A STUDY OF THE SEROLOGICAL RESPONSE TO HELMINTHS IN DOMESTIC ANIMALS.

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Part I.

Introduction.

For many years observers have noted the occurrence of altered body reactivity following the contact of helminthic substances with human or animal tissues. Thus as early as 1759 Hawkins recorded grave symptoms and death in a girl following rupture of a hepatic hydatid cyst. Much later Mourson and Schlagdenhauffen (1882), Debove (1887), Humphry (1887) and Achard (1888a,b) studied the question of hypersensitivity to hydatid disease in humans. These authors held the view that the hydatid fluid was toxic in character, contained "ptomaines" and that sensitivity to the fluid occurred irregularly in persons infested with hydatid cysts.

Sensitivity to the ascaris worm was noted by Huber (1870) who reported sensitivity of himself and other workers to ascaris substances. Much later Goldschmidt (1910) described the allergic syndromes which occurred in humans as a result of working with Ascaris lumbricoides.

No advance was made in the field of serology in helminth diseases until the main serological techniques in bacteriology were developed. Kraus (1897) described the precipitin reaction for the plague bacillus and Bordet and

Gengou (1901) demonstrated the complement fixation technique. Though Edward Jenner described the development of a local skin reaction in smallpox patients in 1801, it was not until 1891, when Robert Koch described the skin sensitivity to the tubercle bacillus, that the skin reaction became widely known. With the development of these tests as applied to bacteriology, work was soon initiated using comparable methods for the determination of serological and hypersensitive responses to helminth parasites.

Review of the Literature.

As it is not practicable, in the scope of this thesis, to give a complete survey of all the literature which has been consulted, only a brief survey has been made to indicate the work carried out in each biological group of helminths.

Phylum. Plathelminthes.

Class. Trematoda.

Fasciola hepatica.

Many workers have been able to show that a certain degree of complement fixation antibody occurred in the sera of animals infested with F.hepatica. Paccanaro (1909) was the first to introduce this method for the diagnosis of liver fluke infestation in sheep and cattle, obtaining positive results in infested animals. Weinberg (1909b) reported that the sera of infested animals

invariably gave positive complement fixation reactions. Similar results were obtained with the sera of infested sheep by Fairley and Williams (1923) and with the sera of infested humans by Lavier and Stefanopoulo (1944). The latter also indicated that antibodies occurring during F.hepatica infestation persisted for some time after the elimination of the parasites. In contrast to the findings of the previous mentioned workers, other workers have found that complement fixation antibodies are not confined to infested animals, but are evident in animals showing no evidence of F.hepatica infestation. Busson (1911) and Brocq-Rousseu, Cauchemez and Urbain (1923) obtained positive complement fixation reactions with the sera of cattle (Busson) and sheep (Brocq-Rousseu et al) which showed no infestation with F.hepatica. These two authors determined the degree of fixation by variation of the quantity of antigen. Höppli (1921), Servanti (1921) and Wagner (1935) reported the occurrence of positive complement fixation reactions not only in the sera of sheep infested with F.hepatica but also in the sera of sheep not infested with the parasite. The last named authors did not determine the degree of fixation but used the fixation of a single volume of complement as the criterion for a positive reaction. Herein probably lies the explanation for the discrepancies

evident in the results of the various reports on complement fixation reactions with the sera cattle and sheep infested and not infested with F.hepatica. Studies on the occurrence of complement fixation antibodies in the sera of humans infested with F.hepatica in isolated instances have met with little success. Thus Heckenroth and Advier (1931) were unable to detect such antibodies in the serum of a person infested with F.hepatica in Corsica and Bacigalupo (1934) obtained only one positive complement fixation reaction out of three sera from infested humans.

Studies using the precipitin test to detect antibodies to F.hepatica were initiated by Hoffman and Rivera (1929). These authors recorded positive precipitin reactions with the sera of infested sheep. Similar reactions were however noted with the sera of sheep which showed no infestation with the parasite. The preparation of the antigens (using ether to remove lipoidal substances) probably accounted for the occurrence of the many false results. Trawinski (1937) obtained positive precipitin results with the sera of all infested animals, while non infested animals all gave negative results. Later, Szaflarski (1946) demonstrated that the sera of sheep suffering from an early invasion of F.hepatica reacted

strongly to the precipitin test while sera from sheep with old standing infestations gave less marked results.

Allergic responses to F.hepatica infestation in sheep were first studied by Sievers and Oyarzun (1932) who reported excellent results with cutaneous scarification and intradermal tests. Currasson (1935) however reported "Il n'y a pas de relation entre l'existence des réactions et celle des parasites" when using allergic tests for the diagnosis of F.hepatica infestation in sheep and cattle. Wagner (1935) and Aygün and Baskaya (1939) reported that hypersensitivity to F.hepatica infestation in sheep could be successfully detected by the intradermal test. Wagner (1935) obtained 73% positive results in infested animals and suggested that this sensitivity persisted for a long time after infestation. Aygün and Baskaya (1939) stated that the results of the skin test paralleled the faecal findings. The allergic response to F.hepatica infestation in humans has been studied by Mazzotti (1942; 1948), Morenas (1943a,b; 1944), Lavier and Stephanopoulo (1944) and Mayer and Pifano (1945a) who obtained marked skin reactions in infested persons. Rukawina (1935), however, obtained negative results with the skin test in humans infested with F.hepatica.

Antibodies which partially protected rabbits against infestation with F.hepatica were demonstrated by Kerr and Petcovitch (1935) who vaccinated rabbits with liver fluke material, thereby protecting the animals to a significant degree against infestation.

Schistosomes.

It has been well established that there is a marked serological response to Schistosome infestation. Fujinama and Nakamura (1909) initiated the work on schistosome serology, reporting negative complement fixation results with the sera of calves infested with Schistosoma japonicum. Later Hayami and Tanaka (1910) reported positive complement fixation with the sera of calves infested with schistosomes, and Yoshimoto (1910) reported similar results with the sera of humans infested with the parasites. Fairley (1919) confirmed the use of the complement fixation test for the diagnosis of Schistosomiasis. He was unable to detect antibodies in the sera of five monkeys dying from the acute form of the disease. Later, Fairley (1926), using an antigen prepared from the livers of snails (Planorbis exustus) infected with the cercariae of S.spindale demonstrated that the sera of goats infested with this parasite showed a marked and sustained complement fixation titre from

the third to the twelfth month. Fairley was of the opinion that this antibody reaction was maintained so long as the host continued to harbour living Schistosomes. Fairley and Jasudason (1930) studied the complement fixation antibody titre in monkeys (Macacus senicus) infected with S.spindale. A preliminary rise in antibody titre was noted during the second week of infestation which corresponded to the cercarial invasion; a secondary rise was observed from the fifth to the ninth week after infestation which was associated with the formation of portal thrombi containing dead schistosomes. In animals where the death of all the schistosomes had occurred (after treatment) the antibody titre gradually fell. Positive results, however, were seen up to three months after the disappearance of living schistosomes. These persisting positive results were attributed to the disintegration of dead schistosomes embedded in portal thrombi. A similar rise of antibody in the sera of infested dogs was noted by Miyagi and Imai (1928) and in the sera of infested humans by Minning (1941).

Hypersensitivity to Schistosome infestation was demonstrated by Fairley and Williams (1927) who described an intradermal reaction in infested individuals. This intradermal reaction was widely used for the diagnosis of schistosome infestation

in areas where the disease is endemic. Satisfactory results with the skin test were obtained by Taliaferro and Taliaferro (1931), Manson-Bahr (1929), Kan-Huai-Chieh (1926), Mayer and Pifano (1945a and b), Guerra, Mayer and Prisco (1945) and Pratt and Oliver-Gonzalez (1947). These authors indicated that skin sensitivity persisted for a long period after infestation had disappeared. Vogel (1932), however, reported that the skin test was unsatisfactory for the detection of schistosome infestation.

Contrary to the findings of Fairley (1919), Papermiester and Bang (1948) demonstrated a precipitate occurring round the cercariae of schistosomes when they were incubated in the sera of humans or animals infested with schistosomes. Liu and Bang (1950) recorded an agglutinin for the cercariae of schistosomes in the blood of Rhesus monkeys which were infested with the parasite. This agglutinin appeared in the serum when eggs were passed in the faeces. No such agglutinin appeared in the sera of monkeys (Macacus philippinensis) which were affected with chronic schistosome infestation, or were not affected. A similar action of serum as mentioned by Papermiester and Bang (1948) had previously been noted in normal serum by Tabangui and Masilungan (1936), Culbertson and Talbot (1935) and Culbertson (1936).

Class Cestoda.

Adult Tape Worms.

Evidence concerning the production of a serological response to tapeworms in humans and domestic animals is conflicting. Many workers have attempted to demonstrate such a response in infested humans and animals using the various serological tests. Precipitin antibody was demonstrated in humans infested with Diphyllobothrium latum by Isaac and Velden (1904). Ghedini (1907) reported positive results with the complement fixation test in persons infested with Taenia solium. Light infestations of tapeworms in horses failed to induce antibody formation, while heavily affected animals did show complement fixation antibody formation, (Weinberg and Parvu,1908a). Kolmer, Trist and Heist (1916) and Ravetta (1937) reported positive complement fixation with the sera of dogs infested with tapeworms, and Jerlov (1919) reported similar results in humans infested with D.latum. Busson (1911), Voille and Saint Rat (1919), Becker (1922), Le Bas (1924), Sievers (1935) and Deschiens and Renaudet (1941) obtained only isolated instances of complement fixation antibody formation in the sera of humans and animals infested with tapeworms.

Antibodies to tapeworm infestations in

laboratory animals have been demonstrated by Hearin (1941) and Larsh (1942; 1943). These authors were able to show that infection of mice with the dwarf tapeworm Hymenolepis nana var. fraterna produced a high degree of immunity. This immunity was humoural in character, being passively transferable to normal animals. The immunity was possessed by young born and nursed by infested mothers and was demonstrable in the serum of mice by the precipitin, agglutination and complement fixation tests, (Larsh, 1943). These antibody responses were produced by the cysticercoid stage alone in natural infestation, but artificial immunisation by vaccination with the tapeworm materials afforded partial protection from infestation (Larsh, 1943). It is noteworthy that a closely related tapeworm, Hymenolepis diminuta, which does not migrate into the villi of the intestine, does not produce this marked antibody response (Chandler, 1940).

Hypersensitivity to tapeworm infestation was noted by Ramsdell (1927) who stated that such hypersensitivity persisted for as long as six years after removal of the tapeworms. Brunner (1928) obtained immediate skin reactions when persons infested with Taenia saginata or D.latum were subjected to intradermal tests with tapeworm material. These reactions were stated to be specific. Turner, Dennis and Berberian (1935),

however, demonstrated that dogs infested with the tapeworm Echinococcus granulosus did not respond regularly to the intradermal injection of tapeworm materials.

Successful artificial immunity against E.granulosus infestation was produced by Turner, Berberian and Dennis (1933; 1936) who immunised dogs with hydatid cyst material prior to infestation with hydatid scolices.

Intermediate stages of Tapeworms.

Echinococcal (Hydatid) Cysts.

Studies on the serological response to hydatid infestation were initiated by Ghedini (1906) who described the complement fixation reaction in persons infested with the cyst. Joest (1907) failed to detect precipitin antibodies in the sera of rabbits infested with the cyst or immunised with cyst material. He concluded that the hydatid fluid was not antigenic and incapable of causing antibody formation. Fleig and Lisbonne (1908) however demonstrated precipitin antibodies in the serum of a patient infested with hydatid cysts.

Following the early work on the serological response to hydatid disease, Weinberg (1909a, b) and co-workers clarified and established suitable serological techniques for the diagnosis of hydatid infestation. Thus Weinberg and Parvu (1908b, c)

reported complement fixation antibodies in the sera of humans and sheep infested with hydatids. The tests were specific. Sheep free from hydatid disease failed to react to the test. In humans the antibodies were found to persist some months after operative removal of cysts. Weinberg and Boidin (1909) found that complement fixation antibodies were not correlated with the degree of eosinophilia and Weinberg (1909c) stated that the degree of antibody production bore no relationship to the size of the hydatid cyst but had a relationship to the site and the degree of fibrosis of the adventitia wall.

Numerous other workers have applied serological methods to the diagnosis of hydatid infestations in humans and animals. In general the presence of an active cyst was associated with the production of antibodies. The rupture of cysts frequently produced a marked rise in the amount of circulating antibody (Fairley, 1922; Graña, 1946 and Pontano, 1941) but suppuration, degeneration or calcification resulted in a loss of antibody (Fairley, 1922; Botteri, 1922; Deusch, 1925; Never, 1936 and Föcking, 1937). Removal of cysts by surgical operation resulted in the loss of circulating antibody (Graña, 1945).

The intradermal hypersensitivity test in hydatid infestation was introduced by Casoni (1911).

Rachman and Stevens (1927) confirmed the existence of a skin sensitivity in persons suffering from Echinococcal cysts and determined that this hypersensitivity could be transferred by means of serum to an insensitive individual. Morenas (1929) reported that operative removal, suppuration or calcification of cysts resulted in a loss of skin sensitivity within two and a half months.

