THE EFFECT OF VARIOUS TISSUE EXTRACTS UPON THE GROWTH OF ADULT MAMMALIAN CELLS IN VITRO.*

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PLATES 27 TO 31.

It was shown in a previous communication[1] that the extent of growth of adult mammalian tissue is to a large extent dependent upon the nature of the plasmatic medium used. It then became necessary to investigate the effects of various tissue extracts upon the growth, for it is possible that the variations in the plasmata of different animals might in part be dependent upon the secretions of various glands. There is already considerable clinical evidence that glandular secretions affect the growth of various tissues *in vivo*. Carrel has also shown[2] that the growth of tissue *in vitro* is stimulated to a marked degree by certain tissue extracts, especially, in the case of the chick, by the extract of chick embryo, but the presence or absence of any specific action of the extracts on different tissues has not been investigated. This communication will deal with the effects of various extracts of adult tissue upon the growth of cells obtained from adult mammals.

TECHNIQUE.

In all cases the plasma and tissues of adult rabbits were used both for cultural purposes and for preparing the extracts. The cultures were prepared by Carrel's technique in the manner I have previously described,[3] but after the piece of tissue had been placed in the

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[1] Walton, A. J., *Proc. Roy. Soc. London*, 1914, series B, lxxxvii, 452.

[2] Carrel, A., and Burrows, M. T., *Jour. Exper. Med.*, 1911, xiv, 244. Carrel, A., *idem*, 1912, xv, 516; 1913, xvii, 14; 1913, xviii, 287.

[3] Walton, A. J., *Jour. Path. and Bacteriol.*, 1914, xviii, 319.

plasma on the sterile cover-slip, a little of the fluid extract, equal in quantity to about one half the amount of plasma, was added with a sterile pipette and the fluids were mixed with a cataract knife. Almost immediately after the addition of the tissue extract the plasma coagulated. The preparation was then inverted over the cell slide and sealed in the usual way. In all cases, both in the primary and in the subcultures, an equal number of controls were made, the tissue in this case being grown in simple plasma. This was essential in every case for, as I have previously shown, the amount of growth varies considerably with the use of different specimens of plasma. It was also necessary to have controls in which the plasma was mixed with some inert fluid so that the changes in growth brought about by dilution should not be confounded with those caused by the addition of the extract. For this purpose a certain number of preparations were made in which the plasma was mixed with about one half of its volume of Ringer's fluid. Under these conditions it was found, as Carrel has previously shown,[4] that growth was somewhat accelerated. Allowance was made for this acceleration in deducing the results of the experiments.

Preparation of the Extracts.—The extracts were prepared by cutting or grinding up the given tissue in sterile Ringer's fluid, leaving the mixture to stand for a short while, and then centrifugalizing it. The supernatant fluid was then pipetted off and used as the extract. The fine division of the tissue in a sterile condition presented some difficulties. In the case of soft tissues such as the testicle, liver, spleen, and kidneys these difficulties were overcome by the use of the following instrument.[5] A brass syringe of a capacity of about seven cubic centimeters was made, the piston of which had two cross bars projecting from its face. These cross bars were made to sink in flush with the rest of the face of the piston, but were kept projecting by means of springs. When so projecting they acted as cutters, but when the piston was pressed down they could sink into it and the face of the piston was then smooth. The nozzle of the syringe was made to unscrew and between it and the barrel was inserted a perforated steel plate. The perforations were ta-

[4] Carrel, A., and Burrows, M. T., *Jour. Exper. Med.*, 1911, xiii, 562.

[5] I am indebted to Mr. H. S. Souttar for assistance in devising this instrument.

pered so that the narrow openings were nearest the piston and the edges on this surface were therefore sharp. The syringe was sterilized and a piece of the required tissue inserted into it. On rotating the piston the cross bars forced the tissue round so that it was cut on the perforations in the steel plate. The piston being at the same time slowly pushed home, the cut fragments of the tissue were forced through the plate. The cross bars sinking in as the piston was forced home, no dead space was left and all the tissue was thus used up. A small amount of Ringer's fluid was now sucked up into the syringe and formed an emulsion with the cut tissue. The mixture was then ejected into a sterile glass tube, corked, and, after standing for a short while, centrifugalized. For the larger pieces of tissue the small mincing machine devised by Haaland[6] would be equally efficacious.

Certain tissues, such as muscle, were too tough to be cut up by this instrument; they were therefore minced as small as possible with sterile scissors in Ringer's fluid and then centrifugalized.

The experiments were carried out in two groups. In the first group the animal was anesthetized, blood removed from the carotid artery, and the tissue required for making the extract then cut out. Small pieces of the viscera which were to be cultivated were then removed and placed in a sterile bowl of Ringer's fluid. By this means cultures were made of several tissues in the same extract which was autogenous to the tissues. 805 cultures were made in this group.

In the second group an animal was killed on a certain day, several of its organs were removed, and extracts made from them. The extracts were kept on ice for twenty-four hours and then centrifugalized. Subsequently one viscus was removed from another animal and cultured with all the extracts. By this means cultures were made of one tissue in several extracts which were homogenous to the tissue. Two hundred and sixteen cultures were made in this group.

This second group served not only as a control to the first group and thereby decreased the possibility of experimental error, but also served to show whether there was any alteration when homogenous instead of autogenous extracts were used.

[6] Haaland, M., *Berl. klin. Wchnschr.*, 1907, xliv, 713.

The results obtained by the former method will be first considered.

SPLEEN EXTRACT.

In this investigation 238 cultures were made, the effect of the extract being tried on the growth of spleen, thyroid, testicle, liver, and kidney. It was found that the tissues reacted somewhat differently, so that the effects on each tissue must be described.

