The effects of various fixatives on the brain of the albino rat : with an account of a method of preparing this material for a study of the cells in the cortex / Helen Dean King.

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HELEN DEAN KING

Associate in Anatomy at The Wistar Institute

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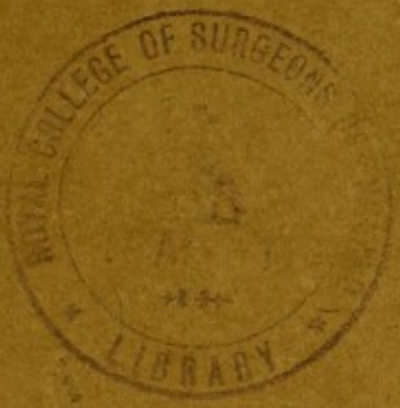

THE EFFECTS OF VARIOUS FIXATIVES ON THE BRAIN OF THE ALBINO RAT, WITH AN ACCOUNT OF A METHOD OF PREPARING THIS MATERIAL FOR A STUDY OF THE CELLS IN THE CORTEX

HELEN DEAN KING

Associate in Anatomy at the Wistar Institute

WITH FIFTEEN FIGURES

While endeavoring to obtain preparations of the brain of the albino rat (Mus norvegicus var. albus) that would be suitable for a study of the cells in the cerebral cortex I have had occasion, this past year, to investigate the histological changes produced in this material by various methods of fixation and of imbedding: the results of this investigation are given in the present paper. There are but few observations regarding the histological action of different fixatives on brain tissue, and none of the recorded investigations dealing with the effects of various preservatives on the weight and volume of the brains of mammals have been accompanied by an account of the structural changes these preservatives produce.

According to the observations of Donaldson ('94), of Hrdlicka ('06), and of Fish ('93), the age and physical condition of an animal, the length of time it has been dead before the brain is put into the fixing fluid, the amount of fluid used and the temperature at which it acts, are all factors which tend to produce variations in the weight and volume of the brain. In all of the experiments on the brain of the albino rat which are recorded in the present paper an effort was made to eliminate as many as possible of the factors which might be supposed to influence the results. The animal selected for each experiment was one that was presumably in a healthy condition. It was killed either by ether or by illuminating gas

and then weighed and measured. The brain was taken out as soon as possible after the death of the animal and placed on absorbent cotton in 40 cc. of the fixing solution whose action was to be tested. Except in one case (rat no. 5), all fixation was done at room temperature which was about 20°C. The brains of adult individuals were taken for all of the experiments but two (rats nos. 20 and 21). The exact age of the animal used was not known in any case; but this factor could have had little, if any, influence on the results, as none of the rats could have been over a year old and the majority of them were much younger. The physical condition of the animals, therefore, is the uncontrolled factor which might have affected the results, and to it can doubtless be ascribed the variations in the results which were obtained when brains of different individuals were subjected to similar treatment.

After remaining in the fixing fluid a given length of time, each brain was drained for a moment on filter paper, to remove the superfluous liquid, and then carefully weighed in a closed weighing bottle. After passing through the various grades of alcohol required by the method of fixation employed, the brains were brought into 70 per cent alcohol, where they remained for forty-eight hours. They were then drained and weighed a second time in order to determine the loss in weight due to the replacement of the water in the brain by alcohol.

In all of the earlier experiments the brains were divided longitudinally after they had been weighed a second time, and each half of the brain was imbedded by a different method in order to ascertain what structural changes could be attributed to the process of imbedding when the same methods of fixation had been employed. It was soon found that methods of imbedding commonly used for neurological material, as well as for other tissues, produce marked alterations in the structure of the cells in the cerebral cortex. Imbedding in paraffine after clearing with either xylol, oil of cedar, bergamot oil, or chloroform, does not give satisfactory preparations of the rat's brain when the details of cell structure are wanted. Celloidin, since it can be used without heat, is a very excellent medium for imbedding brain tissue. There are, however, several disadvantages connected with the

use of celloidin as an imbedding medium, not the least of which is the difficulty of obtaining unbroken series of very thin sections. Equally good results were obtained when brains were imbedded in celloidin according to the methods advocated by Hardesty ('02) and by Lee ('05) as when the very long method devised by Miller ('03) was employed. After experiments had been made with a number of different methods it was finally decided that the most satisfactory results were obtained by double imbedding in celloidin and paraffine according to the method of Bödeker ('08). The details of this method are given in the second section of this paper.

For convenience in description, the data collected in the course of this study are given in six tables. In each of the first five tables the first column gives the index numbers of the rats whose brains were used, while the second column denotes the solutions used for fixation. The next two columns show the weight of each brain on its removal from the fixing solution, together with the percentage gain or loss in weight as a result of the action of the solution; the computed weight of the fresh brain being taken as the standard. The fifth column gives the weight of each brain after it had remained in 70 per cent. alcohol for forty-eight hours; and the last column shows the percentage gain or loss in weight as a result of the replacement of the water in the brain by alcohol. All of the data are brought together in table 6 which gives for each rat, in addition to what is shown in the first five tables, the sex, body weight, body length, the length of time the brain remained in the fixing solution, and also the weight of the fresh brain as computed from body length and body weight according to the method given by Donaldson ('08, '09), which is based on formulas devised by Hatai ('08, '09). [See page 233.]

With the few exceptions noted, all brains were imbedded in celloidin or in celloidin-paraffine. Sections were stained with thionin, except in the two cases (rats nos. 43 and 44), where this stain did not give satisfactory results. The illustrations are from drawings of the large pyramidal cells in the cerebral cortex taken from frontal sections at the level of the optic chiasma. As far as possible cells were selected for drawing which represented the

average condition of the large cortex cells, after the brains had been subjected to a given course of treatment. In the various tables a star (*) is prefixed to the index number of each rat from whose brain cells were selected for illustration.

A. THE EFFECTS OF VARIOUS FIXATIVES ON THE BRAIN OF THE ALBINO RAT

At the present time formaldehyde is very generally used for the fixation and preservation of the brains of man and of the higher mammals. This substance, commonly employed in a 4 per cent. solution (10 per cent. formalin) produces but slight alterations in form or in color and gives a good consistency to the tissues, although it causes a marked increase in weight and in volume. Table 1 shows the various solutions containing formaldehyde that were used as fixatives of the brain of the albino rat and their effects on the brain weight.

TABLE 1[1]

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
*1	4% Formaldehyde	2.5750	+33	1.5706	−19
2	4% Formaldehyde	2.8200	+54	1.6436	−10
4	4% Formaldehyde	2.6778	+50	1.6577	− 7
3	Formol-Müller (cold)	2.2437	+21	1.5537	−16
5	Formol-Müller (warm)	2.1880	+22	1.8711	+ 4
*27	Alcohol-formol	1.6392	−10	1.5147	−16
18	Zenker-formol	1.6040	− 2	1.3297	−18
37	Marina's fluid	1.2219	−33	1.2913	−29
*38	Marina's fluid	1.2146	−35	1.2546	−33
41	Sublimate-formol	2.3315	+21	1.6565	−14
46	Sublimate-formol	2.0512	+17	1.3687	−22
49	Sublimate-formol-acetic	1.7687	− 2	1.5003	−17
50	Sublimate-formol-acetic	1.8944	+ 8	1.5221	−13
*32	Graf's fluid (5% formalin)	2.1520	+23	1.7421	− 0
33	Graf's fluid (10% formalin)	1.9283	+ 7	1.5994	−12
10	Bouin's picro-formol	1.7881	−00	1.4663	−18

[1] In this and in other tables, the percentages given are based on the computed fresh weight of the brain which is shown in table 6.

The brains of three rats (nos. 1,2,4) were fixed for forty-eight hours in a 4 per cent. aqueous solution of formaldehyde which had been made neutral with bicarbonate of soda as, according to Bayon ('05), a formaldehyde solution that has an acid reaction is not suitable for histological purposes. In all three cases there was a large initial gain in the weight of the brain which was followed by such a loss in weight after the brain had been brought into 70 per cent. alcohol that at the second weighing each brain weighed somewhat less than its computed fresh weight. The alteration produced in the brain weight of rats by aqueous formaldehyde solutions are similar to those which this fluid causes in the brains of man and of sheep, according to the investigations of Parker and Floyd ('95), of Flatau ('97), and of Hrdlicka('06).

On making a histological examination of the brains that were fixed in a 4 per cent. solution of formaldehyde, it was found that this substance does not have as injurious an effect on the structure of the cells as do other fixatives that produce much less alteration in the brain weight. One of the large cells from the cerebral cortex of the half of the brain of rat no. 1 which was imbedded in celloidin is shown in fig. 1. There is no apparent shrinkage of the cell body and the cytoplasm stains evenly and appears uniformly distributed. The nucleus, however, has suffered considerably from the action of the fixative, as it is decidedly larger than normal and its reticulum is poorly preserved and stains very faintly.