Goodale and Krischner (1930), Hoder (1933) and Faiguenbaum and Miranda (1949) recorded that the intradermal reaction was a satisfactory method for the diagnosis of hydatid disease in man and animals. However, Never (1936) found that, in cattle, skin tests were quite worthless.

In humans intestinal cestodes and nematodes were found to sensitise patients' skin thus causing skin reactions to hydatid material (Fairley, Fairley and Williams, 1929; Núnez and López, 1934; Rose and Culbertson, 1939 and Culbertson and Rose, 1939, 1941).

The results of various workers show a variation in the value of the different tests used for the diagnosis of hydatid infestation in man and animals viz:-

Complement Fixation Test.

Worker (s).	Percentage of positive Reactions in infested Persons and animals.
Ghedini (1906)	92.0%

Complement Fixation Test. (Cont.)

Worker (s).	Percentage of positive Reactions in infested Persons and animals.
Weinberg et al (1908a,b,1909,1909a,b,c)	88.91% (Aver.)
N.H. Fairley (1922)	89.0%
Zappeloni (1915)	92.0%
van der Hoeden (1922a,b,c,1923,1924 1925)	88.18% (Aver.)
Jthurrat and Calcagno (1922;1923)	88.7%
Herowitz-Wlassowa (1926)	90.0%
Hercus (1926)	58.0%
Cuff (1930)	100.0%
Goodale and Krischner (1930)	59.0% (Aver.)
Hoder (1933)	90.0%
Never (1936)	79.0%
Urbain (1936)	68.5%

Intradermal Reaction.

Worker(s).	Percentage of Positive reactions in infested persons and animals.
Caseni (1911)	87.0%
Hercus (1926)	90.0%
Goodale and Krischner (1930)	86.3%
Hoder (1933)	100.0%
Atanassoff (1936)	89.6%
Grinblatt (1945)	100.0%
Pontano (1941)	90.0%

An increase in the agglutinins and haemolysins for the erythrocytes (Types A and B) of man (and also for other animals, especially sheep) in the sera of humans infested with hydatid cysts following repeated intradermal injections of hydatid fluid was noted by Grana (1942, 1944). This phenomenon was not observed in non-infested individuals.

Cysticerci of Taenia saginata and Taenia solium.

Antibodies or hypersensitivity formed consequent to infestation with Cysticercus cellulosae or Cysticercus bovis were demonstrable in only a percentage of cases (Weinberg, 1909b; Chung and T'Kung, 1939, and Gaehtens, 1941; 1943). However, other authors, Rothfeld (1935), Trawinski and Rothfeld (1935), Trawinski (1936; 1947) and Soltys (1936) have reported the detection of precipitin antibodies in humans and swine infested with C.cellulosae.

Larval stages of other animal Cestodes.

A well marked antibody response to the somatic forms of dog and cat cestodes has been demonstrated in laboratory animals. Henry and Ciuca (1916) demonstrated the production of circulating and anaphylactic antibodies in the sera of rabbits following infestation with Multiceps

serialis. Rats and rabbits, infested with Cysticercus fasciolaris and Cysticercus pisiformis respectively, produced antibodies which, by passive transfer, protected control animals against infestation. Such antibodies were transmitted from mother to offspring, and could be produced by the vaccination of animals with adult tapeworm materials, (Miller, 1930, 1931a,b,c, 1932a,b, 1934, 1935; Miller and Gardiner, 1932, 1934; Miller and Massie, 1932, and Miller and Kerr, 1932).

Campbell (1936a, 1938a,b,c) reported the occurrence of two forms of antibody in animals infested with the somatic stages of cestodes. An "early" immunity antibody, being protective in character, was demonstrable within seven days of infestation. It was also produced by artificial immunisation of animals with the adult and larval forms of the cestode. The naturally and artificially produced "early" antibody could be absorbed in vitro by adult and larval tissues. A "late" immunity antibody appeared several weeks after infestation; it also, was protective in character. The "late" antibody production was thought to be stimulated by the antigenic materials synthesised during the parasitic cysts' metabolism since the antibody could not be produced by artificial immunisation or be absorbed in vitro by adult or larval tissues.

Allergic phenomena were noted in rabbits infested with C.pisiformis by Wharton (1931).

Phylum. Nemathelminthes.

Order. Ascaroidea.

The infestation of experimental animals with ascarid worms of various species has been shown to produce a marked serological response (Coventry, 1929; Blackie, 1931; Oliver-González, 1943, 1946a; Fallis, 1944; Kozar, 1948, and Sadun, 1949). The formation of precipitin or complement fixation antibodies coincided with the migration phase of the parasite and the antibody titre reached a maximum during this phase; after migration was completed the antibody titres fell to a low level. The antibodies stimulated by migration persisted for varying periods of time --4 months, (Oliver-González, 1943) --8 months (Coventry, 1929).

The development of hypersensitivity to experimental Ascaris infestation was demonstrated by Coventry (1929) and Oliver-González (1946a). Skin tests were positive many months after the worms had been eliminated from the host. Fülleborn (1926) has stated that hypersensitivity to Ascaris lumbricoides infestation may persist for at least four years after the loss of the parasite.

Hosts naturally infested with ascarids have shown a great variation in their serological

response. Circulating antibodies in Ascaris infestation have been detected by the complement fixation test by Ghedini (1907), Weinberg and Parvu (1908a), Kolmer, Trist and Heist (1916), Usami and Kamada (1921) and Isbecque (1924). Mild infestations with Ascaris in horses were not associated with antibody formation (Weinbergánd Parvu, 1908a). Busson (1911) was unable to detect complement fixing antibodies in the sera of animals and humans infested with A.lumbricoides.

Many authors have shown that humans and animals may be hypersensitive to ascaris materials but generally such hypersensitivity could not be correlated with present infestation with the parasite, (Weinberg and Julien, 1911; Van Es and Schalk, 1918; Fülleborn, 1926; Brunner, 1928; Höppli and Vogel, 1927; Wilkening, 1937; and Börlin, 1946). Other authors, Ransom, Harrison and Couch (1924) and Franck (1940), have reported that the skin tests are satisfactory for the diagnosis of Ascaris infestation, Franck stressing that the delayed reaction was of diagnostic significance.

Skin tests and precipitin tests carried out on patients in areas where A.lumbricoides is very common were positive in 80% and 61% of cases respectively (Coventry and Taliaferro, 1928), but there was no correlation between the faecal findings and the serological findings.

Recently Oliver-González (1946a) has demonstrated that infection of rabbits with A.lumbricoides produced an increase in the titres of the α and β isoagglutinins in the sera of such rabbits. The isoagglutinins found could be absorbed by parasitic material.

Order. Trichinelloidea.

The serological response to Trichinella spiralis infestation has been adequately demonstrated. The infestation of rabbits with trichinae produced the formation of precipitin and complement fixation antibodies in their sera (Bachman, 1928a, 1929; Bachman and Menendez, 1929; Augustine and Theiler, 1932; Trawinski, 1934 and Cena, 1935). These antibodies were in evidence from the fifth to the twentieth day after infestation and increased thereafter during the migration and establishment of the larvae in the muscles. In humans comparable antibodies persisted for some time after encystment of the Trichinella larvae, disappearing ten months (Hunter, 1931) or a year after infestation (Bachman, 1928a; Gould, 1943).

Circulating antibodies were transmissable from parent to offspring in rats (Mauss, 1939; 1940a; Gaugusch, 1949) and were capable of causing precipitates in and around the larvae and adults of T.spiralis when the parasites were incubated in immune serum (Oliver-González, 1940; 1941; Roth,

1941; 1945). Mauss (1941b) has shown that the infectivity of larvae subjected to the action of precipitating serum was reduced by 66%.

Oliver-González (1941) has demonstrated a dual antibody in T.spiralis infestation. An antibody against the adult form of the parasite appeared about the fifteenth day after infestation, being highest between the twenty-fifth and thirty-fifth and disappearing by the fiftieth day. This antibody on passive transfer protected against infestation by the intestinal phase of Trichinella. An antibody against the larval (muscle) form of the parasite appeared on the thirtieth day after infestation. This antibody gave little or no protection against the intestinal phase of infestation.

Skin hypersensitivity to T.spiralis infestation in experimental animals was demonstrated by Bachman (1928b,c). It appeared as early as the second day after infestation and was characterised by a delayed type of reaction. Skin hypersensitivity to Trichinella infection in humans and swine has been established and utilised as a diagnostic test (Schwartz and McIntosh, 1929; Augustine and Theiler, 1930; 1932; McCoy and Miller, 1931; Maternowska, 1933; Schmid and Schipull, 1937; Lichterman and Kleeman, 1939; MacNaught, Beard and Myres, 1941; Schulz, 1941, and Linneweh and Harmsen, 1943).

Several authors have reported that pigs which showed no evidence of infestation reacted to the skin test (Schwartz, McIntosh and Mitchell, 1930; Heathman, 1936; Spindler and Cross, 1939, and Spindler, Cross and Avery, 1941). The last authors suggested that such positive reactions may be due to ingestion of non-viable Trichinae. However the feeding of killed larvae failed to induce such sensitivity (Gould, 1943, and Avera, Yow, Harrell and Fowler, 1946). Soon after infestation, skin hypersensitivity to T.spiralis is of the delayed (24 hour) type but after a few weeks the reaction becomes "immediate" in nature (Maternowska, 1933; Spink, 1937; MacNaught, Beard and Myres, 1941 and Spaeth, 1942). Hypersensitivity may persist for long periods after infestation has occurred (Spink, 1937). The reaction in human clinical cases may persist for three years but in sub-clinical cases the reaction disappears much earlier (Warren, Drake and Hawkes, 1940). Gould (1943) records a falling incidence of intradermal reactions in successive age decades which is in marked contrast to the rising incidence of trichinosis in the same age periods. Gould suggests either that this is due to a lack of skin response to trichina antigens in older persons with subclinical trichinosis or that the skin sensitivity in older persons is not retained as long as it is in younger persons with

subclinical trichinosis.

Trawinski (1935) commented that immunity to T.spiralis infestation was of an antitoxic nature. Rats which were infested with one to three times the lethal dose of larvae withstood the infestation when immune serum was administered during the migration period of the disease. Previously Flury (1913) isolated a toxic principle from T.spiralis larvae encysted in muscles. When injected into dogs and cats this toxic principle caused damage to capillary walls, hyperaemia and haemorrhages in the internal organs and oedema of the lungs.

Order. Strongyloidea

Family. Strongylidae.

Evidence was produced by Sarles and Taliaferro (1936) and Taliaferro and Sarles (1939) that protective antibodies were produced following infestation of rats with Nippostrongylus muris. The antibody was passively transferable. When N.muris larvae were placed in immune serum, precipitates were formed in and around the larvae. The antibody responsible was developed during the second week of infestation (Sarles, 1938).

Chandler (1935) was unable to transfer immunity to Nippostrongylus infestation either by passive transfer of serum or by active transfer of serum carried out by means of parabiotic twins.

Later, Chandler (1939) expressed the opinion that immunity to intestinal parasites consisted of two phases; a parenteral immunity produced by migration and mucosal burrowing of immature forms and a local intestinal immunity stimulated by the metabolic products of the adult parasites.

Family. Ancylostomidae.

Protective antibodies in hookworm infestation have been demonstrated by Kerr (1938a), and Otto (1939, 1940). The antibodies were shown to bear a direct relationship to the number of larvae administered (Otto, 1941) and their formation coincided with the attack on the intestinal mucosa by the parasite (Sprent, 1946). Serum from infested hosts protected control animals against infection by the parasite (Kerr, 1938a). Stumberg (1930), however, was unable to demonstrate complement fixation antibodies in the sera of dogs infested with Ancylostoma caninum.

When Ancylostoma larvae were incubated in serum from an infested animal, circumlarval precipitates were demonstrable around the larvae after five hours' incubation. One hour's incubation was sufficient to impair by 66% the infectivity of the larvae (Otto, 1939; 1940). A similar reaction was demonstrated with the larvae of Necator americanus and the sera from infested animals (Otto, Schugam and Groover, 1942).

Hypersensitivity to hookworm infestation has been demonstrated by Stumberg and Rodriguez-Molina (1931) and Sprent (1946). In areas where Ancylostomiasis is prevalent, however, no definite relationship was found to exist between infestation and the occurrence of skin reactions (Stumberg and Rodriguez-Molina, 1931). These authors also indicated that following the successful treatment of Ancylostomiasis there was a decline in the skin sensitivity.

Family. Trichostrongylidae and Metastrongylidae.

In intestinal helminth infestation, due to Haemonchus and Trichostrongylus species, there was a definite complement fixation antibody response associated mainly with the mucosal invasion by the larvae but in the case of Trichostrongyles it was also associated with infestation by the adult worms. Reinfestation of sheep with infective larvae produced a sharp rise in the antibody titre, but a decline in the complement fixation antibody was noted after larval migration had been completed. The antibody titre in grazing animals varied inversely with the faecal egg count (Stewart, 1948, 1950a,b,c,d,). Hawkins and Cole (1945) and Filmer (1947) demonstrated that the sera of sheep which had undergone natural infestation with Trichostrongylus spp. caused precipitates around

the exsheathed larvae of these species.

A hypersensitive state appeared to exist in infestations of Trichostrongylidae worms and could be demonstrated by intradermal tests (Stoll and Nelson, 1930 and Kholoshcanov, 1950).

