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STUDIES ON SPECIES SPECIFICITY OF PROTEINS IN ASCARIS LUMBRICOIDES AND ASCARIS SUUM

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Abstract. The separate identity of *A. lumbricoides* and *A. suum* was confirmed by means of the disc electrophoresis method in that differences were established in the protein profile of somatic and ribosomal proteins. In addition differences were found in the representation of lipoproteins, glycoproteins, and in the activity of LDH, SDH, peroxidase, esterase and alkalic phosphatase.

The necessity for an exact determination of species specificity has introduced to biology the use of new, biochemical, analytical methods, which enabled the assessment of signs influenced by the state of expression of genetic information in bacterial-, plant- and animal cells. One of these methods suitable particularly for taxonomic purposes, is the analysis of the spectrum of proteins by disc electrophoresis in acrylamide gels. The method is highly sensitive and the results are reproducible (Clarke 1964, Davis 1962, Maurer 1968, Ornstein 1964, Raymond 1964, Mikhailov and Bogdanov 1970). Earlier studies (Peter 1971, Yoshimura 1969 a, b, Yoshimura et al. 1970, Ruff et al. 1973) obtained from a comparison of somatic proteins in parasitic worms showed that these differences may be used as a criterium for the differentiation of the species under consideration. The present report describes differences in the representation of somatic proteins and their enzymatic activity in various organs of *A. suum* and *A. lumbricoides*. Several experiments have been performed with muscles of *A. suum* and *A. lumbricoides* in order to isolate ribosomes and ribosomal proteins, which are more stable in structure and typical of the species under consideration.

MATERIAL AND METHODS

A. suum was obtained from the intestine of pigs at the abattoir, *A. lumbricoides* from the stool of patients treated with helmirazin.

Preparation of the extracts of somatic proteins. All material was treated three hours after recovery at the latest. The material was washed repeatedly in saline (0.9 % NaCl), rinsed in distilled water, dried, weighed and homogenized in a prechilled Potter-Elvehjen homogenizer with a teflon piston. Proteins were extracted with 0.1 M Tris-glycine buffer, pH 8.3. The homogenate was centrifuged for 25 min at 25.000× g. The supernatant solution was divided into test tubes and stored at −20 °C.

Disc electrophoresis. Disc electrophoresis in acrylamide gels was performed by the methods of Ornstein (1964) and Davis (1962). The gel column (8×0.6 cm) was layered with 20 μg proteins and separation was carried out under a constant flow of 2.5 μA. The gels were stained for 60 min in a solution of 1 % amido black in 7 % acetic acid and destained electrolytically. The concentration of proteins introduced to the gel for specific enzyme detection was increased three times (500—700 μg). Samples were concentrated by means of Aquacid II at 4 °C. Lipoproteins were identified with the

method of Gebott (1969). The method of Arai and Wallace (1969) was used for the staining of glycoproteins. Enzymatic activities were disclosed with modified histochemical methods. Lactic and succinic acid hydrogenases were identified with Schrauwen's method (1966), peroxidase with a modification of Sahulka's (1969) method, arylesterase and alkalic phosphatase with Lojda's (1958) method. The localisation of the individual protein bands was expressed as relative motility (Rp).

Preparation of cell-free extracts and isolation of ribosomes from the muscles of *Ascaris sulim* and *A. lumbricoides*. The muscle sample (5 g with the cuticle) was homogenized by grinding with the same amount of sand for 10 min. The mixture was suspended in 30 ml buffer (10 μm Tris-HCl, pH 7.5 containing 10 mM $MgCl_2$, 60 mM KCl, 6 mM mercaptoethanol and 1 % sodium-dodecyl sulphate). The suspension was stirred and centrifuged at 8,000 × g for 10 min. The sediment was removed and the supernatant containing the membranous and mitochondrial fraction was centrifuged again at 20.000 × g for 30 min. The sediment with the mitochondria and membranes was separated and the supernatant centrifuged at 80.000 × g for 20 min. The supernatant was centrifuged again at 105,000 × g for 2 hr. The sediment containing the ribosomal fraction was washed twice with buffer. The ribosomal fraction was layered on a 30 % sucrose solution in 10 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5. After centrifugation for 15 hr at 105,000 x g, the supernatant was discarded and the pellet containing the ribosomes was suspended in a small amount of buffer and dialyzed over night in the same buffer without sucrose. The ribosomal suspension was stored at −20 °C.

