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THE BIOLOGICAL ESTIMATION OF SUBSTANCES USED IN TREATING CESTODE INFESTATIONS

BY

PAMELA HOLTON

From the Department of Pharmacology, Oxford

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The first experimental observations of the action of anthelminthic drugs were made by von Schroeder (1884, 1885) on annelid worms. He was followed by Trendelenburg (1916), who used round worms, but mainly worked with annelids. Sollmann (1919) and Wasicky (1923) used annelid worms and fish. Hall (1921) was the first to carry out experiments on infested animals. He determined the percentage of parasitic worms which were expelled when dogs were given anthelminthics; his main interest, however, was in nematodes. Rebello, Gomes da Costa, and Toscano Rico (1928; also Gomes da Costa, 1931) showed that cestodes and nematodes behave differently when treated with anthelminthics outside the body, as might be expected since they belong to different phyla of the animal kingdom. Gomes da Costa and his colleagues have developed a method for the observation of isolated segments of *Taenia saginata* and *Dipylidium caninum*. Their results have confirmed clinical findings and they have been able to recommend new drugs for trial (Gomes da Costa, 1930, 1932; Gomes da Costa and Hamet, 1935, 1937). They showed that the survival time of a segment of *Dipylidium caninum* in an oil bath bore an inverse relation to the concentration of filix mas, but made no further attempt to obtain a response graded to the dose (Ettisch and Gomes da Costa, 1937).

Culbertson (1940) worked with mice infested with *Hymenolepis fraterna* (called in this paper *Hymenolepis nana*) and found that doses of atabrine (mepacrine) greatly reduced the number of worms in the intestines of the mice. Again, however, no quantitative results were obtained.

The experiments described in this paper were undertaken to find a convenient method of determining anticestode activity as part of an attempt to isolate the active principles of certain crude drugs. The work has been done with one sample of extractum filicis B.P. Mice supplied to the laboratory were found to be naturally infested by two species of tapeworm, *Hymenolepis nana* and the much larger *Hymenolepis diminuta*. These two species cannot easily be distinguished by inspection of their eggs, so that infested mice may harbour either or

both species, but since *H. diminuta* is so much larger than *H. nana*, the results, which were obtained by weighing the worms, depend almost entirely on the infestation with *H. diminuta*. When fresh mice came to the laboratory, infestation was determined by microscopic examination of the faeces for eggs; as a rule about 30 per cent of the mice were infested. The mice in the faeces of which no eggs were detected were returned to the laboratory stock and were re-examined at intervals of about 3 weeks, when a further 10 per cent were found to be infested.

Details of Procedure

Examination of mice for infestation.—The mice were fed at least an hour before examination. A pellet of faeces was then taken from each mouse and a faecal smear was made on a microscope slide. Using low power focused on the bottom of the smear, it was fairly easy to spot the typical egg (see Fig. 1) containing a hexacanth embryo. When in doubt the high power was used.

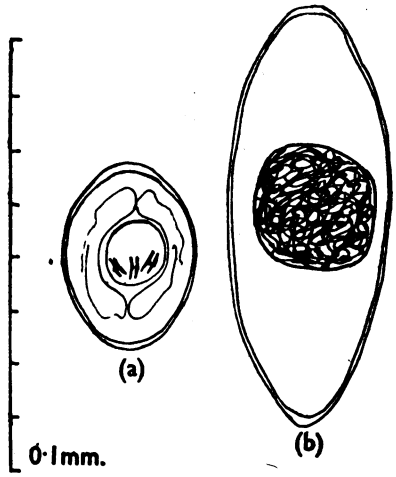

FIG. 1.—Helminth eggs found in mouse faeces. (a) *Hymenolepis* egg containing a hexacanth embryo. (b) Nematode egg. This is the only common egg likely to be mistaken for *Hymenolepis*.

When ext. filicis is used clinically, the patient is starved overnight. The next morning he is given 3–6 ml. of the extract, followed by a saline purge after two hours. This procedure was adopted with the infested mice, and the proportion of worms excreted was found. Preliminary experiments were carried out to find suitable doses of ext. filicis and of purgative.

Oral toxicity of ext. filicis.—The mouse dose, corresponding to a human dose of 6 ml., is 0.002 ml. An aqueous emulsion was made by shaking ext. filicis with sodium glycocholate (1 per cent) solution. This emulsion was not very stable, but was used for the first experiments. Later, when quantitative results were required, a standard emulsion of ext. filicis in ether and sodium glycocholate solution was prepared as described below.

Groups of mice were given by mouth doses of ext. filicis emulsion (1 and 10 per cent by volume). After 24 hours the dead mice were counted. The results are given in Table I.