A cell from the portion of the brain of rat no. 1 which was imbedded in paraffine after being cleared in chloroform is shown in fig. 2. This cell plainly shows the injurious effects produced by this mode of imbedding. The cell body is considerably shrunken, while the nucleus is slightly contracted and very irregular in outline. The smaller cells of the cerebral cortex do not seem to be as adversly affected by the paraffine imbedding as do the larger cells, and most of them appear fully as well preserved as do similar cells in brains that have been imbedded in celloidin or in celloidin-paraffine.

Many investigators have stated that for histological purposes formaldehyde gives the best results when used in combination with other fixing reagents. Of the various formaldehyde mix-

tures that have been devised, the Formol-Müller solution of Orth ('92) has been most highly recommended by Juliusburger ('97), and others as an excellent fixative for the central nervous system. The brain of one rat (no. 3) was fixed for twenty hours in Formol-Müller solution, which was kept at room temperature (20° C.); the brain of another rat (no. 5) remained for three hours in this solution heated to about 35° C. As shown in table 1, each brain had gained about 21 per cent. in weight when it was removed from the solution; the subsequent loss in weight was, however, about 20 per cent. greater in the case of the brain which had been fixed in the cold solution than in that which had been fixed in the warm solution. When these brains were examined histologically the fixation of the cell structures was found to be no better in the one case than in the other. In both brains the large cells of the cerebral cortex appeared very similar to those in brains that had been fixed in 4 per cent. formaldehyde, as there was a slight swelling of the nucleus and a poor fixation of the nuclear contents. As a cell fixative for the brain of the rat, therefore, this fluid seems to have no advantage over the simple aqueous formaldehyde solution.

Parker and Floyd ('95) recommend a solution composed of 6 volumes of 95 per cent. alcohol and 4 volumes of a 2 per cent. solution of formalin as an excellent preservative for the brains of higher mammals. This solution was used as a fixative of the brain of rat no. 27. As the brain had decreased 10 per cent. in weight when removed from the fixing solution (table 1), it is evident that the addition of alcohol to formaldehyde prevents the swelling which is a characteristic action of aqueous formaldehyde solutions on brain tissue. As a cell fixative this fluid does not give satisfactory results. Although there is but little shrinkage of the cell body, the cytoplasm is invariably vacuolated in the vicinity of the nucleus, as shown in fig. 3, while the nucleus itself is somewhat irregular in outline and its contents are vaguely defined and stain faintly.

Zenker-formol was used as a fixative of the brain of rat no. 18. The brain lost but 2 per cent. in weight as a direct result of the fixation; the later shrinkage, after the brain had been brought

into 70 per cent. alcohol, being 18 per cent. The most marked histological effect of this fluid is on the cell nuclei. These structures always appear shrunken and irregular in outline, while their contents are very poorly preserved. Large cells of the cerebral cortex of the brain that was fixed by this method appear much as does the cell shown in fig. 2.

Marina ('97) recommends as a fixative for the central nervous system a solution made as follows:

Alcohol (96 per cent)	100 ccm.
Formol	5 ccm.
Chromic acid	10 cgm.

When used on the brain of the rat (nos. 37 and 38) this fluid produces marked alteration in the brain wieght and also in the cell structures. There is an initial loss of from 33 per cent. to 35 per cent. in the brain weight which is not materially altered by subsequent treatment of the brain with 70 per cent. alcohol. One of the cells from the cortex of the brain of rat no. 38 is shown in fig. 4. There is little apparent shrinkage of the cell body as a whole: the cytoplasm appears uniform, but it stains much more intensely than does the cytoplasm of cells in brains fixed with other formaldehyde solutions. On the cell nuclei this fluid had a very peculiar action. In the great majority of cases the nucleus appears swollen, and it has a very irregular outline with many indentations, as if the fixation had set up an unusual chemical reaction between the fluid contents of the nucleus and those of the cytoplasm. In some cases the nuclear reticulum seems to be entirely broken up so that the nuclear contents, save for the nucleolus, appears to be composed of small, rounded, deeply staining granules; in other cases, as shown in fig. 4, there are a few irregular clumps of nuclear substance scattered among the granules. Marina's fluid produced a much greater distortion of the nuclear structure in the cells of the cerebral cortex than resulted from the fixation with any of the other solutions that were used during the course of these experiments.

Ewing ('98) states that a saturated solution of corrosive sublimate in a 5 per cent. solution of formalin gives a superior fixation

of ganglion cells, bringing out the so-called chromatic network with great clearness. The brains of two rats (nos. 41 and 46) were fixed with this fluid. Each brain gained considerably in weight as a direct result of the fixation, the greater gain (21 per cent.) being made by the brain of rat no. 41 which had remained the shorter time (four hours) in the solution. Both brains later lost considerably in weight, the loss being greater in the brain (rat no. 46) which had remained for twenty hours in the solution. This fluid gives a very much better preservation of the cell structures than might, perhaps, be expected from its effects on the brain weight. Very few of the large cells in the cerebral cortex show any evidence of shrinkage, and the cytoplasm always appears uniform. The nuclear reticulum is fairly well preserved and it stains deeply; but the nucleus itself is usually slightly enlarged. The large cells in the cerebral cortex of the brains fixed by this solution appear very much like that shown in fig. 13.

One of the solutions recommended by Cox ('98) as a fixative for the spinal ganglion cells of the rabbit is made as follows:

Corrosive sublimate (saturated aq. solution)	30 parts
Formalin	10 parts
Glacial acetic acid	5 parts

Comparatively slight alterations are produced in the brain weight as a direct result of fixation in this solution (table 1: rats nos. 49 and 50), although after subsequent treatment with 70 per cent. alcohol the brain loses from 13 to 17 per cent. of its computed fresh weight, depending on the length of time it has remained in the solution. As a cell fixative for the brain of the rat this fluid cannot be recommended. In all cases the nuclei of the large cells in the cerebral cortex are swollen, and the nuclear reticulum appears much like that shown in fig. 1.

The picro-formol solution of Graf ('97) certainly suffers from the omission of acetic acid, as Lee ('05: p. 77) has stated. This solution, made with 5 per cent. formalin, was used as a fixative of the brain of rat no. 32. The brain gained 23 per cent. in weight as a direct result of the fixation; but after remaining in 70 per cent alcohol for forty-eight hours it weighed practically its com-

puted fresh weight. A cell from the cortex of this brain is shown in fig. 5. The cell outline is regular and the cytoplasm appears uniform; the nucleus, however, is swollen and there is a very poor preservation of the nuclear contents.

The brain of rat no. 33 was fixed in Graf's fluid made with 10 per cent. formalin. This fluid produces a very different effect on the brain weight from that which results from fixation with Graf's solution which contains a smaller amount of formalin (table 1 : rat no. 32). The initial increase in the brain weight is but 7 per cent., and the subsequent loss in weight, after the brain has been treated with 70 per cent. alcohol, is sufficiently large to make the final weight of the brain 12 per cent. less than the computed fresh weight. The stronger solution does not give as good a preservation of the cell structures in the cerebral cortex as does the solution that contains the 5 per cent. formalin, as there is a distinct shrinkage of the cell body in addition to an alteration of nuclear structure similar to that shown in fig. 5.

The picro-formol solution of Bouin ('97), which was used to fix the brain of rat no. 10, gave a much better preservation of the nerve cells in the cortex than did any of the other formaldehyde solutions that were tried, and it produced practically no alteration in the brain weight. The brain was imbedded in celloidin-paraffine and sections of it show an admirable preservation both of cell and of nuclear structure. A careful comparison between the cerebral cells in this brain and those in brains fixed in the solution of Ohlmacher ('97) shows that the latter solution gives a slightly better fixation of the nuclei than is obtained with Bouin's fluid. No further experiments were therefore made with Bouin's fluid which is doubtless as excellent a fixative for the central nervous system as it seems to be for many other kinds of materials.

Judging from the results obtained on the brain of the rat, solutions containing formaldehyde give, in general, a good fixation of the cell body, but they tend to produce a swelling of the nucleus which is usually accompanied by a poor preservation of the nuclear contents.

Before the introduction of formaldehyde as a fixing and hardening reagent, bichromate of potassium ($K_2Cr_2O_7$), either in simple

aqueous solution or in combination with sodium sulphate as "Müller's fluid," was very generally employed for the fixation of mammalian brains. Donaldson ('94) studied the action of this preservative on the weight and volume of the brains of sheep. He found that, in general, the weight of a brain increases according to the number of days it is left in the solution; the gain being about 17 per cent. as a result of one day's action of a $2\frac{1}{2}$ per cent. solution, increasing to a maximum of 38 per cent. after an immersion of two years in the fluid.

