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Introduction

Histochemical studies of the glycogen and ribonucleic acid (RNA) have been made by several investigators. Kimura[7] (1934) stated that the greater part of glycogen in the nervous systems of invertebrates and cold-blooded members of vertebrates is observed extracellularly in the interstitial tissue and that of the birds and mammals intracellularly in the tigroid substances of the ganglion cell. This investigator, however, has not given any detail about the componental difference of glycogen found in both cases, though its physiological and biological significances are fully discussed. Schallek[9] (1949) biochemically studied the glycogen in the nervous systems of the spider crabs and squids, and reported that a large amount of glycogen is found in the leg nerve and ganglions of the former, and that the glycogen content of the ganglions in the latter is almost four times to that of the stellete nerve. He also found that axoplasm contains no detectable amount of glycogen.

Caspersson and his co-workers (Landström et al,[8] 1941) studied the nucleic acid distribution in the nervous system and found that the Nissl's bodies contain an enormous large amount of RNA. Hyden[5] [6] (1943 and 1947) also studied the RNA variation of the Nissl's bodies in both the experimental and pathological conditions of animals and stressed the physiological significance of the RNA to the functional activities of the nerve cells. But none of them refered to the glycogen in the Nissl's bodies with special reference to the relation of it to RNA.

As to the relation of the glycogen to the Nissl's bodies of the horse, guinea pig and fowl, one of us has already stated (Toryu,[10] [11] [12] 1937, 1938 and 1948) that the glycogen is contained in the nerve cells as one of the elements of the Nissl's bodies, accordingly the glycogen granules coincide morphologically with the Nissl's bodies. On the other hand, glycogen is not only contained in the Nissl's bodies, but also in such cells, different from the nerve ones, as those of the pituitary body and the pineal body, in which the glycogen exists as pure element as in the liver cells and in the heart muscle fibers as has already been stated by Toryu[10] (1937). Thus the componental relations of the glycogen together with its localizations differs in different organs in the nervous systems of animals.

In the present investigation we have dealt with the distribution and the nature of the glycogen and the RNA in the nervous systems of various animals in order to determine the componental relation of these two substances in the nervous systems of higher and lower animals in connection with the localization of them in the systems.

Materials and Methods

As materials, various species from representative phylums were selected. These animals are given in Table 1.

Table 1. Materials used.

Phylum	Class	Species	No. of individuals	Regions
Coelenterata	Hydroida	*Hydra vulgaris attenuata*	10	Body wall
〃	Anthozoa	*Anthopleura stella*	3	〃
Plathelminthes	Trematoda	*Fasciola hepatica*	3	Brain
〃	Turbellaria	*Dugesia gonocephala*	5	〃
Nemethelminthes	Nematoda	*Parascaris equorum*	5	Nerve ring
〃	〃	*Parascaris lumbricoides*	5	〃
Chaetognatha	Sagittoidea	*Sagitta* sp.	2	Brain, abdominal ganglion
Annelida	Polychaeta	*Nereis* sp.	3	Ventral nerve cord
〃	Oligochaeta	*Pheretima communissima*	15	〃
〃	Hirudinea	*Hirudo nipponia*	3	〃
Mollusca	Bivalvia	*Ostrea gigas*	8	Visceral ganglion
〃	Gastropoda	*Bradybanea* sp.	5	〃
〃	〃	*Cipangopaludina japonica*	3	〃
〃	〃	*Semisulcospira libertina*	3	〃
〃	Cephalopoda	*Loligo bleekeri*	3	Stellete ganglion
Arthropoda	Crustacea	*Atydae* sp.	3	Thoracic ganglion
〃	〃	*Megaligia exotica*	3	〃
〃	Chilopoda	*Scolopendra* sp.	3	Abdominal ganglion
〃	Arachnida	*Araneus* sp.	3	Brain, thoracic ganglion
〃	Insecta	*Paratenodera sinensis*	1	Thoracic ganglion
〃	〃	*Oxya japonica*	15	〃
〃	〃	*Locusta migratoria*	3	〃

(continued)

Arthropoda	Insecta	*Pieris rapae curcivora*	3	Thoracic ganglion
〃	〃	*Sympetrum frequence*	10	〃
〃	〃	*Orthetrum albistylum speciosum*	5	〃
〃	〃	*Musca domestica*	5	〃
〃	〃	*Eristalomya tenax*	3	〃
Echinoderma	Asteroidea	*Asterina pectinifera*	2	Peristomial nerve ring
Protochordata	Urochordata	*Cynthia roretzi*	4	Brain
Vertebrata	Cyclostomata	*Lampetra planeri*	1	Spinal cord
〃	Pisces	*Acanthogobius flauimans*	2	〃
〃	〃	*Cyprinus carpio*	2	〃
〃	〃	*Cyprinus auratus*	3	〃
〃	〃	*Anguilla japonica*	2	〃
〃	Amphibia	*Rana japonica*	5	〃
〃	〃	*Rana nigromaculata*	3	〃
〃	〃	*Triturus pyrrhogaster*	1	〃
〃	Reptilia	*Natrix tigrina tigrina*	2	〃
〃	〃	*Clemmys japonica*	2	〃
〃	Aves	*Passer montanus saturaius*	1	〃
〃	〃	*Gallus gallus var. domesticus*	5	Spinal cord, spinal ganglion
〃	Mammalia	*Rattus norvegicus var. albinus*	5	Spinal cord
〃	〃	*Cavia cobaya*	3	〃
〃	〃	*Mesocricetus auratus*	1	〃
〃	〃	*Canis familiaris*	1	〃
〃	〃	*Capra hircus hircus*	2	〃
〃	〃	*Sus scrofa var. domesticus*	5	Spinal cord Spinal ganglion
〃	〃	*Equus caballus var. orientalis*	10	Spinal cord
〃	〃	*Bos taurus var. domesticus*	5	〃
〃	〃	*Ovis aries*	1	〃