A limited serological response to Dictyocaulus spp. infestation in cattle and sheep has been demonstrated by Hudson (1951) using the complement fixation and intradermal tests. This response was most marked on reinfestation. The period during which the hypersensitive response was evident was limited.

Order. Filaroidea.

Precipitin and complement fixation antibodies and hypersensitive skin reactions have been demonstrated during the course of filarial diseases (Rodhain and Dubois, 1932; Bozicevich and Hutter, 1944; Oliver-González and Bercovitz, 1944 and Bozicevich, Donovan, Mazzotti, Diaz and Padilla, 1947). Fairley (1931b, 1932) has reported that the complement fixation test in Filariasis afforded an index to the more active infestation. Stefanopoulo and Daniaud (1940) reported that complement fixation antibodies in filariasis disappeared in from five to eight months after cure while the skin reaction persisted for a much longer period.

Franks (1946) who passively sensitised

normal persons with the sera of filarial patients was able, by this method, to demonstrate the presence of two antibodies in the sera of filarial patients. One antibody reacted specifically with microfilariae and the other with the adult filarid. This author also determined the presence of a circulating soluble filarial antigen in the sera of humans suffering from microfilaraemia. The amount of circulating antigen appeared as a tide whose ebb and flow were related inversely to the concentration of microfilariae in the circulation.

Summary of the Serological Response to Helminth Infestation.

Circulating antibodies and hypersensitivity occurring in helminth infestations are, in general, similar to those occurring in bacterial diseases. Helminths differ from bacteria in that they do not generally propagate themselves in the host. Consequently, within limits, the degree of the immune response will parallel the intensity of the infestation.

The production of antibodies in helminth infestation appears to follow one of, or a combination of, two main processes, Viz.

a. The migration of immature (or occasionally mature) forms through the tissues of the host.
b. The absorption of antigenic materials by the host from active or degenerating parasites residing in the somatic tissues of the host.

Antibody production associated with the migration of immature forms through the tissues of the host is limited to the migratory phase of the parasite's life cycle. The antibodies disappear shortly after the migration has been completed. This has been demonstrated in Ascaris (Blackie, 1931) Haemonchus (Stewart, 1950b) and Ascaridaa infestation (Sadun,1949). Even where

the adult parasite is somatic (e.g. Schistosomes) the initial invasion of immature forms is responsible for the greatest stimulation of antibody (Fairley and Jasudason, 1930).

The agencies concerned in the stimulation of antibodies by migrating forms have not been clearly determined. Dried and powdered parasites elicit an antibody production when administered parenterally (Kerr, 1938b; Oliver-González, 1943) and such antibodies protect against subsequent infestation. The absorption by the host of antigens from the body tissues of the parasite is therefore an established mechanism in the stimulation of antibodies. Oliver-González (1941) has suggested that the active synthesis of antigens by the metabolism of the immature parasites is an important factor in the stimulation of antibody production. He also suggested that the migration of immature forms through different tissues may produce a variation in the character of the antibodies produced, owing to the difference enzymes elaborated by the parasite for the digestion of various tissues. Antibodies produced by the migration of immature forms through the tissues caused the formation of precipitates in and around the larvae in vitro (Mauss, 1940b; Oliver-González, 1940, 1943; Roth, 1941, 1945; Sarles and Taliaferro, 1936), while larval precipitates have been observed histologically

in the livers and lungs of resistant animals (Sarles and Taliaferro, 1936).

Non-migrating bowel parasites, such as the majority of tapeworms, elicit little or no antibody response. In contrast, the tapeworm Hymenolepis nana var. fraterna provokes a marked antibody response associated solely with the invasion of the host's villi by the cysticercoid stage (Hearin, 1941; Larsh, 1942). It is probable that purely intestinal parasites are unable to induce any great degree of antibody formation as they do not come into intimate contact with the host's tissues and as a result little or no antigenic material reaches the host's circulation. Invasion of the host tissue (bowel epithelium) by migrating parasites has been shown to be associated with the appearance of antigen in the blood stream. Thus Bozicevich and Detre (1940) reported the presence of T.spiralis antigen in the blood of rabbits shortly after they were fed Trichinella larvae. Serum containing this antigen was capable of producing antibodies against T.spiralis when injected into normal rabbits. The authors considered that the antigen was the initiator of the subsequent antibody response in T.spiralis infested animals. Stumberg (1933) has demonstrated a comparable antigen in the sera of sheep infested with Haemonchus contortus.

b. The absorption of antigenic materials from parasites residing in the somatic tissues.

In many cases (intermediate stages of cestodes) it would appear that antibody stimulation is associated solely with the absorption of antigenic materials present in the body of the parasite, as opposed to the synthesis of antigens by metabolism. Conditions which interfere with the permeability of the helminth limiting membrance (fibrosis, calcification etc.) cause a loss in circulating antibodies (Weinberg, 1909c), while excess liberation of antigenic material (rupture of cysts) produces a sharp rise in antibody production, (Fairley, 1922).

In other cases, however, it has been demonstrated that somatic forms produce antibodies associated with the metabolism and growth of the parasite. These antibodies, which are not absorbed by dried parasitic material, are distinct from antibodies produced by the invasion of the immature forms or antibodies produced by vaccination with parasitic material, (Campbell, 1938a,b,c).

When the parasites recide in the blood or lymphoid tissues of the host (Schistosomes and Filarial worms) the active metabolism of the parasite contributes greatly to the stimulation of antibodies, (Fairley, 1931b, 1932; Stephanopoulo and Daniaud,

1940). Disintegration of dead forms may maintain antibody production for some time after cure, (Fairley and Jasudason, 1930). The daily liberation of immature forms into the blood stream (Filarial worms) maintains the antibody response in infested hosts (Culbertson, 1941). In such helminth diseases where immature forms are liberated into the blood two distinct antibodies can be demonstrated, one against the adult and one against the immature form; in addition circulating antigen may be present with circulating immature parasites, (Franks, 1946).

Hypersensitivity has been demonstrated in the majority of helminth diseases were tissue invasion occurs. This hypersensitivity differs from bacterial hypersensitivity in that when established it is usually manifested by an immediate skin reaction, which is in contrast to the delayed (24 hours) skin reaction of bacterial hypersensitivity (Boyd, 1946). It differs from the tuberculin type of hypersensitivity in that it is passively transferable by serum to the skin of normal individuals (Prausnitz Küstner, 1921, reaction). The hypersensitivity is produced at the same time as the circulating antibodies demonstrated by <u>in vitro</u> tests but persists for a much longer period than these antibodies, sometimes for many years after elimination of the causal parasite (Fülleborn, 1926). Hypersensitivity appears to be very pronounced in the

tissues through which the immature forms have migrated. Thus Taliaferro and Sarles (1939) demonstrated histologically that on reinfestation with N.muris the livers and lungs of rats underwent marked reactions which were attributed to hypersensitiveness.

Antibodies induced by helminthiasis are similar to other antibodies in that they reside in the globulin portion of the serum (Mauss, 1940c, 1941). Electrophoretic mobility studies have shown that there is an increase in the proportion of gamma globulin in the sera of animals developing antibodies against helminth parasites, thus apparently comparing with formation of antibodies in bacterial and viral infections (Wright and Oliver-González, 1943).

Antitoxins which occur to a great extent in bacterial disease, have not been demonstrated to any extent in helminth infestations. Trawinski (1935), however, has reported the development of antitoxins in T.spiralis infestation.

Summary of the observations carried out in the present work.

The present work was carried out at the Edinburgh Corporation Abattoir. The abattoir facilities allowed a large number of animals to be used in the various tests and in addition it was possible to carry out a relatively detailed post mortem examination on all the animals used.

It was considered that such facilities afforded an excellent opportunity to correlate serological findings with post mortem evidence of parasitic infestation, since the lack of post mortem examinations has been a feature of many previous studies of the serological response to helminth infestation.

A study has been made of the circulating antibodies and the skin hypersensitivity existing in domestic animals as a result of natural infestation by helminth parasites. The parasites chosen for the study are the commonly occurring representatives of the various biological classes and orders which are of economic or public health importance in Great Britain.

The parasites chosen are presented below.

Class. Trematoda.

Fasciola hepatica (Common liver fluke of sheep and cattle)

Class. Cestoda.

Cysticercus bovis. (Intermediate stage of Taenia saginata)

Class. Nematoda.

Order. Ascaroidea.

Ascaris lumbricoides. (Round worm of pigs)

Order. Strongyloidea.

Dictyocaulus viviparus. (Lung worm of Cattle)

Order. Trichinelloidea.

Trichinella spiralis (Muscle parasite of man, swine and rats)

Part II.

Fasciola hepatica.

Materials and Methods.

Precipitation Reaction.

Precipitin reactions were carried out with great success by Trawinski (1937) who utilised a saline extract of the dried and powdered F.hepatica. The successful use of this antigen was further demonstrated by Szaflarski (1946). The antigen used by Hoffman and Rivera (1929), which consisted of saline extracts of the ether or alcohol insoluble portions of F.hepatica, proved less satisfactory for the precipitin reaction.

In the present examination, the antigen used was prepared according to the method described by Trawinski (1937). Mature flukes, obtained from the bile ducts of infested sheep, were washed in running water and were then cut into four portions by two incisions at right angles, and the caecal contents allowed to escape. Following further washing the portions were then placed in a sterile petri dish and dried in an incubator at 37°C for four days. Subsequently the dried residue was ground to a fine powder in a sterile mortar; the powder was then suspended in sterile saline (0.85% Na Cl in distilled water) in the proportions 1 gram of power to 100c.c. of saline and the suspension was extracted at 4°C for ten days, the mixture being

shaken at frequent intervals. After extraction the suspension was filtered through filter paper and the resulting solution was centrifuged at a speed of 3,000 r.p.m. for half an hour. The supernatant fluid was then heated in a water bath at 55°C for 45 minutes. The final solution was stored in the frozen state at -10°C.

A non-specific, non-helminth solution was utilised in the precipitin test in order to detect non-specific precipitin reactions which might occur. This consisted of a 1% solution of peptone which was sterilised and stored similarly to the F.hepatica extract.

The sera used in the test were obtained from blood drawn from the venticles of the heart of selected sheep immediately after slaughter. The sera was allowed to separate off overnight and before use were centrifuged. The majority of the sheep selected for examination were lambs. The reason for this selection was to ensure that the animals examined had been exposed to one season's infestation only and the infestations found were probably the initial infestation with F.hepatica.

For the test the individual sera were placed at the bottom of narrow tubes (5x32mm) and the antigenic solution in various dilutions was carefully layered over the serum, by means of a finely drawn pipette, so as to produce a clear line

of demarcation between the two fluids. The tubes were then incubated as stated below. After incubation the tubes were examined for the presence of a fine white ring of precipitate at the interface of the two fluids. This reaction was considered as a positive reaction and the degree of reaction was estimated according to the density of the ring of precipitate.

Samples of the antigenic solution were diluted with saline from the initial dilution of 1-100 by doubling dilutions to 1-1,600 and each diluted sample was incubated with undiluted serum. Each sample of serum was tested with similar dilutions of the peptone solution and with the diluting saline.

Initially the tubes were incubated for 25 minutes at 37°C and subsequently at room temperature for 20 minutes, according to the method of Trawinski, (1937). However with this incubation precipitin formation occurred in several of the tubes containing peptone solution. Other non-specific or "anomalous" reactions were observed in the tubes containing the specific antigen and control solution incubated in this manner. Such reactions have been noted in the precipitin test in Trichinellosis, (Bachman, Rodriguez-Molina and Oliver-González, 1934). These authors noted that

with the sera of non-trichinous patients and various helminths proteins a percentage (4.8%) of "anomalous" precipitin reactions occurred. This reaction, which has also been described by Augustine and Theiler (1932), is characterised by the formation of a broad, light brown haze or zone at the interface of the serum and the test antigen. This is readily distinguished from the sharply defined narrow zone of precipitate evident in the true positive reactions. These "anomalous" zones were observed in the higher dilutions of the test antigen, but only rarely in the lower dilutions. Any sera which showed any evidence of these reactions were discarded as unsuitable.

It was found that incubation at room temperature for one hour eliminated precipitin formation in the peptone solution and greatly reduced the number of sera showing "anomalous" reactions. Consequently in the majority of the precipitin tests the tubes were incubated at room temperature before reading.

The Complement Fixation Reaction.

The use of the complement Fixation reaction for the diagnosis of F.hepatica infestations in man and animals has met with varying results. The most satisfactory results were obtained by Fairley and Williams (1923) who utilised an alcoholic extract of fresh liver flukes as antigen, and by

Wagner (1935) and Lavier and Stephanopoulo (1944) who utilised an alcoholic extract of dried and powdered parasites. Earlier workers, Paccanaro (1909) and Weinberg (1909b) utilised saline extracts of F.hepatica, obtaining variable results. However Busson (1911) and Meyer (1913) demonstrated that alcoholic extracts of parasites were superior to the saline extracts for the complement fixation test and since then alcoholic extracts have been generally used.