TABLE 2

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
22	$2\frac{1}{2}$ % $K_2Cr_2O_7$	2.8445	+73	2.1409	+31
*23	$2\frac{1}{2}$ % $K_2Cr_2O_7$	2.5594	+52	1.7518	+ 4
*24	$2\frac{1}{2}$ % $K_2Cr_2O_7$ + alcohol	2.5073	+40	1.8885	+ 6
25	$2\frac{1}{2}$ % $K_2Cr_2O_7$ + alcohol	2.8169	+50	2.1797	+16
*8	Zenker then Müller	1.8716	+ 3	1.6666	− 8
19	Zenker (20% acetic acid)	1.7451	+ 5	1.3167	−21
*9	Dahlgren then Müller	1.9000	+ 3	1.7273	− 7
43	Tellyesniczky's fluid	1.9643	+ 6	1.6372	−12
44	Tellyesniczky's fluid	1.7981	+ 3	1.4906	−14
3	Formol-Müller (cold)	2.2437	+21	1.5537	−16
5	Formol-Müller (warm)	2.1880	+22	1.8711	+ 4
18	Zenker-formol	1.6040	− 2	1.3297	−18

In table 2 is shown the effects on the weight of the brain of the albino rat of various solutions containing bichromate of potassium.

The brains of two rats (nos. 22 and 23) were subjected to the action of a $2\frac{1}{2}$ per cent. solution of bichromate of potassium for forty-eight hours. The gain in weight as a result of the absorption of the fluid by the brain tissue was enormous, being 73 per cent. in one case and 52 per cent. in the other. The brain of rat no. 22, which made the greater initial gain in weight, still weighed 31 per cent. more than its computed fresh weight after remaining

in 70 per cent. alcohol for two days; while the brain of rat no. 23 weighed 4 per cent. more than the computed fresh weight after a similar course of treatment. In spite of the fact that both brains were considerably swollen when they were imbedded in celloidin-paraffine, the tissues appeared greatly shrunken when prepared sections were examined under the microscope. In each brain the large cells of the cerebral cortex were greatly contracted and the cytoplasm much vacuolated, as shown in fig. 6. The nuclei of these cells were also distorted in shape and their membranes appeared abnormally thick. Only traces of a nuclear reticulum could be found even in the most favorable cases. The smaller cells of the cortex were also contracted and badly preserved. This solution had a more injurious effect on the cell structures in the cerebral cortex that did any of the other fixing solutions that were used during the course of these experiments.

Donaldson ('94) found that if the brains of sheep are put into a solution made by adding $\frac{1}{6}$ volume of 95 per cent. alcohol to a $2\frac{1}{2}$ per cent. solution of bichromate of potassium the initial increase in the brain weight is somewhat less than when the $2\frac{1}{2}$ per cent. solution of bichromate of potassium alone is used. Thinking that this mixture might give better preparations of the brain of the rat than were obtained with the simple bichromate of potassium solution, I used it as a fixative for the brains of two rats (nos. 24 and 25). The effects of this fixative on the weight of the brains of rats are similar to those which are produced on the brains of sheep, as the increase in weight, although large, is less than that caused by the bichromate of potassium solution (table 2)). This mixture gives a better fixation of the cell structures in the cerebral cortex of the brain of the rat than does the aqueous solution of bichromate of potassium, but it is by no means a satisfactory fixative for histological purposes. The structure of one of the large cells in the cerebral cortex of the brain of rat no. 24 is shown in fig. 7. The cell body is somewhat shrunken and the nuclear outline is much distorted. In the nucleus of this cell, as in the nuclei of the great majority of the large cells in the cortex of brains fixed by a $2\frac{1}{2}$ per cent. solution of bichromate of potassium, there is no indication of a nuclear reticulum, the greater part of the

chromatin being collected around the nucleolus. The cytoplasm of the cell is not vacuolated, but it appears denser in some places than in others.

At the present time Zenker's fluid is much used for fixing material for cytological study, and it seems to give very excellent preparations of many kinds of materials. The value of this fluid as a preservative of brain tissue was tested on the brain of rat no. 8. After remaining for six hours in this fluid the brain was put into Müller's solution for forty-eight hours in order that it might be properly hardened. As shown in table 2, the weight of the brain was increased but 3 per cent. as a result of the fixation, and the subsequent loss in weight was only about 8 per cent. When this brain was examined histologically it was found that the cells in the cerebral cortex had been poorly preserved in spite of the fact that the mode of fixation employed had produced but a slight alteration in the weight of the brain. The structure of one of the large cells in the cerebral cortex of this brain is shown in fig. 8. The cell outline is fairly regular, but the greater part of the cytoplasm is condensed around the nucleus. Although the nucleus has maintained its normal shape and size, the nuclear contents stains rather faintly and only traces of a nuclear reticulum can be found.

If the amount of acetic acid in Zenker's fluid is increased from 5 per cent. to 20 per cent. and the solution thus modified used as a fixative for the brain of the rat, there is an initial increase of about 5 per cent. in the weight of the brain, which is followed by a loss of about 20 per cent. in weight after the brain has been brought into 70 per cent. alcohol (table 2: rat no. 19). This fluid gives a better fixation of the cell structures in the cerebral cortex of the brain of the rat than does Zenker's fluid, but it is by no means a satisfactory fixative for brain tissue. After fixation with this fluid the large cells in the cerebral cortex appear much like that shown in fig. 3.

The brain of rat no. 9 was fixed in Dahlgren's ('97) fluid and then hardened in Müller's fluid. Cell structures are much better preserved by this mode of fixation than by the Zenker-Müller treatment, although practically the same alterations in brain

weight are produced in both cases (table 2). As shown in fig. 9, which is a drawing of one of the large cells in the cerebral cortex of the brain of rat no. 9, there is no contraction of the cell body and no vacuolization or condensation of the cytoplasm after this method of fixation. The deleterious effects of the fixation manifest themselves only in the nucleus of the cell. This body appears shrunken and irregular in outline, and the nuclear reticulum is not clearly defined.

After ascertaining that the fixatives commonly employed for cytological purposes produce various artefacts in the testis cells of the salamander, Tellyesniczky ('98) devised a theoretically good fixative made as follows:

Bichromate of potassium	3 grms.
Glacial acetic acid	5 cc.
Distilled water	100 cc.

The brains of rats nos. 43 and 44 were fixed in this fluid. The initial increase in the weight of the brains was comparatively slight, being 6 per cent. in one case and 3 per cent. in the other; both brains lost about the same amount (17 per cent.) after being treated with 70 per cent. alcohol. Thionin did not prove to be a satisfactory stain for this material; and, therefore, the sections were stained with Delafield's hæmatoxylin which brought out the nuclear reticulum with great distinctness but did not give particularly sharp outlines to the cell body. Tellyesniczky's fluid gives a fixation of the cells structures in the brain of the rat fully as good as that obtained with Bouin's picro-formol solution; neither solution, however, gives quite as fine a fixation or permits of as brilliant staining as does the solution of Ohlmacher ('97), whose action will be described in detail later on.

The alterations produced in the brain of the rat by fixation with fluids containing both formalin and bichromate of potassium have been already described (rats nos. 3, 5, 18). With the exception of Tellyesniczky's fluid, all of the various solutions containing bichromate of potassium that were used as fixatives of the brain of the rat gave a very inadequate fixation of the cell structures in the cerebral cortex. Owing, doubtless, to the fact that it penetrates

tissues very slowly, bichromate of potassium causes a contraction of the cell body and fails to preserve the nuclear structure.

Corrosive sublimate, either in concentrated water solution or combination with other fixing reagents, has been used successfully by various investigators as a preservative of the cell structures in the central nervous system of the vertebrates. von Lenhossék ('95) and Flemming ('96) recommend a concentrated solution of corrosive sublimate in water as a fixative for nervous tissue. This solution was used on the brains of rats nos. 39 and 40. The swelling of the brain as a result of the fixation was practically the same whether the brain remained for four hours (rat no. 39) or for twenty hours (rat no. 40) in the solution (table 3). After treatment with 70 per cent. alcohol, each brain was found to weigh about 23 per cent. less than its computed fresh weight. A much better fixation of the cells in the cerebral cortex is obtained when a brain is subjected to the action of the solution for twenty hours than when the fluid acts for only four hours. Entirely satisfactory preparations are not obtained in either case, however, as the cytoplasm of the cells is invariably vacuolated, much like that shown in fig. 6. The nuclei are very well preserved by this method of fixation, and details of structure appear with great clearness after the sections have been stained with thionin.