Most of these animals were collected from the summer of 1948 to the winter of 1951. Large animals were dissected and various portions from the nervous systems were obtained and fixed. Small animals, however, were fixed as the entire bodies. Alcohol-formalin saturated with sodium acetate (Toryu), or with magnesium sulphate (Sato) were used as fixing fluids. All materials were embedded in celloidin and sectioned 20μ thick.

The following stains were used: Best's carmine stain, Bauer-Feulgen's stain and Hotchikiss' stain for glycogen; methylene blue, toluidine blue and thionin for RNA; hematoxylin-eosin for general histology; and occasionally, Golgi method for the detection of nerve cells.

Identification of glycogen was made not only by means of salivary test, but also by digestion of amylolytic, proteolytic and lypolytic enzymes. These enzymes were added to the buffer solution of a known pH and used for the digestion of glycogen as has been already stated by Toryu[10] (1937).

Identification of RNA was made by means of ribonuclease digestion (0.03%, 60°C) without using any buffer solution according to the recommendation by Boivin[2] (1947).

Results

1. *Results Obtained for the Distribution of the Glycogen and RNA.*

The results obtained for the distribution of the glycogen and RNA in the nervous systems of various animals are given in Table 2. As shown in that Table, the distribution of the glycogen and RNA in the nervous systems differs in the different groups of animals used, and four types are distinguished according to the comparative histochemistry of the glycogen in the tissue elements of the nervous systems. These types are described as follows:

Table 2. Distribution of the glycogen and RNA in the nervous systems of various animals.

Species	Glycogen			RNA		
	Nerve cells	Interstitium	Neurium	Nerve cells	Interstitium	Neurium
Hydra vulgaris attenuata	−	+	−	+	−	−
Anthopleura stella	−	++	−	+	−	−
Fasciola hepatica	−	+	−	+	−	−
Dugesia gonocephala	−	+	−	+	−	−
Parascaris equorum	* Nerve cells were not determined.					
Parascaris lumbricoides						
Sagitta sp.	* 10% formalin fixation; glycogen was not found.			+	−	−
Nereis sp.	−	+++	−	++	−	−
Pheretima communissima	−	+++	−	++	−	−
Hirudo nipponia	−	+++	−	++	−	−
Ostrea gigas	+++	−	−	+++	−	−
Bradybanea sp.	+	−	−	++	−	−
Cipangopaludina japonica	+	−	−	++	−	−
Semisulcospira libertina	+	−	−	++	−	−
Loligo bleekeri	++	−	−	++	−	−
Atydae sp.	−	−	+	++	−	−
Megaligia exotica	−	−	++	++	−	−
Scolopendra sp.	−	−	++	++	−	−
Araneus sp.	−	−	++	++	−	−
Paratenodera sinensis	−	−	++	++	−	−
Oxya iaponica	−	−	++	++	−	−
Locusta migratoria	−	−	++	++	−	−
Pieris rapae curcivora	−	−	++	++	−	−
Sympetrum frequence	+	−	−	+++	−	−
Orthetrum albistylum speciosum	+	−	−	+++	−	−
Musca domestica	±	−	−	+++	−	−
Eristalomya tenax	±	−	−	+++	−	−

(continued)

Asterina pectinifera	−	+	−	+	−	−
Cynthia roretzi	+	−	−	+++	−	−
Lampetra planeri	++	−	−	+++	−	−
Acanthogobius flavimans	++	−	−	++	−	−
Cyprinus carpio	++	−	−	++	−	−
Cyprinus auratus	++	−	−	++	−	−
Anguilla japonica	++	−	−	++	−	−
Rana japonica	++	++ *1.	−	++	−	−
Rana nigromaculata	++	+ *1.	−	++	−	−
Triturus pyrrhogaster	++	+ *1.	−	++	−	−
Natrix tigrina tigrina	+	+ *1.	−	+	−	−
Clemmys japonica	+	+ *1.	−	+	−	−
Passer montanus saturatus	+++	−	−	+++	−	−
Gallus gallus var. domesticus	+++	−	−	+++	−	−
Rattus norvegicus var. albinus	+++	−	+++ *2.	+++	−	−
Cavia cobaya	+++	−	+++ *2.	+++	−	−
Mesocricetus auratus	+++	−	+++ *2.	+++	−	−
Canis familiaris	+++	−	+++ *2.	+++	−	−
Capra hircus hircus	+++	−	+++ *2.	+++	−	−
Sus scrofa var. domesticus	+++	−	+++ *2.	+++	−	−
Equus caballus var. orientalis	+++	−	+++ *2.	+++	−	−
Bos taurus var. domesticus	+++	−	+++ *2.	+++	−	−
Ovis aries	+++	−	+++ *2.	+++	−	−

* 1. Glycogen in the grey substance of the spinal cord.

