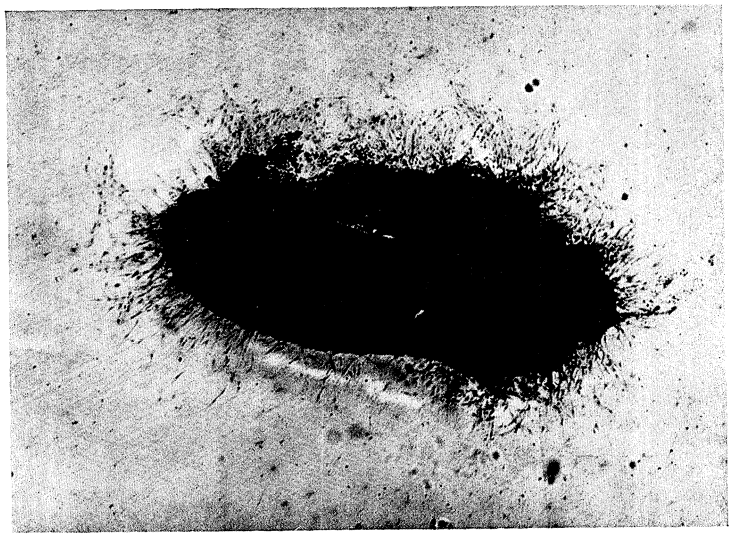

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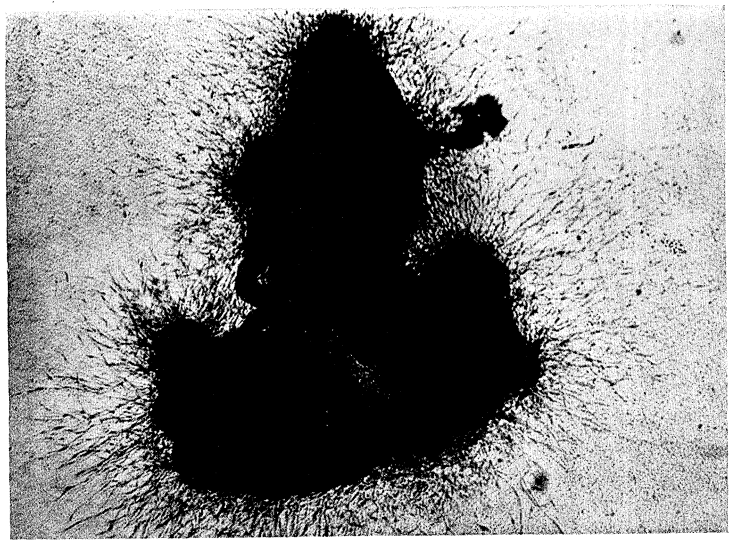

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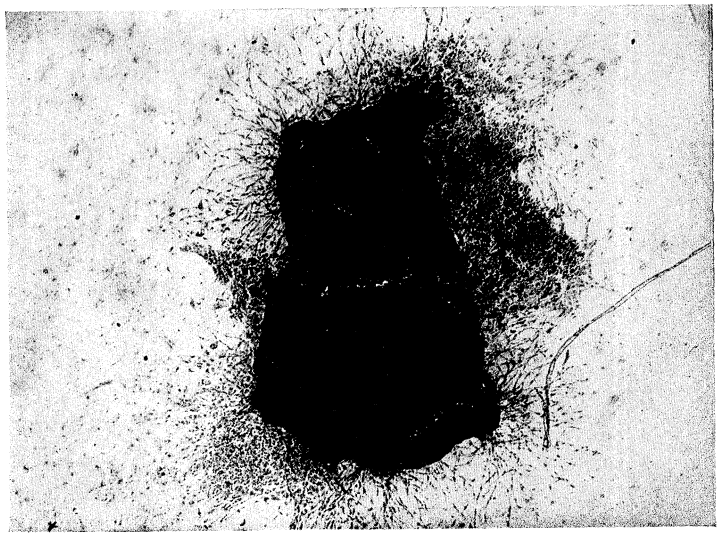

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DESCRIPTION OF PLATES.

