CULTIVATION OF BRAINTUMOURCELLS AND THE RELATIONSHIP OF SOME OF THEM WITH SCHWANN CELLS IN VITRO

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(WITH 12 PLATES)

VERHANDELING DER KONINKLIJKE NED. AKADEMIE
VAN WETENSCHAPPEN, AFDEELING NATUURKUNDE

TWEEDE SECTIE, DEEL XLII, No. 3

1946
N.V. NOORD-HOLLANDSCHE UITGEVERS MAATSCHAPPIJ
AMSTERDAM

Kon. Ned. Akad. Wet., Verh. (Tweede Sectie), DI. XLII, No. 3, p. 1—31, 1946

Although the histological methods are more and more ameliorated, they do not yet eliminate the many divergences of opinion about the histological diagnose and classification of the braintumors. This may be due to the fact that tumourcells generally have a lack of differentiation and do not show the features which, potentially, they possess.

For the purpose of developing the characteristic features of the neoplastic cells, we tried the cultivation of these elements in vitro. Cultivation, however, does not differentiate in the first place those properties which in vivo are indispensable for the building up of organized tissue. Otherwise the development of the form, the modus of growth in vitro, the relations between the neoplastic cells mutually, eventually with other kinds of cells, their mode of reaction on chemical excitation etc. in vitro, may reveal characteristics of the neoplastic cells by which they can be distinguished not only from other kinds of cells, but also from cells that are classified in the same group.

From the features shown by the cells developed in vitro, one may conclude which potencies are harboured in the undeveloped tumour cell.

By "differentiation" we understand the development of specific attributes of the neoplastic cell, failing in the mothercells e.g. the development of gliafibrils and of neurofibrils.

Although the different investigators may have conflicting views about differentiation, they all agree that at least one distinct difference between the cells is also maintained in vitro, viz. according to their origin from the ectoderm or the mesoderm, epithelial and mesenchymal cells and celltypes. Both kinds of cells, within definite circumstances maintain their own features and do not merge into each other.

So we could expect at least to discern tumours of ectodermal and mesodermal origin. Moreover we purposed to get a distinct differentiation of the neoplastic cells and to search for methods which prevent dedifferentiation and benefit differentiation.

METHOD.

Usually small glass- or asbestos-slides, arranged in CARREL-flasks, were used for the cultivation. The solid phase consisted of 0,4—0,6 ccm of bloodplasma (adding 0,25—0,5 ccm heparin 1 : 1000 to each 20 ccm of blood), the fluid phase consisted of heparin-bloodplasma or human ascites-fluid. Though the different ascites-fluids gave different results, they were always better than the results with the usual saltsolutions.

It seems to be of the highest importance, that always the same kind of medium is used. Therefore, each fifth or seventh day, when refeeding with the fluid took place, there was no washing applied and the heparinplasma remained on the culture up to the next refeeding (different from the method of FISCHER and PARKER [1]). As no tissue-extracts were used (all tissue-

extracts proved to be unfavourable) the coagulation of the medium had to be performed by the tissue of the explant or spontaneous.

However, coagulated bloodplasma, though not unfavourable for histological purposes, does not form a very solid medium. Therefore replanting of parts of the culture is only possible when the outgrowth consists of a thick layer of cellular elements, occurring only in cases of mesenchymal tumours. In other cases it also needs the excision of fragments of the motherpiece.

Though it has not been our purpose to make permanent cultures, each tumour had to be cultivated for a definite period, as particularly nervous tissue needs rather a long period of latency before neuroectodermal tumourcells are growing out (up to three weeks). For the histological examination the cultures were stained in toto. A much diluted solution of hematoxylin-DELAFIELD was used for the mesenchymal tumours. The presence of bloodplasma made most of the impregnation techniques unsuitable, therefore the following impregnationmethod, which avoids precipitation was worked out:

Fixation in the CARREL-flask in 4 % formalin at least four days.

Wash in distilled water with some drops of ammonia 12—24 hours.

Take the glass- or asbestos-slides out of the CARREL-flask and transfer to a glassdish containing HORTEGAs silver carbonate solution (20 cc aq dist + 12 drops of HORTEGA silver + 2 drops of piridin). Leave in incubator at 38 C. until the densiest part of the cultures become brown (15—20 min.).

After pouring the fluid out of the glassdish, rinse with distilled water two times and reduce with 10 % formalin at least 50 min.

Alcohol, Xylol, mount in canadabalsam.

MATERIAL AND OBSERVATIONS.

The classification of our material, based on the histological diagnose of the original tumour, generally follows the nomenclature of BAILEY [2]) and CUSHING.

We examined and cultivated:

3 meningeomas
1 oligodendroglioma
5 gliomas
1 angio-glioma
1 ologodendroglioma
4 medulloblastomas
1 ependymoma
3 neurofibromas

and as comparable material:

SCHWANN cells, originating from the sciatic nerve of a rabbit in order to compare them with the neoplastic cells of neurofibromas

3 tumours of the brain, not arising from the nervous tissue

Healthy human braintissue

Cultures of rabbits tissue

MENINGEOMAS.

Tumours, arising from the meninges and classified by CUSHING as meningeomas often show great differences in histological structure, because the meninges are built up by different cellular elements e.g. fibroblasts, endothelial cells and the specific arachnoidea-cells which differ from the lining-endothelium cells. Cultivation of these tumours might bring forward the question whether the leptomeninx contains ectodermal elements conforming to OBERLING [3]), HARVEY and BURR [4]); the more so as in vitro all ectodermal cells differ in a distinct way from the mesenchymal elements.

If it is true that the leptomeninx springs from the neural crest (HARVEY and BURR) as do the SCHWANNcells (HARRISON [5])), the latter, according to recent investigations, giving rise to the mothercells of neurofibromas, there would be a relationship between the neurofibromas and the meningeomas and congenial elements should be present in the cultures of both these tumours. We will try to answer this question. However, we had only the disposal of three meningeomas, a number too small, with regards to the diversity in structure, to establish a differentiation within this big group of neoplasmas, especially concerning the arachnoidcells.

1. *Tumour Aa,* man. 40 years. Tumour springing from the occipito-medial part of the dura mater.

Histological structure: An amount of nuclei, lying in a protoplasmatic syncytium; a loose connective tissue stroma, (red with von GIESON's stain) in which occur numerous bloodsinus, lined by the neoplastic cells. Whorled arrangement of the tumourcells. The oval- or bean-shaped nuclei are rather small, the nuclein granules are arranged along the membrane of the nucleus. Most of the nuclei contain one big nucleolus. (Fig. 1.)

Examination of cultures:

In the first stage of migration, numerous small roundcells occur. As usually, they disappear in due time. Then follows the migration of wedge-shaped cells or spindle-cells, characteristic for cultures of mesenchymal origin (fig. 2). The spindle-cells arrange themselves radially. Development of processes only occurs at the poles of the bipolar cells, whereas fusing of the different elements occurs only by means of these processes. Consequently the neoplastic cells are *polar-cytotrop.*

As usual the borders of the cultures show more wedge-shaped cells, and especially in old cultures there appear rather large multinucleated and giant-cells, which, as a rule is of no importance.

The nuclei of these outgrowing cells are similar to the cells of the original tumour, they are oval- or bean-shaped and not seldom contain but one single big nucleolus.

The duration of the cultures could be extended to 42 days.

So the cultivation of this tumour reveals a differentiation of the neoplastic

cells in the direction of mesodermic connective tissue cells. Apart from the big amount of outgrowing cells, the characteristic features of the nuclei prove that the cultures were built up by tumourcells.

2. *Tumour Hey,* woman 35 years.

In contrast with the first tumour, this meningeoma is composed of more developed mesodermal elements. Arrangements of the cells in columns, separated from each other by a dense layer of connective tissue. The cells are polygonal, elongated or spindle-shaped. The well-developed cellprotoplasma shows a fibrillary structure. This, with the presence of an amount of connective tissue, pleads for a certain degree of maturity of the tumour (fig. 3).

Examination of cultures.

After a short period of latency we observe the migration of small mesenchymal cells and, in an increasing way, more plump spindle-cells and irregular-shaped elements. The rate of growth is a slow one. After some days, big multinucleated giantcells display themselves. Vacuoles appear in the protoplasm and the nuclei contain small or large inclusions, most times circularly arranged (fig. 4). Staining with haematoxylin-DELAFIELD reveals a fibrillar structure of the protoplasm. Sometimes the neoplastic cells form an epithelium-like covering, whereas protoplasmatic processes of the cells, passing through the intersticial spaces, connect the cells with each other. A few cultures disclose a dense, fibrillar reticulum, arranged round the neoplastic cells (fig. 5). Though we are fully aware of the difficulty of the problem, whether reticulin or collagen can be formed in vitro by the outgrowing cells, the difference between these reticular fibres and the fibrous coagulum of fibrin, that occurs sometimes in the embedding plasmatic medium, was so obvious, that we do not hesitate to take this reticulum as derived from the outgrowing cells.

The duration of the cultures amounted to 68 days.

In this case the more or less mature connective tissue cells of the original tumour dedifferentiate in vitro, partly in immature mesenchymal cells, partly in cells of the endothelial-type which occasionally form a fibrous reticulum.

Tumour Ger. man 50 years.

The third meningeal tumour shows an alveolar structure with a dense layer of connective tissue between the clusters of cells. The cellprotoplasm is more or less fibrous.

Examination of cultures:

From a necrotic part of the explant there arises after three days of incubation, an amount of spindle-cells of the well-known mesenchymal type. Cultures, made from a part of the neoplasm that was characterized by the

abundance of blood, within a short time, produced an amount of active granulated wandering cells, which soon disappear. Then follows the migration of wedge-shaped or spindle-cells. They grow fast and form large cultures, which are easily divisible.

By adding 0.03 ccm of isotonic primary sodium phosphate solution, the rate of growth decreases, 0,03 ccm of potassium chlorid-solution stops any further migration, contrary to the behaviour of glioma-cultures (see pag. 10).

Apart from few large plump triangular cells in the periphery of the outgrowth, cells similar to those of tumour 2, these cultures are composed exclusive of slight, radially arranged mesenchymal cells. They form a typical culture of fibroblasts. The protoplasm is fibrous-striped; the nuclei are slight oval.

The duration of the cultures amounted to 31 days.

The fast growing cultures, made from this neoplasm are similar to the cultures of mesenchymal cells, cultivated in embryonic juice, though in our case the medium consisted only of bloodplasma.

Summarizing our experiences on meningeomas we conclude:

The cultures of the three meningeomas equal the cultures of mesenchymal cells. So far, the difference between these outgrowths and those of tumours of neuro-ectodermal or epithelial origin is obvious. Regarding the ectodermal arachnoid-cells we could not find one single element in the cultures that showed epithelial features.

HAEMANGIO-ENDOTHELIOMA.

Tumour West. man, 21 years.

This intracerebral tumour is composed of an amount of neoplastic cells, similar to the lining endothelium of the blood-vessels, and of numerous large or smaller bloodvessels, embedded in a loose connective tissue containing collagen fibres. The nuclei are irregular-shaped and show vacuoles. Not seldom the nucleus is divided in two half-moon-shaped parts by a large vacuole. Some cells show processes similar to vascular shoots (fig. 6).

Examination of cultures.

After two or three days of incubation numerous spindle-cells and small polymorphic cells, as well as large triangular or quadrangular wandering-cells migrate from the motherpiece. The spindle-cells persist during the whole time of cultivation. The large triangular and polygonal cells increase continuously in size and attain totally uncommon sizes, e.g. 310 μ long and 67 μ broad with a vacuolized nucleus of 67 μ long and 44 μ broad (fig. 7). This surface of 21000 μ^2 is about 25 times as large as the surface of the average mesenchymal cell. The polymorphic cells in these cultures are always plate-shaped. The borders of these protoplasmatic plates are excavated at one or more sides, most times however, one large excavation extends over the

whole cell, the nucleus lying close to the deepest point of the excavation. Moreover, from both the peripheric points of the excavation arise processes, going in arched direction, a phenomenon which, with regards to the conditions in vitro, in a high degree reminds of the endothelial cells provided with processes in the original tumour (fig. 8). So the impression is made that the neoplastic cells try to form a canal or bloodvessel. As in the original tumour tissue, the cultures reveal many a vacuolized nucleus and many inclusions in the protoplasm of the cell.

The duration of the cultures amounted to 42 days.

The outgrowing cells show a distinct differentiation as far as they disclose a tendency to form cavities.

GLIOMAS.

Neoplastic glia-cells, due to their relation with the bloodvessels, as to the structure of their protoplasm and their reaction on different staining-methods, are most times clearly distinct from other neoplastic elements. However there remain neuro-ectodermal tumours, composed of cells which lack the slighest degree of differentiation. In those cases it appears impossible to distinguish spongioblasts from medulloblasts or immature neuroblasts, as both kinds of cells arise from the same neuro-ectodermal matrix. It might be that cultivation of these neoplasma would reveal us the different potencies of the elements by which they are built up.

Apart from the diverse functions of the glia-cells, it seems to be a fact that certain glia-cells in the central nervous system play a part equivalent to that of the SCHWANN-cells in the peripheric nervous system. Though it is generally assumed that HORTEGA's oligodendroglia-cells play that part, the question remains whether these cells essentially differ from the astrocytes.

By cultivation of glious neoplasms we hoped to differentiate the undeveloped neoplastic cells into more mature glia-cells and to throw a light on the question in how far there is a relationship between glia-cell and SCHWANN-cell, the latter arising not only from the neural crest, but also from the neutral tube (RAVEN [6]).

We cultivated: 3 spongioblastomas
2 astrocytomas
1 oligodendroglioma.

1. *Tumour Wo.* Spongioblastoma from the occipital lobe.

Oval or round nuclei, containing an amount of nuclein, are arranged radially round the bloodvessels and embedded in a syncytial protoplasm. Some cells have the shape of unipolar spongioblasts. Not a single differentiated gliacell was found in the neoplastic tissue (fig. 9).

