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# THE ANTIGENIC FRACTIONS OF *HYMENOLEPIS DIMINUTA*; THEIR USEFULNESS IN IMMUNOLOGICAL EXAMINATIONS

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In the older literature the intestinal tapeworms were considered as weakly immunogenic. At the same time the digestive tube was regarded as a place where immunologic reactions were not expressed. Now, a number of evidence shows that the tapeworms are good immunogens stimulating hosts to production of antibodies (Coleman and de Sa, 1964; Machnicka and Zwierz, 1966), and cell-mediated immunity (Boroń-Kaczmarska et al., 1978).

In some nematode infections caused by *Nippostrongylus brasiliensis, Haemonchus placei, Trichinella spiralis* the damaging influence of antibodies on the parasites was noted. The similar action of antibodies on *H. diminuta* was also suggested by Befus and Threadgold (1975). The „darkened areas" described by these authors in the tegument of proglottids were of the opinion to be the sign of damage and appeared in number which were parallel to the intensity of infection. According to Befus (1977) immunoglobulins: IgA, IgM, $IgG_1$, and $IgG_2$ were present on the surface of *H. diminuta* what was connected with intensity of infection; in more numerous infections immunoglobulins were registered earlier.

From the above quoted examinations it follows that infection with *H. diminuta* initiates the immunological response in its host. But for the further researches which claim to demonstrate in vivo or in vitro the presence of humoral or cell-mediated reactions of hosts the use of suitable antigens is necessary. The antigens should be specific and applicable in immunological tests. This work presents the evaluation of the usefulness of antigenic fractions of *H. diminuta* purchased in chemical way, in immunologic tests to assay humoral and cellular reactions in infected animals.

## Material and methods

The experiments were carried out on Wistar rats, males, 10 weeks old, weighing 140-150 g and on inbred CFW mice, males, $10 \pm 1$ week of age

weighing 30-32 g. The animals were infected with cysticercoids of *H. diminuta* with the aid of the stomach tube, being under light ether anaesthesia. The mice were given two cystcercoids each, while the rats were infected with five cysticercoids each.

The white rabbits from Popielno, males, weighing 4 kg, being 6 months old at the beginning of experiment were immunized subcutaneously with *H. diminuta* homogenate in 2 week intervals. Each injection contained 200 mg of fresh homogenate with 2.5 ml of Freund's adjuvant. The complete Freund's adjuvant was used in every second injection. The rabbits were bled five months since the immunization had begun. The sera were stored at −30°C untill use. The sera of experimental rats and mice were kept in the same conditions.

Preparation of antigenic fractions. The tapeworms freshly purchased from rats were washed many times with tap and distilled water, homogenized in knife homogenizer at 0°C then lyophilized. The lyophilized homogenate was submitted to fractionation according to the method described by Machnicka-Roguska (1965) and illustrated by Fig. At first the homogenate was delipidized with 10 vol. ethanol : ether, 1 : 1, then with 10 vol. of chloroform and finally with 10 vol. of ethanol : ether, 1 : 1, with addition of 1% HCl. Each supernatant was poured out, sediment aerated before the next extraction. After the extraction of lipids (which were discarded not being the subject of examination) the sediment was treated with 1% $CH_3COOH$. From the supernatant fraction C was precipitated with 3 vol. of ethanol, and from the remaining supernatant by the action of 2 vol. of aceton fraction $C_{acet}$ was precipitated. The sediment was subsequently extracted with 10 vol. of 0.1n. NaOH. The supernatant from this step of preparation after treatment with 15% $CH_3COOH$ gave the precypitate named fraction $N_{0.1}$, the remaining supernatant yielded fraction $C_{alk}$ when treated with 4 vol. of ethanol. The sediment after extraction with 0.1n. NaOH was in turn submitted to the action of 1.0n. NaOH. The supernatant treated with 10% $CH_3COOH$ gave the sediment — fraction $N_{1.0}$, while the remaining supernatant subjected to the action of 40% $CCl_3COOH$ precipitated forming fraction $B_2$. All steps of preparations were carried out at 4°C, each extraction with constant stirring lasted 8 hr. The fractions were dissolved (in water or in 0.1 or 1.0n. NaOH respectively) precipitated again, dialized against bidestilled water for 48 hr and finally lyophilized.

