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By

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Introduction

The presence of acid phosphatase activity in the normal nerve cells has been reported by many investigators (4-7 and 10). It was also reported, by Bodian and Mellors (1), and Smith and Luttrell (8), that the enzymatic activity increases in the nerve cells under the stress condition, coinciding with the disappearance of Nissl's bodies from them. These investigations strongly indicated that the acid phosphatase in the nerve cells may have very important significance on the metabolic changes associated with Nissl's bodies, since the ribonucleic acids, a potential substrate for acid phosphatase, are one of the main contents of the bodies.

In order to assess the significance of the acid phosphatase mentioned above, it is first necessary to investigate the enzymatic activity in the developing nerve cells. The appearance of Nissl's bodies in the nerve cells of chick embryos has been studied histochemically by Toryu (9) in connection with the appearance of glycogen in the cells. No one has reported, however, the occurrence of acid phosphatase in the chick embryos.

The present study was designed to clarify histochemically the possible relationships between acid phosphatase and Nissl's bodies during the development of nerve cells of the spinal cord and the spinal ganglion in the chick embryos, hoping that it might provide further evidences on the close relationships between these two factors.

Materials and Methods

All materials were chick embryos of the White Leghorn breed. The embryos from three days to 16 days of incubation were used. One half of the embryos was fixed in Carnoy's fluid and cut into 8μ paraffin sections for the

demonstration of Nissl's bodies. For the demonstration of acid phosphatase, another half was fixed in cold acetone for 24 hours, and then placed in benzol for 15 minutes. Special care was taken to preserve the enzymatic activity during the paraffin infiltration. Namely, the infiltration was done at 58°C under reduced pressure. Specially designed glass chambers connected with a tap-aspirator were used as the paraffin bath for infiltration. The time needed for this procedure was only 12 minutes in most cases. The tissues were then embedded in paraffin and cut into 8μ sections. The mounted sections were dried for 30 minutes, deparaffinized in xylol, and passed through acetone to water. The acid phosphatase activity was demonstrated by the revised lead nitrate method of Gomori (2), using sodium glycerophosphate as a substrate. The incubation time was generally two hours at 37°C, though it was as long as 24 hours in two to five days embryos.

The demonstration of RNA in Nissl's bodies was conducted by staining the sections from the embryos fixed in Carnoy's fluid with 0.1 per cent thionin, with or without ribonuclease digestion (37°C, two hours).

Results

1. Result Obtained for Acid Phosphatase Activity

Acid phosphatase activity in the nerve cells of the spinal ganglion and spinal cord of the chick embryos at various stages of development was histochemically examined, and the results are as follows:

A. Acid Phosphatase Activity in the Spinal Ganglion

In the three days old embryos the rudiments of the spinal ganglia appeared between the neural tube and the surface ectoderm. No acid phosphatase activity was demonstrated in the cells of the rudiments in this stage.

In the four to five days old embryos the ganglia were broken into a metameric series of cell-groups containing neuroblasts and situated laterally between the ectoderm and the mesodermal somites. The cells in the ganglia thus formed were small and somewhat elongated or irregular in shape. The acid phosphatase was not demonstrated as yet in the cells of the ganglia.

In the six days old chick embryos the neuroblasts showed marked modification: they increased in size and sent out their axis cylinder processes, acquiring the typical form of the ganglion cells, though their size was much smaller than the normal cells and their nuclei were omnipresent. This type of the nerve cells were mostly confined in the distal region of the ganglions. Very weak activity of the acid phosphatase was demonstrated in the cytoplasm of the cells mentioned above, whereas a strong activity in the Golgi zone which is adjacent to the medial surface of the nucleus (Fig. 1). No enzymatic activity was found in the remainder of the neuroblasts which were yet smaller in size.

In seven to ten days of incubation, the nerve cells in the spinal ganglion increased in number as well as in size. The nucleus was located at one pole of oval or rounded cell. The acid phosphatase activity in the Golgi zone increased in most of the nerve cells at this stage. The activity was also demonstrated in the adjacent area of cytoplasm to the zone. A moderate activity was also observed in the periphery of the large nerve cells (Fig. 3).

In 11 to 14 days of incubation, the size of the nerve cells reached nearly the normal level, the nucleus being located in the center of the cytoplasm. An extremely high activity of acid phosphatase was demonstrated in the whole cytoplasm of these cells. In 11 days of incubation, the activity was limited in the Golgi zone of the smaller ganglion cell with excentric nucleus. In 16 days of incubation, however, most of the nerve cells became normal morphologically and had a strong phosphatase activity throughout the cytoplasm.

