FOLIA PARASITOLOGICA (PRAHA) 28: 359–364, 1981.

HISTOCHEMISTRY OF POLYCEPHALIC LARVA OF HYDATIGERA KREPKOGORSKI (SCHULZ ET LANDA, 1934)

J. SCHRAMLOVÁ, Z. ŽĎÁRSKÁ and L. I. LAVROV*

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, and* Zoological Institute, Academy of Sciences of the Kazakh SSR, Alma-Ata

Abstract. Histochemical studies of polycephalic larva of *Hydatigera krepkogorski* (Schulz et Landa, 1934) revealed the presence of acid mucosubstances in microtriches and neutral mucosubstances, proteins with sulphydryl and disulphide groups, tyrosine, tryptophan, histidine and phospholipids in the tegument. The scolex parenchyma contained a large amount of glycogen, whereas the glycogen content in the bladder was very low in this stage.

This paper is a continuation of our previous studies on some enzymes in the larva of *H. krepkogorski* (Schramlová et al. 1980). Although the uni-scolex larvae have been studied in detail, there are only few papers dealing with the histochemistry of polycephal larvae, as those by Race et al. (1965) in *Multiceps serialis* and by Hulínská et al. (1976, 1978a, b) in *Multiceps multiceps* and *Multiceps endothoracicus*. Results of some histochemical reactions in a developmental stage of *H. krepkogorski* larva are summarized in the present paper.

MATERIAL AND METHODS

The larva of *H. krepkogorski* (Schulz et Landa, 1934) originated from ventral cavity of spontaneously infected *Rhombomys opimus* Lichtenstein, 1923. It was a bladder with 15 scoleces on the periphery and was enclosed in a cyst.

The material was fixed in Baker's neutral formol and embedded in paraffin using a standard technique.

The presence of various mucosubstances was detected by PAS reaction (Pearse 1960) combined with acetylation, desacetylation and saliva test for neutral mucosubstances. Alcian blue (Alcianblau 8 GS Fluka), pH 2.6 (Quintarelli et al. 1964) was used for the detection of acid mucosubstances. Alcian blue (pH 2.6) (Scott et al. 1964, Quintarelli et al. 1964) combined with methylation after Fisher and Lillie (1954) and demethylation (Spicer and Lillie 1954) was employed for a detailed differentiation of acid mucosubstances. Neutral and acid mucosubstances were demonstrated by modified AB-PAS reaction by Mowry. DDD (2,2-dihydroxy-6,6-dinaphthyldisulphide) test (Barnett and Seligman 1954) and a control with N-ethylmaleinimide blockade were used for detecting the proteins with SH groups. SS groups were demonstrated by DDD test combined with thioglycolic acid (Pearse 1960) and PFA-AB (performic acid — Alcian blue) after Pearse (1960) using Alcian blue (pH 0.2) control. Tyrosine was detected by Morel-Sisley's diazotization method after Lillie (1957), tryptophan with DMAB (dimethylaminobenzaldehyde) after Adams (1957). Tetrazonium reaction modified after Müller and Chytil (1962) was used as a group test for the detection of tyrosine, tryptophan and histidine.

RESULTS

Results of histochemical reactions used for the elucidation of histochemical nature of individual structures in *H. krepkogorski* larva are summarized in Tables 1 and 2. The morphology of this larva was described elsewhere (Schramlová et al. 1980).

Table 1. Results of histochemical reaction for identification of mucosubstances of *Hydatigera krepkogorski*

Reactions	Scolex										Bladder			
	Rostellar tegument		Sucker tegument		Tegument of remaining scolex part						Bladder tegument			
	microtriches	homogeneous layer	microtriches	homogeneous layer	microtriches	homogeneous layer	subtegumental cells	calcareous bodies	globules in rostellar part	parenchyma cells	microtriches	homogeneous layer	subtegumental cells	parenchyma cells
PAS	–	++++	–	++++	–	+++	++	–	++++	+++	–	+++	++	+++
Schiff	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Saliva test + PAS	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Acetylation 58 °C 48 hr + PAS	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Desacetylation + PAS	–	++	–	++	–	(+)	–	–	++(+)	++	–	(+)	–	+
AB—PAS	blue ++++	rose +++	blue ++++	rose ++++	blue ++++	rose +++	rose +++	–	rose ++	rose ++	blue +++	rose ++	rose ++	rose +
AB pH 2.6	++++	–	+++	–	+++	–	–	–	–	–	+++	–	–	–
AB pH 2.6 + methylation	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AB pH 2.6 + demethylation	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Best's carmin	–	–	–	–	–	–	+	++++	–	++++	–	–	–	+
Hale	++++	–	++++	–	+++	–	–	–	–	–	++	–	–	–

