"THE INDEPENDENCE OF THE PERIPHERAL SENSORY NEURONE IN VIEW OF THE RESULTS OF EXPERIMENTAL SECTION OF THE OPTIC NERVE IN THE RABBIT"

THESIS FOR D.Sc. EXAMINATION

by

JANIE HAMILTON McILROY

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JANIE HAMILTON McILROY, M.A., B.Sc., M.B.

(From the Physiological Laboratory, University of Glasgow)

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PART I - INTRODUCTION

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Although one may accept in the general sense the neurone theory that the nervous system consists of innumerable anatomically independent cellular units, the processes of which exist in contiguity, but not continuity, with each other, the mutual relationship and interdependence of the neurones is a problem as yet unsolved. This is specially the case as regards the peripherally placed neurones. The object of the present investigation was to throw light upon the question; and the retinal neurones were selected for the purpose of experiment, the plan being to separate them from their central connection by section of the optic nerves, without interference with the vascular supply of the retina.

In the retina the peripheral neurones are the cells of the outer and inner nuclear layers, and, although the small size of these neurones renders them more difficult of observation than could be desired, yet the comparative simplicity of their structure makes the histological examination tolerably easy.

The cells of the ganglionic layer on the other hand are conspicuous by their size and structural definition, and al-

though they are part of the neurones injured by section of the nerve their condition was studied in each experiment.

AUTOGENY OF THE VISUAL PERIPHERAL SENSORY NEURONE:

The retina develops as a hollow outgrowth from the primitive fore-brain, the stalk which connects these two structures persisting as the optic nerve. The ganglion cells are the cells of the innermost cellular layer and are in connection with the optic nerve fibres which conduct stimuli through the various stations of arborisation (corpora quadrigemina, corpora geniculata externa, and optic thalamus) to reach the visual cortex.

The retina, optic nerve and optic tract are thus developmentally part of the central nervous system.

With regard to the relations of the retinal elements, two views are held. According to the one which is advocated by Barker,[13] the bipolar cells of the inner nuclear layer are analagous to the spinal peripheral neurones of the dorsal roots, the ganglion cells of the retina corresponding to neurones of the second order (i.e., the neurones of the grey matter of the spinal cord).

One must not drive such a comparison too far, and the alternative view, that the ganglion cells of the retina are analagous to the dorsal spinal ganglia is more generally accepted. The bipolar cells of the inner nuclear layer are

the peripheral sensory neurones which have their peripheral processes in contact with the central process of the still more peripherally situated cells of the external nuclear layer.

It is the aim of the present investigation to follow the fate of the neurones situated distal to the site of injury in the optic nerve.

Owing to limitations of space it is impossible to give a critical review of the general literature bearing upon the present research. A bibliography of the more essential of the published works will be found in the Appendix. With regard to the experiments performed by other workers, section of the optic nerve has formed the subject of numerous publications. The results of these experiments are of little or no assistance to us, since the purposes for which the investigations were instituted were other than the present, and the histological methods - of the earlier workers at least - were inappropriate. An exception, however, is to be made in the case of Birch-Hirschfeld[8] who published in 1900 the first of a series of retinal experiments, including section of the optic nerve.

The value of his work is enhanced by the fact that he employed the Nissl method and described the fine histological appearances in minute detail so that his results have been taken as a guide in the present investigation. He cov-

ered a wide field in his experiments, investigating the behaviour of the retina in the rabbit under the influence of different pathological conditions, viz., electrical and thermal stimulation, section of the optic nerve and poisoning with various drugs (methyl alcohol, nicotin, quinine etc.).

The results of his optic nerve sections must now be summarised more or less in detail.

His operations were performed as far back as possible in the orbit in order to avoid injury to the vessels. In some cases he operated from above, by means of a knob-pointed knife, and, although the operation was practically bloodless, the ophthalmoscope showed almost immediate contraction of the retinal arteries. In one case he cut the nerve intracranially, close to the optic foramen.

In his anatomical investigation he found considerable divergence from the normal 55 hours after operation, chromatolysis having commenced in the case of the ganglion cells. The chromatin clumps had lost their sharpness of outline. The nucleus was large, swollen, diffusely stained, and often resting almost upon the cell wall. The cells of the internal nuclear layer were small, full of chromatin, and often irregular in outline. The cells of the outer nuclear layer were roundish, devoid of edge-crenation, and showed only partial cross-striping. Cases examined at 5, 10 and 15 days

after operation revealed still further advanced chromatolysis, shrinking of the ganglion cell (shown by the increased pericellular space) and its nucleus. The normal Nissl granules had been replaced by a darkly staining, badly defined, crumbling mass, or were altogether wanting, their place being taken by vacuoles of different sizes. He emphasised the fact that these vacuoles were absent from the control.

Valuable as are these results, it was felt that as his attention had been chiefly directed to the ganglion cells, while the object of the present investigation was to study the peripheral neurones, a further series of experiments was desirable.

PART II - PRESENT INVESTIGATION

A. RESULTS OF EXPERIMENTAL SECTION OF THE OPTIC NERVE

(1) Operative Technique:

The operation was performed with the usual aseptic precautions. The animal having been placed in a good light on the operating bench, the eye to be operated upon (in all cases - except one - the right) was douched with tepid sterile water. The conjunctiva was picked up in dissecting forceps and was incised by a line parallel to the corneal margin and situated about 4 m.m. from the latter. This incision extended for about 1 c.m. on either side of a point opposite the middle of the supra-orbital margin.

The wound was then deepened by gentle separation of the conjunctiva from the underlying structures, as far back as possible. The superior rectus muscle was occasionally divided, but in many cases this was not necessary. The "corneal" flap of the wound was then grasped in fixation forceps, and the eyeball rotated downwards. By gentle manipulation the eye was raised out of the orbital cavity (it was sometimes necessary to divide the outer canthus at this stage, if the palbebral fissure were small), turned sharply downwards and

retained in this position by the assistant. In this way the optic nerve (which in the rabbit enters the sclera above the horizontal meridian) came to lie high up on the wound and the nerve, after a little careful dissection, could be isolated. The dense tissue of Tenon's capsule which forms a funnel-shaped investment for the nerve and its adnexae is liable to cause trouble at this stage, the delicate vessels being easily damaged in making way through it. The nerve appeared as a slender glistening white cord and, having been lifted up on the tenotomy hook, was divided far back (i.e., clear of the central vessels) by means of scissors inserted, closed, and then opened merely enough to include the nerve. When traction was removed, the eyeball readily slipped back into the orbital cavity. Haemorrhage, when it occurred, as a rule, took place at the stage of deepening the original incision, i.e., in the region of the anterior vessels of the globe. It was usually easily controlled by pressure. The haemorrhage, which was met with when the nerve was divided, was no doubt due to the inclusion of some of the branches of the central artery, but was of infrequent occurrence, and was soon controlled. In a few cases ulceration of the cornea ensued, being due either to slight abrasion at the time of operation or to an atrophic condition, as the result of lesion of some of the long anterior ciliary nerves. The ulceration subsided in a few days (under bor-

acic douching), leaving a small leucoma.

In all cases total and permanent blindness of the eye appeared to follow from the operation, although the difficulty of estimating the amount of visual perception in a rabbit makes one reserved in any statement regarding the physiological condition.*

The pupil which usually contracted to a pin-point at the moment of division of the nerve, gradually dilated, and remained semi or fully dilated and inactive to light. Ophthalmoscopic examination of the fundus never revealed more than some slight narrowing of the retinal vessels, with pallor of the "medullary rays" in the late stages.

The experiments recorded are those in which section of the optic nerve was uncomplicated by injury to the retinal vessels, or by lasting inflammation. The cases in which the results were considered vitiated, for any reason, were rejected.

(2) Histological Methods:

In every case the same treatment was adopted for the control as for the experimental material.

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*NOTE: The method adopted was that of flashing the brightly illuminated ophthalmoscopic mirror on to the eye in the dark room. The response which, in the case of the control was immediate, although not very vigorous, and consisted in twitching of the eyelids and shunning of the light, was entirely absent in the operated eye

Fixation: For successful demonstration of the Nissl granules, the most satisfactory fixative was alcohol (50% - 75%). The tissue remained in this from a half to several hours, according to the size. Dehydration in several changes of absolute alcohol. Clearing was least injurious when carried out in cedar-wood oil. Other fixatives employed, with less satisfactory results, were - $HgCl_2$ (saturated saline solution), formol-saline (10% formalin in normal saline solution), and picro-formol, (pic. acid, aq. sat. sol. 75 pts., formol 25 pts., acetic acid 5 pts).

Staining: Nissl granules in every case were stained by Toluidin blue, no counter stain being employed. (Methylene blue and Thionin did not yield such good results).

Other stains for general demonstration were haemalum and eosin; and Weigert's rapid iron-haematoxylin method.

Silver impregnation: The chrome-silver method of Golgi was found to be unsuccessful in these experiments, although a fair amount of success was obtained in the case of ox-retina.

The method of Cajal (formula II) produced very beautiful results in many cases, but was seldom satisfactory in control and experimental of the same case.

Cox's method of sublimate and bichromate impregnation was not found satisfactory.

Ehrlich's intra-vitam method (Dogiel's modification) of methylene blue impregnation was employed successfully in one or two cases, but was not of much practical value in these experiments.

(3) Experimental Details

Experiment I: Adult albino, 24 hours after operation.

No microscopic changes were discovered in the operative eye.

Experiment II: Adult albino, 48 hours (Fig. I)

Operation practically bloodless. No inflammation ensued. Fixation of retina in 50% alcohol, and in 10% Formalin. Sections stained by Toluidin blue and by Weigert's iron-haematoxylin method. Impregnation by Cajal's silver method was unsuccessful.

Microscopic Examination: The outer layers of the retina showed no abnormality in the operative eye. The nuclei of the rods showed the chromatin arranged in the usual form of twin masses separated by a considerable interspace - an arrangement which may be described by the term "cross-striping". The fine serration of the edges of these masses, due to the presence of fine strands of chromatin passing out to the cell membrane across the intervening

space, for convenience of description may be designated as "cog-wheel" markings. In the operative eye these features were as well marked as in the control. The larger and more complex nuclei, generally looked upon as belonging to the cones, showed no variation from the normal, having the typical arrangement of the chromatin (i.e., three or four masses) with cruciform interspace. They were much less numerous than the rod-nuclei. The cells of the internal nuclear layer were normal and showed the chromatin arranged in the form of a loose tangled granular skein or network.

With regard to the cells of the ganglionic layer, a certain amount of blurring of the outline of the Nissl bodies was met with in the operative eye. Considerable variation in staining power was to be seen in the control, examples of cells in which the protoplasm was deeply and diffusely stained being fairly common. Most of the cells, however, in the control showed a high degree of differentiation of chromatin which was practically never met with in the operative.

The fact that one found in the normal many cells exhibiting the phenomena which, in the case of the cells of the central nervous system are usually classified as pathological (viz., eccentric position of the nucleus, diffuse staining of the protoplasm with blurring of Nissl bodies, vacuolation, enlargement of pericellular spaces) led one to

the conclusion that such phenomena must either be due to artefacts or that they were themselves an expression of phases of functional activity.

Experiment III: Adult black and white rabbit. 48 hours.

Operation practically bloodless. No inflammation ensued. Fixation in 10% Formol-saline and in Müller's solution. Staining by Toluidin blue and Weigert's iron-haemloxylin method.

Microscopic Examination: The ganglion cells in both retinae were frequently profusely vacuolated, and pericellular spaces were well-marked. The chromatin granules showed little or no variation from the normal, but the number of atypical cells (i.e., those which show diffuse staining of the protoplasm, vacuolation etc.), was greater for the operative than for the control. The outer layers were unchanged.

Experiment IV: Adult albino. 72 hours.

There was some haemorrhage early in the operation, which was easily controlled. No inflammation ensued. Fixation in 50% alcohol. Staining by Toluidin blue and Haemalum and Eosin. Silver preparations unsatisfactory.

Microscopic Examination: In the sections the line of division of the nerve was seen to be behind the entrance of

the vessels into the nerve. The ganglion cells were on the whole well-preserved, some of them showing fairly well-defined chromatin bodies. There was, however, evidence of a certain amount of shrinking in the cells of the operative as compared with the control. In the cells of the latter there was a clear space around the nucleus, whereas in the operative this space was often encroached upon by the shrinking of the cell so that there was a more or less dense ring-like band of chromatin encircling the nucleus. The cells of the outer layers showed no variation from normal.

Experiment V: Adult black and white rabbit. 4 days. (Fig. II).

Operation without haemorrhage. No inflammation followed. Fixation in 50% alcohol. Staining with Toluidin blue. Silver impregnation successful for the operative, unsuccessful for control.

Microscopic Examination: There was on the whole no great abnormality in the operative tissue. There were often tolerably well-preserved cells in the ganglionic layer, but the occurrence of "normal" cells occurred only in the control. Definition of the chromatin bodies was not at any time so sharp in the operative as it was in the control and there was evidence of condensation and shrinkage in many of the cells. The other layers were normal. The silver prep-

arations in the operative showed numerous fine varicosities on the fibrils of the internal plexiform layer. The broad, ribbon-like bands of the external layer were well demonstrated. The preparations of the control also were successful.

Experiment VI: Adult black rabbit. 7 days (Fig. III).

There was slight retrobulbar haemorrhage at time of operation and slight transient conjunctivitis ensued. At the time of death the eye appeared normal. Fixation in 50% alcohol and Müller's solution. Staining by Weigert's iron-haematoxylin method and Toluidin blue. Silver preparations were successful in both control and operative, but no striking difference was revealed.

Microscopic Examination: In the ganglion cells of the operative the chromatin granules were small and had a tendency to become broken up into fine dust. When the granules were large, they had rather badly defined edges and lacked sharpness of definition. Occasionally large fairly well-preserved ganglion cells were met with, but they were never so large or well differentiated as the large cells of the control. The other layers were normal.

In the silver preparations the varicosities on the fibrils of the cellular processes were most marked in the operative, being almost absent in the control. The processes of the "horizontal cells" of the internal nuclear layer were

specially well demonstrated in the control.

Experiment VII: Adult albino. 8 days (Fig. III).

Operation bloodless. Fixation in 50% alcohol and Müller's solution. Silver impregnation successful in the operative, failed for the control. Staining with Toluidin blue and Weigert's iron-haematoxylin.

Microscopic Examination: Well-preserved ganglion cells occurred much less frequently in the operative than in the control. The cells of the operative were usually pale-staining, with finely divided chromatin. The pericellular spaces were more pronounced in the control than in the operative. The outer retinal layers were normal. In the silver preparations varicosities were occasionally met with in the fine fibrils of the inner plexiform layer. The fibrils of the external plexiform layer were seen to end in a rich anastomosis around the cells of the external nuclear layer. The protoplasmic processes of the ganglion cells were devoid of varicosities.

Experiment VIII: Adult albino. 3 weeks. (Fig. IV).

There was slight haemorrhage during the operation and a corneal ulcer developed. This cleared up and, at the time of death, only a small scar remained on the otherwise healthy looking cornea. Fixation in 50% alcohol. Staining

with Toluidin blue, Haemalum and Eosin.

Microscopic Examination: In the control the number of "atypical" ganglion cells in this case was considerable, but at the same time there was a preponderance of quite typical cells. In the operative on the other hand one rarely if ever met with a typical ganglion cell, the best preserved cell consisting usually of a peripheral ring of small bead-like granules surrounding a densely stained nucleus. The outer layers were normal.

(Sections stained with haemalum and eosin showed the presence of a considerable amount of inflammatory exudate with giant cells around the stump of the optic nerve. It is probable that the pronounced atrophy of the ganglion cells in this case was partly due to the inflammatory conditions and not solely to division of the nerve, although from the fact that the outer layers were unaffected one did not consider that the experiment was to be rejected).

Experiment IX: Adult albino. 3 weeks. (Fig. V.)

Operation almost bloodless. No inflammation ensued. Fixation in 50% alcohol, in sat. sol. $HgCl_2$, and in 10% Formol-Saline. Staining with Toluidin blue.

Microscope Examination:

(1) Tissues fixed in $HgCl_2$. The ganglion cells were

smaller in the operative than in the control. The Nissl granules were almost entirely absent from the operative. Vacuolation and enlargement of the pericellular spaces occurred with no greater frequency in the operative than in the control. The outer layers were normal.

(2) Fixation in 50% alcohol. There was a general tendency to clearer definition in the cells than was the case in fixation with $HgCl_2$, but the same relative differences existed between the operative and the control as in the former.

(3) Fixation in Formol-Saline. There were appearances of pronounced vacuolation in the ganglion cells both of operative and control. There was less sharpness of intranuclear definition than that obtained by employment of either of the preceding methods, but the difference between the ganglion cells of the two retinae was maintained as in the former. By the formol-saline method of fixation one saw particularly well demonstrated the cells of the innermost stratum of the internal nuclear layer (Amacrin cells of Cajal) which were arranged at intervals from one another, each cell showing a well marked downgoing protoplasmic process containing chromatin. These cells were slightly more conspicuous in the operative than in the control, and were possibly in connection with centrifugal fibres.

Experiment X: Small white rabbit. 6 weeks. (Fig. VI)

Operation practically bloodless. Slight corneal inflammation ensued which resulted in a small leucoma at the lower part of the cornea. At the time of death the eye seemed otherwise normal.

Fixation in 50% alcohol. Staining with Toluidin blue and with Haemalum and Eosin. Silver impregnation unsuccessful.

Microscopic Examination: In the haemalum and eosin specimens the ganglion cells of the operative were seen to be much reduced in size. The nucleus was often all that remained to represent the cell. These nuclei were irregular in outline and were very densely stained. In the Toluidin blue specimens the cell body was diffusely stained, or the chromatin was in the form of dust-like particles. The cell processes were attenuated and tapered off sharply close to the cell body. The cells of the internal and external nuclear layer appeared normal, unless for the fact that occasionally the cone nuclei in the operative appeared somewhat smaller than in the control.

Experiment XI: Adult albino. 15 weeks. (Fig. VII).

There was practically no haemorrhage during the operation. No inflammation ensued. Fixation in 50% alcohol, in sat. sol. of $HgCl_2$ and in Formol-Saline 10%. Staining with

Weigert's iron-haematoxylin, and Toluidin blue.

Microscopic Examination:

(1) $HgCl_2$ fixation. The ganglion cells of the operative eye were very much shrunken and were diffusely stained. Differentiation into granules was practically never met with, the ground substance being always diffusely stained. Vacuolation when present was less frequent in the operative than in the control. Pericellular spaces were not well seen. There was slight loss of "cogwheel" marking in the cells of the outer nuclear layer, but "cross-striping" was still present. Inner nuclear layer normal.

(2) Fixation in 50% alcohol. Occasionally the operative ganglion cells showed indication of granular structure, but at best the granules were small and dust-like. The pericellular spaces were more conspicuous than in (1) and were of less frequent occurrence in the operative than in the control. Vacuolation was rarely seen in either. The cells with their processes were on the whole more sharply outlined both in operative and control than was the case in fixation with $HgCl_2$. No abnormality was to be made out in the inner nuclear layer. The outer nuclear layer showed slight loss of "cogwheel" marking and "cross-striping".

(3) Fixation in Formol-Saline. Vacuolation was rather well-marked, but occurred on the whole with greater frequency

in the control. Staining was not so satisfactory for operative or control as in (1) or (2).

Experiment XII: Adult Albino. 16½ weeks. (Fig. VIII). There was slight haemorrhage during the operation. No inflammation ensued. Fixation in 50% alcohol. Staining with Toluidin blue, Haemalum and Eosin. Silver impregnation was highly successful in the control, but failed in the operative.

