

HISTOLOGICAL STUDIES OF THE MUSCULAR TISSUE IN *TRICHINELLA SPIRALIS* INFECTION

BY

JAN ZARZYCKI

Department of Histology and Embryology, Veterinary Faculty, Wrocław, Poland

The histochemical studies of the muscular tissue in *Trichinella spiralis* Infestation have been started in 1956 with observations of the behaviour of glycogen. Now, the distribution of nuclein acids and inorganic salts are dealt with.

The material was made up of white mice infested by the injection of about 40 Trichinellae. Beginning from the 5th day after infestation sections from the muscular portion of the diaphragm were taken for examination, first daily, from the 14th day for 3 weeks at some days' intervals and finally after a years period. The sections to be used in the test for the detection of nuclein acids were fixed in Carnoy's fluid. Desoxyribonucleinic acid (DNA) was detected by Feulgen's method, ribonucleinic acid (RNA) according to Brachet. The mice to be used for experiments on the distribution of inorganic compounds were divided into two groups: the animals of the first group were administered, beginning from the 14th day after infestation, a daily oral dosis of 300 international units of vitamin D<sub>2</sub>, 0,01 calcium carbonate and 0,01 calcium phosphate. The animals of the second group served as control. The sections from the diaphragmatic muscles of mice from both groups were fixed in the alcohol-formalin mixture and in Carnoy's fluid, the paraffin embedded sections, 3-5 thick, being burned in oven, type PE M-2 at about 550°C for 15 minutes; Spodograms were examined in dark field. The results obtained are as follows:

## 1. Distribution of nucleinic acids:

The amount and distribution of RNA are subject to clear-cut changes both in the larval body and muscular fibres, depending on the time period which had elapsed since infestation. With the appearance of larvae in the muscular tissue and their penetration into the muscular fibres, the amount of RNA is very slight not only in the muscular fibres but also in the cells of larves which at this time show a somewhat higher level of DNA. Within he half of the second week after infestation the content of DNA in the body of larves is reduced, whereas that of RNA is increased. The diffused staining, typical for this acid is also seen in the muscular fibres, which contain larves in areas involved by granulation. The concentration of RNA in muscular fibres shows a tendency to gradual increase up to the half of the 3d week after infestation. At this time it makes its appearance in the form of clumps; there after the amount of RNA decreases in a fairly rapid way, so that beginning from the 23d day after infestation, it almost disapears from the muscular fibres. Unlike is the behaviour of RNA in larves, where its content rapidly increases, reaching its maximum approximately by the 15th day after infestation, and persists on this level even after the formation of the capsule, surrounding the parasite; it occurs mostly within the cytoplasm of cells of the cellular body. A great amount of RNA also appears in the nucleoli which become enlarged in the muscular

fibres containing larvae. However, in distinction to the sarcoplasm, RNA, present in the nucleoli, persists for a long period of time, even after the formation of the capsule had been completed. As to DNA, its level in the larval body is maintained up to the half of the second week after infestation until the larvae become encapsulated. On the other hand, its amount in the nuclei of muscular fibres, which undergo multiplication and enlargement, shows a decreasing tendency. The cell nuclei of the infiltrate, present in the vicinity of larvae, show a high level of DNA; 2 weeks after infestation a considerable amount of RNA could be detected in the cytoplasm of cells, situated at the poles of the capsule; it was found to persist for some days.

The results obtained suggest that the process of protein synthesis in the *Trichinella* larvae, appearing in the muscular tissue, is inhibited or proceeds at a slow rate. Only when they settle down and in the muscular tissue find favourable life conditions, does the synthesis of proteins take an intense course. —

The increased RNA content in the sarcoplasm of the muscular fibres, into which larvae had penetrated, may be explained on the basis of some theories (Brachet, Trifonowa) which claim that a small degree of damage to cells accompanied by an increased level of RNA, an increase in damage to muscular fibre being followed by the disappearance of RNA.

## 2. Distribution of inorganic salts:

The appearance of larvae in the muscular fibres brought about no essential changes in the distribution of inorganic salts as compared with normal muscular tissue. Areas, containing *Trichinella* larvae, which showed a slight amount of inorganic salt deposits, while penetrating into the muscular tissue, were scarcely found. By the 12th day after infestation some muscular fibres were found to contain a few deposits; these areas corresponded with those showing a staining typical for RNA. Particularly conspicuous are white translucent deposits in the infiltrate cell nuclei and in the nucleoli of enlarged nuclei of the muscular fibres. Later, approximately by the 23d day after infestation the inorganic salt deposits were found to disappear from the neighborhood of larvae, whereas they could be found in great amount and for a long time in their body. The presence of inorganic salt deposits was also found within the capsule, its inner and outer layer being clearly distinguishable. In spodograms, prepared from the muscular sections of mice, treated with calcium salts and vitamin D<sub>2</sub>, the amount of deposits in the larval body and capsule is only slightly higher than in control groups, this occurring as late as 3 weeks after infestation.

Numerous deposits are to be seen in the close vicinity of the larvae, particularly at both poles of the capsule, whereas they are lacking in the control group.

The observations of spodograms confirm a generally accepted theory, claiming that the content of calcium deposits proportional to the reserve of nucleinic acids. The increase in the amount of deposits in the muscular sections of mice receiving calcium salts and vitamin D<sub>2</sub> imply a certain acceleration in the process which results in calcification of encapsulated *Trichinella* larvae.