B. Acid Phosphatase Activity in the Spinal Cord

The activity of acid phosphatase was not demonstrated in any part of the spinal cords of the embryos younger than four days of incubation. In five days of incubation, the ependyme cells showed the activity. It was negative in the remainder of the tissue such as gray and white matters, and the neuroblasts in the anterior horn.

In six days of incubation, the acid phosphatase activity appeared in the neuroblasts of the anterior horn. The distribution of the activity in these cells was similar to those observed in the neuroblasts of the spinal ganglia, *i.e.*, it was mostly limited in the Golgi zone. The enzymatic activity of the ependyme cells was much stronger than that in five days old embryos.

In seven to ten days of incubation, the acid phosphatase activity was demonstrated not only in the nerve cells of the anterior horn, but also in the posterior horn. The activity increased considerably coinciding with the increase in the cell size, and finally (Fig. 5), in 16 days of incubation, it was very strong in most cells.

2. Result Obtained for the Relation of Acid Phosphatase Activity to Nissl's Bodies

Preceding the investigation on the relation of the acid phosphatase activity to Nissl's bodies, the appearance of the bodies were examined morphologically. The observations are as follows:

In about six days of incubation, Nissl's bodies first appeared as fine granules at the periphery of the nerve cells in the spinal ganglia and spinal cords. The nuclei of these cells were located excentrically. In such cells, Nissl's bodies were not present in the Golgi zone, though very fine granules of RNA were demonstrated in this region (Fig. 4). The size and amount of the bodies increased with age, agreeing with the previous observation (9). In 14 to 16 days of incubation, Nissl's bodies were distributed throughout the

whole cytoplasm containing nucleus in its center.

The results described above indicated that the appearance of Nissl's bodies generally agreed with those of the acid phosphatase activity. This will be fully discussed in the next section.

Discussion

The recent investigations on the relation of acid phosphatase activity to Nissl's bodies (3-6 and 10) have indicated that the high activity of this enzyme was demonstrable in the cytoplasm of the nerve cells which contain a large amount of Nissl's bodies. Namely, very high activity was found in the large neurons abundant in Nissl's bodies (3, 4, 6 and 10), whereas it was moderate in the Purkinje cells containing a less amount of the bodies (4 and 10). The activity was negative in the cerebellar granule cells free from Nissl's bodies (3, 4 and 10). It was suggested, from these investigations, that the functional significance of the acid phosphatase in the nerve cells may be regarded as a "carrier" or "donor" of phosphate to Nissl's bodies.

The results in this study revealed that the appearance of acid phosphatase activity in the nerve cells of six days chick embryos is coincident with that of Nissl's bodies at the same period, and that the activity became very strong throughout the whole cytoplasm when Nissl's bodies reached their normal state in about 13 to 16 days of incubation.

The results just stated seemed to be in good accord with the above hypothesis that the acid phosphatase may work during the synthesis of RNA in Nissl's bodies. It should be also pointed out, from the present study, that the site of the acid phosphatase activity was not the same as that of Nissl's bodies at the time of their appearance in the nerve cells. Namely, high enzymatic activity first appeared in the Golgi zone of the neuroblast which was free from Nissl's bodies.

According to Smith (7), the reaction of the acid phosphatase in the normal nerve cells is highest in the axon hillock, the part of cytoplasm which is free from Nissl's bodies. The results in this study and that of Smith mentioned above seemed to strongly support the view of LaVelle *et al.* (3) that the acid phosphatase probably work on the breakdown of RNA in the Nissl's bodies. Moreover, it is the present authors' opinion that their observations on the appearance of the acid phosphatase and Nissl's bodies in the nerve cells of chick embryos indicated that the enzyme surely has a very important role on the synthesis of RNA in the nerve cells, the formation of the Nissl's bodies.

Summary

The activity of the acid phosphatase first appeared in the Golgi zone of the nerve cells of the spinal cords and spinal ganglia in six days old chick embryos. The activity increased with the age. In 16 days of incubation, it was observed in the whole cytoplasm. The site of the acid phosphatase activity was not the same as that of Nissl's bodies before 14 days of incubation, after which they were coincident with each other. The possible role of the acid phosphatase on the formation of Nissl's bodies through the synthesis of RNA was discussed.

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Plate 1

Explanation of Figures

Figs. 1-2. The spinal ganglion of the six days old chick embryo. ×1000.

Figs. 3-4. The spinal cord of the eight days old chick embryo. ×1000.

Figs. 5-6. The spinal ganglion of the 12 days old chick embryo. ×1000.

Fig 1. Gomori's revised method for acid phosphatase. The phosphatase acitivity appeared in the Golgi zone of the nerve cells.