Microscopic Examination: The ganglion cells in the operative retina were much shrunken (although no enlargement of the pericellular spaces was visible). "Normal" ganglion cells were entirely absent. Staining was as a rule very diffuse, although occasionally a dust-like condition of the chromatin occurred. In the latter case the dust-like particles were met with in the centre of the cell and were surrounded by a row of somewhat larger bead-like chromatin granules near the cell margin. The cell processes were much attenuated, being often unrecognisable. The cells of the internal nuclear layer in the operative were perhaps paler than in the control, and the cells which were described (in Experiment IX) as being in connection with centrifugal fibres (amacrine cells) were particularly well marked. The cells of the outer nuclear layer appeared unchanged, unless for a slight impairment of definition in the chromatin as

compared with the control.

Silver impregnation was successful in the control, and in this it is to be noted that the fibres of the plexiform layers were devoid of varicosities.

Experiment XIII: Long-haired adult grey rabbit. 38 weeks. (Fig. IX)

Operation was practically bloodless. Fixation in $HgCl_2$ and 50% alcohol. Silver impregnation was unsuccessful. Staining with Toluidin blue and Weigert's iron-haematoxylin.

(1) $HgCl_2$ fixation. (In the case of the control beautiful fixation of the ganglion cells was obtained by this method, the Nissl granules being plumper and slightly less well-defined than in alcohol fixation). At some parts of the retina the ganglion cells were practically non-existent. At other parts of the retina they were represented, but only by small cells diffusely stained, with eccentric nuclei; or by paler cells with fine dust-like particles of chromatin. The processes of these cells were tapering and ended off sharply close to the cell. The internal nuclei were well-preserved. "Amacrine" cells were not conspicuous in these specimens. The cells of the external nuclear layer showed well-marked "cross-striping" and "cogwheel" arrangement, and appeared almost normal.

(2) Formol-Saline fixation. Vacuolation was very pro-

nounced, especially in the ganglion cells of the control. Pericellular spaces were no less pronounced in the control than in the operative. The ganglion cells in the operative were almost universally shrivelled and diffusely stained. Occasionally a large ghost-like cell was met with. Owing to somewhat pale staining of the cells of the internal nuclear layer the "amacrine" cells were often conspicuous. The cells of the external nuclear layer were often lacking in the typical features of cogwheel marking and cross-striping, but as the same characteristics were met with in the control the defect is probably one of fixation only.

NOTE: Of the other experiments performed in this series, three were rejected on account of severe haemorrhage at the time of operation, the nerve having been divided of necessity rather blindly owing to difficulty in isolating it from the adjacent structures. Two experiments ended in phthisis bulbi. Three cases were discarded on account of unsatisfactory fixation (picro-formol) and eight were lost through the occurrence of incidental disease in the rabbits.

A numerical estimate of the cells of the ganglionic layer in the case of the operative as compared with the control appeared to be of some value in furnishing a calcula-

tion of the ganglion cells which had perished in the course of the experiment. The method of carrying out the attempt was the following:-

(1) As nearly as could be accomplished the same part of the retina was taken for control and operative.

(2) Owing to the difficulty in arriving at an idea of what were cells in a state of perfect preservation as compared with others less well preserved, the plan was adopted of including all the ganglion cells which were present and could be recognised as such, with a view to finding if there were reduction in their number in the operative.

(3) Estimation was made by means of the eye piece micormeter the lineal unit being .2 m.m. with ultimate calculation for 1 m.m.

TABLE

Number of Experiments	Duration	Number of Fields examined		Average Cells per .2 m.m.		Average per 1 min.		Av. per 1 min. in round nos.		Descriptive Result in operative
		Control	Operative	Control	Operative	Control	Operative	Control	Operative	
I	24 hrs.	89	89	2.78	2.74	13.9	13.7	14	14	Practically the same
II	48 "	45	45	9.20	4.28	46.5	21.4	46	21	Markedly fewer
III	48 "	40	40	3.52	3.70	17.6	18.5	18	18	No change, or slightly more numerous
IV	72 "	30	30	2.23	3.33	11.1	16.6	11	17	Much more numerous
V	4 days	52	52	12.56	4.32	62.8	21.6	63	22	Markedly fewer
VI	7 "	60	60	2.30	1.82	11.5	9.1	11	9	Fewer
VII	8 "	64	64	6.48	5.29	32.4	26.4	32	26	Fewer
VIII	3 weeks	43	43	8.41	1.93	42.0	9.6	42	10	Markedly fewer
IX	3 "	523	523	4.56	3.62	22.8	18.1	23	18	Fewer
X	6 "	30	30	3.53	2.43	17.6	12.1	18	12	Fewer
XI	15 "	1023	1023	3.85	3.04	19.2	15.2	19	15	Fewer
XII	$16\frac{1}{2}$ "	30	30	2.50	2.06	12.5	10.3	12	10	Fewer
XIII	38 "	482	482	6.10	2.83	30.5	14.1	30	14	Markedly fewer
						Aggregate		339	206	

NOTE: (1) Out of an aggregate of 339 for the control, and 206 for the operative, the average in each of the 13 cases may be taken as 26.7 and 15.84 respectively (indicating a marked reduction for the operative)

(2) The variability in the control ranges from 63 as highest, to 11 as lowest, whereas in the operative the range is only from 26 to 9

(3) In the control 5 are above 26.7, in the operative all are under 26.7. In the control 4 are under 15.84, in the operative 7 are under 15.84

Thus we see that throughout the series a considerable reduction was the rule, a reduction which was a confirmation of the descriptive results given in the text. That some disappearance of cells had taken place is to be assumed from a scrutiny of the table given above. When degeneration of the ganglion cells took place the first to disappear were the smallest cells. These cells were not always distinguishable from the other cells of the ganglion and nerve fibre layer, and even from small inflammatory cells (lymphocytes) so that in the early cases one may find somewhat conflicting results, e.g., in Experiment IV in which an increase in cells was obtained. This increase may be explained by the fact that the region of retina examined in the operative was not strictly analagous to that of the control. In the later cases where there was less probability of the presence of inflammatory cells, whatever error there might be in the inclusion of extraneous cells was lessened, but always one was inclined to make one's enumeration somewhat larger than the actual, owing to the difficulty of differentiating degenerated ganglion cells from small neuroglial nuclei and lymphocytes.

In the case of the control, however, such confusion was not met with, since the ganglion cells were better defined and more easily distinguished. In this way the numerical results may be taken as somewhat <u>under-estimated</u>, so that the reduction obtained in the operative retina was even more

substantial than it appears.

(4) Discussion of Results:

Thus we see that as early as 48 hours after operation certain changes in the ganglion cells were to be recognised. These changes consisted in a loss of perfect definition in the chromatin granules, probably due to the fact that the ground substance of the cell had taken up the stain to a slight extent. Degeneration apparently did not progress with extreme rapidity, the condition not being much more marked at 8 days than at 48 hours. (This is of significance when compared with the early chromatolytic changes described by Nissl and others in case of peripheral nerve section). At 3 weeks, however, degeneration was definite and became more pronounced with the lapse of time, till at 38 weeks (the longest time allowed) degeneration was practically complete.

Owing to the large range of variability shown by the retinal ganglion cells in the control eyes, one is diffident about drawing conclusions regarding the significance of the so-called "phenomena of degeneration". Adopting, however, as normal the appearances which occurred in the control with the greatest frequency, one endeavours to estimate not only the frequency with which the frankly abnormal types occur in the operative but also (which is of more importance)

whether any "normal" cells exist in the operative.

Degeneration apparently attacks the medium and small cells at an early stage, but my observations were throughout the investigation chiefly confined to the large ganglion cells. The smaller cells are especially liable to accident in the course of histological preparation, and are frequently of abnormal appearance in the control. In any case, however, one must neglect the condition obtaining in the smaller cells owing to the difficulty in recognising the gradual changes in any but the largest ganglion cells. The type of normal cell, therefore, with which one makes comparison is the large multipolar ganglion cell in which the Nissl granules of various sizes are sharply stained, and are arranged in somewhat concentric rows around the more or less central nucleus. The nucleus contains numerous chromatin granules and one or more nucleoli. The ground substance of the cell body is unstained. Granules of chromatin are to be found for a considerable distance along the protoplasmic processes. Vacuoles may or may not occur. (With regard to the occurrence of vacuoles Birch-Hirschfeld emphasises the fact that in his cases vacuolation was confined entirely to the operative material. In the present series, vacuolation was never more conspicuous in the cells of the operative than in those of the control - in many cases less so).

In recapitulating, therefore, one finds as the result

of optic nerve section that degeneration has set in as early as 48 hours and is evidenced by the absence of entirely normal ganglion cells. Degeneration advances steadily and is estimated by the complete absence of normal cells, as well as by the progressive increase in the number of frankly abnormal or degenerated cells. At a period of 38 weeks practically all the cells are atrophic.

Passing from the above degeneration of the sensory neurone of the higher or second order, one comes to a consideration of the fate of the more peripheral neurones of the first order situated in the outer retinal layers. Judging from all authoritative accounts the neurones of the retinal layers are in intimate relationship with one another. Birch-Hirschfeld described well marked changes in the nuclear layers as result of optic nerve section. These changes consisted, in the case of the inner nuclear layer, of reduction in size, irregularity of outline of the cell and condensation of chromatin. The cells of the outer nuclear layer showed rounding of cells, reduction in the size of the interspace tending to obliteration of the "cross-striping", and loss of chromatin of edges ("cogwheel" markings).

In the present series no essential changes in these layers could be made out. In some cases pale-staining of the inner nuclei, or dense staining of the outer nuclei with slight loss of definition might be described, but these con-

ditions are manifest in the control with so much frequency that their occurrence often appears to be due to differences of intensity of stain rather than to pathological factors. In fact one is often able to demonstrate from one slide in the control differences of staining which, if they were to occur in the operative as distinguished from the control, might be looked upon as significant. (The fact, however, that Birch-Hirschfeld mounted his control and operative sections upon the same slides probably eliminated error in this direction to a large extent).

More positive features than differences of staining power are those of reduction in size, and irregularity of outline and loss of the discrete nature of the chromatin granules. In the present series practically no evidence of such changes was to be made out. One may therefore assume with fair confidence that the integrity of the outer neurones in these experiments has been preserved, and that the operation of section of their axon which produced serious damage in the case of the higher neurone (connecting the organ with central nervous system) was accomplished by little or no disturbance of the contiguous outer neurones. The slight loss of definition met with in the outer neurones in Experiment XI is compensated for by the absence of this feature in Experiment XIII (a much later period). Having thus become acquainted with the facts of degeneration in regard to the

neurones of the retina, and having established the point that the peripheral neurones remain intact for such prolonged periods after separation from the central neurones, the question arises whether they are intrinsically more resistant than the outer neurones. To determine this it was decided to study the progress of post mortem changes in the retinal neurones.

B. RESULTS OF AUTOLYTIC EXPERIMENTS:

(1) General Remarks:

Most of the previous work done upon post mortem changes in nerve cells has been concerned with the post morten changes in ganglion cells of the brain and cord. In the following experiments one sought to investigate the changes occurring in the retinal neurones.

Birch-Hirschfeld (53) described post mortem changes in the ganglion cells of the retina. He used the sublimate-thionin method and found evidence of change as early as 2 hours after death. There was slight folding of the nuclear membrane and loss of definition in the Nissl granules. The outer nuclear layer showed rounding off of the nuclei, especially of the rods, with commencing loss of "cross-striping" and edge-crenation. After 3½ hours the ganglion cells

showed enlarged vacuoles, especially at the periphery of the cell. The chromatin was still more or less in the form of granules. The nuclei of the internal nuclear layer were partially degenerated. There was no evidence of "cross-striping" in the nuclei of the external nuclear layer. After 5 hours there was deep staining of the ground substance with blurring of the nuclear and cell-outline. After 7 hours the Nissl granules had disappeared and the ground substance no longer stained deeply. The nucleus had practically vanished. The outer nuclear layer showed rounding of its nuclei with here and there a hint of "cross-striping". After 18 hours the changes had progressed further and little or no chromophilic substance remained.

In the foregoing experiments it is not clear whether some of the changes described were not due to putrefactive conditions. In the present series, in order to eliminate as far as possible the influence of micro-organisms occurring in the surroundings, the following method of examination was adopted. The enucleated eyeball having been thoroughly cleared of all extrinsic structures (muscles, fat etc.) so that a smooth contour might be obtained, the shortened stump of the optic nerve was grasped in forceps and the eyeball was plunged in melted paraffin (50^{0}) for a few seconds. The thin layer of paraffin which adhered to the surface of the eyeball rapidly congealed, and the naked nerve was easily

covered by a drop of paraffin poured on its surface. In this way the eyeball was sealed from the atmosphere by a layer of sterile material. The changes therefore which would take place within the eyeball were presumably those of autolysis and not putrefaction. The "enveloped" eyes were allowed to stand at atmospheric temperature (or in one or two cases at incubator temperature) for the desired period. When this had elapsed the layer of paraffin was easily chipped off and the eyeball treated henceforth in the same manner as the control. In the cases in which ox eyes were used, the eyeballs were obtained from the slaughterhouse with as little delay as possible, and were looked upon for all practical purposes as fresh. In the case of the other animals (dog, cat, rabbit) the eyes were obtained in the laboratory and fixation took place immediately after the death of the animal.

(2) Experimental Details:

Experiment I: Ox eye, autolysis for 16 hours at room temperature. Fixation in picro-formol solution (a fixative which was successful in the case of the ox-eye although less satisfactory in the delicate rabbit retina).

Sections stained by Weigert's iron-haematoxylin method showed marked disorganisation of the rods and cones. The "palisade" arrangement of this layer was almost unrecognis-

able. Under low magnification the other layers of the retina appeared practically unchanged when compared with the control. The external and internal nuclear layers were intact and separated from one another by the outer plexiform layer (which was of apparently normal thickness). Under higher magnification the cells of the external nuclear layer were seen to be densely stained and somewhat smaller than normal. The "cogwheel" arrangement was still recognisable. The cells of the internal nuclear layer showed a reticulum in the form of more or less radiating strands around the deeply stained central nucleolus, and were on the whole slightly less sharply defined than in the control. The innermost cells of this layer ("centrifugal cells") with their downward-passing processes were somewhat densely stained and stood out in strong contrast to the more feebly stained cells in the rest of the layer. The cells of the ganglionic layer were well preserved. Their processes stood out distinctly and the nuclear reticulum was well defined.

There was some blurring of the edges of the chromatin granules of the ganglion cells which were stained with Toluidin blue. The fibres of Müller stood out distinctly both as regards their nuclei and their fibrous expansions in the nerve fibre layer.

Taken as a whole the changes which occurred in the ox-eye as the result of autolysis for 16 hours are not well-

marked and resolve themselves into:-

(1) Disorganisation of the rods and cones.

(2) Slight reduction in size of the rod and cone nuclei with a tendency to diffuseness of staining, although "cogwheel" was still manifest.

(3) Slight loss of prominence in the cells of the internal nuclear layer, with prominence of Müller's fibres and the centrifugal cells of the layer

(4) Slightly increased prominence of the protoplasmic expansions of the ganglion cells which are thrown into relief by the commencing disorganisation of the ground substance of the retina.

Experiment II: (Fig. XI). Ox-eye. Autolysis for 24 hours (room temperature).

A. Fixation in 50% alcohol. Staining by Weigert's iron-haematoxylin and Toluidin blue.

Under a low power the layers of the retina still appeared separate from one another. Under higher magnification the rods and cones were distinctly disorganised although a "palisade" formation was still suggested. The nuclei of the external nuclear layer showed a fair amount of detail, but there was a tendency to blurring of the edges and loss of "cogwheel" marking.

The internal nuclei showed a well defined chromatin network but there was a tendency to enlargement of the spaces between the threads and a general "rarefaction". Many of these spaces appeared full of fat. The nuclei of

Müller's fibres were deeply stained. The ganglion cells both in the iron-haematoxylin and Toluidin blue preparations showed commencing degeneration. There was erosion of the inner aspect of the cell, and clear fat-like bodies could be discovered in many. There was a tendency to diffuseness of staining, and the Nissl granules had commenced to coalesce. The processes were not so well-marked as in Experiment I (picro-formol fixation).

Spaces which looked like vacuoles were in all probability fat-globules.

B. In another specimen in which fixation was (1) by $HgCl_2$ (sat. sol.) and stained by Toluidin blue, practically the same appearances were obtained, viz:- the ganglion cells were pale and had eroded inner margins. The cell processes were not well defined. The nuclei were well preserved. The outer and inner nuclear layers were not much changed. The rods and cones, however, were much disorganised.

(2) In the part of the same retina fixed in 50% alcohol the ganglion cells showed rather more shrinkage and diffuse staining than existed in (1). The external and internal nuclear layers had become approximated owing to the collapse of the outer plexiform layer, and the cells of these layers showed diffuse staining.

The discrepancy which exists between B (2) and A may be explained by the fact that in the former a somewhat longer interval had elapsed before the eye was transferred to the laboratory, and that post morten changes had already set in. The difference between B (1) and B (2) probably lies in the fact that, whilst fixation in the one case was procured by means of $HgCl_2$, in the other it was by alcohol. The latter reagent in our experience is the most satisfactory fixative for the Nissl method in fresh tissue, but in the autolytic retina, where the ground substance is unduly fragile and liable to shrinkage, the unfavourable aspect of alcohol as a fixative is more pronounced than is the case in fresh, resistant tissues. (One does not necessarily conclude from this that in the case of easily damaged tissues corrosive sublimate is to be preferred to alcohol).

C. Another sample of autolysis for 24 hours showed still more pronounced changes in the outer layers - the differentiation between the outer and inner nuclear layers having become lost owing to the almost complete disappearance of the outer plexiform layer. The ganglionic cells, however, exhibited practically the same features as in the other specimens.

Experiment III: (Fig. XII) Ox-eye. 48 hours. (room temperature).

Fixation in picro-formol solution. Staining in Toluidin blue.

In the autolytic retina the layers were seen to be well differentiated, the plexiform layers being still recognisable.

The layers of rods and cones, however, were entirely devoid of palisade structure. The external nuclei were densely stained but there was still indication of the chromatin network ("cogwheel"). The cells of the internal nuclear layer also were deeply stained, and the details of structure were much more obscure than in the corresponding cells of the control. The ganglion cells showed well-defined, diffusely stained processes which stood out against the surrounding tissue. The Nissl granules which were to be observed in the control, were sometimes scarcely indicated in the autolytic retina, their place being taken by diffusely stained cell-contents.

Preservation of the ganglion cells in this specimen was on the whole little inferior to that in Experiment II, a fact which showed that autolysis, even after 48 hours, had produced marvellously little change in the ganglion cells.

Experiment IV: Ox-eye. Autolysis for 24 hours at 37° C. The "enveloped" eye was placed in the incubator for 24

hours, after which fixation was carried out (at room temperature) in picro-formol solution. The control was left in picro-formol for 24 hours in the incubator, after which it was treated at room temperature. Staining by Weigert's iron-haematoxylin method. In the control, by the picro-formol method, the rods and cones are well defined. The external nuclear layer showed the usual ganglion reticulum ("cogwheel") etc. The nuclei and processes of the ganglion cells were well stained and the retinal vessels with their capillaries passing obliquely across the inner layers were particularly well demonstrated.