Protein and sugar determinations were carried out in lyophilized homogenate or fractions. Protein was determined by Folin method according to Kabat and Mayer (1961), taking bovine albumine (Serva) as standard. Sugar (hexoses) were estimated by anthron reaction as des-

cribed Dżułyńska and Piekarska (1956); Blaska (1966) with glucose as standard.

For immunological examinations the polysaccharide fractions were dissolved in saline. Protein fractions were solved in minute amounts of NaOH 0.1 or 1.0n. according to which it was extracted, adjusted to pH 7.0 with HCl and completed to the needed volumne with saline.

Double diffusion in 0.9% agar gel on plates 9×6 cm was performed according to Ouchterlony (1949). The protein contents in the antigenic fractions used in this method was 2.5 mg/ml.

Passive hemaglutination test was done according to Stavitsky (1954) using sheep erythrocytes from Alsever's solution. 5% erythrocytes were sensitised with antigenic fractions containing 2 mg of protein per 1 ml for 1 hr at 37°C. After triple washing in PBS pH 7.2 containing 1% of inactivated calf serum the test was made in plastic plates taking; 0.25 ml inactivated and diluted serum, and 0,05 ml of 2% sensitized erythrocytes. Each time the control were included; with positive and negative sera, and with normal erythrocytes.

To examine cell-mediated immunity the indirect test of inhibition of macrophage migration from capillary tubes according to George and Vaughan (1962) with modifications of Kozar and Piotrowski (1971) was used. The test was performed in the medium pH 7.0 containing Parker solution with 10% of calf serum and canamycin 100 μg/ml. The antigenic fractions were prepared to contain 25.50 or 100 μg of protein per 1 ml of medium. Macrophages were purchased from peritoneal cavity of the healthy rats or mice 48 hr after stimulation with liquid paraffin. Intestinal lymph nodes being the source of lymphocytes were taken immediately cut with scissors to chilled Parker solution and gently pressed through a nylon sieve. The lymphocytes as well as macrophages after three washings in Parker solution were counted in hemocytometer. The mixture macrophages: lymphocytes, 4 : 1 were prepared to fill siliconized capillary tubes 75 mm long, 1 mm in diameter. Then one end of each tube was sealed with paraffin wax and the tubes were centrifuged for 5 min in 1000 r.p.m. Further the tubes were cut precisely on the border of cellular sediment. The pieces containing the cells were fastened with silicon glu into plastic incubation chambers 1.5 cm in diameter which afterwards were filled with incubation medium, closed with cover glasses and kept in humid conditions for 18-24 hr at 37°C. Each test run with controls; examined cell mixture in medium without antigen and cell mixture containing the lymphocytes from uninfected animals in medium with antigen. The projection microscope was used for reading results. The projection fields formed by migration of cells from capillary tubes were recorded on the sheets of paper. The fields afterwards

were measured by planimetry. The calculation of the migration for experimental animals was done according to equation:

$$\% \text{ migration} = \frac{\text{average area of migration with antigen}}{\text{average area of migration without antigen}} \cdot 100\%.$$

## Results

The chemical fractionation of 11 000 mg of lyophilized *H. diminuta* homogenate (Table 1 and Fig.) yielded 6 antigenic fractions in the amounts sufficient for chemical determination and immunological examinations. The chemical composition of the fractions is shown in Table 1. Fraction $N_{1.0}$ contains the highest amount of protein — 74.6% and the smallest amount of polysaccharides. The fraction C has highest amount of sugar — 80.6%. Another polisaccharide fraction $C_{alk}$ contains a very high quantity of protein — 44.8%.