TABLE I

ORAL TOXICITY OF EXTRACTUM FILICIS

Dose	Volume of emulsion administered	Result
50 mg.	0·5 ml. 10 per cent (v/v)	9 out of 15 mice died
20 mg.	0·2 ml. 10 „ „	0 „ 3 „ „
10 mg.	1·0 ml. 1 „ „	0 „ 3 „ „

Since the lethal dose was about 50 mg., one-fifth of this dose (10 mg. ext. filicis) was used as the highest therapeutic dose.

Choice of purgative.—It is usually taught that a saline purgative should be given after ext. filicis, since when castor oil is used the extract dissolves in the oil and is absorbed. The first experiments were therefore made with sodium sulphate as a purgative; this was found to produce diarrhoea when 0.1 g. (anhydrous) was given orally in 1 ml.; half this dose was not effective. Since the purgative effect was delayed in some experiments and might have been due to hypertonicity, 1 ml. of a solution of 6.5 g. $MgSO_4.7H_2O$ in 100 ml. water, which is isotonic, was used instead.

Examination of material.—Examination of faeces or gut contents was carried out in water on a black developing dish. The white fragments of worms were picked out with forceps and rinsed free of debris. At first all fragments were examined microscopically and the number of scolices of each species (*H. diminuta* and *H. nana*) was counted. It was then decided to weigh the worm fragments in a small Gooch crucible after they had been roughly dried with cotton wool and filter paper. An attempt to determine the dry weight after heating to 100°C. for 1 hour was unsuccessful because this weight was never more than a few milligrammes and consequently was too small to be an accurate measure of worm content. The wet weight was of the order of 100 mg. per mouse and was adopted as a convenient measure of the amount of worms. The results of an early experiment are given in Table II.

TABLE II

Material	Scolices			Weight of worm fragments	
Faeces	*H. nana*	*H. diminuta*	Total	Wet, mg.	Dry, mg.
10 Treated mice ..	5	18	23	711	44
10 Control mice ..	1	0	1	33	<1
Gut contents					
10 Treated mice ..	2	4	6	167	3
10 Control mice ..	122	13	135	787	38

Percentage of worms (wet weight) discharged by:

(1) Treated mice 81.

(2) Control mice 4.

In the early experiments the control mice often died, and the conclusion was drawn that the period without food was too long. Glucose injections were first tried as a means of providing calories without giving solid food, but these made no difference. Lumps of sugar were then put in the boxes, and this device was successful; the experiments were carried through without deaths in the control or treated mice provided that mice of not less than 18 g. weight were used.

The next difficulty was that mice receiving a high dose of ext. filicis often discharged a smaller percentage of tapeworms than mice receiving a lower dose. It was assumed that the worms were affected by the higher dose, but the mice were weakened and so did not excrete them in the five hours of the experiment. The solution of this problem was suggested by the fact that worms from the intestines of mice in control groups moved vigorously in water and could easily be separated from those which did not move and which were assumed to be killed or paralysed by the drug. To give the worms every chance of movement, the intestines were opened in warm saline. The " dead " worms and live worms from each group were picked out as described before and weighed separately. This division could always be made without hesitation when the worms were touched with forceps. As well as the " dead " worms in the small intestine, worms were sometimes found in the large intestine, including the caecum; these were obviously about to be excreted, because *Hymenolepis* does not inhabit this part of the gut, and they were included in the total of affected worms.

This revised technique was used in eleven experiments. In each experiment groups of five infested mice (each mouse weighing over 18 g.), which had been fed on lumps of sugar from 6 p.m. the previous day, were given doses of freshly prepared filicis emulsion, followed by magnesium sulphate as a purgative after two hours. Three hours later the faeces of each group were examined as already described. Each mouse was then killed and its intestine was removed. The small intestine was put into warm (38°C.) saline in a black dish and slit open longitudinally with scissors. The mucosa was scraped with a microscope slide and the intestinal contents were examined as described above. The large intestine was treated in the same way. The three categories of worms from the intestines of each group were weighed separately. The weight of worms affected by the drug was expressed as a percentage of the total weight of worms in each group. The method is illustrated by the following experiment:

Exp. 17. Preparation of ext. filicis emulsion.—After being stirred the ext. filicis (230 mg., or four drops) was weighed in a 50-ml. conical flask and dissolved in 1 ml. of ether; distilled water, containing about 1 per cent sodium glycocholate, was added so that 1 ml. of the resulting emulsion contained exactly 10 mg. ext. filicis. The emulsion was shaken before use and diluted when necessary with a solution of glycocholate and ether in the same proportions as above so that a volume of 1 ml. was administered to each mouse.

