Department of Histology and Embryology of the University in Budapest
(Director : Prof. I. Törő)

Department of Anatomy and Biology of the University in Debrecen
(Director : Prof. I. Krompecher)

The Physical Institute of the University in Debrecen
(Director : Prof. S. Szalay)

QUANTITATIVE ESTIMATION OF ALKALINE PHOSPHATASE IN SYMPATHETIC GANGLIONS AND OF ITS CHANGES DURING PREGNANCY

Béla Aros, Tibor Barka, Zoltán Pósalaky and *Gabriella Gerecze*
(Received : 21. April 1951)

We have been examining the action of procaine upon sympathetic ganglions for some time, among other items, we intended to examine the action of procaine upon alkaline phosphatase in the ganglion. The data of literature are very unsatisfactory on the quantity, variability of concentration and exact localisation of the alkaline phosphatase in the ganglions. Thus we were compelled to examine these items first of all. It is the result of this research which we want to report here briefly.

Since the method for estimation of phosphatase had been established by *Gömöri* and *Takamatsu* in 1939, the phosphatase activity of the nervous system has been often dealt with, by *Gömöri*, *Bourne* & al., *Landow* & al., etc. Most of the examinations concern the central nervous system, very few data are found about the phosphotase contents of sympathetic ganglions. Recently the alkaline phophatase activity of the latter was discussed by *Sulkin* and *Kuntz*. The localisation of the enzyme in the ganglions was described by them and no attention was paid to the fluctuations of the enzymatic activity, to differences found between the different ganglions.

Material and Methods

Investigations into the localisation of the enzyme were carried out with the *Gömöri—Takamatsu* method. As fixative for the ganglions 80 per cent. alcohol, at 3° C was used wherein the ganglions were placed immediately after sacrificing the animals. Embedding in paraffine, phosphatase estimation, using the usual standard methods.

For quantitative estimation of the enzyme in sympathetic ganglions the quantitative histochemical method devised by *Barka*, *Szalay*, *Pósalaky* and *Kertész* was used. Essentially, it consists of using a lead tracer (charged by Thorium B) in the *Gömöri—Takamatsu* reaction. Hereby it is possible to estimate quantitatively the lead sulfide deposits, formed proportionally to the enzymatic activity, deposited at the site of such activity. The method proved to be especially suitable for estimating the enzymatic activity in small specimens.

In our experiments, ganglions of rabbits, cats and guinea-pigs were used.

Experimental Results

1. *The localisation of alkaline phosphatase in the superior cervical ganglion of the rabbit.* In describing the localisation of alkaline phosphatase we rely upon findings in the superior cervical ganglion of the rabbit. As regards the localisation, other species examined (cat, guinea pig) do not display any remarkable divergence.

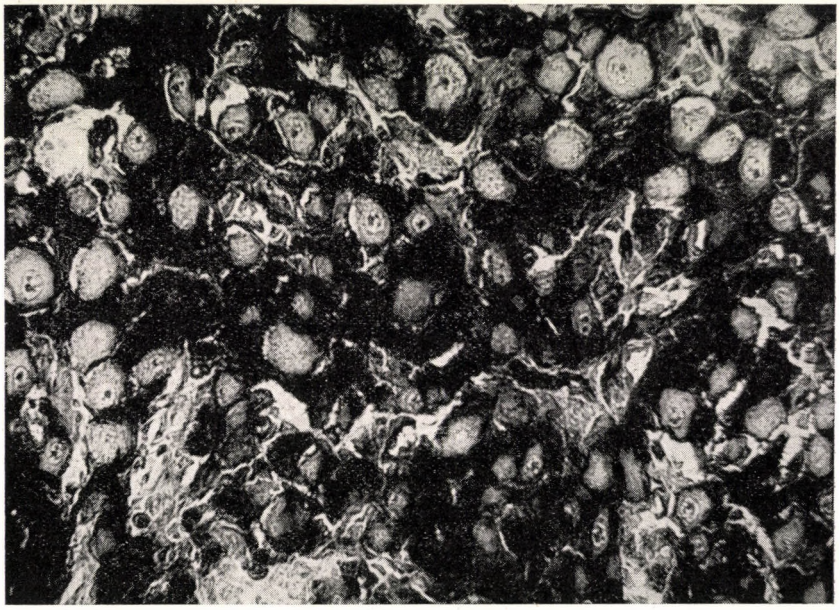

Fig. 1. Superior cervical ganglion, rabbit. Incubation: 10 min. at pH 9,4 at 37° C.