A. HISTOCHEMISTRY OF SCOLEX PORTION

The mucosubstances in microtriches of scolex were detected by Alcian blue (pH 2.6) staining. This reaction suggested the presence of acid mucosubstances, which was confirmed also by the positive result of Hale's raction. As the Alcian blue staining after methylation and demethylation was negative, it is supposed that sulphogroups (HSO_3) and not carboxyl groups are responsible for the acid character of the mucosubstances. During the detection of proteins and their components the microtriches were found to contain a small amount of sulphydryl groups. The reaction was more pronounced if a method sensitive to both sulphydryl (SH) and disulphide (SS) groups was used. Several reactions (Morel-Sisley, TK, DMAB) demonstrated the presence of tyrosine, tryptophan and histidine.

The homogeneous layer of tegument was PAS-positive even after saliva test, which indicated the presence of neutral mucosubstances. The blue staining obtained with Luxol blue, Sudan III B and Giemsa showed the presence of phospholipids. The reaction for SH groups was weak, but that for SS groups was somewhat stronger. The homogeneous layer of tegument was found to contain also tyrosine and tryptophan. The tegument of suckers was the only site where histidine was detected together with tyrosine, tryptophan and phospholipids which showed a higher percentage of positivity than in the remaining tegument. The subtegumental cells contained neutral mucosubstances and gave a weak reaction for tyrosine, tryptophan and SS and SH groups. A small amount of glycogen was here detected by Best's reaction.

The parenchyma portion contained a large amount of glycogen, as determined by Best's test. Dispersed calcareous bodies were positive in Best's reaction for glycogen, the reaction being most intensive in young calcareous bodies. The age of these bodies could be most easily differentiated in Luxol blue and Sudan III B, where the older calcareous bodies were intensively stained blue and in Sudan III B they possessed a dark blue coat on the surface.

Under the tegument of the rostellum was a zone of PAS-positive globules which were positive even in reactions for tyrosine, tryptophan and SS groups of proteins.

B. HISTOCHEMISTRY OF BLADDER WALL

Compared to the scolex portion, the microtriches of the bladder contained a small amount of acid mucosubstances with HSO_3 groups and gave a very weak reaction for tyrosine and tryptophan. Other reactions for phospholipids and proteins with SH and SS groups were so weak that they could be hardly regarded as positive. The remaining tegument was again a homogeneous, non-differentiated layer, which was PAS-positive and contained phospholipids, tyrosine and tryptophan. The presence of SH groups and, particularly, SS groups of proteins was also detected. The subtegumental cells were weakly PAS-positive and contained small amounts of tryptophan and tyrosine. The parenchymal portion, compared to the scolex portion, contained a relatively small amount of glycogen, as revealed by Best's reaction.

DISCUSSION

The results of our histochemical studies agree with the previous histochemical studies of other cestodes.

The tegument contained phospholipids, a small amount of SS and SH groups of proteins, tryptophan, tyrosine and neutral mucosubstances; acid mucosubstances were associated with the microtriches. Acid mucosubstances could be derived from neutral mucosubstances of the tegument. Some authors as Lumsden (1966) associated the

Table 2. Results of histochemical reactions for identification of proteins and phospholipids of *Hydatigera krepkogorski*

Reactions	Scolex										Bladder			
	Rostellar tegument		Sucker tegument		Tegument of remaining scolex part						Bladder tegument			
	microtriches	homogeneous layer	microtriches	homogeneous layer	microtriches	homogeneous layer	subtegumental cells	calcareous bodies	globules in rostellar part	parenchyma cells	microtriches	homogeneous layer	subtegumental cells	parenchyma cells
DMAB	++	++++	+++	++++	++(+)	+++	+++	–	+++	–	+	++	+	+
Coupled tetrazonium reaction	+++	++	++++	++++	++	+	++	–	+++	++	++	+++	++	++
Morel-Sisley	+	++	++	+++	+	+	++	–	++	+(+)	+	++	+	–
DDD	+	+	++	+++	+	+	++	–	–	++	–	+	–	–
Thioglycollic acid + DDD	++	+++	+++	+++	+	++	+++	–	+	++	–	++	–	+
PFA — AB	–	–	–	–	–	–	–	+	–	–	–	–	–	–
AB pH 0.2	++	–	++	–	+	–	+	+	+	+	++	–	+	–
Peracetic acid + aldehyde fuchsin	+	–	+	–	+	–	+	+	+	+	–	–	–	–
Luxol blue	+	++++	+	++++	+	++++	++++	++++	–	++	–	+++	++	–
Sudan black III	–	++++	–	++++	–	++++	++++	++++	–	++	–	+++	++	–
Giemsa	–	++++	–	++++	–	+++	+++++	+++++	–	–	–	++++	+++	–

