THE BEHAVIOUR OF NUCLEAR STRUCTURES DURING DEPLETION AND RESTORATION OF NISSL MATERIAL IN MOTOR NEURONS

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INTRODUCTION

Electrical stimulation of motor cell axons is a convenient and effective method of producing extensive depletion of Nissl material in the parent cell bodies (Barr & Bertram, 1949). In the present study, this method has been used in order to observe the behaviour of certain nuclear structures during depletion and restoration of Nissl material. With the basic dye cresyl violet, components of the cell which contain large amounts of nucleic acid stain intensely, due to the phosphoric acid component of the nucleic acid molecule. The Nissl material of the cytoplasm is known to contain nucleic acid of the ribose type, in the form of nucleoprotein. The same type of nucleic acid is present in the large nucleolus, or plasmasome, so characteristic of the nerve cell nucleus. In the cat, the nucleolar satellite is the only intranuclear structure, other than the nucleolus, which appears well defined with the cresyl violet stain. The satellite, although much smaller than the nucleolus, is large enough to be readily examined in nerve cells of female cats, but lies at the limit of visibility, with an oil-immersion objective and ordinary illumination, in male cats (Barr & Bertram, 1949; Barr, Bertram & Lindsay, 1950). The nucleolar satellite, unlike the nucleolus and Nissl material, contains nucleic acid of the desoxyribose type. The neuron, therefore, lends itself especially well to a study of the relationship between cytoplasmic nucleoproteins and certain nuclear structures of similar chemical composition.

Interest in the present experiments arises from the fundamentally important role of the nucleic acids in cellular physiology. It is likely that information derived from the neuron concerning the structures responsible for the synthesis of cytoplasmic nucleic acids will apply to other cells characterized by large nucleoli and abundant cytoplasmic nucleoproteins. Obvious examples are to be found among rapidly growing and dividing cells such as occur in embryonic tissues, haematopoietic areas and malignant tumours.

MATERIALS AND GENERAL METHODS

In addition to three female cats, which served as unstimulated control animals, twenty cats were used in the experiments (seventeen females and three males). Under nembutal anaesthesia and with aseptic precautions, shielded bipolar platinum electrodes were applied to the right hypoglossal nerve as it crosses the carotid artery. The nerve was stimulated continuously for 8 hr. (50 cyc./sec.) by means of a vacuum tube stimulator with a square wave output, designed and constructed by

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Mr R. W. Luke, of the Department of Physiology. The initial output voltage was 10, with a gradual increase during the first hour to a maximum of 20 V. which was maintained during the succeeding 7 hr. There was a strong deviation of the tongue to the right throughout the period of stimulation.

Survival periods varied between 0 and 450 hr. (approx. 19 days). The animals were perfused, under nembutal anaesthesia, with isotonic 10 % formalin, according to the method of Koenig, Groat & Windle (1945). Serial, paraffin-embedded, 12μ sections were cut from the portion of the medulla containing the hypoglossal nuclei. The sections were stained routinely with cresyl violet (B.D.H.) in 0·5 % solution for 0·5 hr. at 56° C., followed by differentiation with alcohol under visual control. Sections of normal material, prepared by Mr Hugh Lindsay with the Feulgen and methyl green-pyronin methods, have been valuable in establishing the type of nucleic acid present in the nucleolar satellite.

SPECIFIC METHODS AND RESULTS

A. *Depletion and restoration of Nissl material*

A detailed description of changes in the cytoplasm, and the bearing of these observations on the important problem of the function of Nissl material, will be presented in a separate report. For our present purpose it is sufficient to note the following main points.

The alterations in the cells of the right hypoglossal nucleus (stimulated side) were assessed against the appearance of the cells in the left hypoglossal nucleus (unstimulated control side) in each animal. There was no visible change in the Nissl material in the two animals killed at the conclusion of stimulation. From 12 to 72 hr. following stimulation a progressive depletion of Nissl material occurred. The Nissl bodies became smaller until, in many cells, the Nissl material took the form of fine granules scattered diffusely and evenly throughout the cytoplasm. A cell with full complement of Nissl material is shown in Pl. 1, fig. 1. Cells with moderately severe depletion of Nissl material are illustrated in Pl. 1, figs, 2, 3.