In the autolytic retina the rods and cones as such were no longer recognisable. The external nuclear layer was very much broken in outline, encroaching frequently upon the internal nuclear layer. The cells of the former were very deeply stained and exhibited practically no detail of structure. They were small and globular, the "cogwheel" marking being absent. The internal nuclei showed a fair amount of structural detail but were considerably less well preserved than those of the control. The ganglion cells showed erosion of their inner edges. The nuclei were densely stained and fat-globules were present in the cell. Under high magnification there was a general tendency to deposition of fat in the form of fine globules. The cell processes were conspicuous. Müller's fibres were reduced to the form

of granular masses, their nuclei being densely stained and devoid of detail.

Thus we find in this experiment that subjection to heat has hastened the process of autolysis to a certain extent. The condition of the supporting structures is as much advanced as is the case in autolysis for 48 hours at atmosphere temperature (as evinced by the condition of Müller's fibres) and the degeneration of the nerve elements is likewise much in advance of the condition found in autolysis for 24 hours at the lower temperature.

Experiment V: (Fig. XIII) Ox-eye. Autolysis for 72 hours at room temperature.

Fixation in Müller's fluid. No control preparation. The rods and cones were reduced to an amorphous debris. The retinal elements were fairly well differentiated into layers, but there was encroachment of the external nuclear layer inwards at places. The nuclei of this layer were circular, small and densely stained, being devoid of all detail ("cog-wheel" etc.).

The cells of the internal nuclear layer were not well differentiated, only here and there was there an indication of the usual chromatin network. The nuclei of the blood capillaries were to be seen at places throughout the inner layers. The nerve fibre layers showed indication of fibrous

structure. The ganglion cells had persisted well and exhibited very fair nuclear structure. (The granules of the cell body were not brought out well by the fixative). Vacuolation (fat globules) was present in many of the cells. Müller's fibres were scarcely visible.

Experiment VI: (Fig. XIV) Ox-eye. Autolysis for 90 hours at room temperature. Fixation in 50% alcohol. Stained by Toluidin blue and by Haemalum and Eosin. No control preparation.

The external and internal nuclear layers were more or less fused together and the normal stratification of the retina was often lost to view. It was not always easy to determine the layer to which the various cells belonged, since the cells of the nuclear layer had lost all internal detail and appeared merely as small round spherical homogeneous bodies. The ganglion cells were still recognisable, and under low magnification appeared to show fairly good preservation. They were, however, more rounded in outline than normal, the processes having almost entirely disappeared. The nuclei were often eccentric and were densely stained. Under high power the ganglion cells were seen to contain numerous fat globules, the whole cell having a "stippled" appearance. There was occasional evidence of Nissl granules but these bodies, when present, were blurred and washed out

at the edges. Occasionally a long distorted dendritic process was seen in the degenerated ground substance. Here and there one found a well preserved nucleus showing the typical chromatin network, the cell body of which was almost absent. Erosin apparently always began on the inner aspect of the ganglion cell. The capillary framework was in many places particularly well demonstrated, the vessel walls apparently persisting for a considerable time.

In this experiment autolysis had been allowed to go on longer than in any of the others. We find in it that collapse of the outer layers is almost complete; persistence of the innermost layers (ganglion and nerve fibre layer) is marked. Degeneration of all the "supporting" tissue evidently occurred before that of the true nerve elements, a point which emphasises the differentiation between these structures. Degeneration occurred from the periphery of the cell inwards, the processes and cell body disappearing in many cases when the nucleus and central portion of the cell were tolerably intact. The cases in which the processes of the ganglion cells were conspicuous were to be explained by the fact that the ground substance being much degenerated, the comparatively resistant ganglion cells showed up with marked distinctness.

Experiment VII: Rabbit eye. Autolysis for 18 hours.

Room temperature. Fixation in 50% alcohol. Stained with Toluidin blue.

The external and internal layers were still fairly separate from each other by the external plexiform layer. The rods and cones were no longer recognisable, having entirely lost their "palisade" arrangement. Müller's fibres were prominent. The cells of the external nuclear layer were densely stained but often showed chromatin network ("cog-wheel" marking and "cross-striping"). The nuclei of the internal nuclear layer were "rarefied", the chromatin threads being attenuated and the interspaces enlarged. The ganglion cells showed well marked Nissl granules, but there was a blotchiness of the granules, especially of those at the periphery of the cell. There was a ragged appearance of the cell margins, which was not - as was the case in the ox - chiefly confined to the inner aspect. The nuclei of the ganglion cells were somewhat densely stained, at others they were "rarefied" with only an indication of the normal structure. On the whole the retina was well-preserved as compared with the following two experiments.

Experiment VIII: (Fig. XV) Rabbit eye. Autolysis for 24 hours at room temperature. No control preparation of this experiment was kept. Fixation in 50% alcohol. Stained by Toluidin blue and Weigert's iron-haematoxylin.

The rods and cones had practically disappeared. The fibres of Müller in their outer segments were almost invisible, but were well marked in the region of the nerve fibre layer. The external nuclei showed slight evidence of cross-striping. The external plexiform layer was more or less collapsed but showed occasional "cogwheel" markings. The chains of chromatin, forming the network in the bipolar cells, were broken up and the spaces between them enlarged so that the cells often appeared devoid of contents. The ganglion cells were much eroded at the edges and were rarefied. Their nuclei were generally fairly well preserved, but the cell-bodies were very fragmentary. Nisll granules were almost completely absent.

Experiment IX: (Fig. XVI) Rabbit eye. Autolysis for 45 hours at room temperature. Fixation in 50% alcohol. Stained in Toluidin blue.

The retina had collapsed as a whole and the portion available for examination was considerably reduced. The differentiation into layers at places was completely lost.

The nuclei of the external nuclear layer occasionally showed cross-striping, but there was no "cogwheel" to indicate the network of chromatin. Müller's fibres were still more or less existent. There was a general tendency to diffuse staining in the cellular elements. The number of recognisable ganglion cells was small, their nuclei often

being all that was left to mark their existence and the cell body, if present, was much vacuolated. The processes were almost non-existent. The nerve fibre layer was on the whole well preserved. Owing to the greater delicacy of the retina of the rabbit, the autolytic tissues were very liable to injury from technical manipulation. The finer graduations between the fresh condition and the more pronounced stages of autolysis were not demonstrable. As compared with the ox-eye one observed that the fibres of Müller were more resistant in the rabbit and the ganglion cells slightly less so.

Experiment X: (Fig. XVII) Kitten's eye. Autolysis for 48 hours at room temperature. Fixation in 50% alcohol. Stained by Toluidin blue and by Haemalum and Eosin. (This animal had been anaesthetised for several hours and the eyes were obtained about an hour after death).

In the control we found that the rods and cones showed a slight fusion of their outer extremities. The nuclei of the external nuclear layer were arranged in many tiers (from 4 to 6) the nuclei being elongated and rather densely stained. The chromatin network was very well demonstrated ("cogwheel" and "cross-striping"). The internal nuclei were full of chromatin arranged in a fine network. The ganglion cells showed discrete well stained Nissl granules arranged in con-

centric lines around the nucleus. The nucleolus was small and deeply stained. The processes of the ganglion cells were often very conspicuous. The blood capillaries were seen passing along the inner border of the outer plexiform layer, their endothelial nuclei being sometimes difficult to distinguish from those of the horizontal cells of the internal nuclear layer.

In the autolytic retina there was considerable collapse of all the elements. The rods and cones were no longer visible. The nuclei of the external nuclear layer were represented by small circular densely stained masses of chromatin with almost complete loss of detail. The nuclei of the internal nuclear layer were devoid of cell-body and showed only slight evidence of a chromatin nuclear network. The nuclei of Müller's fibres were often densely stained. The ganglion cells had, as a rule, rounded outer margins and eroded inner margins. The protoplasm of the cell body had a "stippled" appearance. Nissl granules, when present, were blurred in outline. The nuclei of the ganglion cells often appeared practically normal, except for some wrinkling of the nuclear membrane. There was not much vacuolation in any of the cells. Some cells appeared almost normal except for a slight loss of sharpness of detail.

Experiment XI: Kitten's eye. Autolysis for 90 hours. (Room temperature) Fixation in 50% alcohol. Stained in

Toluidin blue, and in Haemalum and Eosin. (This animal had been the subject of a prolonged metabolic experiment).

Control: Haemalum and Eosin. The rods and cones were somewhat disorganised, and the external nuclei were densely stained showing little internal detail, although a "cross-striping" was present.

The internal nuclei showed fair chromatin network in the haemalum and eosin specimens. The ganglion cells showed vacuolation (fat globules) in many of the cells, even where there were typical appearances of Nissl granules and absence of diffuse staining. The edge of the cells, however, were often ragged and the processes were short. The small type of ganglion cell with eccentric nucleus and scanty protoplasm generally showed well stained Nissl granules, although there was often a "washed-out" appearance of the chromatin which indicated that the cells were in a more or less abnormal condition.

In the autolytic eye the appearance were very different. The rods and cones were invisible as such, and the various layers of the retina had become fused. The external and internal nuclei under low power view were not to be distinguished from one another, both being represented by small shrunken densely stained spheres. Higher magnification did not reveal any differentiation of the chromatin into a net work.

No Müller's fibres were to be found, and the nuclei of these fibres were indistinguishable from the others of the internal nuclear layer. The ganglion cells were much reduced in number. When present, they were seen as large, somewhat spherical, isolated cells, having a very finely granular, densely stained nucleus. The processes had disappeared. In some cells the inner margin had eroded (as in the ox-eye). Fat globules occurred frequently and Nissl granules were generally absent. The nearest approach to the normal consisted in a very finely granular condition of the cell protoplasm, with faintly outlined dendritic processes.

The preservation in this retina was on the whole much the same as that of the ox-eye for the same period of autolysis. (Expt. VI).

Experiment XII: (Fig. XVIII) Dog's eye. Autolysis for 46 hours (room temperature). Fixation in 50% alcohol. Stained by Toluidin blue, Thionin and Haemalum and Eosin.

Control: The preparations stained by Thionin showed fairly well defined Nissl granules, but there was slightly more staining of the ground substance than was the case in Toluidin blue. In the latter the granules stood out sharply defined against the colourless background. The dendritic processes of the ganglion cells were excellently stained in all the preparations. The nucleus was somewhat more densely

stained by Thionin than by Toluidin blue. Vacuolation was, as a rule, absent. The blood capillaries appeared conspicuous as they crossed the internal nuclear layer to reach the external plexiform layer.

In the autolytic eye the rods and cones had become completely granular and unrecognisable as a palisade structure. The nuclear layers had become almost fused, the outer plexiform layer being greatly reduced in breadth. The external nuclei were densely stained and exhibited practically no structural detail (no "cogwheel" or "cross-striping"). The "centrifugal" cells of the internal nuclear layer were fairly well preserved and showed considerable nuclear detail. The "horizontal" cells also were to be recognised. The ganglion cells showed erosion of the edges of the largest cells. The differentiation of the chromatin into granules was lost, the ground substance being diffusely stained. The nuclei were often diffusely stained and lacking in detail. There was vacuolation, especially in the medium sized cells. The cell processes were sometimes well preserved and stood out against the degenerated supporting elements.

On the whole, however, the ganglion cells in this specimen were well preserved, in many the presence of fairly typical Nissl granules being demonstrable. Erosion of the cells apparently began on the inner aspect, i.e., at the side towards the axis-cylinder, although the nerve fibres

themselves were comparatively resistant. It was the epithelial-like elements of the retina (rods and cones) which perished early, the more purely nervous structures (nerve fibres, ganglion cells) being preserved for a considerable time. The delicate terminals, however, of the external plexiform layer early succumbed, allowing of fusion of the nuclear layers.

The principal points to be noted in these experiments are erosion of the margin of the ganglion cell with diffuse staining of the protoplasm, absence of definite Nissl granules and degenerative changes in the nuclei. Shrivelling of the cells took place and was probably the ultimate fate of all the cells, but one was struck with the absence of marked shrinking of the cells, when compared with the results of Birch-Hirschfeld and others whose experiments were concerned frequently with a shorter period of post morten degeneration.

(3) Discussion of Results:

It is seen from the foregoing that the appearances in the retina which are associated with post morten changes are very different from those which occur as the result of operative conditions during life. We have seen that the ganglion cells which, in the case of section of their axons, readily tend to degenerate, are comparatively resistant to post mortem changes, cells which show tolerably good preserva-

tion being found at a period of 90 hours after death (in the case of the ox eye) 46 hours (in the case of the dog), and 45 hours (in the case of the rabbit). In the outer layers, however, we find degeneration setting in with considerable rapidity in the direction of from without inwards. The rods and cones are the first of all the elements to degenerate, disappearing in the case of the ox before 16 hours and in the rabbit before 18 hours, in the dog not entirely disappeared at 46 hours. The nuclei of the rods and cones begin to show degenerative changes about the same time and the nuclei of the inner nuclear layer somewhat later.

In the experiments described, the short periods given for each animal are those at or about which the first definite changes were made out. For example, in the case of the ox, where the changes of 16 hours are defined, it is to be understood that changes may occur at a period less than that but they are so slight and show so little progression that one has not placed undue importance on their recurrence. Several hours seemed to produce little effect on the cells when the eye was sealed from the atmosphere, and one's object was not so much to set a limit upon the onset of changes as to investigate the various layers. In the case of the rabbit retina it is true that changes set in before 18 hours, but they are not so pronounced and one has selected the periods at which definite changes are to be observed.

In the series described by Birch-Hirschfeld changes are met with in all the elements as early as 2 hours after death, and have ended in complete destruction at 18 hours. The difference between such results and those of the present series in which well preserved ganglion cells are met with at a period of 45 hours (see Fig. XV) is probably accounted for by the fact that in the former case putrefaction was not excluded as a causal factor whereas in the latter the possibility of its occurrence was reduced to a minimum.

PART III - CONCLUSIONS

1 The range of variability amongst the so called normal ganglion cells of the retina, is considerable, and the differences are so marked as to lead to the conclusion that the features described under the term chromatolysis are not necessarily pathological.

2 The smaller ganglion cells of the retina seem to be specially liable to changes, and abnormal small cells are prevalent in the control experiments. They are neglected in the present investigation. The large multipolar cell, which in the healthy retina is of most frequent occurrence, appears much more stable. This cell contains a central nucleus with one or more nucleoli, and shows when treated by the alcohol - toluidin blue method - sharply defined Nissl granules of different sizes arranged more or less concentrically around the nucleus, the ground substance being quite colourless. Departure from this type to any marked extent may be taken to indicate abnormal changes.

3 It is the absence of truly normal cells in any retina, rather than the occurrence of isolated abnormal cells that

indicates a pathological condition. When the number of normal cells is nil, and that of abnormal cells is excessive, degeneration is fairly established.

4 The gradual sequence of chromalytic change (swelling of the cell-body, eccentric position of the nucleus, diffuse staining of the protoplasm, vacuolation etc.) is not demonstrated by the retinal ganglion cells. One is not always able to demonstrate the changes between the first trace of degeneration in a cell (loss of definition in the Nissl granule) and complete atrophy. Almost normal cells may be found side by side with others in which there is diffuse staining of the protoplasm and nucleus, or others in which the cell is pale and contains small dust-like particles. Degeneration of any cell is indicated by the early loss of definition in Nissl granules and by the final or atrophic stage of the process. Vacuolation is met with in the control with at least as much frequency as in the operative, and probably does not represent a pathological condition in vivo.

5 As a result of section of the optic nerve the fibres of which constitute the axons of the ganglion cells of the retina, degeneration of the ganglion cells sets in as early as 48 hours after operation. Degeneration advances with the lapse of time, is marked at 3 weeks and is complete at

38 weeks, all the cells being atrophic. The ganglion cells are the central sensory neurones of the retina and are therefore seen to perish upon section of their central processes.

6 Practically no change takes place in the cells of the outer and inner nuclear layers which are the more peripherally situated neurones existing in contiguity with the ganglion cells. They are therefore seen to remain unchanged when connection with the central neurones is severed, thus showing that there is independence of such peripheral neurones.

7 The fact that these peripheral retinal neurones have no special power of resistance is illustrated by the series of autolytic experiments in which degeneration was seen to occur in the reverse order from that of the optic nerve section experiments. The layer of rods and cones with their nuclei degenerate first, the inner nuclei being next to perish, the ganglion cells being comparatively resistant.

In conclusion I wish to record my sense of great indebtedness to Professor D. Noël Paton under whose constant supervision this work has been carried out; also to Dr. G. Herbert Clark of this Laboratory for assistance in the operations.

The photo-micrographs were taken by Mr. Richard Muir, Edinburgh. The camera lucida drawings are my own work.

ADDENDUM

EXPERIMENTAL SECTION OF THE OPTIC NERVE IN A MONKEY

The following experiment was not completed in time to allow of the results being incorporated with the preceding series, but one feels justified in making a note of the experiment by reason of its having been performed on one of the higher animals.

I have not come across any description of experimental section of the optic nerve in the monkey which would bear upon the present investigation, and although the results in this case were not uncomplicated, I feel it may be of interest to record the history of the experiment. Two facts are to be noted in connection with this experiment, viz:- That it is somewhat difficult to obtain healthy apes for experimental purposes, and also that the operation of section of the optic nerve in the small monkeys at my disposal is extremely difficult, the nerve being almost inaccessible owing to the depth of the orbital cavity. One operation was performed in the orbital cavity and resulted in buttonholing of the globe, and had to be discarded. In the present

operation (Kraulein's) the nerve was approached from the cranial cavity, a portion of the temporal bone being removed.

In the manipulation of this difficult operation I was assisted by Dr. T. Graham Brown of the Physiological Department, and I gratefully acknowledge his help.

HISTORY OF EXPERIMENT:

The animal was a small elderly female, badly nourished. Ether anaesthesia was used. Due antiseptic precautions being observed, a large wedge-shaped portion of the external wall of the orbit was removed, and the optic nerve isolated and divided far back in the orbital fossa. There was slight haemorrhage after division of the nerve, but this was easily controlled and appeared to come from the extrinsic vessels. There was slight contraction of the pupil after section.

Examination of the fundus showed little or no change in the vessels of the disc (see Fig. 18d) which shows that the vessels of the retina remained full of blood. The animal recovered from the anaesthetic immediately. There was some redness and effusion of the eyelid for a day or two after the operation, but this condition passed off and the eye appeared normal except for the presence of ptosis which had resulted from dragging of the eyelid into the wound on the temple.

The animal did not appear at any time much inconven-

ienced by the operation, the left eye apparently being able to do duty for both. Nine weeks later the eye looked clear and healthy. There was still slight ptosis of the upper eyelid. Ophthalmoscopic examination of the fundus with homatropine showed extreme pallor of the disc. Vision in the eye was nil. The animal was killed 25½ weeks after operation.

HISTOLOGICAL EXAMINATION:

The eyes were removed immediately after death and were fixed with as little delay as possible. There was no sign of phthisis bulbi in the operated eye. The cornea in each was removed as well as lens vitreous etc. The retina in the operated eye appeared in parts considerably attenuated, especially towards the ora serrata. The cup thus formed was in each case divided into four parts and these were treated by various fixatives, the corresponding portions being taken from both for the same fixative. The fixatives employed were (1) $HgCl_2$ sat. sol. (2) 75% alcohol. (3) absolute alcohol + 0.75% of ammonia for silver impregnation and (4) Müller's solution. The tissues were embedded in paraffin. Silver impregnation was unsuccessful in both cases. In the sections fixed by $HgCl_2$ and 75% alcohol Toluidin blue was the stain employed. In (4) Weigert's rapid iron haematoxylin method was used. In the case of

(2), i.e., the alcohol material, the retina was so thinned out that little could be made out from it. The observations were mostly taken from the material which was fixed in $HgCl_2$.