presence of acid mucosubstances in microtriches with the pinocytic activity. In our case it would explain the different intensity of the reaction for acid mucosubstances in the microtriches of scolex, which is stronger than the reaction in microtriches of bladder. On the other hand, these substances can form micellar colloids on the surface of the tegument and enable thus the penetration of small molecules of aminoacids and monosaccharides, whereas the large molecules of the host enzymatic proteins are filtrated away. This again indicates that at this stage of development the bladder is already a rudimentary organ and therefore the amount of acid mucosubstances is much smaller than in the microtriches of the scolex.

Hulínská et al. (1978) demonstrated tyrosine, tryptophan and phospholipids also in the tegument of rostellum in *M. multiceps* and *M. endothoracicus*. The tegument of *H. krepkogorski* larva differed only in the positive reaction of sucker tegument for SS groups of proteins. Hulínská et al. (1978a) described globules with PAS-positive mucosubstances and tyrosine on the tegument surface in both species. The authors assume that the globules are produced by the secretory activity of rostellar tegument. In our case the globules did not get to the surface of rostellum, but formed a zone directly under the tegument of the rostellum. They were rich in PAS-positive mucosubstances and contained also tyrosine and proteins with SS groups. The intensity of histochemical reactions of calcareous bodies was associated with their age. This has already been stressed by Žďárská (1975) who detected, like Nieland and von Brand (1969), acid mucosubstances and strong positivity in Luxol blue reaction in the centre of young calcareous bodies. In our case the calcareous bodies never contained acid mucosubstances. It cannot be determined whether this difference in the histochemical nature is related with the species or age of the lavra, as only one developmental stage was studied. Hulínská et al. (1978b) described histochemical reactions in bladder tegument of various developmental stages of *Multiceps endothoracicus* larva. Young stages exhibited a great accumulation of acid mucosubstances with HSO_3 groups both in microtriches and in the homogeneous layer of tegument and, on the contrary, a very small amount of phospholipids. In older stages, when the bladder is reduced, the positivity for acid mucosubstances decreases and the amount of phospholipids increases. The reaction for tyrosine and tryptophan was also weaker in the tegument of young stages than in the tegument of partly reduced mother bladder, whereas the positivity of reaction for disulphide groups of proteins increased. We have studied only one stage and this comparison suggests that the stage with reduced mother bladder was involved. The tegument contained a large amount of phospholipids, PAS-positive mucosubstances, tyrosine, tryptophan and proteins with SS groups, but acid mucosubstances were not demonstrated. The low content of glycogen in the bladder wall supports this opinion as well. Most probably the glycogen is deposited in the parenchyma of scolex portion in this larva and not in the bladder wall like in *Cysticercus bovis* (Žďárská 1975). Hulínská et al. (1978b) demonstrated a large amount of glycogen in the bladder wall of young larvae of *M. endothoracicus*, but no glycogen was found in the bladder walls of older stages.

ГИСТОХИМИЯ ПОЛИЦЕФАЛЬНОЙ ЛИЧИНКИ *HYDATIGERA KREPKOGORSKI* (SCHULZ ET LANDA, 1934)

Я. Шрамлова, З. Ждярска и Л. И. Лавров

Резюме. При изучении полицефальной личинки *Hydatigera krepkogorski* (Schulz et Landa, 1934) с помощью гистохимических методов обнаружены кислые мукосубстанции в микротрихах и нейтральные мукосубстанции, белки с сульфгидрильными и дисульфидными

группами, тирозин, триптофан, гистидин и фосфолипиды в тегументе. Паренхима сколекса содержала большое количество гликогена, тогда как содержание гликогена в пузырке было очень низко у этой стадии.