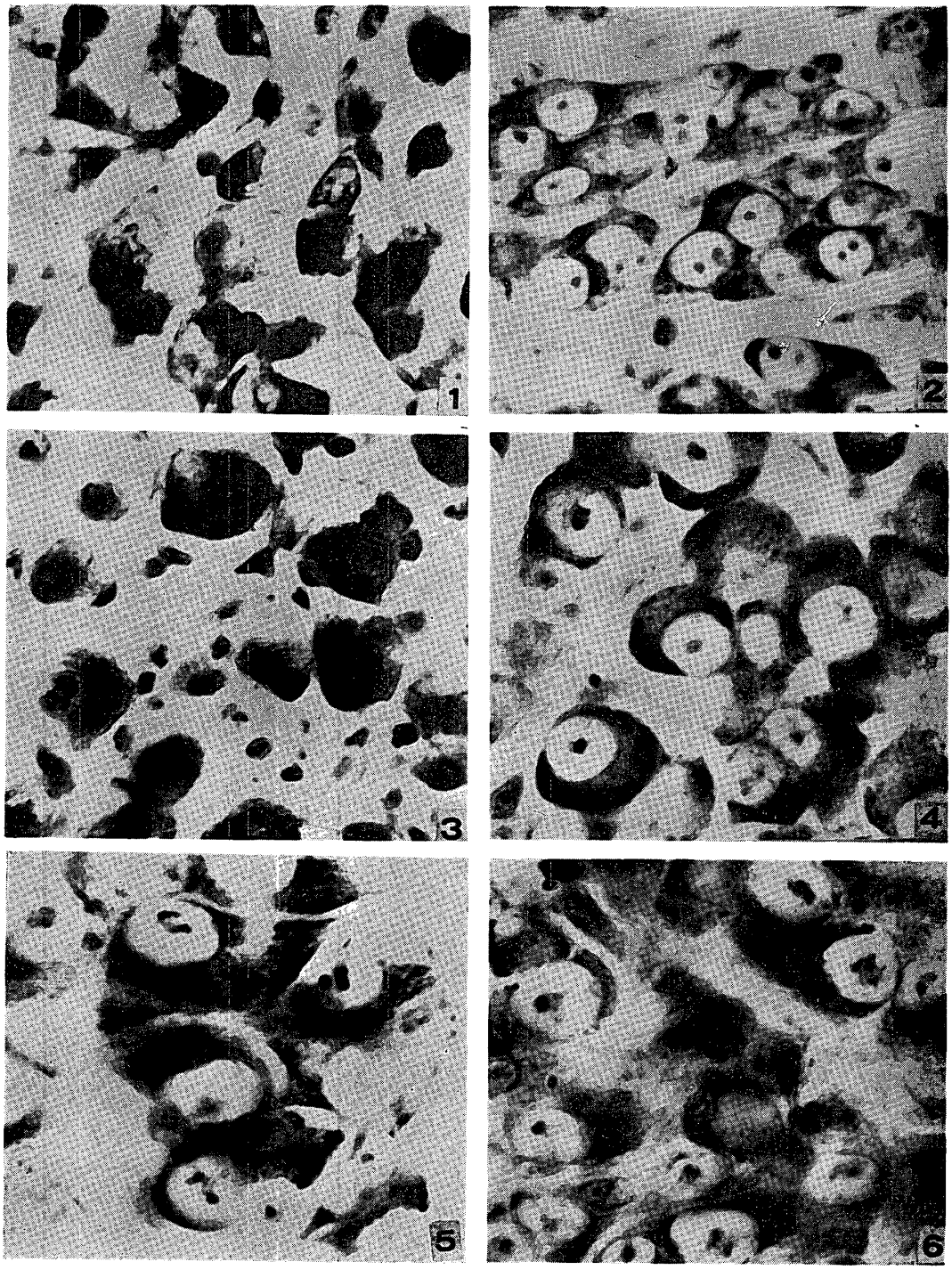
Fig. 2. 0.1 per cent thionin stain. The Nissl's bodies appeared in the periphery of the nerve cells.

Fig. 3. Gomori's revised method for acid phosphatase. The activity increased in the cytoplasm of the anterior horn cells.

Fig. 4. 0.1 per cent thionin stain. The Nissl's bodies increased in number in the anterior horn cells.

Fig. 5. Gomori's revised method for acid phosphatase. The activity increased throughout the cytoplasm of the ganglion cells.

Fig. 6. 0.1 per cent thionin stain. The Nissl's bodies were still few in the Golgi zone of the ganglion cells.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