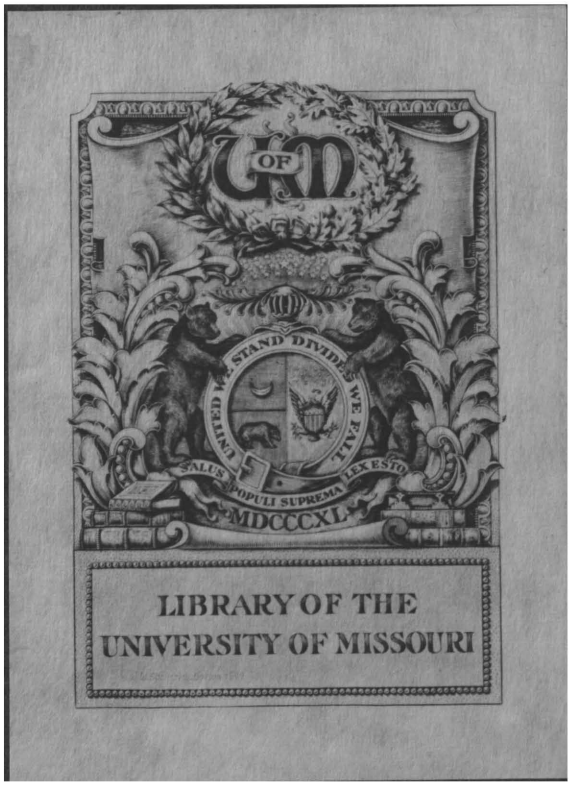

A CYTOLOGICAL STUDY OF THE DEVELOPMENT OF THE FUNCTIONAL ACTIVITY OF NERVE CELLS IN FOETAL AND INFANTILE ANIMALS

by

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SYNOPSIS

Introduction--

Morphological changes corresponding to functional activity have been found by numerous investigators. The purpose of this investigation was in the first place to determine, if possible, at what period functional activity might begin. The second aim was to investigate to what extent early changes of function correspond to adult changes and to trace the progress of development. This has been done principally in terms of the nucleus-plasma relation theory of Richard Hertwig.

SOURCE OF MATERIAL.--This article is based principally on the study of puppies from the foetus to four weeks. In addition to dogs, cats and rabbits have been used. The kitten at birth was used principally for comparison and its cells were not studied in detail. The rabbits served largely for the purpose of learning technique. Over one hundred cells were measured in addition to drawing the several stages. The animals used are listed below in order of advancing age.

Experiment 1. Male dog foetus at term. Weight one hundred and eighty five grams, removed from uterus of well fed, white bitch of medium size, by Cesarean section. She was calculated to have one week more, but as a result of the operation she began to abort.

Experiment 2. Female kitten. Age, thirty minutes after birth. Mother a little larger than the average size of domestic cat and in good condition.

Experiment 3. Rabbit. Age, eighteen hours. Born after shipment of mother and probably prematurely.

Experiment 4. Pup. Age, thirty six hours. Probably born prematurely.

Experiment 5. Male pup. Age, six days, weight about seven hundred grams, in fine condition.

Experiment 6. Litter of four puppies.

(a). Bitch pup. Age, said to be one week. Weight, four hundred and twenty grams.

(b). Male pup. Age, one and a half weeks. Weight, three

hundred and eighty grams.

(c). Male pup. Age, two weeks. Weight, three hundred and eighty grams.

(d). Male pup. Age, two and a half weeks. Weight, three hundred and thirty grams. Eyes not open in any one of the litter. They were taken from the mother at the same time and fed milk from a bottle. The indications are that the feeding did not add to their weight.

Experiment 7. Litter of two puppies.

(a). Female pup. Age, two and a half weeks. Weight, eight hundred and fifty grams.

(b). Female pup. Age, three weeks. Weight, nine hundred grams.

Experiment 8. White female pup. Age, four weeks. Weight, thirteen hundred grams.

Experiment 9. Rabbit. Age, approximately one year.

All the animals were killed by ether. The brain tissue was removed and put into fixatives within ten to twenty minutes after death.

MICROSCOPIC TECHNIQUE.--The routine fixing fluid was a saturated solution of corrosive sublimate 95 parts and 5 parts of 40 percent solution of formaldehyde. Vom Rath's fluid was used in several experiments. The chemicals and proportions of this fluid are as follows:

Sat. aq. Picric	200 cc.
Plat. Chlor. (in 10 cc. H_2O). .	1 grm.
Glacial Acetic Acid	2 cc.
2% Osmic Acid	25 cc.

In addition to the above two, a mixture of Flemming and Hermann's fluid, which gives an excellent nerve cell picture, was employed. The proportions are as follows:

One per cent Chromic Acid	7 1/2 cc.
" " Platinic Chloride .	7 1/2 cc.
" " Osmic Acid	4 cc.
Glacial Acetic Acid . . .	1 cc.

After a fixation of 24 hours in the formalin-sublimate solution the tissue was run thru the graded alcohols with 12 hours in each. The alcohols from 50% to 80% were iodized. From the alcohols the tissue was imbedded in paraffin. Tissues from the Vom Rath's and Flemming-Hermann solutions were washed twenty four hours in running water before running thru the alcohols and imbedding.

The sections were cut five micra thick. Erythrosin-toluidin blue was employed as the routine stain. After removing the paraffin in the usual way, the sections were stained about 1 1/2 minutes in 1% Erythrosin, heated to 40°. After washing

thoroughly, they were transferred to saturated aqueous solution of toluidin blue from five to seven minutes. Differentiation was done in 95% alcohol. Sections were then passed thru absolute alcohol and xylol. Leaving them in the xylol for about ten minutes they were then mounted in balsam. The Flemming-Hermann fixation was followed by staining in Heidenhain's iron-hematoxylin in the usual way. It afforded identical results with the toluidin blue preparations.