As the retina of the monkey presents features somewhat different from those of the rabbit a slight sketch of the various elements may be outlined. As will be seen from fig. 18a, the normal retina consists of three layers of cells - the outer and inner nuclear layers and the layer of ganglion cells. The nuclei of the outer layer are in rows, three or four deep, and are oval or roughly spherical in outline. Their chromatin is distributed in the form of 2 to 5 small ragged masses irregularly grouped in the cell. The outer layer therefore differs from the cross striped arrangement met with in the rabbit.

The nuclei of the inner nuclear layer are arranged in rows uniformly 3 deep. These nuclei are slightly larger than those of the outer layer and stain less intensely. Their chromatin is strewn somewhat irregularly throughout the cell.

The ganglion cell layer contains numerous blood vessels and small glial cells. The ganglion cells occur at frequent intervals and are seen to be of varying sizes. The most conspicuous type are the large multipolar cells with more or

less centrally placed nucleus. The inner plexiform layer is a well developed closely granular layer occurring between the ganglion cell layer and the inner nuclear layer. In the outer plexiform layer two distinct strata are to be seen, viz:- a narrow inner band which is dense in character, and a broader outer band containing large spaces traversed by trabeculae which pass upwards to the outer nuclear layer. Towards the region of the ora serrata these spaces are very large and give the appearance of cysts.

OPERATED EYE: From the absence of any definite signs of inflammation in the eyeball and no phthisis bulbi no undue loss of nutrition such as would result from section of the ciliary nerves etc., we would be justified in inferring that the changes produced in the retina were those resulting from section of the optic nerve alone. In many places the retina is very attenuated, being reduced to the merest membrane. At the parts, however, where the retina appears most intact one is able to examine the various layers and compare with the corresponding layers in the control. In the outer nuclear layer at these parts little or no change is to be seen in the operated eye (fig. 18e). There is perhaps a slight loss of definition in the chromatin granules, but it is not marked. Similarly in the case of the inner nuclear layer practically no change is to be seen in the cells themselves as result of the experiment. There is, however, con-

siderable thinning of the layer as a whole, the layer consisting sometimes of only one row of cells. The ganglion cells are practically absent. When found they are atrophic in the extreme. In this layer, however, many other cells are seen, among which are the extruded cells of the inner nuclear layer, and many neuroglial cells.

The blood vessels are of good calibre and full of blood, which indicates that the blood supply of the retina has not suffered (see fig. d).

The specimens which were fixed in Müller's fluid and stained by Weigert's method, do not add anything to the above.

This case, while not entirely satisfactory owing to the thinning of the retina and age of the animal concerned, shows however in the well preserved parts of the retina similar features to those met with in the case of the experiments in rabbits and may therefore be included with the latter as confirmatory of the general result of the investigation, viz:- that the peripheral sensory neurone is capable of independent existence.

PART IV - APPENDIX

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(1) REFERENCES:

(1) Apathy S., Mitth. aus d. zool. Stn. 2 Neapel 1897 Bd. XII 4

(2) Bach, Trans. 8th Internat. Ophth. Cong. Edin. 1894

(3) Barbacci v. Campacci. Centralb. f. Nervenhlk. 1897
" " " Riv. di patol. nerv. e ment. 1897 F. 8

(4) Barker A., Text Book, The Nervous System London 1901

(5) Benda, Neurol. Centralb. 1895.

(6) Berlin R. Sitzungsber. der Ophth. Gess. zu Heidelburg. 1871

(7) Bethe A., Morphol. arb. Jena 1898. Bd. VIII.

(8) Birch-Hirschfeld, Arch. f. Ophth. 1900 Bd. L.

(9) Bolton J. S., Phil. Trans. Roy. Soc. 1900, vol. 193

(10) Cajal, S. Ramon y., Die Retina d. Wirbelt ü. stzg. v. Greef 1894
Cajal, S. Ramon y., Trab. del Lab. de invest. biol. 1903 Tom. II. f 4
Cajal, S. Ramon y., ibid. 1906. Tom. IV
" " " ibid, 1907. Tom. V f. 3

(11) Campbell A. W., Histol. Studies on Localis. of Cerebral Function, Camb. 1905

(12) Colucci, Arch., ital. de biol. 1895, T. XXIII. f. i.

(13) Dean and Usher, Brain, 1896 vol. XVI; ibid 1903 vol. XXVI.

(14) Ewing, Arch. of Neur. and Psychopath. 1898, vol. I. 3

(15) Flemming, Anat. Hfte. 1896. Bd. VI

(16) Ford-Robertson W. Critical Digest. Brain 1899. vol. XXII.

(17) Forel A., Arch. f. Psych. v. Nervenhlk. 1887. Bd. XVIII.

*(18) v. Gerlach J., Manual of Histology (S. Stricker) New York, 1872

†(19) Goldscheider v. Flatau., Norm. v. Path. anat. d. Nervenzellen, Berlin 1898

(20) Golgi C. Riv. sper. di freniatr. 1882. vol. VIII, ibid 1883 vol. IX., ibid. 1885. vol XI.

(21) v. Gudden. For complete list see Barker (4) p. 800.

(22) Halliburton. Sc. Prog. 1908, No. VII.

(23) Hamburger. (Proc. of) Physiol. Lab. d. Utrecht Hoogesch, 1889, XI

(24) Hertel. Arch. f. Ophth. 1898, Bd. XLVI 2. ibid 1901. Bd. LIII 2. Arch. of Ophth. 1898 Vol. XXVII p. 549

(25) His., For list see Barker (4) p. 164.

(26) Krause. W. Die. memb. fenest. d. Tetina. Leipz. 1868

(27) Krenchel. Arch. f. Ophth. 1874. Bd. XX

(28) Knies. Arch. f. Aug. - v. Ohrenheilk. 1878, Bd. VII.

(29) Leber. Handbuch v. Graefe Saemsch Bd. II. 1

(30) Lehrmann, Exp. quaed. de nervi opt., Inaug. Dissert. Dorpat. 1857

(31) v. Lenhossek. Neurol. Central. 1897.

(32) Lent. E., Ztsch. f. Wiss. zool. 1856. Bd. VII.

†(33) Levi Giulio. Riv. di patol. nerv. e ment. 1896 f. 4. ibid. 1898. f. 1

†(34) Lugaro, E., Riv. di Patol. nerv. e ment. 1896. f. 12 ibid 1897

(35) Mann., Journ. of Anat. and Phys. 1895. vol. XXIX

(36) Marchwort, Arch. f. Augenhlk. 1881. Bd. X

(37) Marinesco. G. Neurol. Centralbl. 1898

(38) v. Monakow, For list see Barker (4) p. 784

(39) Mott, F. W., Pres. add. to Roy. Soc., Brit. Med. Jour. 1909 Nov. 13

*(40) Neppi, Riv. di Patol. nerv. e ment. 1897 vol. II f. 4

(41) Nissl F., Allg. Ztsch. f. Psych. 1892 Bd. XLVIII.
" Neurol. Centralbl. 1894
" Centralbl. f. Nervenhlk. v. Psych. 1894
" Neurol. Centralbl. 1895

(42) Onuff. J. of Nerve and Ment. Dis. 1895. vol. XX 10

(43) Parsons J. H., Text Book, Pathol. of the Eye. Lond. 1905, Pt. II

(44) Pergens. Annal de la Soc. Roy. des Sc. Med. et Nat. de Brux. 1897. vol. VI

(45) v. Poncet, Gaz. med. de Paris. 1882. nr. 52

(46) Quain. Text book of Anat. 1909. vol. III. 2 p. 359

(47) Redard, Recueil d'Ophth. 1880; also Nagels Jahresber. 1880. S. 192

(48) Remak. R., Arch. f. Anat. Phys. v. Wissensch. med. Berlin 1844

(49) Rosin. Deutsche med. woch. 1898

(50) Rosow. B. Sitzsber. d. math. natur. class d. kaiserl. acad. d. Niss., Wien 1864. Bd. XLIX 1., ibid Bd. L 11

(51) Russi. Inaug. Dissert. Bern. 1880

(52) Schultze, M. Arch. f. Mikros. anat. 1866. Bd. II

(53) Wagenmann A., Arch. f. Ophth. 1890. Bd. XXXVI, 4

(54) Waldeyer, W., Deutsche med. Wehnschr. Leip. 1891 Bd. XVII

(55) Waller A., Lond. Edin. and Dub. Philos. Mag. 1850, vol. XXXVII

(56) Warrington, Jour. of Phys. 1898. vol. XXIII. p. 112

(57) Wright H., Journ. of Phys. 1900. vol. XXVI

*Quoted by Barker (4)

† " " Ford Robertson (16)

" " Birch-Hirschfeld (8)

" " Wagenmann (53)

(2) DESCRIPTION OF PLATES:

Fig. I: Experiment II. 48 hours after section of the optic nerve.

(a) Operative. Tol. Blue. Photomic. X1000. showing ganglion cells somewhat diffusely stained. In the one to the left the nucleus is eccentric. The outer retinal layers are normal.

(b) Control. Tol. Blue. Photomic. X1000. showing large ganglion cell with discrete chromatin granules, and centrally placed nucleus. The outer layers are not seen.

(c) Operative. Tol. B. Drawing (cam. luc.) oil m.m. 1/12. ganglion cells selected from different fields to show the best preservation. Note, diffuse staining in most of the cells. The cells are smaller than in (d)

(d) Control. Tol. B. Drawing (c.l.) oil m.m. 1/12. different types of ganglion cells which occur normally, (a) is a so called 'normal' cell, (b) is a rarefied or 'ghost' cell, (c) shows the nucleus eccentric.

Fig. II: Experiment V. 4 days after section of the optic nerve.

(a) Operative . Tol. B. Photomic. X.700, showing large ganglion cell with somewhat diffuse staining. The outer layers are normal.

(b) Control. Tol. B. Photomic X.500. Although under lower magnification than (a) the ganglion cells show much greater detail of structure. The outer layers contrast rather unfavourably with those of (a)

(c) Operative. Tol. B. Drawing (c.l.) oil m.m. 1/12. showing ganglion cells from different fields. Most of the cells are small and stain feebly The chromatin granules are dust-like. No quite normal cells appear. (compare control).

(d) Control. Tol. B. Drawing (c.l.) o.i. 1/12. ganglion cells from different fields. (a) "normal" cell, (b) small cell with eccentric nucleus and peripherally-situated granules.

(e) Operative. silver impreg. Drawing (c.l.) o.i. 1/12. showing (a) ganglion cell, (b) bipolar cell from inner nuclear layer. Staining was less successful than in control. Varicosities not well marked.

(f) Control. as in (e) showing (a) ganglion cells with optic nerve fibres, (b) "horizontal cell" from outer plexiform layer. Note frequent varicosities on the fibrils.

(g) Operative. Tol. B. Photomic. x 950. To show normal outer layers.

(h) Control. Tol. B. Photomic x 950. Outer layers compare unfavourably with (g)

Fig. III: Experiment VII. 8 days after section of the optic nerve.

(a) Operative. Tol. B. Drawing (c.l.) o.i. 1/12. ganglion cells selected from different fields. Note, pale staining, and dust-like condition of the Nissl granules

(b) Control, as in (a) ganglion cells from different fields illustrating various types of cell, some of which are not "normal". Normal cells are frequent in the sections, however.

(c) Operative. Tol. B. Photom. X.1000, showing outer layers normal. Note well-marked "cross-striping" and "cog-wheel" in the outer nuclear layer.

(d) Control. Tol. B. Photomic. X.1000, showing outer layers which are if anything less "normal" than in (e)

(e) Operative silver impreg. Photomic X800, shows large ganglion cell with dendrite passing upwards to inner plexiform layer. The axon becomes continuous with an optic fibre (below).

(f) Operative silver impreg. Drawing (c.l.), o.i. 1/12. showing ganglion cells with their processes. Note absence of varicosities.

Fig. IV: Experiment VIII, 3 weeks after section of the optic nerve.

(a) Operative. Tol. B. Photomic X1000. Note the diffusely staining ganglion cell with what appears to be the extruded nucleus (above).

(b) Control, as in (a). Large typical ganglion cell in centre; small cell to the right, showing degeneration (eccentric extruded nucleus and rupture of cell)

Fig. V: Experiment IX, 3 weeks after section of the optic nerve.

(a) Operative. Tol. B. Photomic X400, shows small ganglion cells which are somewhat diffusely stained and have feeble processes. The outer layers are normal

(a^1) Same, X1000

(b) Control, as in (a). Showing normal ganglion cells and outer layers.

(b^1) Same, X900.

(c) Operative. Fixation 50% alc. Tol. B. Drawing (c.l.) o.i. 1/12. Showing small diffusely stained ganglion cells. The larger cells seen to the left show a dust-like condition of the chromatin.

(d) Control, as in (c). Showing sharply defined Nissl granules in the ganglion cells.

(e) Operative. Fixation in $HgCl_2$. Tol. B. Drawing (c.l.) o.i. 1/12. ganglion cells from different fields. The cells are smaller than in control and have greater tendency to diffuse staining. No "normal" cells are to be found.

(f) Control, as in (e). Ganglion cells from different fields. These cells illustrate the atypical cells often met with in the controls.

(g) Operative. Fixation in formol saline. Tol. B. Drawing (c.l.) o.i. 1/12. Ganglion cells showing somewhat diffuse staining. Vacuoles are frequent. The cells are smaller than in (h).

(h) Control, as in (g). Ganglion cells showing large Nissl granules. Vacuoles are met with.

Fig. VI: Experiment X, 6 weeks after section of the optic nerve.

(a) Operative. Tol. B. Photomic. X1000. The ganglion cell in the lower part of the field shows somewhat diffuse staining. The outer layers are practically normal, the nuclei of the rods and cones showing a well-marked "cross-striping" and "cogwheel" arrangement.

(b) Control. Tol. B. Photomic X500, showing typical ganglion cell to left. In the cell to the right the nucleus is eccentric and the pericellular space is conspicuous.

(b^1) Same, X950. To show outer layers with normal appearances.

(c) Operative. Tol. B. Drawing (c.l.) o.i. 1/12. Note the much diminished ganglion cells as compared with control. Staining is diffuse or there is a dust-like condition of the granules. (a) represents the nearest approach to a "normal" cell.

(d) Control, as in (c). Note large typical ganglion cells (a) and (b), with atypical forms (c) and (d).

(e) Control. Silver impreg. Drawing (c.l.) o.i.1/12. Showing ganglion cells (a) and (b) with adjacent optic nerve fibres. (c) represents the varicose fibres of the internal plexiform layer. (d) fibres of the outer plexiform layer with a large "horizontal cell" in the centre.

Fig. VII: Experiment XI, 15 weeks after section of the optic nerve.

(a) Operative. Tol. B. Photomic. X1000. Note the solitary ganglion cell having a scanty ring of protoplasm around the centrally situated nucleus. The cells of the inner nuclear layer are normal. The cells of the outer nuclear layer are densely stained and show less definition than in the control, but still show indication of "cross-striping" and "cog-wheel" arrangement.

(b) Control, as in (a). Showing deeply stained ganglion cells alternating with others which tend to become rarefied. Note well defined "cogwheel" markings of outer nuclear layer. In this case the slides to be photographed were not from strictly the same regions in both as will be seen from the marked preponderance of ganglion cells in the control, but the points illustrated are borne out in all the other sections examined.

(c) Operative. Fixation in Formol Saline. Tol. B. Drawing (c.l.) o.i. 1/12. Showing ganglion cells much diminished in size. Diffuse intense staining is the rule. There is no vacuolation. Pericellular spaces not enlarged. No normal cells are to be found.

(d) Control, as in (c). Ganglion cells from different

fields. Note vacuolation in (a) enlarged pericellular space in (b).

(e) Operative. Fixation in 50% alcohol. Tol. B. Drawing (c.l.) o.i. 1/12. Ganglion cells from different fields. Note small size of cells, diffuse staining and absence of vacuolation. Pericellular spaces occasionally seen (a).

(f) Control, as in (e). Showing many normal ganglion cells (a) with others (b) less typical.

(g) Operative. Fixation in $HgCl_2$. Tol. B. Drawing (c.l.) o.i. 1/12. Showing differentiation even less well marked than in (e)

(h) Control, as in (g). Differentiation less pronounced than in (g).

(i) Control. Silver impreg. Drawing (c.l.) o.i. 1/12. (a) Ganglion cell with axon towards the right. (b) Ganglion cell with optic nerve fibres in juxtaposition. (c) Group of cells, the large cell in centre being a "horizontal cell" from the outer plexiform layer, the cells on the right being those of the inner nuclear layer, those on the left from the outer nuclear layer. Varicosities are nowhere well demonstrated.

Fig. VIII: Experiment XII. $16\frac{1}{2}$ weeks after section of the optic nerve.

(a) Operative. Tol. B. Photomic. X1000. Showing shrunken ganglion cell. The protoplasm is diffusely stained and the nucleus is somewhat eccentric. The pericellular space is seen. Protoplasmic extensions are scarcely visible (compare (b)).

(b) Control, as in (a). Showing "normal" ganglion cell, with well defined upgoing process.

(c) Operative. Tol. B. Photomic. X950. Showing outer layers which appear normal.

(d) Control. Tol. B. Photomic. X950. Showing outer layers which if anything compare unfavourably with (c)

(e) Control. Silver Impreg. Photom. X700. Showing large ganglion cell with well-stained processes. The axon is seen passing out (below) to become continuous with an optic nerve fibre. No varicosities are evident.

(f) Operative. Tol. B. Drawing (c.l.) o.i. 1/12. Ganglion cells much diminished in size. The chromatin, when granular, is lightly stained and is in the form of fine dust-like particles. Usually, however, there is a condition of dense diffuse staining of the protoplasm.

(g) Control, as in (f). Ganglion cells show large well defined Nissl granules. A few rarefied cells may be seen (a).

(h) Control. Silver impreg. Drawing (c.l.) o.i. 1/12. Showing successful staining of the fibrous structures in the various cells.

Fig. IX: Experiment XIII. 38 weeks after section of the optic nerve.

(a) Operative. Formol saline fixation. Tol. B. Photomic X1000. Showing shrunken hyaline ganglion cells with eccentric nuclei. The chromatin is diffusely and densely stained. Their pericellular spaces are not enlarged. Note that although definition of the chromatin in the external nuclear layers is not well marked the corresponding elements in the control show similar characteristics.

(b) Control, as in (a). Note ganglion cell showing large well defined Nissl granules. The cells of the outer and inner nuclear layers show scarcely better definition than those of the corresponding layers in (a).

(c) Operative. Fixation in $HgCl_2$. Tol. B. Drawing. (c.l.) o.i. 1/12. Ganglion cells from different fields. Note the small size and absence of well defined Nissl granules. There is wrinkling of the nuclear membrane, pericellular spaces are not enlarged.

(d) Control, as (c). Shows various types of ganglion cell. In (a) there is diffuse staining. Note presence of large cells with well defined Nissl granules, a type never found in (c).

(e) Operative. Fixation in Formol Saline. Tol. B. Drawing (c.l.) o.i. 1/12. Illustrates the condition of atrophy in the ganglion cells. Note shrunken cells and lack of vacuolation. Pericellular spaces not enlarged. Diffuse staining is prevalent, practically no granular structure being indicated.

(f) Control, as in (e) ganglion cells from different fields, showing large well-defined granules. Note vacuoles and conspicuous pericellular spaces.

Fig. X: Ox eye, autolysis 24 hours (room temperature)

I (a) Autolytic Eye: Fixation in 50% alcohol. Haemalum and Eosin. Photomic. X700. Large ganglion cell showing granular condition of the chromatin. The nucleus is somewhat eccentric. The protoplasmic processes are prominent. The cells of the external and internal nuclear layers are considerably more degenerated than the ganglion cells.