Examination of cultures:

Few hours after explantation numerous small ameboid cells migrate from the motherpiece and, within 3 days, disappear. Then there appears an-

other kind of wandering cells, slowly but constantly increasing in number (fig. 10) about twice as large as the first migrating cells, viz: 20—100 μ length and 5—25 μ broad. The cells are polymorphic and the shape varies continuously. Some cells posses processes, smooth and rectilinear, growing out to about 1 mm length (fig. 11) and directed centrifugally from the explant. Not seldom a process is withdrawn to proceed in other direction.

Generally the outgrowing neoplastic cells do not form dense colonies, but they lay more or less scattered, sometimes in small groups. In some of the cultures there is a so sumptuous growth that the outgrowing cells lay in a dense layer. In these cases a big change in the cell-shape is revealed (fig. 12). The cells become elongated, cylindric, their length amounts to 180 or 200 μ, their width 25 μ. They possess small undulating membranes or small or long processes. Contrary to the arrangement of mesenchymal cells they never arrange radially, but always alongside of each other. Consequently there is no polar cytotropy. Moreover, each cell remains isolated. The transition of the wandering cells into these cells is obvious.

The fixated and stained or impregnated cultures show small round cells, more ample polygonal cells and, finally, elongated pear-shaped cells. The latter possess the characteristic features of well-developed gliacells viz: polygonal cells from which radially protrude one or more processes. The cells are often multinucleated, the nuclei sometimes being arranged circularly, sometimes lying in the periphery of the cellbody. The cytoplasm has a reticular aspect, due to dense stripes of protoplasm running through the cell body. The processes can be very long, with rectilinear course, they do not make anastomoses with other cells (fig. 11).

The duration of the cultures amounted to 43 days

2. *Tumour Ro.* woman, 42 years. Spongioblastoma multiforme.

The tumour is composed of uni- and bipolar spongioblasts and polymorphic multinucleated elements. The cytoplosm has no fibrillar structure, but is rather homogeneous. The structure of the nucleus in the large cells is often atypic viz: fragmentation of the nucleus, or conglomerates of nuclein-granules (fig. 13).

Examination of cultures: After 4 days of incubation the migration of granular wandering-cells begins. At first these cells are round, afterwards they become polygonal and reveal a vivid activity, changing their shape constantly. Small processes protruding from the pointed angles of the cells elongate more and more and connect with other cells of the same kind (fig. 14).

These processes are smooth without visible nodules. They can be extremely long in cultures that have ascites-fluid added to the fluid medium.

Some cells contain bullet-shaped inclusions arranged in rosettes, which after staining reveal themselves as fragmentations of the nucleus. The size of the cell amounts to 70—80 μ length.

The addition of 0,03 ccm of isotonic sodium carbonate-solution or primary sodium phosphate-solution stimulates the growth and gives rise to very large cultures.

Impregnated cultures show, next to the motherpiece, large multi-nucleated cells, from which protrude three or more delicate rectilinear processes in centrifugal direction. More peripheric parts of the cultures contain cells of peculiar structure (fig. 15) viz.: peripheric from a multi-nucleated inner area of the cell lies a zone of fibrillar protoplasm passing into a transparent thin protoplasmatic vacuolized membrane. From the fibrillar protoplasmatic layer arise prolongations which run through the membrane and through the culture as a thread-like fibrillary-structured formation. At their end, these very long processes expand fan-shaped, to connect with other elements of the same kind. The nuclei disclose a diversity of shapes. Bullet-shaped and pear-shaped stalked nuclei, as well as all possible constrictions and indentations of the nucleus, up to the unbellated shapes are displayed. These forms equal those, described by ACHUCARRO [7]) in cases of glioblastoma multiforme (fig. 16). Apart from these atypical cells the cultures contain smaller typical gliacells.

The duration of the cultures amounted to 31 days.

3. *Tumour Schr.* man, 50 years. *Spongioblastoma multiforme.*

Histologically the structure of this tumour is similar to the former one. The neoplastic tissue contains many giant cells, multinucleated and with conglomerates of nuclein-globules. Specific impregnationmethods do not reveal any gliafibrils.

Examination of cultures.

After three days of incubation a vivid migration of wandering cells, similar to those of the former case, occurs. The cells form long processes which make anastomoses with the processes of other cells, building a widespread loose reticulum throughout the culture (fig. 17 and 18).

From the explant itself protrudes an amount of radially directed fibres. At points where the degree of proliferation is very high, the cells push together and become spindle-shaped, similar to those cells described in the first glioma (Wo). Here too, contrary to the behaviour of mesenchymal cells, the cells place themselves alongside each other and never lay polarly behind each other. The protoplasm of the outgrowing cells is granular and transmutes at the periphery of the cellbody into a thin membrane. The nuclei disclose the most peculiar shapes as in the former case.

Adding 0,03 ccm of isotonic primary sodium phosphate-solution or potassium chloride-solution causes the development of a very slightly built reticulum.

The duration of the cultures amounted to 26 days.

4. *Tumour* Bro. man, 30 years. *Astrocytoma.*

Histologically the tumour is composed of small cells embedded in an

somewhat fibrous substratum. The nuclei are small and round. The cytoplasm does not easily stain, so that the thick wavy glia-fibres which are present in the stroma seem to have no connections with the cell-bodies. Only the gold-sublimate-method of CAJAL made it obvious that the neoplasm was a fibrillar astrocytoma with local disintegration of the tissue.

Examination of cultures.

After 6 or 7 days of incubation, large, polygonal granular cells, disposing of a vivid activity, migrate from the explant. Within a few days many of them show short processes.

Stained or impregnated cultures display polymorphic granular cells with small circle-round nuclei which, although there may be an increase of the cytoplasm, preserve their shape and size. They are fully equal to the nuclei of the original tumour (fig. 19). The protoplasm not seldom encloses one or more vacuoles. Only a small number of cells show short and blunt processes. A reticulum is not formed.

The duration of the cultures amounted only to 12 days. Perhaps due to this fact, the development of long fibres, as we saw them in the original tissue, failed to turn up, perhaps the neoplastic cells were too highly developed to form a culture. We leave this point undecided.

5. *Tumour Bou.* w. 1 year. Astrocytoma of the brain. Highly disintegrated tissue. Astrocytes with very long processes.

Examination of cultures.

After two days of incubation begins the migration of an amount of cells, which gradually become elongated, and with short or more extending processes. Within a fortnight most of the cells are triangular or polygonal, enclosing vacuoles, whereas one or more processes, in every direction, make anastomoses with the processes of other cells (fig. 20). Very large cells with spindle-shaped body of 40 μ breadth and 350 μ length also occur. From the centrifugal pole of some of these cells there protrudes large and finer processes of 200—400 μ length, forming a loose reticulum.

0,03 ccm of isotonic calciumchlorid-solution and of secundary sodium phosphate solution impede each migration of cells. Primary sodium phosphate and particularly potassium chlorid stimulate the development of wide-spread networks in extremely beautiful cultures.

In the fixated and stained cultures all the transitional stages, from small wandering cells to ample, protoplasmatic, polygonal or fan-shaped cells with short thread-like processes and finally to large spindle- or bullet-shaped cells with long branching processes (fig. 21) are present. Many cells are multinucleated.

Cultivation of the explants was stopped on the 26th day, though many cultures were in excellent condition.

From this patient we got, apart from the neoplastic tissue, a piece of apparently healthy white matter of the brain with the remark that the

tissue was lying at a rather great distance of the neoplasm. By cultivation of this tissue we got the same results as with the tissue of the neoplasm. Histological examination of that material afterwards revealed that the apparently healthy tissue contained an amount of neoplastic cells.

OLIGODENDROGLIOMA.

Tumour F. man, 26 years. *Oligodendroglioma* of the brain.

Histologically the tumour is composed of small round cells with scanty protoplasm and round dark nuclei. The cells show but very small processes. Some large glia-cells occur. The tissue contains areas of calcifications.

Examination of cultures.

Only after 12 days appear the first cells in the cultures. They are polymorphic wandering cells which, partly, take rectangular shapes. From the peripheric poles of the cells protrude small pseudopodes.

After this stage triangular or pear-shaped cells with but one or two smooth processes appear. The processes are lightly waving and 4—5 times as long as the cell-body (fig. 22). The cells are similar to those of the original tissue.

The cultures were only examined during cultivation, because, unfortunately, we postponed the fixation for too long a time.

The duration of these cultures amounted to 61 days.

Recapitulation of our results with gliomas.

Four of the five gliomas display, when cultivated, a distinct differentiation of the immature cells of the neoplasm into fully developed glia-cells, whereas the fifth case (Bro), perhaps due to the too short period of cultivation does not develop beyond the stage of isolated wandering cells.

All cultures of the five neoplasms begin with the migration of isolated cells, which only for the trained eye can be recognized as prospective glia-cells. The structure of the cells does not become characteristic until the size of the cells increases and the typical membraneous ectoplasma-forms are present. Only from these cells starts the development of elements which make it obvious that all these different outgrowing cells are to be taken as glia-cells.

Our attempt to cultivate glia-cells out of a piece of braintissue of a child of one year gave no migration at all.

Cultivated glia-cells, arising from the explanted neoplasmas, are similar to those in the human organism, with respect to the shape of the cell-body, the fibrous structure of the cytoplasm and the excentric position of the nucleus. The fragmentation of the nuclei in the outgrowing cells correspond with those fragmentated nuclei, described by different authors in cases of glioblastoma multiforme.

However in respect to the cellprocesses, the histological structure in vivo differs from that in vitro. In vivo the processes may make connections with

the walls of the bloodvessels, or as to HELD [8]), build up the membrana limitans gliae. In vitro the processes do not have the opportunity to fulfill this task. Therefore it is of importance that we could ascertain that the glia-cells in vitro make anastomoses and form a meshwork, adverse to the general opinion that glia-cells in vivo do not make syncytial connections.

Though our experience with oligodendroglioma is limited, we can state that the one examined tumour also in vitro proved to be structured by a peculiar type of glia-cell.

ANGIO-GLIOMA.

1. *Tumour Hul.* man 50 years. *Angio-glioma* at the base of the brain.

The neoplasm is composed of an amount of large and ample blood-vessels, surrounded by protoplasmatic glia-cells (fig. 23). The number of bloodvessels is so large, that it is dubious wether this neoplasm belongs to the haemangiomas with non-neoplastic reactions of the neuroglia, or to the gliomas with non-neoplastic proliferations of the bloodvessels, or with the neoplastic development of glious as well as mesodermal tissue.

Examination of cultures.

After some hours of incubation occurs the migration of a great number of wandering cells, which, within few days, disappear. Then follows an abundant migration of spindle-cells, some of which distinguish themselves by their largeness and plate like shape (endothel-cells). But also small pear-shaped or rhombic cells with long and bifurcating prolongations disclose themselves (glia-cells).

In the stained cultures three types of cells are present: 1. Cells with lumpish cell bodies with indented and regularly curved processes (fig. 24). The nuclei are pale and ovoid-shaped. The protoplasm contains coarse granules and many inclusions. These cells resemble those, present in the cultures of the angioma West, apprehended by us as endothel-cells. As was the case there, the cultures of this neoplasm also reveal small spindle-cells of the usual mesenchymal type. In the third place, bipolar, triangular or pear-shaped cells with long rectilinear branching and anastomosing processes occur. Probably these cells are glia-cells.

The duration of the cultures amounted to 24 days.

The fact that in these cultures the greater part of the outgrowing cells must be taken for endothelium-cells and only a small part for glia-cells pleads for the conception that in this case the angioma has developed as a neoplastic structure and that the surrounding glia-cells are reactive cells rather than neoplastic ones.

MEDULLOBLASTOMAS (NEUROSPONGIOMAS).

By these names we understand neoplastic formations composed of cells, identical with the most undeveloped elements of the neuro-ectodermal

matrix (indifferent cells of SCHAPER [9])). They must be bivalent elements giving rise to glia-cells as well as to nerve-cells. ROUSSY and collaborators [10]) called the neoplasmata, built up by the cells *"neurospongiomas"* in comparison with the neurospongium of the embryonic brain. Presuming that differentiation of the outgrowing cells of cultures, made from this material might occur, we could expect the development of glia-cells as well as of nerve-cells.

1. *Tumour Ja. Medulloblastom* of the cerebellum, built up by round- or oval-shaped medulloblasts. No arrangement in rosettes. In none of the examined preparations of the neoplastic tissue a developed nerve or glia-cell is to be found.

Examination of cultures.

Besides the migration of wandering cells, there are three celltypes present in the cultures. 1. Small cup-shaped cells of 25—60 μ length and 3—20 μ breadth, possessing many short branching processes of fibrous structure, protruding from the border of the cup and with one, sometimes two longer prolongations, arising from the base of the cup. All processes seem to make anastomoses with the processes of cell-bodies of other elements. All processes are beaded, e.g. disclose bud-shaped thickenings along their course.

When these cells move, all processes are retracted, whereas the frontpole displays pseudopodic prolongations. Mitosis frequently occurs. Remarkable is the fact, that during the whole period of cell-division the long process or the two diametrical-lying processes are not retracted (fig. 25). One of the daughter-cells keeps the processes, whilst the other forms new ones.

The second type of cell appears only after 17 days of incubation and is represented by very large pear-shaped cells, 75—100 μ length and 45—50 μ br. From the apex of the cell grows a rather thick process of 1 mm length into the explant, whilst diametrically opposite to this, 8—12 shorter (200—100 μ) processes protrude polyp-like in centrifugal direction, like the neurit and dendrites of the nerve-cell. The cellbody and the parts of the processes next to the cellbody contain an amount of granules. The foremost pole of the cell displays vivid movements of the pseudopodic protoplasm (fig. 26 and 27).

The third type of cell reveals all transitional stages between glioblasts and mature gliacells. The latter possess long rectilineal prolongations, an excentric lying nucleus, sometimes lobated, and fibrillar protoplasm (fig. 28). The duration of the cultures amounted to 36 days.

2. *Tumour Jo. Ja.* man, 6 years. *Medulloblastoma.*

The material, acquired by punction of the cerebellum consists exclusively of neoplastic tissue. This is built up by round-cells, arranged in rosettes. The nuclei possess a heavy chromatinic network, regularly distributed (fig. 29).

Examination of cultures.