A saturated aqueous solution of corrosive sublimate, to which 5 per cent. of acetic acid had been added, was used as a fixative of the brain of rat no. 29. As shown in table 3, the brain increased but 5 per cent. in weight as a direct result of the action of the solution, and it subsequently lost about 20 per cent. of its computed fresh weight after being washed and passed through the lower grades of alcohol into 70 per cent. alcohol. Sublimate-acetic is a somewhat better fixative for the cell structures in the cortex of the brain of the rat than is the concentrated aqueous solution used on the brains of rats nos. 39 and 40, and it gives a remarkably good preservation of the nuclei, as is shown by an examination of fig. 12. The rest of the cell, however, is not preserved in an entirely satisfactory manner, as the cytoplasm is invariably vacuolated, although there is no evident shrinkage of the cell body as is the case in many of the cells in the brains of rats nos. 39 and 40.

A physiological salt solution saturated with corrosive sublimate was used as a fixative of the brains of rats nos. 42 and 45. There was an increase in the brain weight as a result of the fixation comparable to that produced in other brains preserved in solutions containing corrosive sublimate (table 3). The initial increase in the brain weight, however, was over twice as great (16 per cent.) when the brain remained in the solution for twenty hours (rat no. 45) as when the solution acted on the brain for only four hours (rat no. 42). The appearance of the cells in the cerebral cortex of brains fixed by this method is about like that shown in fig. 12. After a brain has remained twenty hours in this solution the nuclei of the large cerebral cortex cells appear slightly enlarged, and their contents stain less sharply than when a shorter time (four hours) has been employed for the fixation of the tissue.

The solution employed by Lang ('78) for the preservation of planarians has recently been used with apparently good results as a fixative of nervous tissues. For use on the central nervous system this solution, according to Ewing ('98), is made as follows:

Corrosive sublimate	5 grm.
Sodium chloride	6 "
Glacial acetic acid	5 cc.
Distilled water	100 cc.

The effects of this fluid on the weight of the brains of rats nos. 35 and 36 are shown in table 3. The initial increase in the weight of the brains was not very large, being 15 per cent. in the case of the brain which had remained in the solution for twenty hours (rat no. 35) and 10 per cent. when the solution acted for four hours only (rat no. 36). After being treated with 70 per cent. alcohol, these brains lost a comparatively small amount (table 3), yet the fixation of the cell structures in the cerebral cortex was not as good as that obtained by fixation with other corrosive sublimate solutions which produce a much greater alteration in the brain weight. One of the large cells from the cerebral cortex of the brain of rat no. 35 is shown in fig. 11. The nuclear reticulum is well preserved and stains very clearly; but in many cells the

nucleus itself is slightly swollen, although it retains its rounded form. The cell body is contracted and the greater part of the cytoplasm is condensed around the nucleus.

The sublimate-osmic-acetic mixture of Cox ('98), which was used as a fixative for the brains of rats nos. 47 and 48, produces a much greater increase in the brain weight if it is allowed to act for three days than if the brain is removed from the solution at the end of two days (table 3). The brain of rat no. 48 was the only one fixed in a solution containing corrosive sublimate that

TABLE 3

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGAT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
39	Saturated aqueous sol. $HgCl_2$ ..	2.0760	+ 8	1.4695	−23
40	Saturated aqueous sol. $HgCl_2$ ..	2.0229	+11	1.4087	−23
*29	Sublimate-acetic.....	1.8604	+ 5	1.4414	−19
42	NaCl + sublimate.....	1.9927	+ 7	1.3947	−25
45	NaCl + sublimate.....	2.1549	+16	1.5074	−19
*35	Lang's fluid.....	2.0670	+15	1.6794	− 7
36	Lang's fluid.....	2.0429	+10	1.7970	− 3
47	Sublimate-osmic-acetic.....	1.9917	+ 2	1.5483	−12
48	Sublimate-osmic-acetic.....	2.1555	+22	1.8365	+ 4
41	Sublimate-formol.....	2.3315	+21	1.6565	−14
49	Sublimate-formol-acetic.....	1.7687	− 2	1.5003	−17
50	Sublimate-formol-acetic.....	1.8944	+ 8	1.5221	−13

did not weigh less than its computed fresh weight after treatment with alcohol. Owing to the presence of osmic acid, this solution blackens the tissues considerably and sections must be bleached with hydrogen dioxide before they can be stained with thionin. The histological effects of this solution on the brain tissue is somewhat better than that obtained with any of the solutions of corrosive sublimate previously described. Very few of the large cells in the cerebral cortex show any signs of a contraction of the cell body or of a vacuolization of the cytoplasm; and the nuclei are well preserved in all cases. This solution does not give

a uniform fixation of the cell structures, however, and therefore it is not the best solution that can be selected for the preservation of brain tissue.

Other corrosive sublimate solutions used in the course of these experiments contained various amounts of formaldehyde, and their action on the weight of the brain of the rat as well as on the structure of the cells in the cerebral cortex have already been noted. All of the corrosive sublimate solutions that were used give a very good fixation of the nuclei in the large cells of the cerebral cortex; but they have a tendency to produce a vacuolization in the cytoplasm, and so do not give a fixation of the cell body at all comparable to that of the nucleus.

The effects of various corrosive sublimate solutions on the weight of the brain of the albino rat are shown in table 3.

For comparative purposes the brains of two rats (nos. 26 and 28) were fixed in alcohol, although this fluid is very little used at the present time for cytological work unless one is employing the technique used to bring out the so-called "Nissl substance" in the cytoplasm of the nerve cells The effects of this mode of fixation on the weight of the brain of the albino rat are shown in table 4.

TABLE 4

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
*26	Alcohol (30%)	1.7753	− 0	1.6201	− 9
28	Alcohol (95%)	1.4418	−22	1.4611	−21
30	Carnoy's fluid	1.8192	− 2	1.4077	−24
31	Carnoy's fluid	1.7575	− 3	1.3042	−23
*34	Carnoy's fluid	1.7416	− 2	1.3110	−28

As shown in the above table, there is less initial loss in weight when the brain of a rat is fixed in weak alcohol than when strong alcouol is used. These results accord with those that Donaldson

obtained by preserving brains of sheep in alcohols of different strengths. Practically the same cytological changes are produced in the brain by fixation in alcohol, whether a strong or a weak solution has been employed. As shown in fig. 10, which is a drawing of one of the large cells in the cortex of the brain which was fixed in 30 per cent. alcohol, this mode of fixation causes a very slight shrinkage of the cell body as compared with that produced by a $2\frac{1}{2}$ per cent solution of bichromate of potassium. The nucleus, however, is very greatly contracted and it is surrounded by a fluid vacuole. The cytoplasm appears uniformly distributed throughout the rest of the cell body although it stains more deeply in some regions than in others.

The solution most in vogue at the present time for the fixation of the cell structures in the central nervous system of the vertebrates is the chloroform-alcohol-acetic mixture devised by Carnoy ('87), which is known to many neurologists under the name of van Gehuchten's ('88) fluid. This solution was used as a fixative for the brains of three rats (nos. 30, 31, 34). Although acting on these brains for different lengths of time, the solution produced about the same alterations in the brain weight (table 4) and in the structure of the cells of the cerebral cortex in all three cases. The initial loss in the weight of a brain as a result of fixation by this solution is very slight, varying from 2 per cent. to 3 per cent. in different cases; subsequently the brain loses from 23 per cent. to 28 per cent. of its computed fresh weight when brought into 70 per cent. alcohol. The histological action of Carnoy's fluid on the cell structures in the cerebral cortex of the brain of the rat is shown in fig. 13. The cell has seemingly retained its normal size and shape and the cytoplasm appears uniformly distributed. The nucleus, however, is somewhat swollen; yet it has retained its rounded form, and the nuclear reticulum is well preserved and stains sharply. Carnoy's solution does not give quite as good a fixation of the cell structures in the cerebral cortex as can be obtained with other fluids, especially with the Ohlmacher solution described below.

Of all of the various fluids that were used as fixatives of the brain of the albino rat, the solution of Carnoy as modified by

Ohlmacher ('97) gave the best preparations for a study of the size and structure of the cells in the cerebral cortex. Table 5 shows the effects of this solution, acting for various lengths of time, on the weight of the brains of different individuals.

TABLE 5

RAT NO.	LENGTH OF TIME IN HOURS SOLUTION ACTED	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM THE FIXING SOLUTION	PER CENT. LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. LOSS IN WEIGHT
11	6	1.8267	− 8	1.6248	−18
6	5	1.6100	−12	1.4471	−22
13	4	1.5787	−17	1.4498	−25
14	3	1.5458	−16	1.4633	−20
15	3	1.3978	−16	1.3099	−21
16	3	1.4590	−18	1.4000	−21
*17	3	1.6390	−11	1.4875	−20
7	2	1.7389	− 2	1.4099	−21
12	2	1.6924	−10	1.5748	−16
21	2	0.2489	−14	0.2011	−30
20	1	0.2523	−16	0.2074	−31

In this, as in other series of experiments, brains of various individuals reacted differently although subjected to the same course of treatment. These variations in the results can doubtless be attributed, in great part, to differences in the size of the brains and to the physical condition of the animals at the time that they were killed. There is no swelling of the brain after fixation in Ohlmacher's solution; on the contrary, there is a loss of about 15 per cent. in the weight of the brain of an adult rat as a direct result of the fixation, which is followed by a further loss of about 5 per cent. after the brain has been brought into 70 per cent. alcohol. The alterations produced in the brain weight are practically the same whether the brain remains for two or for six hours in the solution.