In this examination alcoholic extracts of the liver fluke were utilised since they had proved more satisfactory than the saline extracts in the hands of other workers. However a small number of tests was carried out with a saline extract to determine what degree of fixation occurred with this extract. The alcoholic antigen was prepared after the method described by Wagner (1935). Mature liver flukes were obtained from the livers of infested sheep and were washed, dried in an incubator at 37°C for four days and were then reduced to a powder in a mortar. The powder was then extracted in the proportions of 1-20 with absolute alcohol for four days at 37°C. After filtration the extract was evaporated and the subsequent residue was suspended in saline in the proportion of 1-5. This antigenic material was examined for anticomplementary activity. It was found that the 1-5 dilution of

suspension in saline possessed an anticomplementary action of 1 Minimal Haemolytic Dose of complement. When the 1-5 dilution was diluted with an equal volume of saline no anticomplementary action was noted; consequently this dilution was utilised in the test.

As Fairley and Williams (1923) have obtained very good results with an alcoholic extract of fresh flukes, an extract was prepared as described by these authors. This consisted of a 10% alcoholic extract of fresh mature liver flukes which were ground with fine sand and alcohol and extracted for 48 hours at 37°C. The extract was then removed by filtration and subsequently diluted to 1 in 10 with saline. This final solution possessed no anticomplementary action and was utilised as prepared for the part of the examination which was carried out as described by Fairley and Williams (1923).

The saline extract used was a 1:100 saline extract of dried and powdered liver fluke, prepared as was the antigen for the precipitin reaction.

As it was thought that results obtained in preliminary tests were non-specific in character, a non-specific antigen was prepared and used in conjunction with the tests with the specific antigen. This non-specific antigen was an alcoholic extract of sheep heart prepared similarly to the Wasserman antigen but without the addition of cholesterol.

The sera were obtained from the heart blood of selected sheep after slaughter. The sheep selected were lambs, thus ensuring that the animals had been exposed to only one season's infestation with F.hepatica and were probably undergoing initial infestation. The sera were allowed to separate off overnight and prior to use in the test were inactivated at 55°C for 30 minutes. The sera were examined for anticomplementary action and it was found that a dilution of 1 in 5 in saline frequently produced a slight anticomplementary action, while a dilution of 1 in 10 of the sera gave no anticomplementary action. This latter dilution was used in the test.

Complement (Preserved guinea pig serum) and anti-sheep-blood-cells haemolytic serum were obtained from Burroughs Wellcome and Co. The minimal haemolytic dose of each reagent was determined daily prior to each set of examinations being carried out. A 3% suspension of washed sheep cells (R.B.C.s) was made up daily for use in the test. For the test the haemolytic system consisted of 0.5cc of each of the 3% R.B.C. suspension and the haemolytic serum (Diluted to 1:500 with saline, representing 10 M.H.D. per 1cc). The haemolytic system was incubated at 37°C for 30 minutes prior to being added to the other reagents of the test.

The test, using the method of equal

volumes of reagents was carried out as indicated in the table below:-

	Test Proper.				Complement Control.	
	Tubes.				Tubes.	
	1.	2.	3.	4.	1.	2.
Saline.	-	-	-	-	1cc.	1cc.
Antigen (1 : 2)	0.5cc.	0.5cc.	0.5cc.	0.5cc.	-	-
Serum (1 : 10)	0.5cc.	0.5cc.	0.5cc.	0.5cc.	-	-
Complement (Contained in 0.5cc of saline)	1MHD	2MHD	3MHD	4MHD	1MHD	2MHD

After incubation for 1 hour at 37°C in a water bath 1cc of the prepared haemolytic system was added to each tube. The tubes were then incubated for a further 30 mins. when the degree of haemolysis was ascertained.

	Serum Control.		Antigen Control.	
	Tubes.		Tubes.	
	1.	2.	1.	2.
Saline.	0.5cc.	0.5cc.	0.5cc.	0.5cc.
Antigen. (1 : 2)	-	-	0.5cc.	0.5cc.
Serum. (1 : 10)	0.5cc.	0.5cc.	-	-
Complement. (Contained in 0.5cc of Saline)	1MHD.	2MHD	1MHD.	2MHD.

After incubation for one hour the haemolytic system was added and the tubes were incubated for a further 30 mins. when the degree of haemolysis was ascertained.

In order to examine the results reported by Fairley and Williams (1923) the complement fixation test was carried out by their method using the alcoholic extract of fresh liver fluke diluted 1 : 10 in saline as indicated below.

Reagents.	Tubes.				
	1.	2.	3.	4.	5.
Antigen (1:10)	0.5cc	0.5cc	0.5cc	0.5cc	-
Serum (Inactivated) (1 - 5)	0.5cc	0.5cc	0.5cc	-	0.5cc
Complement (0.02cc=1MHD)	0.06cc	0.09cc	0.12cc	0.02cc	0.02cc
Saline.	-	-	-	0.5cc	0.5cc

After 1 hour's incubation at 37°C 1cc of the prepared haemolytic system was added and the whole incubated for a further 30 mins. when the degree of haemolysis was ascertained.

The Action of Serum on the Miracidia of the Liver Fluke.

Eggs of F.hepatica were collected from the gall bladder of infested sheep, the eggs were centrifuged from the bile at low speed and were then well washed in rain water. The eggs thus collected were placed in a large petri dish and incubated at 27°C for 14 days in the dark. Daily the culture was aerated by means of a large pipette. When the miracidia were required the plate was exposed to the daylight. The miracidia hatched and being photo-trophic were attracted in large numbers to the side

of the petri dish near the light. This made it easy to pipette off the miracidia into a test tube.

In the test a suspension of 15,000 miracidia per 1cc of distilled water was used (saline caused immobilisation and death of the miracidia). Counting was carried out by diluting a sample of the suspension 1 to 10 with distilled water, adding a drop of concentrated HCl to kill the miracidia and then the dead forms were counted in the chamber of the MacMaster slide. According to the numbers in the suspension it was adjusted to contain the required number. For the test proper 0.5cc of the suspension was used.

Complement was obtained from Burroughs Wellcome and Co. (M.H.D. = 0.02cc). For the present test this was diluted arbitrarily to 1:10 with saline and 0.1cc of this solution was used in the test.

Sera used in the test were collected from the heart blood of selected sheep immediately after slaughter. The sera were diluted by doubling dilutions in distilled water from 1:10 to any dilution required. In the test 0.5cc of each dilution of serum was used.

As it was thought that the concentration of proteins or allied substances might have an effect on the miracidia, solutions of egg white and peptone were used instead of the serum in some tests. The egg white was diluted with distilled water by

doubling dilutions from 1:10 to 1:320. The peptone utilised was commercial peptone and was used in concentrations of from 10% falling to 0.625% by doubling dilutions with distilled water.

Two control tubes were put up in all tests, one control consisting of distilled water, in place of the serum, but with complement, the other control tube containing neither serum nor complement and serving as a control on the viability of the miracidia.

The test was performed in a series of 2cc test tubes, each tube containing the reagents as indicated below. After all the reagents had been placed in the tubes, the tubes were incubated at 37°C in a water bath for 15 minutes. After this time the tubes were rapidly examined under the low power of a microscope and the degree of lethal effect of the serum determined. The following stages in lethal effect were recognised:-

Negative ... Miracidia not affected

\+ ... Slight effect; miracidia slowed down, some deformity, but all still motile.

++ ... Majority of miracidia moribund and lying at the bottom of the tube; a few still motile but showing deformity and "tail" precipitates.

+++ ... All the miracidia moribund and lying at the bottom of the tube; amoeboid shaped and scarcely recognisable as miracidia.

The ++ stage was taken as the end point in the reaction.

Arrangement Of Test.

Reagents.	Test Proper. Tubes.					
	1.	2.	3.	4.	5.	6.
Dilutions of Serum.	1:10	1:20	1:40	1:80	1:160	1:320
Serum.	0.5cc	0.5cc	0.5cc	0.5cc	0.5cc	0.5cc
Complement (Diluted 1:10)	0.1cc	0.1cc	0.1cc	0.1cc	0.1cc	0.1cc
Suspension of Miracidia (15000/cc)	0.5cc	0.5cc	0.5cc	0.5cc	0.5cc	0.5cc

Tubes incubated for 15 minutes at 37°C

Reagents.	Complement Control. Tubes.	Aqueous Control. Tubes.
	1.	1.
Complement (1:10)	0.1cc	-
Distilled Water.	0.5cc	0.6cc
Suspension of Miracidia (15000/cc)	0.5cc	0.5cc

Tubes incubated for 15 minutes at 37°C

In order to determine whether the serum component responsible for the miracidal action was thermo-labile, sheep serum was heated at 56°C for 30 minutes and was then examined for lethal action. Heated and unheated complement were added to this inactivated serum in order to determine whether inactivation of serum alone produced a loss of lethal action.

The Intradermal Test.

Satisfactory skin tests for the diagnosis of F.hepatica infestations have been carried out by many workers. A saline extract of the dried and powdered adult F.hepatica has been most frequently satisfactorily used as antigen. (Wagner, 1935; Mazzotti, 1942, 1948; Morenas, 1943a,b, 1944; Lavier and Stephanopoulo, 1944; Szaflarski, 1950; Sobiech, 1951). However, saline extracts of the lipoid free F.hepatica material have been used in some instances with good results, (Sievers and Oyarzun, 1932; Aygun and Baskaya, 1939). Kellaway (1928) determined that two antigenic substances existed in extracts of F.hepatica. One, present in the saline extract, showed decreasing solubility in increasing strengths of alcohol and was in nature a true anaphylactic antigen. This antigen was heat stable and protein in nature. The other substance, probably a lipin, was soluble in absolute alcohol and did not possess anaphylactic properties.

In this examination a saline extract of dried and powdered F.hepatica was used being prepared as was the saline extract for the precipitin test after the method described by Trawinski (1937). The antigen was used in the strength of 1:100 (original strength of the extract) or in the strength of 1:1000. The 1:100 dilution was used in the majority of the tests in cattle as Szaflarski (1950) has reported excellent results using this dilution. However, Mazzotti (1948, 1951) has stated that high dilutions of F.hepatica extract were as satisfactory as lower dilutions for the intradermal test in humans. Consequently a series of tests was carried out in cattle using a dilution of 1:1000. Wagner (1935) utilised a dilution of 1:1000 of the saline extract for intradermal tests in sheep with satisfactory results. This dilution was used for the skin test in sheep.

An attempt was made to produce a more purified form of antigen for the skin test in cattle and therefore a purified protein of F.hepatica was prepared and employed.

The purified protein component of F.hepatica was prepared according to the method used by Dennis (1937) for the preparation of such a protein from hydatid fluid. Mature flukes were obtained from the bile ducts of infested sheep and after washing they were frozen overnight at -10°C.

The brittle frozen flukes were then ground to a smooth paste in a mortar, this paste being diluted to ten times its own volume with distilled water. To this suspension was added trichloracetic acid to give a concentration of 5%. This mixture was placed in a refrigerator at 4°C for 12 hours. After this time flocculation was complete and the precipitate was collected by means of a centrifuge. This was washed three times in distilled water to remove excess acid. The precipitate was then suspended in 50cc of water and 10% NaOH was added drop by drop with constant agitation until solution had been completed. Any insoluble residue was removed by centrifuging and the clear supernatant fluid was chilled and subjected to reprecipitation by 1/N glacial acetic acid until precipitation was complete. After standing for 12 hours at 4°C the precipitate was recovered by centrifuging; it was washed free of acid by distilled water and was then dried in an incubator at 37°C. The dried powder was ground in a mortar and dissolved in slightly alkaline saline in proportions of 1:1000. The solution was centrifuged to remove any insoluble matter and it was then sterilised at 55°C for 45 minutes in a water bath. The yield or dried purified protein from 10 grams of fresh F.hepatica was 200 milligrams.

A 1% solution of peptone was used as a control solution in all tests to ascertain the

degree of skin reactivity, if any, to simple trauma or the presence of foreign substances.

In several cattle (13) a saline extract of hydatid scolices and membranes was used in addition to the peptone control solution. The hydatid extract was prepared in a similar manner to the F.hepatica extract and was used in a dilution of 1-100. This hydatid extract was used to determine the relative specificity of the reactions in cattle to the Fasciola antigen.

Intradermal tests with extracts of Dictyocaulus viviparus (Reported in Part V) were frequently carried out in cattle in conjunction with the tests with Fasciola antigen. Though these intradermal injections constituted a separate study, it was found that the reactions produced by the D.viviparus antigen were independent of sensitisation by F.hepatica, since reactions occurring to either antigen showed no constant relationship.

Intradermal tests with an extract of Cysticercus bovis were also carried out in cattle in conjunction with tests with F.hepatica antigen. The results of these tests are presented in part III. They indicate that the C.bovis antigen reacted in a manner similar to the F.hepatica antigen.

All the antigens prepared and sterilised above were passed through a "pyrex" micro filter and subsequently placed in small 1 cc bottles with

rubber stoppers and the solutions were then stored in the frozen state at -10°C.

The intradermal injections were made with a "Record" type dental syringe with a graduated plunger shaft so that a regulated amount of fluid could be delivered. Separate syringes were used for the different antigenic solutions and the syringes were cleaned and sterilised after use daily.

Injections were made intradermally, in 0.1cc amounts, at the selected sites. The site in the sheep was the wool-free area on the inside of the right hind leg. This site was clipped free of hair if necessary and cleaned with saline prior to injection. The injection sites were marked with an indelible pencil, the sites of the test and control injections being at least three inches apart.