When the change was at its maximum, the cells of the experimental side appeared a little larger than the cells of the control side. Possibly some dilution of the cytoplasm occurred, similar to that characteristic of axon reaction (Barr & Hamilton, 1948). However, the loss of stainable substance in the cytoplasm was too extensive to be accounted for by dilution alone. A considerable decrease in the total amount of Nissl material in the cell body must have occurred. This has been demonstrated quantitatively, under somewhat similar circumstances, by Hydén (1943) and Hamberger & Hydén (1945) using the method of selective absorption in the ultraviolet range.

A recovery phase followed, which lasted for approximately 14 days. Restoration of Nissl substance throughout the cytoplasm was preceded by the appearance of deeply basophil material at the nuclear membrane. These nuclear caps increased in frequency and size from the second day following stimulation well into the recovery period. Pl. 1, fig. 2, shows thin nuclear caps at several places along the nuclear membrane. The nuclear cap is better developed in Pl. 1, fig. 3. As recovery proceeded, the perinuclear region of the cytoplasm became filled with densely stained

particulate material (Pl. 1, fig. 4). Later, the entire cytoplasm became more deeply stained than in control cells, the normal Nissl pattern and density of staining being gradually restored. It is significant that restoration of the Nissl material proceeded from the nuclear membrane toward the cell membrane. All cells recovered.

The broken line in Text-fig. 3 represents the approximate time course of depletion and restoration of Nissl material, based on a careful examination of 500 nucleoli-containing cell sections in the control and experimental hypoglossal nuclei of each animal.

B. *Changes within the cell nuclei*

(i) *Nucleolus*

The nucleoli of normal neurons were Feulgen-negative and stained with the pyronin component of the methyl green-pyronin mixture. This was to be expected since it is well known that the nucleolus or plasmasome contains nucleic acid of the ribose type.

Table 1. *Changes in profile area of nucleolus (mean of fifty nucleoli ± S.E. of mean)*

Survival Time (hr.)	Sex	Profile area of nucleolus (μ^2) Control (left)	Exper. (right)	Change (%)	S.E. of difference	Significant ? (critical value > 2·0)
Control	F.	13·0±0·4	13·4±0·4	+3·1	0·6	No
Control	F.	11·4±0·2	11·4+0·2	0·0	0·3	No
Control	F.	11·4±0·2	12·2±0·3	+7·0	0·4	No
0	F.	11·5±0·2	11·4±0·2	−0·8	0·3	No
0	F.	11·9±0·2	12·3±0·3	+3·3	0·4	No
12	F.	11·6±0·2	11·7±0·2	+0·9	0·3	No
24	F.	10·9±0·2	10·6±0·2	−2·7	0·3	No
36	F.	11·1±0·2	10·7±0·2	−3·6	0·2	No
48	F.	11·0±0·3	11·9±0·3	+8·2	0·4	Yes
60	M.	11·4±0·2	12·9±0·2	+13·2	0·3	Yes
72	F.	11·1±0·2	14·0±0·2	+26·1	0·3	Yes
72	M.	11·6±0·2	13·6±0·2	+17·2	0·3	Yes
84	F.	11·4±0·2	13·7±0·4	+20·2	0·4	Yes
96	F.	10·9±0·2	14·1±0·2	+29·3	0·3	Yes
108	F.	10·3±0·3	14·2±0·3	+37·9	0·5	Yes
120	F.	10·7±0·2	13·5±0·2	+26·2	0·3	Yes
136	F.	11·4±0·2	13·9±0·3	+21·9	0·3	Yes
156	F.	11·4±0·3	13·7±0·3	+20·2	0·4	Yes
192	M.	11·3±0·2	14·9±0·3	+31·9	0·4	Yes
214	F.	12·6±0·3	14·8±0·3	+17·5	0·4	Yes
288	F.	11·7±0·2	13·2±0·3	+12·8	0·4	Yes
384	F.	9·9±0·2	10·2±0·2	+3·0	0·3	No
450	F.	12·4±0·2	12·9±0·3	+3·9	0·3	No