1. Three days' growth in fresh homogenous plasma.
2. Three days' growth in homogenous plasma three days old.
3. Three days' growth in homogenous plasma ten days old.
4. Three days' growth in fresh autogenous plasma.
5. Five days' growth in fresh autogenous plasma.
6. Five days' growth in fresh homogenous plasma.

The Decomposition of Formates by Bacillus coli communis.

By EGERTON CHARLES GREY, 1851 Exhibition Scholar.

(Communicated by Dr. A. Harden, F.R.S. Received February 19,—Read March 26, 1914.)

(From the Biochemical Department of the Lister Institute.)

Many observations have been made on the variability of gas production by intestinal bacteria under natural conditions (see Penfold (1911) and Arkwright (1913), where literature is quoted).

Penfold has found that by artificial selection of *Bacillus coli communis* in the presence of sodium chloroacetate, strains may be isolated which produce no gas from glucose and gas in lessened amount from mannitol, although in both cases acid is produced as with the normal organism. The writer has also shown that by artificial selection of *B. coli communis* by the chloroacetate method of Penfold, various stages between the original gas-producing and the selected non-gas-producing strain may be obtained, and the changes have been found to be associated in part with the disappearance of the enzyme which decomposes formic acid (1914). It was found that two kinds of artificially selected strains could be produced from the original strain of *B. coli communis*; one unable to decompose formic acid, and the other still able to bring about this decomposition provided glucose were present. The artificially selected organism, which could not decompose formates even in the presence of glucose, was likewise unable to produce gas from mannitol, whereas the organism which still retained the power of decomposing formates was also able to produce gas from mannitol, although it produced this gas in an amount approximately equal to one-half of that produced under the same conditions by the original *B. coli communis* from which it was derived. It seemed, therefore, likely that by a closer study of

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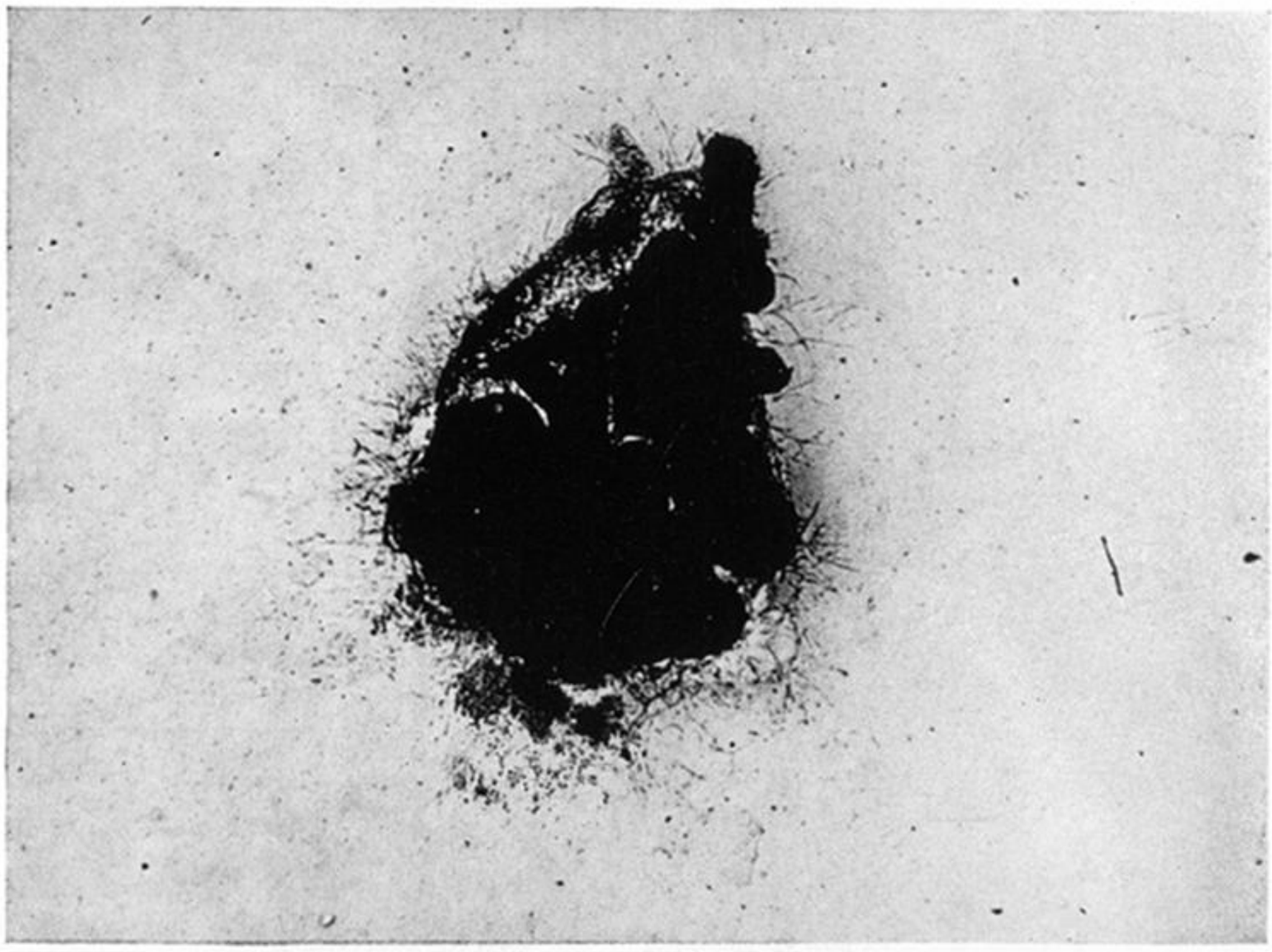