REFERENCES

BARRNET R. J, SELIGMAN A. M., Histochemical demonstration of sulphydryl and disulphide groups of protein. J. Nat. Cancer. Inst. 14: 769—803, 1954.

FISHER E. R., LILLIE R. D., The effect of methylation on basophilia. J. Histochem. Cytochem. 2: 81—87, 1954.

HULÍNSKÁ D., FEDOSEENKO V. M., LAVROV L. I., Histochemistry and fine structure of the bladder tegument of a larval *Multiceps endothoracicus*. Folia parasit. (Praha) 25: 149—155, 1978a.

—, LAVROV L. I., GUBAYDULIN N. A., Comparative study on the histochemistry and the fine structure of the suckers and the rostellum of larvae of *Multiceps multiceps* Leske, 1780 and *Multiceps endothoracicus* Kirschenblatt, 1948. Folia parasit. (Praha) 25: 247—255, 1978b.

—, ŽĎÁRSKÁ Z., GUBAYDULIN N. A., Localization of some enzymes in polycephalic larvae of two species of the genus *Multiceps* (Cestoda). Folia parasit. (Praha) 23: 227 to 236, 1976.

LUMSDEN R. D., Cytological studies on the absorptive surfaces of cestodes. I. The fine structure of the strobilar integument. Z. Parasitenkd. 27: 355—382, 1966.

MÜLLER J., CHYTIL F., Introduction into the histochemistry of proteins. Stát. zdrav. naklad., Praha, 1962. (In Czech.)

NIELAND M. L., von BRAND T., Electron microscopy of cestode calcareous corpuscle formation. Exp. Parasitol. 24: 279—289, 1969.

PEARSE A. G. E., Histochemistry theoretical and applied. J. A. Churchill Ltd., London, 1960.

QUINTARELLI G., SCOTT J. E., DELLOVO M. C., The chemical and histochemical properties of alcian blue. III. Chemical blocking and unblocking. Histochemie 4: 99 to 112, 1964.

RACE J., LARSH J. E., ESCH G. W. Jr., MARTIN J. H., A study of the larval stages of *Multiceps serialis* by electron microscopy. J. Parasitol. 51: 364—369, 1965.

SCOTT J. E., QUINTARELLI G., DELLOVO M. C., The chemical and histochemical properties of alcian blue. I. The mechanism of alcian blue staining. Histochemie 4: 73—85, 1964.

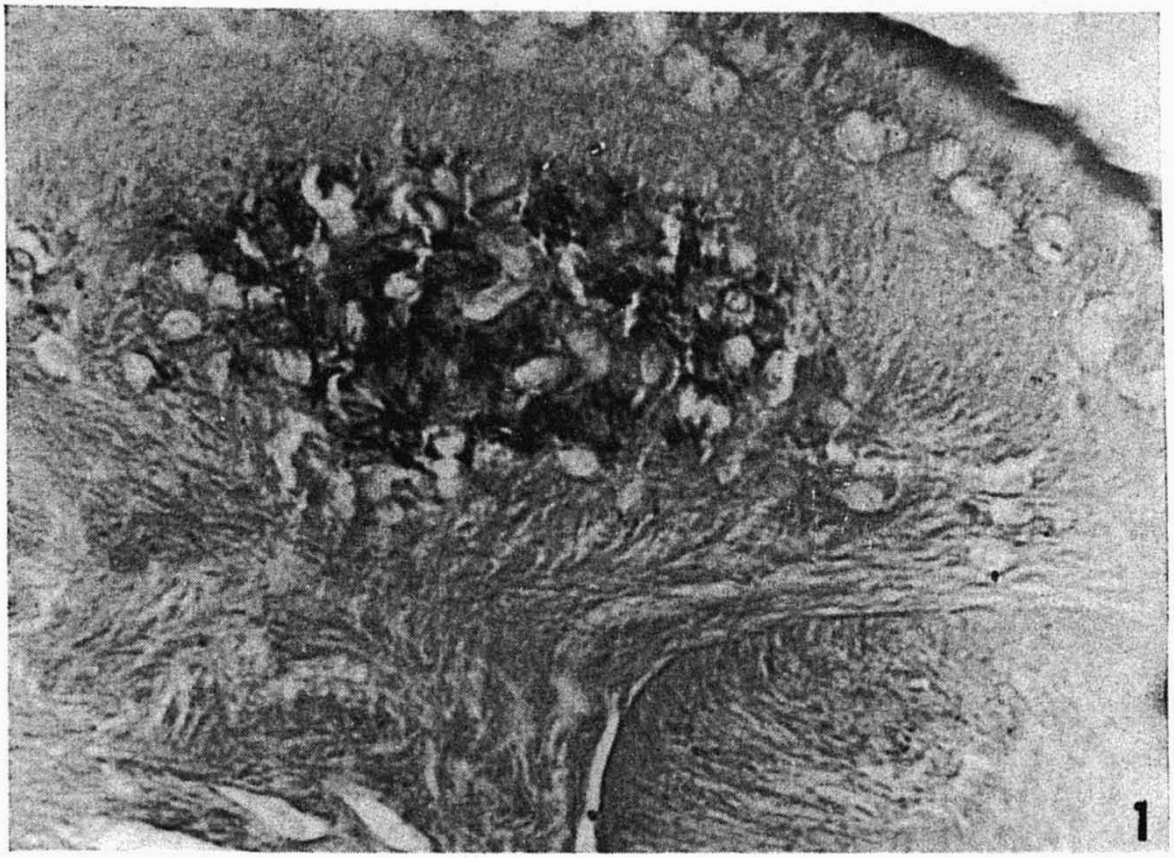
SCHRAMLOVÁ J., ŽĎÁRSKÁ Z., LAVROV L. I., Localization of some enzymes in polycephalic larva of *Hydatigera krepkogorski* (Schulz et Landa, 1934). Folia parasit. (Praha) 27: 47—51, 1980.