Whilst the greater part of the cells in the explant remains inactive and gradually degenerate, there appears after 5—6 days after incubation an amount of characteristic cells, building up cellcolonies of 7—10 $\mu\mu^2$ surface. The cells in these colonies always lie at a distance one from another with wide interstices. The development goes as follows: From the motherpiece arise thin, zig zag running fibres which compose a delicate network by anastomosis. Along this meshwork walk the small round cells of the explant (fig. 30). These cells partly remain small and round, however, they do not leave the meshwork and they seem to take part in the formation of it, so that this reticulum gradually spreads out towards the periphery. Another part of these cells develop into large cells, the size of which gradually increases, so that the peripheral-lying cells in the meshwork are the most developed cells (fig. 31). Like the small cells they form an integrative part of the reticulum. The cell-body is polygonal and about 40—100 μ great. The protoplasm is granular, the nucleus is pale. The fibrils composing the reticulum are smooth and double contoured. Along their course they display small nodules and triangular branching-points of the same structure as the cytoplasm (fig. 31 and 32). Mitosis is not observed. The duration of the cultures amounted to 59 days.

3. *Tumour Nu.* man, 28 years. *Neurospongioma.*

Cystic degenerating neoplasm. The nuclei, containing a heavy chromatinic network are spherically arranged. Conglomerates of nuclei occur. The protoplasmatic stroma does not reveal any nerve- or glia-fibres. Many cavities are filled with colloid. A small amount of mesodermic tissue is found.

Examination of cultures.

After a latent period of 3—4 days, there occurs the migration of many bullet-shaped or pear-shaped cells, partly with very long (ca. 500 μ) more or less branching processes, which disclose themselves very well when they run over an area of liquefaction.

In the neighbourhood of the motherpiece, where the cells lie close together, they arrange as epithelcells. The original tissue gradually mortifies from within to the periphery, whereas the living cells withdraw to the border of the explant, arranging themselves in arches or semi-circles. After four weeks, most of the cells have withdrawn their processes and are rounded.

Fixated and impregnated cultures reveal bullet- and pear-shaped cells, from which arise one or two long fine fibres, branching along their long course (fig. 33).

The duration of the cultures amounted to 42 days.

4. *Tumour Do.* man, 5 years. *Medulloblastoma* of the fourth ventricle.

Very weak and decayed tissue. As there was a lack of any structure and

arrangement of the elements and the protoplasm was not at all differentiated, it was difficult to classify the neoplasm.

Examination of cultures.

Very soon after explanting the migration of wandering cells begins. Heavy granulated cells migrate in dense groups, but the cells always remain isolated and increase but little in size. After about ten days the cells show processes which try to connect with those of other cells. Some cells reveal triangular or hand-shaped forms with rather long processes. In the stained cultures one sees groups of small wandering-cells with coarse protoplasm as described above. Some cells have short processes, other possess long, ramificating prolongations whilst large cells, rectangular and hand-shaped, similar to those described at the SCHWANN cell cultures (see pag. 22) also are present (fig. 34).

The duration of the cultures amounted to 29 days.

EPENDYMOMA.

Tumour Ha. man, 49 years.

Ependymoma, consisting of polygonal or cubic protoplasmatic cells with circle-round nuclei, arranged as neuro-epithelium. The tissue contains many large or smaller cysts and areas of calcareous degeneration.

Unipolar cells with a short or long process are revealed by the HORTEGA method. These cells may be spongioblasts as well as neuroblasts (fig. 35).

Examination of cultures.

Only when the abundance of blood in the explants has for the greater part disappeared, the migration of large triangular cells begins. These cells arrange themselves as a mosaic and assume the shapes of gothic fish-bladder-models (fig. 36), a shape characteristic for SCHWANN cells as we shall see later on. Besides these cells, pear-shaped or triangular cells occur. They have 3—6 rather long processes, arising with a broad base, but rejuvenating into smaller, smooth and rectilinear fibres without buds or nodules. It is regrettable that the shapes and structures of these cells, which we take for glia-cells, only were fixed by photography in the living cultures (fig. 37) and that fixated and stained cultures containing these cells, fail.

In the stained cultures the cells that did develop show no mesodermal features, but are similar to those, described by us as SCHWANN cell cultures (see pag. 22).

The duration of the cultures amounted to 55 days.

The four examined medulloblastomas reveal in the original tissue not the least sign of differentiation, whereas by cultivation of three of them the neoplastic tissues give rise to cells whose differentiation goes in the direction of glious or nervous elements.

As far as results, acquired on pathological cells, hold good for normal

cells, our results in the cases of Jo. Ja. (case 2) where the development of a syncytial meshwork by the outgrowing cells is obvious, support the opinion of BOEKE [11], concerning the syncytial nature of the nervous system.

In the fourth case, the immature cells of the original tumour, are, by cultivation, differentiated into elements, showing in the first stages similarity to glia-cells; at farther development, however, taking the shape and features of SCHWANN cells, as we shall prove afterwards.

Regarding the origin of SCHWANN cells (from the neural crest only or from the neural tube as well) it is interesting to observe that cultures of the fourth ventricle-neoplasm, as well as of the ependymoma, arising from the first ventricle, reveal cells similar to the SCHWANN cell in vitro.

This fact supports the opinion that SCHWANN cells have relationship with neuro-ectodermal cells.

NEUROFIBROMAS.

The cultivation of these tumours was the starting point of our researches. The histological structure of neurofibromas, after all, does not reveal in an obvious way that the ectodermal SCHWANN cell, as is supported by several investigators, gives rise to these neoplasms. For the often abundant fibrous stroma of these tumours stains more or less like mesodermic tissue and the features of the nuclei do not reveal whether these cells belong to the SCHWANN celltypes or to mesenchymal groups of cells.

It is here not the place to put forward the many arguments that try to solve this problem. We only mention the view of HERINGA [12] that the SCHWANN cell is of mesodermic origin and the view of NAGEOTTE [13] taking the mesodermal LAIDLAW's sheath as a product of the SCHWANN cell protoplasm.

By cultivation of neurofibromas we expected to throw a light on this problem.

1. *Tumour v. Str. Neurofibroma* of the acustic nerve.

Characteristic neurofibroma-structure, though no palisading of nuclei. Scanty amount of bloodvessels. Ovoid-shaped cells lie arranged in bundles or sheaths, embedded in a non-fibrous stroma (fig. 38).

Examination of cultures.

Soon after incubation appear small polymorphic cells with pointed angles and of high activity. Some of them possess rather long processes. However, these cells are different from leucocytes, blood-monocytes or mesenchymal spindle-cells. After five days, all cultures reveal, in the nearest surroundings of the motherpiece, many conglomerates of cells or syncytial protoplasmatic plates, which contain different numbers of nuclei (fig. 39) amounting to 600. The surface of the conglomerates amounts to 1100: 400 $\mu\mu$. In this case we did not observe the arising of these formations (contrary to the

next case). However we could observe that isolated small cells fused with each other or with small or large conglomerates; moreover, that parts of the conglomerates are detached to remain independent or to fuse again with other conglomerates. All these changes proceed extremely fast, e.g. within half an hour. The isolated cells increase in size and give rise to more prolongations, which run in different directions and connect with those of other cells, so that a loose meshwork is built up (fig. 40).

These formations were specially found peripherically from the explant. Some cells, however, which migrated far from the explant and could fix themselves on the bottom of the CARREL-flask, were able to form new colonies within an extremely short time. Though mitosis was never observed, it must have occurred, as within 3 or 4 weeks the whole bottom of the CARREL-flask was covered with cellular elements.

The small wandering-cells, most resembling the elements by which the mother-piece is built up, must be taken for the original neoplastic cells. These are small cells with scanty protoplasm, forming pseudopodical processes and showing vivid movements. From these cells up to the final stage of large reticulum-building cells, all transitional stages occur. The amount of protoplasm increases; not seldom it passes into an extensive membraneous extoplasm. Particularly in multinucleated cells this ectoplasm extends concentrically round the endoplasm; in reticulum-building cells, however, it extends unilaterally, so that the typical shape of the papyrus-umbel is displayed (just as in the next case and in the case of tumour Wo. Fig. 10). These cells make anastomoses with other cells in different directions. The endoplasm is coarse-grained, the largest granules lying in the neighbourhood of the nucleus. The whole picture makes it obvious that cultivation of this neurofibroma has given rise to the development of very peculiar cells.

The duration of the cultures amounted to 28 days.

2. *Tumour Po.*, man, 40 years. Neurofibroma-structure with palisading of nuclei. The stroma is fibrous, the fibres stain but faintly with VON GIESON, they impregnate well following the HORTEGA-method. The structure is similar to that of the former case.

Examination of cultures.

After two days of incubation we find in all the cultures a number of small isolated, polymorphic wandering-cells, very active, with many ramificating processes, which elongate during cultivation and connect in all directions with those of other cells, forming a reticular cellular syncytium (fig. 41).

After a few days already appear conglomerates of cells as in the former case. However, in this case extremely large and grotesque or fanciful shaped cells with very large or gigantic vacuoles are displayed (fig. 42), similar to the giant-cells in SCHWANN cell cultures (pag. 24).

In this case too, the conglomerates contain hundreds of nuclei. Here too

we saw a continuous detaching, followed by fusing of fragments of the conglomerates as well as the fusing of a few isolated cells with a conglomerate.

The seventh day after explanting we observed for the first time that these conglomerates are not only built up by cell-division or by fusing, but that the explant expels them as a whole. Like a lava-stream smaller or larger conglomerates flow out at an extremely high rate (fig. 43). Their smallest number of nuclei is 20—50. As such formations are not present in the tissue of the original tumour, it is obvious that they arose during cultivation within the motherpiece, a phenomenon unknown up to now.

In this case too the protoplasm of the cells is honeycomb-like and provided with large areas of ectoplasm, not seldom unilaterally situated, so that papyrus-umbel-shaped cells are displayed (fig. 41).

The duration of cultivation amounted to 51 days.

3. *Tumour Do. De.* woman, 33 years.

Neurofibroma of the cauda equina, arising from the dorsal root of SI or SII.

The tumour was encapsulated and contained obviously the remainings of the root.

Histological examination reveals many well-maintained nerve-fibres. The bundles of fibres are accompanied by elongated large plasmatic cells with elongated nucleus. The peripheric part of the cytoplasm contains vacuoles and fatgranules. The arrangement of the cells is bundle-like. Obviously these cells have the characteristics of SCHWANN cells. However, the tumour is for the greater part built up by smaller elements, which disperse the bundles of fibres (fig. 44). The cytoplasm of these cells, not staining with haematoxylin, is filled up by fatty degenerated granules. Probably due to the accumulation of these products of disintegration, the cells are rounded and show indented nuclei. However, it occurs that the cytoplasm shows very small processes which form, together with processes of other cells, a small syncytial loose reticulum, as described by ANTONI. Palisading of nuclei was lacking.

The presence of many healthy fibres, the amount of products of disintegration, the lack of mesenchymal structures, and the clinical fact too that symptoms of the disease appeared only a short time before operation, justify the view that the neoplastic growth was of recent date.

Examination of cultures.

After 24 hours already the migration of numerous polymorphic wandering cells is disclosed. The cells are irregularly shaped and differ obviously from leucocytes and lymphocytes. After two or three days an amount of small or larger cell-groups appears. We could observe that these conglomerates as a whole are detached from the explant. The dispersed conglomerates give rise to the above described wandering cells. From them pro-

trude processes which however generally do not reach those of other cells, so that at a few places only a reticulum is formed.

In impregnated cultures the polymorphic character of the isolated cells is clearly revealed. They show small cells with scanty protoplasm and short processes, as well as large cells containing non-fatty granules with fan-shaped ectoplasm. They are similar to the glia-cells or SCHWANN cells in vitro (fig. 45 and 46).

The duration of the cultures amounted to 24 days.

The cultivation of the three neurofibroma's teaches us that, as far as we know, there are no mesenchymal cells arising from the explants, but cells of the following characteristics:

1. Agglomerating, e.g. forming of small or greater groups of cells, as it sometimes occurs in cultures of glioma's.

2. Honeycomb-like protoplasm, like the protoplasm of SCHWANN cells in vitro.

3. Membraneous ectoplasm, similar to that of glia-cells in vitro and of SCHWANN cells in vitro, when they are isolated.

4. Cellbody is found triangular, to papyrus umbel-like shaped, as is the case with cultivated glia-cells or SCHWANN cells.

5. No excentric laying nucleus contrary to the position of the nucleus in glia-cells.

6. Formation of reticulum. The development of the reticulum differs from that of mesenchymal networks in so far as the processes of neurofibroma-cells, arising from any possible point of the case, extend in all directions to fuse with other cells at any possible point, whereas the mesenchymal processes always arise from the cell poles to fuse with the pole of the next cell. Consequently the neurofibroma-cells disclose an *all-sided cytotropy*, whereas the mesenchymal cells disclose a *bipolar cytotropy*.

However, the reticulum of the neoplastic neurofibroma-cells differs from that developed by glia-cells in vitro, viz, the processes of the neurofibroma-cells do not run a long way before connecting with other cells, but make connections as soon as possible in the next surroundings of the cell. Consequently the cytotropy between neighbouring cells must be of a high degree, causing real anastomoses between the cells, though, after due time they can be loosened. Comparison e.g. with the very active and mobile cells in cultures of the spleen, reveals that these latter cells continuously make contacts with other cells, however, after some seconds, release themselves, or are expelled; a phenomenon which also occurs in cultures of sarcoma-cells.

Resuming, we state that the cultures of neurofibromas show relationship with the cultures of SCHWANN cells derived from the sciatic nerve of a rabbit, but that they are not identical with them. The SCHWANN cell cultures lack the agglomeration of cells, the neurofibroma-cultures lack the density

of the outgrowing tissue. Finally we may only conclude that the cultivated neurofibroma-cells do not behave as mesenchymal, but as neuro-ectodermal elements.

COMPARABLE MATERIAL.

The following normal and pathological material was available:

1. *Tumour Schou. m.* Metastasis of adeno-carcinoma in the brain.

Examination of cultures. From the sanguineous explant first migrate an amount of small wandering cells, which partly degenerate, partly transmute into macrophages and giant cells. Some spindle-cells too occur. However, after two weeks, all mesenchymal cells in the cultures have disappeared. Between the seventh and the eighteenth day characteristic epithelium-cells are disclosed and epithelial membranes are formed (fig. 47). The cells are irregular-shaped.