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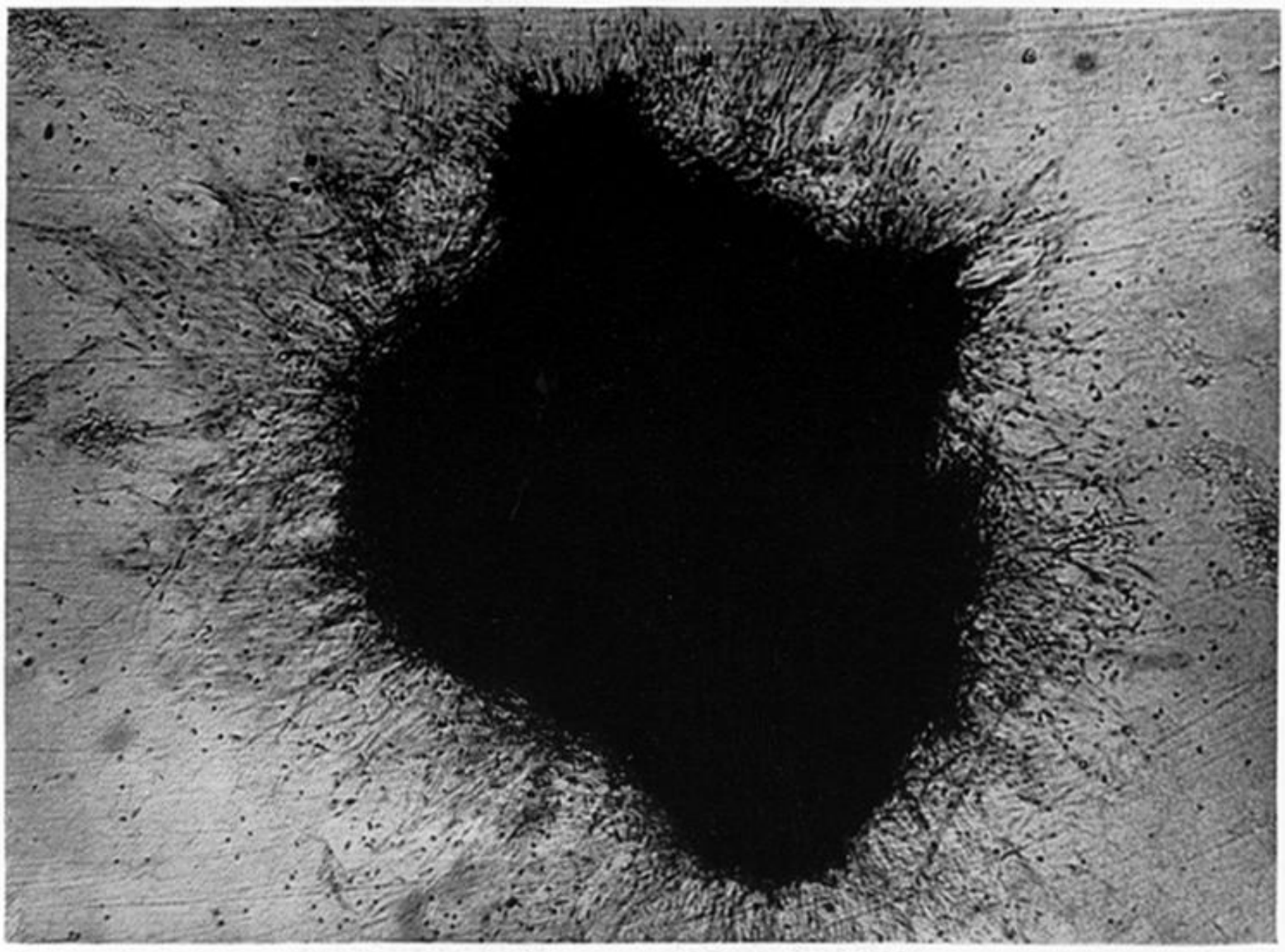