TECHNIQUE OF SIZE-COMPUTATION AND COMPARISON.--Measuring the separate stages and determining the volume of cell body and nucleus and the nucleus plasma relation were carried out according to the procedures given by Dolley. For volume calculation, the cell and nucleus were first outlined with camera lucida and the major and minor diameter of each were measured with a standard ruler in millimeters. The major diameter multiplied by the square of the minor (the two minor axes being approximately equal where three dimensions are involved) gives the comparative volume of the cell and nucleus. To obtain the plasma mass the nuclear mass is subtracted from the total cell mass. The plasma mass divided by the nuclear mass gives the size ratio between the two and this value is the nucleus-plasma coefficient. This follows Herwig's idea that for every cell the volume of the cell body bears a constant relation to the nucleus. The volumes of the cells and nuclei and the nucleus-plasma coefficients for these experiments are recorded in tables I, II, and III.

Based on Table I, the curve Fig. IA of cellular and nuclear growth was constructed by using the figures representing the ratio between the cell and nucleus of the 36 hours pup, Table I, as the necessary divisors for reduction in each of the other puppies. The resulting quotients are the ordinates in centimeters. In Fig. IB on puppies of the same litter, Table II, the curve begins with the one week dog, Table II, and the ratio value of the cell and nucleus of this animal is used in the succeeding reductions. In both series by this means both the absolute and the relative growth to the youngest dog are shown. Figures II and III represent the stages of activity in the 2 1/2 weeks pup, Table III, and the 4 weeks pup respectively. In each, the upper curve gives the volumetric relations, the lower the nucleus-plasma relation. The curves of volume were plotted after the reduction just indicated. For the nucleus-plasma curve the ordinates are constructed above or below the base line according as the stage figures differ from the resting cell (Stage 1).

REVIEW OF LITERATURE.

A. <u>The changes ascribed to function</u>. Hodge in 1892 began the study of changes in nerve cells due to functional activity. The nerve cell at this time was not thought of as changing morphologically when stimulated, as does the gland cell in producing secretion or a muscle in performing its work. "Its secretion is consciousness." In studying cellular activity his principle was to seek for changes in the nucleus and in the protoplasm of the cell--"a cell becomes specialized to perform a certain function only by an increased growth of certain of

its parts." Hodge's chief observations were made after stimulating with an induced current the spinal ganglia of the dog, cat, and frog, and from the normal stimulation (daily fatigue) of the cerebrum and cerebellum of the honey bee and pigeon. In these first experiments he noted in the fatigued cell that the nucleus decreased in size, stained darker, and had an irregular outline. The cell protoplasm showed marked shrinkage in size and vacuolation. In his most recent communication Hodge has compared nerve cells of a child at birth with those of a man of 92 years. Also he has compared nerve cells of young and old bees. In the cerebellum of the old person he notes the Purkinje cells are shrunken and are scarce--twenty five per cent had disappeared. A more striking divergence is noted in the spinal ganglion cells. In the individual of ninety-two there is strong pigment in the protoplasm, while in the foetus there are no pigmented bodies. The nucleus of the foetus is described as being large, round, and clear.

Lugaro made a large number of measurements of the sympathetic ganglion cells in different states of function. It is worth while to summarize his results:

1. "The activity of the nerve cell is accompanied by a state of swelling in the protoplasm of the cell body.

2. Fatigue produces a diminution in the size of the cell body.

3. In the moderate degrees of activity, altho the protoplasm of the cell body becomes swollen, the nucleus is not subject to modifications of volume.

4. When the activity is continued and prolonged for a long time the nucleus submits to modifications analogous to those of the cell body, but less intense and slower.

5. The quantity of the chromatic substance in the cell body varies especially according to the individual characteristics in ratio to the size. While it is probable that the first phases of activity determine a slow augmentation of the chromatic substance, the later phases accompanied by fatigue show a diminution and more diffuse distribution.

6. The activity of the cell determines in the nucleus an augmentation of volume which gives way slowly to the action of the reduction of fatigue." Lugaro stimulated the sympathetic a short distance above the superior cervical ganglion.

Vas stimulated the sympathetic ganglion cells of the rabbit and noted an increase in size of both the cell and the nucleus. The chromatin moves away from the nucleus to the periphery of the cell, leaving the center of the cell a distinctly lighter color. Whether or not the amount of chromatin diminishes with cell activity, he did not say.

Lambert and Mann repeated the experiments of Vas. Lambert observed changes in chromatin similar to Vas. He failed to make out an increase in size of cells and their nuclei. Mann notes there is a change in size of the cell, the nucleus and nucleolus with activity. The stored up chromatic materials in the resting cell are used up, Mann states, in the performance of function.

These studies on ganglion cells represent the principal investigations. In addition, the retinal nerve cells have been studied by Pergens and Chiarini, and cortical cells by Mann,

Pugnat, and Van Durme. In general they have found changes in size and disappearance of chromatin corresponding in kind to those of the lower cells, though they are more marked in degree.

Dolley has carefully studied the changes in the Purkinje cell by exercising the dog in the tread mill, by bleeding (trophic stimulation), by inducing abnormal states of traumatic shock (mechanical stimulation), and by injection of chemicals (chemical stimulation), such as caffein, alcohol, and certain bacterial toxins. In each of these the nerve cell goes thru the same regular and orderly sequence of events. He has divided these changes in the cell into thirteen stages. Stimulation only increases the intensity of the changes; that is, a normal animal exhibits the same changes that occur in the stimulated animal, but fewer cells are affected in the former. The writer has confirmed the results of Dolley by measurement and drawing in water color of the cells (Plate I) in the different stages of a normal rabbit. The cells are numbered according to stage from 1 to 12.