In order to ascertain whether Ohlmacher's solution would give as satisfactory preparations of the brains of young as of adult individuals, the brains of two rats (nos. 20 and 21), killed when

they were about forty-eight hours old, were fixed in this fluid. Each brain had lost about 15 per cent. in weight on removal from the solution. This loss in weight was subsequently increased to about 30 per cent. after the brains had been treated with 70 per cent. alcohol. The fact that the brains of young individuals lose more weight than do those of adults after fixation with Ohlmacher's solution is doubtless to be attributed, in part at least, to the differences in the percentage of water in the brain tissue of rats of different ages. The brain of a very young rat contains about 10 per cent. more water than does that of an adult animal (Donaldson), and the replacement of this larger amount of water by alcohol would necessarily produce a greater alteration in the brain weight.

In brains of young rats, as well as in those of adults, there is such a uniform shrinkage of the brain substance after fixation with Ohlmacher's solution that only very slight traces of it can be detected on examining prepared sections of brains that have been properly imbedded. That the method of imbedding that has been employed can produce marked alterations in the cell structures of tissues that have been well fixed is shown by a comparison of figs. 14 and 15. These drawings are of cells in the cerebral cortex of different halves of the same brain (rat no. 17) that were imbedded in different ways. When a brain that has been fixed in Ohlmacher's solution is imbedded in paraffine after being cleared with chloroform or with any of the other substances commonly used for this purpose, there is invariably a shrinkage of the cell body, as shown in fig. 14, and a condensation or vacuolization of the cytoplasm. If, however, the brain is imbedded in celloidin or in celloidin-paraffine, the large cells in the cerebral cortex have the appearance of the cell shown in fig. 15. There is no shrinkage evident anywhere in the cell. The cell outlines are regular and the protoplasmic processes stand out with great clearness; the cytoplasm is uniform in appearance and evenly distributed throughout the cell. The nucleus always maintains its normal relations with the cell body and its contents are well preserved and stain very sharply.

According to Ohlmacher, this solution gives an adequate fixation of the human brain, subdivided by Meynert's section, in twenty-four hours. It seems probable, therefore, that this method of fixation would give satisfactory preparations of the brain of any mammal if allowed to act for the proper length of time. There seems to be no disadvantage whatever connected with the use of this solution as a fixative of brain tissue, unless it be the cost of the ingredients of which the solution is composed.

TABLE 6

Summary of Data Collected

RAT NO.	SEX	BODY WEIGHT IN GRAMS	BODY LENGTH IN MM.	NORMAL WEIGHT OF FRESH BRAIN COMPUTED	SOLUTIONS USED FOR FIXATION	NO. HOURS SOLUTIONS ACTED	WEIGHT OF BRAIN IN GRAMS WHEN REMOVED FROM SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
1	♂	277	219	1.94	4% Formaldehyde.	48	2.5750	+33	1.5706	−19
2	♂	163	196	1.83	4% Formaldehyde.	48	2.8200	+54	1.6463	−10
3	♀	158	199	1.85	Formol-Müller (cold).....	20	2.2437	+21	1.5537	−16
4	♀	129	183	1.78	4% Formaldehyde.	48	2.6778	+50	1.6577	− 7
5	♀	164	188	1.80	Formol-Müller (warm)	3	2.1880	+22	1.8711	+ 4
6	♂	187	198	1.85	Ohlmacher	5	1.6100	−12	1.4471	−22
7	♀	137	184	1.78	Ohlmacher	2	1.7389	− 2	1.4099	−21
8	♂	160	190	1.81	Zenker.....	6	1.8716	+ 3	1.6666	− 8
					Müller.....	48				
9	♀	170	197	1.84	Dahlgren.....	4	1.9000	+ 3	1.7273	− 7
					Müller.....	48				
10	♂	182	186	1.79	Picro-formol	4	1.7881	− 0	1.4663	−18
11	♂	275	228	1.98	Ohlmacher	6	1.8267	− 8	1.6248	−18
12	♂	206	207	1.88	Ohlmacher.....	2	1.6924	−10	1.5748	−16
13	♂	228	210	1.90	Ohlmacher.....	4	1.5787	−17	1.4498	−25
14	♂	169	194	1.83	Ohlmacher....	3	1.5458	−16	1.4633	−20
15	♂	126	157	1.65	Ohlmacher.....	3	1.3978	−16	1.3099	−21
16	♂	158	181	1.77	Ohlmacher.....	3	1.4590	−18	1.4000	−21
17	♂	232	199	1.85	Ohlmacher.....	3	1.6390	−11	1.4875	−20
18	♀	111	154	1.63	Zenker-formol	1½	1.6040	− 2	1.3297	−18
19	♀	106	159	1.66	Zenker (modified)..	1¼	1.7451	+ 5	1.3167	−21
20	♂	6		0.30	Ohlmacher.....	1	0.2523	−16	0.2074	−31

TABLE 6—Continued

RAT NO.	SEX	BODY WEIGHT IN GRAMS	BODY LENGTH IN MM.	NORMAL WEIGHT OF FRESH BRAIN COMPUTED	SOLUTIONS USED FOR FIXATION	NO. HOURS SOLUTIONS ACTED	WEIGHT OF BRAIN IN GRAMS WHEN REMOVED FROM SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
21	♀	6		0.29	Ohlmacher	2	0.2489	−14	0.2011	−30
22	♂	108	156	1.64	2½% $K_2Cr_2O_7$	48	2.8445	+73	2.1409	+31
23	♂	88	163	1.68	2½% $K_2Cr_2O_7$	48	2.5594	+52	1.7518	+ 4
24	♂	162	187	1.79	Alcohol $K_2Cr_2O_7$	48	2.5073	+40	1.8885	+ 6
25	♂	190	207	1.88	Alcohol $K_2Cr_2O_7$	48	2.8169	+50	2.1797	+16
26	♂	174	184	1.78	Weak alcohol	27	1.7753	−00	1.6201	− 9
27	♂	168	191	1.81	Alcohol-formol	24	1.6392	−10	1.5147	−16
28	♂	221	198	1.85	95% Alcohol	24	1.4418	−22	1.4611	−21
29	♂	151	184	1.78	Sublimate-acetic	1½	1.8604	+ 5	1.4484	−19
30	♂	213	202	1.86	Carnoy's fluid	3	1.8192	+ 2	1.4077	−24
31	♂	181	194	1.82	Carnoy's fluid	4	1.7575	− 3	1.3042	−23
32	♀	141	178	1.75	Graf (5% formalin)	2½	2.1520	+23	1.7421	−00
33	♂	165	191	1.81	Graf (10% formalin)	1½	1.9283	+ 7	1.5994	−12
34	♀	149	184	1.77	Carnoy's fluid	19	1.7416	− 2	1.3110	−28
35	♀	167	189	1.80	Lang's fluid	20	2.0670	+15	1.6794	− 7
36	♂	208	203	1.86	Lang's fluid	4	2.0429	+10	1.7970	− 3
37	♀	173	194	1.82	Marina's fluid	72	1.2219	−33	1.2913	−29
38	♂	197	201	1.86	Marina's fluid	96	1.2146	−35	1.2546	−33
39	♂	259	214	1.92	Cor. sublimate	4	2.0760	+ 8	1.4695	−23
40	♂	177	195	1.83	Cor. sublimate	20	2.0229	+11	1.4087	−23
41	♂	265	216	1.92	Sublimate-formol	4	2.3315	+21	1.6565	−14
42	♂	213	203	1.86	NaCl + sublimate	4	1.9927	+ 7	1.3947	−25
43	♀	213	204	1.86	Tellyesniczky	48	1.9643	+ 6	1.6372	−12
44	♀	137	177	1.74	Tellyesniczky	24	1.7981	+ 3	1.4906	−14
45	♂	196	200	1.85	NaCl + sublimate	20	2.1549	+16	1.5074	−19
46	♀	135	179	1.75	Sublimate-formol	20	2.0512	+17	1.3687	−22
47	♂	141	179	1.75	Cox (osmic)	48	1.9917	+ 2	1.5483	−12
48	♂	150	182	1.76	Cox (osmic)	72	2.1555	+22	1.8365	+ 4
49	♂	171	192	1.81	Cox (formol-acetic)	48	1.7687	− 2	1.5003	−17
50	♂	137	178	1.75	Cox (formol-acetic)	72	1.8944	+ 8	1.5221	−13

B. A METHOD OF PREPARING THE BRAIN OF THE ALBINO RAT FOR A STUDY OF THE CELLS IN THE CEREBRAL CORTEX

Experience has shown that considerable time is often consumed in adapting a general method of preparation to the particular material with which one is working, and that in many cases comparatively slight variations in the lengths of time different fluids act on the tissues produce marked structural effects. For these reasons it has been thought advisable to give in detail a method of preparing the brain of the rat which produces satisfactory preparations for a study of the cells in the cerebral cortex, although in this method there is very little that is new. This method should give equally good preparations of the brain of any other small mammal, and it would doubtless be applicable also to small pieces of the brain of any of the larger animals.