SPICER S. S., LILLIE R. D., Saponification as a means of selectively reversing the methylation blockade of tissue basophilia. J. Histochem. Cytochem. 7: 123—125, 1959.

ŽĎÁRSKÁ Z., Histochemistry of the parenchyma of *Cysticercus bovis*. Folia parasit. (Praha) 22: 345—354, 1975.

Received 4 February 1981.

J. Sch., Parasitologický ústav ČSAV,
Flemingovo nám. 2, 166 32 Praha 6,
ČSSR



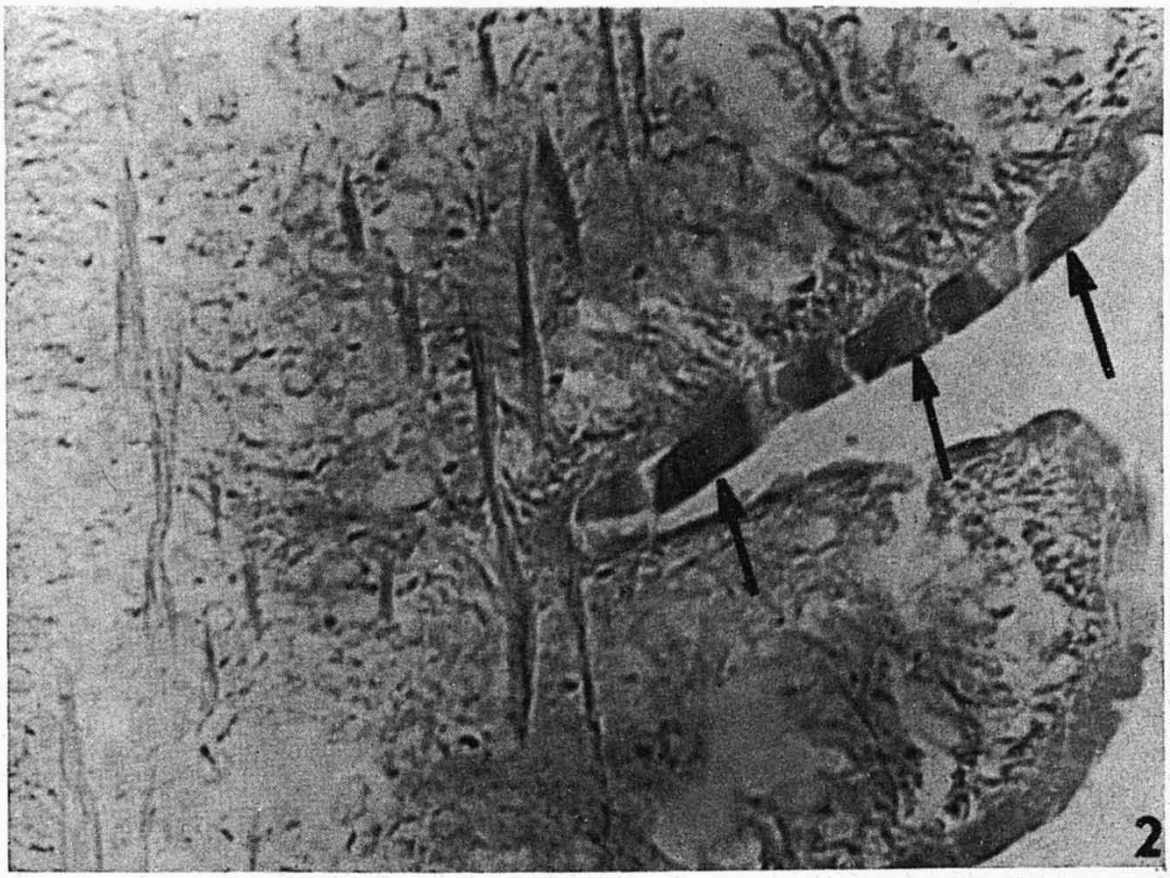
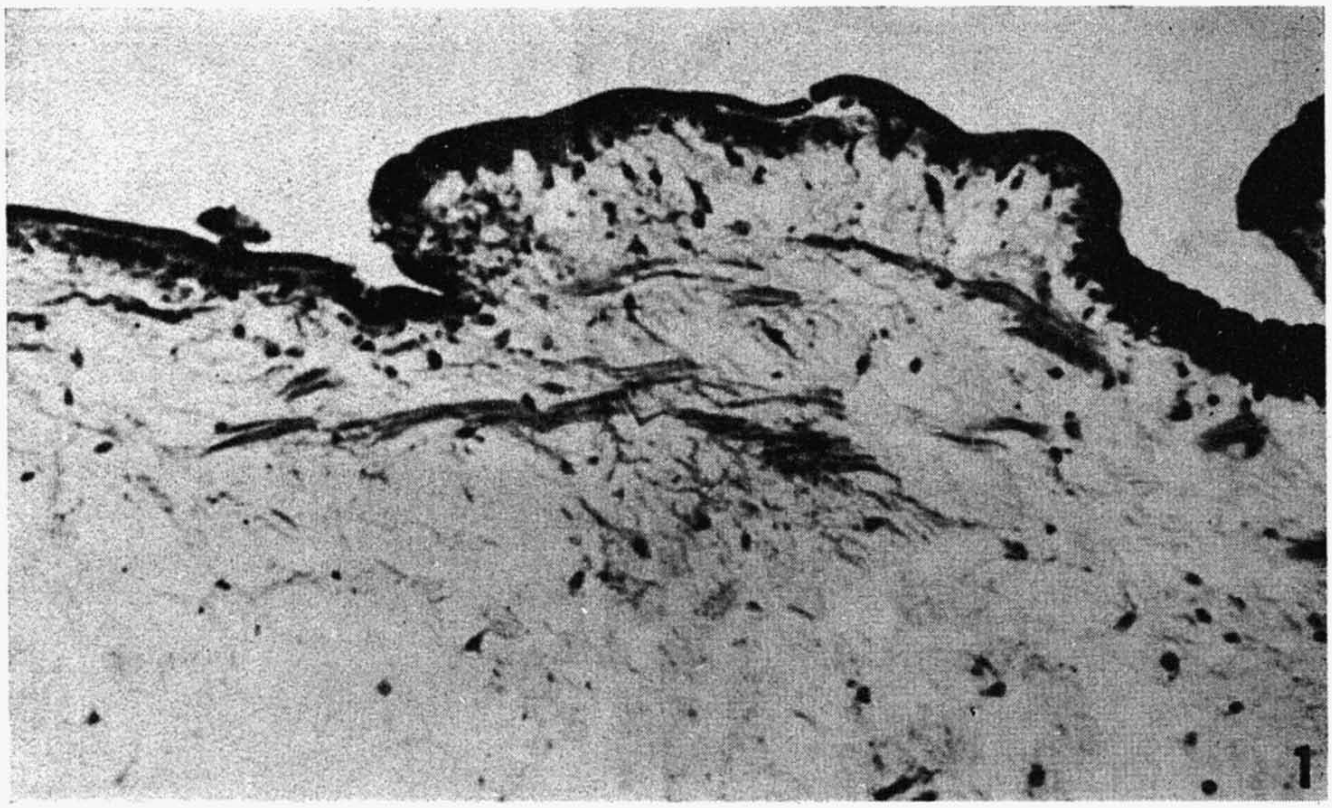


Fig. 1. Globules in rostellar portion of larva of *Hydatigera krepkogorski*. AB-PAS (250×). **Fig. 2.** Detection of acid mucosubstances in microtriches of tegument in the scolex portion. AB-PAS (350×).