* 2. Glycogen in the meninges.

Type 1 (Annelida type). Four phylums of invertebrates, Coelenterata, Plathelminthes, Annelida and perhaps Echinoderma belong to this type. In this type the glycogen is found in the interstitial cells of the nervous systems, and the RNA in the nerve cells. A small amount of the glycogen is found in the interstitial cells of the diffuse nervous systems of Coelenterata and in those of primitive concentrated ones of Plathelminthes and Echinoderma. On the other hand, however, a large amount of glycogen is found in the interstitial cells of the developed concentrated nervous systems of Annelida. The RNA in the nerve cells is abundant in Annelida, but little in the lower animals in this type (Fig. 1).

The glycogen in the interstitial cells is easily digested by saliva (two hours, 37°C) and the RNA in the nerve cells by ribonuclease (two hours, 60°C). The nature of the glycogen and RNA described above must be, therefore, considered as pure elements.

Two phylums, Nemathelminthes and Chaetognatha, are possibly involved in this type, though no detailed conclusions were obtained in this work. The two species of Nemathelminthes were found to contain a large amount of glycogen in their bodies, so that the exact localization of it in their central nervous systems was not clearly decided. In the case of Chaetognatha the glycogen in their nervous systems was not observed, since the animals were fixed and preserved in formalin for some days and the glycogen in the animal bodies was lost before staining.

Type 2 (Mollusca type). Only one phylum, Mollusca, belongs to this type. In this type the glycogen and the RNA are found in the nerve cells of the visceral and stellete ganglions, and not in any other part of the tissues. The glycogen in the nerve cells is easily digested by saliva, and the RNA by ribonuclease. The nature of the glycogen and RNA in the nerve cells of this type, therefore, must be pure substances as in the type 1 (Fig. 2 and 3).

Type 3 (Arthropoda type). Only one phylum, Arthropoda, belongs to this type. In most species of this type the glycogen is found in the cells of neurium and connective tissue of the ganglions and brain, and also in the neurium of the peripheral nerves.

In some species, however, the glycogen is found in the nerve cells, especially in the large ones of the abdominal and thoracic ganglions, though the amount of it is relatively small. Thus the type 3 is divided into two sub-types, the one consists of most species of this type (Sub-type 3a), and the other consists of four species of insects (Sub-type 3b). The glycogen is contained in the neurium and in the connective tissue in the type 3a, and in the nerve cells in the type 3b. In two species of type 3b (Diptera) the amount of the glycogen in the nerve cells is very small and no detectable amount of it is found in any other part of the ganglions. The amount of the RNA in the nerve cells of the type 3b is much larger than that of other species of type 3a (Fig. 4).

Since the glycogen in the neurium and in the nerve cells mentioned above is easily digested by saliva and the RNA in the nerve cells by ribonuclease, the nature of these two substances must be pure elements.

Type 4 (Vertebrata type). Two phylums belong to this type. They are six classes of Vertebrata and one class of Protochordata. The glycogen and RNA are found in the nerve cells as the Nissl's bodies without exception. In some species the glycogen is also found in other tissue elements such as the glia cells of the grey substance of the spinal cord or in the meninges. Thus the four sub-types are distinguished as follows:

Type 4a (Pisces type). In this type the glycogen and RNA are exclusively found in the nerve cells and not in any other part of the tissue. The amount of these substances is relatively small. Three classes of the lower chordates, Urochordata, Cyclostomata and Pisces, belong to this type.

Type 4b (Reptilia type). In this type the glycogen exists both in the nerve cells and in the glia cells of the grey substance of the spinal cord, while the RNA exists only in the nerve cells. The glycogen in the glia cells is most abundant in the central grey substance and easily digested by saliva. The glycogen and RNA in the nerve cells exist as elements of the Nissl's bodies and not digested by saliva, but easily by ribonuclease. Two classes, Amphibia and Reptilia, are involved in this type (Fig. 5).

Type 4c (Aves type). In this type the glycogen and RNA are found limited in the nerve cells of the spinal ganglion and spinal cord in large amount, and not in any other part of the tissue. To this type belongs one class, Aves. These substances are contained in the Nissl's bodies and not digested by saliva. After the digestion of ribonuclease the glycogen as well as the RNA in the Nissl's bodies disappear (Fig. 6).