The site in cattle was the skin at approximately the middle third of the neck on a line parallel to the spine of the scapula. The sites to be injected, four inches apart, were clipped free of hair and the thickness of the fold of skin at the site measured by means of callipers and recorded. The injection of the antigenic or control solution was then made. The subsequent reaction was observed after 4 hours and the thickness of the skin fold again measured and recorded.

Desensitisation injections of antigenic solutions were given intradermally into the skin of

the neck. In order to detect local skin desensitisation the same site was subjected to reinjection with 0.1cc. of antigen solution three times at intervals of 24 hours. The reaction was measured in each case four hours later. In order to induce generalised desensitisation 3cc of the antigen solution were injected intradermally on one side of the neck and 24 hours later the usual intradermal test was carried out on the opposite side of the neck.

Experiments in passive transfer of skin sensitivity by means of serum (Prausnitz Küstner reaction) were carried out as follows. Serum was obtained from the heart blood of animals which had shown a marked reaction to the intradermal test or had failed to react. This serum was injected intradermally in 1cc amounts into the skin of the neck of non-reacting animals. The site of the injection was marked by an indelible pencil. Twenty four hours later the same site was measured by callipers and injected with 0.1cc of the antigenic material and the subsequent reaction was measured four hours later.

Reactions

After injection of the antigenic material the site was marked with a small pea like swelling about 4mm in diameter. In animals which reacted, this initial swelling increased in size until after

an hour it consisted of a flat elevated wheal which in sheep was surrounded by an area of erythema which varied in diameter. In sheep the reaction was examined and noted one hour after the injection. The size of the wheal and the extent of the erythema were measured by means of callipers.

In cattle, during and after the initial wheal formation there occurred to a varying extent an oedematous infiltration at the site of the injection. This infiltration reached its maximum in four hours and was maintained for a further two to four hours; after 24 hours the reaction had resolved, the site being marked only by a slight thickness of the skin. In white-skinned animals the reaction was surrounded by a zone of erythema but in other animals it was not possible to observe such erythema. The reaction in cattle was measured in four hours' time, the fold of the skin where the reaction had occurred being measured by callipers.

On histological examination the skin reaction is characterised by a marked perivascular infiltration of polymorphonuclear leucocytes, the majority of which were eosinophils, together with marked oedema of the dermis. This infiltration of eosinophils is noticeable as early as 20 minutes after the injection of the antigenic material. Botteri (1922) and Tanturi (1939) have reported an infiltration of eosinophils at the point of a

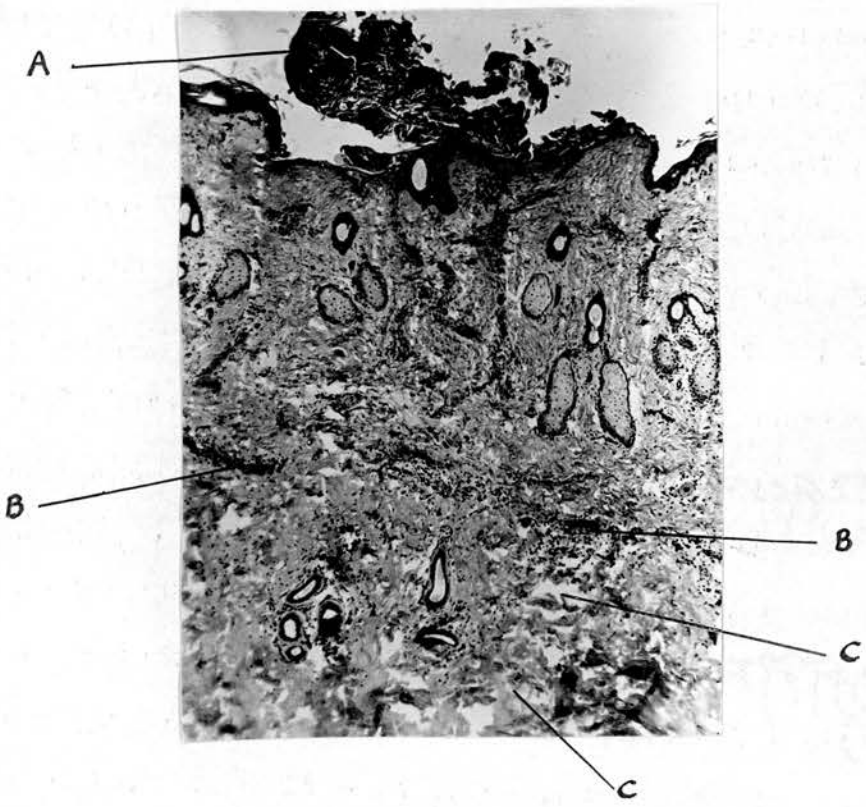


PLATE I.

Section of bovine skin 20 minutes after the intradermal injection of 0.1cc of 1-100 saline extract of F.hepatica. (Stained H. and E.) (x 50)

Key.

A. Serous exudate at site of skin puncture.

B. Early perivascular cellular migration (eosinophils)

C. Early oedema separating collagen fibres.

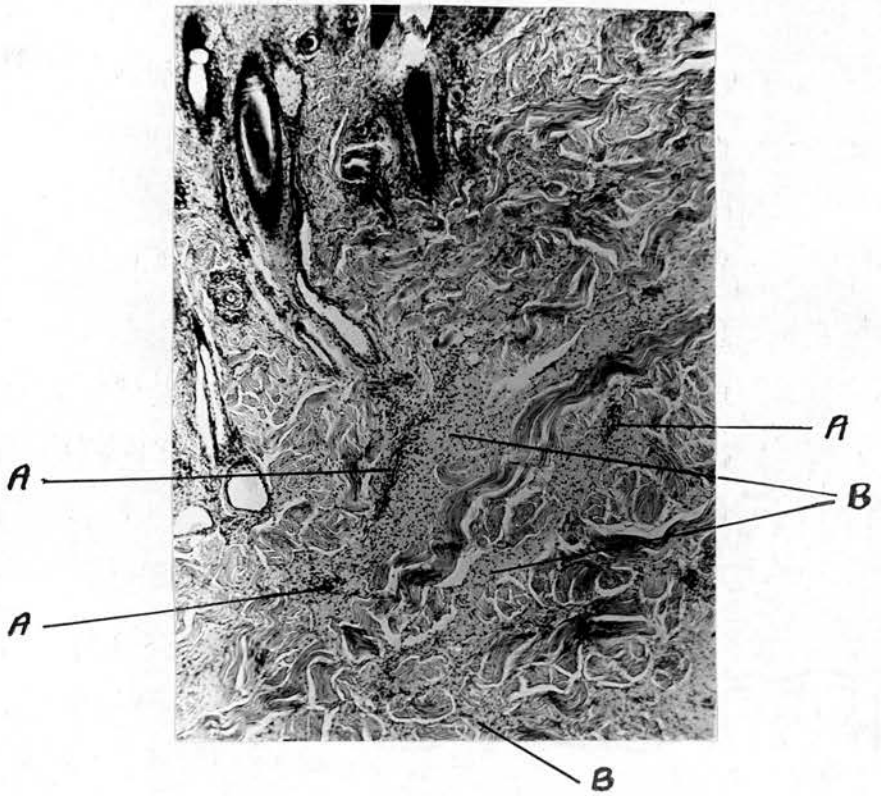


PLATE II.

Section of bovine skin 4 hours after the intradermal injection of 0.1cc of 1-100 saline extract of F.hepatica. (Stained H. and E.) (x 50)

Key.

A. Perivascular cellular migration (eosinophils).

B. Oedema, containing many cells, causing marked separation of collagen fibres.

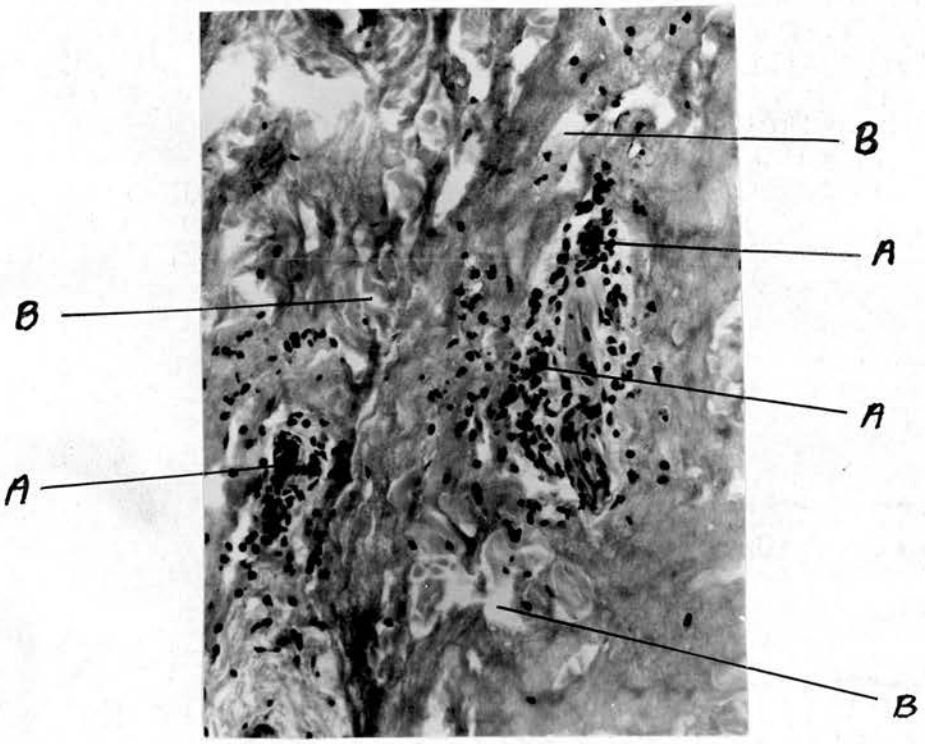


PLATE III.

Section of bovine skin 20 minutes fater the intradermal injection of 0.1cc of 1-100 saline extract of F.hepatica. (Stained H. and E.) (x 250)

Key.

A. Early perivascular cellular migration (eosinophils).

B. Early oedema causing separation of collagen fibres.

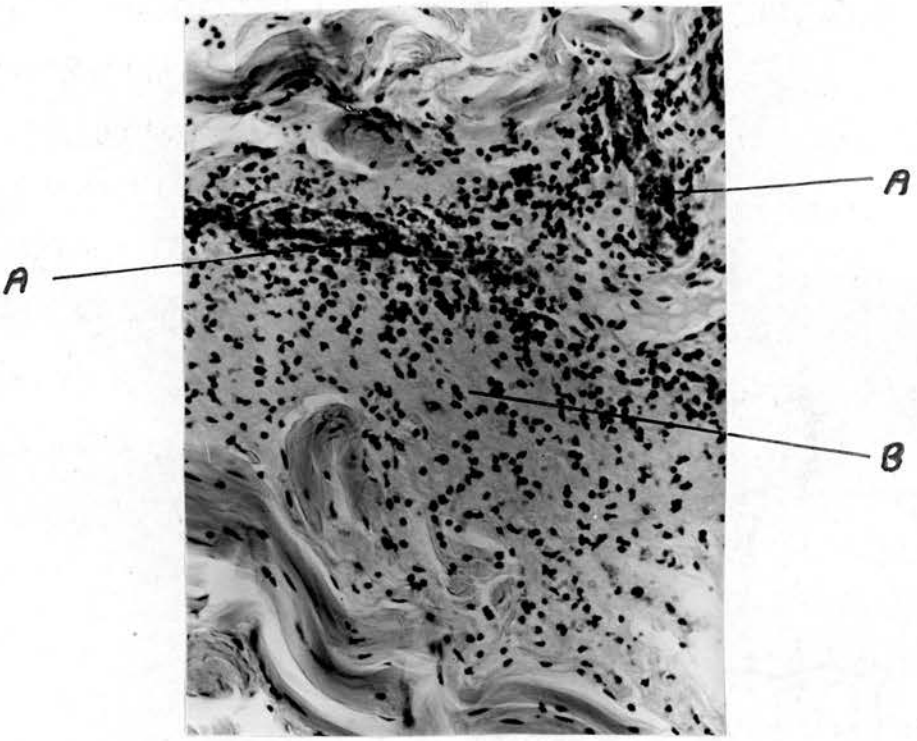


PLATE IV.

Section of bovine skin 4 hours after the intradermal injection of 0.1cc of 1-100 saline extract of F.hepatica. (Stained H. and E.) (x 250)

Key.

A. Marked perivascular cellular migration (eosinophils).

B. Oedema, containing many cells, causing marked separation of collagen fibres.

positive skin reaction in echinococcus infestation. Dienes (1930) states that skin reactions of the anaphylactic type are characterised by a quickly developing oedema and an intense accumulation of leucocytes. Rich (1944 pp.414) is of opinion that eosinophilia is a characteristic phenomenon at the site of injections in hypersensitive reactions of the anaphylactic type. It therefore appears that the reaction observed in F.hepatica infestation is of a true hypersensitive nature, being of the anaphylactic type.