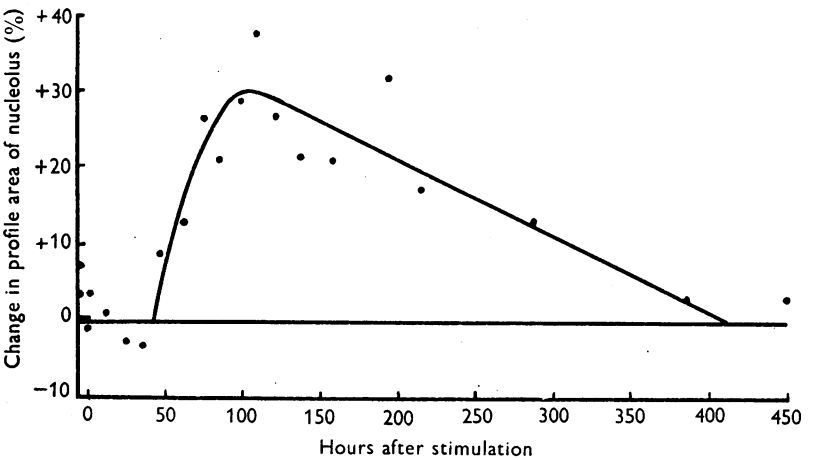
In sections stained with cresyl violet, the nucleoli of stimulated neurons were examined for alterations in size, position and internal structure.

Fifty nucleoli were measured in the control and experimental hypoglossal cell groups in each animal. Two diameters of each nucleolus were measured with a filar micrometer ocular, and the profile area determined by the formula for the area of an ellipse. The results are shown in Table 1. The variations in control animals and up to 36 hr. after stimulation were due to errors in sampling and measurement. However, in the interval between 48 and 108 hr. following stimulation, the nucleolus enlarged rapidly and significantly. There followed a gradual return to normal size which was reached about 400 hr. following stimulation. An approximation of the

time course of changes in nucleolar size may be obtained by constructing a smooth curve through the points derived from the data in column 5 of Table 1 (see Text-fig. 1).

Considering the series as a whole, the mean value for normal nucleolar profile area in the three male cats was $11{\cdot}4\mu^2$ and in the twenty female cats $11{\cdot}4\mu^2$. Dr W. H. Cook, working in this laboratory, had occasion to measure nucleoli in cells of the lateral geniculate body of the cat. The mean profile areas were as follows: females (seven animals), $4{\cdot}1\mu^2$; males (five animals), $4{\cdot}0\mu^2$. There appears, therefore, to be no significant difference in nucleolar size between the sexes.

Although the position of the nucleolus was variable in normal cells of the hypoglossal nucleus, it was usually near the centre of the nucleus, as seen in cell sections. No alteration in the position of the nucleolus in experimentally altered cells could be detected at any stage by general inspection. In order to make this observation



Text-fig. 1. Time course of nucleolar enlargement following stimulation of the axons of motor neurons.

more reliable, the distance between the centre of the nucleolus and the centre of the nucleus was measured, on camera lucida tracings, in a sample of fifty cells of the left and right sides, in three animals. The mean values were as follows: 48 hr.—control $2{\cdot}0\mu$, exper. $1{\cdot}8\mu$; 108 hr.—control $1{\cdot}8\mu$, exper. $1{\cdot}8\mu$; 288 hr.—control $1{\cdot}3\mu$, exper. $1{\cdot}4\mu$. These measurements confirm the general impression of a normal nucleolar position following stimulation of the nerve.

When stained with cresyl violet, nucleoli of normal cells in the control hypoglossal cell group showed little detail other than an occasional vacuole. The high nucleic acid content of the nucleolus caused it to stain so intensely with basic dyes that details of internal architecture were largely obliterated. The enlarged nucleoli of stimulated cells, however, showed extensive vacuolation. Either pre-existing vacuoles were unmasked by chemical changes in the swollen nucleoli or an increase in size and number of the vacuoles occurred. The latter alternative seems the more probable.

(ii) *Nucleolar satellite*

Since the nucleolar satellite was too small in nerve cells of male cats for satisfactory observation with the ordinary light microscope, only three males were included in the series. The results will be presented separately according to sex.