1. "The resting cell. It is lacking in intranuclear chromatin except within the Karyosome (nucleolus), and the amount of extranuclear chromatin varies with the individual.

2. The stages of progressive hyperchromatism, in which in the pure type, the initial enlargement of the whole cell reaches its maximum.

3. The stage of maximum hyperchromatism, which is associated with the beginning of shrinkage.

4 & 5. The stages of regressive hyperchromatism together with the maximum of shrinkage. Coincident in place but separated originally to denote difference in shape, stage 4 being more attenuated and spindle. Both stages 4 and 5 are to be further divided into an early, the pure Hodge type, and a late division, characterized by the sharp beginning of nuclear edema.

6. The return of the cytoplasmic chromatin in its continued reduction to the average normal level. This stage is principally distinguished by the maximum disproportion in the size of the nucleus, owing to its much greater edema.

7 & 8. Two stages leading to the primary disappearance of cytoplasmic chromatin.

9 & 10. The stages of secondary re-appearance of cytoplasmic chromatin. The chromatin is first formed about the nuclear membrane and then passes out.

11. The stage of secondary disappearance of cytoplasmic chromatin. With the complete using up of the previous supply, the karyosome is left containing the only vestige of basic chromatin in a much more exhausted-looking cell.

12. The disintegration and passing out of the ultimate content contained within the karyosome.

13. The exhausted cell, with complete dechromatinization."

B. <u>On time of appearance of chromatic material in developing nerve cells</u>. It is well demonstrated by the work of Scott, Hatai, and Collins that the extranuclear chromatin of

the nerve cell (the Nissl substance) arises developmentally from the nucleus. They have contented themselves, however, with the general fact. Working either upon a very early stage of development or upon segregated parts, they have not furnished definite data as to the time it makes its appearance in various portions of the system. It will be shown that the vast majority of Purkinje cells in the foetus at term are practically devoid of extranuclear chromatin (Nissl substance). This opens up a new field in its indication of successive assumption of function by different parts in the course of their development. The discussion of this functional relationship and its bearing on functional growth are beyond the scope of this paper, but it is too important to pass over without mention.

Thus Hatai, working on the spinal ganglion cell of the white rat, found Nissl substance at a very early stage, namely, in embryos 10-13 mm. long. Scott, who only mentions the spinal cord specifically, found that the extranuclear chromatin developed in the pig at about 15 mm. Again Mühlmann, who most lately has investigated the development of the nerve cell, notes its first appearance in the ox in embryos 8-14 cm. long, but he deals with no higher specialized cells than cord or ganglion. Such cells as these must assume function very early. On the contrary, there is no reason in the light of general knowledge why the Purkinje cell should function before birth. The relation here indicated will be further investigated.

THE DEVELOPMENT OF FUNCTION IN TERMS OF NUCLEUS-PLASMA RELATION.

A. The Transition Period from Embryonic State.

I. The foetal condition.--Sections were taken as far as could be determined from the worm, uvula and lateral sides of the cerebellum. The foetal cerebellum as studied in the dog, Experiment I, is approximately one centimeter in diameter in any direction. It is very soft and delicate, and extremely difficult to section preparatory to fixation.

The Purkinje cell of the foetus at this age is immaturely developed. The first and most striking feature of the cell is the large nucleus. The nucleus is more or less oval; its long axis runs parallel with the long axis of the cytoplasm when the latter appears. It contains minute granules which were demonstrated by all three stains, namely, erythrosin-toluidin blue, Grenacher's borax-carmine, and iron-hematoxylin. Two types of granules are observed with both the erythrosin-toluidin blue and the iron-hematoxylin. The former stains both basic and acid granules, the latter stains some granules intensely black and others a lighter grey. This agrees with the results for the iron hematoxylin as obtained by others, the black granules being basi-chromatin. The acid and grey staining granules are plastin or nucleolar substance.

In the majority of cells the cytoplasm is minimal in amount, though in a very small number it is distinct. It consists of a rounded mass placed at the dentrite pole of the nucleus, giving to the latter an eccentric position. In mea-

suring the cells the plasma volume is omitted because of its undeveloped state, and only the nuclear volume was considered (Table I.). At the juncture of the nucleus and cytoplasm, when the latter is present, there is almost always blue staining chromatic material after toluidin blue. At this stage the dendrites become in part evident. The karyosome stains well in a majority of the cells. It is either a blue black or red with blue material imbedded upon this. The number of karyosomes in a cell may be two, but only one most generally appears.

Having the above description of the embryonic character of the Purkinje cell, we will now describe briefly the embryonic condition of associated structures. Practically all histologists have divided the adult cerebellum into three layers; namely, the outer molecular, the middle granular, and the inner medullary. These structures in the foetus, Experiment I, are not easily distinguished as definite layers. The thickness of the granular layer amounts approximately to three cells only. The molecular layer proper is almost without cells. Situated upon the outer border of this layer a granular stratum is noted and has been described by others as being present in early development. The middle granular layer is composed of a few small granular cells arranged so irregularly as to prevent its being called a distinct layer. A few cells in this layer are in mitosis.

The medullary layer is considerably more distinct, being broader than either of the other two described. In it are

medullary nerve fibers and small round granular cells. The breadth of the medullary layer is only relative to the deficient development of the other structures. The brain is quite smooth and free from folds. Not only are the Purkinje cells embryonic in character at this age, but the associated structures too are equally embryonic and show very slight development. The brain of the kitten, Experiment 2, taken ten minutes after birth shows ~~almost~~ almost an appearance identical with the foetus just described.