As demonstrated by double diffusion in agar gel antisera of immunized rabbits gave only precipitin band with fraction $N_{1.0}$. The sera of infected rats (taken 15 d.p.i.) showed the precipitin line with fraction $C_{alk}$, then with fraction $N_{1.0}$ and again after 72 hr of incubation the second precipitin band with fraction $C_{alk}$. The sera of infected mice formed two bands of precipitation with fraction $C_{alk}$ only. The second band appeared with sera received 15 d.p.i. from mice. The other fractions did not from the precipitation bands with the above enumerated sera when double diffussion plates were watched during one week.

The results of comparative studies on usefulness of the antigenic fractions in passive hemagglutination test are shown in Table 2. The highest titres were received with the sera of mice infected H. dininuta 1 : 80. That was the case with erythrocytes coated with fraction $C_{alk}$. The other fractions gave evidently smaller titres.

TABLE 1

The antigenic fractions of *H. diminuta* purchased by chemical fractionation

| Fraction | Weight [mg] | Protein [%] | Polysacharides [%] |
|---|---|---|---|
| C | 1410 | 20.20 | 80.62 |
| $C_{acet}$ | 130 | 38.00 | 60.72 |
| $N_{0.1}$ | 20 | 64.71 | 4.25 |
| $C_{alk}$ | 810 | 44.80 | 38.34 |
| $N_{1.0}$ | 460 | 74.64 | 3.46 |
| $B_2$ | 30 | 50.76 | 5.45 |