(*a*) *Females.* In cells of the control side, the satellite was roughly spherical in shape and approximately 1 μ in diameter (compared with a mean diameter of 4·0 μ

Table 2. *Incidence and position of nucleolar satellite in* 12 μ *sections containing the nucleolus and stained with cresyl violet (female cats)*

Survival time (hr.)	Hypoglossal nucleus	Position of satellite (%)			Total (%)
		Adjacent to nucleolus	Free in nucleoplasm	Adjacent to nuclear membrane	
Control	Left	64	9	2	75
	Right	62	11	1	74
Control	Left	37	11	9	57
	Right	39	9	11	59
Control	Left	51	12	6	69
	Right	54	9	6	69
0	Control	59	15	9	83
	Exper.	49	17	11	77
0	Control	43	15	7	65
	Exper.	40	18	6	64
12	Control	70	10	4	84
	Exper.	70	9	5	84
24	Control	61	9	7	77
	Exper.	55	18	8	81
36	Control	62	7	2	71
	Exper.	52	23	4	79
48	Control	72	10	1	83
	Exper.	54	29	2	85
72	Control	54	10	4	68
	Exper.	36	25	8	69
84	Control	42	17	4	63
	Exper.	24	41	7	72
96	Control	56	8	3	67
	Exper.	36	34	4	74
108	Control	55	7	2	64
	Exper.	40	28	4	72
120	Control	56	16	6	78
	Exper.	37	35	5	77
136	Control	68	11	7	86
	Exper.	38	42	7	87
156	Control	62	6	2	70
	Exper.	50	23	2	75
214	Control	63	7	10	80
	Exper.	41	27	8	76
288	Control	50	11	2	63
	Exper.	36	22	3	61
384	Control	53	10	5	68
	Exper.	50	12	8	70
450	Control	66	8	5	79
	Exper.	65	9	6	80

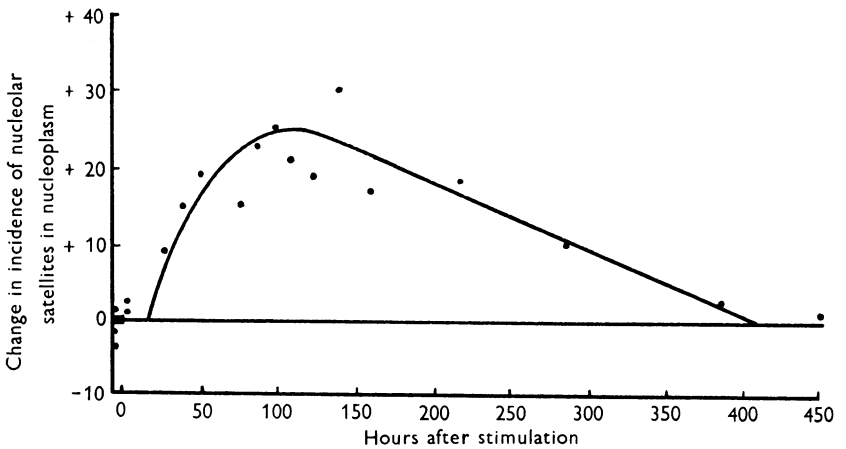
for the nucleolus). The satellite stained deeply with cresyl violet, due probably to its nucleic acid content. The satellite was Feulgen-positive and stained with the methyl green component of the methyl green-pyronin mixture. Its nucleic acid, therefore, is principally of the desoxyribose type, unlike the nucleolus and Nissl material, which contain ribose nucleic acid.

As shown in Table 2, the satellite of normal cells was situated most frequently adjacent to the nucleolus (Pl. 1, fig. 1). It was almost always spherical in this situation. When viewed tangentially with respect to the nucleolus, an exceedingly narrow clear interval was seen frequently between the nucleolus and its satellite. There may be a shallow concavity of the nucleolar surface at this point.

In a proportion of normal cells the satellite was free in the nucleoplasm, touching neither the nucleolus nor the nuclear membrane. Occasionally, such satellites departed from their usual spherical shape.

Still less frequently, the satellite lay adjacent to the nuclear membrane. In some instances the satellite was clearly flattened against the membrane.

The data in Table 2 are based on an examination of 500 cell sections in the right and left hypoglossal nuclei in each animal. All cell sections contained a nucleolus.



Text-fig. 2. Time course of movement of the nucleolar satellite away from the nucleolus following stimulation of the axons of motor neurons.

