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ZOOLOGY

THE USE OF HORSE STRONGYLE LARVAE IN SCREENING COMPOUNDS FOR ANTHELMINTIC ACTIVITY

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Since the discovery of the sulfonamides, research in chemotherapy has gone on at an ever-increasing rate. One phase of this expansion has been an intensification of the search for new and better anthelmintics.

The pattern of this type of research is essentially that which was used during World War II in searching for new antimalarial and antifilarial drugs (cf. Wiselogle, 1946, and Conference on the Chemotherapy of Filariasis, 1948) Hundreds and even thousands of compounds, selected more or less at random, are tested for activity When a promising compound is found, other related compounds are synthesized and studied.

Because so many compounds are studied in the initial screening test, a rapid, cheap technic is essential. The host which one wishes to treat is usually unsatisfactory because it is too large, too expensive to maintain, or not available in sufficient numbers. Hence workers have turned to *in vitro* tests or *in vivo* tests in small laboratory animals.

Neither of these types of test is completely satisfactory Both miss compounds which would be effective against parasites of economic importance; both give promising results with compounds which are later found to be valueless against these parasites. Brackett and Bliznick (1949), for instance, using *Nippostrongylus muris* in the mouse (*Mus musculus*), screened over 1625 compounds for anthelmintic activity Among the known nematocides which they tested, carbon tetrachloride was fairly active, but tetrachlorethylene, hexylresorcinol, and phenothiazine were not satisfactory Santonin and beta-naphthol were practically worthless; gentian violet, thymol, and n-butyl chloride were completely ineffective. On the other hand, trichloracetamide and some of its relatives were highly effective. Unfortunately, this compound turned out to be ineffective against *Strongyloides ratti* in the rat (*Rattus norvegicus*), *Syphacia obvelata*, *Aspicularis tetraptera*, and the adults and larvae of *Trichinella spiralis* in the mouse (*Mus musculus*), and *Litosomoides carinii* in the cotton rat (*Sigmodon hispidus*)

In the last analysis, if one wants to find a compound which is effective against a particular parasite in a particular host, he must test it against that parasite in that host. No other procedure will give definitive results. The screening tests simply help in the hunt.

In vitro tests are usually faster and cheaper than *in vivo* ones. It is not necessary to have facilities for

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housing and handling many experimental animals, and the tests can be done conveniently on a table top. On the other hand, the results obtained in an *in vitro* test cannot necessarily be carried over without modification to the living animal. The drug-helminth reaction is a relatively simple, clear-cut one *in vitro*, but introduction of a host into the reaction brings in many modifying factors. A compound which is active *in vitro* may not be active *in vivo*, and *vice versa*. And an effective compound might turn out to be too toxic for the host to be used.

In vitro tests can be carried out either on the adult worm or on larvae. The advantage of using the adult is that it is the stage for which animals are treated. The metabolism of the larva often differs from that of the adult, so that a drug which kills larvae might not kill adults, and *vice versa*. Nevertheless, larvae are often so easy to obtain and to use that screening with them is justified. Otto and Maren (1948, 1949), for instance, discovered the new antifilarial compound, arsenamide, as a result of screening with *Dirofilaria immitis* larvae.

The screening test which I shall describe is a modification of the test developed by Parnell (1936) It uses the larvae of horse strongyles as test organisms. These nematodes belong to the family Strongylidae. Dikmans (1945) lists three species of large strongyles (genus *Strongylus*) and 36 species of small strongyles of 11 different genera as occurring in horses and other equids in North America. Practically all horses have multiple infestations. It does not seem to matter a great deal which species is used in the test.

The eggs of these nematodes are passed in the feces. First-stage larvae hatch in about a day, feed in the manure, and molt to long-tailed second-stage larvae in another day These also feed in the manure. In a few days they molt again to become short-tailed third-stage larvae. The second-stage cuticle is not completely cast, but is retained as a protective sheath around the third-stage larvae. These larvae do not feed. They leave the manure, crawl onto the vegetation, and are eaten by horses when they graze.

This test was originally designed to be used in searching for compounds which would kill the larvae on pasture. However, it is also applicable to screening drugs to be used against the adults.

Strictly speaking, this is not an *in vitro* test, since it is carried out against larvae in their natural environment. However, since no host is involved, the test is generally grouped with the *in vitro* tests.

The chemical compound to be tested is mixed with talcum powder to give it body One-half gram of this mixture is mixed thoroughly with 4.5 g. of fresh horse manure containing strongyle eggs. The manure-talc-chemical mixture is placed in a small cheesecloth bag, which is suspended in a closed, 2-oz. wide-mouth glass jar. The jar is set aside in a dark cupboard for a week. By this time the larvae have reached the third stage, and many of them have migrated out of the feces. They collect in the droplets of condensation water which appear on the sides of the jar, and can be recognized by examining the condensation water with a powerful hand-lens (I use a 15-X microscope ocular)

If no larvae can be found, the bag is placed in a Baermann apparatus (a funnel with a piece of rubber tubing closed by a pinch-clamp)

Enough warm water is poured into the funnel to half cover the bag. If larvae are present, they migrate into the water. After an hour or two some of the water is withdrawn through the rubber tubing at the bottom of the funnel, and examined for larvae with a low-power microscope or hand-lens.

The presence of living larvae indicates that the compound was ineffective. In the work cited above, both Parnell and I counted the larvae, but I now feel that this is not necessary in a screening test of this type.

In the preliminary screening test, each compound is set up in concentrations of 0.01, 0.005, 0.0025 and 0.001 M. If these give promising results, further studies are carried out. In my earlier work, I used standard percentages of compounds, but comparisons on an equimolar basis are preferable.

Untreated controls are essential, since in an occasional manure sample the larvae failed to develop.

One advantage of using talcum powder as a diluent instead of attempting to dissolve the compounds is that both soluble and insoluble compounds can be studied without changing the technic. In addition, a compound whose solubility is not known can be screened without taking time to determine if it is soluble.

Because of the nature of this technic, it is impossible to tell whether a compound acts on the eggs or on the first-, second-, or third-stage larvae. This does not affect the value of the test for screening purposes. For more precise information, the effect on each stage could be determined separately

The selection of a manure donor is important. A strongyle egg count of at least 1000 eggs per gram of feces is desirable. Horses will sometimes be found in whose manure larvae do not develop well; such horses cannot be used as donors.

So far over 150 compounds have been screened against horse strongyle larvae. The results on 70 have already been reported (Levine, 1949), and those on the others will be reported later. A number of promising compounds have been found, including several iodine compounds.

Among known nematocides which were tested, 1% sodium fluoride, nicotine sulfate, and phenothiazine killed all larvae in the manure, while 1% hexylresorcinol killed 99.5% (compared with the number of larvae present in the controls) When present in a concentration of 0.1% in the manure, crystal violet killed 53% of the larvae; hexylresorcinol, 79%; phenothiazine, 82%; sodium fluoride, 84%; and nicotine sulfate killed them all. This last compound killed 99 to 100% of the larvae in four tests at a concentration of 0.01%.

Summary

A cheap, convenient technic is described for screening chemical compounds for possible anthelmintic value, using horse strongyle larvae as test organisms. The compound to be tested is mixed with horse manure containing strongyle eggs. The mixture is suspended in a cheesecloth bag in a glass jar. The jar is set aside in a cupboard for a week, and the jar and manure are then examined for larvae.

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