Sedimentation analyses were carried out in the Beckman Spinco model L at 37,020 rev/min. The ribosomal suspension (0.95 mg/ml) was centrifuged in the rotor AN-D at 20 °C and bar angle 60°.

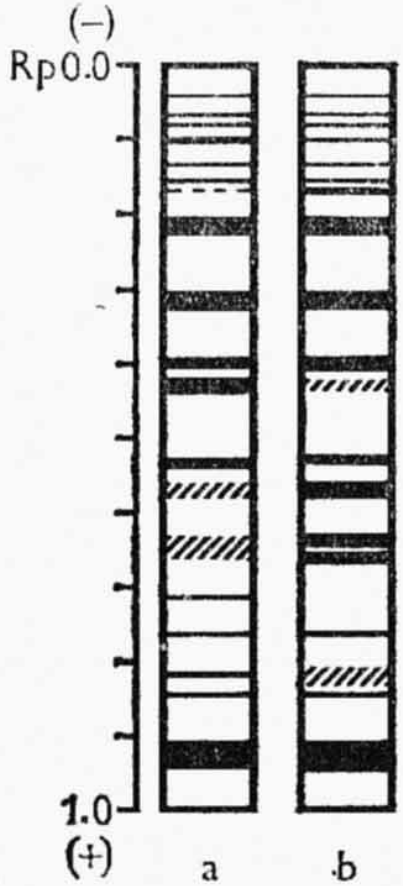

Fig. 1. Electrophoretic profile of proteins: a — *A. suum*, b — *A. lumbricoides*

Preparation of ribosomal proteins and electrophoresis. Ribosomal proteins were extracted from purified ribosomes with 66 % CH_3COOH in 8 M urea (100 mg ribosomes were mixed with 1 ml of extraction mixture). The suspension was stored for 1 hr at 0 °C, and proteins were obtained from the supernatant by centrifugation at 20,000 x g for 20 min. The sediment containing ribosomal RNA was removed. The supernatant was dialysed against 6 M urea. Electrophoresis of ribosomal proteins was performed by the method of Gesteland and Staehlin (1967).

RESULTS

Qualitative differences in somatic proteins of *A. suum* and *A. lumbricoides* were disclosed solely in the protein fraction Rp 0.07 (Fig. 1). In order to obtain a more detailed information on differences between the two species, we used electrophoresis for the study on the profile of proteins and on enzymatic activity in the isolated organs (muscles with cuticle, muscles, gonads, perienteric fluid). The results indicated that the composition of proteins in the isolated organs differed in the number and intensity of the protein bands. In view of the fact that the majority of enzymatic reactions occurs in the helminth cuticle, we compared extracts of muscles, with a cuticle with those of muscles without a cuticle. In extracts of *A. suum*, we identified 19 proteins in muscles with a cuticle, 10 in muscles without a cuticle, 17 in gonads and 10 only in the perienteric fluid (Fig. 2A). The Rp value of proteins was analogous to that occurring in proteins of the homogenate of the whole worm. The composition of proteins in the isolated organs of *A. lumbricoides* differed in both quality and quantity in the various organs. We identified 18 proteins in muscles with a cuticle, 14 in muscles without a cuticle, 17 in gonads and 10 in the perienteric fluid (Fig. 2A). Comparable were only the corresponding organs of each species. The perienteric fluid of the two species was composed of proteins with

a similar Rp value. The same applied to a comparison of the electrophoretic profile of lipoproteins and glycoproteins, and of enzymatic activity in the isolated organs of *A. suum* and *A. lumbricoides* (Figs. 2B—D, 3). Our examples indicated that several of the identified enzymes such as esterase (Fig 3C) and dehydrogenase of malic acid (SDH) (Fig. 3A) were similar in both *A. suum* and *A. lumbricoides*. On the other hand, differences were found in the remaining enzymes detected, i.e., dehydrogenase of lactic acid (LDH) (Fig. 2D) and peroxidase (Fig. 3B). Although in muscle- and gonad homogenates of *A. suum*, alkalic phosphatase activity could be detected on several sites in the gel (Fig. 3D), we did not succeed in demonstrating it in the extract of muscles with a cuticle and without a cuticle, and in the extract of gonads of the species *A. lumbricoides*.