The frequency with which the satellite could be seen in 12μ sections stained with cresyl violet varied between 57 and 86 % (mean value 73 %). Slight differences in staining may have been responsible for this variation from one animal to another. There was likely one satellite to a cell, the organelle being invisible at times when eclipsed by the nucleolus, or when it was some distance from the nucleolus and in an adjacent section. Table 2 also shows the percentage incidence of the satellite adjacent to the nucleolus, free in the nucleoplasm, and adjacent to the nuclear membrane. While there was some variation from animal to animal, a definite pattern existed, typical of motor neurons of the hypoglossal nucleus. The mean values were: adjacent to nucleolus, 58 %; in nucleoplasm, 10 %; adjacent to the nuclear membrane, 5 %. (Total, 73 %.)

In the experimentally altered cells the most obvious change was the position of the satellite. During the second, third and fourth days following stimulation, an increasing number of satellites moved from the nucleolus for variable distances into the nucleoplasm (Table 2). The satellite is successively farther from the nucleolus in Pl. 1, figs. 2–4. The normal position was gradually restored during the succeeding 12 days.

Inspection of Table 2 shows that there was little change in the incidence of satellites adjacent to the nuclear membrane. The excursion of the satellite was for variable distances into the nucleoplasm, short of the nuclear membrane, and back to the nucleolus. The time course of satellite migration is best appreciated by constructing a smooth curve through points representing the difference in the incidence of satellites in the nucleoplasm in control and experimental cells (Text-fig. 2).

The satellite continued to stain deeply with cresyl violet throughout the period of experimental alteration. Although accurate measurement of such a small object proved difficult, the satellite clearly increased in size coincidentally with the increase in size of the nucleolus. Rarely, two enlarged satellites were encountered in experimentally altered cells.

It seemed of interest to learn whether the position of the satellite was influenced by the polarity of the neuron. It was found, from camera lucida tracings, that the axon arose predominantly from the medial and ventral aspects of the cell body. The nucleolar satellite, on the other hand, appeared to lie at random on all sides of the nucleolus. This random distribution applied to satellites adjacent to the nucleolus, free in the nucleoplasm and next to the nuclear membrane, in both control and experimentally altered cells. The polarity of the neuron, therefore, bore no obvious relationship to the position of the nucleolar satellite.

(*b*) *Males.* The nucleolar satellite in normal hypoglossal motor neurons of male cats was so small that there was difficulty in identifying it with certainty except in a small proportion of cells. This statement applies to the examination of cells with the ordinary binocular light microscope, with a Leitz fluorite oil-immersion objective 95×, aper. 1·32, and 10× oculars.

Table 3. *Incidence and position of nucleolar satellite in* 12 *μ sections containing the nucleolus and stained with cresyl violet (male cats)*

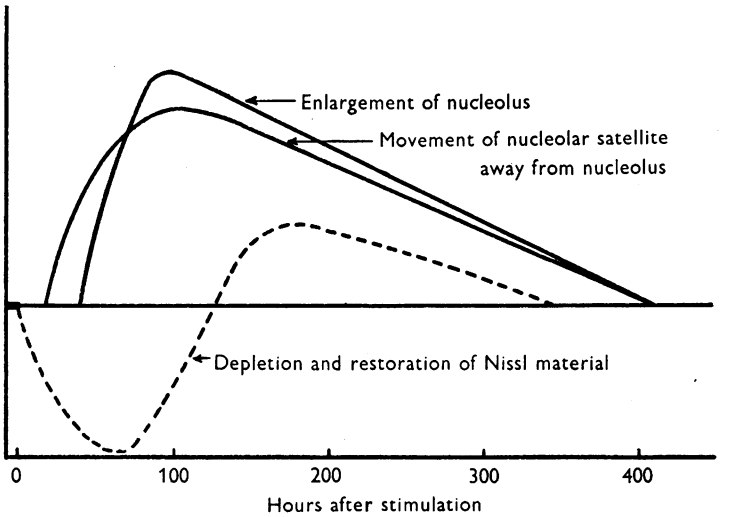
Survival time (hr.)	Hypoglossal nucleus	Position of satellite (%)		Adjacent to nuclear membrane	Total (%)
		Adjacent to nucleolus	Free in nucleoplasm		
60	Control	5	2	0	7
	Exper.	16	8	0	24
72	Control	2	1	0	3
	Exper.	12	7	0	19
192	Control	4	1	0	5
	Exper.	24	14	0	38

The figures in Table 3, based on an examination of 500 cells in each instance, are only approximate because of the minute size of the satellite in the male. However, comparison of the data for normal cells in Tables 2 and 3 clearly illustrates the difference in nuclear morphology according to sex. Pl. 1, fig. 5, represents a typical normal neuron in the male cat.