Type 4d (Mammalia type). The highest class of vertebrates, Mammalia, belongs to this type. The glycogen and RNA exist as the elements of the Nissl's bodies in the nerve cells of the spinal cord, spinal gnaglion and brain, the amount of which is the largest of all four sub-types. A considerable amount of the glycogen is also found in the meninges, especially in Pia mater of the spinal cord and in Dura mater of brain. Since the glycogen in the meninges is easily digested by saliva, it must be pure substance. The glycogen in the nerve cells, however, is not digested by saliva and so must be some other form of glycogen as already stated by Toryu[10](1937). The further details for the nature of the glycogen and RNA of the Nissl's bodies will be described in the following chapter (Fig. 7, 8, 9 and 11).

2. *Results Obtained for the Nature of the Glycogen and RNA in the Nervous Systems.*

To obtain further details for the nature of the glycogen and RNA in the nervous systems of various animals, 10 species were selected from the four

Table 3. Digestive power of saliva and ribonuclease on the RNA and glycogen in the nervous systems of representative animals of four types.

Type	Species	Ribonuclease		Saliva	
		RNA	Glycogen	RNA	Glycogen
1	*Pheretima communissima*	−	+	+	−
2	*Ostrea gigas*	−	+	+	−
2	*Loligo bleekeri*	−	+	+	−
3a	*Locusta migratoria*	−	+	+	−
3a	*Megaligia exotica*	−	+	+	−
3b	*Orthetrum albistylum speciosum*	−	+	+	−
4a	*Cyprius auratus*	−	−	+	+
4b	*Rana japonica*	−	−(+)*2	+	+(−)*2
4c	*Gallus gallus domesticus*	−	−	+	+
4d	*Rattus norvegicus var. albinus*	−	−(+)*1	+	+(−)*1

* 1. Glycogen in the meninges. * 2. Glycgen in the gray substance.
− : Digested. + : Not digested.

types described in the previous chapter.

First, sections of the nervous tissues of these animals were treated with ribonuclease or saliva for two or four hours and then stained with Best's carmine fluid or methylene blue. The results are given in Table 3.

As already seen in Table 3, the ribonuclease solution digests the RNA in the nerve cells of all species selected from four types. The glycogen in the interstitium in the type 1, in the nerve cells in the type 2 and type 3b, in neurium in the type 3a and in the meninges and gray substance in the type 4b and 4d is easily digested by saliva, but is not affected by ribonuclease digestion. On the other hand, the glycogen in the nerve cells in the type 4 is not digested by saliva, but is easily by ribonuclease. Accordingly it is considered that the RNA and glycogen in the nervous systems of the type 1, 2 and 3, and the glycogen in the meninges and gray substance in the type 4 are pure substances, but those in the Nissl's bodies of the type 4 may be some conjugated state of the glycogen and RNA.

To ascertain the view just stated for the nature of the glycogen and RNA in the Nissl's bodies of chordates, sections of the spinal cord were treated with various enzyme solutions. The results are shown in Table 4.

As will be seen in Table 4, the glycogen in the nerve cells is digested by the co-operation of pepsin and saliva, though it resists pepsin or saliva when used separately. The mixture of these two enzymes is found relatively inactive. It also resists lipase solution, but is easily digested by the co-operation of lipase, pepsin and saliva, the time required for the complete digestion being almost one half of that required for it by the co-operation of pepsin and saliva (Fig. 10 and 12).

Table 4. Digestive power of various enzymes on the RNA and glycogen in the Nissl's bodies in the type 4 (horses, pigs, dogs and rats).

Enzyme solution	PH	Hours	RNA	Glycogn
Saliva 50%	7	12	+	+
Pepsin 1%	2	6	+	+
Pepsin 1% Saliva 50%	2 7	3 3	+	+
Pepsin 1% Saliva 50%	2 7	6 6	−	−
Pepsin and Saliva (mixture)	5	12	±	±
Lipase solution	7	12	+	+
Lipase/Pepsin/Saliva	7/2/7	1/2/3	−	−

− : Digested, + : Not digested. ± : Almost digested.

The results obtained above show a good agreement with that of Toryu[10] (1937), who found that the three elements, glycogen, lipoid and basophilic protein are contained as a conjugated state in the Nissl's bodies. Namely, the glycogen and RNA in the Nissl's bodies are only digested by the co-operation of proteolytic, amylolytic and lypolytic enzymes. The digestive power of ribonuclease for the glycogen as well as RNA in the Nissl's bodies seems to provide a positive evidence for the conjugated nature of the glycogen with the RNA in the nerve cells of the type 4.

The stainability of the glycogen in the nervous systems of the animals was determined by Bauer-Feulgen's stain and Hotchikiss' stain. The glycogen in the nervous systems of the type 1, 2 and 3, and those of the meninges and grey substance of the spinal cord of the type 4 can stain intensely by these methods. The glycogen in the nerve cells of the type 4 also stain by them, though the reaction are not so strong as that of Best's stain.