II. The Growth of the Cytoplasm.--Having studied the Purkinje cell just previous to birth it will be taken up now after birth. A decided difference becomes at once noticeable. There occurs a marked development of the cell body. The nucleus also continues to increase in size but at a much slower rate. This growth is illustrated in three ways: (a) by comparison of cytoplasmic and nuclear growth from the foetus to four weeks in the curve, text-figure IA (Table I); (b) by comparison of cytoplasmic and nuclear growth from one week to two and one-half weeks, in curves text-figure IB (Table II); (c) by a series of diagrams of the cells from each of the dogs given in Table I, text-figure Ia. The diagrams are based upon the measurements of the cell and nuclear diameters as recorded in the table. The method of curve construction is given under "Technique of size-computation and ~~measurements~~ comparison." Both curves show a striking increase of cytoplasm with increasing age. In the curve, figure IB, all the dogs are from the same litter. This growth in curve A is more rapid than in curve B, it will be noted

Two reasons are offered to account for this: In curve A thirty cells were measured in each dog and the average was taken. In curve B only ten cells were measured and the average used. The dogs of curve A were killed immediately after separation from their mother. The dogs in curve B were taken from a bitch at a week old and fed milk from a bottle up to the time of killing. In actual weight these puppies are less than those of corresponding age in curve A. They developed outwardly very poorly, and the comparatively less marked growth of the cytoplasm would be sufficiently explained by this, though ten cells are not entirely sufficient for an average. The fact that both curves start from different stages, that is, from a foetal puppy and a seven days' puppy respectively, makes the curves look more different. It will be noted that the nucleus varies very little in size. Especially is this true in curve A, where a larger number of cells were measured and the average taken. The fact that it has remained practically constant is in keeping with the view that these cells have not functioned to a marked degree. This will be taken up in the following paragraph. The curves clearly show the rapid cytoplasmic growth from a foetus to four weeks. Figure Ia gives a diagrammatic survey of this growth.

III. Evidence that changes of full functional activity are not present up to two and one-half weeks in the present material.--We begin first by examining the foetal Purkinje cell morphologically. A brief summary will be given:-

The cell is small, with a large granular nucleus and practically

no cytoplasm. When the cytoplasm is present the basic chromatic material is massed around the nuclear rim at the juncture of the two. At this stage the dendrite is in part evident. The picture just after birth, Experiments II and III, shows a cell a step in advance of this. The size is increased, not markedly however, and the basic chromatic material is more distinct at the junction of the nucleus and cytoplasm. The growth in size of cell and the distribution of chromatin is found still further advanced in the one and one-half weeks pup, Experiment 6 . The latter animal shows a cell approaching the normal resting cell, Plate I, stage 1, more closely than any previously described. It has, however, not reached that stage thus far, because the nucleus is eccentrically located and the extranuclear basic material is stored only around the outside of the nuclear membrane. Taking stage 1, Plate I, as the resting cell of Dolley type, a similar cell is not to be found in any of the material used up to two and one-half weeks. Further, there is an absence of all stages of activity. This is in keeping with the statement made by Adami: "In the embryo, growth is at its maximum, function at its minimum."

B. The Period of Incidence of Activity.

I. The morphological indications of functional reaction.--As has been stated by Dolley, in substance, there are no clear clean-cut jumps from one stage to another, but they are more or less arbitrary, serving as a means of attack. Now taking up the study of the two and one-half weeks dog, Exper-

iment 7a, Table III, we for the first time in the series find Purkinje cells comparable to the stages of the normal cells, Plate I. The normal resting Purkinje cell of this animal in agreement with the usual description conforms in shape fairly well to that of a pear. Its nucleus is not eccentrically located, as previously described in the dog of two weeks, but more centrally within the cell body, and frequently two dendrites are seen coming off. The nucleus contains in addition to the karyosome, the red staining plastin material arranged in a somewhat loose reticulum. The karyosome stains rather an intense blue. The extranuclear chromatin is uniformly arranged in spindle-like masses over the cytoplasmic area.

Morphologically in respect to chromatin it is a typical resting cell of the adult dog; though it may be said that in measurement the cell and nuclear volumes and the nucleus-plasma relation fall behind, as will be noted later in detail. The number of these cells to be seen is not large. Approximately eight to ten occur in a section one-half centimeter by one centimeter.

Cells showing a hyperchromatic state are present, but fewer in number than stage I. The chromatic material both intra- and extra-nuclear is diffuse. In a few cells it forms irregular clumps around the nuclear membrane in addition to the diffuse appearance. This has been stated as being indicative of greater nuclear activity. This cell is classed as stage III, Plate I.

The spindle-like Hodge type, Plate I, stages IV and V, when seen, shows a decrease in chromatic material. The cells of the later Hodge type, Plate I, stage V, are not so irregular in outline as noted in the adult dog.

Stage VI is more frequently seen than are any of the preceding stages. Edema in the nucleus is especially striking.

Stage VII and stage VIII are many similar at this age. The writer could not distinguish the two separately until measurements were taken. The complete absence of stages above VIII is noted so far as this material goes. The limiting to stage VIII can mean nothing else, it seems, than that a functional activity has advanced no further than this point. Apparently none of the cells at this age show a secondary restoration and disappearance of chromatin. The karyosomes remain well intact and stain a striking dark blue color in most of the sections. It further bears more on the evidence that the cell is still growing, and from the morphological data just cited its full functional capacity has as yet not been attained.

II. The evidence of partial development of functional capacity in terms of the nucleus-plasma relation. As regards the outward appearance of the chromatin ~~and the relative size of the nucleus to the plasma~~, the cells correspond, yet a study of Table III shows that the two and one-half weeks pup is not developed nearly so much as the four weeks.