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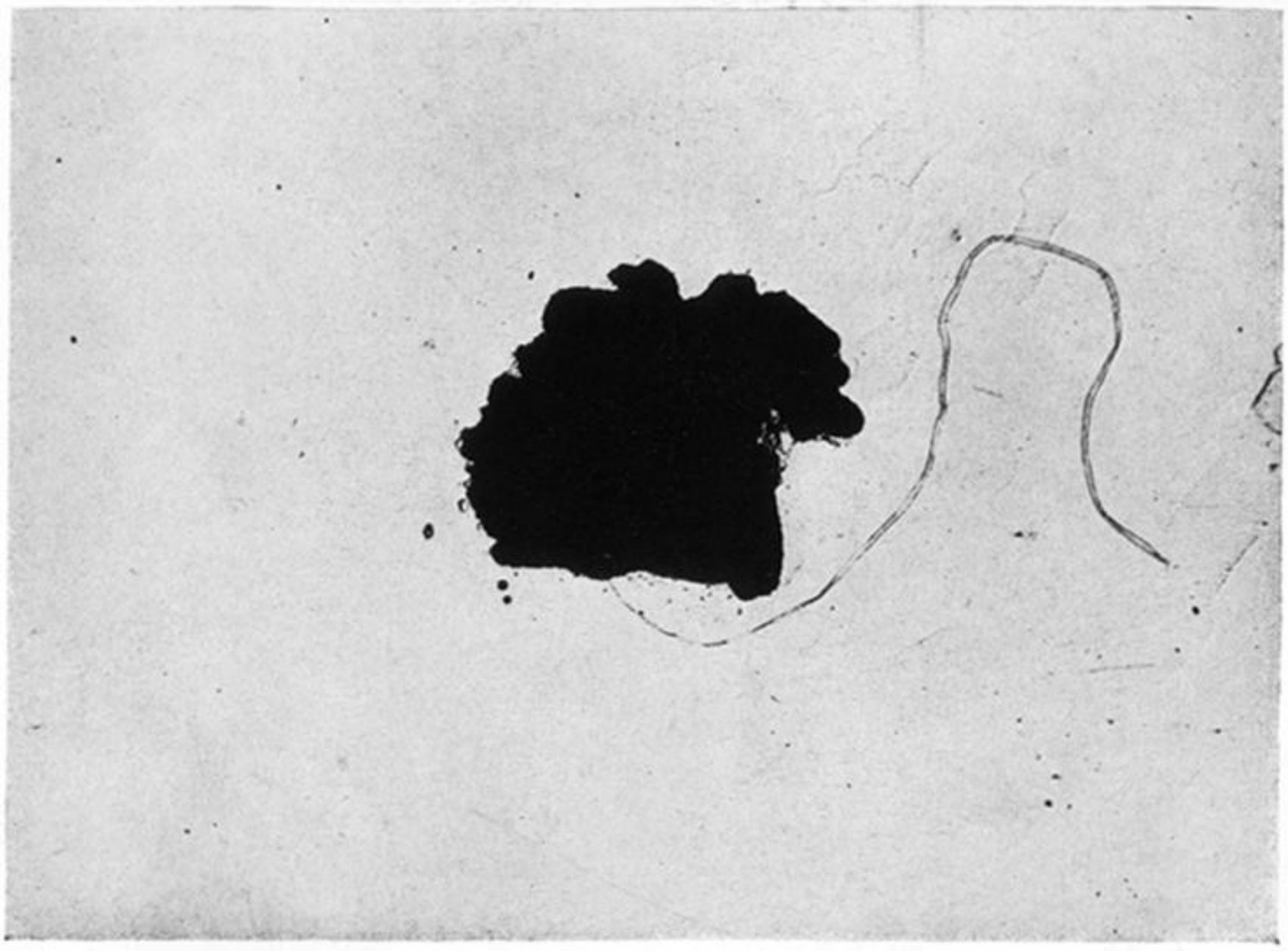

