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Parasitology

STUDIES ON THE TOXICITY OF LARVAL *ANISAKIS SIMPLEX* (RUD.). PART I. BIOLOGICAL TEST ON WHITE MICE

BADANIA NAD TOKSYCZNOŚCIĄ LARW *ANISAKIS SIMPLEX* (RUD.). CZĘŚĆ I. PRÓBA BIOLOGICZNA NA BIAŁYCH MYSZKACH

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The purpose of the present work was to examine the toxicity of fish flesh infected with dead *Anisakis* larvae by feeding it to white mice at different qualitative and quantitative compositions.

The toxicity of fragmented fresh larvae and those previously subjected to the action of different temperatures was also studied.

INTRODUCTION

There is a great possibility of the direct contact of man with *Anisakis* larvae, since they are common parasites in very many species of sea-fishes. They occur, as a rule, in the abdominal cavity of fishes and are well seen with the naked eye on the intestine, but happen to pierce into the muscles and then are hard to perceive.

There are fish species in which exceptionally many larvae penetrate into the skeletal muscles. One of them is the blue whiting (*Micromesistius poutassou* Risso, 1826), infested in nearly a hundred per cent, in whose muscles we found several tens of larvae per fish.

The exposure of fishes to low (below −20°C) and high (above +60°C) temperature and also adequately concentrated brine (above 20° Bé) or primary marinating bath (7% of acetate acid and 15% of common salt) kill the *Anisakis* larvae and thus people are out of danger to became infected with them. Nevertheless, toxic properties of the tissues and body fluids of the nematodes remaining dead in the muscles of fishes may come into play and in the case of heavily infested fishes they may cause poisoning in man.

This is of particular significance considering the planned production of fish mince for ready-to-cook products aiming at the most economic utilization of the large amount of fish flesh left after filleting or from fishes unsuitable for filleting. A great many fragmented *Anisakis* larvae may occur in such mince.

The aim of this paper is to clear out this problem.

MATERIAL AND METHODS

Standard class CFN mice, females, red in colour acc. to Lene-Petter's scale, approximately one month old and on the average 16.0 g in weight, bred at the Laboratory of Experimental Animals at Przeźniewo near Poznań were used in this experiment.

Frozen mince of blue whiting (*Micromesistius poutassou*) was used for our study. It was very heavily contaminated with pieces of *Anisakis* larvae of various size, there being also occasional whole larvae in it. Assuming that on average 10 pieces corresponded to a whole larva, 100 g of mince contained 4.7. *Anisakis* larvae.

In our experiment fresh larvae of *Anisakis* simplex, removed from Baltic herrings and stored alive in common saline at 2–4°C in the refrigerator were also used.

The mice after an adaptation period were divided into four groups with respect to the diet fed and its quantitative composition:

I. The control group (20 mice) was given a basic diet: oat flakes, milk powder and water – ad libitum.

II. The animals of this group were fed on blue whiting mince given in different weight proportions in three sub-groups of four mice each:
   1. thawed blue whiting mince and oat flakes in the weight ratio of 1:2, water – ad libitum;
   2. thawed blue whiting mince and oat flakes in the weight ratio of 1:1, water – ad libitum;
   3. thawed blue whiting mince and oat flakes in the weight ratio of 2:1, water – ad libitum.

III. The mice of this group were supplied with the basic diet of oat flakes, milk powder and water with an addition of fragmented living *Anisakis* larvae, put into their oesophagus by means of a pipette. The experiment was carried out in three subgroups of 11 mice each. Each mouse received:
   1. one larva in subgroup 1,
   2. three larvae in subgroup 2, and
   3. five larvae in subgroup 3.

IV. The mice of this group were given feed with *Anisakis* larvae in the form of homogenate subjected to thermal action. The experiment was made in three subgroups of 9–11 mice each. The mice received respectively:

1. blue whiting mince dried for 48 hours at 70°C and water ad libitum;
2. the basic diet as in the control group and homogenate of 3 living *Anisakis* larvae after treating them with the temperature of 100°C for 2 hours. The homogenate was administered into the oesophagus of mice by means of a pipette;
3. the basic diet and homogenate of 3 *Anisakis* larvae already treated with the temperature of minus 25°C for 72 hours.

