

metabolic products are present in the bile within 15 min of administration.

The results indicate that the anthelmintic action of carbon tetrachloride against adult *Fasciola hepatica* in the sheep is not wholly direct but probably involves metabolic products of the drug, and/or materials produced by the liver when it is affected by the drug.

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Food Medium of Prepared Dog Biscuit for the Mass-production of the Nematode DD136 (Nematoda; Steinernematidae)

THE use of nematodes for biological control of pest insects requires mass-culture techniques that can produce large numbers of nematodes quickly, cheaply and simply. Mass-culture techniques developed for *Neoaplectana glaseri* Steiner permitted the use of this nematode for biological control of the Japanese beetle, *Popillia japonica* Newm., in the New England states¹.

The nematode-bacteria complex known as DD136 (ref. 2) was tested as a potential agent for biological control³⁻⁵. Dutky *et al.*⁶ developed mass-culture techniques by rearing the nematodes on larvae of the wax moth, *Galleria mellonella* L., and obtained up to 2×10^8 infective stage nematodes per insect. This technique involved first rearing wax moth larvae in quantity. At Belleville, various media were tested for mass rearing of DD136. Of several kinds tested the most satisfactory was a dog food, 'Ken-L' (Quaker Oats Company of Canada, Peterborough, Ontario), which is kibbled in manufacture into 1/8-3/8-in. pieces.

The technique is simple. 20 ml. of the dog food is mixed with 20 ml. of distilled water in a 9-cm Petri-dish. This is then autoclaved for 15 min at 15 lb. pressure and cooled overnight under sterile conditions. The nematodes for inoculum are rinsed five times with 0.1 per cent formalin to remove most of the contaminants. Each plate is inoculated with $1-2 \times 10^8$ nematodes. A few days incubation at about 24° C gives rise to large numbers of nematodes, indicated by the shiny grey appearance of the culture. When the nematodes become heavily concentrated in the culture they align and remain motionless until disturbed. They may be gathered from 8 to 14 days after inoculation, but some samples may take up to 20 days to develop to this stage. When the inoculum is too sparse ($1-5 \times 10^8$ nematodes per plate), collection may be delayed a week or more. The nematodes are washed from the medium into a large container and are allowed to settle. The suspended debris from the medium is decanted and discarded. The nematodes are then stored in 0.1 per cent formalin at 5°-10° C in litre flasks.

Generally a single plate yields about 3×10^8 nematodes at one time, but as many as 7.1×10^6 have been obtained in a single collection. But each plate can usually be used six times, so that the total yield per plate in a two-month period may be at least 1.8×10^6 nematodes. Several plates were used up to twelve times. It is estimated that 60-80 per cent of the nematodes collected are in the infective stage.

After 2-4 days the medium usually becomes lightly contaminated with bacteria that remain throughout the life of the culture. Plates of medium that become too

heavily contaminated with bacteria or fungi may be discarded. Bactericides are not added, as the bacteria are probably necessary for the success of the culture. Fungicides need not be added as it is more economical to discard the contaminated plate.

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Isolation of *Trypanosoma theileri* Laveran 1902, from Cattle in Scotland

WHILE *Trypanosoma theileri* is considered to be a cosmopolitan parasite of cattle, it has yet to be recorded from Scotland¹. During September and October 1964 the milking herd of Ayrshire cattle belonging to the Royal (Dick) School of Veterinary Studies, Edinburgh, was screened for the trypanosome using the blood agar culture technique recommended by Herbert². The herd is situated at the Veterinary Field Station eight miles south of the city.

Six cultures were inoculated with 2 ml. of defibrinated blood from each animal examined. One out of 25 cows sampled yielded positive cultures, typical critidial forms being found in one culture on the third day and in the remaining five by the sixth day. This cow was in its twelfth lactation and had been bought from Lanark cattle market in July 1959. The history prior to that time is uncertain.

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ENTOMOLOGY

Incorporation of Labelled Thymidine into the Silk Gland of the Silkworm

It has already been established that the silk gland of the *Bombyx mori* larva grows bigger without cell division^{1,2}, that each gland cell nucleus is gradually ramified, showing a strong affinity to Feulgen reaction (unpublished observation) and that both chromatins and nucleoli increase in number and volume with age³. It is not yet known, however, whether deoxyribonucleic acid in the nuclei of the glandular cells is synthesized during larval growth without cell division. It is reported by Amano *et al.*⁴ that labelled thymidine is an adequate tracer of newly formed deoxyribonucleic acid in autoradiographs. We have investigated this problem by electron microscopic autoradiography.

Silkworm larvae in the fifth day after the fourth moult were used as specimens. Each silk gland was dissected out from both series of larvae, either 30 min or 2 h after injection of 50 µc. of thymidine-6-³H.