Spleen.—Of this tissue fifty-eight cultures were made and the results were found to be constant. Even in the early stages the emigration of cells was somewhat more marked in the case of the cultures with the extract than in the controls. At the end of twenty-four hours the number of round cells in the plasma was distinctly greater and they had passed further into the medium. It is doubtful, however, if these cells should be described as parenchymatous cells. They appear to be polynuclear and mononuclear blood cells, and never in any of the preparations have mitotic figures been demonstrable in them, either in the controls or in the preparations with the extracts. The presence of these cells in large numbers to a certain extent obscures the changes of true growth and gives rise to some difficulty in the comparison of any two specimens. They must not be confounded with other cells which appear after two or three days. These are much larger, cuboidal in shape, and grow in solid masses from the edge of the tissues. They have a well defined nucleus with a wide zone of protoplasm. They are probably parenchymatous in nature and arise from the spleen pulp. They are quite distinct from the radiating, branching, and more deeply staining connective cells which on the third or fourth day begin to grow into them from the edge of the tissue. In the preparations to which spleen extract had been added the growth of these cells was always more marked than in the controls, so that whereas on the fourth day specimens grown in simple plasma would often show only irregular patches of these cells the corresponding specimens with the extract would not infrequently show a wide mosaic-like sheet as wide as, or wider than, the original piece of tissue. After four or five days the growth of cells of the connective tissue type was also more advanced in the stimulated specimens. The increase was not very marked, but was constant in all cases. Not only was the

growth of this type of cell more extensive, but it commenced at an earlier date so that they were seen on the second or third day, whilst in the controls they were not definite until the third or even the fourth day. In the subcultures both the parenchymatous and connective cells were more marked in the specimens with the extract for the first subculture, but in the later subcultures the parenchymatous cells were overgrown by the connective tissue type, the growth of which, however, was always more extensive in the specimens containing the extract.

Thyroid.—Fifty-four cultures were made of this tissue. In some of the earlier experiments no growth occurred with the extract, but in all these there was very slight growth in the controls. In the later experiments the growth was much more marked in the preparations with the extract than in the controls. The cells grew rapidly from the edge of the tissue and were mainly of the parenchymatous type, so that after five or six days large solid masses of cuboidal cells were seen projecting into the surrounding medium. Although the greater number of the cells were of the parenchymatous type there was also an increase of the connective tissue cells, these in all cases being more abundant than in the controls. In the case of the controls the majority of the cells were of the connective tissue type, there being very few parenchymatous cells present.

When subcultures were made the cells even of the stimulated specimens became more and more of the connective tissue type. That is to say, when life is prolonged the connective tissue cells, both in stimulated and unstimulated specimens, tend to overgrow the parenchymatous cells (figures 1, 2, and 3).

Testicle.—Of this tissue thirty-six cultures were made and in every case the extract caused marked stimulation of growth. Even after twenty-four hours the difference between the stimulated specimens and the controls was visible. In the case of the controls only a few branching cells were observed spreading from the edge of the tissue, but in the stimulated specimens these formed a well marked ring around the tissue. Growth rapidly progressed and by the fourth day the new tissue formed a mass four or five times the diameter of the original tissue (figure 7). Close to the cut edges the newly growing tissue

formed a solid mass indistinguishable from the original tissue, so that this appeared to have markedly increased in size. This is well shown in the figures of the stimulated and unstimulated specimens, the former of which is only half the magnification of the latter (figures 4 and 7). Outside the solid masses so formed the cells were widely spreading in all directions, so that a mosaic was formed apparently only one cell thick. Two types of cells were discernible: branching, irregular connective tissue cells, and more cuboidal cells with a dark staining nucleus and more distinct protoplasm. These latter cells were always grouped together to form tongue-shaped masses radiating from the sides of the tissue. In the controls the growth of the cells which were apparently parenchymatous in nature was much less marked, nearly all the cells being branching, elongated, and apparently of connective tissue, although even these cells were not growing to nearly so marked an extent as the similar type of cell in the stimulated specimens.

It is interesting to note that with this tissue there was no vacuolation of the plasma. If the cultures were left for a relatively long period without subculturing the cells became granular and degenerate, so that on staining only a mass of debris was seen, but in no case were large vacuoles formed such as are common with degenerate specimens of other tissues.

In the subcultures the connective type of cell rapidly overgrew the parenchymatous in the usual way, but the growth of the connective tissue type of cell was more marked and life could be prolonged to a greater extent in those cases where an extract had been used.

Liver.—Of this tissue thirty-six cultures were also made. In them the variation between the growth of parenchymatous and connective tissue cells is much more evident, for the parenchymatous cells of the liver form solid, deeply staining masses which can readily be distinguished from the connective tissue type. In all cases it was found that in the controls the characteristic feature was the presence of large club-shaped masses of cuboidal cells radiating from the cut edge into the plasma, while the connective tissue cells were present only in small numbers (figure 10). In the specimens to which the extract had been added there was a considerable in-

crease in the growth of the branching spindle cells, but only a few cuboidal cells were present. It is important to note that the parenchymatous cells were not only relatively but actually less in number in the tissues to which the extract had been added. The same characteristics were seen in subcultures which had been fixed in a relatively early stage. In the specimens with the extract there were well defined connective tissue overgrowth and only a few parenchymatous cells, whereas in the controls the amount of parenchymatous growth was extensive and the connective tissue growth less marked. Later, even in the specimens without extract, the cuboidal cells were overgrown by those of the connective tissue type.

Kidney.—Of this tissue fifty-four cultures were made. It was found necessary to exercise caution in drawing conclusions, for of all tissues this seems to be the one most likely to die, and in a series of cultivations several may die from no apparent cause. It was found, however, that in all cases where growth was present this was more marked in the stimulated specimens and that a large proportion of them showed growth. Not only was there an increase of parenchymatous growth, but the connective tissue cells were present also in large numbers. As I have previously shown, the growth of kidney tissue in normal plasma is characterized by the presence of cuboidal parenchymatous cells, very few connective tissue cells being present.

TABLE I.

Effect of Spleen Extract.