The suspension of larval fragments and homogenate were prepared in distilled water, not exceeding 1 ml per mouse. They were introduced into the oesophagus of mice using a narrow bent pipette or one-millimetre syringe ending in a fine catheter.

A daily check for feed consumption was made in each experimental group.

After 10 days of such experiment, feeding was stopped and the mice were weighed. The increase in body weight was calculated and the animals were killed by means of decapitation.

A dissection was carried out for close anatomo-pathological inspection of the internal organs: the liver, kidneys, spleen and alimentary canal. Finally, the percentage ratio of the weight of these organs to the body weight of the mice examined was calculated. The results obtained were subjected to the statistical analysis by the formula

$$\frac{M_1 - M_2}{\sqrt{m_1^2 - m_2^2}} > 3$$

where M_1 is the arithmetic mean of the weight of particular organs in control mice, M_2 – the arithmetic mean of the weight of these organs in experimental mice, and m_1^2 and m_2^2 the mean errors of standard deviation.

The results obtained are presented in graphs (Figs. 1 and 2) and diagrams (Figs. 3, 4 and 5).

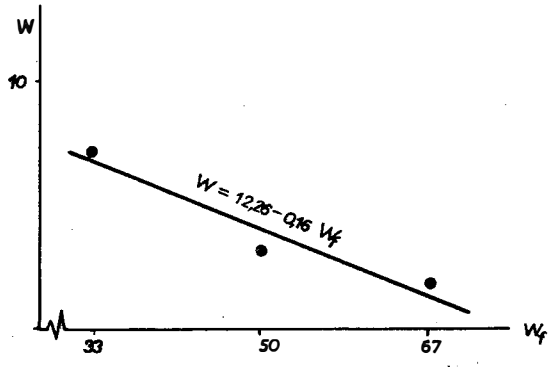

Fig. 1. A relationship between the increase in the body weight of mice and the share of fish mince in the diet (experimental group II). W – increase in the body weight of mice in percentage; Wf – percentage share of fish mince in the diet of mice

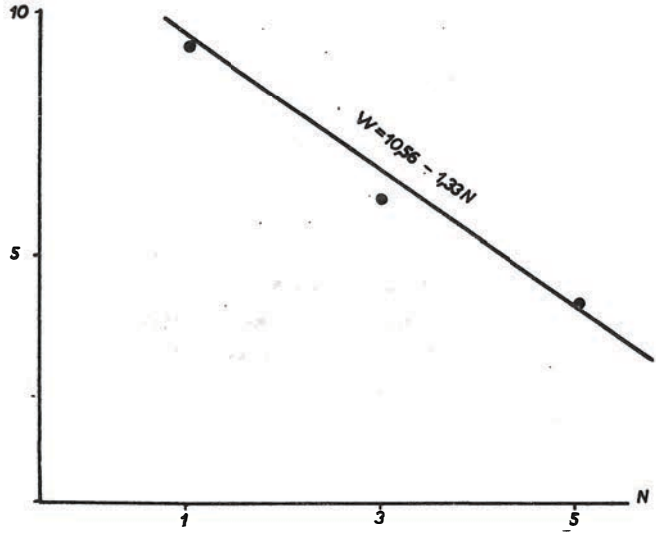

Fig. 2. A relationship between the increase in the body weight of mice and the number of killed *Anisakis* larvae introduced into the oesophagus of mice (experimental group III). W – increases in the body weight of mice (in %); N – number of *Anisakis* larvae

