THE BIOCHEMICAL CHARACTERIZATION OF A SEROLOGICALLY ACTIVE LIPID FRACTION OF THE NEMATODE *HAEMONCHUS CONTORTUS*

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Summary

Protein, polysaccharide, and lipid fractions were prepared from *Haemonchus contortus*. The lipid fraction appeared to be the only one essentially concerned in reactions with natural antisera from sheep, and further work was carried out to determine the nature of the serologically active lipid. It was found to be hydrophilic, acidic, susceptible to oxidation, free from protein and polysaccharide, and to have a molecular weight greater than 1000. Its biochemical significance, rôle in the activity of the boiled antigen, and possible relation to other lipoidal antigens are discussed.

I. INTRODUCTION

Stoll (1932) showed that sheep could develop a pronounced resistance to infestation with *Haemonchus contortus* and this has been confirmed and extended by Gordon (1948). Earlier workers (see Stumberg 1933) had commented on the presence or absence of antibodies to *Haemonchus contortus* in sheep sera, and Hawkins and Cole (1945) had found that when exsheathed strongyle larvae were placed in sera from resistant sheep, a precipitate formed at various sites on the larval surface; however, Stewart (1948) was the first to demonstrate clearly by classical methods the presence of antibodies against *Haemonchus contortus* and *Trichostrongylus* spp. in sheep sera and to study their rôle in the resistance of sheep to these parasites.

The immunochemical studies here described had as their objective the identification of the biochemical fraction of the parasites responsible for the serological reaction.

The polysaccharide fractions of helminths have been examined by several workers, especially by Campbell (1936, 1937, 1939) and Gonzalez (1943, 1946), who showed that helminth polysaccharides have many of the characteristics of bacterial polysaccharides. Others (Brisou 1946; Culbertson, Rose, and Gonzalez 1947; Warren 1947) have studied the serological activity of aqueous extracts of helminths. Apart from the work of Fairley (1925, 1927), Kellaway (1928), Wharton (1930), and earlier workers, on the lipids of helminths, little is known of their nature, specificity, and importance in immunology. These workers found that alcoholic extracts of the helminths reacted with

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corresponding antisera; Fairley and Kellaway found that these lipids could function as antigens, whereas Wharton considered they could act only as haptens and also noted that the specificity of the lipids which he prepared was low.

In this Laboratory, protein, polysaccharide, and lipid fractions were prepared from *Haemonchus contortus*. As only the lipid fraction showed pronounced serological activity, it was the only fraction investigated in detail. The serological activity of the various fractions was titrated by means of a complement fixation reaction and the author is indebted to his colleague, Mr. D. F. Stewart, for carrying out these tests.

II. Materials and Methods

(*a*) *Collection of Material*

The collection and treatment of biological materials in the fresh state was achieved by the following techniques, designed for the rapid collection of large amounts of material.

A method devised by Mr. H. V. Whitlock of this Laboratory was employed for collecting and cleaning nematode eggs. It may be described briefly as follows:

A large quantity (up to 2 or 3 kg.) of freshly passed faeces containing a large number of ova is thoroughly broken down with pestle and mortar and then mixed with a quantity of water to make a free-flowing suspension. This is allowed to stand overnight in a refrigerator. The suspension is then poured into a fine mesh sieve (approximately 30 meshes per inch) and washed several times to obtain the maximum number of ova free from the larger pieces of debris. It is then mixed with an equal volume of sugar solution (150 g. sugar in 100 ml. water). Large shallow enamel trays 14 by 10¼ by 2 in. are fitted with glass plates cut so as just to fit inside the tray, with one corner cut off to permit the introduction of rubber tubing, and with handles cemented to the upper surface at each end so as to overlap the ends by 1 in. and to serve as supports. The glass plate in position lies just below the level of the rim of the tray. The glass plate is placed in position. The suspension is poured into the tray until it makes contact with the entire surface of the glass plate. The preparation is allowed to stand for approximately 30 minutes, when the ova will have floated to the surface and will be adhering to the under surface of the glass plate. It is essential that the glass plate is thoroughly dry and clean before use.

A length of rubber tubing is introduced through the opening at one corner of the glass plate and portion of the faeces-sugar suspension siphoned off. The plate with an adhering film of fluid containing large numbers of ova may then be lifted from the tray. The ova are then washed off and the washings transferred to a narrow container to sediment, or alternatively they may be centrifuged. The ova should be washed with water several times. Concentration between washings is achieved either by sedimentation or centrifugation.

