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CONTROL OF IMMATURE FASCIOLA HEPATICA USING DI-[2-(4-ACETAMIDO PHENOXY) ETHYL] ETHER

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Abstract. The drug di-[2-(4-acetamido phenoxy)ethyl]ether*) at a dosage of 100 mg/kg is shown to be highly effective against *Fasciola hepatica* in sheep three weeks or 6 weeks after infection. At these times essentially all parasites were in the tissue of the liver. Possible modes of action of drugs effective against immature *Fasciola* are discussed.

The control of fascioliasis in domestic animals continues to depend mainly on chemotherapy and remains unsatisfactory because at recommended dose rates there is very little effect on immature parasites within the liver tissue and an erratic response during the period when movement into the bile ducts occurs (in sheep at about 6 weeks). Several drugs have been shown to have an effect on parasites of about 4—6 weeks but usually at very high dosage. Drugs with a specific effect against immature parasites are very few. Emetine hydrochloride at 3.0 mg/kg has been shown to be effective against 3 or 4 weeks old parasites in sheep (Grant and Jaggers 1969) but has not been developed commercially, possibly because of cost.

A new drug di-[2-(4-acetamido phenoxy)ethyl]ether had been shown in commercial screening to show promise and was made available to us by the Wellcome Foundation England. This paper describes tests against experimental infections in sheep at Weybridge.

MATERIALS AND METHODS

The experiment was carried out in two parts using sheep or lambs known to be free from *Fasciola* at the time of experimental infection. Metacercariae of *Fasciola hepatica* were produced from experimentally infected snails at the laboratory and used 2—6 weeks after emergence. The drug di[2-(4-acetamidophenoxy)etlhy]ether was given orally as an approximately 19 % w/v watery suspension at the rate of 100 mg a.i./kg.

FIRST EXPERIMENT

14 female and 10 castrated male cross-bred Clun sheep about 4½ years old were assigned, on the basis of similar weights, to three experimental groups. Each sheep was infected with 3 000

*) "diamphenethide" "Coriban"—Burroughs Welcome

metacercariae. Three weeks later 8 sheep of Group 1 were treated with the drug and at 6 weeks Group 2 was treated. The remaining 8 sheep acted as untreated control (Group 3) — Table 1.

SECOND EXPERIMENT

This was essentially the same as the first experiment but involved 10 Dorset horn lambs 17—22 weeks old at the time infection. They were divided into 3 groups on a weight basis and each lamb was infected with 2 000 metacercariae. Group 4 (Table 1) was treated with drug at 3 weeks, Group 5 at 6 weeks and four untreated lambs comprised Group 6.

One untreated sheep from Group 3 and one lamb from Group 6 were slaughtered at 6 weeks after infection to assess the stage of development of the parasites at this time. All the rest of the

Table 1. Effect of the drug at three and six weeks after infection with *Fasciola hepatica*

Number of Animal	Group and time treated	Parasites recovered	Total parasites in Group
4124		0	
4088		0	
4095		0	
4119	1	0	30
4105	(three weeks)	1	
4082		1	
4085		2	
3032		26	
4108		1	
4116		1	
3025		5	
4084	2	6	305
4097	(six weeks)	10	
4103		10	
4079		30	
4107		242	
4118		589	
3027*)		661	
4080		738	
4077	3	844	7176
4096	(untreated)	1015	
4089		1074	
4090		1108	
4113		1147	
1775	4	0	
1708	(three weeks)	4	80
1827		76	
1702	5	0	
1773	(six weeks)	25	73
1770		48	
1774**)		740	
1703*)	6	786	3625
1707	(untreated)	870	(4 animals)
1701		1229	

*) Killed at 6 weeks after infection
**) Died at 7 weeks 6 days

animals were killed at about 12 weeks after infection. The carcases and livers were given a general examination and the livers then carefully examined for the presence of parasites. No abnormalities, apart from those usually associated with infection with *Fasciola* were noted.

The numbers of parasites recovered are recorded in Table 1.

RESULTS

As will be seen from the Table 1 an average of about 900 parasites was recovered from each untreated animal and one lamb (No 1774) died from fascioliasis at about 8 weeks after infection. Examination of the two animals slaughtered at six weeks (Nos. 3027 and 1703) showed that practically all the parasites were at that time still within the liver tissue. Only one parasite, in either instance, was recovered from the bile ducts.

Treatment at 3 weeks or at 6 weeks after infection was very effective and the numbers of parasites recovered were reduced to 5% or less of those recovered from the untreated sheep.

DISCUSSION

In our experiments there was no gross evidence of toxicity arising from the use of the drug which clearly has a very high degree of efficacy against *Fasciola hepatica*, at least while parasites are in the tissue of the liver. Such high efficacy against such young parasites is very rare. The drug belongs to a group of compounds known to have activity against schistosomes and the structure activity and structure toxicity relationships have been discussed by Standen (1963). Standen mentions that in experimental infections the diaminodiphenoxy alkanes behave as do most schistosomicides by inducing a shift of worms from the mesenteric veins to the liver. This is followed by ensheathment in inflammatory tissue and by subsequent phagocytosis. Such a sequence of events may well be a function of the worms rather than of the drug. Activity with primary, secondary or tertiary p-aminodiphenoxy alkanes increases with the age of the schistosomes. This may be contrary to what happens with *Fasciola*. Standen suggested that activity lay not in the compounds themselves but in one or more metabolites liberated during their breakdown in the host tissues.