No differences were found, as regards localisation of the enzyme, between the superior and inferior cervical and coeliac ganglions.

The nerve tissue is regarded in literature as one containing but small quantities of alkaline phosphatase. That is why it was surprising to see a marked phosphatase reaction after the incubation for 30—60 minutes. The marked reaction is indicative of a considerable enzymatic activity. For examination of enzymatic localisation, cuts incubated for 5—15 minutes, proved to be most suitable.

The connective tissue capsule of the ganglion and connective tissue bundles traversing the capsule are free of any enzymatic activity. On the other hand some parts in the ganglion contain great quantities of the enzyme (Fig. 1, 5, 6). The plasma of the nerve cells cannot be regarded as free of enzymatic activity since a slight granular reaction is displayed there too. The reaction is appearently

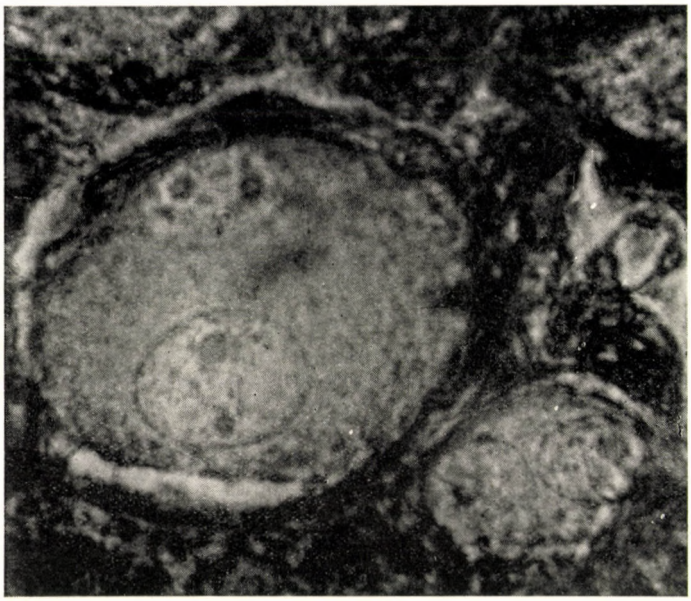

Fig. 2. Nerve cell from the ganglion with capsular cell. Magnification 1200×. Magnification of a party of Fig. 1.

more marked in the peripherial parts of the nerve cell protoplasm, where there are Nissl granula in a greater number too (Fig. 2, 3). The dendrits of the nerve cell do not contain phosphatase, therefore no enzymatic reaction is displayed by them. The plasma of different nerve cells does not display differences in the intensity of the phosphatase reaction that could be taken seriously. There are no differences in the tingibility of the cells comparable to those found in hematoxylin-eosin specimens (dark and bright cells). A markedly positive reaction is found both in the membrane of the nucleus and in the nucleolus. Within the nerve cells the most marked reaction is displayed by the nucleolus. The nucleolus and nucleus-membrane are well stained in those specimens too where prior to the reaction, the enzyme was inactivated by heat, etc.; in these specimens the reaction is surely due to an aspecifical linkage of the lead-ions. Taking into consideration this non-specific lead fixation, a high enzymatic activity of the nucleolus is nevertheless, obvious. It is a well-known fact that the enzyme is contained in greatest quantity in those tissue elements which display the highest non-specific fixation of lead, and *vice versa (Newman* et al.*)* Within the nucleus a few granula with enzymatic activity are to be seen. The reaction of binuclear nerve cells is similar.