Discussion

Kimura[7] (1934) studied the histochemical distribution of the glycogen in various members of animals and reported that considerable amount of glycogen is found in the nervous system of some species in four phylums, Nemathelminthes, Mollusca, Arthropoda and Vertebrata. Concerning the localization of the glycogen he also stated that the substance in the nervous systems of invertebrates representing the former three phylums mentioned above is found inter- or extracellularly in the interstitial tissue. The results obtained in the present investigation, however, show some disagreement with that of Kimura ; namely the glycogen in the nervous systems is found in the interstitial tissues of Coelenterata, Plathelminthes, Annelida and Echinoderma, in the neurium and connective tissue of ganglions of Arthropoda, and in the nerve cells of Mollusca and some species of Insecta.

As to the glycogen in the nervous systems of the vertebrates, Kimura stated that it is contained in the Nissl's bodies of the birds and mammals, and extracellularly in the interstitial tissue of the cold-blooded members of the vertebrates. In the present investigation, however, the glycogen in the nervous systems of the vertebrates is contained in the Nissl's bodies of all six classes of the vertebrates, though the amount of which in the lower classes is relatively small. It has been found by Toryu[10] (1937) that the fixatives containing chroloform, such as Carnoy's fluid, are not available for the fixation of the glycogen in the Nissl's bodies, because chroloform dissolves the lipoidal component of the Nissl's bodies and makes other components such as glycogen and RNA unstable for histochemical examinations. The difference between the results obtained by us and by Kimura probably due to the fixative used, because the latter used Carnoy's fluid for glycogen in the Nissl's bodies.

The amount of the glycogen in the Nissl's bodies of the lower classes of vertebrates such as Reptilia, Amphibia, Pisces and Cyclostomata is smaller than that of higher ones such as Aves and Mammalia, suggesting that the nervous systems of the latters have higher functional activities.

The glycogen in the meninges of Mammalia and in the grey substance of Reptilia and Amphibia is pure substance as that in the liver. This shows that the glycogen contained in the interstitial or connective tissue cells will be utilized for only combusion, but that in the Nissl's bodies for functional activities.

Schallek[9] (1949) found by chemical analysis that the glycogen contents of the ganglions and nerves differ in different species of animals. He stated that the amount of glycoyen in the ganglions of squids is much larger than that of stellate nerve and that the amount of the glycogen in the ganglions of spider crabs is almsot equal to that of the leg nerve. Holmes[4] (1929) also studied the glycogen in the nerves of crab cancers and reported that the glycogen concentration is many times to that of mammalian nerve. This agrees with our results obtained by histochemical analysis as follows: The glycogen in Crustacea such as spider crab and crab cancer is selectively contained in the neurium, the tissue element in the nerves as well as in the ganglions. On the contrary, however, the glycogen in Mollusca such as squids is limited in the nerve cells in the ganglions. Almost no glycogen is contained in the mammalian nerves, but relatively a large amount of it in the nerve cells.

It will be considered that there are some important relations between the amount of the glycogen in the nervous systems and its functional activities in different ranks of animals. To support the idea just stated the following facts will be noticed: First, very small amount of the glycogen is found located in the interstitium of the primitive nervous systems of the lower invertebrates such as Coelenterata, Plathelminthes and Echinoderma, while a large amount of it is found located not only in the interstitium but also in the neurium and nerve cells of the concentrated ones of the higher animals such as Annelida, Mollusca, Arthropoda, Protochordata and Vertebrata. Second, the same relations are also found even in one phylum, i.e., the nervous systems of the lower vertebrates such as Reptilia, Amphibia, Pisces and Cyclostomata contain relatively a small amount of the glycogen, but those of the higher ones such as Aves and Mammalia contain a large amount of it. Third, the nervous systems of vertebrates contain the glycogen in the Nissl's bodies conjugated with protein (RNA), such as the so-called desmoglycogen by Brachet[3] (1950), but those of the lower animals in the interstitium or neurium as pure substance.

Notwithstanding the localization of the glycogen in the nervous system differs in different types of animals, that of the RNA is strictly limited intracellularly in the nerve cells. The RNA in the nerve cells of the lower animals appears diffusely in small amount and that of the higher ones as granules in abundant and often conjugated with the glycogen.

Toryu[11] (1938) studied the histochemical changes of glycogen in the Nissl's bodies of the nerve cells of guinea pigs after a section of their axones and reported that the so-called chromatolysis is accompanied by glycogenolysis, and that when the recovery of the Nissl's bodies takes place the granules of the same form and arrangement as Nissl's bodies appear, showing that the glycogen in the nerve cells is contained in the Nissl's bodies. The same relation was found by the same author (Toryu[12], 1948) in the nerve cells of horses affected with paralysis and of hen with B_1 avitaminosis.

Bodian[1] (1947) studied the nucleic acid changes during the chromatolysis and found that RNA disappears during the two weeks after a section of axones of the nerve cells, and then recovers within one month, suggesting that the so-called chromatolysis is nothing else than the RNA changes in the Nissl's bodies.