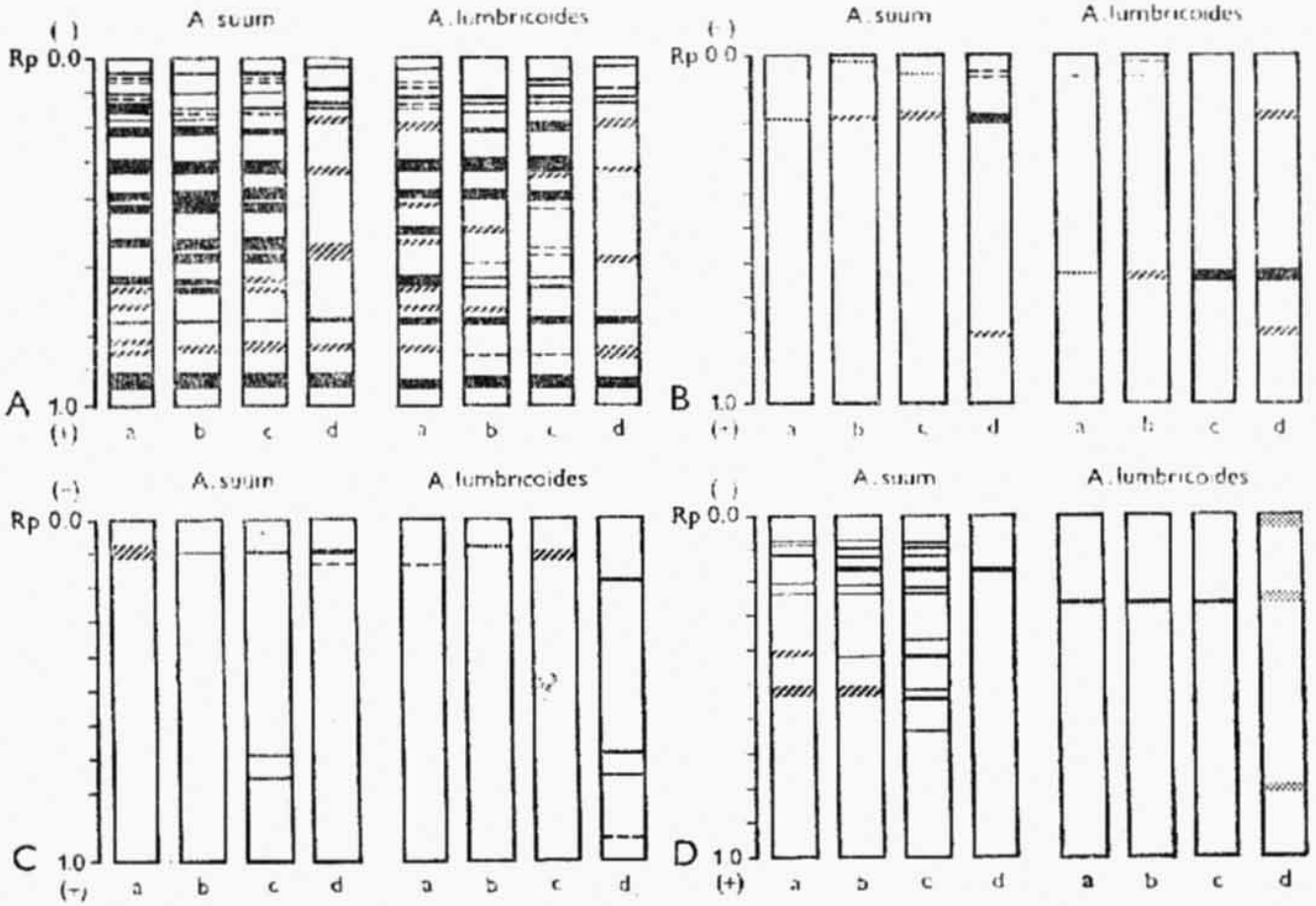

Fig. 2. Electrophoretic profile of proteins isolated from the individual organs of *A. suum* and *A. lumbricoides*, and the identification of LDH in the protein spectrum: A — total picture of somatic proteins; B — lipoproteins; C — glycoproteins D/LDH; a — muscles with a cuticle; b — muscles without a cuticle; c — gonads; d — perienteric fluid

In view of the fact that differences in the isosymes of *A. suum* and *A. lumbricoides* were not unanimous, I decided to compare the electrophoretic profile of ribosomal proteins, which are typical of the species under consideration, in order to determine differences in the two species (Girolamo 1968, Bielka and Welfe 1968, Low and Wool 1967). Since no literary data were available on these problems in parasitic worms, I had to start to isolate ribosomes and ribosomal proteins from both worm species in a rather laborious fashion.