The greatest quantities of alkaline phosphatase are found in the sympathetic ganglions in the layers around the nerve cells. All parts of the ground-

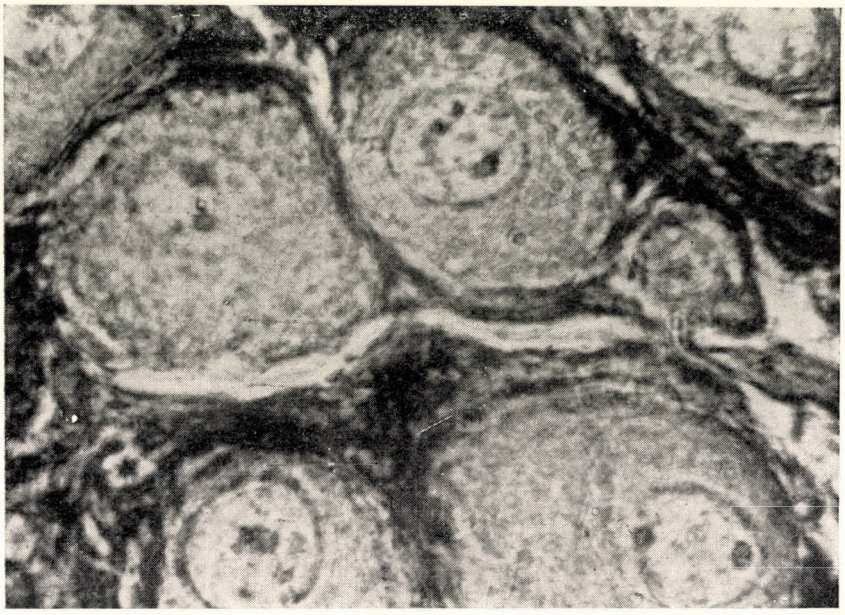

Fig. 3. Another group of nerve cells from the same specimen.

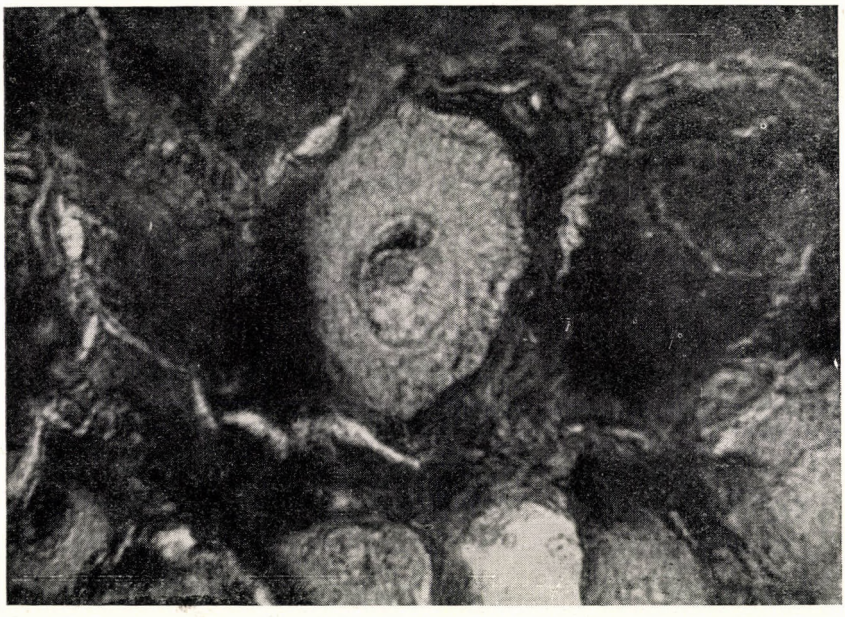

Fig. 4. Nerve cell from the inferior cervical ganglion, rabbit. Marked reaction of interstitium.

substance display an equally high enzymatic activity, to which the typical phosphatase reaction in the sympathetic ganglions is due. A positive reaction is, thus, given by the nerve fibers, by the nerve seaths, the capsular cells adjacent to the ganglions, and the interstitial glia cells in a somewhat greater distance. The reaction of these elements is so marked that a differentiation between them is hardly possible (Fig. 4). The capsular cells adjacent to the ganglion do not display any difference from interstitial cells, similarly to their behaviour in cuts prepared in other ways. No different reaction is seen between the plasma of