From the results of Toryu and Bodian it will be suggested that the glycogenolysis coinciding with RNA changes during the chromatolysis is to be more easily explained if the existence of the conjugated state of the glycogen and the RNA in the Nissl's bodies is admitted as their componental elements in the nerve cells.

Summary

Various animals from ten phylums including fourty-six species are collected and their nervous systems are studied for the histochemical distributions of the glycogen and RNA (Ribonucleic acid). The results can be summarized as follows:

1. The glycogen distribution in the nervous systems of these animals differ in different types of animals. Four types and six sub-types are distinguished concerning their glycogen contents in various tissue elements as follows:

Type 1. The glycogen is found in the interstitium. Four phylums belong to this type. They are Coelenterata, Plathelminthes, Annelida and perhaps Echinoderma.

Type 2. The glycogen is found in the nerve cells of the ganglions, separated from the RNA. One phylum, Mollusca, belongs to this type.

Type 3. Arthropoda belongs to this type. In most species the glycogen is contained in the neurium or connective tissue of the ganglions as well as nerves (sub-type 3a), but in some species of Insecta (Odonata and Diptera) in

the nerve cells (sub-type 3b).

Type 4. Two phylums, Protochordata and Vertebrata, belong to this type. The glycogen is mainly contained conjugated with the RNA in the nerve cells, but in some species is found in other parts of the tissues. Four sub-types are distinguished as follows:

Sub-type 4a. The glycogen is contained in the nerve cells, the amount of which is relatively small. Pisces, Protochordata and Cyclostomata belong to this type.

Sub-type 4b. The glycogen is contained in the glia cells of the grey substance of the spinal cord as well as in the nerve cells. Two classes of Vertebrata, Amphibia and Reptilia, belong to this type.

Sub-type 4c. The glycogen is selectively found in the nerve cells, the amount of which is very large. One class, Aves, belongs to this type.

Sub-type 4d. The glycogen is contained in the meninges as well as in the nerve cells of the spinal cord and brain. Mammalia belongs to this type.

2. The RNA is found in the nerve cells with no exception, though the amount of it differs in different types of animals.

3. The amount and localization of the glycogen and RNA in the nervous systems have important relation to the functional activities and ranks of the animals. The primitive nervous systems of the lower animals contain very small amount of it in the interstitium, while concentrated ones of the higher animals contain a large amount of it not only in the interstitium but also in the neurium and in the nerve cells.

4. The conjugated state of glycogen with RNA in the Nissl's bodies of the vertebrates was studied by enzyme digestion as well as by staining methods and highly possible existance of the conjugated substance was fully discussed.

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References

(1) Bodian, D. 1947. Symp. Soc. Exp. Biol., Vol. 1, p. 163.
(2) Boivin, A., R. Tulosne, R. Vendrey and R. Minck. 1947. Arch. sci. physiol., Vol. 1. p. 307.
(3) Brachet, J. 1950. Chemical embryology, p.327. Interscience Publ., New York.
(4) Holmes, E.G. 1929. Biochem. Journ., Vol. 23, p. 1182.
(5) Hyden, H. 1943. Acta Physiol. Scand. Vol. 6, p. 17.
(6) Hyden, H. 1947. Symp. Soc. Exp. Biol., Vol. 1, p. 154.
(7) Kimura, T. 1934. Trans. Japan. Path. Soc., Vol. 24, p. 593.

(8) Landstrôm, H., T. Caspersson and G.Z. Wohfart. 1941. Zeit. mikr. anat. Forsch., Vol. 49, p. 96.
(9) Schallek, W. 1949. Biol. Bull., Vol. 97, No. 1, p. 252.
(10) Toryu, Y. 1937. Sci. Rep. Tohoku Imper. Univ. Biol., Vol. 12, No. 1, p. 1.
(11) Toryu, Y. 1938. ibid. Vol. 12, No. 4, p. 483.
(12) Toryu, T, and K. Chiba. 1948. Japan, Journ. Veterin. Soc., Vol. 11, No. 1, p. 1.

PLATE 1.

EXPLANATION OF FIGURES

1. Section of ventral nerve cord from common earthworm (*Pheretima communissima*). Best's carmine stain. ×400.
   The glycogen is contained in the interstitial tissue.
2. Section of visceral ganglion from oyster (*Ostrea gigas*). Methylene blue stain. ×625.
   A large amount of RNA is contained in the nerve cells.
3. Section of visceral ganglion from oyster. Best's carmine stain. ×310.
   A large amount of glycogen is contained in the nerve cells'.
4. Section of thoracic ganglion from locust (*Locusta migratoria*). Best's carmine stain. ×350.
   The glycogen is selectively contained in the neurium and connective tissue of the ganglion and nerve.
5. Section of spinal cord from turtle (*Clemmys japonica*). Best's carmine stain. ×310.
   A considerable amount of glycogen is contained in the nerve cells and in the glia cells of the central grey substance.
6. Section of spinal cord from chick (*Gallus gallus domesticus*). Methylene blue stain. ×610.
   Very large amount of RNA is contained in the nerve cells.