The flotation process may be repeated several times on the material in the tray; further quantities of ova will thereby be obtained. At each repetition the glass plate is carefully washed and dried before use.

Infective larvae from faecal cultures were cleaned and washed in distilled water by a modification of a technique described by Hamilton (1950) for the collection of *Trichostrongylus* spp., a No. 2 sintered-glass filter being used instead of metal gauze filters. After the larvae had been washed off the filter, concentration was effected by centrifuging.

Small species of nematodes such as *Haemonchus contortus* and *Trichostrongylus* spp. were collected by the method described by Hamilton (l.c.) and then dried between filter papers. Larger helminths, such as *Moniezia expansa* and *Fasciola hepatica,* were merely washed in saline and dried with filter paper before use.

(*b*) *Treatment of Material*

It was found that the cleaned nematode eggs and larvae could be macerated satisfactorily in a conical, ground-glass bacterial mill without the use of powdered glass. Adult nematodes and animal tissues were ground in a mortar and pestle, without the addition of sand.

In some experiments an aqueous suspension of macerated material or a boiled antigen preparation were lyophilised before extraction with the appropriate solvent. Lyophilisation was effected by the technique of Flosdorf and Mudd (1935).

Conditions favouring the rancidification of fats caused deterioration of the serological activity of the lipid fractions, hence all such material was stored and treated under nitrogen and kept in the cold as much as possible.

(*c*) *Preparation of Fractions*

Lipid fractions were prepared from helminths and animal tissues by extraction with absolute alcohol. *Haemonchus contortus* was extracted with other solvents in order to study the nature and solubility of the serologically active lipid. After the first experiments, a standard empirical method was used for the preparation of all lipid fractions.

One part of the material to be extracted was macerated and transferred to a gas-tight, ground-glass stoppered Erlenmeyer flask together with one hundred parts of the appropriate solvent. "Quickfit" apparatus was always used to avoid possible contamination from cork or rubber. The contents of the flask were thoroughly gassed with nitrogen and the extraction was then left in the refrigerator at 4°C. The actual time of extraction ranged from 24 hours to two weeks in different experiments without any apparent effect on the potency of the final preparation.

The extraction was centrifuged and the supernatant filtered through filter paper to remove any remaining particulate matter. The solvent was removed by vacuum distillation under nitrogen. With absolute alcohol as solvent, the

temperature of distillation was usually of the order of 25°C. Temperatures of distillation above 30°C. caused a deterioration of serological activity of the lipid fractions.

After removal of the solvent, the brownish-white greasy lipid residue was suspended in 25 parts of 0.9 per cent. sodium chloride solution and stored under nitrogen in the cold. The lipid suspension so formed was white, opaque, and physically stable, at no time showing any tendency to precipitate, and it was used in this form for serological tests. For extraction, 100 parts of solvent to one part of fresh material were used to keep the water content as low as possible. The lipid residue from one part of material was suspended in 25 parts of physiological saline so that the results in the serological tests would be directly comparable with those obtained with the boiled antigen used by Stewart (1948).

All solvents used in this work were the purest obtainable and all were redistilled before use. The redistilled anaesthetic ether was stored in the dark over sodium.

Polysaccharides were prepared from *Haemonchus contortus, Nematodirus, Ascaris lumbricoides, Moniezia expansa,* and *Fasciola hepatica* by the method of Melcher and Campbell (1942), followed by treatment with chloroform (Sevag, Lackman, and Smolens 1938), until free from protein and negative to the biuret test.

In some experiments protein solutions were prepared from saline extracts of *Haemonchus contortus.* An equal volume of saturated ammonium sulphate solution was added to the saline extracts, then 10 per cent. trichloracetic acid to the point of maximum precipitation. The precipitate was collected and dialysed against saline till free from sulphate and trichloracetate ions.

Thorough absolute alcohol extractions of various helminths was followed by saline extraction of the alcohol-insoluble residue in another series of experiments, the saline-soluble extracts being used as protein fractions without further treatment.

Nematodes were also extracted under nitrogen by concentrations of aqueous alcohol ranging up to 50 per cent. alcohol, followed by treatment with ammonium sulphate and trichloracetic acid, as above, and dialysis under nitrogen of the supernatants and precipitates against 5 per cent. alcohol in distilled water till free of sulphate. After centrifuging, both supernatants and precipitates were used as protein fractions. The alcohol content did not interfere in the complement fixation test. In all experiments solutions were restored to isotonicity before testing or injection.