Figure 4 illustrates soluble basic proteins after centrifugation of the cellular extracts at 8.000 x g (Fig. 4A), at 20.000 x g (Fig. 4B), and of ribosomal proteins (Fig. 4C). For *A. lumbricoides* I identified 28 basic proteins, for *A. suum* 26 only. Purified ribosomes

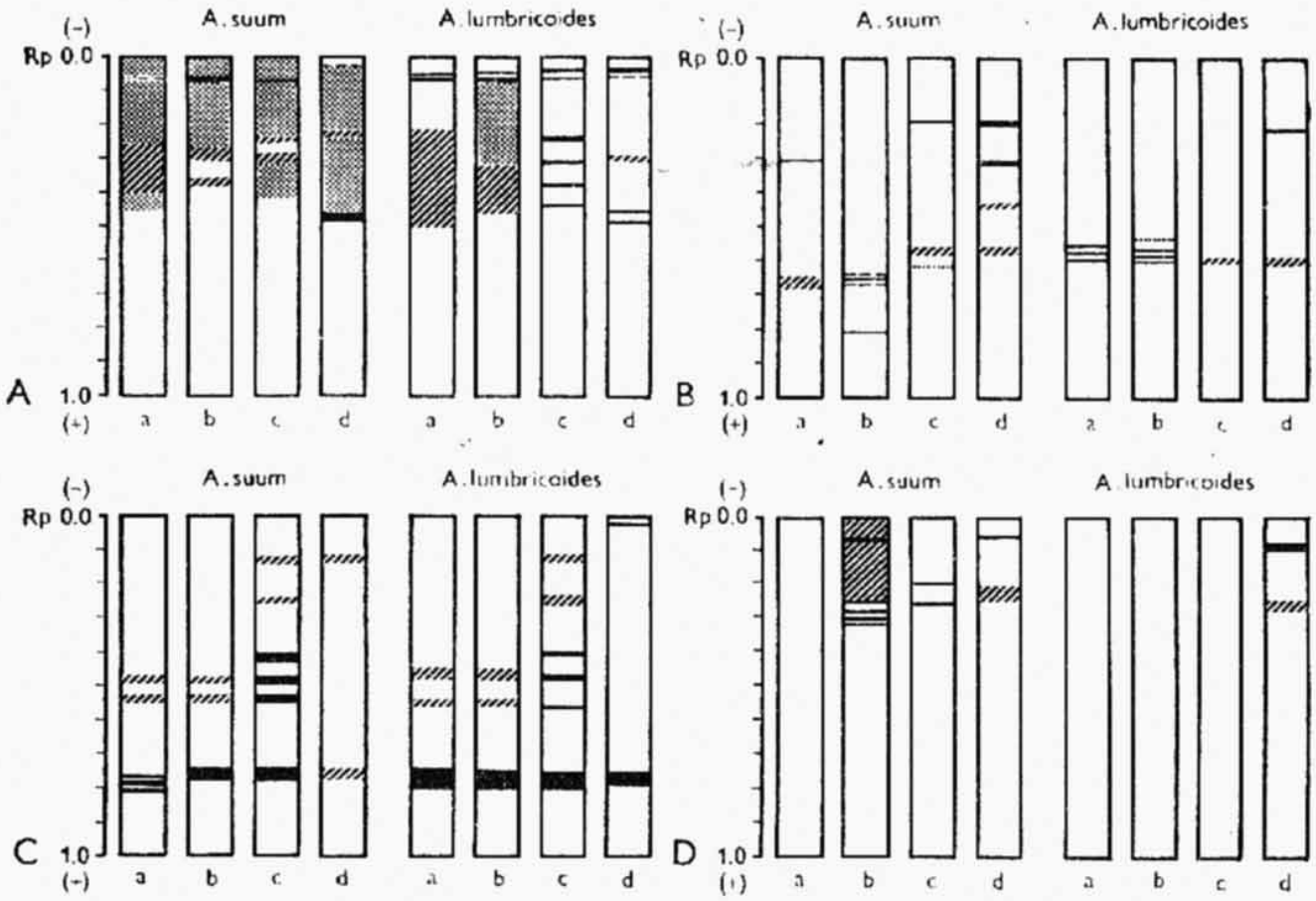

Fig. 3. Identification of several enzymes in the protein spectrum of organs isolated from *A. suum* and *A. lumbricoides*. A — SDH; B — peroxidase; C — esterase; D — alkalic phosphatase; a — muscles with a cuticle; b — muscles without a cuticle; c — gonads; d — perienteric fluid