It has been proved beyond a reasonable doubt that there is a constant N/P relation in resting cells between correspond-

ing types in animals of the same species, Dolley, in a recent work on the crayfish, confirms this statement and finds that "for resting cells, of both sensory and motor types in Cambarus virilis, there is a definite relation of nuclear mass to cell mass. The coefficient of this relation is an identical constant both among the four types of primal resting cells in the same animal and for all animals of the species, whatever their size." In addition to this he has found a constant relation in the resting cells of corresponding types within each of three other species, namely, man, dog, and rabbit. All dogs have been found to have for the resting Purkinje cell a nucleus-plasma coefficient of approximately 11, Table III. During the course of activity, the same constant and orderly shifts and final upset of this relation occur in corresponding cells, such as the Purkinje, in any species.

Now comparing the resting cell, stage I, ~~Plate I~~, of the two and one-half weeks dog, Table III, with the corresponding type in Dolley's adult dog, Table III, we see the N/P coefficient in the former is 5.8, in the latter 11.8. This is a striking difference. Observing the table again it is seen that the same course of relation is run thruout the eight stages, which is further proof that the cells were in function. The trend of the ~~figure~~ curve is the same, but the actual figures are lower. In the younger puppy the N/P relation is lower, and the whole sequence of events is founded on this lower relation. With this it is evident that function began before full development was reached.

C. The Attainment of Complete Functional Activity.

Now if we again make a comparison of the resting cell of the 2 1/2 weeks dog, Table III, stage I, with the corresponding cell of the 4 weeks dog, Table III, stage I, the N/P coefficients are 5.8 and 10.1 respectively. This latter figure is practically that for the adult dog. The subsequent shifts in the relation likewise closely correspond to the figures for the adult thru all the stages. The cell has gone thru the identical sequence of events in both cases, but the 2 1/2 weeks pup has a lower N/P relation, which means it began functioning before reaching full development. On the other hand, the 4 weeks pup starts with approximately a normal N/P relation and holds the adult relation thru its whole sequence of events. We conclude therefore that full functional capacity is reached in the pup of 4 weeks. Tho it is based on a lower N/P relation the fact that the trend of the curve in the 2 1/2 weeks pup is identical with that at full development is illustrated in Text figures 2, 3, and 4. Figure 4 is copied from Dolley's curve of the Purkinje cell of man. Comparison will show the close identity of the 2 1/2 weeks dog with both the 4 weeks and the adult. This applies both to the volumetric curves, upper, and the curves of nucleus-plasma coefficients, lower. The minor deviations are of little importance and are due to the smaller number of cells averaged in the puppies. The trend of the curves is identical. The only difference is that the 2 1/2 weeks pup is founded on a lower coefficient figure and holds to this relation thruout.

Post Embryonic Growth from 2 1/2 Weeks to 4 Weeks as an Attribute of Function. The question will now be discussed as to what relation exists between the growth of the cell and its function. This discussion will be limited to the process from 2 1/2 weeks to 4 weeks. All cells at 2 1/2 weeks have been shown to be in function. The only difference from fully developed cells that they exhibit is found in a different nucleus-plasma relation. The only changes subsequent to this point are the known changes of function. The essential factor is a further increase in size of the cell body; that is, the cell body grows. Even a greater growth of cell body takes place in the process of activity from the resting cell to more advanced stages. By this growth it accomplishes no more than the more immature cell accomplishes, if we can accept Dolley's view that the result of inter-action of cell body and nucleus is the formation of Nissl substance. It merely comes to a more efficient, that is, more abundant, formation. Therefore it is held that this growth in line with other functional hypertrophies is a functional growth and does not depend on an inherent growth power.

CONCLUSIONS.

1. The Purkinje cell before birth is an embryonic cell. Its nucleus is large and remains practically constant in size up to the beginning of functional activity. The cytoplasm is evident in only a very few of the cells. Its growth after birth is rapid up to two and a half weeks.

2. The functional activity begins in the dog at about two and a half weeks. The sequence of events is the same, the only

difference is a lower nucleus-plasma coefficient.

3. Complete functional activity is reached at the age of four weeks in terms of the nucleus-plasma relation; that is, the nucleus-plasma coefficient at this age is approximately the same as in the adult dog. In actual size the cell is smaller than a corresponding stage in the adult dog.

4. Post embryonic growth of the cell from two and a half weeks to four weeks is a functional growth.

Table I.

The growth from the foetus to four weeks.

Age of dog	Number of cells measured	Average diameters of cells	Average diameters of nuclei	Average volume of cell body	Average volume of nucleus	Nucleus-plasma coefficient
Foetus	20		17X12		2448	
36 hrs	25	35X18	17X13	8467	2873	2.9
6 days	20	38X19	16X14	10582	3136	3.3
2 1/2 wk*	40	43X23	17X14	19415	3332	5.8
3 wks*	30	43X25	17X14	23543	3332	7.
4 wks*	30	48X27	16X14	31856	3136	10.1

* Resting type cells.

Table II.

The growth from one week to two and a half weeks.

Age of dog	Number of cells measured	Average diameters of cells measured	Average diameters of nuclei	Average volume of cell body	Average volume of nuclei	Nucleus plasma coefficient
1 wk	10	34X15	14X13	5284	2366	2.2
1 1/2 wk	10	32X16	14X14	5448	2744	1.9
2 wks	10	43X19	17X14	12191	3332	3.6
2 1/2 wk	10	45X23	19X16	18941	4864	3.8