Tissue.	With extract.		Without extract.	
	Parenchymatous.	Connective.	Parenchymatous.	Connective.
Spleen...	Fair, slightly increased	Very good, considerably increased	Fair	Fair.
Thyroid..	Very good	Good	Fair	Moderate.
Testicle..	Very extensive	Extensive	Fair	Good.
Liver....	Slight	Good	Very good	Slight.
Kidney ..	Good	Good	Fair	Very slight.

Spleen extract would therefore seem always to stimulate the growth of connective tissue to a considerable extent. Its effect on the parenchymatous cells appears to differ with the tissues used. Of

the organs investigated, growth of these cells was markedly stimulated in the case of the testicle and thyroid, slightly stimulated in the kidney and spleen tissues, and inhibited in the case of the liver. These results are perhaps more clearly shown by table I.

MUSCLE EXTRACT.

In this investigation 259 cultures were made, the same tissues being used as with the spleen extract. As already mentioned, this extract had, owing to the toughness of the tissue, to be made by mincing the tissue with a pair of scissors. For this reason it was probably not so concentrated as were the other extracts, but here, as in other cases, controls were grown in plasma diluted with Ringer's fluid and it was found that the changes about to be described were not due to the fact that the plasma was diluted.

Spleen.—Seventy-eight cultures of this tissue were made. The effect of the extract was noticeable after growth had proceeded for a few hours. Thus after four hours the number of round cells which had emigrated into the plasma was in all cases more marked when the extract had been used. After twenty-four hours this difference was more evident, so that in the cultures with the extract the cells had wandered nearly to the limit of the plasma and formed a large mass around the tissue. The increased emigration of cells is liable to give rise to the belief that growth has been stimulated by the extract, but when care is taken to observe only the cuboidal parenchymatous cells this is seen not to be the case. In the preparations which have had the muscle extract added to them the growth of cuboidal cells is much less, and only a few of these cells are seen at the time when the same cells form a big area in the preparations without extract. On the other hand, the branching connective cells grow more rapidly in those cases in which the extract is added. In the case of the subcultures the preparations without extract show in the first subculture an overgrowth of the cuboidal parenchymatous cells, but those with the extract showed only radiating connective cells. In the third or fourth subculture the connective tissue cells are alone seen in both preparations. It would therefore appear that the muscle extract stimulates the growth of connective tissue cells but inhibits the parenchymatous cells of the spleen.

Thyroid.—Seventy-three cultures of this tissue were made. The results given were very definite. The specimens without the extract showed after two or three days the outgrowth of cuboidal cells which formed a mosaic around the tissue. The extent of the growth of these cells varied considerably with the animals used, but in all cases it was visible. Shortly after their appearance a few branching connective tissue cells were seen which soon grew out as radiating branches. In the case of the preparations with the extract the growth of the cuboidal cells was always much less, and in the majority of cases no such cells could be seen. The growth of the connective tissue cells was, however, much more rapid, so that by the fifth or sixth day a very wide network of branching cells was seen around the original tissue. The distinction between this wide branching network of connective tissue cells and the mosaic of cuboidal cells seen in the preparations without extract and in those treated with spleen extract was very clear.

Testicle.—Thirty-six cultures were made. The effect of this extract was not nearly so marked as that of the spleen. In all cases, however, there was a stimulation of the connective tissue type of cell. The branching cells wandered to a considerable distance into the plasma, forming an open network. The cuboidal parenchymatous cells also showed a distinct overgrowth in the stimulated specimens. In the unstimulated ones only small tongue-shaped processes of these cells were seen, whereas in the ones with the extract it was not uncommon to find a mosaic of these cells as large as the original piece of tissue. This overgrowth was observable both in the primary cultures and in the first subcultures. In the later subcultures these cells were overgrown in the usual way by the connective tissue cells, the growth of which was, as in the primary cultures, more marked in the stimulated specimens.

Liver.—Twenty-four cultures were made. In all cases the preparations containing the muscle extract showed more marked growth of the connective tissue type of cell, but again not to so great an extent as when the spleen extract was used. The effect upon the parenchymatous cells was not so definite. Those preparations with no extract again showed in all cases the characteristic masses of deeply staining cuboidal cells. In the preparations with the extract

these masses were also present, but they were never so extensive, the connective type of cell always preponderating. Similar changes were seen in subcultures. In the first subcultures both types of cell were seen, but the parenchymatous type of cell was more abundant in the specimens without the extract, the connective type in those with the extract. In later subcultures the connective tissue cell was more abundant in both.

Kidney.—Forty-eight cultures were made. As usual, a certain number of specimens died in both preparations. In cases in which growth took place, it was, however, clearly demonstrated that this was more marked in the specimens to which the extract had been added. Not only was the amount of growth more extensive, but in two parallel series those with the extract always showed a larger percentage of successful cultivations. As was to be expected from the results obtained with other tissues, the growth of connective tissue was more marked with the extract, but here again it was not nearly so extensive as when spleen extract had been used. In addition there was a very evident increased activity of the parenchymatous cells. As is usual with this tissue, these cells formed a definite mosaic apparently only one cell thick around the original piece of tissue, but, whereas in the controls these masses were small and projected only from certain points of the tissue, in the stimulated specimens fixed at a corresponding period of time they were much larger and often surrounded the tissue on all sides.

It would appear then that muscle extract stimulates the growth of connective cells whatever type of tissue be used, but this stimulation is not so marked as with spleen extract, although this difference may be due to the fact that the extract is weaker. On the other hand, some parenchymatous cells are stimulated and some are inhibited. It is important to note that the effects upon these parenchymatous cells differ from those of the spleen extract. Thus this type of cell is strongly stimulated in the case of the kidney, only slightly stimulated in the testicle, slightly inhibited in the liver, and more strongly inhibited in the thyroid and spleen. These results are shown in table II.

TABLE II.

Effect of Muscle Extract.