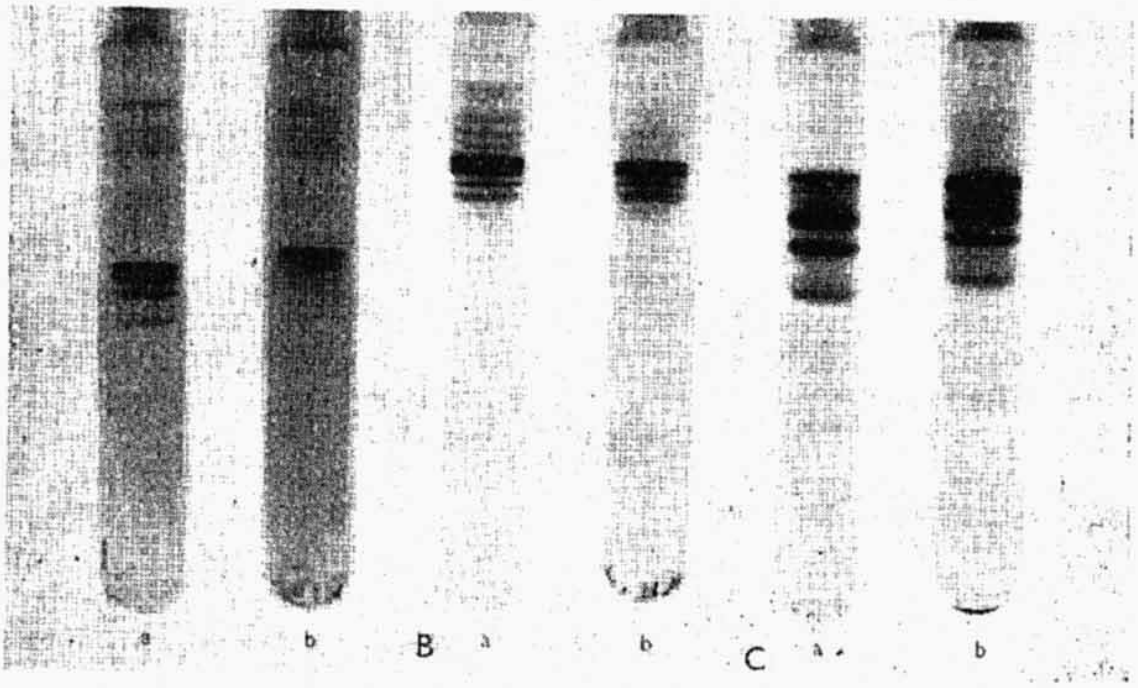

Fig. 4. Comparison of the electrophoretic profiles: A — supernatant after centrifugation at 8,000 x g for 15 min.; B — supernatant after centrifugation at 20,000 x g for 30 min; C — basic ribosomal proteins; a — *A. suum;* b — *A. lumbricoides.*

of both species were used for the sedimentation analysis. The values of cytoplasmic ribosomes (S_{20} 78) were identical in both species. The presence of peroxidase (Fig. 5A, B), with a maximum of 416, 545 and 583, was found in the supernatant fraction of both species. Similar values were obtained by Chance (1952).