The reactions with peptone solution in sheep consisted, in the majority of cases, of only a small spot of erythema indicating the injection site, however in some sheep a more marked reaction was evident. This consisted of a larger zone of erythema but without any wheal formation. In cattle no wheal formation was evident with the peptone solution; in some animals however there was a slight infiltration at the injection site in four hours (increasing the thickness of the fold of skin by 1-2mm). In some animals however which showed a marked reaction to the F.hepatica extract the site of the peptone injection developed an increased oedematous infiltration but this was in no way comparable with the reaction at the specific antigen site. Lewis and Loomis (1928) have reported an increased reactivity of the skin to non-specific irritants in animals

which possess a marked degree of skin sensitivity to tuberculoprotein. It is possible that in this examination a comparable mechanism was the cause of the oedematous infiltration at the peptone injection site in animals which reacted strongly to the specific antigen.

Post Mortem Examination.

All animals were numbered before intradermal injections were made. After slaughter the viscera and carcase of each animal were examined. The liver was examined for the presence of F.hepatica. An incision was made into the substance of the liver and across the main bile ducts. Where no microscopic evidence of liver fluke was found the contents of the gall bladder were collected and centrifuged and any deposit examined for the presence of liver fluke eggs. A note was made of the existence of parasitic nodules on the surface of the liver. The presence of other parasitic forms such as cysticerci of cestodes was noted.

The lungs were examined for the presence of F.hepatica and other helminths.

A note was made of any bacterial disease which was present in the carcase or its viscera and the state of nutrition and pregnancy (if present) were noted.

Results.

Precipitin Reaction.

In the precipitin test the results were read after the tubes had been incubated for one hour at room temperature. A narrow, whitish plane or ring of precipitate at the junction of the two fluids was considered a positive reaction. The degree of reaction was estimated from the thickness, density and time of appearance of the ring of precipitate and was designated as follows:-

?	doubtful reaction.
+	weak positive reaction.
++	moderately strong positive reaction.
+++	Strong positive reaction.

TABLE I.

Frequency distribution of Reactions occuring at different dilutions of Antigen with the sera of sheep not infested with F.hepatica.

Dilution of Antigen.	Degree of Precipitation.				
	?	+	++	+++	Neg. in all dilutions.
1-100	2	2	-	1	
1-200	2	2	-	1	21
1-400	1	-	1	-	
1-800	-	-	1	-	
1-1600	-	-	-	-	

(A total of 26 animals examined)

The moderate and strong reactions which

occurred with the various dilutions were given by the serum of one animal. This was an aged ram which showed fibrosis of the liver but no evidence of F.hepatica infestation.

TABLE II.

Frequency distribution of the reactions occurring at Different Dilutions of Antigen with the Sera of sheep showing hepatic lesions of early F.hepatica infestation. (22 animals examined).

Dilution of Antigen	Degree of Precipitation.				
	?	+	++	+++	Neg.in all dilutions.
1-100	-	-	7	15	
1-200	-	2	10	10	
1-400	4	5	5	8	-
1-800	3	2	4	2	
1-1600	2	4	1	1	

TABLE III.

Frequency Distribution of Reactions occurring at different dilutions of antigen with the Sera of sheep infested with mature F.hepatica. (32 animals examined).

Dilution of Antigen	Degree of Precipitation.				
	?	+	++	+++	Neg.in all dilutions.
1-100	-	6	5	10	
1-200	-	7	8	9	
1-400	-	9	9	2	2
1-800	1	8	2	3	
1-1600	1	3	1	3	

Histogram showing the percentage of reactions at various dilutions of antigen with various groups of sera.

Animals not infested with F. hepatica. (26 animals)

Animals showing early invasion of the liver by F. hepatica. (22 animals)

Animals infested with mature F. hepatica. (32 animals)

Percentage of reactions occurring at different dilutions of the antigen with the various groups of sera.

80.76%

7.6% 7.6% 3.8% 7.6% 7.6% 3.8% 3.8% 3.8% 3.8%

68.17% 31.73% 9.1% 45.45% 45.45% 17.2% 22.72% 22.72% 13.6% 9.09% 18.18% 9.09% 9.09% 18.18% 4.54% 4.54%

6.25% 18.75% 15.62% 31.25% 21.87% 25.08% 28.12% 28.12% 28.12% 6.25% 3.12% 25% 6.25% 9.37% 3.12% 9.37% 3.12% 9.37%

Negative in all dilutions.

? + ++ +++

1-100 1-200 1-400 1-800 1-1600

Dilutions of antigen and degree of reactions.

Dilution of antigen and degree of reactions.

Dilution of antigen and degree of reactions.

The Complement Fixation Reaction.

TABLE IV.

Frequency Distribution of the Fixation of Complement with the sera of Sheep infested with F.hepatica. (Wagner,1935, method using alcoholic extract of dessicated parasite).

Degree Affected.	No. of sera.	Fixation of Minimal Haemolytic Doses of Complement				
		No Fixation.	Less than 1 MHD.	1 MHD.	1.5 MHD.	2 MHD.
Lightly affected						
Sheep.	20 (6)	5 (2)	7 (2)	7 (2)	1	-
Moderately affected						
Sheep.	19 (3)	-	4 (3)	15	-	-
Heavily affected						
Sheep	11 (4)	1 (2)	5 (2)	5	-	-
Total.	50 (13)	6 (4)	16 (7)	27 (2)	1	-

(The figures in parenthesis denote the reactions occurring with the saline extract of F.hepatica).

TABLE V.

Frequency distribution of Fixation of Complement with the sera of sheep not infested with F.hepatica (Wagner, 1935, method).

No.of sera Examined.	Fixation of Minimal Haemolytic Doses of Complement.				
	No Fixation.	Less than 1MHD.	1MHD.	1.5MHD.	2MHD.
20 (2)	8 (2)	10	2	-	-

(The figures in parenthesis denote the reactions occurring with the saline extract of F.hepatica.)

TABLE VI.

Frequency distribution of Fixation of Complement with the sera of sheep infested and not infested with F.hepatica using an alcoholic extract of Sheep Heart (Non-specific Antigen).

Animals.	No.of Sera.	Fixation of Minimal Haemolytic Doses of Complement.			
		No Fixation.	Less than 1MHD.	1.5MHD.	2MHD.
Not Affected.	12	-	10	2	-
Lightly Affected	9	-	2	6	1
Moderately Affected.	13	-	6	4	2
Heavily Affected.	6	-	2	2	2
Total.	40	-	20	14	4

TABLE VII.

Frequency distribution of fixation of complement with the sera of sheep infested with F.hepatica, using an alcoholic extract of fresh F.hepatica. (Fairley and Williams, 1923, technique).

Animals.	No.of Sera.	Fixation of Minimal Haemolytic Doses of Complement.			
		No Fixation.	3M.H.D.	4.5 M.H.D.	6M.H.D.
Lightly Affected	3	3			
Moderately Affected	10	9	1(Slight)		
Heavily Affected	5	3	2 (1 Slight)	1	1(Slight)
Early infestation.	2	1	1(Slight)		
Total.	20	16	4(3 Slight)	1	1

Action of sheep serum on the miracidia of F.hepatica.

When sheep serum was added to a suspension of miracidia the following changes took place. The motility of the miracidium was impaired, the speed of the movement being reduced. Concomitantly with this slowing a collection or "precipitate" of granular material developed at the posterior end of the miracidium and later encroached along the sides of the organism. This "precipitate" produced a further decreased motility and a slowing of the beating cilia which at a later period began to beat slowly and in an incoordinated manner. Later deformity of the miracidia took place, the anterior extremity becoming enlarged and globular. With this deformity motility was lost and the miracidia gravitated out of suspension, taking on an amoeboid form. Later the deformed miracidia clumped together.

This reaction occurred to a slightly greater extent with the sera of animals infested with F.hepatica. From the results of the action of serum on miracidia it was thought that it might be possible to establish a test by which it would be possible to titrate the antibody present in the sera of sheep infested with F.hepatica. The details of the test have been presented in the previous section.

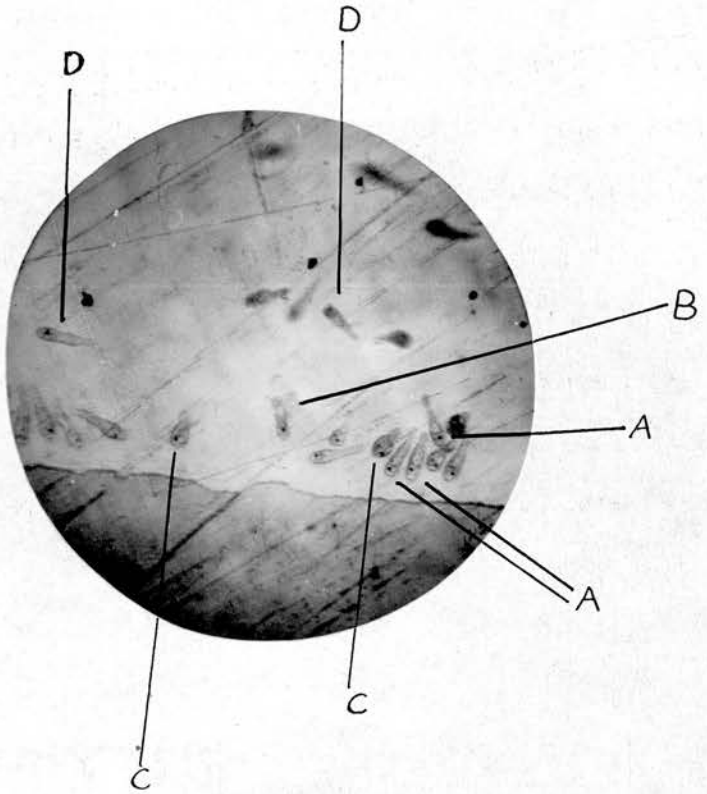


PLATE V.

Miracidia 5 minutes after the addition of an equal volume of serum (0.5cc) (diluted 1-10 with distilled water) and 0.1cc of complement (diluted 1-10 with saline) to the suspension. (Unstained) (x 100).

Key. A. Miracidia showing "tail" of precipitate.

B. Precipitate encroaching along the side of the organism.

C. Miracidia showing deformity with enlargement and globular appearance of the anterior extremity.

D. Apparently normal miracidia.

Table VIII.

Frequency distribution of the reactions occurring with sheep serum and the miracidia of F.hepatica.

Sera.	Numbers of sera reacting at different dilutions.							
	Initial Dilutions of Sera.						Controls.	
	1/10	1/20	1/40	1/80	1/160	1/320	Complement Control.	Aqueous Control.
Sera from infested animals which had a lethal effect on miracidia (25 sera examined)	25	25	24 (1)	21 (3)	5 (8)	1	-	-
Sera from animals showing early lesions of F.hepatica which had a lethal effect on miracidia (11 sera examined)	11	11	11	11	4 (4)	- (2)	-	-
Sera from non-infested animals which had a lethal effect on miracidia (16 sera examined)	16	16	12 (3)	5 (5)	- (3)	-	-	-

The figures in parenthesis denote the sera which had only a slight (+) effect on the miracidia at the given dilution.

(The miracidia in the negative and control tubes were still active after 18 hours at room temperature).

Polygon comparing the percentage of sera which had a lethal effect on the miracidia of F. hepatica at the different dilutions of sera in the three groups of animals.

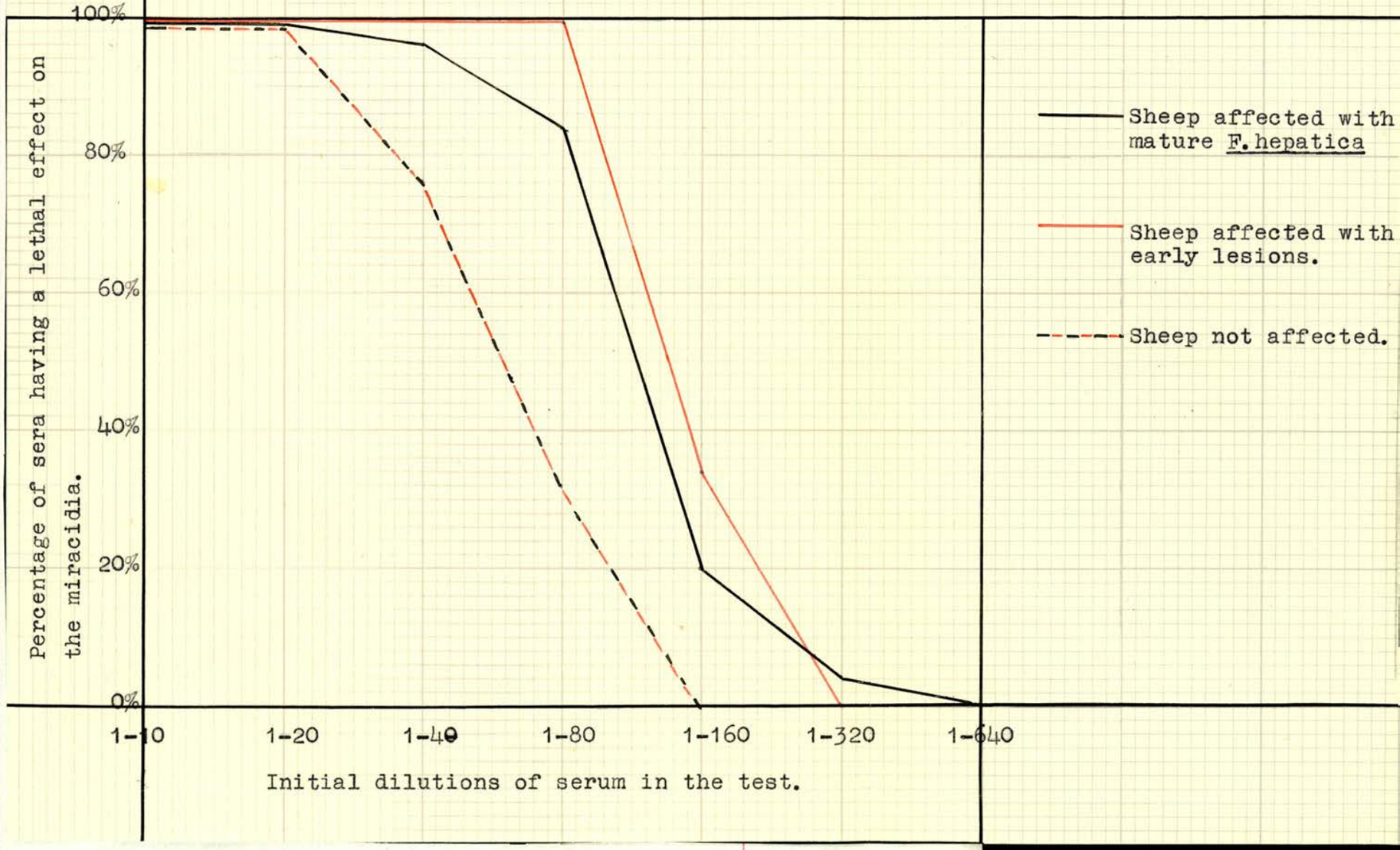


TABLE IX.