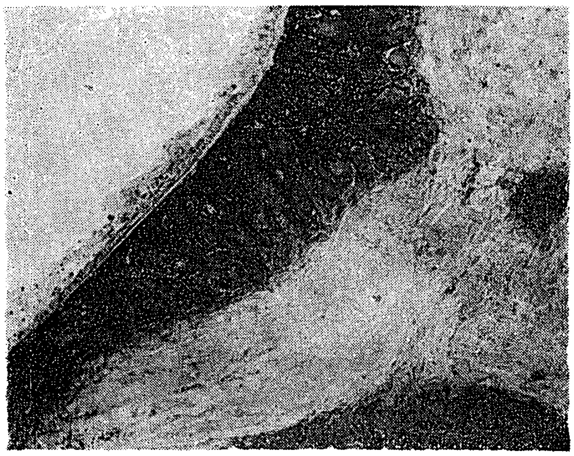


Fig. 1.

Fig. 2.

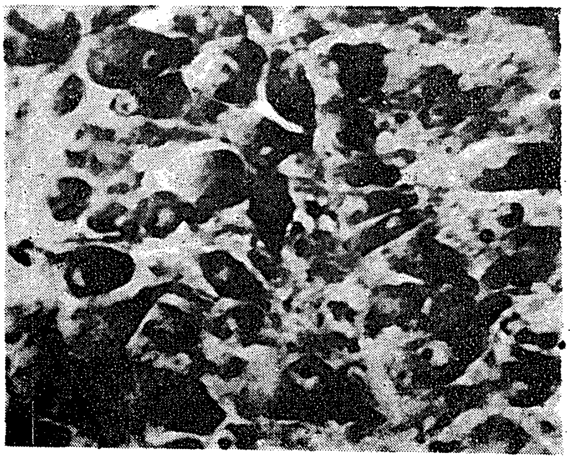
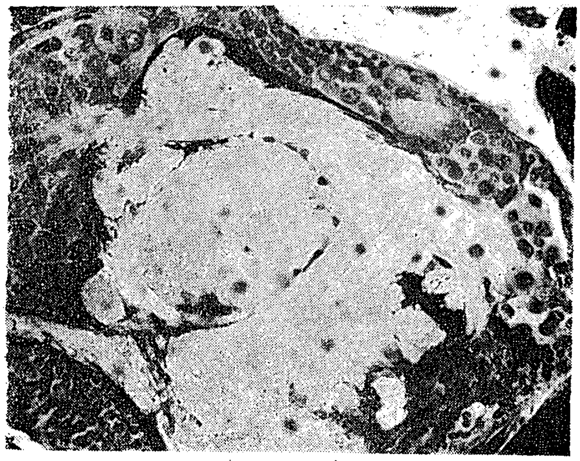


Fig. 3



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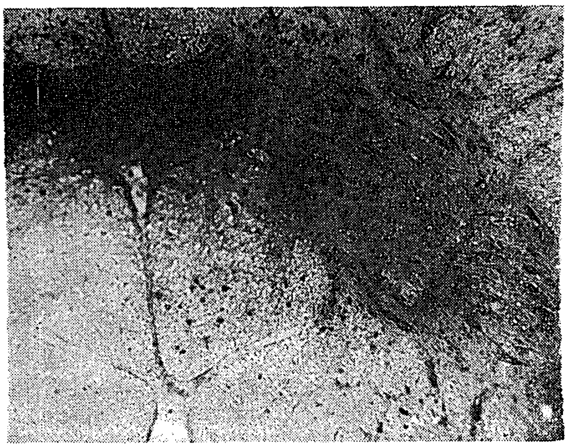

Fig. 5.

Fig. 6.

PLATE 2.

EXPLANATION OF FIGURES

7. Section of spinal cord from horse (*Equus caballus orientalis*). Best's carmine stain. ×350.
   A large amount of glycogen is contained in the pia mater.
8. Section of spinal cord from horse. Hotchkiss' stain. ×100.
   Large motor cells stain diffusley.
9. Section of spinal cord from dog (*Canis familiaris*). Best's carmine stain. ×625.
   Very large amount of glycogen is contained in the Nissl's bodies of motor cell.
10. Section of spinal cord from dog. Best's carmine stain after digestion of co-operation of pepsin, saliva and lipase. ×625.
   The glycogen in the Nissl's bodies disappears.
11. Section of spinal cord from horse. Methylene blue stain. ×625.
   Very large amount of RNA is contained in the Nissl's bodies of motor cell, but not in axonhillock.
12. Section of spinal cord from dog. Methylen blue stain after digestion of ribonuclease. ×625.
   RNA in the Nissl's bodies disappears.