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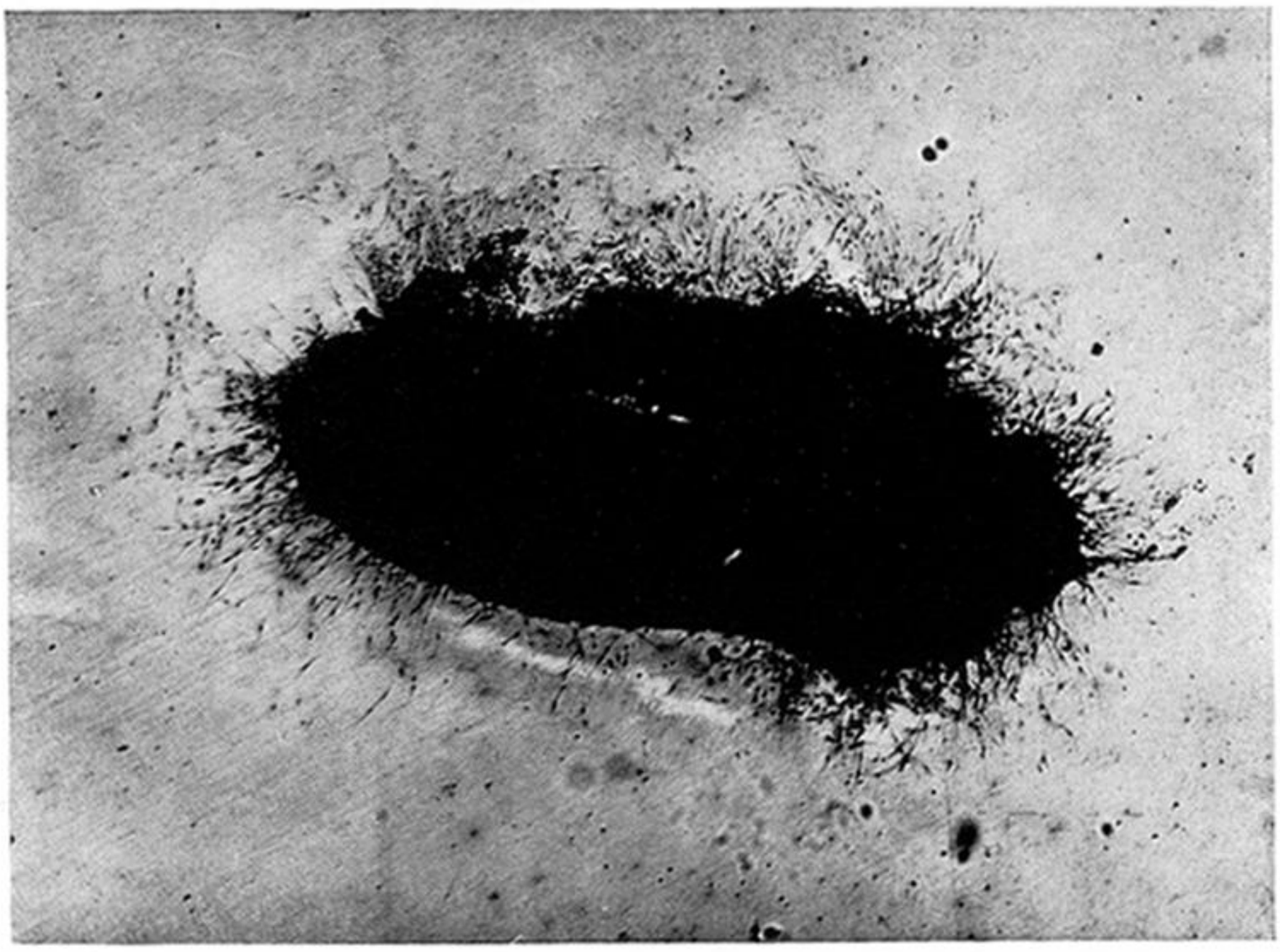