For fixation the solution devised by Ohlmacher ('97) is used. This solution is made as follows:

Absolute alcohol	80 parts
Chloroform	15 parts
Glacial acetic acid	5 parts
Corrosive sublimate to saturation (about 20 per cent.)	

As the corrosive sublimate dissolves rather slowly, it is necessary to make up the solution a few days before it is required for use.

Brains of adult rats are well fixed after being subjected to the action of this solution for three hours: for the fixation of the brains of very young individuals an immersion of two hours in the liquid is sufficient. On removal from the solution the brain is placed in 85 per cent. alcohol, where it remains for about one hour. It is then transferred into iodized 70 per cent. alcohol, where it is kept until the corrosive sublimate has been extracted from the tissues. This latter process requires at least twenty-four hours, and if the brain has not been subdivided it is necessary to renew the liquid and keep the brain in it for two or three days. The brain is then brought into 80 per cent alcohol where it can remain as long as necessary. It is advisable to imbed the material as soon as pos-

sible, since long immersion in alcohol is injurious to any tissue and greatly lessens it staining powers.

For imbedding the celloidin-paraffine method of Bödeker ('08) gives quite as satisfactory preparations of brain tissue as does celloidin, and it has the great advantage of imbedding this material so that it can readily be cut in very thin serial sections, which can subsequently be treated as if paraffine alone had been the imbedding medium. The directions for this method as given by Bödeker are rather general, and the method as finally adapted to the brain of the rat is as follows: From 80 per cent. alcohol the brain is passed through 95 per cent. alcohol, absolute alcohol, and ether-alcohol, remaining in each solution for twenty-four hours. It is then transferred into 2 per cent. celloidin where it is left for two or for three days, depending on the size of the brain. After six hours immersion in chloroform the brain is put into benzole for one hour, and is then carried over into benzole saturated with soft paraffine where it remains eighteen hours. In order to facilitate the penetration of this solution into the brain tissue it is advisable to keep the liquid slightly warm (about 35°C.) The brain is then placed in melted soft paraffine (melting point about 45°C.), which is kept just above the melting point for the three hours that the brain remains in it. Subsequently the brain is brought into melted hard paraffine (melting point about 54°C.), which must be kept as near the melting point as possible since heat is very in-injurious to brain tissue. After remaining in the hard paraffine for two hours the brain is ready to imbed in hard paraffine. Brains thus prepared can be cut with a Minot microtome into serial sections which can be made as thin as 5μ if desired.

The sections are mounted in the usual way with albumen fixative and the paraffine removed with xylol. In further treatment one must avoid the use of absolute alcohol, as this substance tends to loosen the sections from the slide. In place of absolute alcohol a mixture composed of equal parts of chloroform and of absolute alcohol can be used with safety. After passing through the various grades of alcohol into distilled water the mounted sections are stained for two or three minutes in a 1 per cent. solution of carbolic acid saturated with thionin. They are then washed for a

moment with distilled water and differentiated in 95 per cent. alcohol. The process of differentiation can be watched under a microscope, as it does not take place very rapidly. If a counterstain is desired a small amount of eosin can be added to the alcohol in which the sections are differentiated. The slides are then passed quickly through the chloroform- alcohol mixture into xylol, and the sections are finally mounted in Canada balsam.

Although thionin is known to be an excellent stain for cytological purposes, it is little used when preparations are to be kept for any length of time, as it fades rapidly if used in an aqueous solution. As a stain for the cell structures in the cerebral cortex of the brain of the rat, thionin has been found to act more energetically and to give somewhat sharper outlines when dissolved in a weak solution of carbolic acid than when used in an aqueous solution. In order to test the permanency of the stain, prepared slides were exposed for three months on a well lighted laboratory table. At the end of this time the sections were somewhat faded, but structural details could still readily be made out. Other slides similarly stained have been kept for over a year in slide boxes and the sections do not appear to have faded in the slightest degree. If the sections are not exposed to the light unnecessarily, it is probable that the stain will be as permanent as that given by the great majority of the anilin dyes.

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EXPLANATION OF FIGURES

All figures were drawn with the aid of a camera lucida under a Zeiss apoc. 1.5 mm obj.; oc. 8. They have been reduced to give a magnification of about 800 diameters.

1. Cell from the cerebral cortex of a brain (rat no. 1) imbedded in celloidin after fixation in 4 per cent formaldehyde.

2. Cell from the cerebral cortex of a brain (rat no. 1) imbedded in paraffine after fixation in 4 per cent formaldehyde.

3. Cell from the cerebral cortex of a brain (rat no. 27) imbedded in celloidin-paraffine after fixation in alcohol-formol.

4. Cell from the cerebral cortex of a brain (rat no. 38) imbedded in celloidin-paraffine after fixation in Marina's fluid.

5. Cell from the cerebral cortex of a brain (rat no. 32) imbedded in celloidin-paraffine after fixation in the picro-formol solution of Graf.

6. Cell from the cerebral cortex of a brain (rat no. 23) imbedded in celloidin-paraffine after fixation in a 2½ per cent solution of bichromate of potassium.

7. Cell from the cerebral cortex of a brain (rat no. 24) imbedded in celloidin-paraffine after fixation in alcohol-bichromate of potassium.

8. Cell from the cerebral cortex of a brain (rat no. 8) imbedded in celloidin after fixation in Zenker's fluid followed by hardening in Müller's fluid.

9. Cell from the cerebral cortex of a brain (rat no. 9) imbedded in celloidin after fixation in Dahlgren's fluid followed by hardening in Müller's fluid.

10. Cell from the cerebral cortex of a brain (rat no. 26) imbedded in celloidin-paraffine after fixation in 30 per cent alcohol.

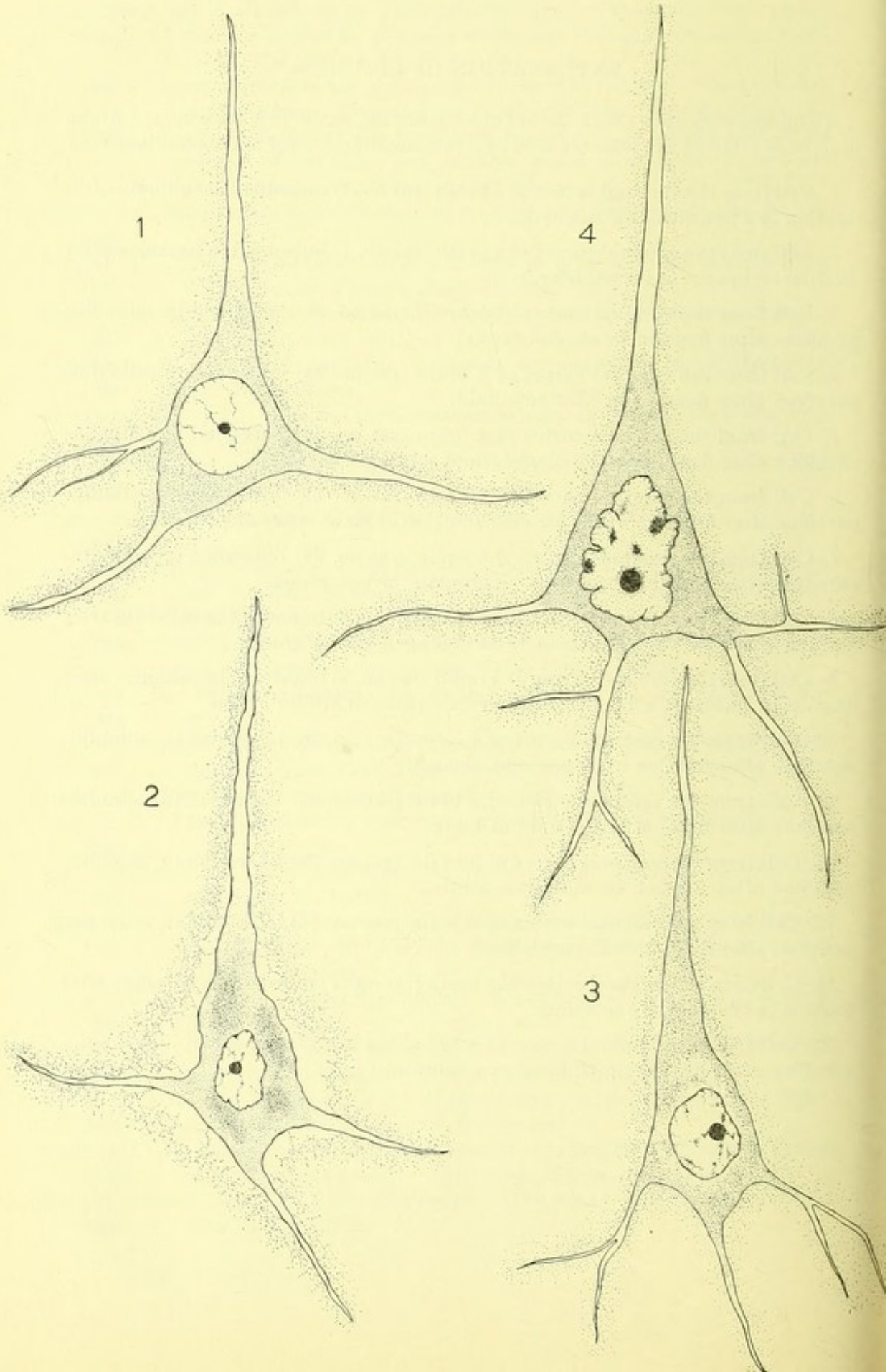
11. Cell from the cerebral cortex of a brain (rat no. 35) imbedded in celloidin-paraffine after fixation in the fluid of Lang.