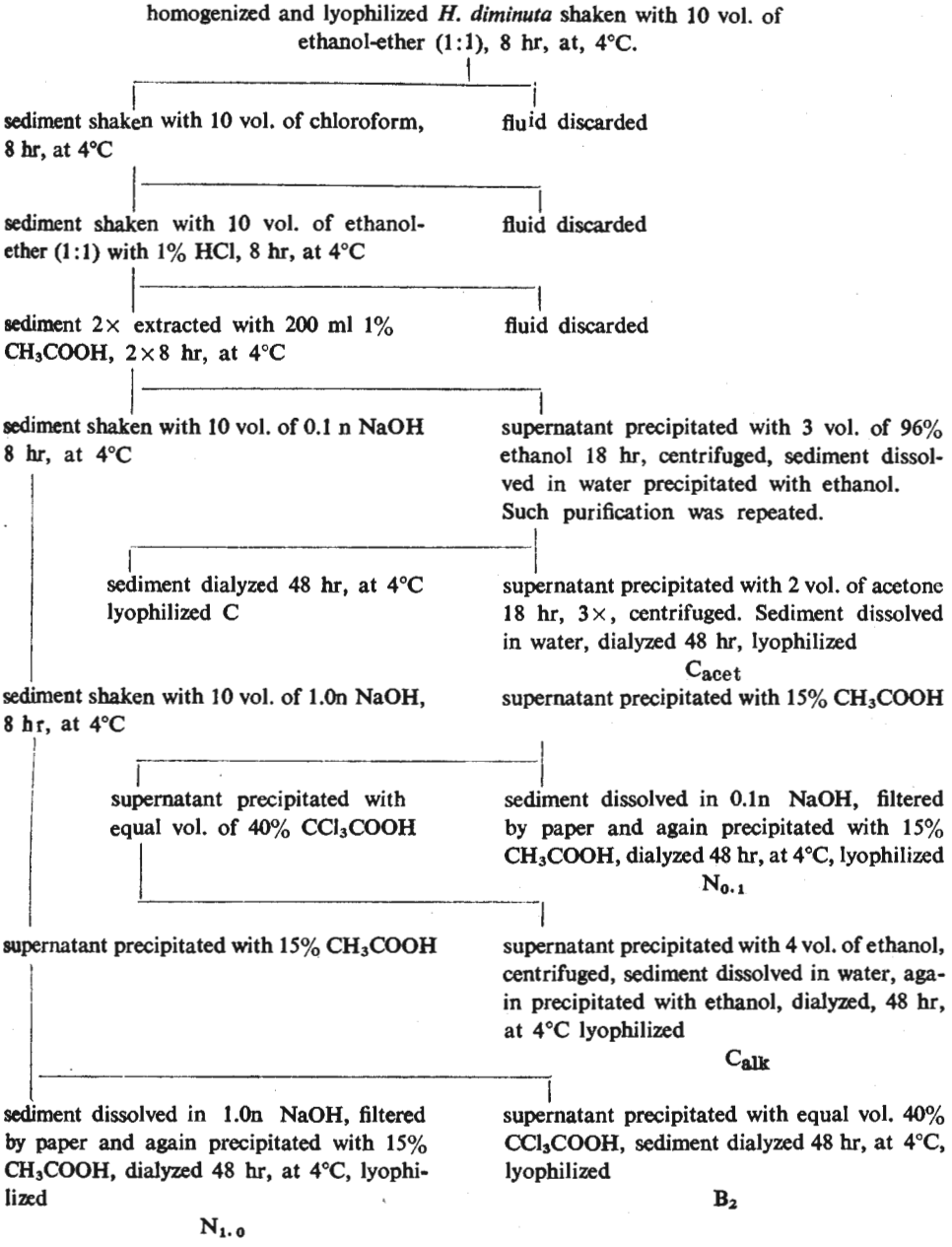


Fig. Preparation of antigenic fractions

The examinations in which all antigenic fractions each in three protein concentration 25, 50 and 100 μg/ml were tried in the indirect test of inhibition of macrophage migration were demonstrated in Tables 3-5. As it is visible the specific and repeatable results were obtained with fractions $N_{1.0}$ and $C_{alk}$ in concentration of fraction protein 50 μg/ml of medium. The other fractions gave unsatisfactory results similar to these received in tests with lymphocytes deriving from uninfected animals. The higher 100 μg/ml or smaller 25 μg/ml protein of antigen con-

TABLE 2

The results of passive hemaglutination test with sera of mice infected with *H. diminuta*. The comparative studies on antigenic fractions

| d.a.i. | Number of sera | Mean serum titre with antigenic fraction | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | $C_{acet}$ | $N_{0.1}$ | $C_{alk}$ | $N_{1.0}$ | $B_2$ |
| 7 | 2 | 1:20 | — | 1:20 | 1:80 | 1:40 | 1:10 |
| 9 | 2 | 1:10 | — | 1:40 | 1:80 | 1:40 | 1:5 |
| 11 | 2 | 1:20 | — | 1:20 | 1:20 | 1:20 | — |
| 13 | 2 | 1:10 | 1:5 | 1:10 | 1:20 | 1:20 | 1:5 |
| 15 | 2 | 1:10 | 1:5 | 1:5 | 1:40 | 1:40 | — |
| 17 | 2 | 1:10 | 1:5 | 1:5 | 1:40 | 1:10 | 1:10 |
| 20 | 2 | 1:10 | 1:5 | 1:10 | 1:40 | 1:10 | — |
| 22 | 2 | 1:10 | 1:5 | 1:10 | 1:40 | 1:10 | 1:5 |
| 26 | 2 | 1:10 | 1:5 | 1:10 | 1:40 | 1:10 | 1:10 |
| 29 | 2 | 1:10 | — | 1:20 | 1:80 | 1:20 | — |
| 31 | 2 | 1:10 | 1:5 | 1:20 | 1:80 | 1:20 | 1:10 |

TABLE 3

The results of indirect test of macrophage inhibition migration with lymphocytes of mice infected with *H. diminuta* and control. Comparative studies on antigenic fractions with protein concentration 25 μg/ml of medium