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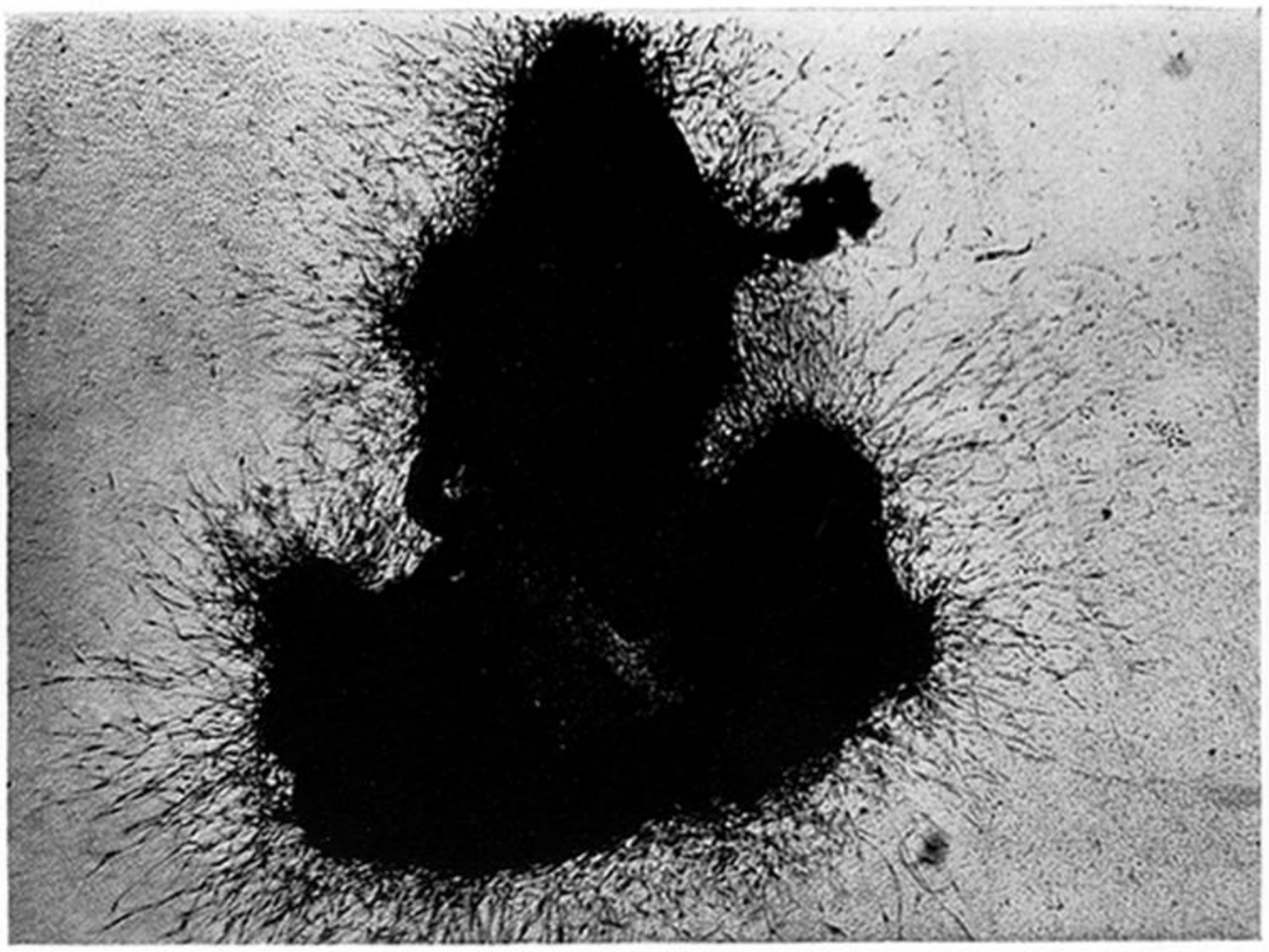