Tissue.	With extract.		Without extract.	
	Parenchymatous.	Connective.	Parenchymatous.	Connective.
Spleen . . .	Slight or absent	Good, increased	Fair	Fair.
Thyroid . .	Very slight	Good	Fair	Moderate.
Testicle . .	Good, slightly increased	Good	Fair	Fair.
Liver	Good	Good	Very good	Slight.
Kidney . .	Very good	Good	Fair	Very slight.

TESTICLE EXTRACT.

In investigating this extract 160 cultures were made, the same tissues being used as with the previous extracts. As the testicle of the adult rabbit is soft, an extract is readily prepared.

Spleen.—Thirty-six cultures were made. As in the case of the splenic and muscular extracts, the early emigration of cells was more marked than in the control specimens. The cuboidal cells of the parenchyma showed, however, no increase in any of the specimens. These cells were present, but, if anything, their growth was slightly less than that of the controls. In no case was the effect marked. On the other hand, the growth of connective tissue was considerably increased in all specimens. The extract was distinctly more active in this respect than the muscle extract, but was not so powerful as that of the spleen.

Thyroid.—Twenty cultures were made. In every case there was an increase in the growth of the parenchymatous cells, but not to so marked an extent as when the spleen extract was used. In all there was overgrowth of the connective tissue as compared with the controls. In the subcultures the connective tissue was also more marked when the extract was used, and more rapidly overgrew the parenchymatous cells.

Testicle.—Forty-eight cultures were made. As in the case of the other tissues, there was a considerable increase in the growth of the connective tissue, but never to so marked an extent as when the spleen extract was used. The growth of parenchymatous cells is also distinctly increased, these cells forming large mosaic-like areas when the corresponding controls showed small areas projecting

from the edge of the tissue (figure 8). Not uncommonly by the third day these masses of cuboidal cells formed in the stimulated specimens an area with a diameter as large as, or larger than, that of the original tissue. In no case, however, were these masses nearly so extensive as in the case of the preparations stimulated with splenic extract. In the subcultures the cuboidal cells were in the usual way rapidly overgrown by the connective tissue cells.

Liver.—Thirty-six cultures were made. In all cases there was some increase of the connective tissue cells, these cells being present at an earlier date and growing more rapidly. The parenchymatous cells were definitely decreased in numbers, there being only slight growth of small cuboidal masses from the edge of the tissue (figure 14). The same increased growth of the connective tissue was seen in the subcultures.

Kidney.—Twenty cultures were made. In all cases the tendency for vacuolation which is so marked with this tissue was more in evidence when the extract was used, and in such cases very little growth was seen. If, however, this vacuolation occurred early the cells might continue to grow in the plasma, and owing to the vacuole, were then completely separated from the original tissue. Under such circumstances no connective tissue cells were to be seen, it being probable that the cells are separated from the tissue before this type of cell has commenced to grow. If the growing portion remained attached to the original tissue there was always an increase in the numbers of connective tissue cells in those specimens with the extract. In all cases the growth of parenchymatous cells was much less in the specimens with the extract than in those without it.

Testicular extract appears, then, to stimulate the growth of connective tissue in all cases. This stimulation is more marked than is the case with muscle extract, but is not so extensive as when spleen extract is used. Certain of the parenchymatous cells are stimulated and some of them are inhibited. Thus the growth of this type of cell was always more marked in the case of the testicle and thyroid, was diminished in the case of the kidney and liver, and was but slightly if at all affected in the case of the spleen. These results are shown in table III.

TABLE III.

Effect of Testicle Extract.

Tissue.	With extract.		Without extract.	
	Parenchymatous.	Connective.	Parenchymatous.	Connective.
Spleen.....	Fair	Good	Fair	Fair.
Thyroid.....	Good	Good	Fair	Moderate.
Testicle.....	Very good	Good	Fair	Fair.
Liver.....	Very slight	Good	Very good	Slight.
Kidney.....	Slight	Good	Fair	Very slight.

THYROID EXTRACT.

In the investigation of this extract 148 cultures were made, the same tissues being again used. Owing to the small size of the thyroid gland, only small quantities of the extract could be prepared from each animal.

Spleen.—Sixteen cultures of this tissue were made. As with the other extracts, the specimens to which this extract was added showed an increase in the number of cells emigrating in the early stages. The growth of both parenchymatous and connective tissue cells was also definitely increased. By the third day the specimens with the extract already showed many branching connective tissue cells and masses of cuboidal parenchymatous cells, the corresponding cells being much less marked in the controls. By the fifth day the specimens with the extract were surrounded by large masses of parenchymatous cells, while close to the edge of the original tissue the connective cells formed a branching network difficult to distinguish from the tissue itself.

Thyroid.—Thirty-four cultures were made. The connective tissue cells were again increased, but not so extensively as when spleen extract was used, the amount of growth of this type of cell resembling that found when testicular extract was used. The parenchymatous cells were also somewhat more in evidence than in the controls, but the amount of stimulation of these cells was slight. The growth was not nearly so extensive as when splenic extract was used, and in fact in some cases it was but little more marked than in the controls.

Testicle.—Forty cultures were made. In all the experiments

there was a very marked increase in the growth of the parenchymatous cells. The growth of these cells was as extensive as with the use of splenic extract, so that by the fourth or fifth day large masses of these cuboidal cells were seen extending widely from the edge of the tissue into the surrounding medium. The connective tissue cells were, however, as in the case of the other tissues, not stimulated to nearly so marked an extent as by the spleen extract, although the growth was always more extensive than in the case of the controls. For this reason the masses of cuboidal cells were more clearly visible than when spleen extract was used (figure 6). In the subcultures the connective tissue grew more rapidly, so that the growth of the parenchymatous cells was not nearly so marked.

Liver.—Thirty-six cultures were made. The connective tissue growth was again increased to a moderate extent. The characteristic masses of deeply staining cuboidal cells were also more extensive with the use of the extract, the specimens in this particular differing markedly from those in which splenic extract was used (figure 12). The cuboidal cells formed large rounded or club-shaped masses growing irregularly from the edge of the tissue, but in some cases where the medium was thinner they formed mosaic-like masses apparently only one cell thick, which completely surrounded the original tissue and extended for some distance into the medium around. In the subcultures the connective tissue growth was more marked.