Following stimulation, the satellite of male cells enlarged so that the incidence of visible satellites increased. This is illustrated by the data in Table 3 (still approximate) for the incidence of satellites in male cells following stimulation. Occasionally, the satellite in the stimulated male neuron approached the size characteristic of the female. A cell in a male cat, with an enlarged satellite following stimulation, is shown in Pl. 1, fig. 6.

Finally, during the later stages of recovery of the Nissl material in both sexes, the basophilia of the nucleoplasm increased. This may be due to an increase in the nucleic acid content of the nucleoplasm.

The time course of changes in the content of Nissl material in the cytoplasm, enlargement of the nucleolus and changes in the position of the nucleolar satellite may be compared in Text-fig. 3.



Text-fig. 3. Combined curves of depletion and restoration of Nissl substance, nucleolar enlargement and movement of nucleolar satellite away from the nucleolus, following electrical stimulation of axons of motor neurons.

DISCUSSION

The morphological changes within the nucleus during depletion and restoration of Nissl material are consistent with the view that the nucleolus and associated structures play an important role in the synthesis of cytoplasmic nucleoproteins (Caspersson, 1947).

Cytoplasmic nucleic acid of the ribose type is abundant in cells which elaborate or maintain large amounts of protein. Typical examples are embryonic cells (Brachet, 1947), cells in haematopoietic tissues (Thorell, 1944), malignant cells (Caspersson & Santesson, 1942; Biesele, 1944; Stowell, 1947), serous secreting gland cells (Caspersson, 1947) and the neuron (Hydén, 1943). In each instance the cell is characterized also by a large nucleolus containing abundant nucleic acid of the ribose type.

The changes in the nucleolus are explicable if it is assumed that depletion of the

Nissl material, and in particular the ribose nucleoproteins of the Nissl material, serves as a stimulus to the nucleolus to elaborate substances necessary for the replacement of the depleted nucleoproteins. According to Hydén (1943, 1947), the nucleolus and associated structures, under similar circumstances, are the site of an increased level of ribose nucleic acid synthesis and also participate in the formation of simple proteins of the histone type, containing hexone bases. These substances, especially the histones, are thought by Hydén to pass from the region of the nucleolus toward the nuclear membrane. Cytoplasmic ribose nucleic acid and nucleoproteins are then laid down in the cytoplasm adjacent to the nuclear membrane. The enlargement and vacuolation of the nucleolus seen in the present experiments may be interpreted as a morphological expression of its increased activity. The chemical reactions necessary to the replacement of the cytoplasmic material appear to gain such momentum that the nucleic acid and nucleoproteins in the cytoplasm attain a level higher than normal before the cytological picture typical of control cells is restored.

The term 'nucleolar satellite' is used provisionally until more information concerning this interesting sex-influenced structure becomes available. This subject is considered in some detail elsewhere (Barr *et al.* 1950).

The behaviour of the nucleolar satellite during altered states of cell metabolism is worth close scrutiny. The movement of the satellite in the nucleoplasm is slow. Two weeks or more may be required for the satellite to make its full excursion for variable distances into the nucleoplasm and back to the nucleolus. If all material in the nucleus were visible and changes in the viscosity gradient between nucleolus and nuclear membrane known, a simple physical explanation for the movement might be apparent. Available evidence points to the flow of histones and nucleic acid from the nucleolus to the nuclear membrane. This flow, when accelerated, might carry the minute satellite along toward the nuclear membrane.

The increase in size of the nucleolar satellite during the restoration of Nissl substance suggests that the satellite may play an active role in the synthesis of some cytoplasmic constituent. If this should prove to be the case there may be a quantitative difference in some aspect of cell metabolism according to sex.