| d.a.i. | Number of mice | Antigenic fractions *H. diminuta* | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | $C_{acet}$ | $N_{0.1}$ | $C_{alk}$ | $N_{1.0}$ | $B_2$ |
| 7 | 2 | 94.23 | 89.20 | 81.80 | 83.23 | 85.54 | 81.83 |
| 9 | 2 | 90.12 | 92.18 | 82.21 | 68.32 | 71.50 | 83.73 |
| 11 | 2 | 87.57 | 81.36 | 91.20 | 61.09 | 60.30 | 84.10 |
| 13 | 2 | 88.46 | 86.98 | 92.00 | 67.85 | 55.45 | 86.66 |
| 15 | 2 | 98.46 | 85.90 | 92.26 | 77.26 | 54.00 | 84.40 |
| 17 | 2 | 90.71 | 86.60 | 91.80 | 78.30 | 72.12 | 83.05 |
| 20 | 2 | 98.61 | 86.98 | 93.43 | 84.13 | 83.34 | 83.65 |
| 22 | 2 | 90.70 | 89.25 | 90.00 | 88.10 | 90.24 | 90.75 |
| 26 | 2 | 89.23 | 96.06 | 98.76 | 88.00 | 90.12 | 85.70 |
| 29 | 2 | 96.20 | 99.08 | 98.32 | 84.06 | 98.40 | 96.10 |
| 31 | 2 | 95.10 | 89.34 | 85.10 | 98.22 | 93.43 | 93.48 |
| Control | 2 | 97.19 | 97.33 | 98.54 | 96.39 | 97.32 | 98.34 |

TABLE 4

The results of indirect test of macrophage inhibition migration with lymphocytes of mice infected with *H. diminuta* and control. Comparative studies on antigenic fractions with protein concentration 50 μg/ml of medium

| d.a.i. | Number of mice | Antigenic fractions *H. diminuta* | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | $C_{acet}$ | $N_{o.1}$ | $C_{alk}$ | $N_{1.0}$ | $B_2$ |
| 7 | 2 | 89.74 | 92.13 | 86.54 | 80.10 | 82.44 | 84.72 |
| 9 | 2 | 92.28 | 90.83 | 82.43 | 62.22 | 69.50 | 78.30 |
| 11 | 2 | 84.75 | 80.43 | 83.28 | 67.80 | 59.00 | 76.21 |
| 13 | 2 | 86.64 | 84.98 | 89.90 | 59.30 | 52.80 | 82.22 |
| 15 | 2 | 89.44 | 85.80 | 86.62 | 68.44 | 52.20 | 88.85 |
| 17 | 2 | 90.14 | 84.36 | 90.70 | 77.65 | 70.10 | 80.80 |
| 20 | 2 | 94.11 | 84.90 | 91.43 | 82.58 | 80.24 | 88.00 |
| 22 | 2 | 92.30 | 88.52 | 92.16 | 88.20 | 87.27 | 90.33 |
| 26 | 2 | 99.10 | 96.61 | 98.70 | 94.24 | 90.24 | 94.43 |
| 29 | 2 | 92.80 | 90.36 | 94.23 | 96.40 | 94.74 | 79.80 |
| 31 | 2 | 94.70 | 91.43 | 93.34 | 96.66 | 94.94 | 85.38 |
| Control | 2 | 99.43 | 96.34 | 98.18 | 97.43 | 98.36 | 95.55 |

TABLE 5

The results of indirect test of macrophage inhibition migration with lymphocytes of mice infected with *H. diminuta* and control. Comparative studies on antigenic fractions with protein concentration 100 μg/ml of medium

| d.a.i. | Number of mice | Antigenic fractions *H. diminuta* | | | | | |
|---|---|---|---|---|---|---|---|
| | | C | $C_{acet}$ | $N_{o.1}$ | $C_{alk}$ | $N_{1.0}$ | $B_2$ |
| 7 | 2 | 92.18 | 99.28 | 89.76 | 85.40 | 67.36 | 86.15 |
| 9 | 2 | 90.40 | 94.82 | 88.11 | 71.30 | 72.28 | 79.34 |
| 11 | 2 | 86.75 | 84.38 | 90.32 | 64.29 | 63.43 | 88.21 |
| 13 | 2 | 88.64 | 89.82 | 96.24 | 69.70 | 59.25 | 90.26 |
| 15 | 2 | 96.64 | 84.82 | 94.62 | 79.30 | 62.00 | 88.76 |
| 17 | 2 | 93.14 | 89.00 | 96.67 | 82.30 | 76.22 | 87.52 |
| 20 | 2 | 94.25 | 96.83 | 91.34 | 87.10 | 86.28 | 88.54 |
| 22 | 2 | 94.40 | 93.37 | 92.16 | 89.87 | 90.10 | 93.58 |
| 26 | 2 | 96.43 | 96.20 | 98.60 | 91.30 | 93.20 | 93.30 |
| 29 | 2 | 95.20 | 90.46 | 90.23 | 94.16 | 95.10 | 91.67 |
| 31 | 2 | 91.48 | 93.43 | 98.84 | 99.24 | 91.88 | 94.28 |
| Control | 2 | 93.68 | 84.48 | 98.12 | 96.42 | 98.18 | 84.18 |