The ext. filicis emulsion and the purgative were administered with a blunted hypodermic needle, used as a stomach tube, and a 1-ml. syringe.

Timetable of experiment

9.40 a.m. Group 1. 5 mice given by mouth 10 mg. ext. filicis.
Group 2. „ „ „ 5 mg. ext. filicis.
Group 3. „ „ „ 2.5 mg. ext. filicis.
Group 4. „ „ „ 1.25 mg. ext. filicis.

Each group was put into a wooden box with a metal floor and supplied with 4 lumps of sugar and water.

11.40 a.m. Each mouse was given by mouth 1 ml. warm 6.5 per cent (w/v) magnesium sulphate solution. The groups were replaced in their boxes.

2.40 p.m. Examination of faeces scraped from the floor of each box.
Examination of intestines.

The results are given in Table III.

TABLE III

Figures are weights of worms (mg.)

	Group 1	Group 2	Group 3	Group 4
Dose of ext. filicis:	10 mg.	5 mg.	2·5 mg.	1·25 mg.
Worms in faeces (*a*)	53	235	286	121
Live worms in small intestine (*b*)	0	75	290	771
" Dead " worms in small intestine (*c*) ..	0	41	0	0
Worms in large intestine (*d*)	65	130	115	0
Total worms ($a + b + c + d$)	118	481	691	892
Total worms affected by drug ($a + c + d$)	118	406	401	121
Percentage wet weight of worms affected by drug	100	85	58	13

The results of the eleven experiments are given in Table IV. A total of 220 mice were used ; 55 for each dose of ext. filicis.

TABLE IV

PERCENTAGE WET WEIGHT OF TAPEWORMS AFFECTED BY DOSES OF EXT. FILICIS GIVEN TO GROUPS OF 5 MICE

	Group 1	Group 2	Group 3	Group 4
Dose of ext. filicis:	10 mg.	5 mg.	2·5 mg.	1·25 mg.
Experiment 14 ..	100	55	48	0
15 ..	91	18	9	0
16 ..	100	88	38	6
17 ..	100	85	58	13
18 ..	100	59	90	0
19 ..	67	85	0	0
20 ..	100	33	67	44
21 ..	96	100	8	0
22 ..	100	45	58	52
23 ..	86	92	40	28
24 ..	85	100	92	14
Mean ..	92	69	46	14

It will be noticed that in any single experiment (e.g., expts. 18, 19) the response is not necessarily graded to the dose, but if the results of any three consecutive experiments are taken together the mean percentage wet weights of tapeworms affected are graded to the doses. This shows that at least fifteen mice must be used for every dose of anticestode drug. The results are shown graphically in Figs. 2 and 3. In Fig. 2 the mean percentage effect was plotted against