Kidney.—Twenty-two cultures were made. In these specimens again there was some increase in the connective tissue growth. The amount of growth of the parenchymatous cells was always less extensive than in the case of the controls. There was also a greater tendency for the liquefaction of the medium to take place, the original tissue being thereby separated by a fluid ring from the growing cells.

Thyroid extract appears, therefore, always to stimulate the growth of the connective tissue type of cell, whatever tissue is used, the amount of stimulation being about equal to that with testicular extract, but being less marked than that found with the use of splenic extract. Of the different tissues employed in this inves-

tigation the majority showed an increase in the growth of the parenchymatous cells when the extract was added. The extent of this stimulation differed, however, with the various cells. Thus, whereas this type of cell was markedly stimulated in the case of the spleen, testicle, and liver, it was only slightly increased in the case of the thyroid, and inhibited in the case of the kidney. These results are shown in table IV.

TABLE IV.

Effect of Thyroid Extract.

Tissue.	With extract.		Without extract.	
	Parenchymatous.	Connective.	Parenchymatous.	Connective.
Spleen.....	Very good	Good	Fair	Fair.
Thyroid.....	Good	Good	Fair	Moderate.
Testicle.....	Very good	Good	Fair	Fair.
Liver.....	Very good	Good	Very good	Slight.
Kidney.....	Slight	Good	Fair	Very slight.

In the second group of experiments one tissue only was cultivated from each animal. The extracts had been previously prepared from the tissues of another animal, as already described. The fluid was pipetted off the emulsified tissue and kept on ice in a sterile tube. In some cases it was used after twenty-four hours, but in other cases only after several days had elapsed. In this way it was possible to determine whether the effects of the extract were in any way altered by preserving it on ice. It will be seen that in all the experiments in this group the extracts were homogenous to the tissues, whereas in the last group they were autogenous. In no case was it evident that the homogenous extracts acted in any way differently from the autogenous extracts. The homogenous extracts were as efficient as the autogenous.

In this group 216 cultures were made.

Thyroid.—The thyroid was removed from a rabbit, and thirty-six preparations were made and cultivated in groups of six each in the following media: plasma, plasma and Ringer's fluid 2 to 1, plasma and thyroid extract 2 to 1, plasma and spleen extract 2 to 1, plasma and testicular extract 2 to 1, plasma and liver extract 2 to 1. All the extracts were one day old.

The results of these cultivations confirmed those made in the previous group. The controls showed a fair amount of growth of both the connective and parenchymatous cells, but with Ringer's fluid there was a slight increase in the growth of the connective tissue. The preparations with the thyroid and testicular extracts both showed a slight increase of the parenchymatous and connective tissue cells, while those with the spleen extract showed a considerable increase of both varieties of cells. Those with the liver extract showed, however, a marked diminution both in the parenchymatous and the connective tissue cells.

Testicle.—The testicle was removed and thirty-six preparations were made. The same culture media were used as in the last experiment, the extracts in this case being eleven days old. Here again the results confirmed those obtained in the first group of experiments. The control specimens showed a fair amount of parenchymatous and connective tissue growth (figure 4). With Ringer's fluid there was a definite increase in the amount of connective tissue growth although the parenchymatous cells were not increased (figure 5). With the thyroid extract there was a marked increase in the amount of parenchymatous growth and a slight increase in the connective tissue growth (figure 6), a similar result being obtained with the testicular extract (figure 8). With the splenic extract both the parenchymatous and connective tissue cells were greatly increased (figure 7). With the liver extract, as in the case with the thyroid tissue, there was no growth of the parenchymatous, and a greatly diminished growth of the connective tissue cells (figure 9).

A second experiment, performed on precisely the same lines, gave identical results, the extracts in this case being thirteen days old.

Kidney.—The kidney was removed and thirty-six preparations were made in groups of six specimens in each of the same culture media as before. The results agreed with those of the first group. In the controls there was good growth mainly of the parenchymatous cells, very few of the connective cells being present. In the specimens with Ringer's fluid the growth of connective cells was

rather more marked, but with this diluent the medium liquefied more readily and hence the growth of parenchymatous cells was less. With the thyroid extract the growth of parenchymatous cells was decreased as compared with the controls, but that of the connective cells was somewhat more marked. A similar result was obtained with the use of testicular extract. With the spleen extract there was a very definite increase in the amount of growth of both types of cell, but with the liver extract again there was very little or no growth.

Liver.—Thirty-six preparations were made in the same varieties of media, the extracts in this case being one day old. Good growth of both parenchymatous and connective tissue cells was obtained in the controls (figure 10). With Ringer's fluid the growth of the connective tissue cells was increased, but that of the parenchymatous cells was somewhat lessened (figure 11). With thyroid extract the connective growth was increased to a moderate extent, while the parenchymatous growth was considerably increased (figure 12). Testicular extract had a similar effect upon the connective cells, but diminished the growth of the parenchymatous cells (figure 14). This action is of interest, for in all the tissues previously considered these two extracts had a similar effect, but in the case of the liver tissue the one inhibits and the other stimulates the growth of the parenchymatous cells. With liver extract the growth of both types of cell was considerably diminished (figure 15). Splenic extract stimulates only the connective tissue growth (figure 13).

A second experiment was carried out on similar lines, but in this case the extracts were all twenty days old. It was found that in this case no growth occurred with any of the extracts, although good growth was seen in the preparations in simple plasma and in those in plasma and Ringer's fluid.

The results, therefore, of this series of experiments confirmed those of the first group, but in addition it was seen that liver extract apparently strongly inhibits the growth of both the connective tissue and parenchymatous cells of all tissues. The effects of other extracts are constant whether they are homogenous or autogenous

to the tissue used. Up to a certain period of time the extracts can be kept unchanged on ice, but after this time they appear to undergo a change.