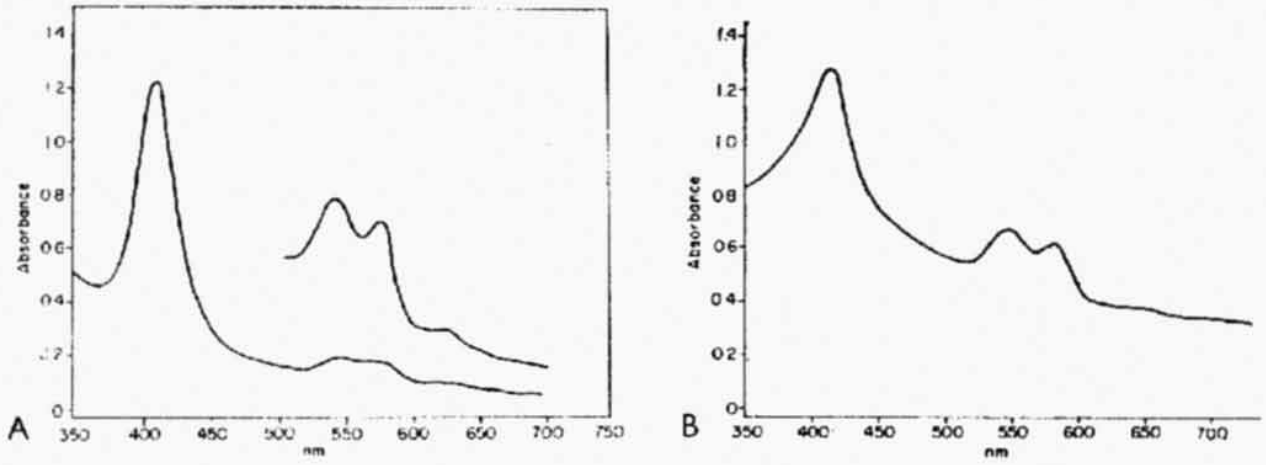

Fig. 5. Visible spectrum of peroxidase and sedimentation profiles of isolated ribosomes sedimentation profile after 12 min. of centrifugation at 37. 020 rpm. A — A suum; B — B. lumbricoides.

DISCUSSION

The method of electrophoresis has been employed most effectively in the classification of microorganisms in both plant- and animal systematics. Little attention has been given to similar studies on parasitic worms. Fundamental work on the identification of morphologically similar species has been performed by Yoshimura (1969 a, b,), Yoshimura et al. (1970). In view of the fact that differences in the protein spectra of morphologically similar species are often minute, special methods such as immunoelectrophoresis, methods for the determination of enzyme activity, various modifications of extraction procedures, etc. are required for these studies. In our experiments we have tried to assess differences in enzyme activities of somatic proteins in the two species under consideration. For these purposes we have used both homogenates of the whole worms and those of its individual organs. Other authors, e.g., Naumycheva and Sushchenko (1969), Evans (1971), Fodge et al. (1972), Tan and Zam (1973) have tried to distinguish and characterize esterases in *A. suum* by means of various extraction methods by employing different substrates and inhibitors. In an attempt to obtain a maximum of reliable, differentiating characters between *A. suum* and *A. lumbricoides*, I have tried to assess differences even in ribosomal proteins of 80 S cytoplasmic ribosomes, which are typical of each species. Differences in the profile of somatic and ribosomal proteins in *A. suum* and *A. lumbricoides* procured with disc electrophoresis confirmed the concept that the two ascarids are independent species.

ИЗУЧЕНИЕ ВИДОВОЙ СПЕЦИФИЧНОСТИ БЕЛКОВ ИЗ *ASCARIS LUMBRICOIDES* И *ASCARIS SUUM*

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Резюме. Методом дискового электрофореза была изучена разница в белковом профиле соматических и рибосомных белков из *Ascaris lumbricoides* и *Ascaris suum*. Кроме того были найдены разницы в содержании липопротеинов, гликопротеинов и в активности дегидрогеназы молочной и яблочной кислот, пероксидазы, эстеразы и щелочной фосфатазы.

REFERENCES

ARAI K., WALLACE H. W., Electrophoretic determination of glycoprotein. An improved method with cleared cellulose acetate membrane. Analyt. Biochem. 31: 71–76, 1969,

BIELKA H., WELFLE H., Characterization of ribosomal proteins from different tissues and species of animals by electrophoresis on polyacrylamide gel. Mol. Gen. Genet. 102: 128–131, 1968.

CHANCE B., The spectra of the enzyme-substrate complexes of catalase and peroxidase. Arch. Biochem. 41: 404–415, 1952.