III. Results

(*a*) *Properties of the Lipid Fraction*

(i) *Extraction and Solubility of the Lipid.*—In the first stages of this work the serologically active lipid of *Haemonchus contortus* was found to be soluble in absolute alcohol. *Haemonchus contortus* was then extracted with other

solvents to determine the solubility and nature of the active material. The extracts were tested as saline suspensions for serological activity. The activity of the extracts is shown in Table 1.

TABLE 1
SEROLOGICAL ACTIVITY OF MATERIAL EXTRACTED BY SEVERAL SOLVENTS FROM FRESH *HAEMONCHUS CONTORTUS*

Solvent	Reaction of Extract with *Haemonchus contortus* Antiserum
Absolute Alcohol	+++++
Alcohol 75%	+++++
Alcohol 50%	+++++
Methyl alcohol	+++++
Propyl alcohol	−
Butyl alcohol	+
Amyl alcohol	−
Anaesthetic ether	++
Petroleum ether (60-80°C. fraction)	+
Benzene	+
Carbon tetrachloride	±
Chloroform	−
Acetone	−
Phenol 2%	+++
Pyridine	+++++
Diethylene glycol	+++++
Absolute alcohol (10N hydrochloric acid to pH 3.0)	−
Absolute alcohol (40% sodium hydroxide solution to pH 10.0)	−
Absolute alcohol 100 ml. + aqueous trichloracetic acid 30 ml. at pH 3.0	+++++
Aqueous N/4 trichloracetic acid	−

+++++ indicates high activity; − indicates no activity.

Certain of the organic solvents caused the macerated worm material to coalesce into gummy lumps and this possibly prevented complete extraction. To overcome this, the macerated worm material was lyophilised before extraction by a selection of these solvents (see Table 2). This method extracted

more material but without much change in serological activity. The activity of these extracts is shown in Table 2.

TABLE 2

SEROLOGICAL ACTIVITY OF MATERIAL EXTRACTED BY SEVERAL SOLVENTS FROM LYOPHILISED *HAEMONCHUS CONTORTUS*

Solvent	Reaction of Extract with *Haemonchus contortus* Antiserum
Absolute alcohol	+++++
Anaesthetic ether	+
Benzene	±
Chloroform	±

+++++ indicates high activity; ± indicates only a trace of activity.

Ether and petroleum ether (60-80°C. fraction) extracted the active material from acidified aqueous suspensions of the lipid, but not from neutral or alkaline suspensions. On the other hand, chloroform extracted the active material from aqueous suspensions at any pH. The serologically active lipid, together with other material, was precipitated from aqueous suspensions on acidification below pH 2.5. Further details of these points are given later in this article.

(ii) *Stability of the Lipid.*—As already noted, the active lipid of *Haemonchus contortus* was unstable under conditions favouring rancidification of fats. This rendered chemical work very difficult and imposed rigorous limits on experiments designed to identify the serologically active lipid, because detection depended on serological activity. Saline suspensions of the lipid of *Haemonchus contortus* only retained their serological activity for two to three weeks in nitrogen in the cold and attempts were therefore made to stabilize it with antioxidants.

Pyrogallol, added to the saline suspensions in concentrations of 0.01 per cent. and 0.005 per cent., and hydroquinone added in a concentration of 0.01 per cent., did not appear to confer stability. However, this approach was abandoned because the antioxidant caused so much darkening of the erythrocytes used in the complement fixation tests that the results could not be read with any degree of accuracy.

The addition of Raney-nickel in a concentration of 0.1 per cent. during extraction with absolute alcohol, and gassing with hydrogen instead of nitrogen, did not enhance the activity of the saline suspension of the lipid when compared with a control extraction.

At first it was thought that exsheathing of the *Haemonchus contortus* larvae would result in better maceration, but the exsheathing was found unnecessary. Incomplete washing after treatment with sodium hypochlorite also gave preparations of the lipid fraction and of boiled antigen which were unsatisfactory for serological work.

(*b*) *Quantitative Aspects of the Lipid Fraction*

In the few experiments with *Haemonchus contortus* eggs, absolute alcohol gave extracts of much higher serological activity than those obtained from third-stage larvae or adults. The third-stage larvae consistently yielded lipid of high activity, whereas the activity of lipid obtained similarly from adults was very variable.

Fresh batches of *Haemonchus contortus* larvae were collected and divided into two equal lots. One lot was immediately macerated and extracted with absolute alcohol as above; the other lot was allowed to age in an incubator at 30°C. for one week, until most of the larvae were very sluggish, before maceration and extraction with absolute alcohol. The saline suspensions of lipid obtained from these extracts were equally active.