CONCLUSIONS.

The cultivation of cells *in vitro* affords a valuable means of estimating the effects of tissue extracts.

Tissue extracts have a definite effect upon the growth of adult mammalian cells *in vitro*.

The majority of tissue extracts stimulate the growth of connective tissue, but liver extract inhibits it.

The extracts are to a certain extent specific in their action upon the growth of parenchymatous cells. Some cells are stimulated by one extract and inhibited by another, and those extracts which inhibit one type of parenchymatous cell may stimulate another type.

Homogenous and autogenous extracts are equally efficacious in their action upon the growth of cells.

The extracts may be preserved for a short period of time without suffering any change in their power of affecting the growth of cells.

EXPLANATION OF PLATES.[7]

PLATE 27.

Growth of adult rabbit thyroid in plasma and spleen extract.

FIG. 1. First culture. Fourth day.
FIG. 2. Subculture. Third day.
FIG. 3. Second subculture. Third day.

PLATE 28.

Growth of adult rabbit testicle in various media.

FIG. 4. Four days' growth in simple plasma.
FIG. 5. Four days' growth in plasma and Ringer's fluid.
FIG. 6. Four days' growth in plasma and thyroid extract.

PLATE 29.

Growth of adult rabbit testicle in various media.

FIG. 7. Four days' growth in plasma and splenic extract.
FIG. 8. Four days' growth in plasma and testicular extract.
FIG. 9. Four days' growth in plasma and liver extract.

[7] I am indebted to Mr. Summers for the photographs of the growing tissues.

PLATE 30.

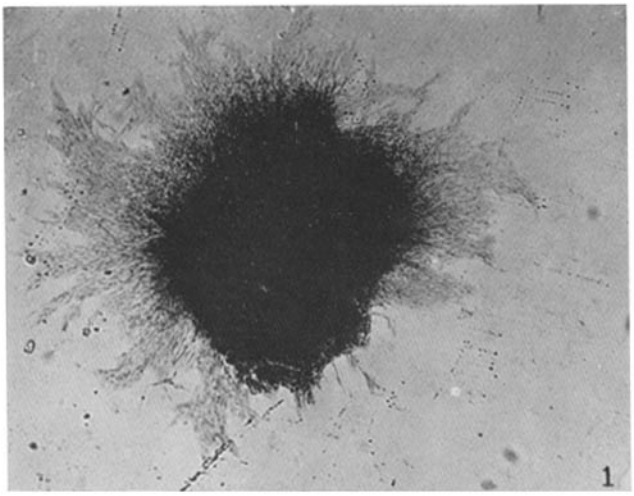
Growth of adult rabbit liver in various media.

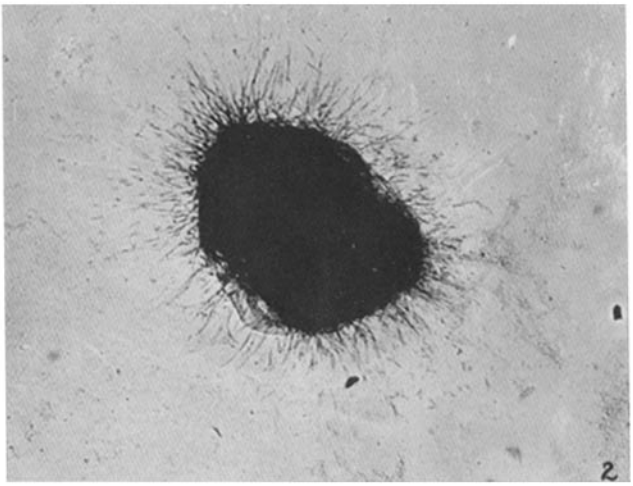
FIG. 10. Four days' growth in simple plasma.
FIG. 11. Four days' growth in plasma and Ringer's fluid.
FIG. 12. Four days' growth in plasma and thyroid extract.

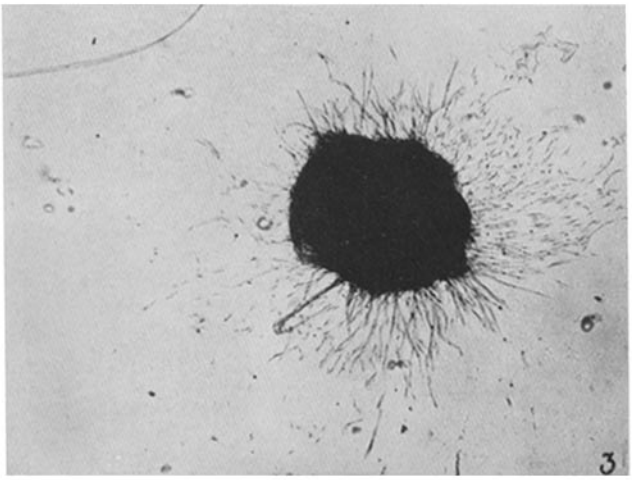
PLATE 31.

Growth of adult rabbit liver in various media.

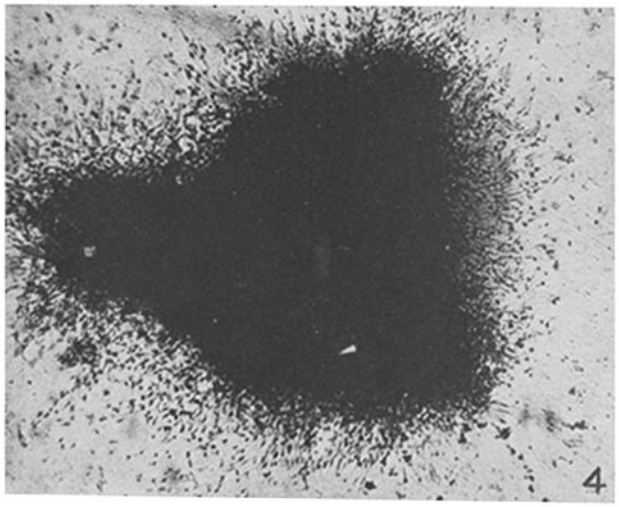
FIG. 13. Four days' growth in plasma and splenic extract.
FIG. 14. Four days' growth in plasma and testicular extract.
FIG. 15. Four days' growth in plasma and liver extract.