CLARKE J. T., Simplified "disc" (polyacrylamide gel) electrophoresis. Ann. N. Y. Acad. Sci. 121: 428–436, 1964.

DAVIS B. J., Disc electrophoresis – Part II: Materials and methods. Reprinted by Destillation Products Industries (Eastman Kodak), Rochester, 1962.

EVANS A. A. F., Tissue specificity of enzymes and proteins in the nematode *Ascaris lumbricoides* with observations on *Aphelenchus avenae*. Int. J. Biochem. 2: 262–264, 1971.

FODGE D. W., Gracy R. W., HARRIS B. G., Studies on enzyme from parasitic helminths. Biochim. Biophys. Acta 268: 271–284, 1972.

GEBOTT M. D., New easier procedure for lipoprotein electrophoresis. Solutions 1: 25, 1969.

GESTELAND R. F., STAEHLIN T., Electrophoresic analysis of protein from normal and cesium chloride-treated *Escherichia coli* ribosomes. J. Mol. Biol. 24: 149–155, 1967.

GIROLAMO M. D., CAMMARANO P., The protein composition of ribosomes and ribosomal subunits from animal tissues, electrophoretic studies. Biochim. Biophys. Acta 168: 181–194, 1968.

LOJDA Z., Azokopulační reakce v histochemickém průkazu enzymů. Stát. zdrav. nakl., Praha, 1958.

LOW R. B., WOOL J. C., Mammalian ribosomal protein: analysis by electrophoresis on polyacrylamide gel. Science 155: 330–332, 1967.

MAURER H. R., Disk-Elektrophorese. Theorie und Praxis diskontinuierlicher Polyakrylamidgel-Elektrophorese. De Gruyter, Berlin, 1968.

MIKHAILOVA Y., BOGDANOV A. A. (Electrophoresis of ribosomes and ribosomal RNA in polyacrylamide gel). Biochimia 35: 3, 403–408, 1970. (In Russian.)

NAUMYCHEVA M. I., SUSHCHENKO N. I., (Tissue protein fractions of adult pig *Ascaris* (muscles, cuticula, uterus, cavity fluid and whole body). Tr. vsesoyuz. inst. gelm. im. akad. K. I. Skryabina, 41–43, 1969. (In Russian.)

ORNSTEIN L., Disc electrophoresis – I. Background and theory. Ann. N. Y. Acad. Sci. 121: 320–349, 1964.

PETER R., Disc-Elektrophoretische Untersuchungen zur Frage der Artcharakterisierung paludicoler Trieladen (Plathelminthes: Turbelaria). Z. f. zool. System. Evolutionsf. 9: 263–318, 1971.

RAYMOND S., Acrylamide gel electrophoresis. Ann. N. Y. Acad. Sci. 121: 350–365, 1964.

RUFF M. D., DAVIS G. M., WERNER J. K., *Schistosoma japonicum:* Disc electrophoretic protein patterns of the Japanese, Philipine and Formosan strains. Exp. Parasitol. 33: 437–446, 1973.

SAHULKA J., Acid phosphatase fractions from various growth zones of borad bean root revealed by disc electrophoresis in acrylamide gel. Biol. Plant. 12: 442–444, 1969.

SCHRAUWEN J., Nachweis von Enzymen nach elektrophoretischer Trennung an Polyacrylamid-Säulchen. J. Chrom. 23: 177 bis 180, 1966.

TAN P., ZAM S. G., Isolation and characterisation of non-specific esterase enzyme of *Ascaris suum* perienteric fluid. Comp. Biochem. Physiol. 46B: 673–682, 1973,

YOSHIMURA K., *Paragonimus westermani*, *P. ohirai* and *P. miyazakii*. Electrophoretic comparison of whole body proteins. Exp. Parasit. 25: 118–130, 1969a.

–, *Paragonimus*. Electrophoretic fractionation of whole body proteins as an aid in specific identification of a species from Sado Island. Jap. Exp. Parasit. 25: 107, 1969b.

–, HISHINUMA Y., SATO M., A preliminary study on the disc electrophoretic patterns of *Paragonimus kellicotti* Ward, 1908 adult worms. Res. Bull. Meguro Parasit. Mus. 3: 12–19, 1970.

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