Comparison of the action of sheep serum on miracidia with and without the addition of Complement (0.1cc of Complement, 1:10 dilution).

Serum.	Initial Dilutions of Serum. 1/10;	1/20;	1/40;	1/80;	1/160.	Controls. C.C.	N.C.
A. With Comp.	+++	+++	+++	++	-	-	-
Wihtout Comp.	-	-	-	-	-	-	-
B. With Comp.	+++	+++	+++	+++	+	-	-
Without Comp.	-	-	-	-	-	-	-
C. With Comp.	+++	+++	+++	+++	++	-	-
Without Comp.	-	-	-	-	-	-	-

(The various reactions were designated -, + ++ or +++ as laid out in the details of the test on page 45)

TABLE X.

Comparison of the action of heated and unheated serum on miracidia with the addition of normal complement and heat inactivated complement.

(Heating was carried out for 30minutes at 56°C)

Serum	Initial Dilutions of serum 1/10;	1/20;	1/40;	1/80;	1/160;	Controls. C.C.	N.C.
Serum C. Unheated without complement	-	-	-	-	-	-	-
Unheated with normal complement.	+++	+++	+++	+++	++	-	-
Unheated with heat inactivated complement.	+	-	-	-	-	-	-
Heated C. Serum with normal complement.	+++	+++	+++	+++	++	-	-
Heated C. Serum with heat inactivated complement.	+	-	-	-	-	-	-

Table X indicates that the lethal action of serum was absent only when complement was removed or destroyed by heating. As complement alone (C.C. control) did not cause any lethal action it is concluded that the component of serum responsible for this action is thermostable at 56°C. and requires the addition of complement before the lethal action is evident.

TABLE XI.

The effect of the concentration of Protein and peptone on miracidia with and without the addition of Complement.

Substance.	Initial Dilutions of Egg White in Distilled Water.					Controls.	
	1/10	1/20	1/40	1/80	1/160	C.C.	N.C.
Egg White With Comp.	+	-	-	-	-	-	-
Without Complement	-	-	-	-	-	-	-
	Initial Dilutions of peptone in Distilled Water.					Controls.	
	10%	5%	2.5%	1.25%	0.625%	C.C.	N.C.
Peptone							
With Comp.	+++	+	-	-	-	-	-
Without Complement	-	-	-	-	-	-	-

TABLE XII.

Frequency Distribution of animals, infested with F.hepatica, which gave the following skin reactions to 1-1,000 saline extract of the F.hepatica.

Animals	No. of Animals	Skin Reactions occurring with the Specific antigen and Peptone.						
		Specific antigen.					Peptone.	
		Wheal 10 mm and over	Wheal 7-9mm	Wheal 6mm & under	Erythema 10mm:5-9mm.	Negative	Erythema. 5-9mm:	10mm and over
Lambs. (Average age 6 months)	3	1	1			1		
Gimmers. (Average age 1½ years)	4	3				1		
Ewes. (Varying age)	25	12	5		4 1	3	4	
Total.	32	16	6		5	5	4	
Percentage		68.75%			15.63%	15.63%	12.5%	

TABLE XIII.

Frequency distribution of animals (sheep), showing evidence of calcified parasitic lesions on the liver, which gave the following skin reactions to 1-1,000 saline extract of F.hepatica.

Animals	Number of Animals	Skin Reactions occurring with Specific antigen and Peptone (in mm's)					
		Specific Anitgen					Peptone.
		Wheal 10mm and over	Wheal 7-9mm.	Wheal 6mm and under	Erythema 10mm ;5-9mm	Negative.	Erythema. 5-9mm;10mm and over
Lambs. (Average age 6 months)	4	3				1	
Gimmers. (Average age 1½ years)	2		2				
Ewes. (Varying age)	6	4			2		
Total.	12	7	2		2	1	
Percentage.		75%			16.6%	8.33%	

TABLE XIV.

Frequency distribution of animals (sheep) showing no evidence of F.hepatica infestation which gave the following skin reactions to 1-1,000 saline extract of F.hepatica (in mm's)

Animals.	Number of Animals.	Skin Reactions occurring with the Specific antigen and peptone.(in mm)					
		Specific Antigen.					Peptone.
		Wheal 10mm and over	Wheal 7-9mm	Wheal 6mm and under	Erythema 10mm ; 5-9mm	Negative	Erythema 5-9mm ; 10mm and over
Lambs. (Average age 6 months)	36	1	1		1	33	2
Gimmers. (Average age $1\frac{1}{2}$ years)	16				3	13	
Ewes. (Varying age)	32	5	2		6	19	1
Total.	84	6	3		10	65	3
Percentage.		10.71%			11.9%	77.38%	3.55%
					89.20%		

In the intradermal tests there was no correlation between the strength of the reaction and the degree of infestation, this being contrary to the findings of Wagner (1935).

The animals presented in Table XIII showed evidence of calcified lesions on the surface of the liver. These were probably parasitic in origin, though it is impossible to state that they were due to F.hepatica infestation. Though 75% of these animals gave a marked skin reaction to the intradermal test, thus suggesting that they may have been due to F.hepatica infestation, the number of animals (12) is too small for conclusions to be drawn.

TABLE XV.

Frequency distribution of animals, infested with F.hepatica, which gave the following reactions to the skin test using 1-100 saline extract of F.hepatica.

Age Group	No. of Animals.	Animals showing the following increases in skin thickness. (in millimetres.)													
		1-2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over
1-2 yrs.	6	-	-	- (2)	-	1	1	-	-	-	1	2	-	-	1
2-3 yrs.	47	-	- (3)	-	2	9	5	4	5	3	4	1	1	3	10
3-4 yrs.	61	- (3)	-	-	7 (1)	14	3 (1)	5	7	4	7	2	2	1	9
4-5 yrs.	22	-	-	1	1	3	-	4	2	1	-	1	-	1	8
5-6 yrs.	13	-	- (2)	- (1)	1	5	1	1*	2	-	1	-	1	-	1
7 yrs.& over	15	2	-	1	1	3	-	5	1	-	-	-	2	-	-
Total.	164	2 (3)	- (5)	2 (3)	12 (1)	35	10	9	17	8	13	6	6	5	29
Percentage.		9.75%				90.25%									

The figures in parenthesis indicate the increase in skin thickness in animals injected with 1-100 hydatid extract. None of these animals ware infested with hydatid cysts. One animal, that marked with an asterisk, was infested with a small number, 6, of Cysticercus tenuicollis cysts.

The most frequently occurring observation in table XV is the increase in skin thickness of 6mm (Modal Value). The percentage of animals which gave skin reactions lower than the modal value is only 9.75% of the total number examined and 75% of the animals reacting less than the modal value reacted with a skin thickness of 5mm.

The two animals which gave an increase in skin thickness of only 1-2mm were aged cows. One animal, slightly affected with F.hepatica was affected with a suppurative mastitis and marked emaciation. Botteri (1922), Spengler (1923) and Deusch (1925) have indicated that cachectic states produce a loss of skin sensitivity in hydatid disease. It is possible that a comparable mechanism produced a loss of skin sensitivity in this animal.

The other animal, which gave an increase in skin thickness of only 1-2mm, possessed marked suppurative lesions in the bile ducts -- a few eggs of F.hepatica were found in the bile. A loss of skin sensitivity has been noted in hydatid disease as the result of suppuration of the parasitic cyst (Botteri, 1922; Deusch, 1925). Suppuration of the bile ducts may be the cause of the loss of skin sensitivity in this case. One animal, aged 4-5 years was similarly affected with suppuration of the bile ducts and gave an increase in skin thickness of 4mm to the F.hepatica extract.

If these three animals are removed from the frequency distribution table of infested animals as being not typically infested animals, the percentage of animals showing reactions below the modal value then is 7.92% of which 92.3% gave skin reactions of 5mm.

The strength of the reaction was generally higher in heavily infested than in lightly infested animals, especially in the age group of 2-5 years. In older animals this correlation was not found.

TABLE XVI.

Frequency distribution of animals, not infested with F.hepatica, which gave the following reactions to the skin test using 1-100 saline extract of F.hepatica.

Age Group.	No.of Animals.	Animals showing the following increases in skin thickness (in millimetres).							
		1-2	3	4	5	6	7	8	9 & over.
1-2 yrs.	7	2	3	1	1	-	-	-	-
2-3 yrs.	6	1	1	1	1	2	-	-	-
3-4 yrs.	15	4	1	7	1	2	-	-	-
4-5 yrs.	9	-	3	1	2	3	-	-	-
5-6 yrs.	6	-	2	2	2	-	-	-	-
7 & over	5	-	1	2	1	-	-	1	-
Total	48	7	11	14	8	7	-	1	1
Percentage.		83.33%				16.66%			

83.33% of the skin reactions occurring in animals not infested with F.hepatica are below

the modal value for the increase in skin thickness occurring in infested animals. No non-infested animals showed the marked increase in skin measurements which was evident in infested animals.

TABLE XVII.

Frequency Distribution of Animals, infested with F.hepatica, which gave the following reactions to the skin test using 1-1000 saline extract of F.hepatica.

Age Group	No. of Animals	Animals showing the following increases in skin thickness (in millimetres)												
		1-2	:	3	:	4	:	5	:	6	:	7	:	8
2 - 3 yrs.	31	4		6		2		4		4		2		4
	(31)							(2)	(7)			(2)	(3)	

		9	:	10	:	11	:	12	:	13:	14	:	15 & over.
2 - 3 yrs.	31	-		1		1		-		1	1		1
	(31)		(1)		(1)						(2)		(13)

(Figures in parenthesis indicate the reactions occurring with the 1-100 saline extract used in conjunction with the 1-1000 extract)

Table XVII indicates that a dilution of 1-1000 of the saline extract of F.hepatica is not as satisfactory as the 1-100 dilution in the skin hypersensitivity test. The results obtained by Mazzotti (1948) in humans could not be confirmed in cattle.

TABLE XVII.

Frequency Distribution of Animals, not infested with F.hepatica, which gave the following reactions to the skin test using 1-1000 saline extract of F.hepatica.

Age Group	No. of Animals	Animals showing the following increases in skin thickness (in millimetres).												
		1-2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8	8 - 9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15 & over.
2 - 3 yrs.	7	6	-	-	1	-	-	-	-	-	-	-	-	-	-
	(7)	(3)	(1)	(3)											

(Figures in parenthesis indicate the reactions occurring with the 1-100 saline extract of F.hepatica used in conjunction with the 1-1000 extract).

TABLE XIX.

Frequency Distribution of Animals, infested with F.hepatica, which gave the following reactions to the skin test using a.) 1-1000 dilution of Purified F.hepatica protein; b.) 1-10,000 dilution of Purified F.hepatica protein and c.) 1-100 saline extract of F.hepatica.

Age Group.	No. of Animals		Animals showing the following increases in skin thickness (in millimetres).													
			1-2	3	4	5	6	7	8	9	10	11	12	13	14	15 & over.
5 yrs. & over.	19.															
Antigen (1-1000)		a)	13	3	1	1	1	-	-	-	-	-	-	-	-	-
Antigen (1-10,000)		b)	18	1	-	-	-	-	-	-	-	-	-	-	-	-
Antigen		c)	1	-	2	3	6	3	-	1	-	1	-	-	1	1

In table XIX the animal which gave only 1-2mm increase in skin thickness to the 1-100 saline extract of F.hepatica and one animal which gave an increase of 4mm to the 1-100 saline extract have both been mentioned in relation to table XV. Both these animals were affected with suppuration of the bile ducts.

Table XIX indicates that the purified protein component of F.hepatica, as prepared in this examination, is unserviceable for eliciting a skin reaction in F.hepatica infestation.

Desensitisation.

The following animals were subjected to reinjection at the same site with 0.1cc of the 1-100 saline extract of F.hepatica. Three injections were made at intervals of 24 hours and the reactions noted in each case, as indicated in the table below.