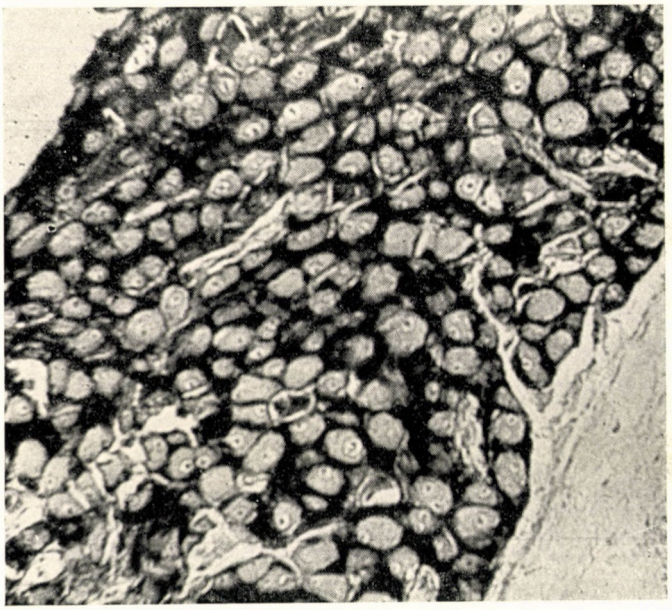

Fig. 5. Phosphatase reaction in the coeliac ganglion, rabbit. There is no enzymatic reaction in the connective tissue.

capsular and glia cells. Accordingly it cannot be stated whether their plasma displays the positive reaction or whether this semblance is due to traversing fibers containing the enzyme. It is, on the other hand, clearly seen, that the capsule of the nerve cell displays a totality of enzymatic activity. Since according to *Kuntz* and *Sulkin*'s statements the glious capsule does not contain anything else than cellular elements, one is justified to state that the plasma of glia cells contains phosphatase too. The interstitial glia cells in the layer between the nerve cells display a marked reaction. The uniformity of the phosphatase reaction of the glia and of the capsular cells is indicative of the non-different nature of these elements.

As already briefly mentioned, a marked reaction is seen in inactivated specimens too, in the nucleus membrane and in the nucleolus of the nerve cells. A tinction is seen in the nuclei of the fibrocytes in the capsule around the ganglion too, while the capsule itself is phosphatase-negative. In inactivated specimens differences are discernible among the plasma of the single nerve cells. It is to be stressed that the reaction of the vascular endothelium — which is generally regarded as of high phosphatase-activity — is less marked than the reaction in the interstitium.

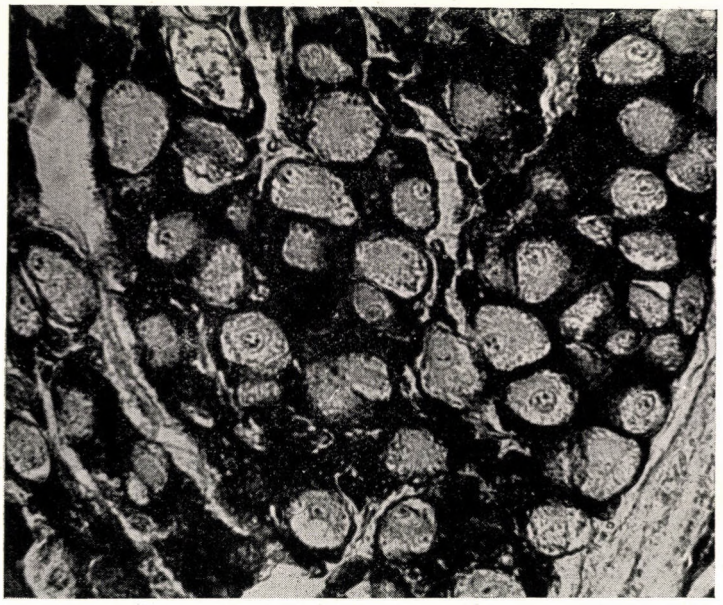

Fig. 6. Coeliac ganglion, rabbit. The connective tissue capsule and connective tissue bundles intruding into the ganglion are free of phosphatase.