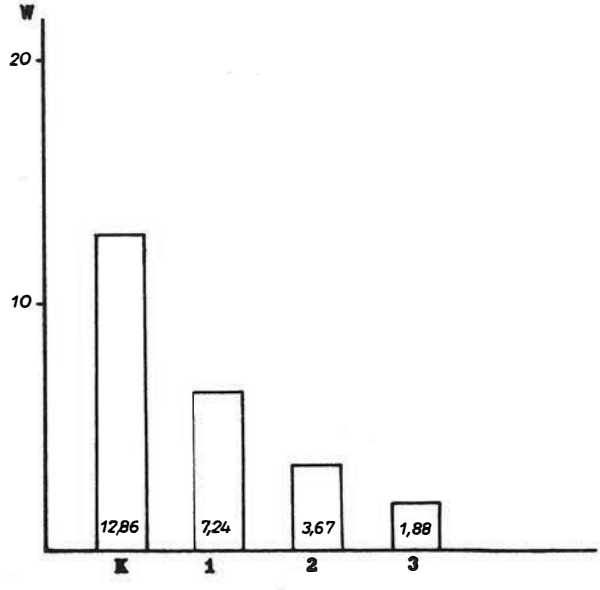

Fig. 3. Effect of fish mince in the diet of mice on the increase in their body weight (experimental group II). W – increases in mouse weight (in %); K – control; 1, 2, 3 – serial number of experiments

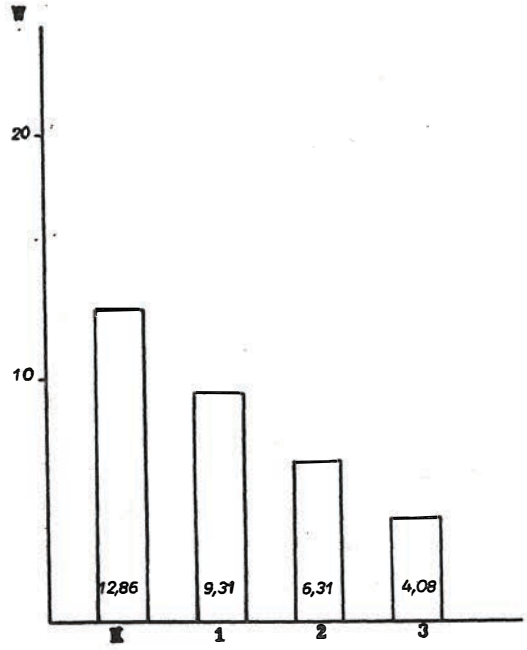

Fig. 4. Increases in the body weight of mice receiving killed *Anisakis* larvae (experimental group III). W – increases in the body weight of mice (in %); K – control; 1, 2, 3 – serial number of experiments

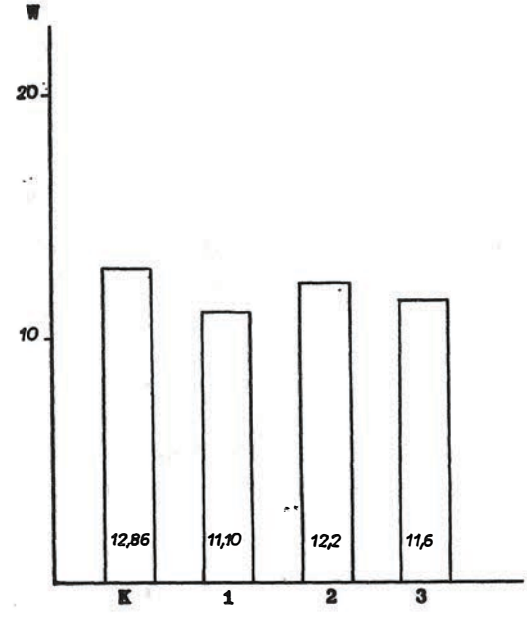

Fig. 5. Increases in the body weight of mice receiving homogenates of *Anisakis* larvae, subjected to the action of different temperatures prior to the experiment (experimental group IV). W – increases in the body weight of mice (in %); K – control; 1, 2, 3 – serial number experiments

RESULTS

In group II of experimental mice fed on thawed blue whiting mince infested with *Anisakis* larvae the normal increase in body weight was found to be lower related to the percentage increase of the mince in the diet. Straight relationship was observed and the equation line is assuming the form:

$$W = 12.26 - 0.16\,Wf$$

where W is the percentage increase in body weight and Wf the percentage share of fish mince in the diet (Figs. 1 and 3).

This result indicates the negative effect of fish flesh on mice, that must be taken into consideration as far as a whole experiment is conceived. Gibson (1970) drew his attention to the analogous effect of fish flesh in food on the condition of rats; he observed a small fall in body weight and the dulling of the fur of these animals.

As it is seen from further part of experiment (group IV of mice), the presence of *Anisakis* larvae in the mince is here of no importance, since the mince was frozen earlier and thus the toxins of the nematodes was destroyed.