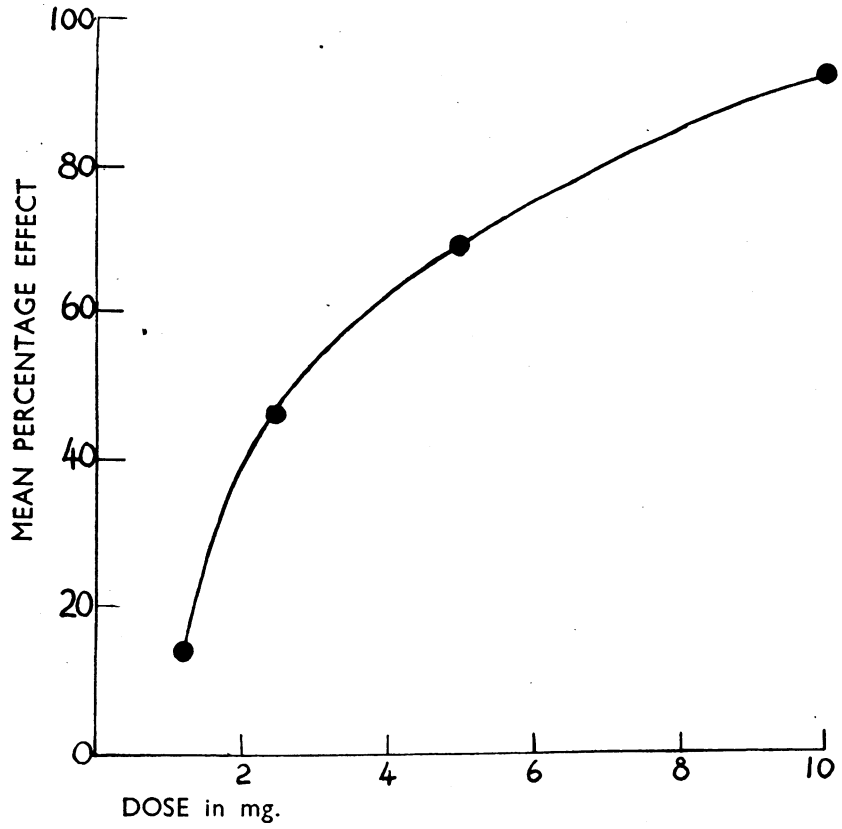

FIG. 2.—Results of 11 experiments using a total of 55 mice for each dose of ext. filicis. Ordinates: Mean percentage wet weight of tapeworms affected. Abscissae: Dose (mg.) given to each mouse.

each dose and a curve was drawn relating dose to effect. In Fig. 3 the expected probits of the mean percentage effect, calculated as described by Bliss (1938), were plotted against the logarithms of the doses. The slope of the line, $b=2.28$. The probability of the line, P, is approximately 0.02.

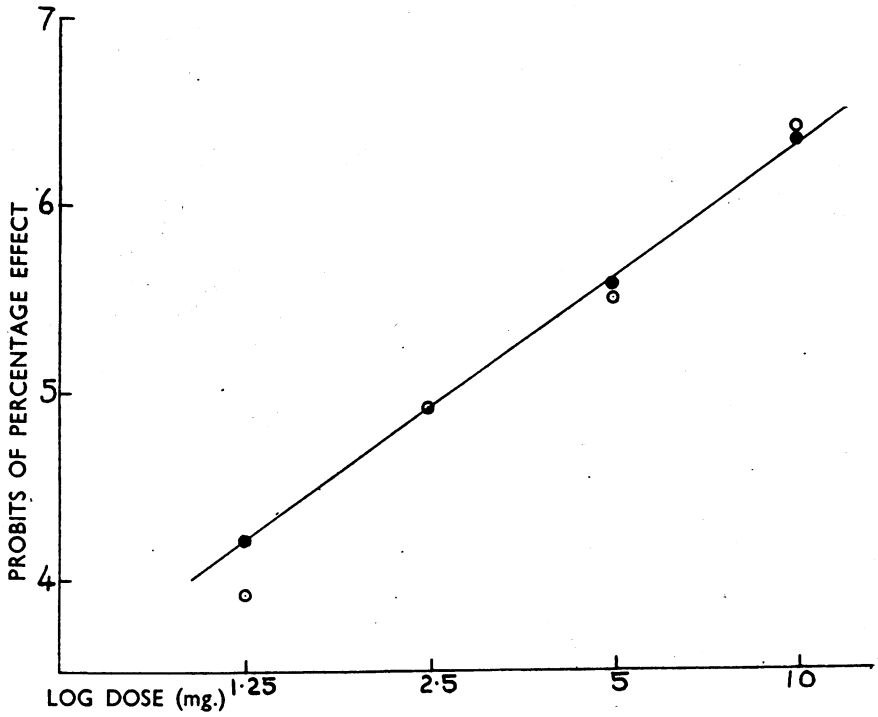

FIG. 3.—Same results as in Fig. 2. Ordinates: Probits of mean percentage wet weight of tapeworms affected. Abscissae: Log dose (mg.) given to each mouse. White circles: Empirical probits. Black circles: Expected probits.

SUMMARY

A method for the standardization of anticestode drugs is described. Mice infested with *Hymenolepis diminuta* were used as experimental animals. The mice were fed on lumps of sugar only for 15 hours. Then each mouse was given a dose of ext. filicis in 5 per cent ether emulsion using a stomach tube. Two hours later each mouse was given a dose of magnesium sulphate solution as a purgative. After another three hours the faeces were examined for *Hymenolepis* fragments, which were collected and weighed. The mice were killed and worms from each group were divided into three categories, which were weighed separately.

1. Worms from the large intestine (not normally inhabited by *Hymenolepis*).
2. " Dead " worms from the small intestine.
3. Live worms from the small intestine.

The weight of the affected worms (categories 1 and 2 and worms in the faeces) was expressed as a percentage of the total weight of worms. Using four different

doses of ext. filicis in a total of 220 mice (55 per dose), it was found that the probits of the percentage wet weight of worms affected increased in linear relation to the logarithm of the dose.

This work was carried out as a result of an inquiry from the Colonial Products Research Council whether substances could be tested for activity against tapeworms. The work has been done at the suggestion and under the supervision of Prof. J. H. Burn. My thanks are due to Prof. R. T. Leiper, who very kindly arranged for me to be shown the details of *Hymenolepis* infestation in mice.

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