2. *Differences in the alkaline phosphatase activity between superior and inferior cervical and coeliac ganglions.* In examining this problem, the quantitative histochemical reaction, relying upon radioactive lead tracer, as mentioned in the methodical part, has been used. The experimental results are summarised in Table I. The enzymatic activity of the rabbit in the superior cervical and coeliac ganglion is expressed in Pb γ/mm^3. tissue. The data of the table display differences between the two aforementioned ganglions amounting up to 50 per cent., the activity in the superior cervical ganglion being more marked. The difference seems to be statistically probably significant ($S = 3{,}1$). A similar difference is found between the superior and inferior cervical ganglions in the cat.

Table I.

The phosphatase activity of the sympathetic ganglions in the rabbit and in the cat, measured in Gamma Pb for 1 mm³ tissue. Incubation at 37° C, at pH 9,4 in a 1 per cent. sodium gylcerophosphate veronal buffer solution

	Phosphatase activity Pb γ/mm³ tissue of ganglion			
	cervicale sup. in control rabbits	coeliacum in control rabbits	Remark	Sign. diff. (S)
In controll rabbits	247,8	115,6	of 2 kg weight	
	253,7	—	1,5 « «	
	147,6	96,3	2 « «	
	129,9	80,5	0,5 « «	
	149,3	—	2 « «	
	120,3	86,0	1,5 « «	
	151,7	120,9	1,5 » «	
	172,9	99,8	Mean	3,1
	57,5	17,8	Standard deviation	
In pregnant rabbits	294,7	117,5	2—3 weeks pregnant	
	236,7	80,0	4 « « (approx.)	
	212,5	150,8	4 « «	
	179,7	152,2	at the end of pregnancy	
	201,3	153,2	at the end of pregnancy	
	201,0	152,0	1—2 weeks pregnant	
	195,0	137,0	early pregnancy	
	217,3	134,9	Mean	4,6
	12,1	27,4	Standard deviation	
	1,7	2,7	Sign. diff. between controll and pregnant group (S)	

The significance of difference is computed :

$$S = \frac{M_1 - M_2}{\sqrt{\delta M_1 - \delta M_2}}$$

Changes in the phosphatase activity of sympathetic ganglions during pregnancy. By chance, the ganglion of a pregnant rabbit was also examined. As it seemed to contain — in comparison with non-pregnant rabbits — increased quantities of phosphatase this problem was examined more closly. The enzymatic activity of 7 pregnant rabbits and two pregnant cats was examined. The results are summarised in Table 1. It is appearant from the data that ganglions of pregnant animals display a higher enzymatic activity. The enzymatic activity of 7 non-pregnant rabbits amounts on the average to 172,9 units (as designed by us) whereas the average enzymatic activity value of the 7 pregnant rabbits is 217,2 Pb γ/mm³. The significant difference amounts to : 1,7 which is of borderline importance. In pregnant animals a higher enzymatic activity

is found in the coeliac ganglion too. This amounts in the controlls to 99,8, in pregnant animals on an average to 134,9. The difference is here more marked, the significant difference 2,7. Similar differences were found between the ganglions of pregnant and non-pregnant cats too. The differences between the enzymatic activity of the superior cervical and coeliac ganglions is demonstrable in pregnant animals, too, it seems in this case rather more marked. The enzymatic activity of superior cervical ganglion in pregnant rabbits amounts to 217,3 of the coeliac ganglion to 134,9 Pb γ/mm^3 The significant difference is : 4,6.

As demonstrated in Table II., there is some difference between male and female animals too as regards phosphatase activity of the ganglions. However, this problem was not further examined.

Table II.