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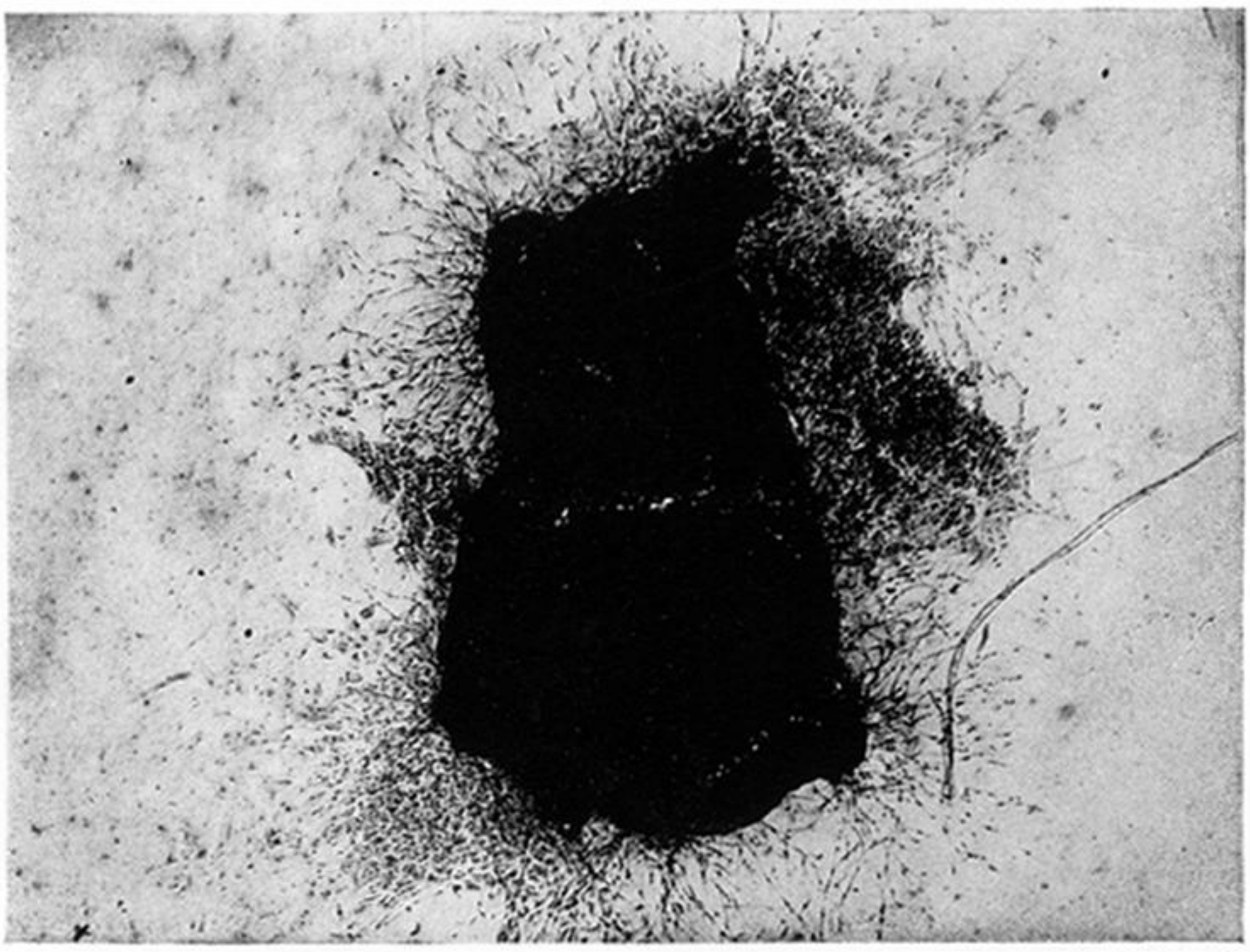

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