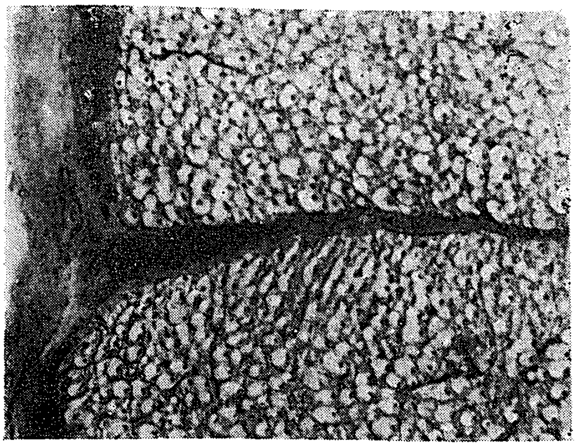

Fig. 7.

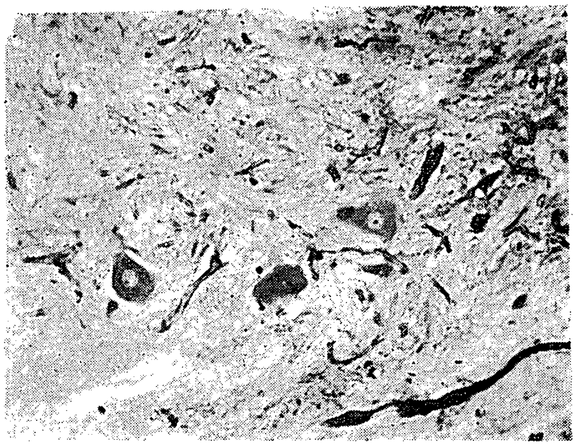

Fig. 8.

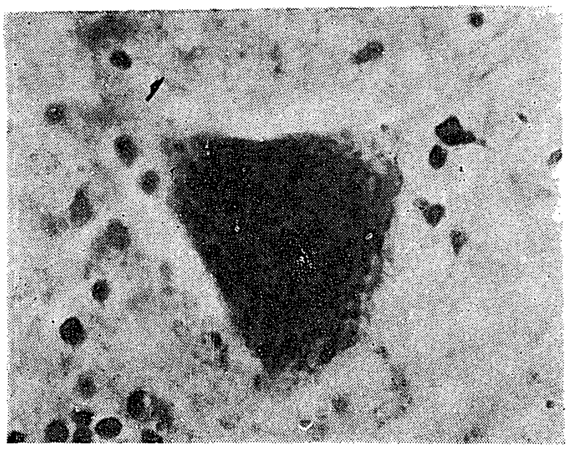

Fig. 9.

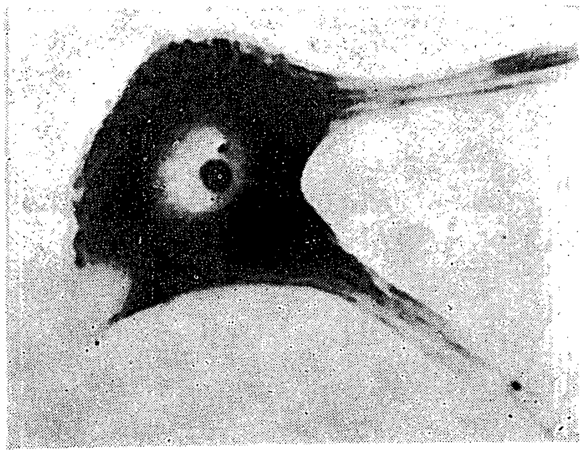

Fig. 10.

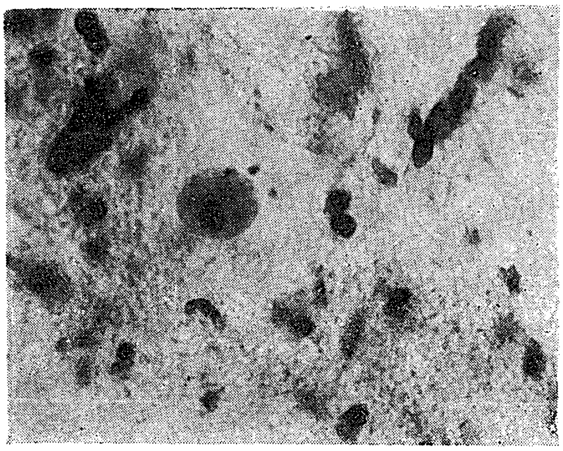

Fig. 11.

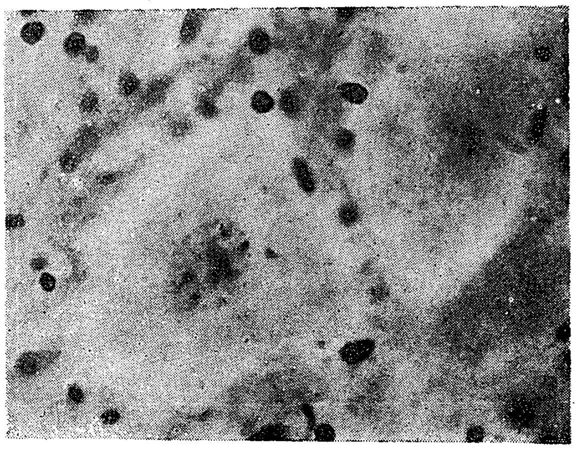

Fig. 12.