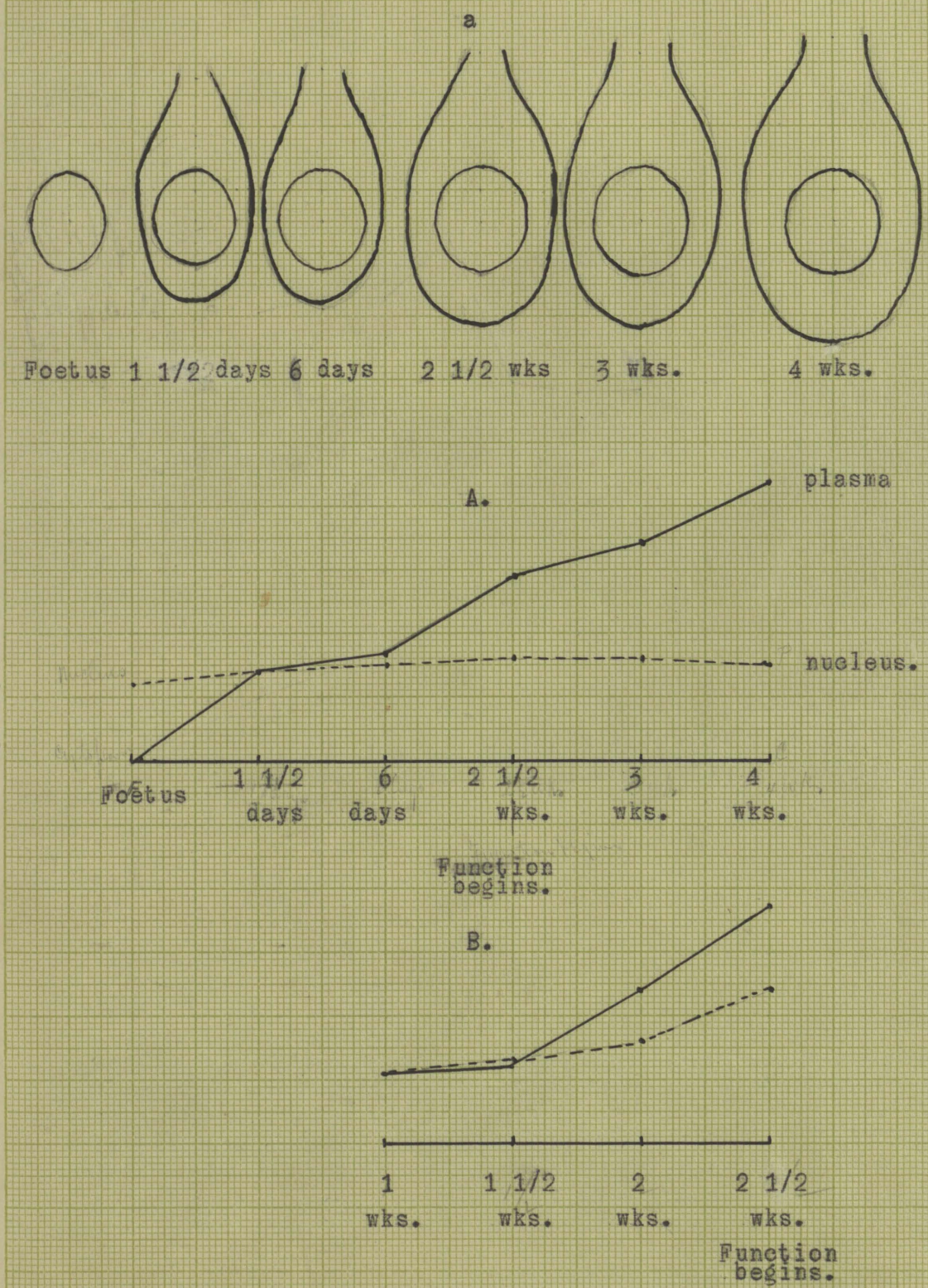
Table I

Comparison of activity in [illegible]c and adult animals.

Stages of activity	Dog 4 weeks old			Dog 2 1/2 weeks old			Adult dog*
	Average diameters of cell and nucleus	Volumes of cell body and nucleus	N/P coefficient	[illegible]ge diameters [illegible] cell and [illegible]nucleus	Volumes of cell body and nucleus	N/P coefficient	N/P coefficient
I	48 X 27 16 X 14	31856 3136	10.1	[illegible] X 23 [illegible] X 14	19415 3332	5.8	11.8
II	47 X 29 17 X 14	36295 3332	10.6	[illegible] X 26 [illegible] X 15	24116 3600	6.7	11.6
III	41 X 26 16 X 12	24412 2304	10.6	[illegible] X 26 [illegible] X 14	23904 3136	7.6	13.3
V	43 X 26 15 X 12	26908 2160	12.4	[illegible] X 23 [illegible] X 11	18816 1815	10.3	15.7
VI	48 X 32 19 X 17	43661 5491	7.9	[illegible] X 26 [illegible] X 17	23866 5202	4.5	8.5
VII	54 X 33 21 X 18	52002 6804	7.6	[illegible] X 30 [illegible] X 17	36809 5491	6.7	9.7
VIII	56 X 37 22 X 20	67864 8800	7.7	[illegible] X 32 [illegible] X 18	45744 6480	7.	10.9
IX	69 X 35 22 X 18	77397 7128	10.8				13.1

* These figures taken from Dolley (1910), being the average[illegible] the measurement of 2200 cells.

Text-figure I.