Duration of the cultures amounted to 51 days.

2. *Tumour Smi. Adenoma* of the hypophysis.

Examination of cultures.

Many small wandering cells, arising within few days from the explant, disappear within a week. After 17 to 20 days the explant forms thick cellular protrusions, developing into large characteristic epithelial membranes with irregular shaped cells as in case I (fig. 48).

The duration of the cultures amounted to 86 days.

3. *Tumour Fle. Cholesteatoma.* Disintegrated tissue with an amount of cholesterin-crystals. No outgrowth within 27 days occurred.

4. *Granulation-tissue Aan.* m. 36 years. Tuberculosis of the spine.

Examination of cultures.

After four days the borders of the explants reveal some uncharacteristic cells, that do not change after prolongated cultivation.

The cultivation lasted 41 days.

5. *Epilepsy Ve.* w. 36 years. Brain-tissue with light inflamatory reactions.

Examination of cultures.

After 20 days of incubation the migration of small wandering cells is disclosed. The cells do not change during two weeks afterwards, they show the typical forms of lymphocytes in vitro.

The cultures lived 44 days.

6. *Grey matter of the brain. Bou.* w. 1 year.

Only one, out of 12 cultures, after 7 days of incubation, revealed one single spindle-shaped cell, which degenerated very soon.

Observed 21 days.

7. *Embryonic brain-tissue.*

Cultivation of embryonic tissue of the brain (3—6 months) too, did not give rise to characteristical nervous elements, when the conditions were the same as in those of the tumours.

8. *Mesenchymal cells.*

To compare with the tumour-cultures of mesenchymal origin, we had the disposal of spindle-cell-cultures and endothel-cell cultures of the omentum, ovarium and thyroid gland of human adults. We also disposed of cultures of leucocytes. The features of these cultivated human cells do not differ essentially from those of animals (fig. 49), only that, with our methods, the cells are sometimes more ponderous and more granular as is usual in cultures of fibroblasts of the chick.

9. SCHWANN cells in vitro.

Since the investigations of INGEBRIGTSEN [14]) this kind of cell has only been cultivated by MURRAY, STOUT and BRADLEY [15]) with MAXIMOWS technique.

Method of cultivation.

Under ether-narcosis the sciatic nerve of an adult rabbit is sectioned, and the central stump ligated. After 7—8 days, pieces of the peripheral stump, and, for comparison, also pieces of the central stump and pieces of a normal healthy nerve are removed and brought in artificial rabbits ascites-fluid, following to HAMBURGER–DE HAAN [16]). With fine tweezers they are stripped of the perineural sheaths. The bundles of fibres are unravelled and stripped of the endoneurial sheaths. The fibres are sectioned in pieces of 2 mm length.

The cultivation, with exception of some cultures in the hanging drop, took place in CARREL-flasks on small pieces of mica or cellophan or celloidin (DEMUTH [17])), with 0,5 ccm rabbits plasma (with heparin, acquired by heart-punction) and 0,5 ccm of artificial rabbit-ascites as solid medium and 0,1 ccm ascites-fluid as fluid medium. This medium is perpetual, that is to say, the cells can be cultivated in it, for an indefinite time.

The great advantage of this material was, that, by using the peripheral stump we got from the beginning pure cultures (in 324 cases 316 times).

The duration of the cultures amounted to 275 days.

Examination of cultures.

The latent time before the first cells are migrating from the motherpiece, varies from some hours to 7 days. After removing in a new flask, the latent time decreases to 2 days.

From the borders of the nervefibres in the explant cells are detaching themselves.

They migrate radially into the solid medium and proliferate very fast, e.g. the extent of the cultures in mm² amounted to:

Nr.	0	4	6	8	11	13	15	17	19 days
28549	2,2	3,5	6,2	12,2	33,2				
28550a	1,4	4,4	9,7	15,0	33,7	37,4	41,4	51,0	85,3
28550b	1,4	2,3	5,2	11,0	12,8	26,6	39,1	89,0	103,6
28559c	1,1	1,1	1,8	2,1	7,8	27,2	38,7	59,4	68,6
28555a	5,0	5,2	8,7	31,5	41,4	45,5			
28555b	3,0	3,8	7,0	22,4	28,7	34,7			
28555c	3,6	3,6	6,0	12,3	20,0	38,6			
28557	1,8	3,1	6,8	10,5	20,2	31,2	41,8	46,8	74,3
28568	3,0	3,0	4,5	6,2	8,2	14,2	29,4	45,3	73,0
28578	2,6	2,6	2,6	3,7	7,3	12,0	30,2	53,6	81,5

When four cultures are incubated together in one CARREL-flask, they push together after about 3 weeks and after the fourth week the whole bottom of the flask is filled up with cellular elements. However in plasma alone too the rate of growth is not essentially lower, and we did not in any way succeed in decreasing it.

In the area of growth the cells arrange themselves bundle-shaped. These bundles always run in faintly curved lines (fig. 50), sometimes whorls are formed. The border of the cultures is smoother as those of mesenchymal cultures, because the occurrence of spindle-shaped cells at the border is rare.

Contrarily the border-cells are wedge-shaped, with the basis of the cellbody turned to the periphery. Whereas in mesenchymal cultures the elongated cells of the border, as a rule, are one by one protruding into the medium, surrounding the explant, the migration of the SCHWANN cells occurs in bundles of cells, lying next to each other, so that large tongues of tissue are formed (also similar to epithelial cultures). When some cells, e.g. in areas of liquefaction, are isolated, as soon as the area is filled up by plasma or ascites-fluid, the cells give rise to new cells, from which, in each direction, processes grow out, forming together a dense meshwork of cellular elements. These cultures make it obvious that the SCHWANN cells are *all-sided cytotropic* (fig. 51). We often found a dense part of the grown-out tissue surrounded by isolated cells. The outgrowing cells in the SCHWANN cell- cultures display the most various shapes, hardly to be found in cultures of other cells. The most characteristic are always the cells lying at the border of the explant. However, among those cells there are but few that show the typical forms in a clear way. The pictures change so fast that only after repeated observation of the same cells, it is possible to acknowledge the less characteristic cells as well.

The most typical shape of the outgrowing SCHWANN cell is like the obstetric-hand-shape or the pointing-hand-shape (fig. 52). The cellbody

is elongated triangular. The point of the triangle is stretched and directed to the centre of the culture. From the basis protude about 5 processes, broad-based, but soon narrowing and pointed, processes which, usually, are of different length, in such a way that the length decreases continuously from one side to the other. Next to these we found transitional stages of such cells to cells with one process or to wedge-shaped cells with short pseudopodes (fig. 55), hardly different from undifferentiated mesenchymal cells ("fibroblasts" of the cultivators). The elements in the inner zone of the cultures as well show a superficial similarity with these "fibroblasts", though generally they are more prolonged-rhombic and not spindle-shaped. Often they are excavated at two or more sides or in the shape of gothic fish-bladders, so that the trained eye can discern them easily, especially in living cultures, whereas by fixating and staining of the cultures the differences in shape become less obvious (DEMUTH [18]).

However, divergences of this groundform occur in three directions. First there are cells with rather long processes, smooth or waving and branched at the end (fig. 53), giving rise to complicated networks. They are similar to nerve- or glia-cells, though by silverimpregnation nerve- or glia-fibres were not revealed. The second diverging feature regards the manner of growing which is characteristic for in vitro growing epithelium (fig. 54) viz: the cells are polygonal or fishbladder-shaped and are arranged as paving-stones. The border of such cultures can be rather smooth as is the case in epithelium cultures. Regarding the origin, the building of epithelium might be possible, but the fact that such growths always are transformed in due time into characteristic SCHWANN cell-cultures, traced by photography, proves that the growth is no real epithelium (fig. 55). The SCHWANN cells all the same have the tendency to arrange themselves pavement-like, viz with the broad sides joining together, contrary to the arrangement of mesenchymal cells (fig. 49).

Finally the third diverging feature regards the cell-form during migration, as can be observed in all kind of cultures. The cells detach themselves from their connections with neighbouring cells and migrate as isolated cells into the medium. Whereas wandering-cells of most kind of tissues, e.g. mesenchymal tissue, have a round ground-form, especially when they transform in macrophages, the SCHWANN cells preserve as wandering cells too their triangular or quadrangular shape. Provided with one or more processes, which generally are more delicate and longer than the pseudopodes of the leucocytes, these wandering SCHWANN cells not seldom resemble nerve- and glia-cells (fig. 56). Transformations of these cells in multinucleated vacuolized giant-cells occur. Their grotesque forms are similar to those described in the cases of neurofibromas (fig. 57). The development of these grotesque cells is provoked by different stimuli.

The protoplasm of all these types of cultivated SCHWANN cells is spongy and coarse. The processes have the same structure. The nucleus is oval-shaped or round; generally it is flattened at the poles and thicker than the

nucleus of mesenchymal cells. The chromatine network is regularly distributed through the nucleus (fig. 58). The following facts prove that the cells, described above and acquired by cultivation, are SCHWANN cells:

1. To get pure cultures of SCHWANN cells, it is necessary to subject the nerve to a WALLERian degeneration. Cultivation in the same way of pieces of a normal nerve only gives rise to a small amount of spindle-cells, some of which may be SCHWANN cells. However, the building up of a real culture of cells fails to turn up.

Cultivation of the central stump of the sciatic nerve, sectioned 7 days before, so that there is also degeneration in the motherpiece, may indeed give rise to extensive cultures, however they are built up partly by SCHWANN cells, partly by mesenchymal spindle cells, the difference between both these elements being obvious *).

2. In regard to the function of both kinds of cells, there is a difference: 0,03 or 0,1 ccm of an extraction of half a rabbits brain with 20 cc of ether, added to a mixed culture of SCHWANN cells and spindle-cells causes within two hours the death of all the SCHWANN cells, whereas the mesenchymal cells are maintained. Should some of them be damaged, which is not to be wondered at in a medium of an big amount of degenerating cells, they are restored after a few days, so that pure cultures of the mesenchymal cells can be developed. 0,03—0,1 ccm of ether alone does not give any reaction. A watery extract of the other half of the rabbits brain (20 ccm of water), or 1 ccm of a watery colloidal solution of lecithin 1 : 1000, does not in the least alter the beautiful SCHWANN cell cultures.

0,1 ccm of a solution of cholesterin in ether 1 : 1000 gives no visible alteration. However, 0,1 cc of a solution of cholesterin in oleum olivarum 1 : 1000 causes a decrease of growth, whereas the cells become large and ponderous. However, after some weeks, when the oil has disappeared, the cells become smaller and disclose e.g. after 47 days particular delicate forms.

0,03 or 0,1 ccm of ergosterin, solved in ether 1 : 1000 damages the cells. 50—100 γ Vitamin B1 does not influence the rate of growth or the form of the SCHWANN cells.

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*) In this point our experiments differ from those of MURRAY, STOUT and BRADLEY. Perhaps our better results may be due to the fact that only after the 7th or 8th day after sectioning cultivation of the nervesubstance was started. After a second week namely it is not so easy to get pure SCHWANN cell cultures, because in the meantime reactive cells and fibroblasts have penetrated between the nervefibres.

Comparison of our experiences with those of these investigators is hardly possible as material and technique are too different. The cells which are taken by these authors as SCHWANN cells might probably have been observed by us, but only in zones of liquefaction, which seldom occurred in our cultures. In such areas, very fine cells, with long bipolar processes are disclosed; the form of these cells is due to the high pressure to which the cells are exposed when bridging the liquefaction-zone. But especially this form resembles that of the mesenchymal cells, so that we might reserve our judgment.

If fresh nervous tissue, e.g. from the lumbal medulla, is placed at a small distance from fresh cultures of degenerated sciatic nerves, generally none of the tissues grow out. Sometimes there arises a small culture of SCHWANN cells at the side turned away from the medullar tissue, and a migration of some mesenchymal cells from the medullar explant.

A piece of fresh medullar tissue added to a beautiful SCHWANN cell-culture causes immediately the death of many SCHWANN cells and the culture restores only after two or three weeks.

Consequently we must state that fresh nervous tissue contains a substance, solvable in ether, which causes a decrease in growth of SCHWANN cells, but becomes inactive in case of degeneration of the nervous tissue. These observations agree with the conditions in the living organism. Indeed these circumstances prevented that nervefibres from the medullar explant penetrated into the SCHWANN cell cultures. They too prevented the SCHWANN cells to assume the shapes and features of those in vivo. Watery extracts of nervous tissue de not give a growth-decrease, but bring about a very fine reticulum, which resembles in a high degree the reticulum, built up by glioma-cells in vitro. As the SCHWANN cells are the pioneers for the regenerating nervefibres, we may have an important hint for the possibility of clearing a way for regenerating nervefibres. In regard to the peculiar circumstances of regenerating nerves to carcinomous tissue (JULIUS [27])), this may of high importance.

3. The caracteristics of the SCHWANN cells in vitro, contrary to the big changes, which undergo other cells by cultivation, e.g. epithelium of the liver, correspond in a high degree with the characteristics of the SCHWANN cells in vivo, viz: spongy protoplasm (DOINIKOW [19]), NAGEOTTE), forming of syncytium or of sheaths (NAGEOTTE), sinus-shaped cell-borders in the case of artificially, by nervesection acquired, SCHWANNoma's (MASSON [20])), relationship, regarding the form, with nervecells (BUNGNER [21]), APATHY [22]), BETHE [23])) and with gliacells (HARRISON, SCHIEFERDECKER [24]), HELD [25])), ovoid-shaped nucleus with an abundance of chromatin (NEMILOFF [26])). It is remarkable that so many of the cytological features are maintained in vitro. Comparison with other cells e.g. cartilaginous- or osteoid-cells, epithelium-cells etc. of which, notwithstanding maintained specific potencies by cultivation, the forms are changed in such a high degree that the cells become unrecognizable, suggests that the SCHWANN cells, next to nerve-cells and glia-cells, in regard to their form, maintain in vitro a high degree of specificity. The capacity to develop forms similar to those of nerve-cells or glia-cells under the particular conditions of cultivation, and also the potency to form epithellike membranes, may support the view that all three the kinds of cells have the same origin, viz. the neuro-epithelium. Only the lack of fibrous structures distinguish the SCHWANN cells from the nerve- and glia-cells

It is obvious that SCHWANN cells must be found in all the tissue material,

containing nerves with SCHWANN-sheaths or their mothercells. Indeed we found in many other cultures e.g. in livercultures and retina-cultures and especially in cultures of the ventral horn of the dorsal and lumbal medulla SCHWANN cell-like elements. However the most characteristic were the cultures of the suprarenal gland and of the sympathetic nerve of the rabbit. Cultivations, made from that material, disclosed nearly pure cultures of SCHWANN cells with all the typical features of them, whereas all transitional stages from epithellike or reticulum-forming cells to hand-like cells were visible.