Phosphatase activity Pb γ/mm^3 tissue of ganglion		
cervicale sup. in *control* cats	cervicale inf.	Remark
282,9	172,4	Male
228,6	139,7	Male
190,0	143,0	Female, non pregnant
234,1	151,7	Mean
323,6	253,0	Pregnant
349,4	220,9	Pregnant
336,5	236,9	Mean

Discussion

The role of phosphatase in cellular metabolism is only partly known. It should not be forgotten that the role of phophatase will not be understood before the functions of the total enzymatic system are disentangled of which the phosphatase is but a part only *(Moog)*. In the ganglions phosphatase plays an important role in metabolic process, so first of all in hydrolysis. The connections between phosphatase and nuclein acid metabolism were also demonstrated *(Jeener)*. Some connection between phosphatase and Nissl granula, as observed by us, was assumed by *Sulkin* and *Kuntz* too. The fact that the greatest activity was demonstrated in the interstitium, is indicative of the important role of glia

cells in preserving the normal function of the nerve cells. Interstitial glia cells, and first of all capsular cells suffer some changes while the nerve cell exerts any action, as has been demonstrated by *Kuntz* and *Sulkin* after irritation of preganglionary sympathetic fibres. Both in the nerve and in the glia cells vitamin C has been demonstrated by *Sulkin* and brought in connection with the Golgi matter by him.

The differences found in the enzymatic activity between upper and lower cervical and coeliac ganglions can be explained in several ways. Probably this finding is partly due to the fact that in the coeliac and inferior cervical ganglion less interstitium is contained than in the upper cervical one. The aforementioned two ganglions contain a larger number of nerve cells, with a poor enzymatic activity. This is a satisfactory explanation of the demonstrated differences. Nevertheless, there are possibly real differences in the enzyme contents, due to the functional differences. This problem is naturally not easy to solve.

A problem of similar difficulty is that of the higher enzymatic activity in the ganglions of pregnant animals. During pregnancy a hyperphosphatasemia occurs, as described multitudinously *(Burthiault, Heredia, Coryn, Du Voil,* etc., see at *Baur)*. This may cause in some way the increased activity. On the other hand, it is well known too that changes of the hormonal equilibrum may activate phosphatases. So the gonadotropic hormone causes a marked rise in the phosphatase contents of the femur in the rat *(Moog)*. The changes in the enzymatic activity may due in this case also to some structural change, i. e. to an increase of the interstitium of the ganglions during pregnancy.

The problem of the different enzymatic activity of male and female animals needs further experimental investigations ; similarly the problems what caused the scattering of the activity values in our material within the single groups, what the effect of age, of the stage of pregnancy etc. It is noteworthy that *Barr* found sexualdimorphic differences in the nerve cells and in the ganglions.

Some change in the enzymatic activity of the ganglions occurs in relation to age. We want to stress that no enzymatic activity was demonstrable in the sympathetic ganglions of rabbit-embryo. Our further investigation of this problem are in progress. By this observation attention is called to some new interesting chemical problems of histogenesis.

Summary

The phosphatase activity in the sympathetic ganglions of the cat, rabbit and guinea pig has been examined histochemically and by a quantitative histochemical tracer method. The localisation of the enzyme is exactly described. A higher enzymatic activity of the superior cervical ganglion in comparison with the inferior cervical and coeliac ganglion is stated. This is probably due to structural differences. During pregnancy some increase of the enzymatic activity occurs in the sympathetic ganglion.

REFERENCES

1. *Barka T., Szalay S., Pósalaky Z.* és *Kertész L. :* 1951. Quantitatív histokémiai phosphatase meghatározás rádióaktív ólom felhasználásával. Kísérletes Orvostudomány. III. évf. 1. sz.

2. *T. Barka, S. Szalay, Z. Pósalaky* and *L. Kertész :* 1951. Quantitative histochemical demonstration of phosphatase by the help of radioactive lead. (In the press.)

3. *M. L. Barr, E. G. Bertram :* 1949. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature. Vol. 163. No. 4148. 676.