centration in medium comparably with 50 μg/ml gave non-valid results of the inhibition of macrophage migration i.e. expressed as smaller per cent of inhibition of migration in infected animals.

## Discussion

The employed scheme of fractionation with mild chemical methods Machnicka-Roguska (1965), allowed to receive antigenic fractions diffe-

rentiated in their chemical composition. The amounts of antigenic fractions were sufficient to perform chemical determinations as well as introductory and proper immunological examinations. The other methods of antigen preparation as gell filtration and column fractionation Kent (1963); Kronman (1965) warrant that the obtained products are in a perfect native form but usually in very small amounts, insufficient for the performance of comparative examinations. As it was underlined by many authors that the removal of lipids increases the specificity of antigenic fractions; for example in Schistosoma mansoni antigens Chaffe at al. (1954), Anderson (1960) in *Trypanosoma cruzi* antigens (Five and Kent, 1960).

Kent (1957) prepared antigenic fractions of *H. diminuta* using gel filtration and column chromatography after delipidization. These fractions differed markedly in their chemical composition from the fractions described in this work, because of different fractionation method employed. The common feature of the fractions deriving from both preparations was the simultaneous presence of proteins and carbohydrates strongly fixed to one an other in different proportions. The same was noted when the antigenic fractions from Taenia saginata and Moniezia expansa were prepared (Machnicka-Roguska, 1965; 1972).

The results of double diffussion in agar gel pointed out the presence of identical antigenic components in fractions $C_{alk}$ and $N_{1.0}$ but in different amounts what showed the different time of appearance of the single precipitin lines. The richest source of antibodies were the sera of rat suitable host for *H. diminuta*. The poorer reactions were noted with the sera of mice and of parenterally immunized rabbits.

Consequently and accordingly to the data deriving from double diffusion the results, the comparison of all fractions in hemaglutination test and inhibition of macrophage migration pointed out the best activity of $C_{alk}$ an $N_{1.0}$. Especially in passive hemagglutination test fraction $C_{alk}$ was useful probably due to high contents of sugar conditioning direct coating of erythrocytes.

The performance of the comparative examinations taking all antigens and the sera received at different times after infection and immunization, should give the best choice of antigens which can be used in experimental examinations. It follows from the described above researches that fractions $C_{alk}$ and $N_{1.0}$ seem to be right antigens to monitor immunity which develops after infection with *H. diminuta*.