(b) Autolytic Eye. Fixation in 50% alcohol. Tol. B. Photomic. X1000. Large ganglion cell with slight erosion of the cell margin. There is still a fair degree of granular differentiation, but the staining tends to be more diffuse than in control. The nucleus is deeply stained and is central. Protoplasmic processes not indicated.

(c) Autolytic Eye: Fixation in $HgCl_2$, Haemalum and Eosin. Photomic X500. The nuclear layers are still separated from each other by the outer plexiform layer. The cells of the nuclear layers are shrunken and densely stained. The ganglion cell to the left and below is somewhat ghost-like. The rods and cones have lost their "palisade" structure.

(d) Autolytic Eye: Fixation in $HgCl_2$, Tol. B. Photomic X700. Note the dense staining in the somewhat shrunken cells of the outer and inner nuclear layers. The ganglion cells tend to become diffusely stained.

(e) Autolytic Eye: Tol. B. Drawing (cam. l.) o.i. 1/12. Ganglion cells selected from different fields. Note their well preserved condition. The processes are often conspicuous. Granular structure is well marked. There is occasionally a tendency to pale staining and vacuolation.

(f) Autolytic Eye: Tol. B. Drawing (c.l.) o.i. 1/12. Ganglion cells selected from different fields. The smaller cells show rather diffuse staining. The larger cells are somewhat ghost-like.

(g) Same as (f)

II Control of I. Tol. B. Drawing (cam. luc.) o.i. 1/12. Showing different types of normal cells (a) rarefied cell with eccentric nucleus. (b) cell containing discrete Nissl granules and eccentric nucleus. (c) cell which is profusely vacuolated, (d) small cell diffusely stained.

Fig. XI: Ox eye. Autolysis 48 hours (room temperature)

(a) Autolytic Eye: Fixation in picro-formol. Tol. B. Photomic X1000. Large ganglion cell with conspicuous processes and well defined Nissl granules. Nucleus and nucleolus still well preserved.

(b) Autolytic Eye: Fixation in 50% alcohol. Tol. B. Photomic. Showing disorganisation of rods and cones. The large ganglion cell in the centre of field is somewhat diffusely stained, but is still granular. The nuclear layers show degenerative changes.

(c) Control of (b). Large ganglion cell in centre of field shows well defined Nissl granules. Outer layers well differentiated.

Fig. XII: Ox eye. Autolysis for 72 hours (room temperature)

(a) Autolytic Eye: Fixation in Müller's solution. Weigert's iron haemalox. Photomic X500. The external plexiform layer is almost obliterated. The cells of the outer and inner nuclear layers are de-

generated (shrunken, densely stained). Large ganglion cell at lower part of field shows somewhat eroded edges and densely stained nucleus.

(b) Autolytic Eye: Fixation in 50% alcohol. Tol. B. Photomic X1000. Large ganglion cell diffusely stained, but still granular. Note protoplasmic process on left. The outer layers are not all shown in this field.

(c) Autolytic Eye: Fixation in 50% alcohol. Haem. and eos. Photomic X1000. Ganglion cells with erosion of cell margins, but otherwise fairly well preserved.

Fig. XIII: Ox eye. Autolysis for 90 hours (room temperature)

(a) Autolytic Eye: Fixation in 50% alcohol. Tol. B. Photomic X1000. Showing fusion of external and internal nuclear layers (the cells of the two layers are scarcely distinguishable from each other) Ganglion cells densely stained but still showing indication of granules. The nuclei are deeply stained.

(b) Control of (a) Large ganglion cell with strong upgoing process. The nucleus is eccentric. Nissl granules lack perfect definition.

(c) Autolytic Eye: Tol. B. Drawing (cam. luc.) o.i. 1/12 Ganglion cells from different fields. Staining is somewhat diffuse and often intense. The cell margins are eroded. Protoplasmic processes generally wanting. There is still indication of granular structure.

Fig. XIV: Rabbit's eye. Autolysis for 24 hours (room temp.)

Tol. B. Photomic X1000. Showing ghost-like ganglion cell with eroded edges. There is still evidence of the protoplasmic extensions. The nucleus is well preserved. Granular structure still visible in these cells.

Fig. XV: Rabbit's eye. Autolysis for 45 hours (room temp.)

Fixation in 50% alcohol. Tol. B. Photomic. X1000. Large ganglion cell with well preserved processes. The nucleus is central and is in a state of good preservation. Nissl granules are present, but there is a tendency to diffuse staining. The cells of the inner nuclear layer are degenerated, but less so than those of the outer nuclear layer.

Fig. XVI: Kitten's eye. Autolysis for 48 hours (room temp.)

(a) Autolytic Eye: Fixation in 50% alcohol. Tol. B. Photomic X1000. Large ganglion cell with densely stained but still granular protoplasm. The nucleus is well preserved. The cells of the outer and inner nuclear layers are almost completely degenerated.

(b) Control of (a). Note the large typical ganglion cell with up-going process. The cells of the outer and inner nuclear layers present marked contrast to those of (a). Note the elongated rod nuclei with several horizontal interspaces between the chromatin masses.

(c) Autolytic Eye: Fixation 50% alcohol. Drawing (cam. luc.) o.i. 1/12. Ganglion cells from different fields. Note the well preserved cell-processes; in (a) the stain tends to be diffuse.

(d) Control of (c). Showing ganglion cells from different fields; (a) typical cell; (b) rarefied or ghost-like cell; (c) cell with tendency to diffuse staining of the protoplasm.

Fig. XVIII: Dog's eye. Autolysis for 46 hours (room temp.)

(a) Autolytic Eye. Fixation in 50% alcohol. Tol. B. Photomic X500. Showing degenerated cells of the outer and inner nuclear layers. These layers are still separated from each other by the outer plexiform layer. The large ganglion cell below shows erosion. of the inner edges and diffuse staining of the protoplasm. Granular structure still recognisable.

(b) Control of (a). Note the large typical ganglion cell with well marked Nissl granules, central nucleus and up-going process. The cells of the outer and inner nuclear layers are well differentiated.

(c) Autolytic eye: Tol. B. Drawing (cam. luc.) o.i. 1/12 Ganglion cells from different fields. In (a) there is profuse vacuolation and the cell margins are eroded; (b) cell with well defined process; (c) almost normal cell, showing tendency to diffuseness of stain.

(d) Control of (c): Ganglion cells from different fields; (a) typical cell with well-defined Nissl granules, central nucleus, and well marked process; (b) somewhat rarefied cell, with eroded margins.

Fig. 18: Monkey's Eye. $25\frac{1}{2}$ weeks after operation.

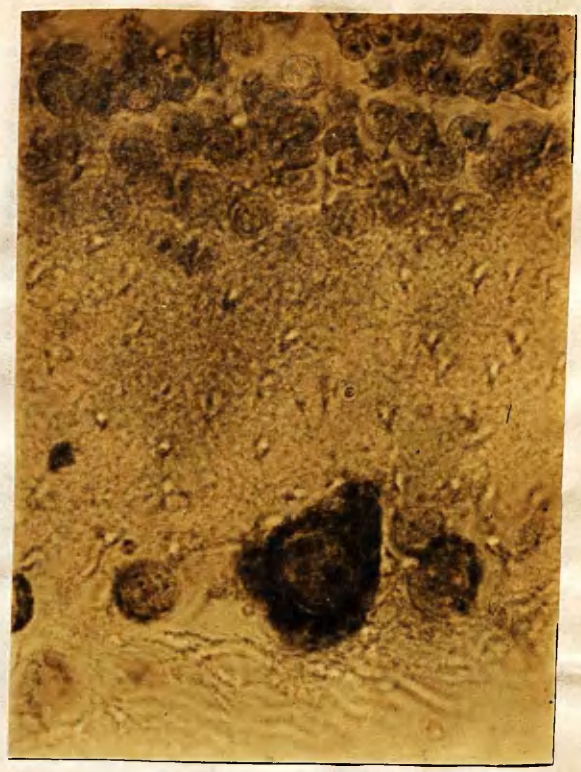
(a) Control: Fixation in $HgCl_2$. Tol. B. Photomic X500 showing the various elements of the normal retina.

(b) and (c) Control as in (a) X1000. Showing the ganglion cells, and inner nuclei. The outer nuclei are seen at the upper margin of (b).

(d) Operative: Fixation in $HgCl_2$. Weigert's Haematoxylin. Photomic X300. Showing well-filled blood vessels in the retina.

(e) Operative: Fixation in $HgCl_2$. Tol. B. Photomic X1000. Showing absence of ganglion cells. Note the much-thinned inner nuclear layer, the cells themselves being well preserved. The cells of the outer nuclear layer also are practically normal.

(a)



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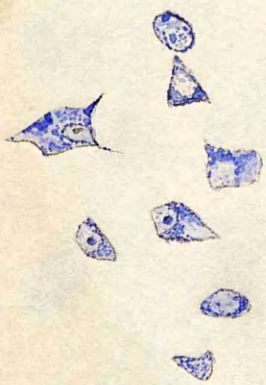

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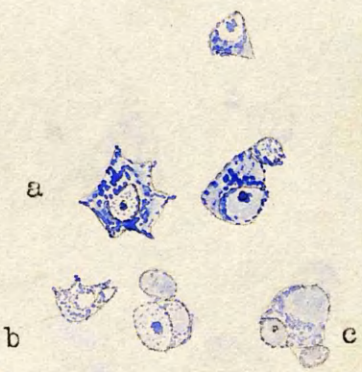
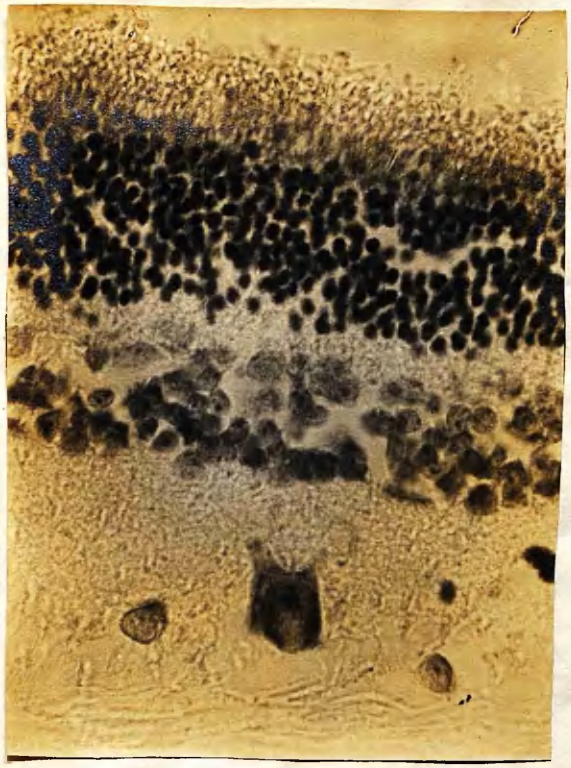
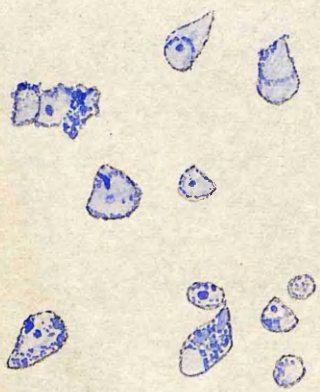


(d)

FIG. 1





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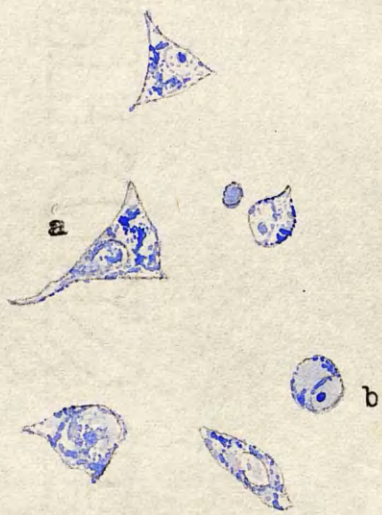
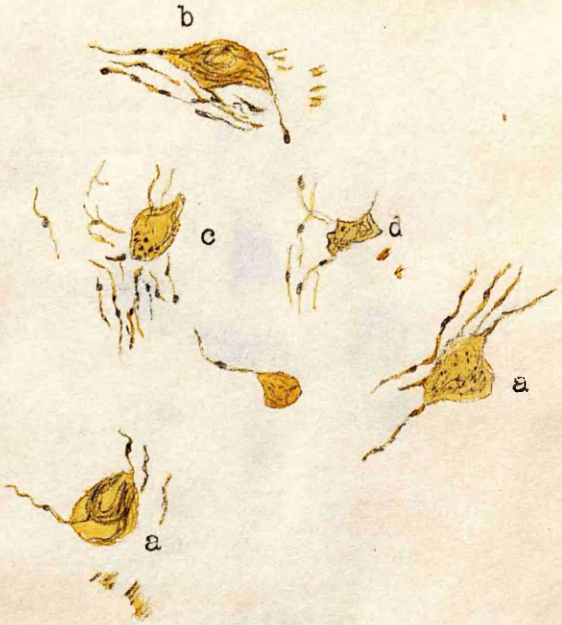
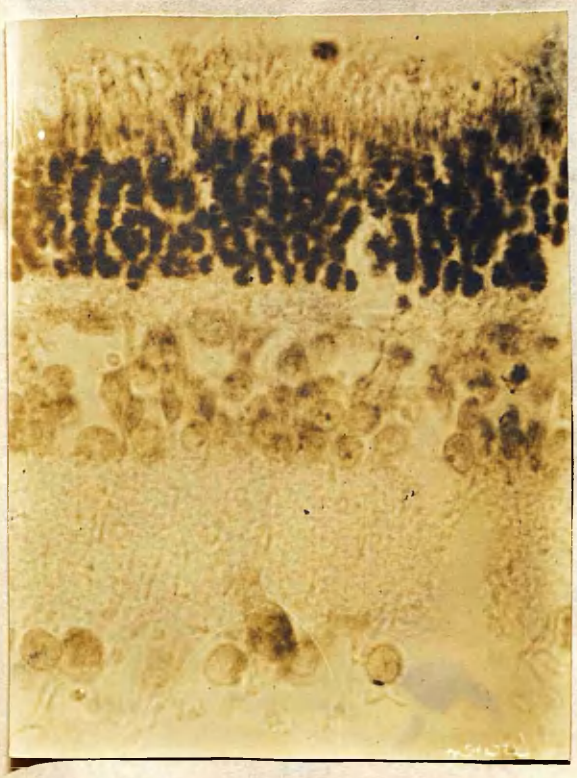


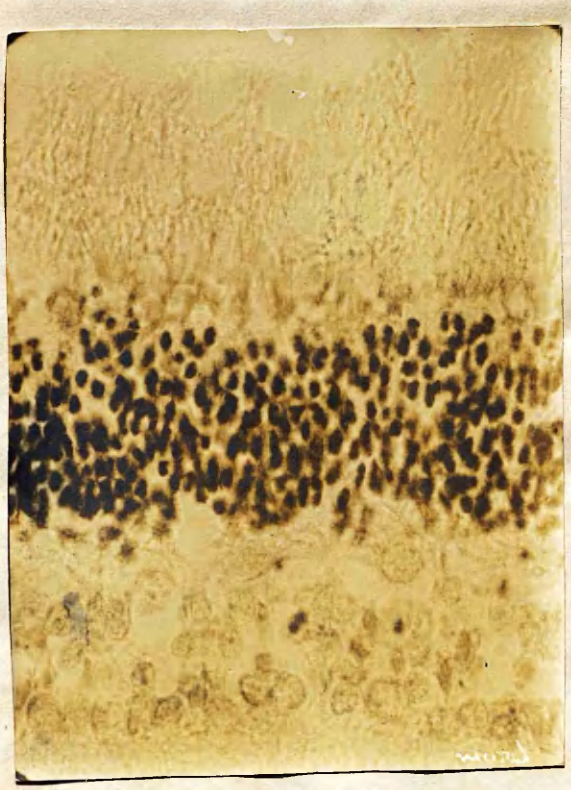
(d)

FIG. 2

(e)







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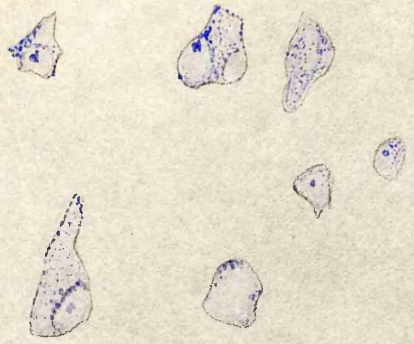

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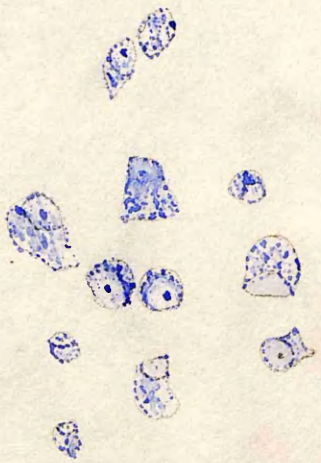

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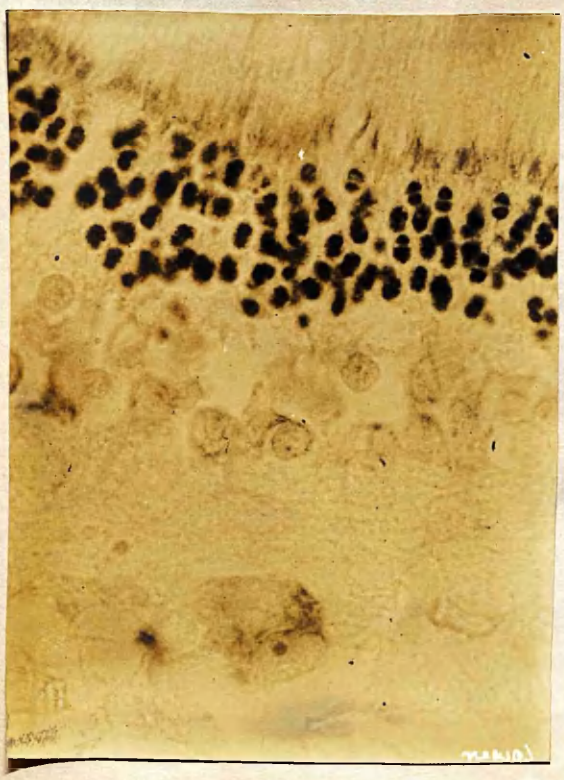

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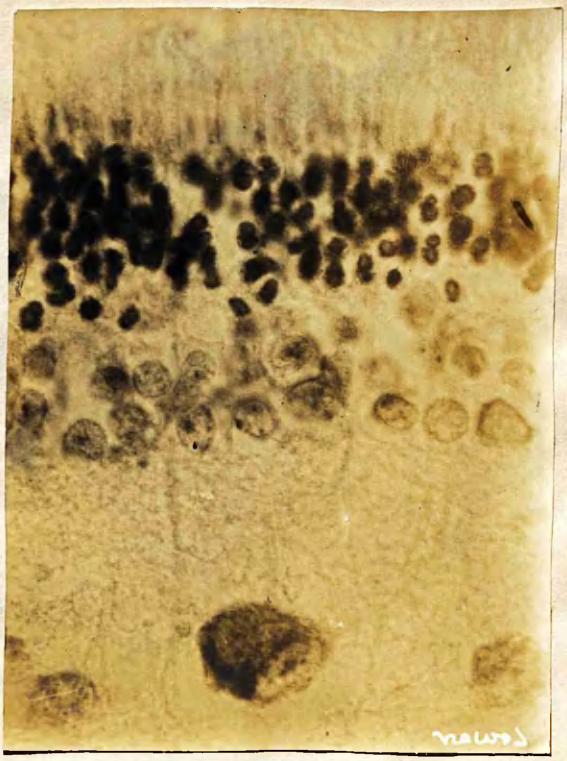