DISCUSSION.

Our results are based above all on the facts that, under the accepted conditions in vitro a morphological visible differentiation occurs. This especially occurs with tumourcells of neuro-ectodermal origin. Less obvious it is with tumourcells of mesodermal origin, whereas the neurofibromas display an exceptional specific cell-form, as has not yet been observed in vitro till now.

Differentiation in vitro facilitates to establish the histological diagnose. The cultivation permits to pursue continuously a development which only can be presumed by histological research, e.g. the observation of the transformation of spongioblasts into mature glia-cells and of medulloblasts into spongio- or neuroblasts.

The observation of the living cell too may give us a decision in questions as e.g. the different kinds of cytotropy, (polar or allsided); the forming of a syncytium by glia- and nervecells. But above all the cultivation teaches us to acknowledge common features of different kinds of cells, e.g. the relationship between SCHWANN cells and glia-cells and the ectodermal origin of neurofibromas.

Besides the possibility to discern morphological differences by cultivation, there seems to be the possibility to discern functional differences of tumour cells of different origin. Primary sodium phosphate and potassium chlorid give a decrease of growth for meningeomas, an increase however for gliomas, whereas it stimulates the development of a network. If these observations would be confirmed further on, they may lead to useful consequences.

Asking, why, by the cultivation of tissue originated from the neuroectoderm, we got advanced differentiation, contrary to many observations of other investigators, so it may be supposed to be due to our cultivation method. It is probable, but not sure that differentiation is stimulated when no embryonic juice is applied. Our conceptions of the activity of embryonic juice, due to year long experience, have changed. But it would lead us too far to expound our view here. We want only to believe that the results acquired by cultivation of the classic chick-fibroblasts are not applicable to the cultivation of human tissue and tissue of other mammals.

The age of the plasmaspender does not matter. Generally the plasma of

one of us (50 years) was taken, in order to work with the same medium.

Though the age of the patient lies between 1 year and 70 years, we could not establish the smallest relation between age and growth.

Other factors, as damaging during operation (electro-coagulation) may be responsible for totally negative results, which otherwise only occurred in two of our cases. A long stay in the refrigerator, up to 3 days, does not harm, on the contrary, it seems to stimulate the over-growth of neuro-ectodermal elements in regard to the mesenchymal ones. Sex and blood-group of patients and bloodspender do not seem to play a particular part, which otherwise, as far as experienced by us, also holds for other non-neoplastic human tissue.

We consider it of great importance for the results acquired, that also apparently hopeless cultures were attended further on, as it turned out that also after a latent-period of three weeks an outgrowth of the explant could be observed.

There finally remains the question whether the cells appearing in vitro are really tumourcells. The experimentum crucis, viz. grafting of pure cultures on a human being was but one time applied. A culture of neuro-fibroma (case Po) was grafted subcutaneous on one of us. The result was after fourteen months a negative one, it did not prove anything.

For the mesenchymal tumours the histological picture of the cultures do not give any evidence. In any case there is often a striking resemblance between the shapes of the cell and the nucleus of the original tumour and those of the cultures.

If, however, a neuroblastic tissue does not contain any nerve-fibre, whereas in the cultures of this tissue there occurs a development of nerve-cells, e.g. of the type of PURKINJE cells, so we may take it for granted that the neoplastic cells of the original tissue have given rise to these cells. The same applies to the arising of glious elements from immature spongioblasts and to the peculiar cells arising from neurofibromas. Moreover there is the fact that non-neoplastic human brain tissue, under the circumstances settled, has not yet given rise to the migration or growth of nerve- and glia-cells in vitro. Our researches might be valued in one practical sense, viz. we could demonstrate that by cultivation of material acquired by brain-punction the diagnose of the neoplastic tissue could be made as well.

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DESCRIPTION OF THE PLATES.

PLATE I.

Fig. 1. Meningeoma Aa. Masson B., 1 : 200.
Fig. 2. Meningeoma Aa. Culture, 20 days, silver-impregnation, 1 : 35.
Fig. 3. Meningeoma Hey. Masson B., 1 : 350.
Fig. 4. Meningeoma Hey. Culture, 16 days, Masson B., 1 : 300. Large plate-shaped concave cell with processes curved in the same direction.
Fig. 5. Meningeoma Hey. Culture 26 days, fix. silver, 1 : 350. Network, built up by the cells.
Fig. 6. Haemangio-endothelioma West. Masson B., 1 : 250.

PLATE II.

Fig. 7. Haemangio-endothelioma West. Culture, 13 days, living, 1 : 225. Very large cell with vacuolized nucleus to compare with spindle-cells of normal size.
Fig. 8. Haemangio-endothelioma West. Culture, 26 days, fix. silver, 1 : 110. Two concave cells with processes curved in the same direction.
Fig. 9. Spongioblastoma Wo. silver, 1 : 175.
Fig. 10. Spongioblastoma Wo. Culture, 6 days, living, 1 : 400. Wandering-cells with short processes. Three cells fan-shaped or papyrus-umbelshaped.

PLATE III.

Fig. 11. Spongioblastoma Wo. Culture, 40 days, fix. Masson B, 1 : 250. Glia-cells with excentric lying nuclei and long processes.
Fig. 12. Spongioblastoma Wo. Culture, 8 days, living, 1 : 400. Dense layer of cells with elongated, cylindric shapes.
Fig. 13. Spongioblastoma multiforme. de Ro., Masson B., 1 : 250.
Fig. 14. Spongioblastoma multiforme de Ro., Culture. 11 days, living, 1 : 225. Proliferation of glia-cells.
Fig. 15. Spongioblastoma multiforme de Ro., Culture, 16 days, fix. silver, 1 : 350. Rosettes of nuclei. See text.

PLATE IV.

Fig. 16. Spongioblastoma multiforme de Ro., Culture, 16 days, fix. silver, 1 : 350. Fibres running through the ectoplasm.
Fig. 17. Glioma Schr. Culture, 18 days, fix. haem DELAFIELD, 1 : 110. Network of long fibres.
Fig. 18. Glioma Schr. Culture, 18 days, living, 1 : 225. Many long fibres.
Fig. 19. Astrocytoma Bro. Culture, 12 days, fix. silver, 1 : 225. Small round nuclei. Vacuolized protoplasm.
Fig. 20. Astrocytoma Bou. Culture, 14 days, living, 1 : 225.
Fig. 21. Astrocytoma Bou. Culture, 10 days, fix. Masson B., 1 : 225.

PLATE V.

Fig. 22. Oligodendroglioma F. Culture, 26 days, living, 1 : 225.Smooth and regularly waving processes.
Fig 23. Angioglioma Huls. Masson B., 1 : 110.
Fig. 24. Angioglioma Huls. Culture, 20 days, fix. silver, 1 : 110. Endothel.
Fig. 25. Medulloblastoma Ja. Culture, 11 days, living, Cell in mitosis, (equatorial plate) with preserved processes, 1 : 450.
Fig. 26. Medulloblastoma Ja. Culture, 18 days, living, 1 : 450. Purkinje-cell typ.
Fig. 27. Medulloblastoma Ja. Culture, 19 days, fix. haem. Del., 1 : 250.
Fig. 28. Medulloblastoma Ja. Culture, 19 days, fix. Masson B., 1 : 225. Glia-cell-types.

PLATE VI.

Fig. 29. Medulloblastoma Jo. Ja. silver, 1 : 170.

Fig. 30. Medulloblastoma Jo. Ja. Culture, 20 days, fix. silver, 1 : 120.
Fig. 31. Medulloblastoma Jo. Ja. Culture, 27 days, living, 1 : 225.

PLATE VII.

Fig. 32. As fig. 30, 1 : 225.
Fig. 33. Neurospongioma Nu. Culture, 11 days, fix. silver, 1 : 250.
Fig. 34. Neurospongioma Do. Culture, 20 days, fix. silver, 1 : 250.
Fig. 35. Ependymoma Ha. Haem., 1 : 110.
Fig. 36. Ependymoma Ha. Culture, 7 days, SCHWANN cell-like elements, 1 : 150.
Fig. 37. Ependymoma Ha. Culture, 10 days, living, 1 : 225. Glia-cell-typ.

PLATE VIII.

Fig. 38. Neurofibroma v. Str. von GIESON, 1 : 110.
Fig. 39. Neurofbiroma v. Str. Culture, 29 days, living, 1 : 80. Various small and one very large conglomerate of 500—600 cells.
Fig. 40. Neurofibroma v. Str. Culture, 12 days fix. silver 1 : 250. Reticulum.
Fig. 41. Neurofibroma Po. Culture, 8 days, fix. silver, 1 : 250. Conglomerates, reticulum and various cells papyrus-umbel-shaped.

PLATE IX.

Fig. 42. Neurofibroma Po. Culture, 21 days, living. 1 : 150. Cell-conglomerates with very large vacuoles.
Fig. 43. Neurofibroma Po. Culture, 9 days, living. A cellconglomerate flowing out of the motherpiece. Other conglomerates are already lying in the surrounding medium.
Fig. 44. Neurofibroma Do. De. Haemat., 1 : 110.
Fig. 45. Neurofibroma Do. De. Culture, 14 days, fix. silver, 1 : 225. Honeycomb-like protoplasm.
Fig. 46. Neurofibroma Do. De., 14 days, living, 1 : 120. Wandering cells partly in the shape of the papyrus-umbel.

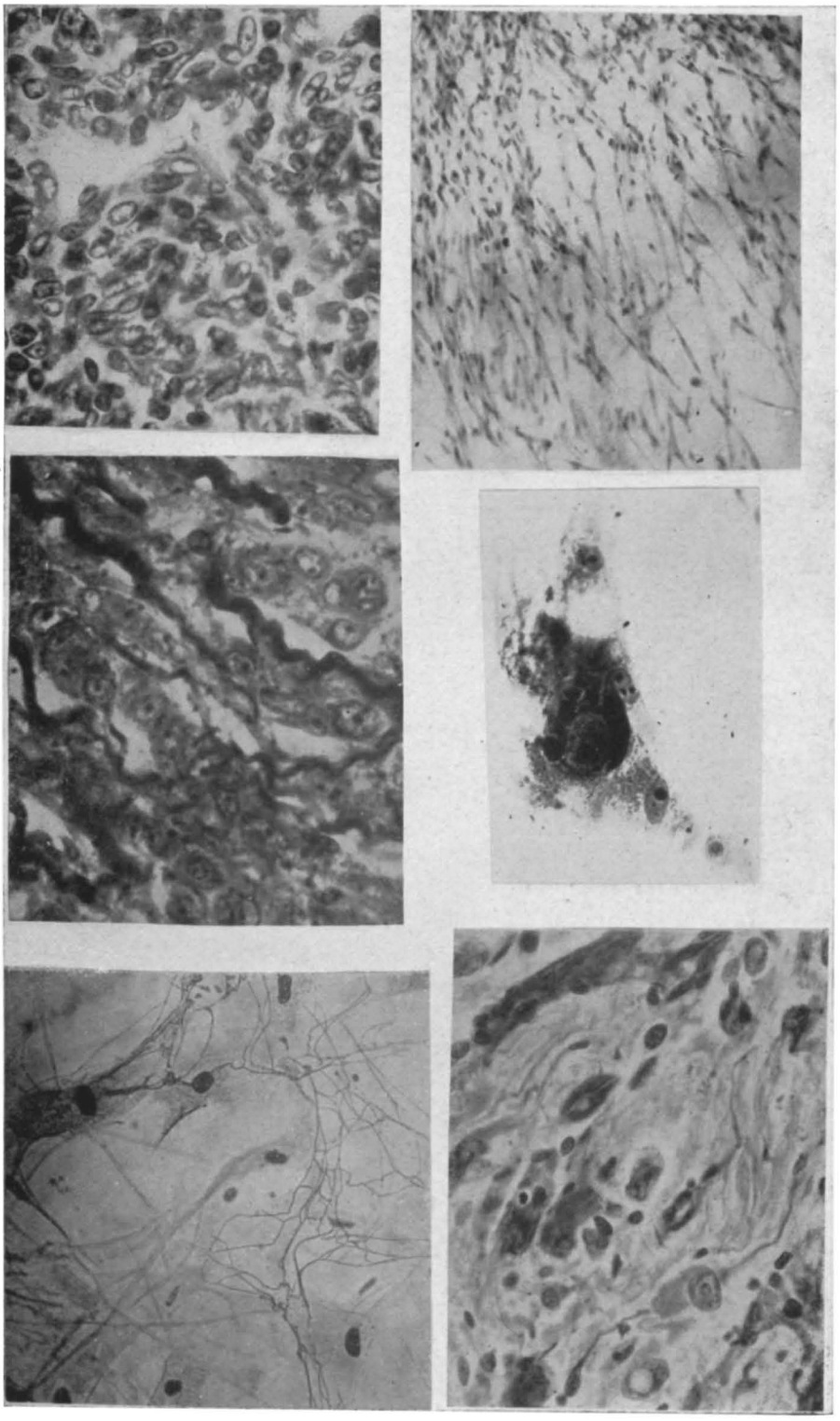
PLATE X.

Fig. 47. Carcinom, metastasis in the brain. Schou.Culture, 26 days, living, 1 : 225. Epithelial membrane. Cells of irregular shape.
Fig. 48. Adenom of the hypophysis. Sm. Culture, 20 days, living. Epithel-tongues.
Fig. 49. Omentum of human adult. Culture, 12 days, fix. silver, 1 : 225. Typical mesenchymal spindle-cells.
Fig. 50. SCHWANN cells of the rabbit. Culture, 56 days, fix. silver, 1 : 110. Outer part of the dense area of growth. Arrangement of the wandering cells in whorls and sheaths.
Fig. 51. SCHWANN cells of the rabbit. Culture, 175 days, living, 1 : 120. Arrangement pavement-like and net-form.