There is an interesting parallel between the behaviour of the nucleolar satellite in the cat's nerve cell and that of certain heterochromatic granules in the toad's egg (Painter & Taylor, 1942). During the development of the ovum, several hundred nucleoli appear which line up along the inner surface of the nuclear membrane. Each nucleolus has attached to it a minute heterochromatic granule which is located between the nucleolus and nuclear membrane. This morphological appearance of the nucleus is characteristic of the stage when large amounts of ribose nucleic acids are being laid down in the cytoplasm of the maturing ovum. One cannot fail to see certain parallels between the intimate relation of the multiple heterochromatic granules and the nuclear membrane in the toad's egg, and the migration of the nucleolar satellite toward the nuclear membrane in the cat's neuron, during a period of intense synthesis of cytoplasmic nucleic acid in both instances.

Finally we wish to point out that the cause of the severe depletion of Nissl material, following electrical stimulation of axons, is still in doubt. Liu, Bailey & Windle (1950) feel that damage to the axons by the stimulating current may be

responsible. However, the time course of the cell changes is shorter than one would expect were the phenomenon simply one of axon reaction. Further, characteristic chromatolysis has been observed in the absence of Wallerian degeneration distal to the site of application of the electrodes. Hydén (1943) described similar changes in spinal cord cells of guinea-pigs following exercise to exhaustion. Chromatolysis of cells of the cochlear ganglion in guinea-pigs was found by Hamberger & Hydén (1945) following exposure of the animals to loud sounds. It is tempting to think, therefore, that intense activity of the cells of the hypoglossal complex may have been at least partially responsible for the depletion of Nissl material which was so conspicuous in these experiments. Regardless of the cause of the depletion of Nissl substance, axon stimulation is a very useful means of producing extensive alterations in the metabolism of the neuron. The application of cytochemical methods to cells so altered may throw new light on the chemical reactions involved in the degradation and synthesis of the complex Nissl substance.

SUMMARY

1. Depletion of the Nissl material of motor neurons was produced by electrical stimulation of the cat's hypoglossal nerve. Chromatolysis in stimulated cells was first seen 12 hr. following stimulation, and increased progressively to the 72nd hour. A recovery period followed, complete at approximately 384 hr. The cells passed through a hyperchromatic stage before the normal Nissl pattern was restored.

2. The nucleolus increased in size progressively from 48 to 108 hr. following stimulation. Normal size was regained by 384 hr. Vacuolation was more apparent in the enlarged nucleoli than in nucleoli of unstimulated cells. Nucleolar position was unaltered.

3. The nerve cells of female cats contained a nucleolar satellite, approximately 1 μ in diameter. On the basis of the Feulgen and methyl green-pyronin stain, the satellite contained desoxyribose nucleic acid in contrast to the ribose nucleic acid of nucleolus and Nissl material. The satellite was rarely visible in normal male nerve cells.

4. Following stimulation, the satellite tended to move from its usual position, adjacent to the nucleolus, towards the nuclear membrane. The normal pattern, as concerns position, was restored by the 400th hour. The satellite, in female and male cells, increased in size following stimulation of the neuron.

5. The observations are consistent with the view that the nucleolus and possibly its satellite play a prominent role in the synthesis of ribose nucleoproteins, which are an important constituent of the Nissl material of nerve cells.