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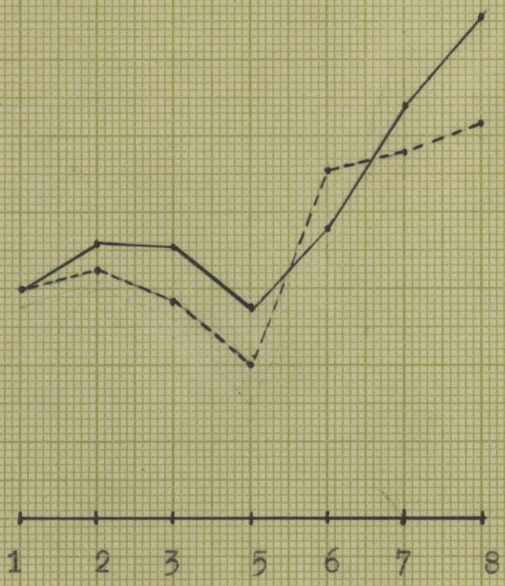
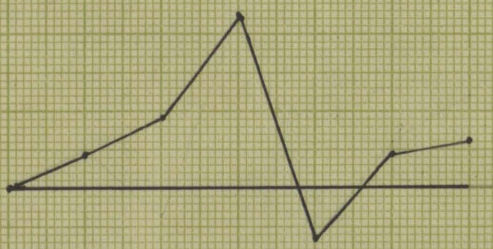
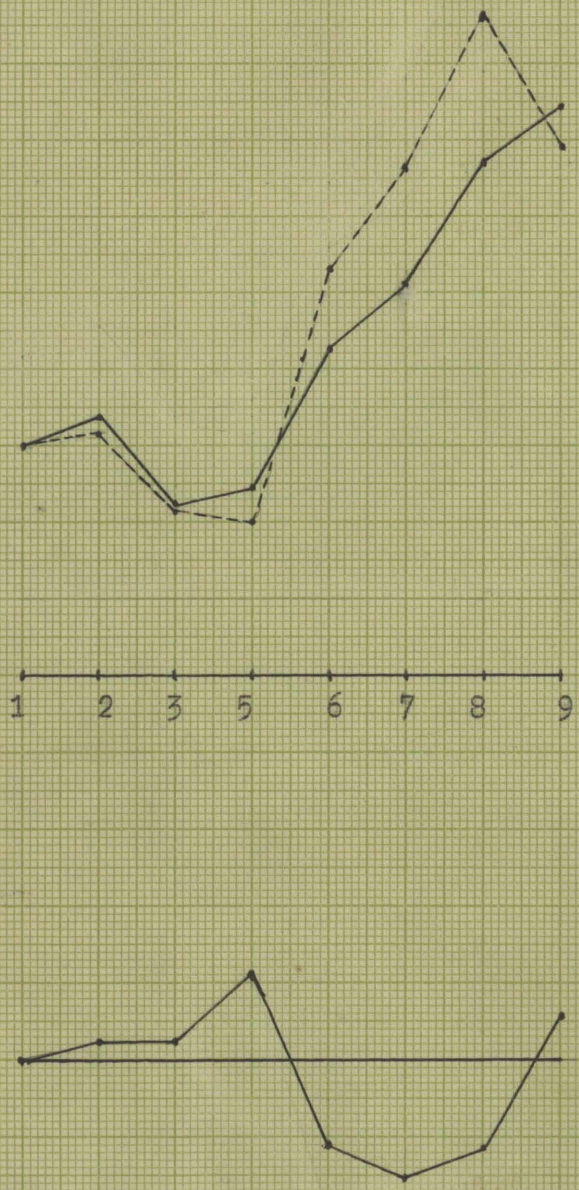
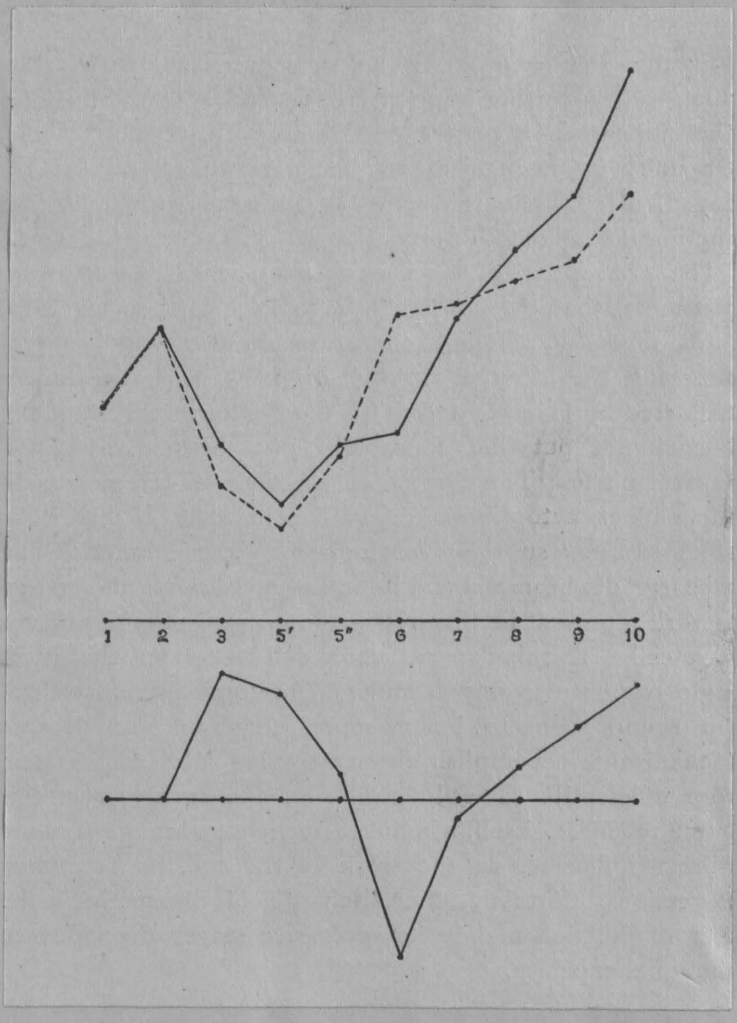


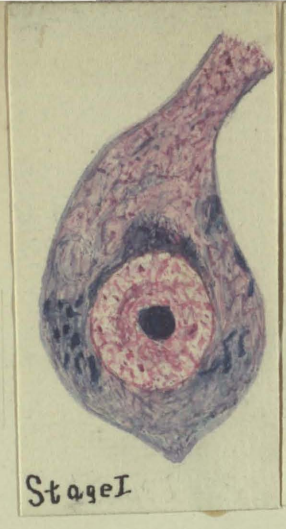


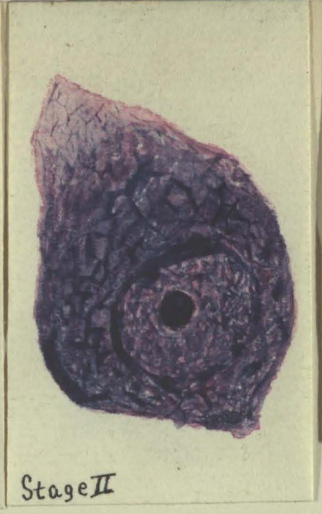
Text-figure III.