In group III the experimental mice were fed on the normal diet with an addition of living larvae cut into pieces, and then the increase in the body weight of the mice was found to be smaller with simultaneous increase in the number of *Anisakis* larvae given to them.

Straight relationship was observed and the equation line is expressed by the formula

$$W = 10.56 - 1.33\,N$$

where W is the percentage increase in body weight and N – the number of *Anisakis* larvae (Figs. 2 and 4).

As regarde group IV, in addition to the normal diet the mice were supplied with the homogenate of living *Anisakis* larvae subjected to the action of different temperatures. The increase in the body weight of the mice was similar to that of the control groups. The differences observed were statistically non-significant (Fig. 5).

A dissection for anatomo-pathological inspection has pointed out the strong hyperaemia of mucosa of the stomach and the first section of the duodenum in mice supplied with fresh *Anisakis* larvae (group III). It looks like the catarrhal state in the inspected pieces of the alimentary tract.

In addition to the above-discussed experiments, three other ones were carried out to examine the influence of fish mince dried at 70°C and added to the basic food (oat flakes) in the ratios of 1:2, 1:1 and 2:1. This sort of food was easy taken by the mice. The stomachs of the mice were closely filled with it and probably these contents had a bearing on the very high increases in the weight of mice, which were respectively 35.20, 29.90 and 11.11%. In this case, as in the group II, we observed the tendency to decrease the gain on the weight of the animals being under the experiment.

For comparison one group of mice were given a mixture of equal parts of mince of fresh Baltic cod, non-infested with *Anisakis* larvae, and oat flakes and then the increases in the body weight of mice were 6.67% In an analogous experiment when the mice were

supplied with thawed blue whiting mince (group II, subgroup 2) this increase was hardly more than a half of that value. This may be due to a lower nutritive value of blue whiting flesh infested heavily by parasites and also to the differences in the quality of the fish products between different fish species.

Statistical analysis of the weights of particular organs of mice in all experimental groups in comparison with the corresponding weights of the control mice showed that the differences between them were not significant. This fact points to the lack of selectivity of the toxins in relation to the organs examined and to their action on the general metabolism of mice.

DISCUSSION

Experimental animals, such as rabbits, rats, guinea pigs and dogs, have been used for studies on the properties of *Anisakis* larvae for a long time.

The experiments were carried out specially in relation to the invasions of larvae (Myers, 1963; Asami and Inoshita, 1967), their pathogenicity (Oyanagi, 1967; Ruitenberg, 1970) and as the effect of different compounds on the metabolism of larvae in connection with the search for effective remedies for anisakidosis in man (Hamajima et al., 1969).

Studies were also performed on the antigenic properties of larvae and on the methods for detecting their invasion *in vivo* (Suzuki et al., 1969; Taniguchi, 1970), on the amino acid composition of these larvae (Oichi et al., 1972) and other problems, but no papers have been hitherto published that investigate the harmful effect of fish body and the fish products contaminated with dead larvae and to determine the degree of their toxicity to the mammalian organism.

Although mice are omnivorous animals, they were not insensible to the presence of both fresh and dried fish mince in their food. As the effect of their reaction was a considerable fall in body weight, when the doses of fish flesh increased in the diet of mice. Such effect was also observed by Gibson (1970), who fed fish to rats infested with living *Anisakis* larvae introduced directly into their stomach by laparotomy.

In further experiments fish mince was eliminated from the diet of mice so that the proper reactions of mice to dead *Anisakis* larvae are not be obliterated (experiments III and IV).

CONCLUSIONS

The following conclusions have been drawn on the basis of these investigations.

1. Freshly killed and divided to pieces *Anisakis* larvae given to mice in feed exert an unfavourable effect on them. They cause a fall in the body weight of mice and that fall grows with the increase of the doses of larvae in feed. They also disturb the alimentary tract of mice. As an effect of this action the catarrhal state of the stomach and the first section of the duodenum was observed.