PLATE XI and XII.

Fig. 52. SCHWANN cell culture, 4 days, living, 1 : 200. Cells hand-like shaped.
Fig. 53. SCHWANN cell culture, living, 22 days. Cell-form as those of glia-cells or nerve cells.
Fig. 54. SCHWANN cell culture, 6 days, living, 1 : 225. Epithel-like arrangement. Gothic fishbladderforms.
Fig. 55. SCHWANN cell culture. The same as Fig. 54, 5 days later, 1 : 120.
Fig. 56. SCHWANN cell culture. 78 days, fix. silver, 1 : 225. Cells similar to wandering glia-cells.
Fig. 57. SCHWANN cell culture, 35 days. Conglomerate of cells with giant vacuoles, living.
Fig. 58. SCHWANN cell culture, 39 days, fix. Masson B., 1 : 450.

PLATE I.



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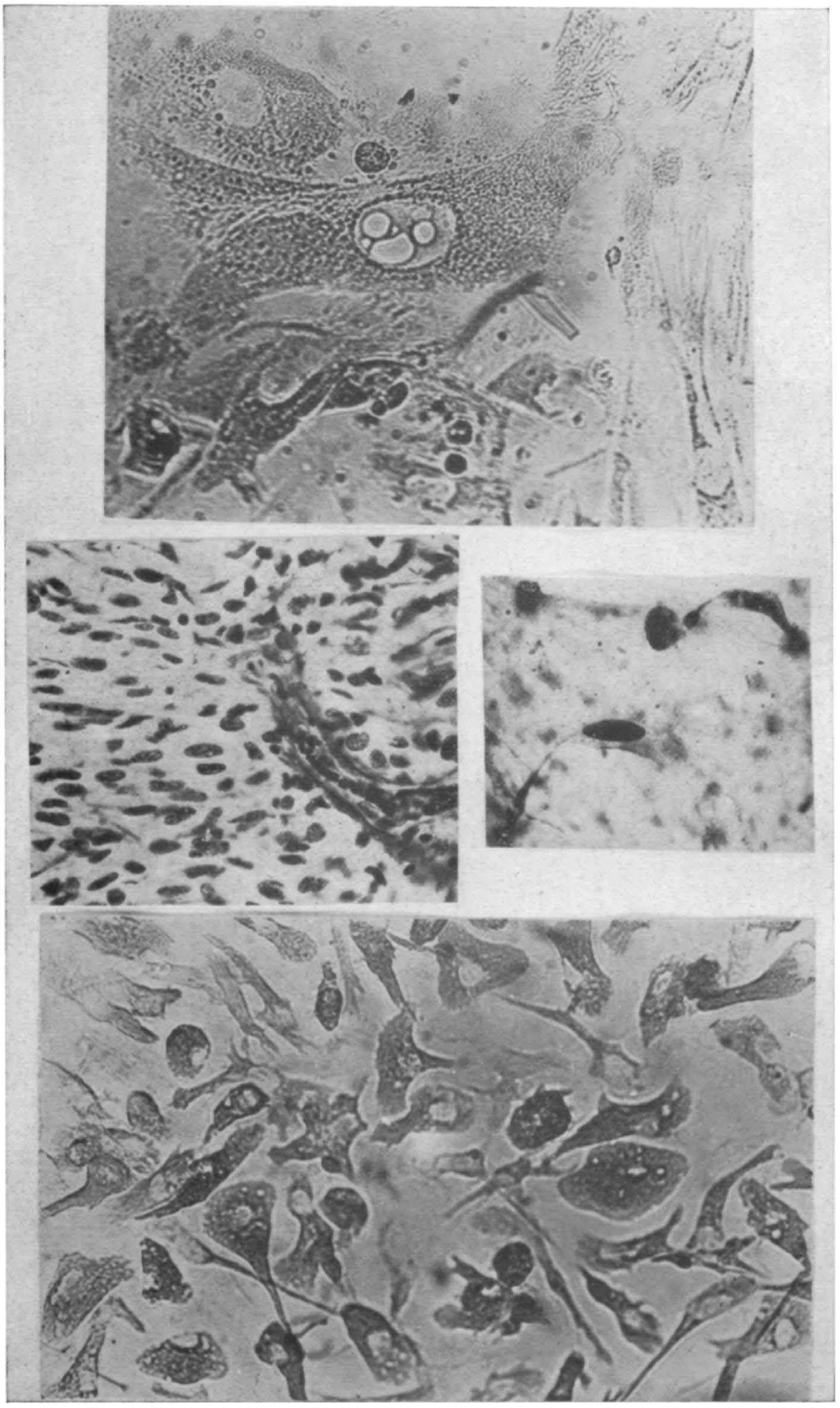

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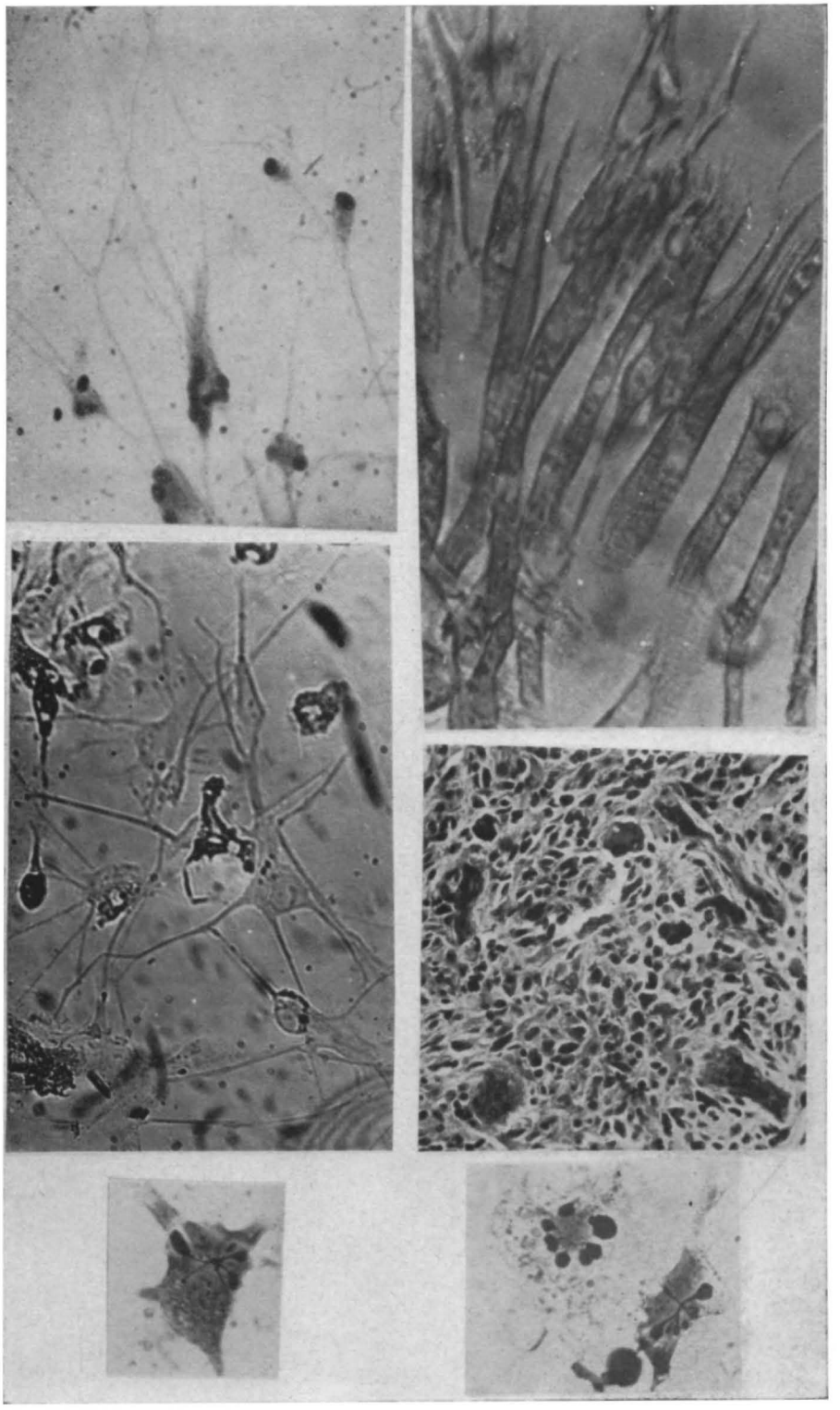

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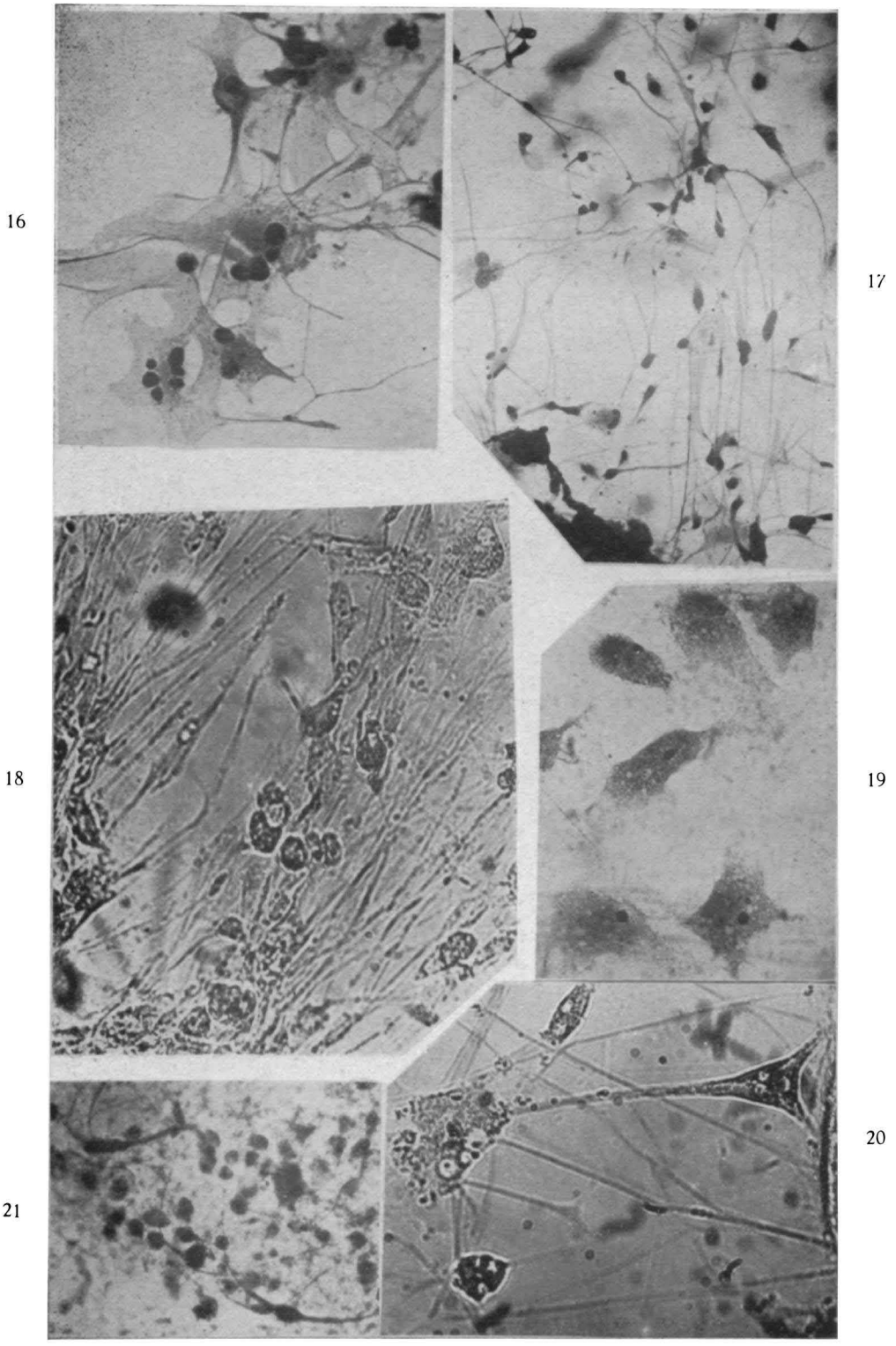