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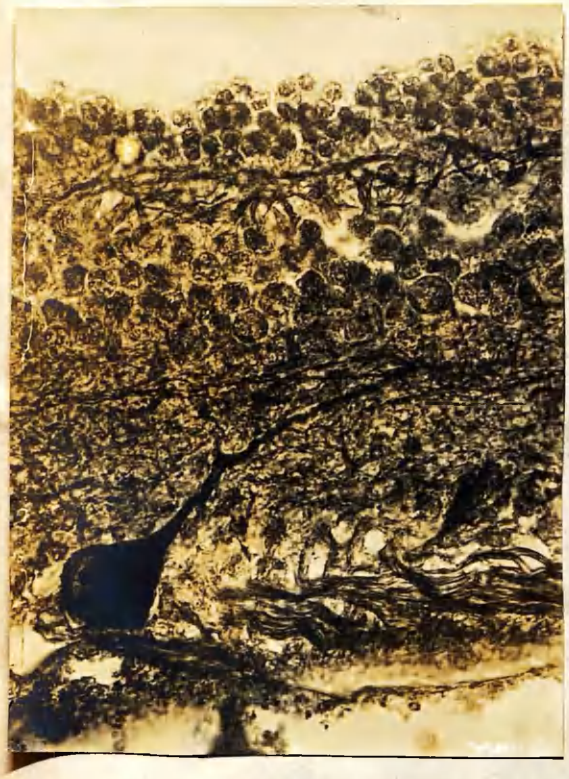
FIG. 3



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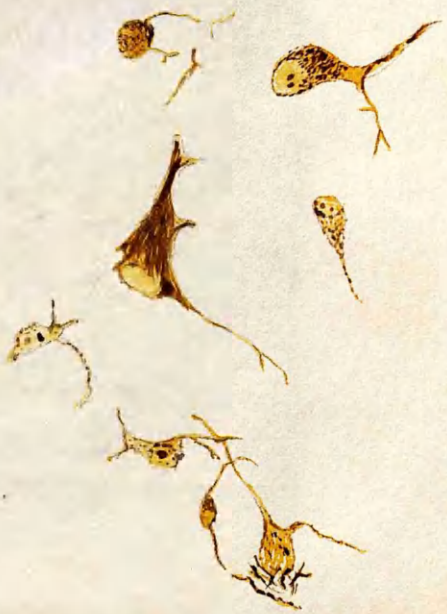
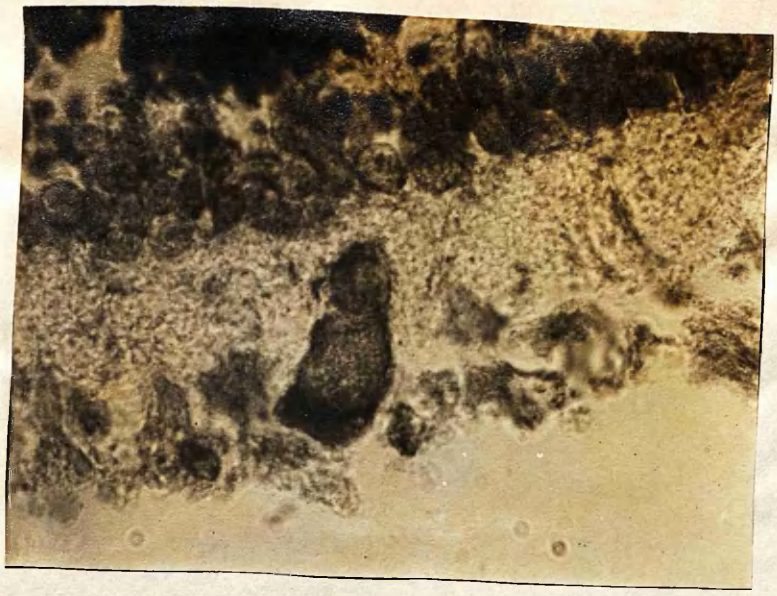


(f)

FIG. 3

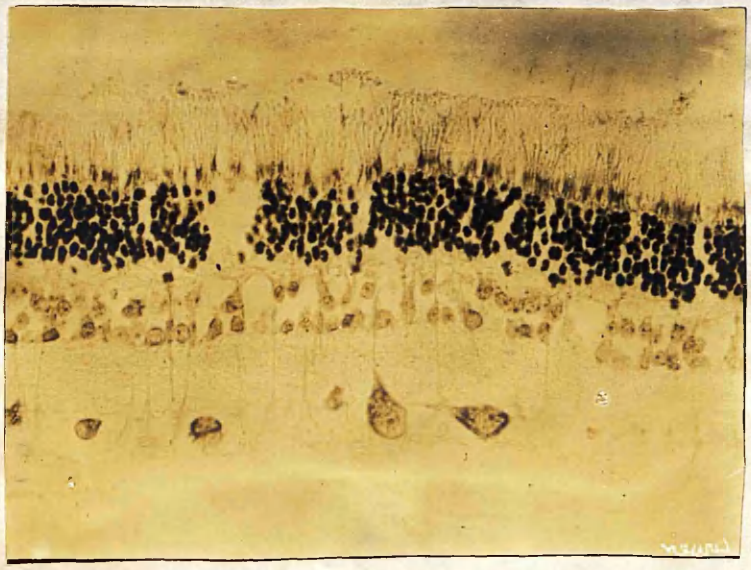


(a)

(b)

FIG. 4

(a)



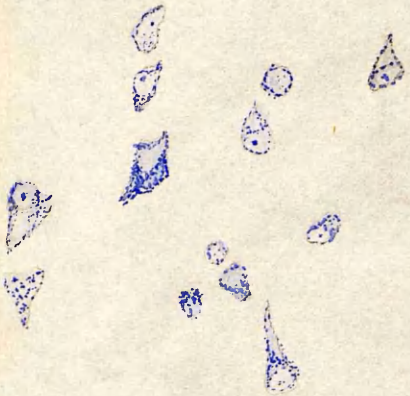
(b)

FIG. 5

(a[1])

(b[1])

FIG. 5



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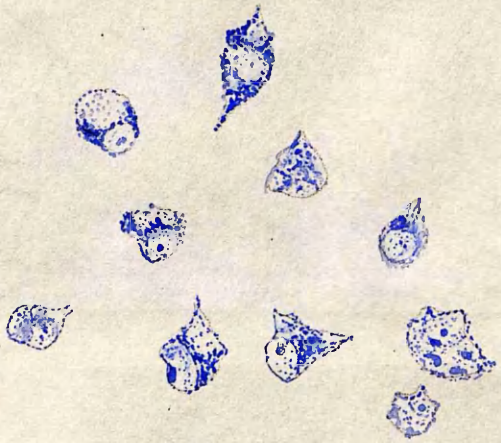

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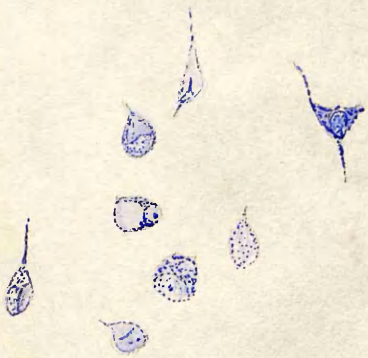

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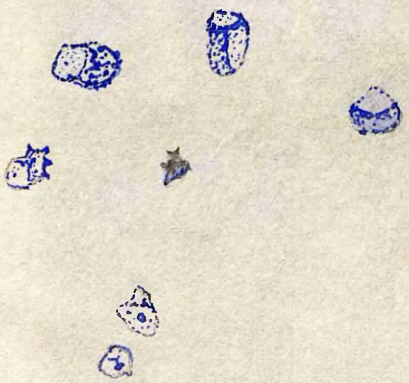

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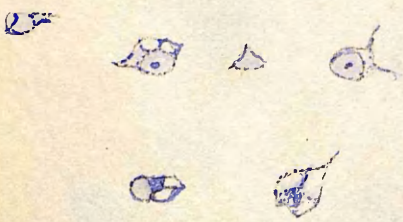
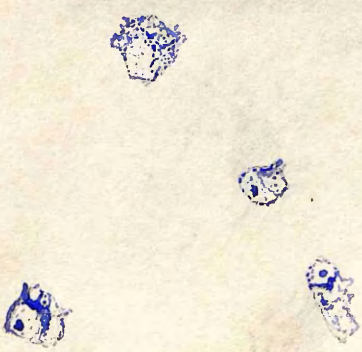
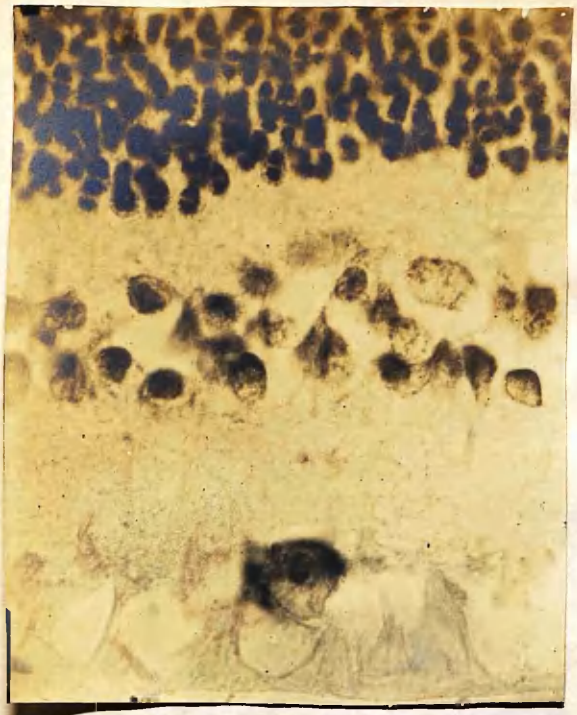


(g)



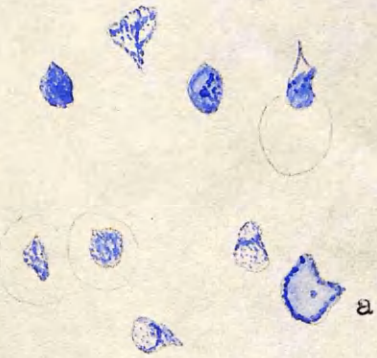
(h)

FIG. 5



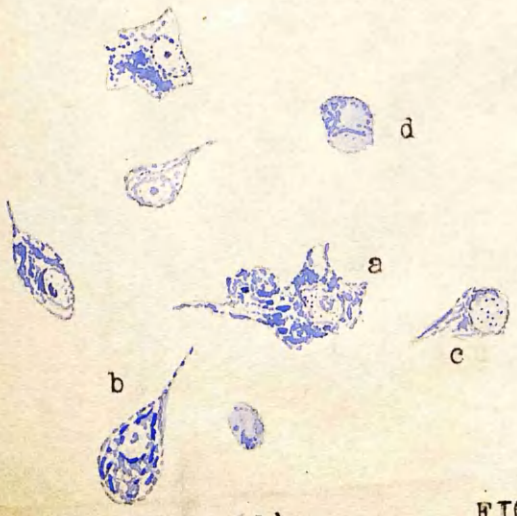
(a)

(b)



(c)

(b1)



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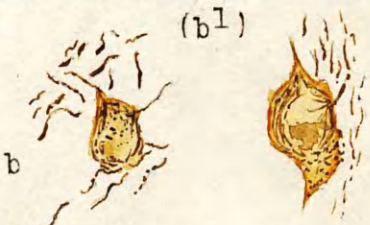
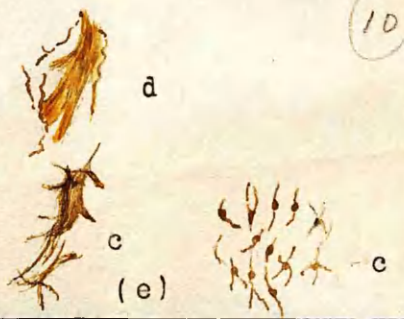
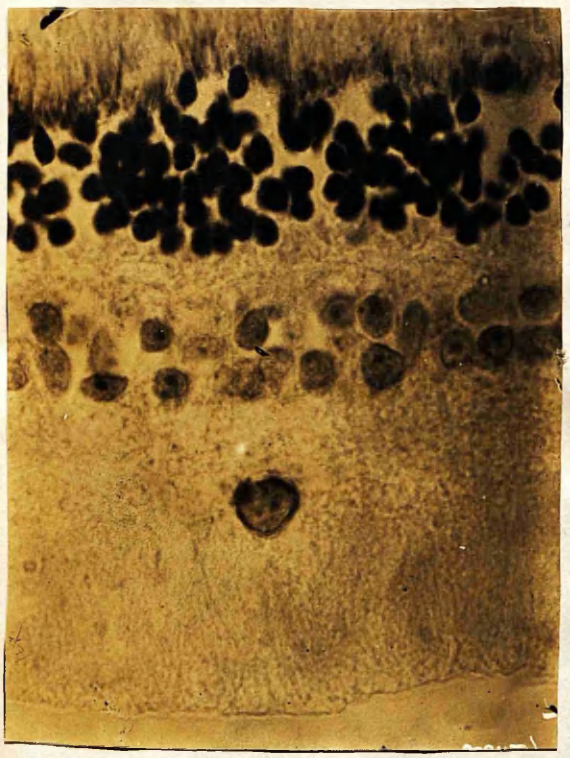




(e)

10

FIG. 6



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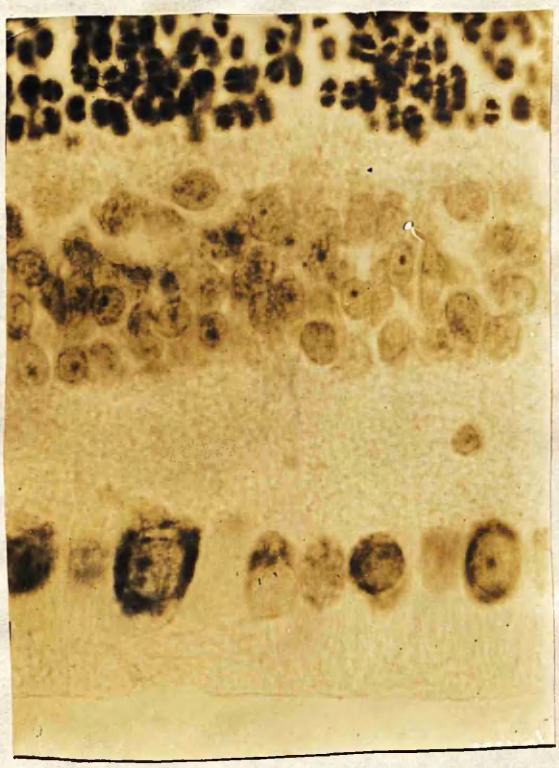


(b)

FIG. 7

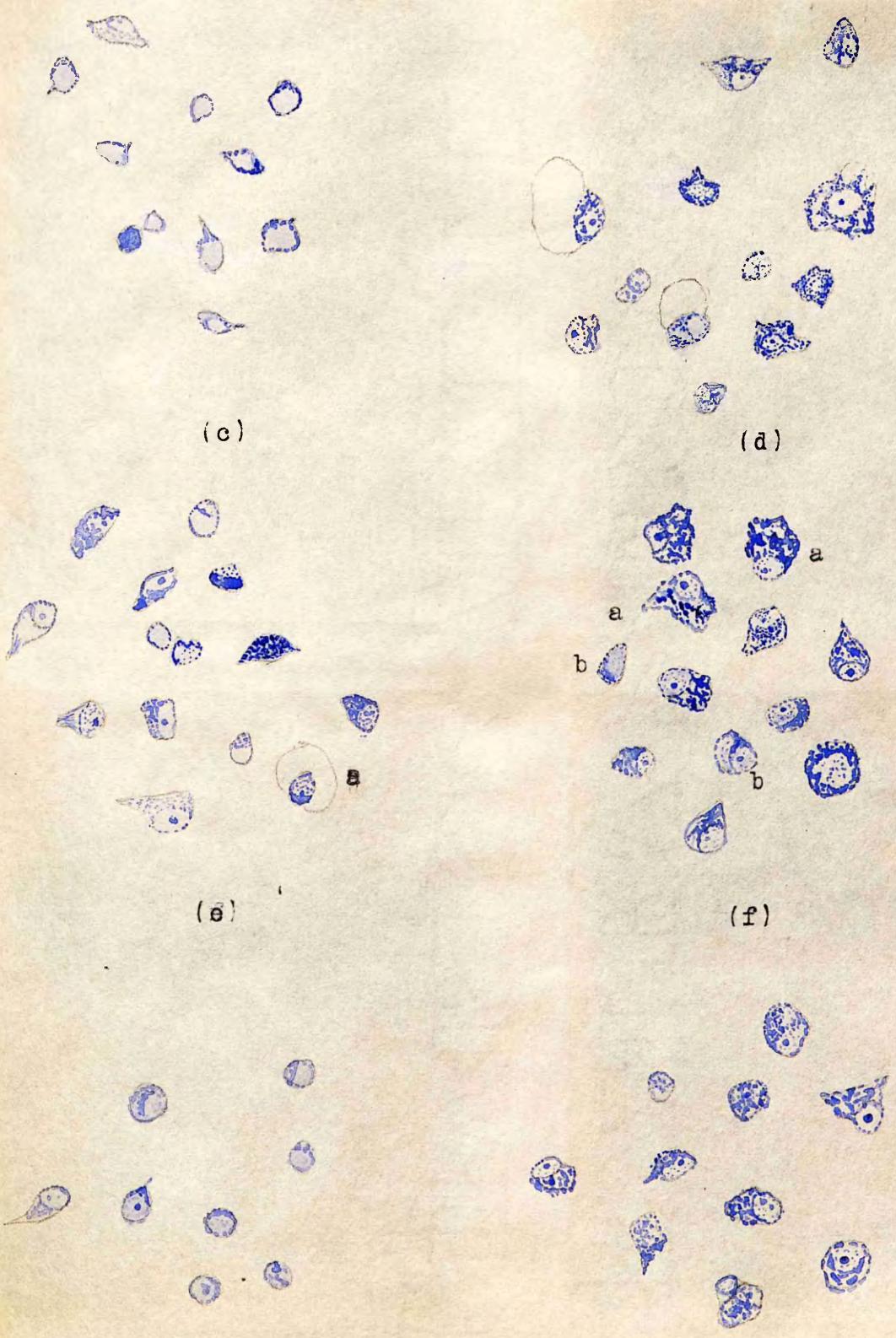
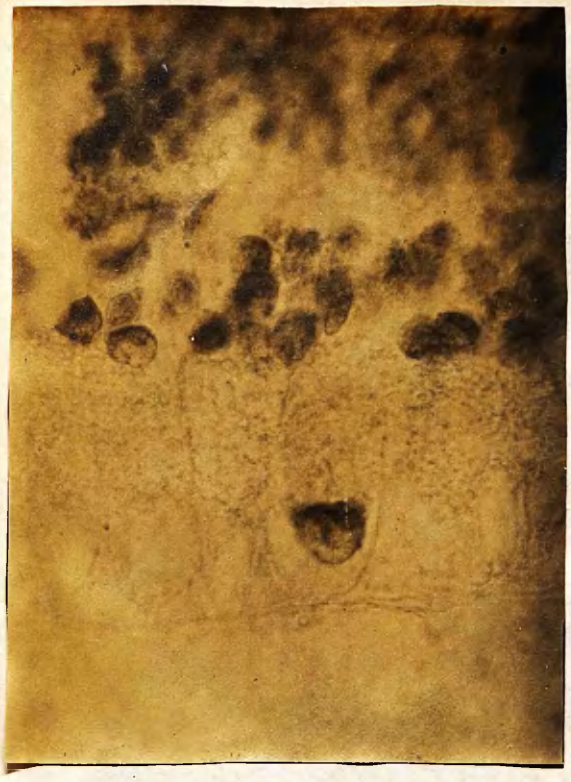
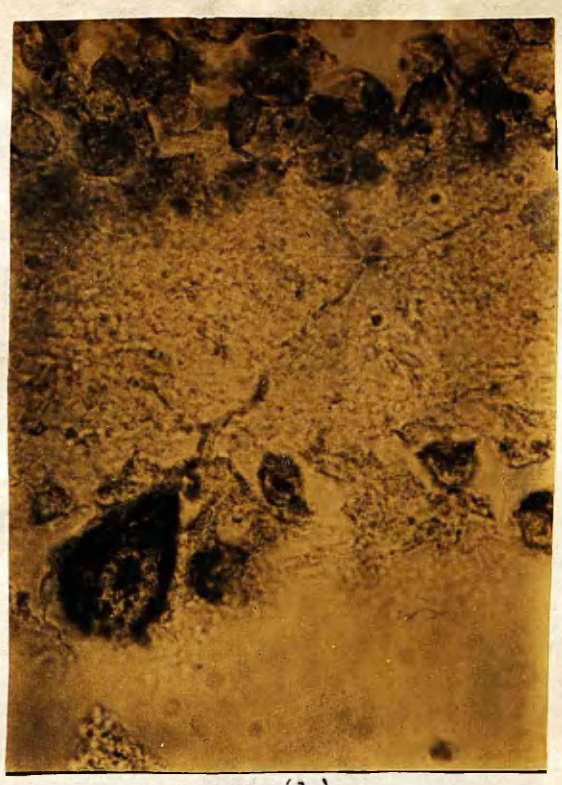