Approximately 0.015 g. of material could be extracted by absolute alcohol from 1 g. of centrifuged *Haemonchus contortus* larvae. If the larvae were first treated with acetone, or were allowed to age, the quantity of material extracted by absolute alcohol was much reduced although its serological activity was not diminished. It follows, therefore, that the serologically active lipid was present only in small amounts in the absolute alcohol-soluble material.

(*c*) *Cross Reactions of Helminth Lipids*

Saline suspensions were also prepared of the absolute alcohol-soluble lipids of other helminths and of some vertebrate tissues, and these were tested for serological activity against sheep sera known to contain complement-fixing antibodies only for *Haemonchus contortus* antigen. The immunological significance of the cross reactions obtained will be discussed elsewhere by my colleague, Mr. D. F. Stewart; the results are summarized in Table 3, merely as an indication of the distribution of the lipid among helminths.

(*d*) *Nature of the Lipid from* Haemonchus contortus

The first fractionation of the alcohol-soluble material of *Haemonchus contortus* was made by extracting aqueous lipid suspensions at different pH levels with ether or petroleum ether (60-80°C. fraction). The fraction obtained by extraction of the alkaline lipid suspension was known as the "unsaponified" fraction. The ether extract obtained after acidification of the suspension to pH 2.0, was called the "fatty acid" fraction.

Both ether extracts, after washing with distilled water, were evaporated to dryness by vacuum distillation under nitrogen. Saline suspensions of the lipid fractions were then prepared as above; when tested by the complement-fixation test the "unsaponified" fraction was inactive, whereas the "fatty acid" fraction was quite active.

The pH of aqueous suspensions of the lipid was usually found to be about 5.0, i.e. slightly more acid than the distilled water used (pH 5.5 to pH 6.0). Ether extraction at this pH did not remove any active material, but on acidifica-

tion to pH 2.0, active material was obtained as before. On the other hand, active material could be extracted by chloroform from aqueous suspensions of lipid at alkaline, neutral, or acid pH.

TABLE 3
CROSS REACTION OF LIPIDS FROM DIFFERENT HELMINTHS WITH *HAEMONCHUS CONTORTUS* ANTISERUM

Material Extracted	Reaction with *Haemonchus contortus* Antiserum
Haemonchus contortus adults	+
Haemonchus contortus larvae	+
Haemonchus contortus eggs	+
Nematodirus—adults	+
Trichostrongylus adults	?
Trichostrongylus larvae	+
Oesophagostomum—adults	+
Moniezia expansa—adults	+
Ascaris lumbricoides—adults	+
Chabertia ovina—adults	+
Dictyocaulus filaris—adults	+
Strongyloides—larvae	+
Fasciola hepatica—adults	−
Paramphistomum cervi—adults	−
Beef heart	−
Beef liver	−
Sheep's brain	−
Sheep's intestinal mucosa	−
Hen's egg yolk	−

The addition of hydrochloric acid or sulphuric acid to pH 2.0 in these experiments, followed by warming in nitrogen, resulted in the formation of greasy globules which floated on the surface and dissolved readily in ether. Treatment of aqueous suspensions of the alcohol-soluble lipid with trichloracetic acid in the cold to a pH less than 2.5 gave a flocculent precipitate containing the active lipid, which could be readily centrifuged off and resuspended in saline. If the solution was made alkaline after acidification with trichloracetic acid, the precipitate redissolved to give the characteristic opaque lipid suspension.

The alcohol-soluble material of *Haemonchus contortus* was also subjected to hydrolysis. Saturated solutions of barium hydroxide, pig pancreatic lipase, and alcoholic solutions of potassium hydroxide were used as hydrolytic agents, all experiments being carried out in nitrogen. No significant results could be obtained, since the loss of serological activity was so great in the control experiments, from which the hydrolic agents were omitted, that the inactivation in the presence of the hydrolytic agents could not be attributed to hydrolysis alone.

A molecular still was used in a further attempt at fractionation. The alcohol-soluble material of *Haemonchus contortus,* after degassing by evacuation of the still without external heating, was subjected to distillation at pressures of the order of 0.01 mm. mercury and at temperatures ranging up to 290°C. The distillates were white or very light brown in colour and, when suspended in saline, were always serologically inactive. The residues showed considerable decomposition, being dark red or black in appearance; after suspension in saline and light centrifugation to remove particulate matter, the light brown opaque supernatants showed serological activity. The stability of the active material under these conditions was probably due to the absence of moisture and oxygen.