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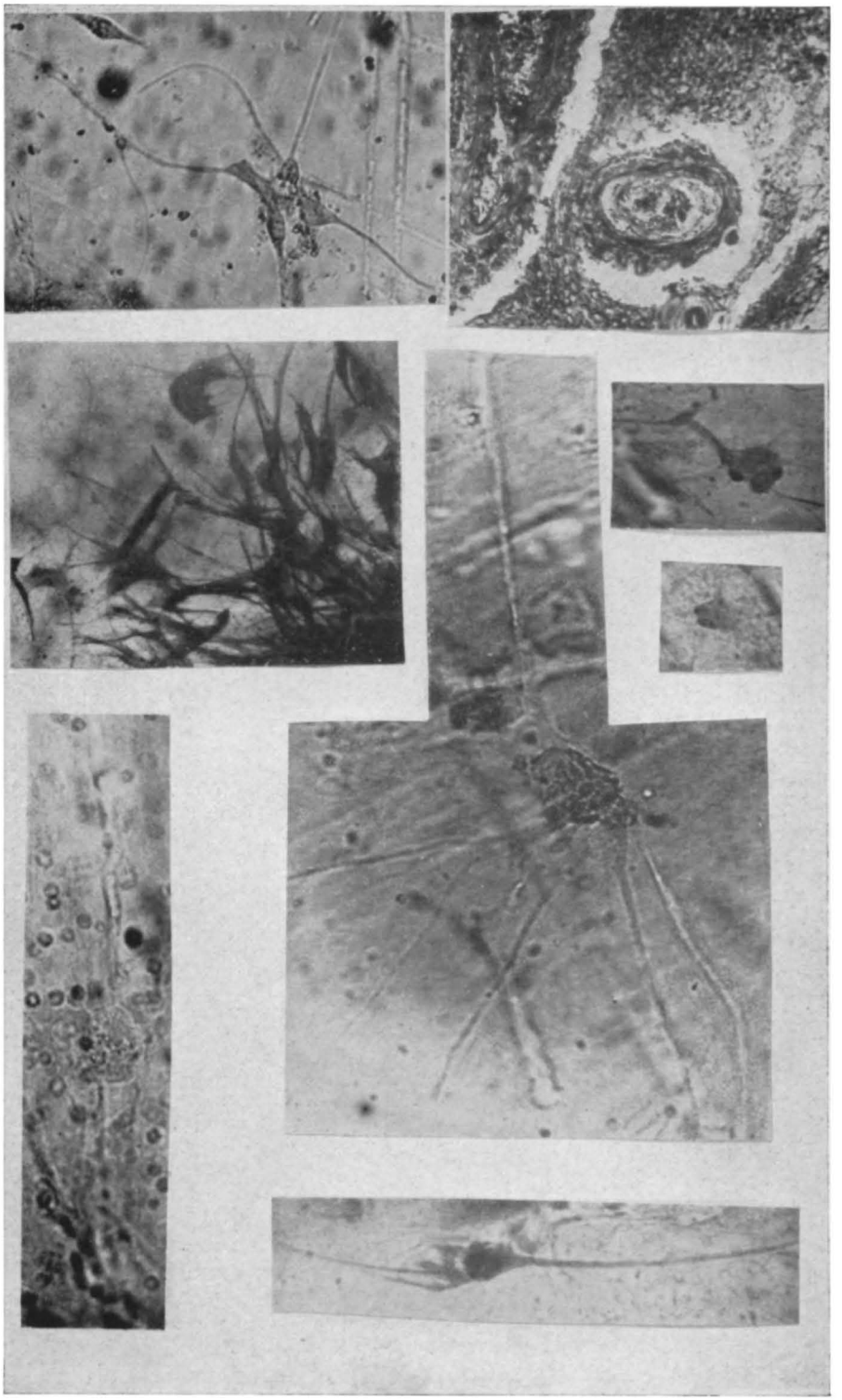

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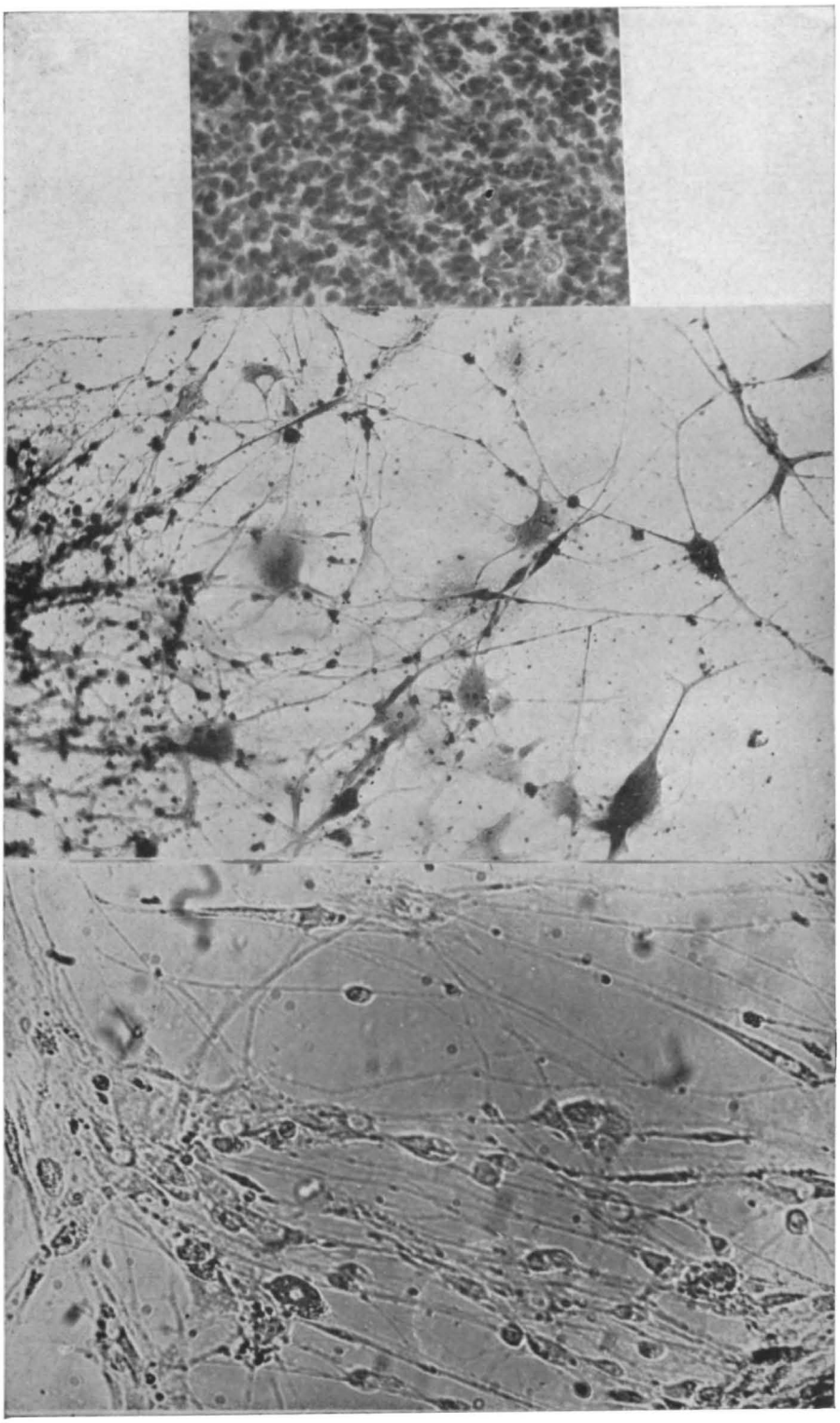

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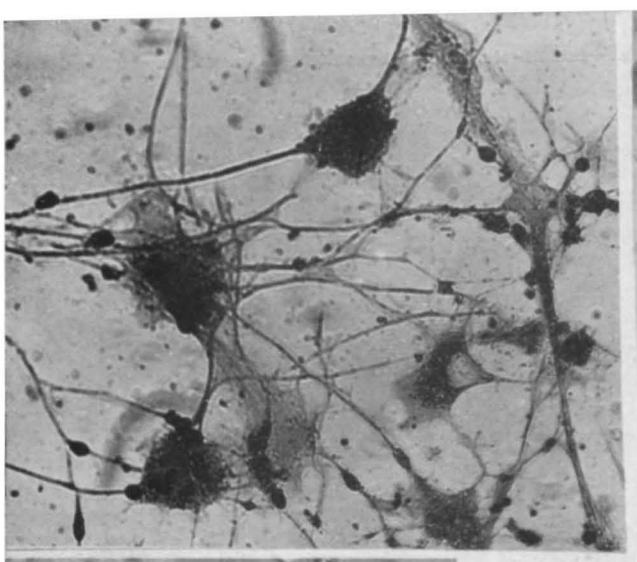

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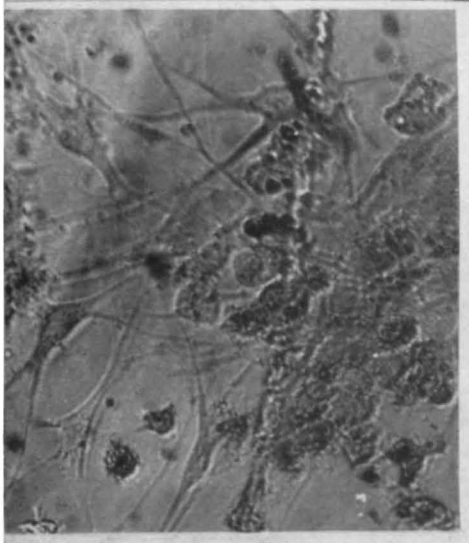

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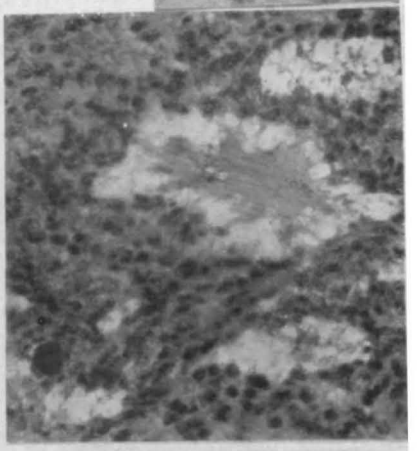

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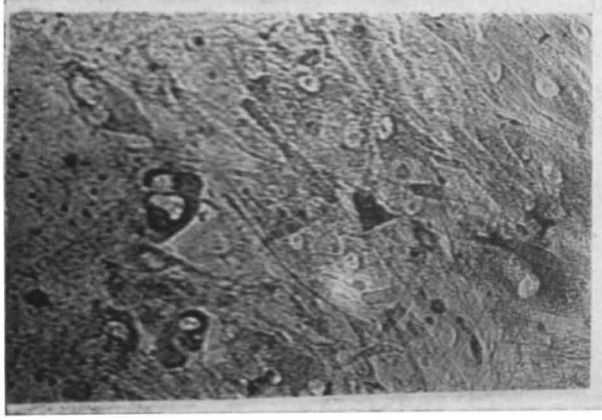

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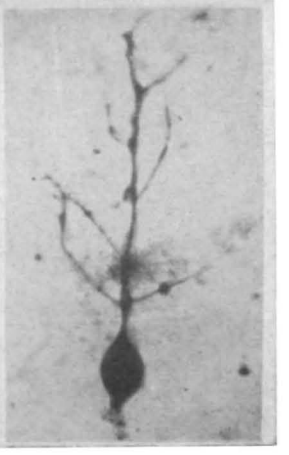

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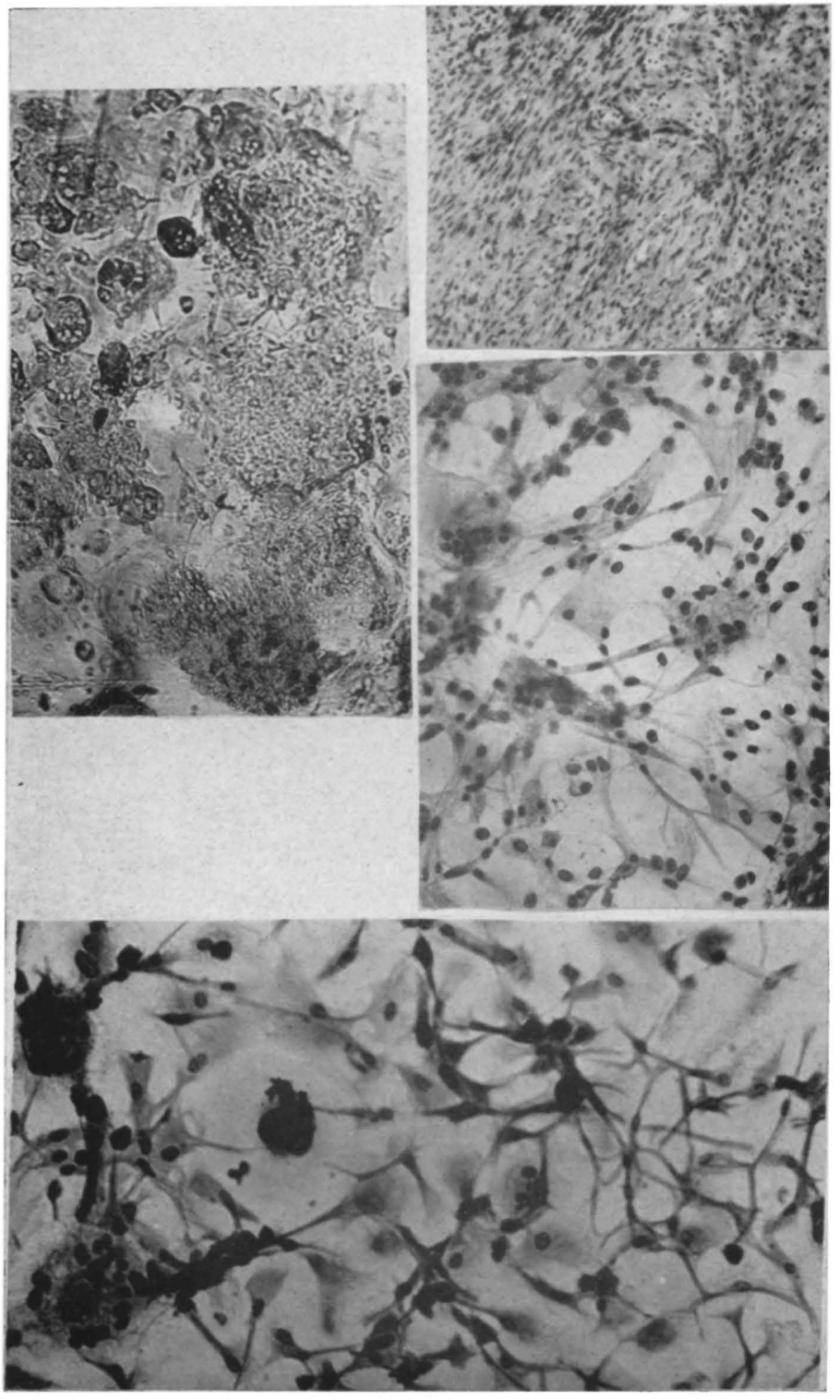