FIG. 7



(a)



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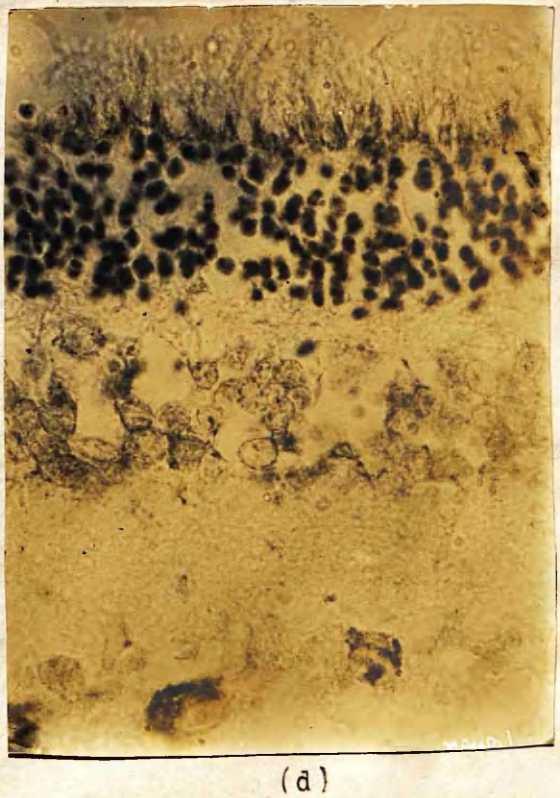
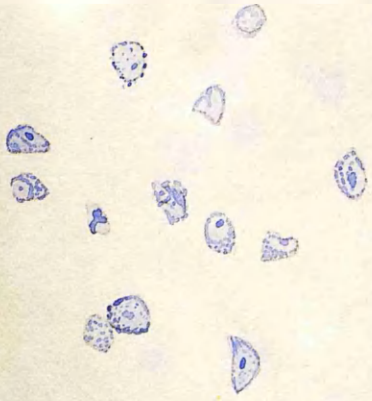
(c)



(d)

FIG. 8

(h)



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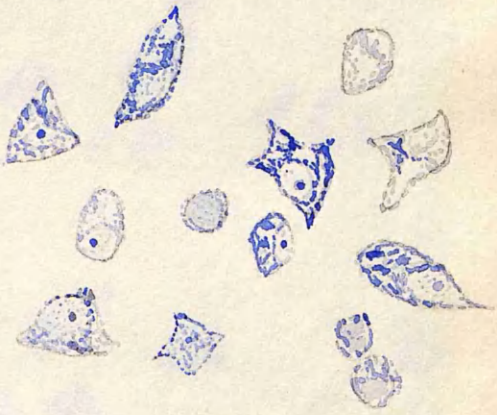
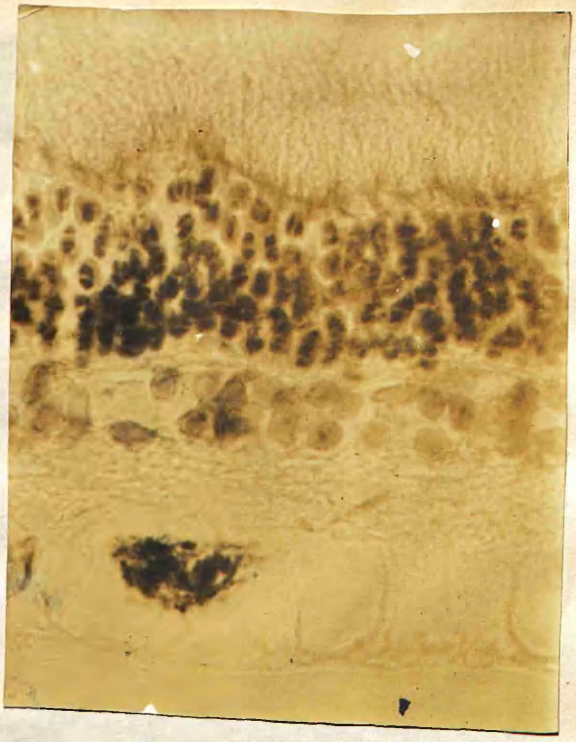
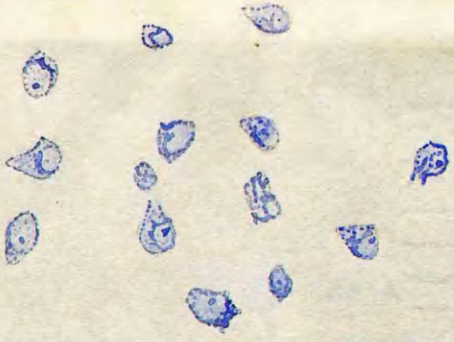


(g)

FIG. 8





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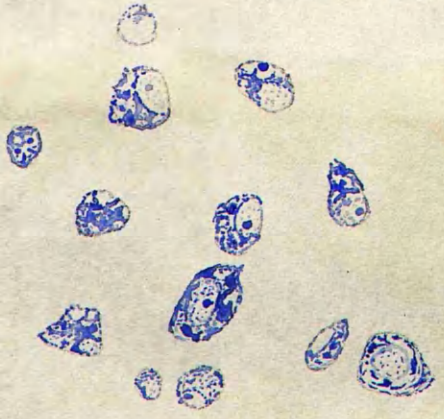

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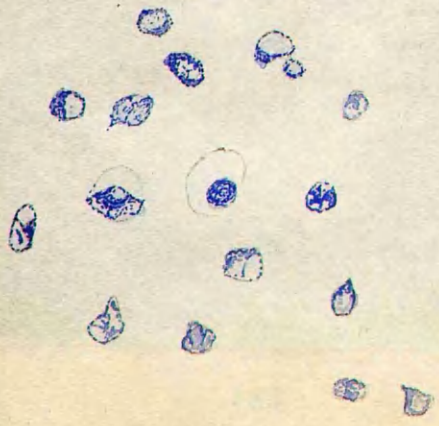

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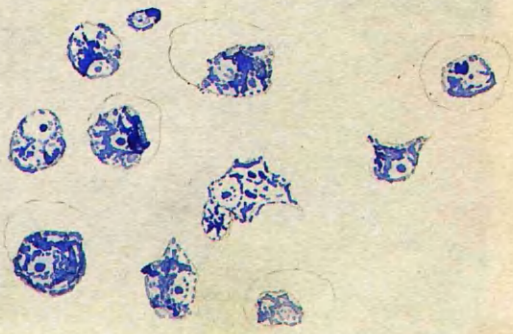
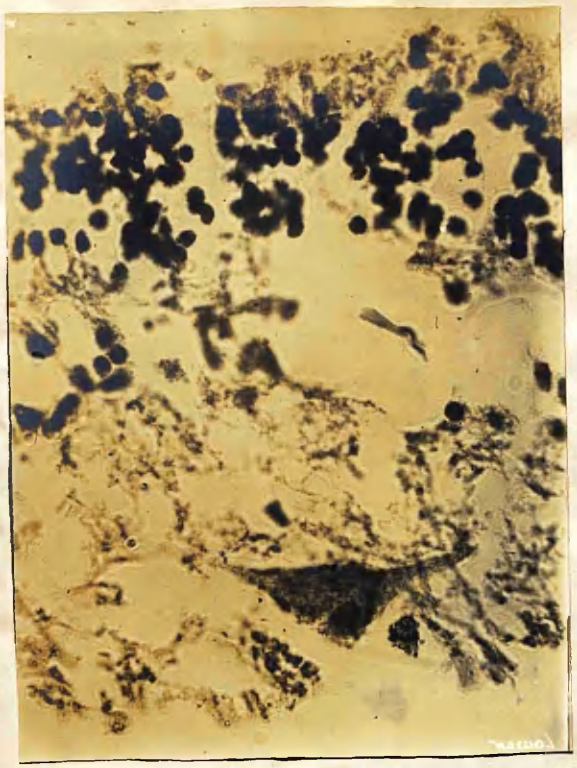


(f)

FIG. 9



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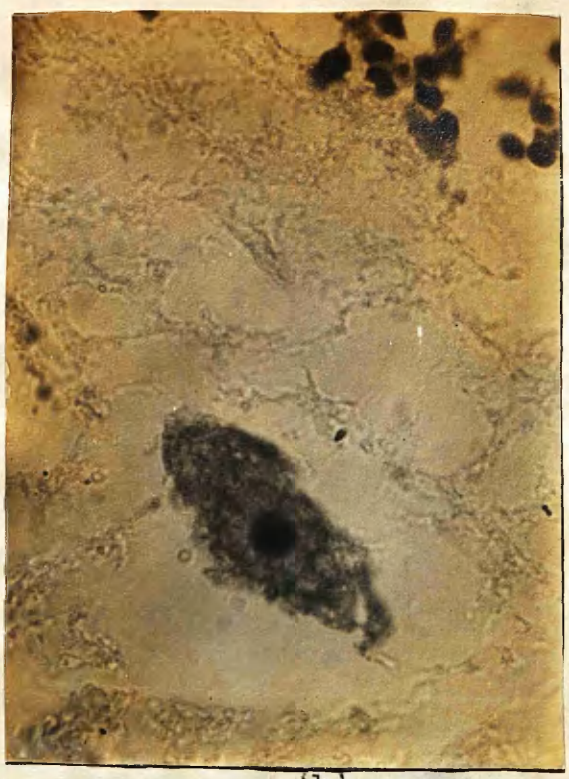
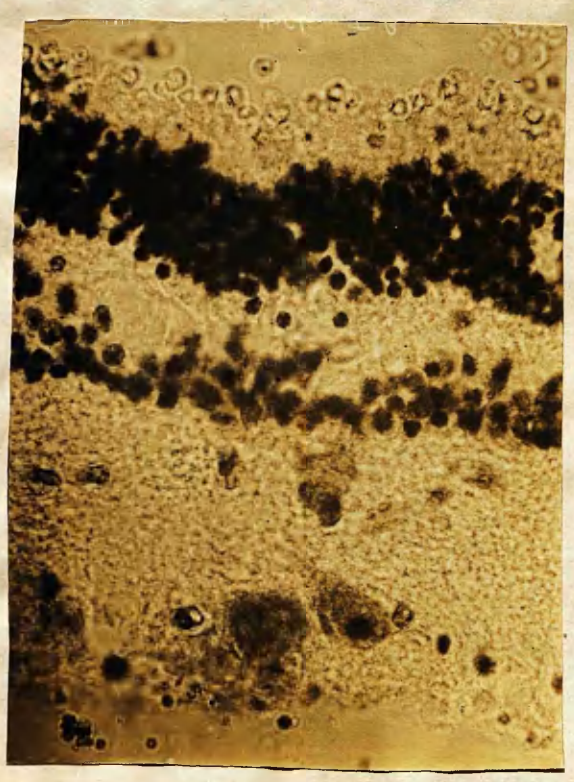


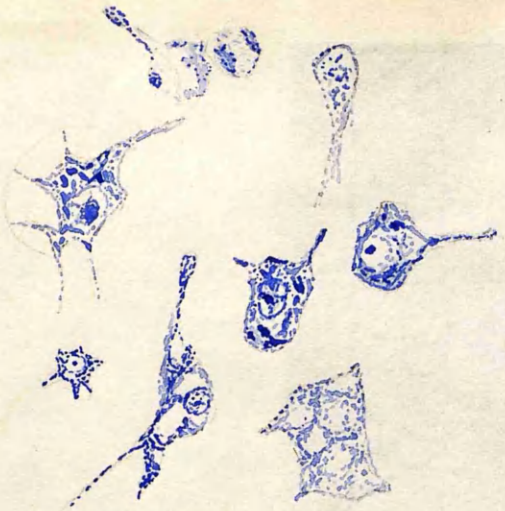
(b)

(c)



(d)

FIG. 10



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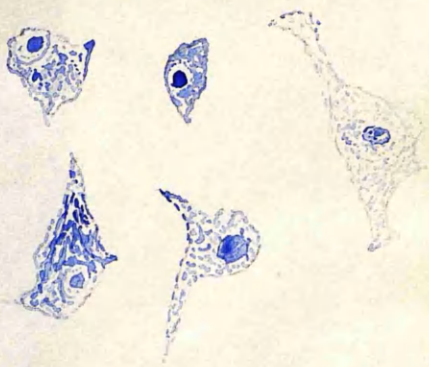

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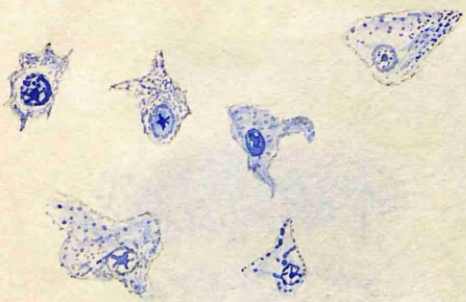

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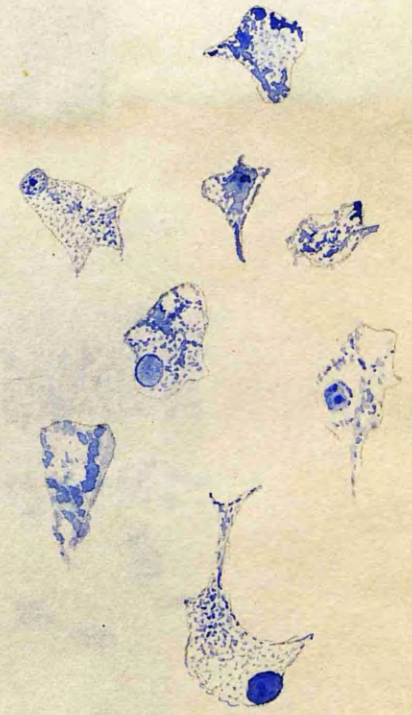

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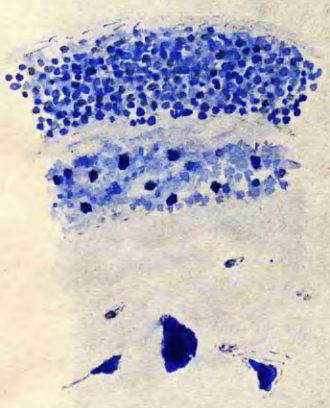
FIG. 10



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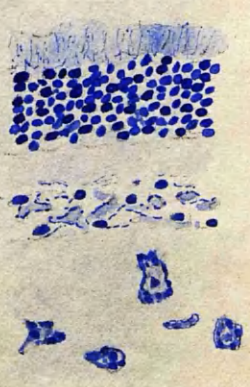

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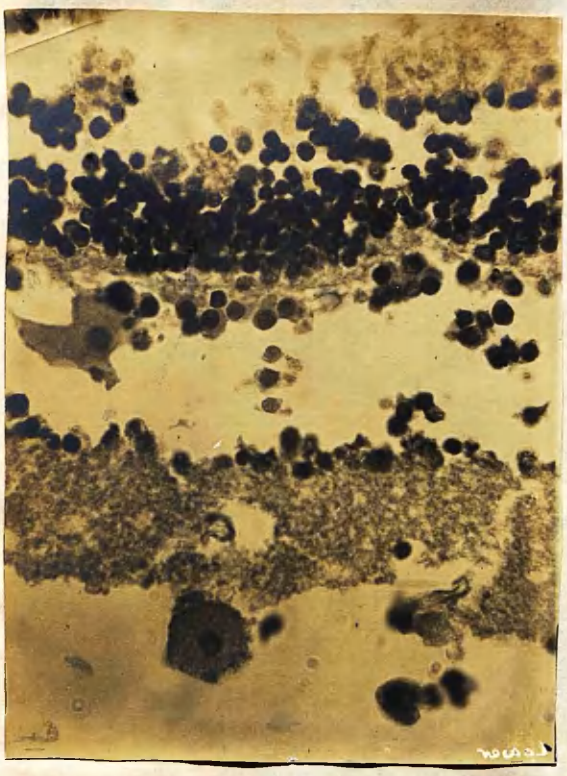
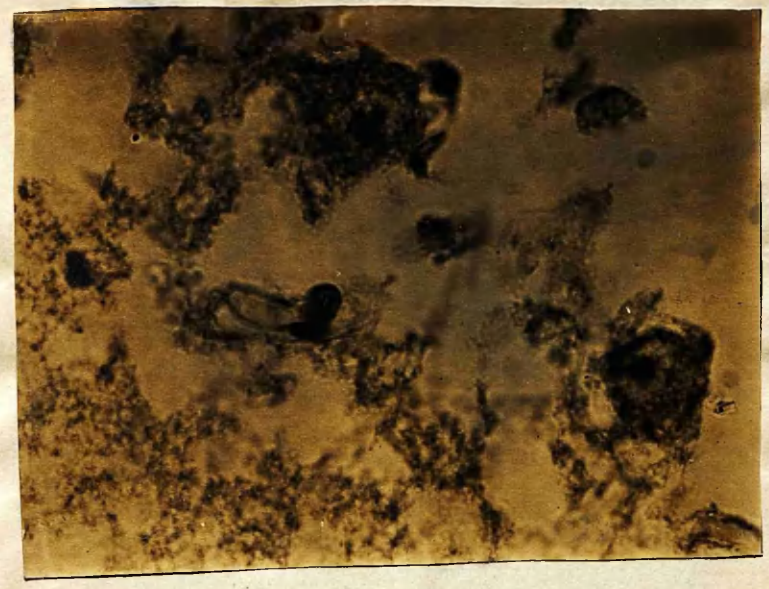
FIG. 11