The material extracted by absolute alcohol from *Haemonchus contortus* was negative to Molisch and "rapid furfural" tests and negative to colour tests for proteins, although some samples gave a doubtful positive biuret test. One sample of material was analysed and found to contain 0.06 per cent. total P, 2.0 per cent. total N, and 1.4 per cent. reducing substances (calculated as glucose) after hydrolysis. These figures have little significance as far as the serologically active lipid is concerned but show that the phospholipid content of the absolute alcohol extract was low and that nitrogenous compounds and possibly carbohydrates were present in the extract.

The active lipid of *Haemonchus contortus* would not dialyse through cellophane tubing and this fact was used in experiments to remove salts formed during neutralization, which otherwise would affect the isotonicity of the saline suspensions of the lipid fractions subsequently prepared. Dialysis was carried out under nitrogen against distilled water.

(*e*) *The Lipid in Relation to the Boiled Antigen*

The close similarity in serological activity between the absolute alcohol extract and the boiled antigen prepared from *Haemonchus contortus* suggested that the active lipid might be concerned in the serological activity of the boiled antigen.

It was known that saline extracts of *Haemonchus contortus* had only slight activity in the complement fixation test, and that saline extracts of *Haemonchus contortus,* previously extracted with absolute alcohol, were without activity. It was found necessary to extract 1 g. lots of *Haemonchus contortus* with four 100 ml. lots of absolute alcohol to remove all serologically active lipid. A boiled antigen prepared from the extracted residue of *Haemonchus contortus* was inactive.

To determine whether the serologically active lipid was present in the boiled antigen and, if so, in what form, a boiled antigen was prepared, but was not made isotonic, and was then lyophilised. It was then extracted with absolute alcohol and the alcoholic extract treated as above. The resultant saline suspension of the lipid was found to be active. It was necessary to extract the lyophilised boiled antigen with at least three 100 ml. lots of absolute alcohol to remove all the extractable active material. The residue was then resuspended in 25 ml. saline and it too, was found to be serologically active.

(*f*) *Protein and Polysaccharide Fractions*

Stewart (personal communication) found that the protein solutions and the polysaccharide fraction of *Haemonchus contortus* would not fix complement with antisera from naturally infested sheep, and for that reason their chemical nature has not been examined.

IV. Discussion

The organic solvents which extracted serologically active material from *Haemonchus contortus* with any degree of efficiency, were miscible with water and the majority were protein denaturants and contained hydroxyl groups. It is possible that a presumably physical combination of lipid with protein must be broken down as a preliminary step in the extraction; this would explain why only slight activity was found in saline extracts of macerated material, especially if a saline-insoluble structural protein were involved.

This is supported by the fact that, although repeated absolute alcohol extraction of *Haemonchus contortus* removed all serologically active lipid, this was not so with the boiled antigen, suggesting that the serologically active lipid entered into varying degrees of physical, rather than chemical, combination with absolute alcohol-insoluble protein in the boiled antigen. Also, the boiled antigen did not have to be stored under nitrogen (Stewart 1948) which meant that the serologically active lipid was stabilized.

That the serologically active lipid of *Haemonchus contortus* was hydrophilic and acidic was shown by the nature of the solvents which extracted it from *Haemonchus contortus;* by the formation of very stable aqueous suspensions; by the precipitation of the serologically active lipid, together with other acid-precipitable material, on acidification of aqueous suspensions below pH 2.5; by its extraction with ether or petroleum ether from acidified aqueous suspensions but not from alkaline or neutral aqueous suspensions. The hydrophilic and acidic properties of the serologically active lipid would facilitate the formation of physical aggregates with protein.

The instability of the serologically active lipid of *Haemonchus contortus* under conditions favouring rancidification rather than disintegration suggests that the lipid was unsaturated and easily oxidized but was not an unstable complex aggregate involving two or more compounds. This suggestion is supported by its stability during molecular distillation. The molecular size of

the lipid was indicated by its failure to distil in the molecular still and its inability to dialyse through cellophane. Fawcett (1939, 1948) points out that the upper molecular weight limit for practical molecular distillation of organic substances is in the region of 1000 and that organic substances with higher molecular weights generally undergo thermal decomposition before the vapour pressure necessary for distillation is achieved; phosphatides and proteins decompose and cannot be distilled. Apparently, then, the serologically active lipid has a molecular weight greater than 1000, and a relatively stable molecular configuration, since it did not readily undergo disintegration.