4. *H. Baur :* 1948. Tierische Phosphatasen. Physiologie, pathologische Physiologie und klinische Bedeutung. Z. f. Vitamin-, Hormon- und Fermentforschung. Bd. 2. 49. 507—596.

5. *G. Bourne, MacKenzie, MacKinnon:* 1943. The distribution of alkaline phosphatase. in various tissues. Quart. J. of exp. Physiol. Vol. 32. No. 1. 1—21.

6. *R. Burthiault, R. Berger, J. Gonnet :* 1948. Étude de la phosphatasémie au cours des avortemens. Gynécologie et Obstétrique. 47/3. 347—48. (Excerpta Med. Obstr. and Gyn. Sec. X. 2566 §).

7. *G. Gömöri :* 1941. The distribution of phosphatase in normal organs and tissues J. of Cell and comp. Physiol. Vol. 17. 71—85.

8. *J. L. Heredia :* 1948. Fosfatasemia y fosfatemia durante el parto y en el cordon umbilical en el momento de nacer. Obstetricia y Ginecologie Latino-Americanas 6/8. 345—55 (Exc. Med. Sec. X. 1949 : 2147 §).

9. *R. Jeener :* 1946. Sur le liens de la phosphatase alcaline avec les nucleoproteides du noyau cellulaire et des granules cytoplasmiques. Experientia. Vol. II. No. 11. 458.

10. *A. Kuntz, N. M. Sulkin :* 1947. Components of supporting tissue and ganglion cell capsules in autonomic ganglia. Anat. Rec. Vol. 97. 395.

11. *A. Kuntz, N. M. Sulkin :* 1947. The neuroglia in the autonomic ganglia : cytologic structure and reactions to stimulation. J. of comp. Neurol. Vol. 86, No. 3. 467—477.

12. *H. Landow, E. A. Kabat, W. Newmann :* 1942. Distribution of alkaline phosphatase in normal and in neoplastic tissues of the nervous system. Arch. of Neurol. and Psychiatr. Vol. 48. 518—530.

13. *F. Moog :* 1946. The physiological Significance of the phosphomonoesterases. Biol. Rev. 21. 41—59.

14. *W. Newmann, E. A. Kabat, W. Abner :* 1950. Histochemical studies on tissue enzymes. V. A. Difficulty in enzyme localisation in the acid range due to selective affinity of certain tissues of lead, its dependence on pH. Am. J. Path. Vol. XXVI. No. 3. 489—503.

15. *N. M. Sulkin, A. Kuntz :* 1950. A histochemical study of the autonomic ganglia of the cat following prolonged preganglionic stimulation. Anat. Rec. Vol. 108. No. 2. 255—279.

16. *N. M. Sulkin, A. Kuntz :* 1948. Distribution of ascorbic acid in autonomic ganglia and its alteration in experimental and pathologic states. Anat. Rec. Vol. 101. No. 1. 33—47.

17. *H . Takamatsu :* 1939. Histochemische Untersuchungen der Phosphatase und deren Verteilung in verschiedenen Organen und Geweben. Transactions Soc. Path. Jap. 29. 492—498.

СОДЕРЖАНИЕ ЩЕЛОЧНОГО ФОСФАТАЗА В СИМПАТИЧЕСКИХ НЕРВНЫХ УЗЛАХ И ИЗМЕНЕНИЯ ЭТОГО СОДЕРЖАНИЯ В ПЕРИОД БЕРЕМЕННОСТИ

Б. Арош, Т. Барка, З. Пошалаки, и Г. Герецe

Резюме

Авторы исследовали содержание энзима (щелочного фосфатаза) в симпатических узлах кошки, кролика, и морской свинки. Исследования производились отчасти путем гистохимических методов, а отчасти — с употреблением радиоактивных изотопов — путем количественных гистохимических методов. Авторы подробно описывают разположение энзима в узлах. Они устанавливают, что верхний шейный узел содержит больше энзима чем нижний шейный узел или чревный узел. Это обстоятельство по всей вероятности связано с различным строением узлов. В период беременности энзиматическая активность симпатических узлов несколько повышается.