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## LITERATURA


1. Anderson, R. J.: Serologic diagnosis of *Schistosoma mansoni* infections. I. Development of cercarial antigen slide flocculatio test. — *Am. J. trop. Med. Hyg.*, 9, 299-303, 1960.
2. Befus, A. D.: *Hymenolepis diminuta* and *H. microstoma*; mouse immunoglobulins binding to the tegumental surface. — *Expl Parasit.*, 41, 242-251, 1977.
3. Befus, A. D., Threadgold, L. T.: Possible immunological damage of *Hymenolepis diminuta* in mice and rats. — *Parasitology*, 71, 525-534, 1975.
4. Blaska, P.: Bestimmung der Kohlenhydrate und Lipide. — *Limnologica*, 4, 403-418, 1966.
5. Boroń-Kaczmarska, A., Machnicka-Roguska, B., Łotocka, K., Sokolewicz-Bobrowska, E.: Untersuchungen über das Verhalten des Macrophagenmigrationshemmtestes mit dem Polysaccharidantigen „C" von *Taenia saginata* in der Menschlichen Taniase. — *Zbl. Bakt. Hyg.*, 240, 538-541, 1978.
6. Chaffe, E. F., Bauman, P. M., Shapilo, J. J.: Diagnosis of schistosomiasis by complement fixation. — *Am. J. trop. Med. Hyg.*, 3, 905-913, 1954.
7. Coleman, R. M., de Sa, L. M.: Host response to implanted adult *Hymenolepis nana*. — *J. Parasit.*, 50, 17, 1964.
8. Dżułyńska, J., Piekarska, Z.: Polysaccharides in serum-protein fractions obtained by salting out and precipitating with methanol. — *Bull. Acad. pol. Sci. Cl. II Sèr. Sci. biol.*, 4, 419-425, 1956.
9. Five, E. H., Kent, J. F.: Protein and carbohydrate complement fixing antigens of *Trypanosoma cruzi*. — *Am. J. trop. Med. Hyg.*, 9, 512-517, 1960.
10. George, M., Vaughan, J. H.: In vitro cell migration as a model for delayed hypersensitivity. — *Proc. Soc. Exp. Biol. N. Y.*, 3, 514-521, 1962.
11. Kabat, E. A., Mayer, M. M.: Experimental Immunochemistry. — Thomas, C. C., Springfield 3, 11, 1961.
12. Kent, N. H.: Biochemical studies on the proteins of Hymenolepis diminuta. — *Expl Parasit.*, 6, 351-357, 1957.
13. Kent, N. H.: Fractionation, isolation and definition of antigens from parasitic helminths. — *Am. J. Hyg.*, 22, 30-45, 1963.
14. Kozar, Z., Piotrowski, R.: Peritoneal macrophage inhibition test in guinea pigs infected with *Trichinella spiralis*. — *Wiad. Parazytol.*, 17, 609-615, 1971.
15. Kronman, B. S.: Immunochemistry of Schistosoma mansoni cercariae — *J. Immun.*, 95, 13-18, 1965.
16. Machnicka-Roguska, B.: Preparation of *Taenia saginata* antigens and chemical analysis of antygenic fractions. — *Acta parasit. pol.*, 13, 337-347, 1965.
17. Machnicka-Roguska, B.: Badania nad antygenami *Moniezia expansa*. — Rozprawa habilitacyjna, 1-22, 1972.
18. Machnicka-Roguska, B., Zwierz, C.: Serological studies on *Taenia saginata*. — *Acta parasit. pol.*, 14, 27-33, 1966.
19. Ouchterlony, Ö.: Antigen-antibody reactions in gel. — *Acta path. microbiol. scand.*, 26, 507-515, 1949.
20. Stavitsky, A. B.: Micromethods for the study of proteins and antibodies. I. Procedure and general applications of hemagglutination-inhibition reactions with tanic acid and protein — treated red blood cells. — *J. Immun.*, 72, 360-367, 1954.

# ANTYGENOWE FRAKCJE *HYMENOLEPIS DIMINUTA* ORAZ ICH PRZYDATNOŚĆ W BADANIACH IMMUNOLOGICZNYCH

by

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Przeprowadzono badania nad przydatnością do reakcji hemaglutynacji biernej i odczynu zahamowania migracji makrofagów frakcji antygenowych *H. diminuta*. Frakcje antygenowe uzyskano za pomocą preparatyki chemicznej. Oznaczono skład chemiczny frakcji oraz ich wzajemne pokrewieństwo antygenowe.

Badania wykazały, że frakcja antygenowa *H. diminuta* $C_{alk}$ dała najwyższe i powtarzalne miana hemaglutynacji biernej, co świadczy o dużej specyficzności tej frakcji.

W odczynie zahamowania migracji makrofagów największą specyficzność wykazały frakcje $N_{1,0}$ i $C_{alk}$ w stężeniu 50 µg białka frakcji na 1 ml płynu odżywczego.

Wyniki przeprowadzonych badań wskazują, iż wymienione frakcje antygenowe mogą być przydatne w oznaczaniu odporności typu humoralnego i komórkowego u szczurów i myszy zarażonych *H. diminuta*.