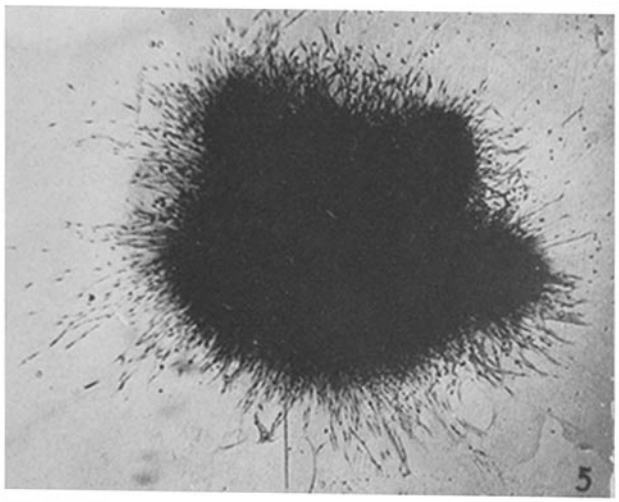


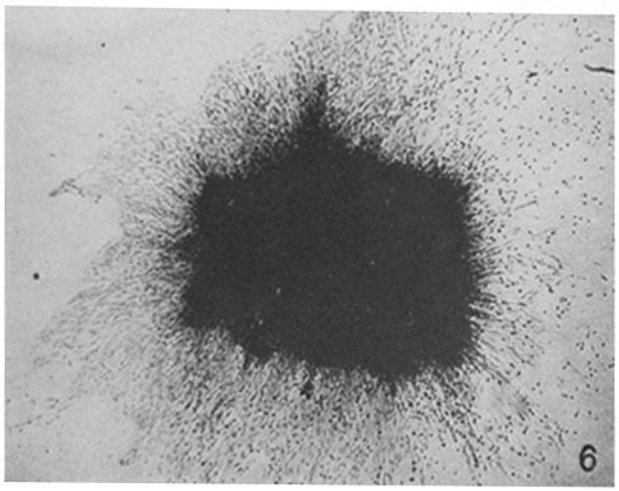




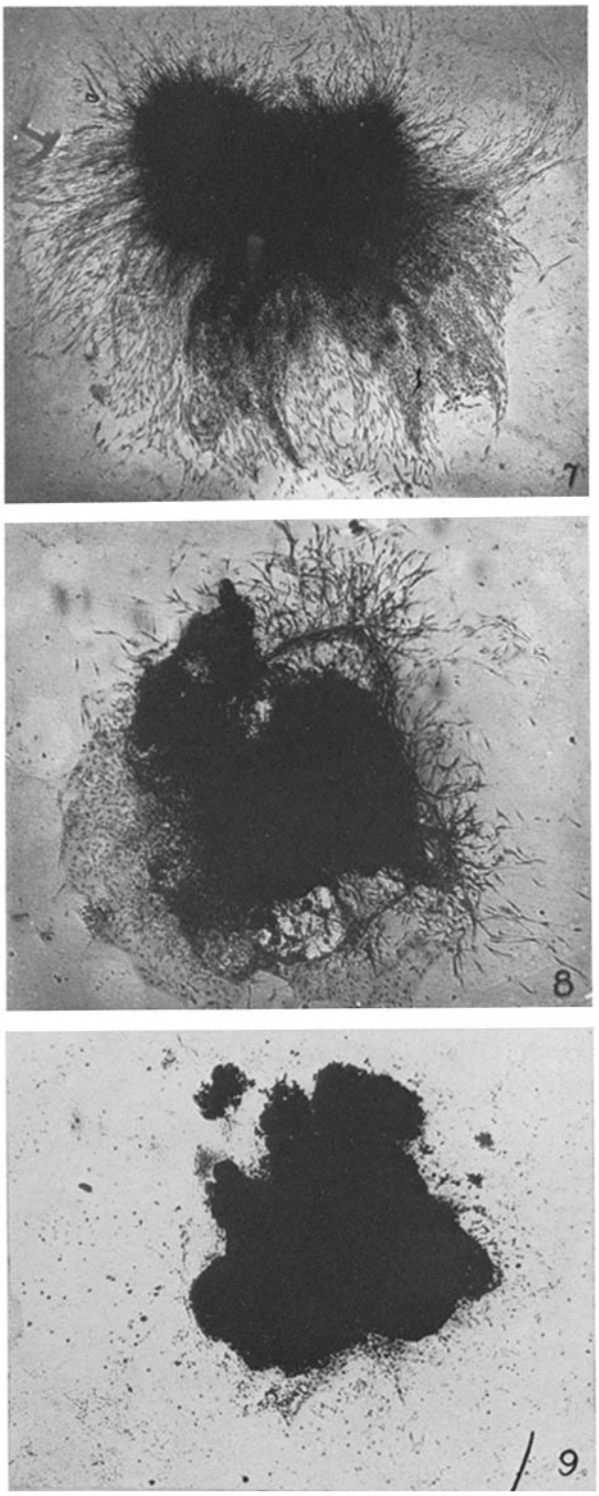
(Walton: Growth of Adult Mammalian Cells *in Vitro*.)