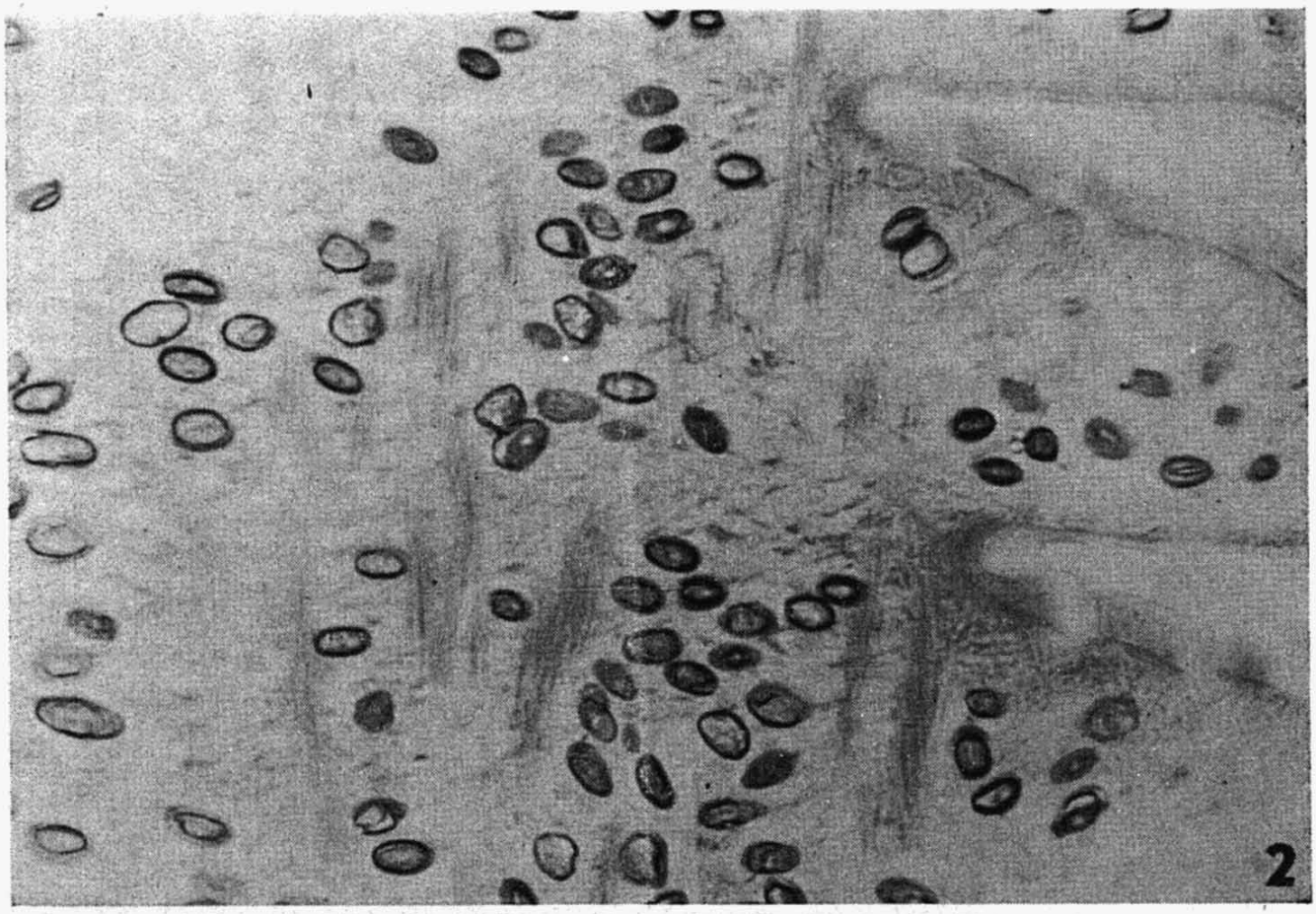


Fig. 1. Tangential section through the wall of bladder. Tetrazonium copulation (250×). **Fig. 2.** Calcareous bodies in the scolex portion of larva of *Hydatigera krepkogorski*. Best's carmine (250×).