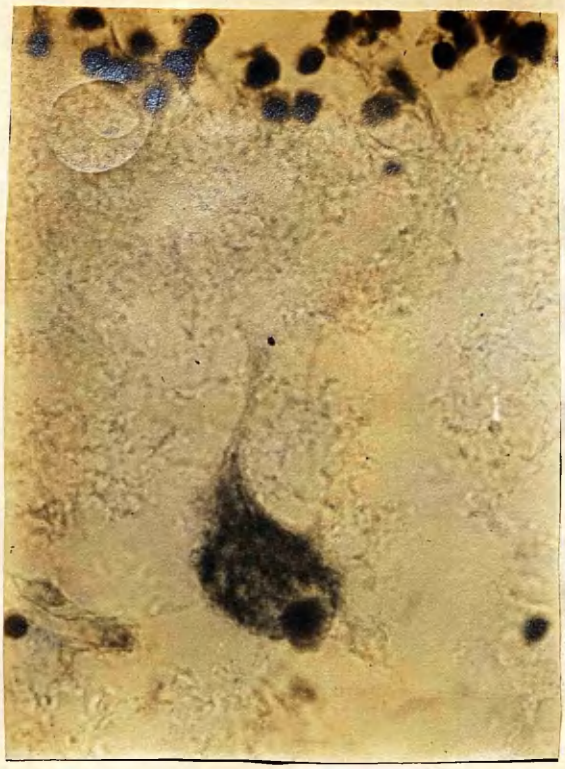
(a)

(b)



(c)

FIG. 12



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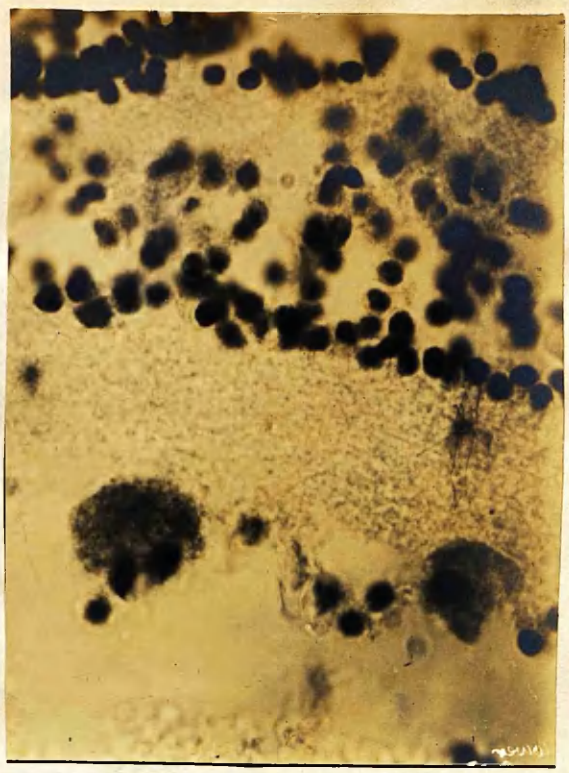

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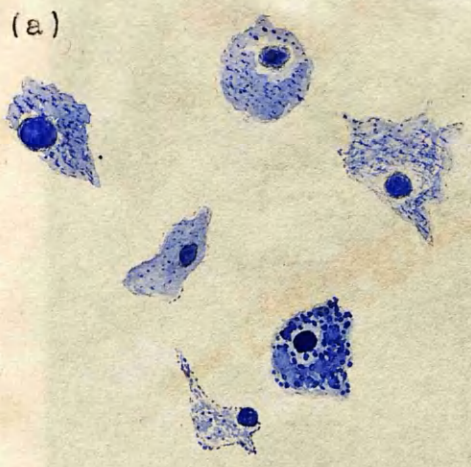
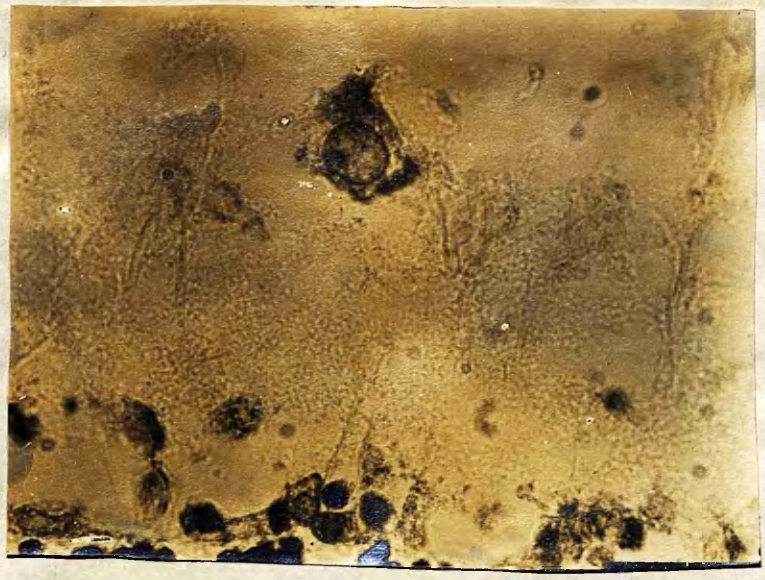


(c)

FIG. 13

FIG. 14



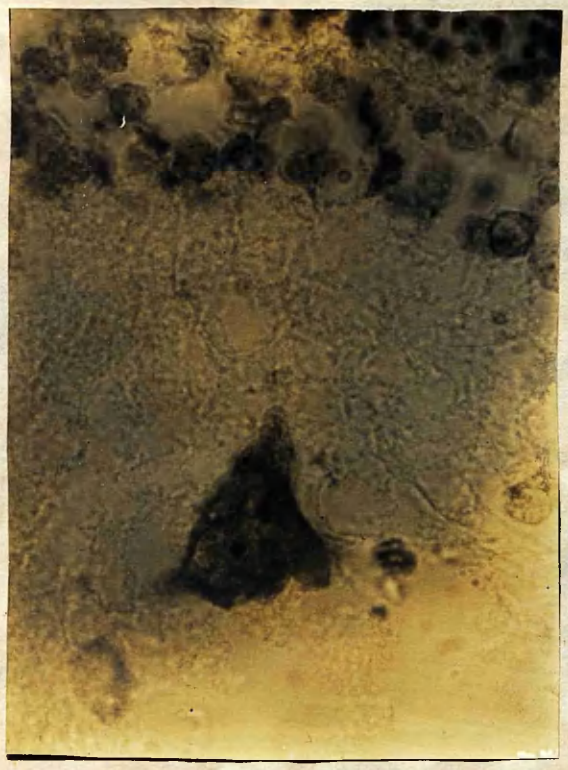
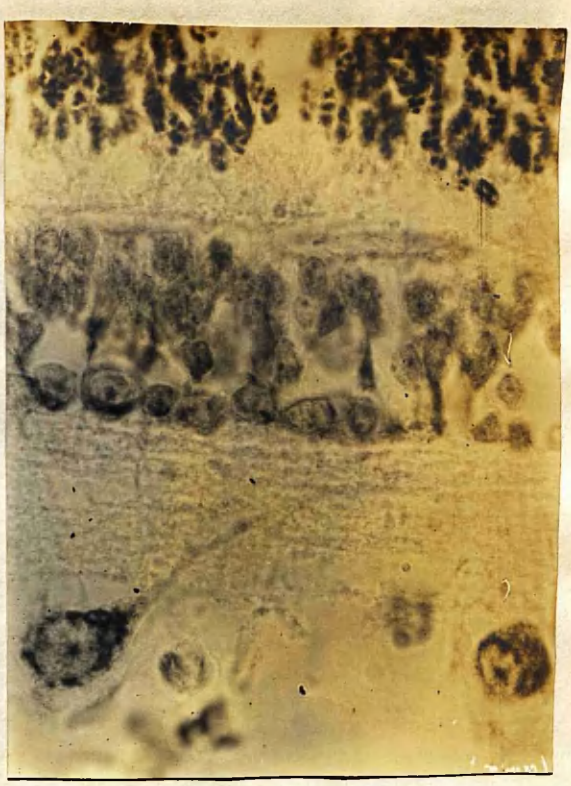
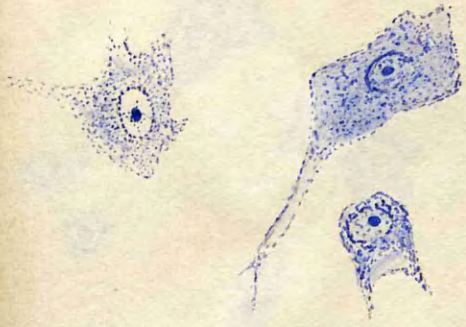
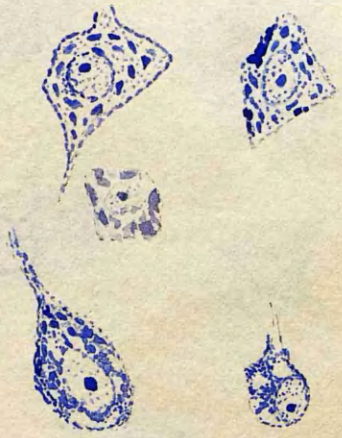


FIG. 15



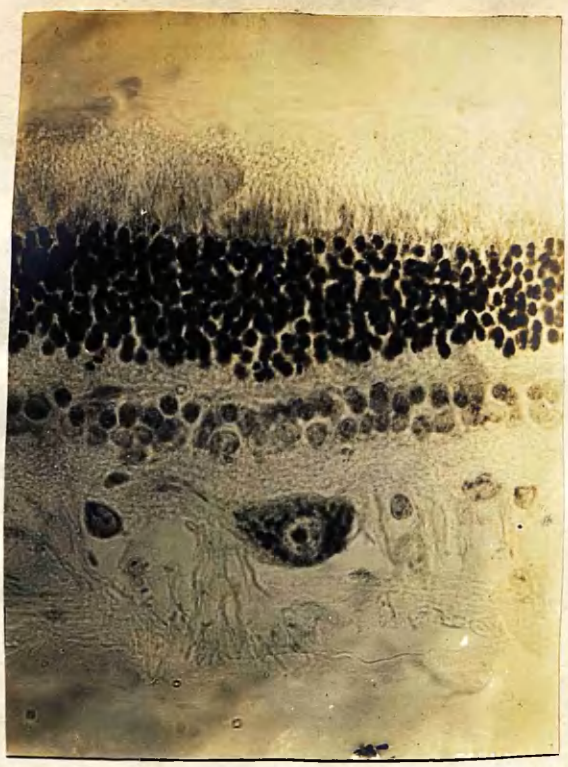


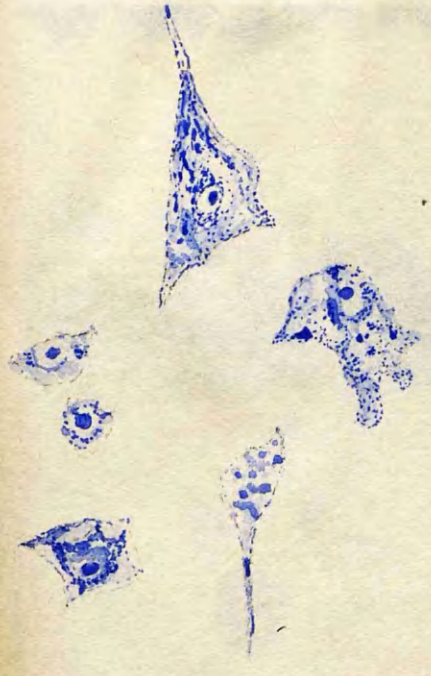
(c)



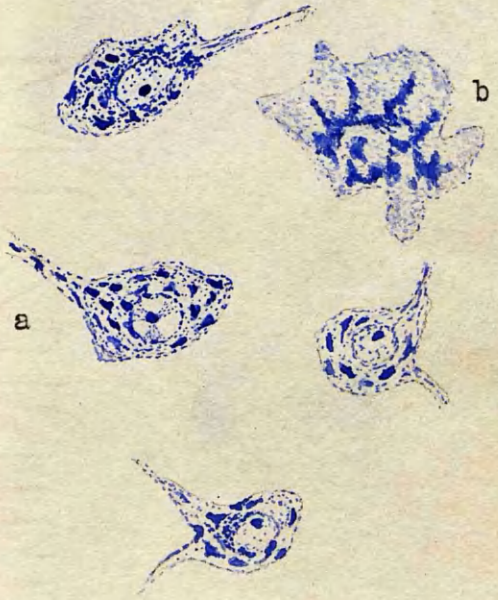
(d)

FIG. 16



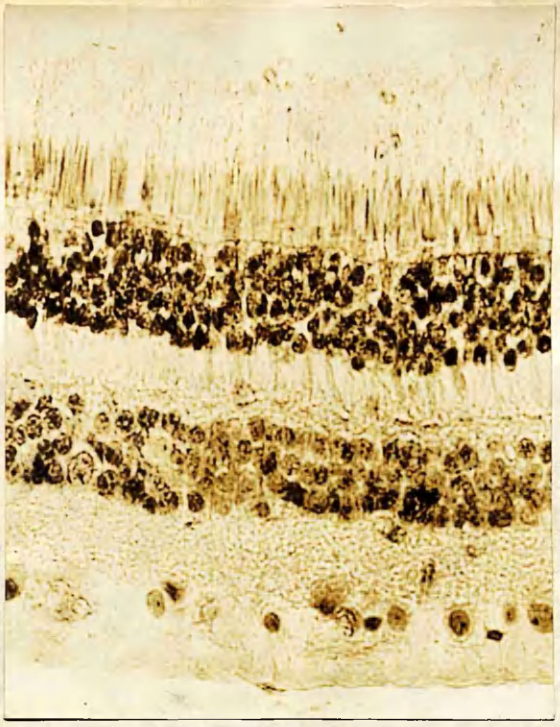


(c)

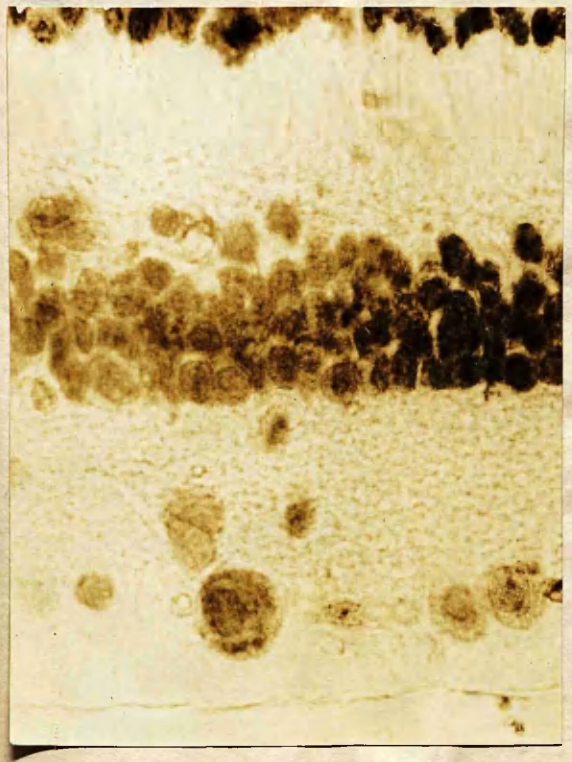


(d)

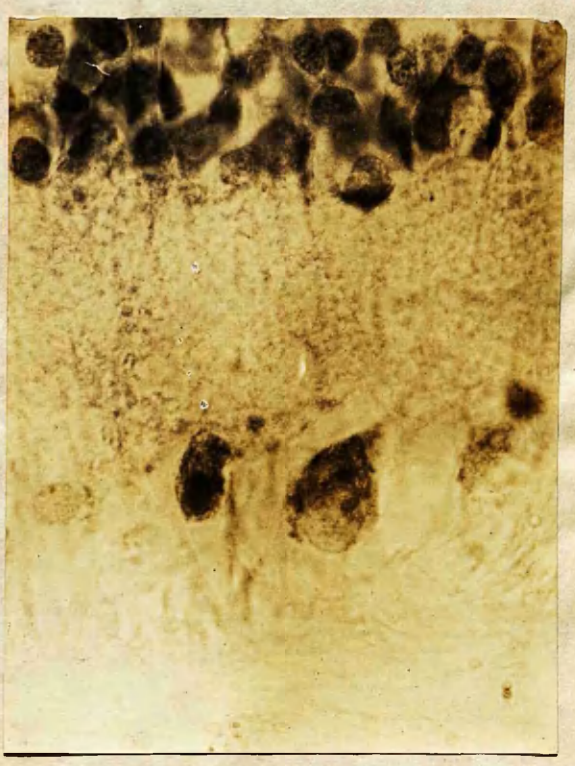
FIG. 17



(a)

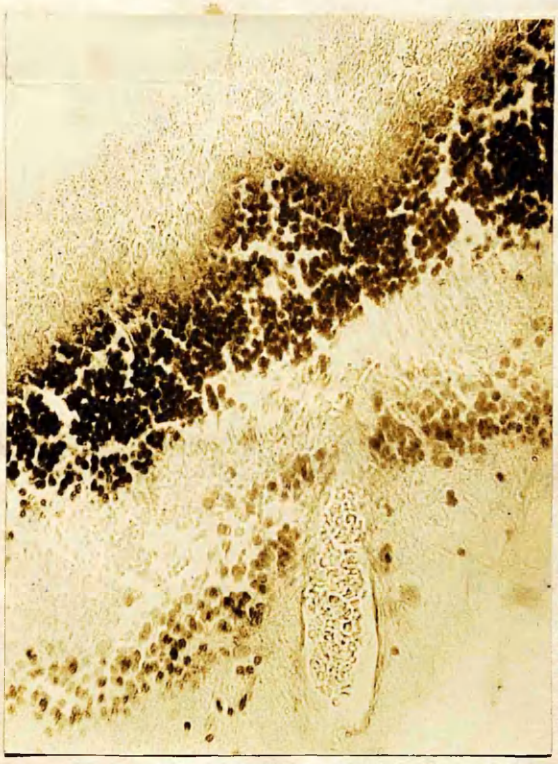


(b)

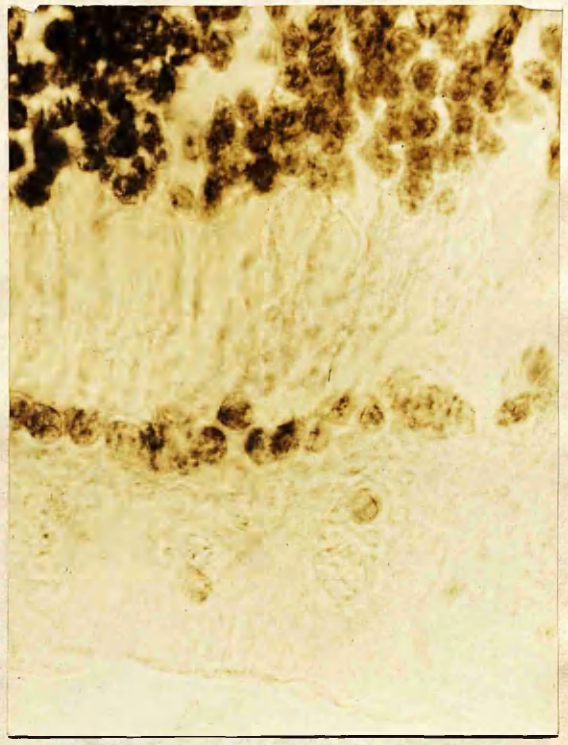


(c)

FIG. 18



(d)



(e)

FIG. 18