2. *Anisakis* larvae in the form of homogenate, exposed to the action of temperature (–25 and +100°C), have no harmful effect on mice. Both, low and high temperature destroy the toxins produced by these larvae.
3. The toxins of *Anisakis* larvae show no selectivity in relation to the abdominal organs (liver, kidney, spleen), but they act on the mouse organism as a whole, bringing about a general fall in body weight.
4. Fishes added to diet of mice in the fresh or dried form are not absorbed by them, so its presence in feed gives a fall in their body weight.

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BADANIA NAD TOKSYCZNOŚCIĄ LARW *ANISAKIS SIMPLEX* (RUD.) CZĘŚĆ I. PRÓBA BIOLOGICZNA NA BIAŁYCH MYSZKACH

Streszczenie

Badania nad toksycznością mięsa rybiego, zanieczyszczonego martwymi larwami *Anisakis*, przeprowadzono na myszkach, którym podawano różne zestawy jakościowe i ilościowe karmy.

Po 10 dniach doświadczeń myszki ważono i obliczano przyrosty ciężaru ciała a następnie zabijano przez dekapitację. Wykonano sekcję anatomo-patologiczną narządów wewnętrznych oraz obliczano w procentach stosunek ciężaru wątroby, nerki i śledziony do ciężaru badanych myszek. Przeprowadzono analizę statystyczną uzyskanych wyników.

Na podstawie przeprowadzonych doświadczeń ustalono, że:

1. Świeżo zabite rozdrobnione larwy *Anisakis* oddziałują ujemnie na myszki przy podawaniu ich w karmie, powodując spadek ciężaru ciała myszek zwiększający się w miarę zwiększania dawki

larw. Wywołują one również silne przekrwienie śluzówki żołądka oraz początkowej części dwunastnicy, wskazujące na nieżyt tych odcinków przewodu pokarmowego.

2. Larwy *Anisakis* w postaci rozdrobnionej poddane działaniu temperatur $-25^{\circ}C$ i $+100^{\circ}C$ nie wykazują ujemnego wpływu na myszki, co świadczy, że ich toksyny uległy zniszczeniu przez działanie termiczne.
3. Toksyny larw *Anisakis* nie wykazują działania wybiórczego na narządy miąższowe (wątroba, nerka, śledziona) lecz oddziałują ogólnie na cały organizm myszki.
4. Mięso rybie w postaci świeżej lub suszonej nie jest przyswajane przez myszki lecz obecność jego w karmie powoduje spadek ciężaru ciała myszek. Fakt ten może zacierać właściwą reakcję myszek na pasożyty.

ИССЛЕДОВАНИЯ НАД ТОКСИЧНОСТЬЮ ЛИЧИНОК ANISAKIS SIMPLEX (RUD.) I БИОЛОГИЧЕСКИИ ОПЫТ НА БЕЛЫХ МЫШАХ

Р е з ю м е

Исследования над токсичностью мяся рыб, заражённого мёртвыми личинками Anisakis, были проведены на белых мышах, которым давали различные по количественному и качественному составу корма.

После 10 дней продолжения опытов мышей взвесили и определили прирост веса тела, а затем умертвили путём декапитации. Затем провели анатомо-патологическое вскрытие внутренних органов и вычислили в процентах отношение веса печени, почки и селезёнки к весу тела исследуемых мышей.

На основе проведенных опытов установлено, что:

1. Свежеумерщвлённые измельчённые личники Anisakis отрицательно воздействуют на мышей при подаче их с кормом, вызывая уменьшение веса тела мышей, возрастающее по мере увеличения дозы личинок. Они вызывают сильную гиперемию слизистой оболочки желудка и начальной области двенадцатиперстной кишки, свидетельствуя тем самым о катаре этих участков желудочно-кишечного тракта.
2. Личинки Anisakis в измельчённом виде, подвергнутые действию температур $-25^{\circ}C$ и $+100^{\circ}C$, не проявляют отрицательного воздействия на мышей, что свидетельствует о том, что их токсины подверглись разрушению при термическом воздействии.
3. Токсины личинок Anisakis воздействуют не на отдельные паренхимные органы (печень, почки, селезёнка), а на весь организм мышей в целом.
4. Мясо рыб в свежем или сушёном виде мыши не усваивают, а наличие его в корме вызывает уменьшение веса тела мышей. Это может ослаблять правильную реакцию мышей на присутствие паразитов.

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