Emetine, another drug known to have a marked effect against immature *Fasciola* (Grant and Jaggers 1969) is structurally unrelated to di-2-(4-acetamido phenoxy) ethyl]ether but in any event published work on the mode of action of emetine (Grollman 1966) gives no indication of a capacity for a special effect against trematodes. Grollman suggested that the action of emetine as a potent inhibitor of protein synthesis was likely to account for both its therapeutic and its toxic properties and he drew an analogy between the action of glutarimide antibiotics and the ipecac alkaloids.

Not much is known about the action of the drugs commonly in use against *Fasciola*. As indicated by Boray and Happich (1968) many do have an effect against young flukes (4 weeks old) but only at potentially toxic doses. There is a well marked dose response i.e. the higher the dose the younger the parasite that is affected. It is possible to augment efficacy, apparently without necessarily augmenting toxicity, by giving certain drugs in combination e.g. hexachlorophane and nitroxynil (Kendall and Parfitt 1971) but this additive effect sheds no light on mode of action. Broome (1966) showed that hexachlorophane itself was active against both mature and immature *Fasciola* in the presence of bile but not of blood. He suggested

that hexachlorophane was excreted into the bile as a metabolite which was the active therapeutic agent. This would explain the greater activity of the drug on parasites in the bile ducts. The observation of Kendall and Sinclair (1969) that extremely young liver flukes in rabbits were unexpectedly susceptible to hexachlorophane could be explained on the same basis because during initial invasion of the host they may well be exposed to bile which contains the metabolite (a glucuronide) in high concentration.

An alternative hypothesis explains the fasciolicidal effect of such halogenated hydrocarbons in terms of liver damage rather that a direct effect of the drug. Such a suggestion was made by Alexander and Macdonald (1960) but their reasoning was based at least in part on a failure to demonstrate the excretion of carbontetrachloride in bile whereas Fowler (1970) found that the drug was in fact present for at least six hours following dosage. Fowler did however agree that there might be an indirect effect through the release of the products of liver damage.

Earlier (1969) Khalidi and Zaki had suggested that the toxic principle was either the direct product of liver breakdown or that toxic compounds might arise through the interaction between carbon tetrachloride and liver lipids. In a slightly different context Kendall and Sinclair (1969) were able to show that the combined effect of a previous infection and therapy with hexachlorophane was able to protect a rabbit against reinfection most probably because of changes in the liver proving deleterious to the invading parasites.

If the general hypothesis, that parasites are killed or prevented from becoming established as the result of changes in the liver be accepted, it is not easy to see why immature parasites within the liver tissue should not be affected by therapy at least to the same extent as are older parasites in the bile ducts. There is some indication (Standon, personal communication) that di-2-(4-acetamido phenoxy)ethyl] ether may be rather less effective against older parasites. If this is so, there is an interesing parallel with the action of emetine and the possibility of further lines of investigation into mode of action.

БОРЬБА С НЕПОЛОВОЗРЕЛЫМИ ТРЕМАТОДАМИ *FASCIOLA HEPATICA* С ПРИМЕНЕНИЕМ ПРЕПАРАТА DI-[2-(4-ACETAMIDO PHENOXY) ETHYL] ETHER

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Резюме. Препарат di-[2-(4-acetamido phenoxy)ethyl]ether*) в дозе 100 мг/кг показал высокую эффективность в борьбе с *Fasciola hepatica* у овец 3 или 6 недель после заражения. В эти периоды все паразиты находились в ткани печени. В работе рассматриваются возможные способы действия эффективных препаратов против неполовозрелых трематод *Fasciola*.

*) „diamphenethide"

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CYSTICERCOIDS OF CESTODES (DILEPIDIDAE) FROM FISHES OF LAKE SKADAR

Helminthological examination of fishes in Lake Skadar (Yugoslavia) revealed the presence of larval stages of cestodes (cysticercoids) of the family Dilepididae in 10 out of a total of 195 fishes examined (5. 1 %). The individual species concerned were identified on the basis of the new classification of the family Dilepididae by Baer and Bona Boll. Inst. Mus. Zol Univ. Torino 6. The cysticercoids recovered belonged to three species:

1. *Valipora campylancristrota* (Wedl, 1855) Baer et Bona, 1960. (Fig. 1). Cysticercoids of this species were found in the gall bladder of *Blenius fluviatilis*, *Alburnus albidus arborella* and *Leuciscus cephalus albus*. Rostellar hooks (20) arranged in two rows; length of the longer hooks 0.023—0.025 mm, of the shorter hooks 0.011 to 0.012 mm. Diameter of suckers 0.080—0.090 mm. The cysticercoids, until the present designated, *Dilepis unilateralis* (Rudolphi, 1819) occurred in *Tinca tinca*, *scardinius erythrophtalmus* and *Cyprinus carpio*.

2. *Neogryphorhynchus cheilancristrotus* (Wedl 1855) Baer et Bona, 1960. (Fig. 2a). These cysticercoids were obtained from the stomach

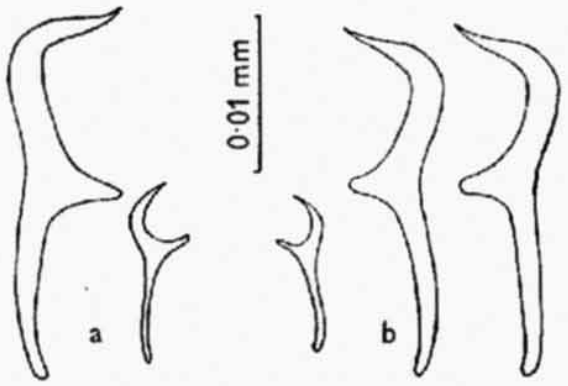

Fig. 1. *Valipora campylancristrota* (Wedl, 1855) a — from the gall bladder of *Blenius fluviatilis;* b — from the gall bladder of *Alburnus albidus arborella*.