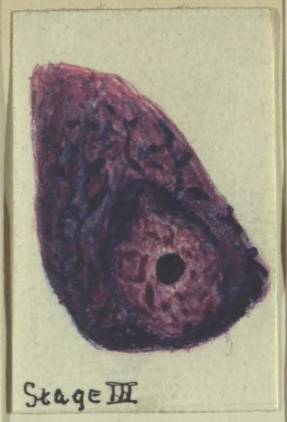


Text-figure IV.




Stage I

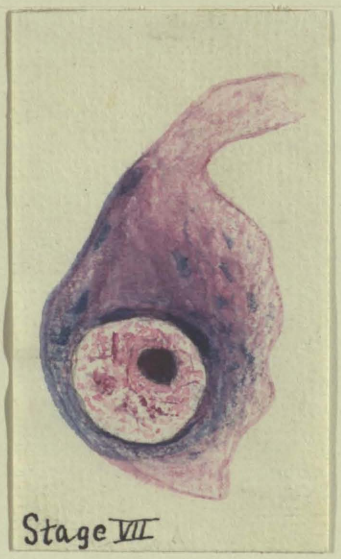

Stage II

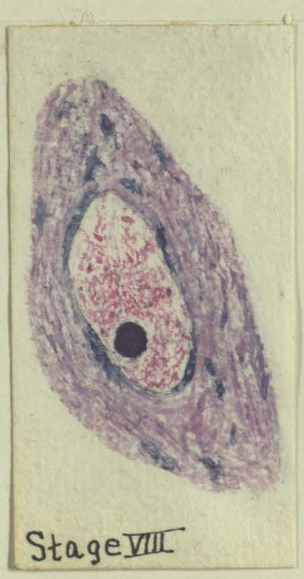

Stage III


Stage IV


Stage V


Stage VI


Stage VII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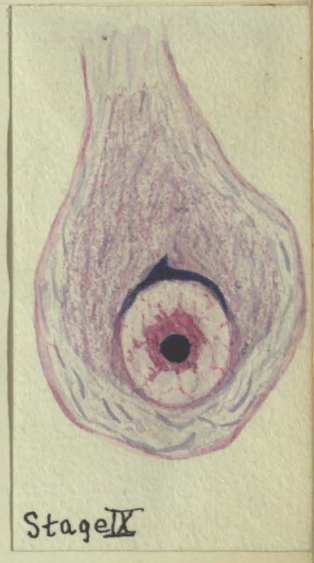
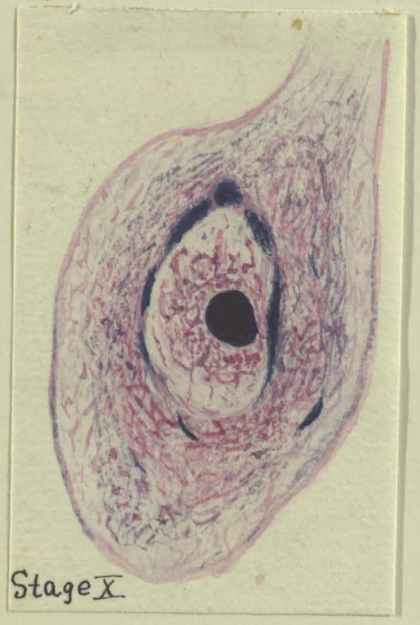
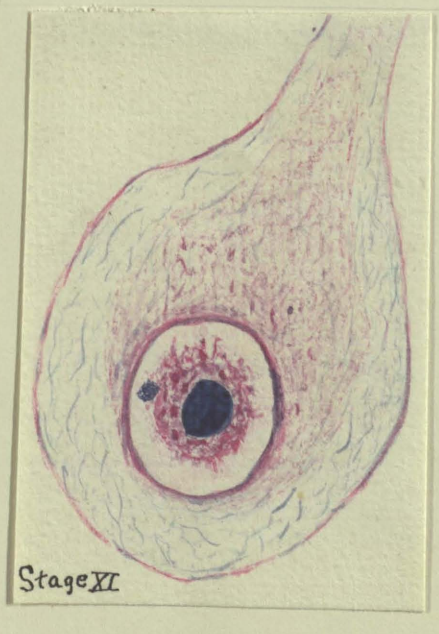

Stage IX


Stage X


Stage XI

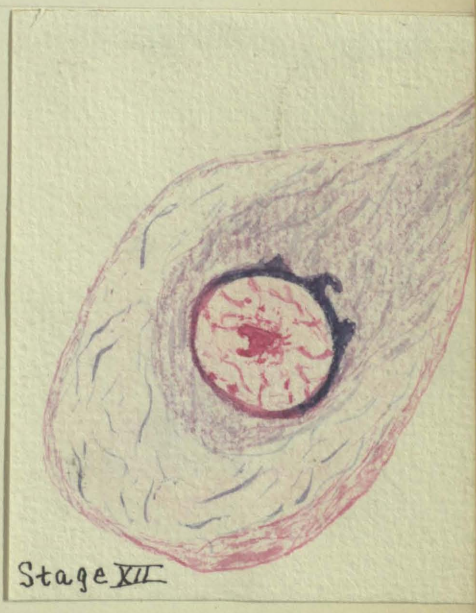

Stage XII

PLATE I.

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