The absence of polysaccharide or protein in the absolute alcohol-soluble serologically active material of *Haemonchus contortus* was indicated by the negative protein and polysaccharide colour tests, the ease of solution in ether and petroleum ether after acidification of equeous suspensions of the absolute alcohol-soluble material, and the behaviour of the active lipid in the molecular still. However, the presence of very small amounts of nitrogenous compounds and simple saccharides in the serologically active lipid cannot be excluded.

To summarize these points briefly, the serologically active lipid probably occurs in *Haemonchus contortus* in loose physical combination with protein, it is hydrophilic, acidic, and susceptible to oxidation, with a molecular weight probably greater than 1000 and is presumably free from protein and polysaccharide; it apparently combines physically with protein in the boiled antigen, in which the serological activity of the lipid is stabilized.

Properties of the Boivin, Forssman, and Brucella (Miles and Pirie 1939; Paterson, Pirie, and Stableforth 1947) antigens which are similar to those of the serologically active lipid may be given as follows:

The Boivin antigen from *Salmonella typhimurium* is extracted by diethylene glycol from dried cells, is non-dialysable, forms opalescent solutions, and is very unstable. The Brucella antigen (Paterson, Pirie, and Stableforth 1947) is readily precipitated from crude solutions with other acid-precipitable material, is extracted from the organisms by 2 per cent. phenol, dissolves in pyridine with dissaggregation, and the antigen is disaggregated and part thereof extracted by chloroform from acetate buffer at pH 4.0. The Forssman hapten may be extracted from tissues by alcohol; the purified hapten is soluble in water, dilute alkali, and pyridine, and is not soluble, or barely so, in most organic solvents. Reducing sugars are detected after hydrolysis, and the Forssman hapten must be mixed with proteins to produce antibodies.

On the other hand, the Boivin antigen is specific, toxic, and antigenic, and on hydrolysis fatty acids and a specific polysaccharide are liberated; the Brucella antigens are precipitated by alcohol, and are resistant to boiling; the *Brucella melitensis* antigen appears to be a complex of at least two phospholipids with protein-like material and the *N*-formyl derivative of a polyhydroxamine. The *Brucella abortus* antigen is probably similar chemically. The Forssman hapten is resistant to boiling, and the purified active moiety is

apparently not soluble, or barely so, in most organic solvents, including alcohol, ether, and chloroform (Landsteiner and Levene 1925); on hydrolysis, fatty acids, hexosamine, and probably a hexose are liberated (Boyd 1947).

Chemically, the serologically active lipid of *Haemonchus contortus* does not appear to belong to any of these types of lipoidal antigens; the common properties discussed are non-specific to each type and are probably shared by all compounds which contain a sufficiently high proportion of lipid.

The biological and available chemical evidence suggests that the serologically active lipids of the helminths studied by the author have a number of common features and are a type of compound not as yet found in serologically active preparations from other classes of organisms.

As regards the work of Fairley (1925, 1927), Kellaway (1928), and Wharton (1930) using bilharzia cercariae (*Schistosoma spindalis*), *Fasciola hepatica,* and *Moniezia expansa,* respectively, the type of material extracted in each case by alcohol (95 per cent. or higher) would be similar, although techniques varied slightly. Fairley and Wharton found that the alcoholic extracts of the helminths they studied could fix complement, and Kellaway suggested that the alcohol-soluble material of *Fasciola hepatica* was capable of complement fixation. For reviews of earlier work, which in the main support this hypothesis, reference should be made to these three authors.

All the serologically active lipids of helminths which have been studied have shown low specificity. Thus Wharton demonstrated the low specificity of tapeworm lipids and the present work shows that the absolute alcohol extracts of the nematodes and cestode studied (see Table 3) all fixed complement in the presence of *Haemonchus contortus* natural antisera from sheep with known histories, whereas alcoholic extracts of *Fasciola hepatica, Paramphistomum cervi,* and various vertebrate tissues did not. The fact that the absolute alcohol extract of *Moniezia expansa* fixed complement in the presence of *Haemonchus contortus* natural antisera in this Laboratory, implies that Wharton was dealing with a very similar, if not the same, lipid.

Since the serologically active lipid of *Haemonchus contortus* was found in greater amounts in the eggs and third-stage larvae and the active lipid content was not diminished in "aged" larvae, it is suggested that it functions as an essential lipid in the metabolism of *Haemonchus contortus.* Because of the similarity between the serologically active lipids of the helminths studied, it is possible that they are essential lipids involved in the basic metabolic structure of the helminths concerned.

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