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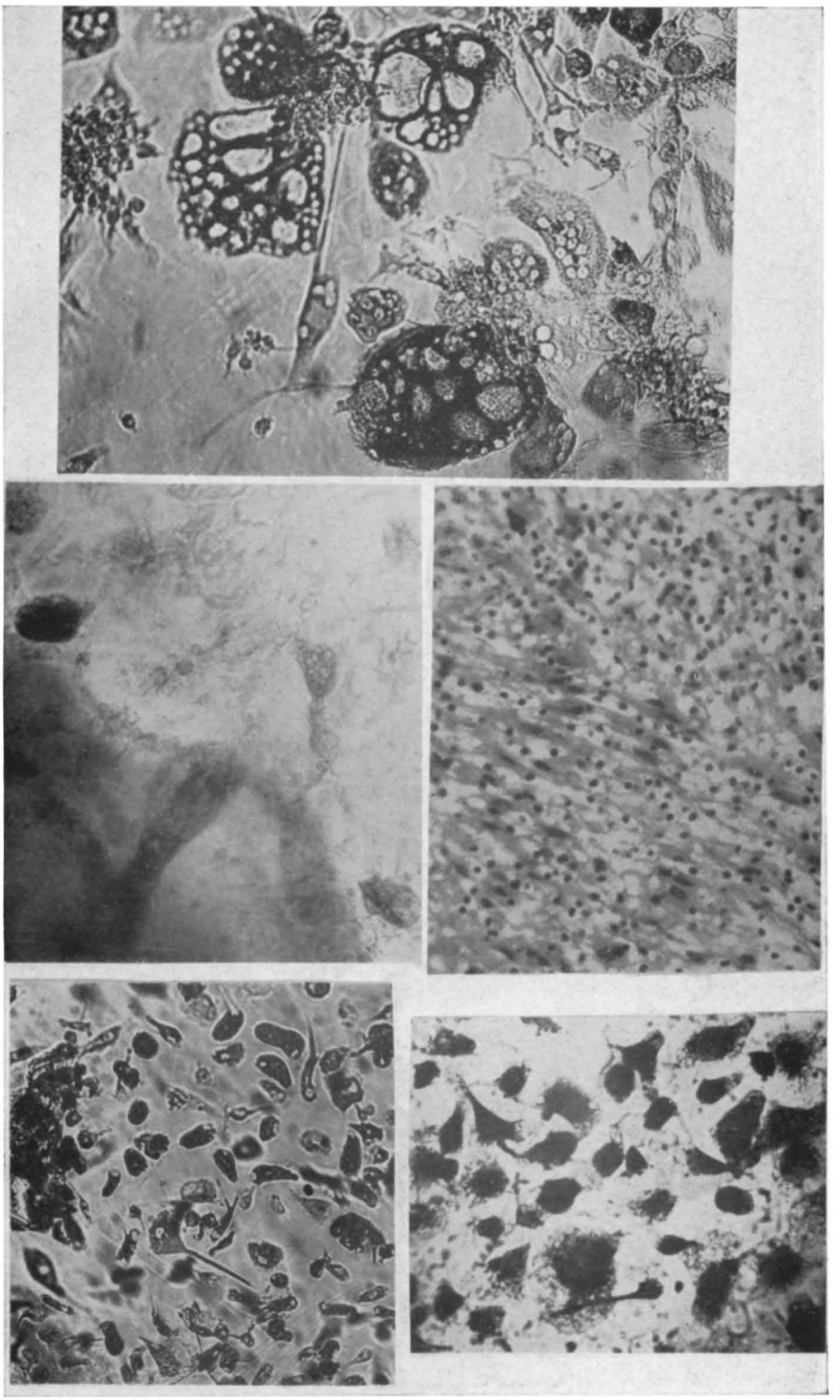

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PLATE X.

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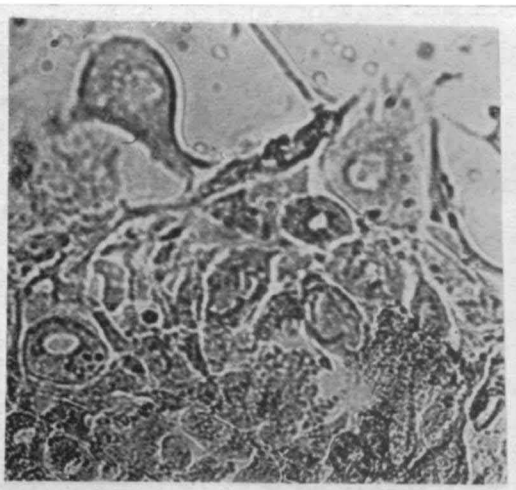

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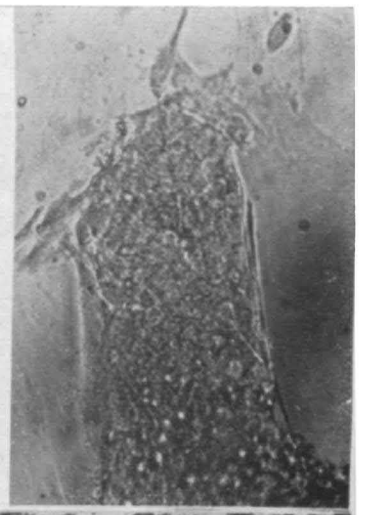

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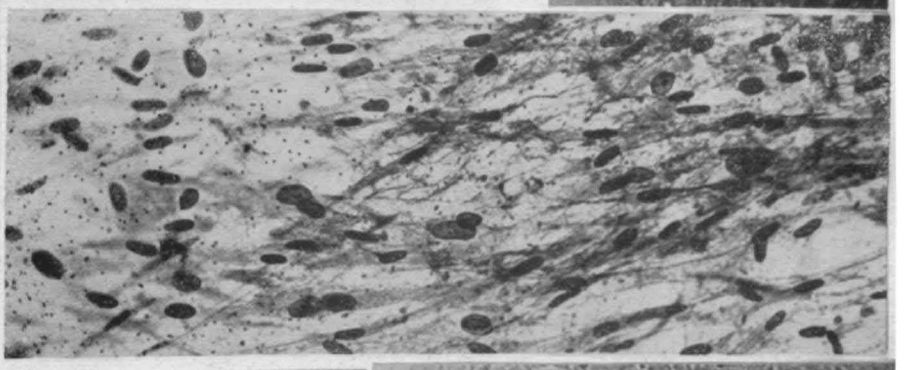

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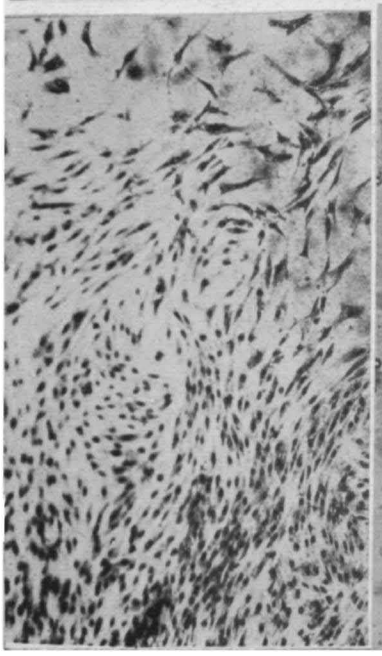

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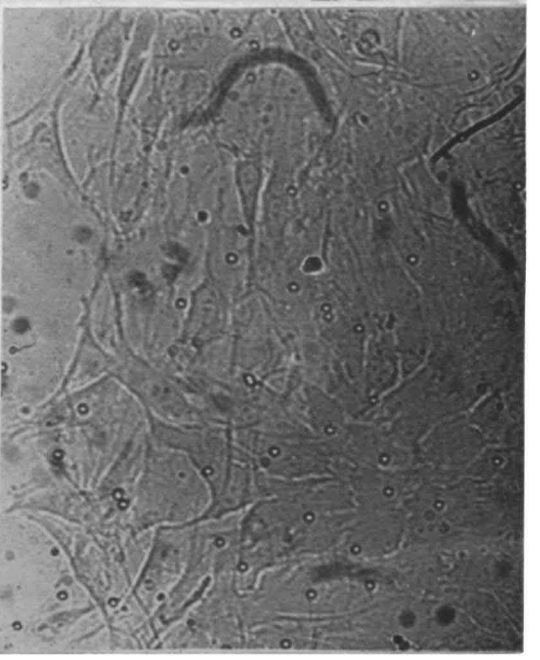

PLATE XI.

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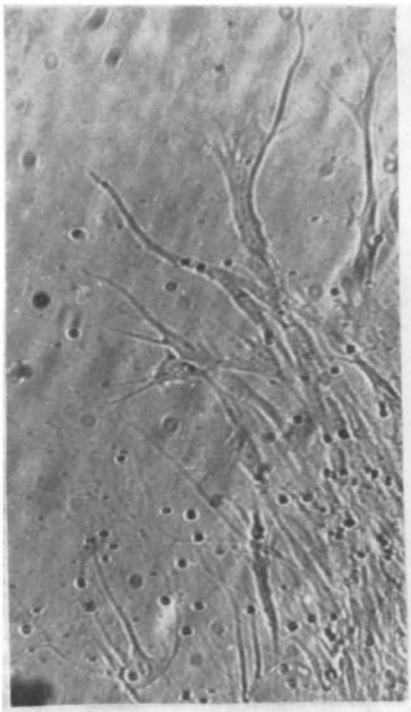

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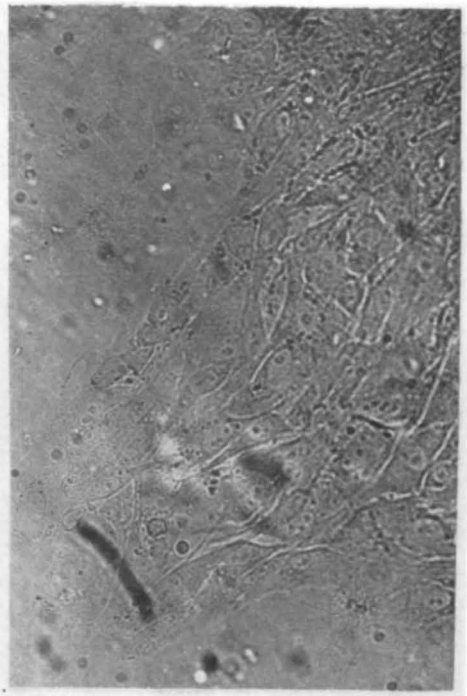

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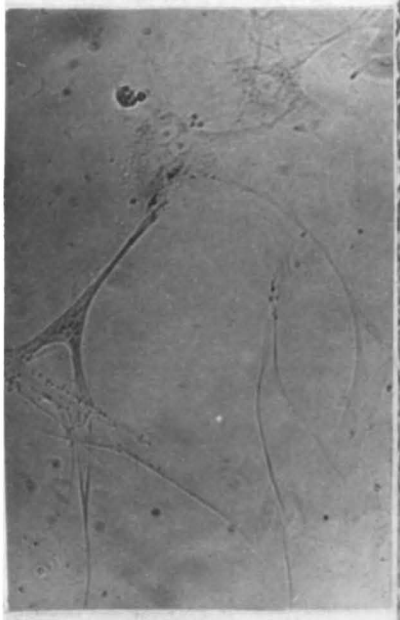

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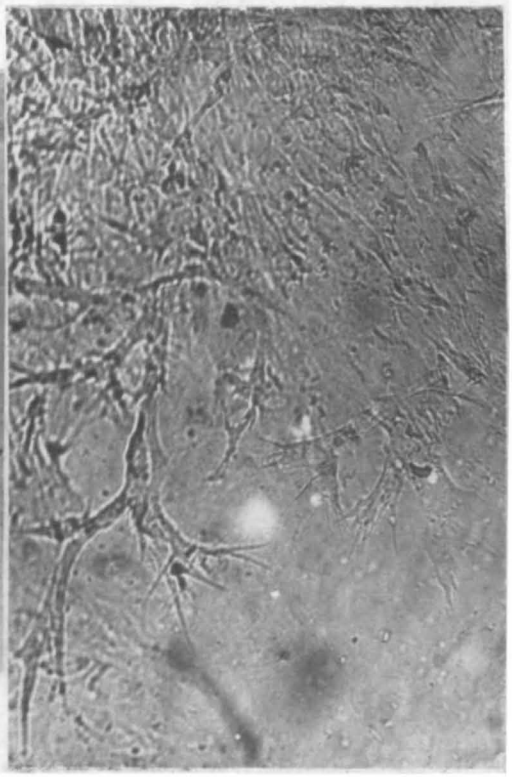

PLATE XII.

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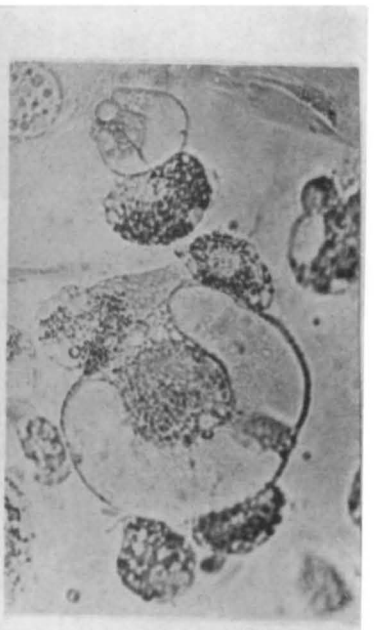

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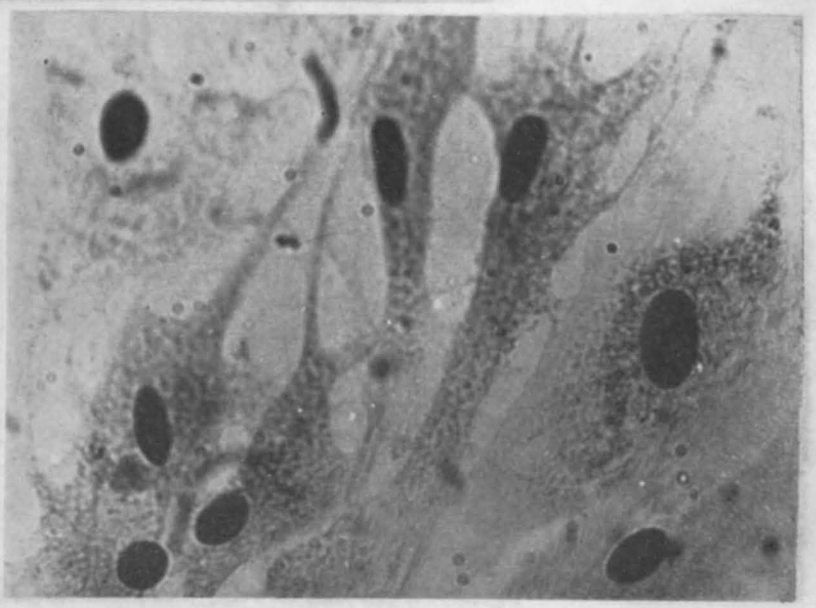

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