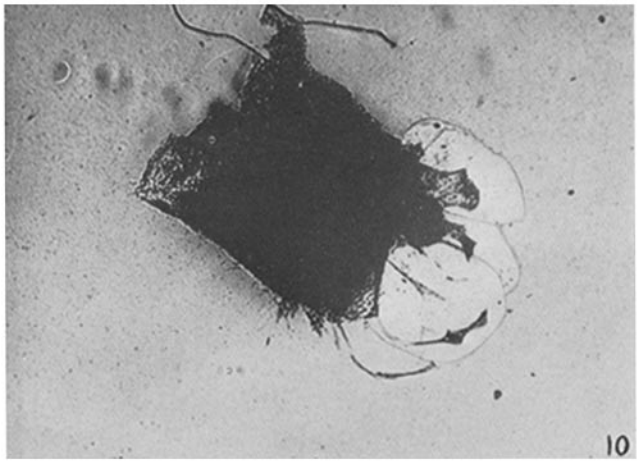


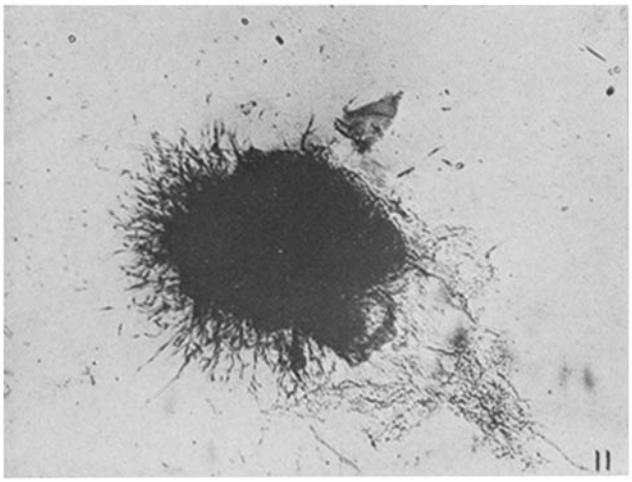


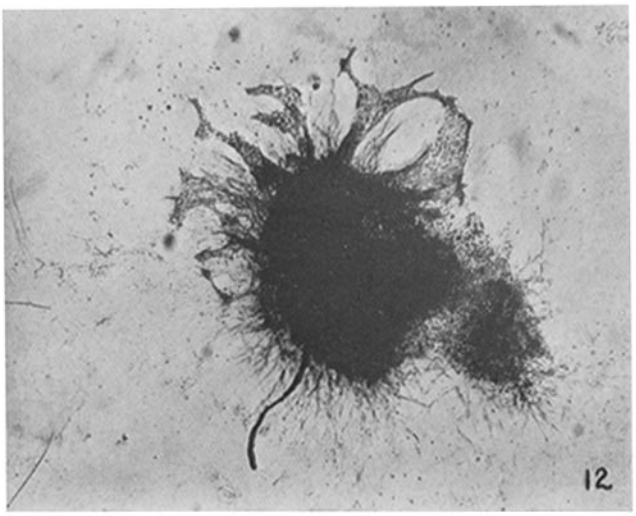
(Walton: Growth of Adult Mammalian Cells *in Vitro*.)



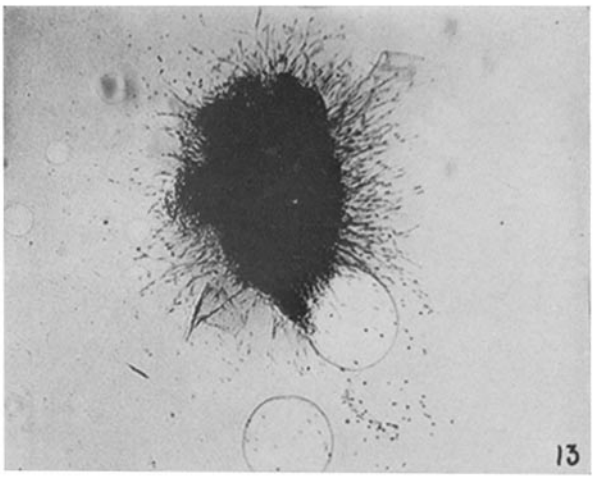
(Walton: Growth of Adult Mammalian Cells *in Vitro*.)

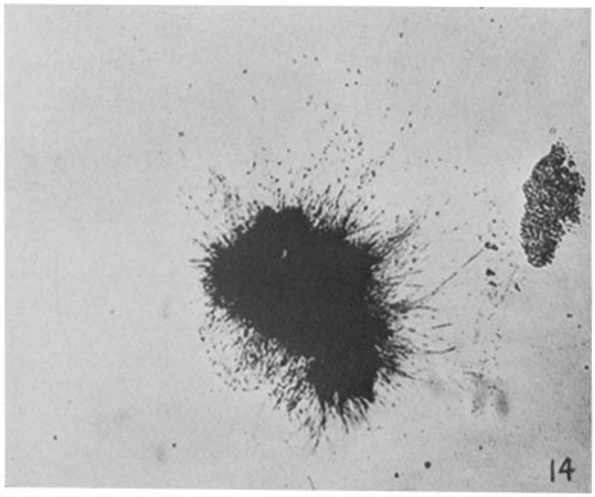


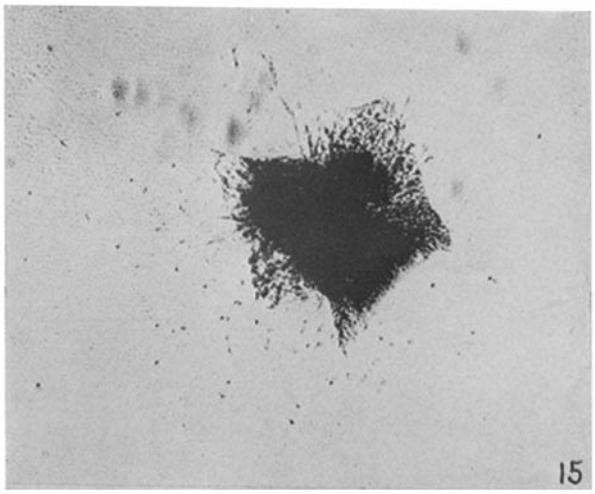




(Walton: Growth of Adult Mammalian Cells *in Vitro*.)







(Walton: Growth of Adult Mammalian Cells *in Vitro*.)