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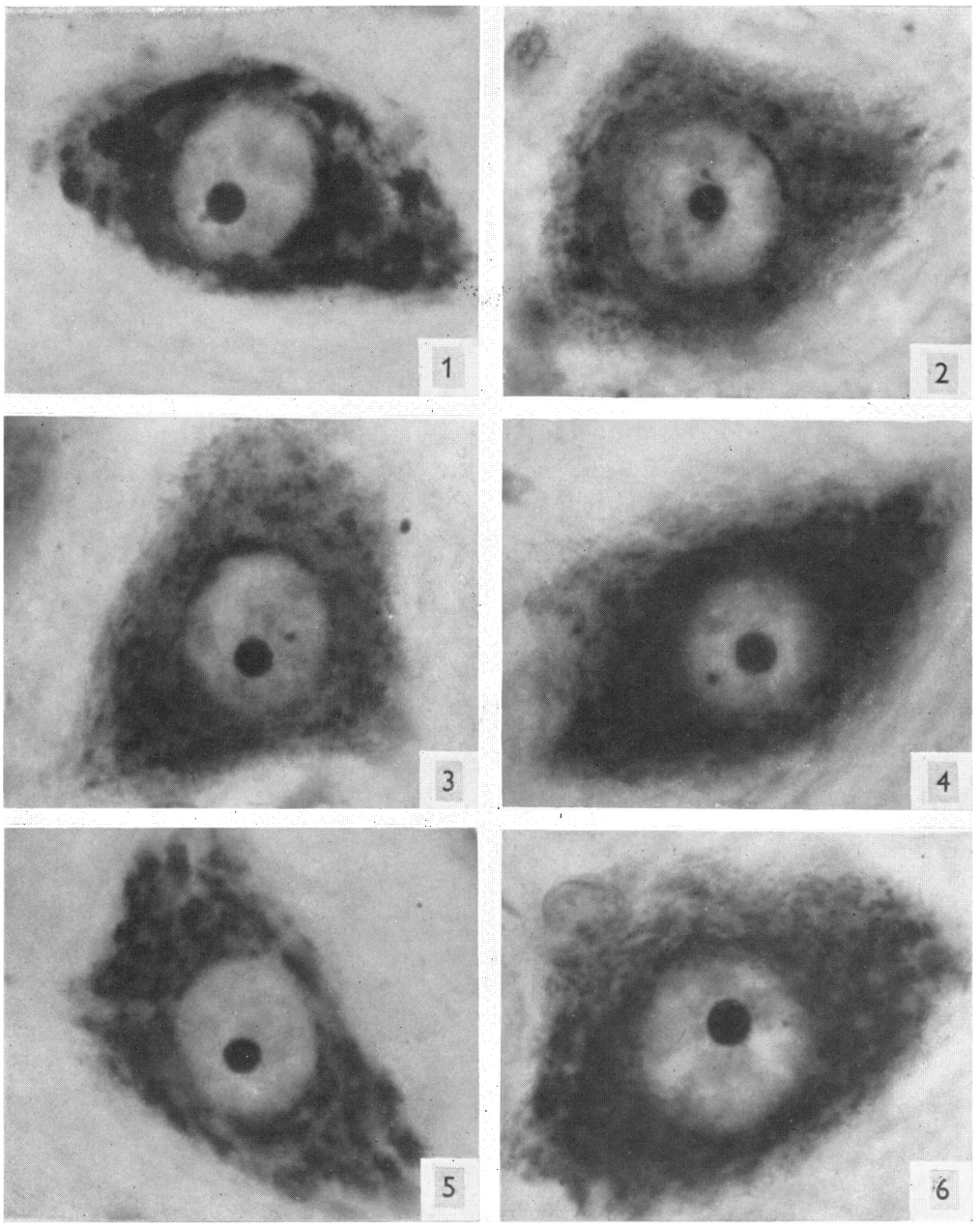

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EXPLANATION OF PLATE

Cresyl violet stain. Magnification × 1400.

Fig. 1. Normal hypoglossal motor nerve cell from control side of a female cat. The well-defined nucleolar satellite is seen adjacent to the nucleolus.

Fig. 2. Nerve cell of female cat 48 hr. following stimulation of its axon. There is moderately severe depletion of the Nissl material. Several thin nuclear caps lie adjacent to the nuclear membrane. The nucleolar satellite has begun to move away from the nucleolus.

Fig. 3. Nerve cell of female cat 72 hr. following stimulation of its axon. The degree of chromatolysis is similar to that shown in fig. 2, but the nuclear cap is more prominent and the satellite has moved farther into the nucleoplasm.

Fig. 4. Nerve cell of female cat well on the way to recovery 108 hr. following stimulation of its axon. The Nissl material is less dense at the periphery of the cytoplasm as compared with the perinuclear zone. The enlarged satellite is a considerable distance from the nucleolus.

Fig. 5. Typical normal male neuron from the hypoglossal nucleus. There is no visible nucleolar satellite.

Fig. 6. Neuron from a male cat 192 hr. following stimulation of its axon. A small nucleolar satellite is visible at about 3 o'clock not far from the nuclear membrane.