12. Cell from the cerebral cortex of a brain (rat no. 29) imbedded in celloidin-paraffine after fixation in sublimate-acetic.

13. Cell from the cerebral cortex of a brain (rat no. 34) imbedded in celloidin-paraffine after fixation in Carnoy's fluid.

14. Cell from the cerebral cortex of a brain (rat no.17) inbedded in paraffine after fixation in Ohlmacher's solution.

15. Cell from the cerebral cortex of a brain (rat no. 17) imbedded in celloidin-paraffine after fixation in Ohlmacher's solution.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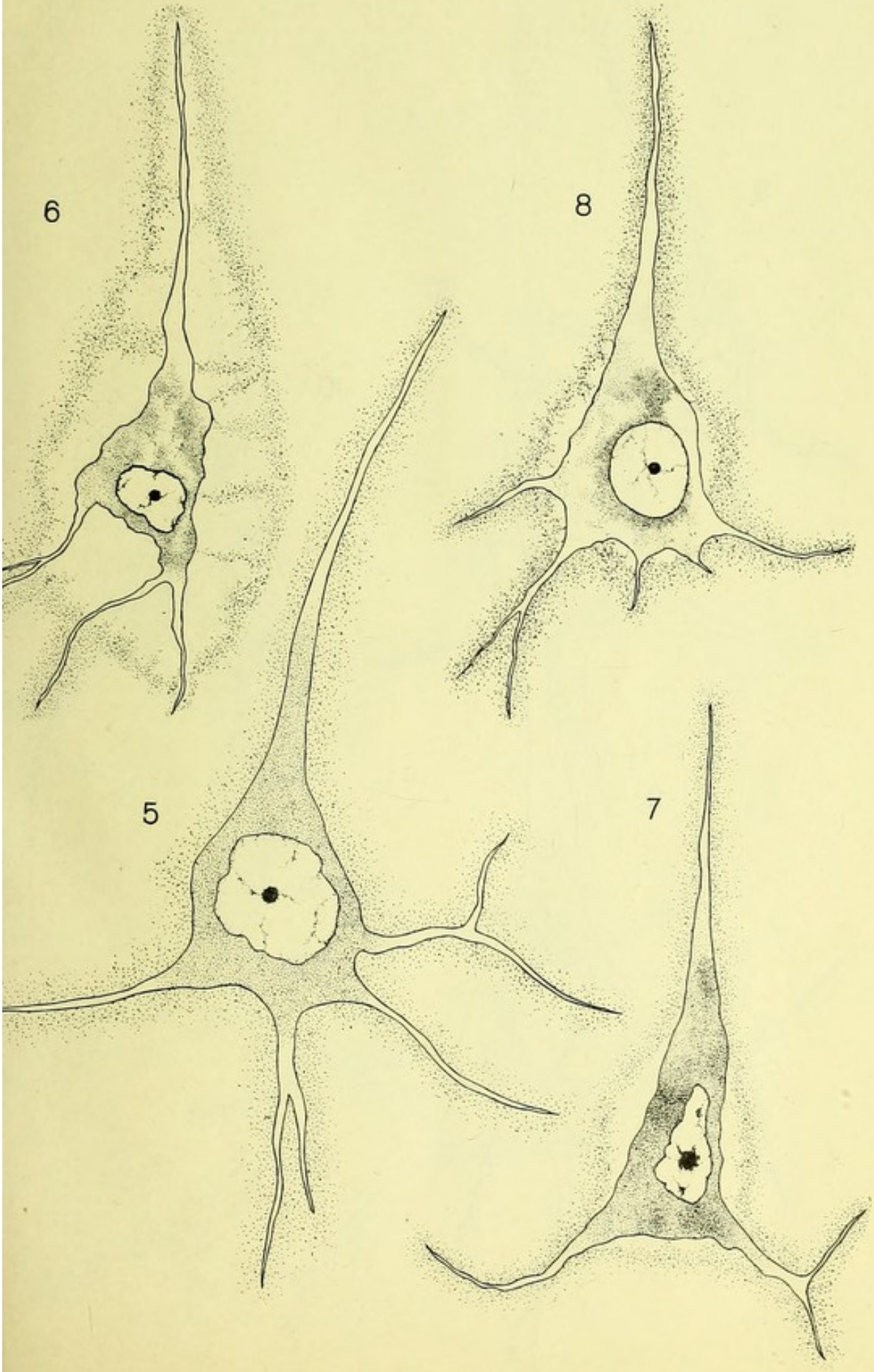
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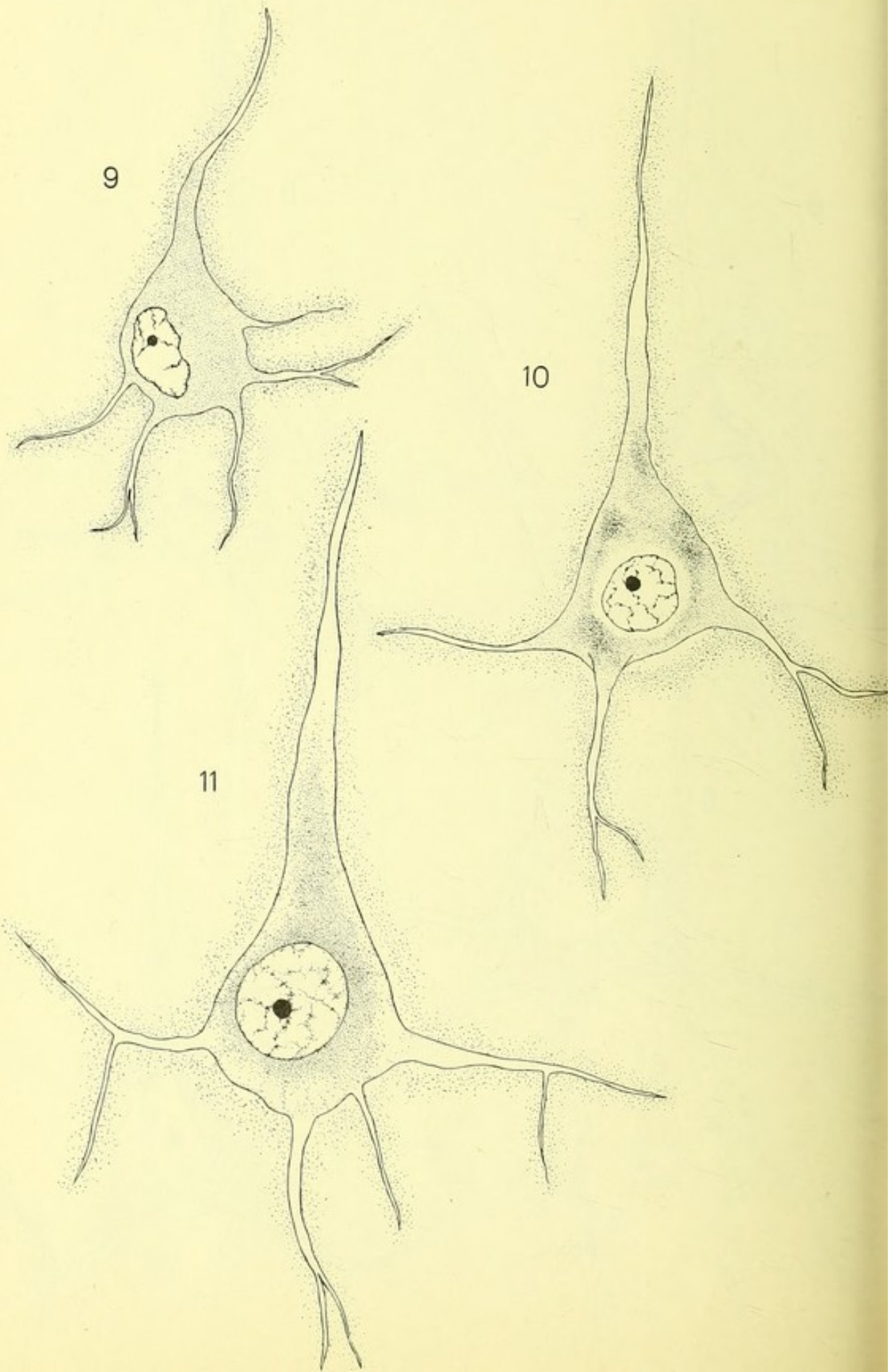
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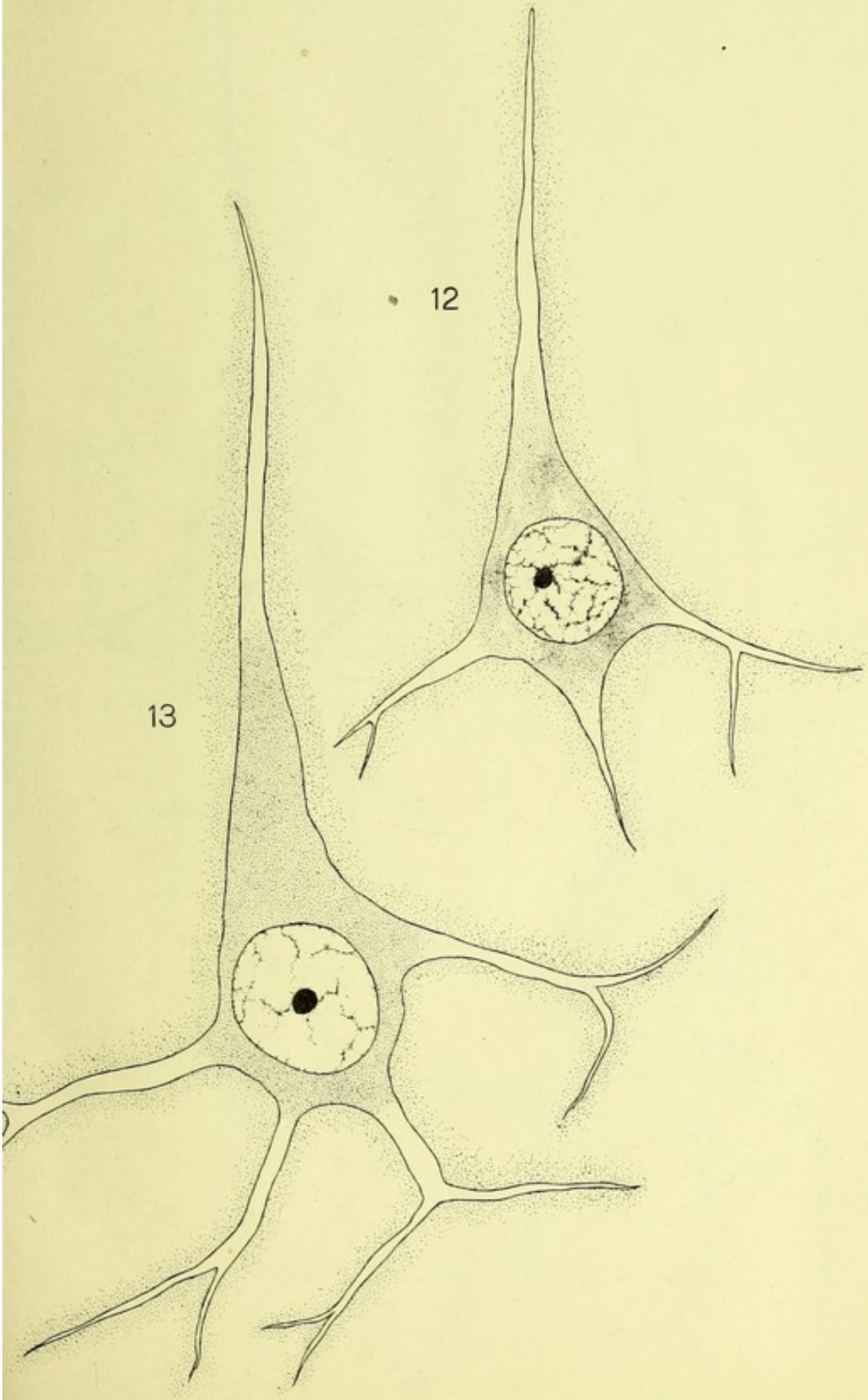
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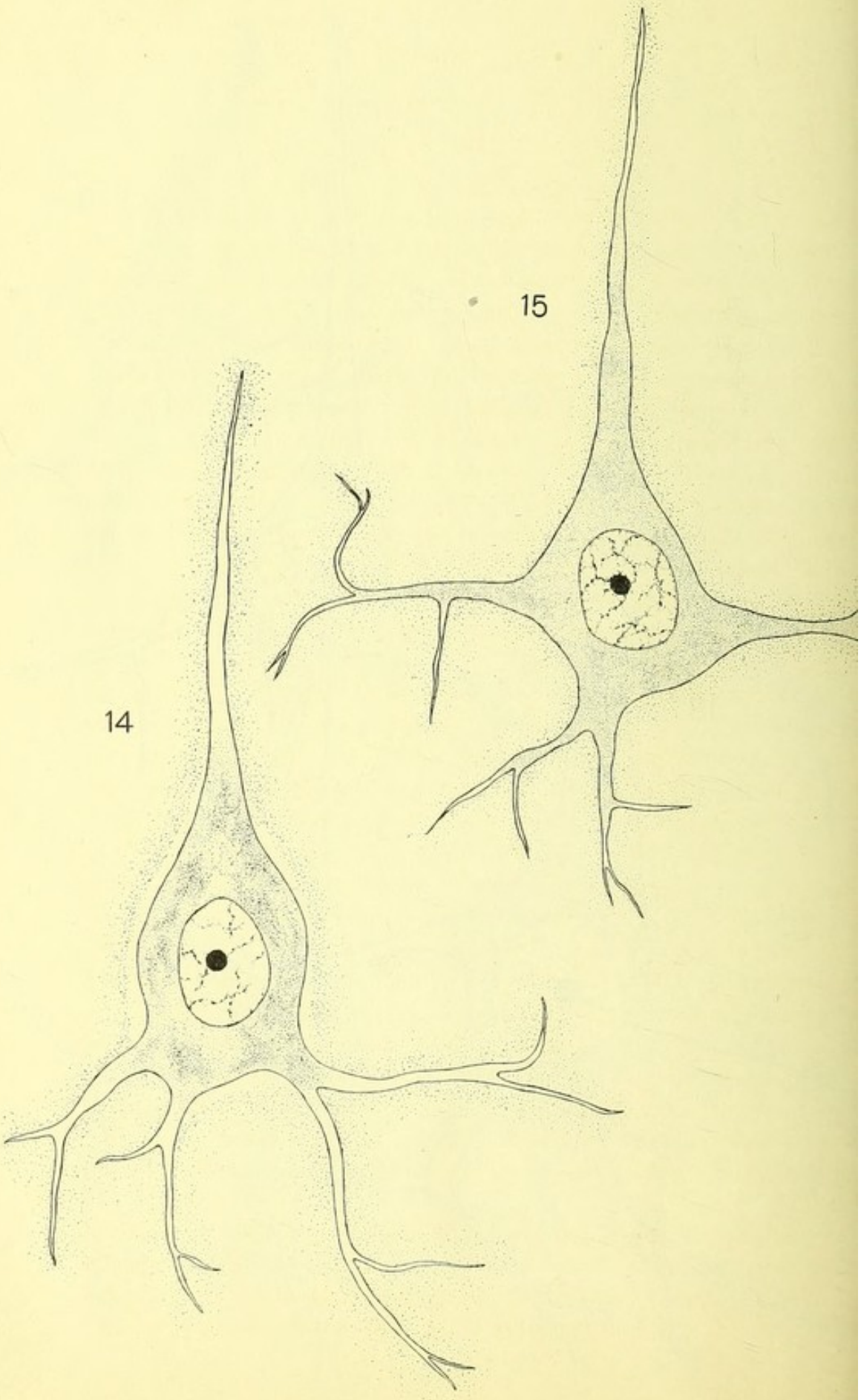
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