TABLE XX.

Frequency distribution of the increase in skin measurements following reinjection of the initial site with 1-100 saline extract of F.hepatica (0.1cc injected).

Initial Increase in skin thickness (in m.m.s)	Increase in skin thickness after second injection (in m.m.s)	Increase in skin thickness after third injection (in m.m.s)
20 mm	13mm	5mm
6 mm	4mm	3mm
19 mm	8mm	8mm
16 mm	9mm	7mm
23 mm	14mm	8mm
8 mm	6mm	3mm
Average Decrease	6.16mm.	9.66mm

The following 15 animals were given 3 cc of the 1-100 saline extract of F.hepatica intradermally on one side of the neck and 24 hours later these animals were subjected to the normal intradermal test (0.1cc) on the opposite side of the neck. The reactions are presented in the following table.

TABLE XXI.

Frequency Distribution of the increase in skin measurements (following the intradermal injection of 3cc of the 1-100 saline extract to produce desensitisation) to 0.1cc of the 1-100 saline extract of F.hepatica

Skin Measurements (in millimetres)

Initial Increase in skin thickness to 0.1cc of 1-100 prior to desensitisation with 3cc	12	18	11	8	17	24	18	19	11	10	9	18	22	19	19
Increase in skin thickness to 0.1 cc. of 1-100 after desensitisation with 3cc.	6	9	11	8	12	18	21	17	9	7	3	21	14	13	9
Decrease in skin thickness, due to desensitisation	6	9	-	-	5	6	-	2	2	3	6	-	8	6	10
Increase in skin thickness after desensitisation							3					3			

Average Decrease in Sensitivity = 4.02mm.

The experiments in desensitisation of skin hypersensitivity to F.hepatica infestation indicated that local desensitisation occurred to a marked degree at the site of the initial intradermal injection, but general desensitisation, produced by large injections of antigenic fluid, occurred to a less marked degree.

Passive Transfer of Skin Sensitivity by means of Serum. (Prausnitz Küstner, 1921, reaction).

Serum which was taken from animals which had reacted to the intradermal injection of the saline extract of F.hepatica, was given intradermally, in lcc amounts, to animals which had not previously reacted to the saline extract. Twenty four hours later the prepared site was injected with O.lcc of the saline extract and the resulting reaction noted. The results obtained are presented in table XXII.

TABLE XXII.

Results of passive transfer of skin sensitivity by means of serum.

Skin Measurements (in millimetres)

Initial Skin increase in donor animal to 0.1cc of saline extract	10	4	10	4	6	11	10	11	10	2	14	11	11	9	8	11	9	12	2	14
Increase in skin thickness at the prepared site after transfer of serum in recipient animal to 0.1cc of saline extract	3	4	5	4	5	8	5	4	6	3	14	8	8	7	8	8	8	11	4	7
Initial increase in skin thickness to 0.1cc of saline extract in recipient animal prior to passive transfer	3	3	4	4	5	5	5	4	4	3	6	4	4	5	4	5	4	3	4	4
Increase in skin measurements as a result of passive transfer of serum	-	1	1	-	-	3	-	-	2	-	8	4	4	2	4	3	4	8	-	3

Table XXII indicates that successful transfer of sensitivity by way of serum was achieved to a marked degree in two cases and partially in four other cases. This indicates that the hypersensitivity in some animals is circulatory in nature. Rachman and Stevens (1927) have reported the successful passive transfer of skin hypersensitivity in humans to Ascaris lumbricoides.

Discussion.

The Precipitin Reaction.

The precipitin reaction proved satisfactory for the demonstration of antibodies in F.hepatica infestation in sheep, using the saline extract of dried and powdered parasites. The results were not as perfect as those reported by Trawinski (1937) who obtained positive reactions with the sera of all (100%) infested animals and negative results with all sera from non-infested animals. The results compare better with those reported by Szaflarski (1946) who obtained less marked reactions than Trawinski in animals infested with mature parasites.

Reactions were found to be stronger when the parasite was in its early migration stage through the liver prior to its establishment in the bile ducts. At this stage of the life history of F.hepatica the microscopic picture of the yellowish white and haemorrhagic tracts is one of cellular infiltration in the periportal areas with degeneration

and necrosis of liver tissue. The cellular infiltration consists of mononuclear cells, red blood corpuscles and especially eosinophilic polymorphonuclear leucocytes (Fairley and Williams, 1923). This picture is similar to that described by Fairley (1920) in monkeys experimentally infested with Schistosomes. The serological response in schistosomiasis exhibited a marked preliminary rise during the initial migration of the cercariae, (Fairley and Jasudason, 1930). It was also at this stage that the serological response to F.hepatica infestation was highest, as determined by the precipitin reaction. This is also the stage when most tissue damage is caused by the invading parasite.

In schistosomiasis there was a marked antibody response so long as the parasites continued to live in the host. A secondary rise in antibody occurred with the formation of portal thrombi containing degenerating worms. Unlike the schistosomes, which are blood stream parasites, F.hepatica exists in the bile ducts of the liver. The walls of the affected bile ducts frequently become fibrosed, thus isolating the mature liver fluke from the body fluids of the host. The passage of antigenic material from the parasite to the host tissue will thus be rendered difficult or impossible. Hite, Banks and Dack (1938) have stated that the passage of antigenic material through granulation tissue is

greatly reduced.

It would appear, therefore, that the migration of immature forms in Fascioliasis was responsible for the initial antibody response. The presence of antibody in animals infested only with the mature form of F.hepatica in the liver was most probably due to the persistence of the initial response, being maintained by the absorption of small amounts of antigenic material from the mature parasites in the bile ducts. Reinfestation may however stimulate antibody production and serve to maintain the antibody level.

No correlation was noted between the degree of serological response and the degree of infestation. This would tend to indicate that the number of mature parasites per se was not the criterion for the degree of antibody response. Rather, it may be that the degree of biliary fibrosis, thus reducing absorption of antigenic material, and the degree of reinfection were responsible for the level of antibody.

With the exception of one animal, the sera of a few non-infested animals showed unimportant reactions only. The exception was that of the sera from an aged sheep which gave marked precipitin reactions. It is possible that this sheep had developed antibodies through repeated small infestations with F.hepatica, though no evidence of infestation was found. Similarly the few non-infested

animals which gave slight reactions may have had an abortive form of Fascioliasis, which left no evidence at post-mortem.

The Complement Fixation Reaction.

The most noteworthy feature about the results of the complement fixation test in Fascioliasis was that the fixation of complement was of a very small order. Such fixation cannot be regarded as evidence of specific complement fixation antibodies occurring during F.hepatica infestation.

It was seen that similar reactions occurred with sera from infested sheep when an alcoholic extract of sheep heart was used as antigen. Mackie and Finkelstein (1928) have reported that the sera of several species of animals, including sheep, react non-specifically with non-antigenic substances (saline, alcohol, peptone and alcoholic extract of sheep heart) in the complement fixation test, sometimes to the degree of 4 M.H.D.s.

In this examination the degree of fixation occurring with the sera of infested and non-infested sheep and with the non-specific antigen (sheep heart) was similar in each case. Consequently no specific complement fixation can be said to have taken place.

When the technique described by Fairley and Williams (1923) was used only one serum gave fixation of over 4.5 minimal haemolytic doses of complement. Four other sera gave fixation of approximately

2.5 M.H.D.'s of complement. These results do not confirm those obtained by Fairley and Williams who demonstrated marked complement fixation with the sera of liver fluke infested sheep.

A survey of the results of other workers who have used the complement fixation test in Fascioliasis indicates that much of the complement fixation noted was of a non-specific type comparable to that reported in this examination. Paccanaro (1909) reported complement fixation with the sera of healthy sheep and lambs when extracts of F.hepatica were used as antigen. Busson (1911), who determined the degree of fixation by varying the quantity of antigen, reported that complement fixation could be detected in the sera of animals not infested as well as the sera of animals infested with F.hepatica. Höppli (1921) detected complement fixation antibodies in the sera of cattle free from liver fluke, while Servanti (1921) demonstrated variable fixation of complement with sera from sheep infested with F.hepatica. Servanti used fixed doses of reagents in the test. Following the work of Fairley and Williams (1923), who reported excellent results with the complement fixation test in liver fluke infestation, Brocq-Rousseu, Cauchemez and Urbain (1923) carried out extensive experiments with the complement fixation test on the sera of sheep infested and free from F.hepatica. The authors determined the degree

of fixation by varying the amount of complement in the test. The results of their work showed that while 85% of sera from liver fluke infested animals gave positive complement fixation, 60% of the sera from non-infested animals also gave similar fixation. Wagner (1935) utilised antigens and serum dilutions which, in this examination, have been shown to possess anticomplementary action. In addition Wagner did not determine the degree of fixation by varying the quantities of one component of the test. Indeed the criterion for the positive reaction was the fixation of a single volume (0.2cc) of complement. While 73% of the sera of infested animals showed complement fixation, of the sera from ten animals, which showed no evidence of infestation, five gave positive complement fixation.

It is to be noted therefore, that where the degree of fixation of complement has been determined by previous workers, with the exception of Fairley and Williams (1923) similar fixation occurred with the sera from infested and non-infested animals. Where the degree of complement fixation was not determined and the criterion for a positive reaction was the fixation of an arbitrary volume of complement, fixation was frequently noted in the sera of healthy animals.

It would appear, therefore, that the fixation reported by previous workers, with the

exception of Fairley and Williams (1923), was probably due to non specific complement fixation properties of the sera comparable to those reported by Mackie and Finkelstein (1928).

In the series of 20 animals examined by Fairley and Williams (1923), the sera of 14 showed marked complement fixation. Nine of these animals showed acute toxic changes in the liver. Though the actual character of these changes was not stated, it is possible that they were due to marked early migration of the immature F.hepatica through the substance of the liver. If this was so, it is probable that complement fixation antibodies would be present in high titre since in the precipitin reaction a rise in precipitin antibody has beennoted at this stage of early invasion.

In this examination the sera of two animals were examined which displayed evidence of early invasion by the immature liver fluke, though only on the caudate lobe of the liver. One of the sera gave partial fixation of 3 M.H.D.'s of complement (approx. 2.5 M.H.D.'s fixed); the other serum gave negative reactions. However, if sera from animals showing similar changes to those examined by Fairley and Williams (1923) had been available comparable results might have been obtained.

The action of sheep serum on the miracidia of F.hepatica.

The results of the examination indicate that sheep serum, even in high dilutions, in the presence of complement, exerted a lethal effect on the miracidia of the liver fluke. The serum principle responsible was not destroyed by heating at 56°C for 30 minutes. However complement was a necessary component in the reaction since the absence of complement or the presence of heat-inactivated complement rendered the serum almost entirely ineffective. A very slight lethal action was evident with heat-inactivated complement and this possibly was indicative that the heat-stable components of complement (3rd. and 4th. components)played a small part in the lethal effect.

High concentrations of protein and peptone in the presence of complement, were seen to exert an adverse effect on the miracidia, as indicated by the action of high concentrations of egg albumen and commercial peptone, (Table XI). When these concentrations of protein and peptone are compared with the protein concentrations of the dilutions of serum which cause death of miracidia it is significant that protein concentration alone could not be held responsible for the lethal action. From the results shown in the frequency distribution table (Table VIII) it was seen that there was a slight difference between the effects of the sera from animals infested with F.hepatica and the sera from non-infested animals.

Animals presenting lesions of early invasion showed the highest lethal effect. The difference between the various groups of animals was so small, however, as to be of no significance.

Reactions causing precipitates and death with the intermediate stages of nematode helminths have been noted by several authors. Otto (1939) recorded oral, anal and oesophageal precipitates around the larvae of Ancylostoma caninum when they were placed in immune serum. Roth (1941), Oliver-Gonzalez (1940) and Mauss (1940b) have reported similar precipitates around the larvae and adults of Trichinella spiralis when placed in immune serum. Otto, Schugam and Groover (1942) noted this phenomenon with the larvae of Necator americanus.

While the reaction took 12 hours to 4 days to develop in the larvae of nematodes, only a few (15) minutes were necessary for the action of sheep serum on the miracidia.

The precipitate occurring with the larvae of nematodes appeared to be a reaction between the excretions and secretions of the larvae and the antibody in the serum, (Oliver-Gonzalez, 1940). In contrast, the action of serum on miracidia was not confined to immune serum but occurred to almost the same extent in normal serum. No precipitate was evident at the anterior extremity of the miracidium as occurred with the larvae of nematodes. Since the

physiological openings of the miracidium are situated posteriorly (excretory pores draining the flame cells) it is possible that the posteriorly developing "tail" of precipitate occurring on the miracidium may be the result of the combination of excretions and some substance occurring in the normal blood serum. This substance needed the presence of complement, whereas the larval precipitates in nematodes developed in the absence of complement, (Oliver-Gonzalez, 1940).

A cercaricidal effect of normal serum on the cercariae of Schistosoma japonicum has been reported by Tabangui and Masilungan (1936). Complement was necessary for this reaction which took one hour at 37°C to be completed. The action was increased by the use of serum from an animal which was infested with the parasite. Culbertson and Talbot (1935) also recorded such a reaction with the cercariae of schistosomes when incubated in serum. The parasites became distorted, lost their motility and finally were unrecognisable as cercariae. The serum constituent responsible for this reaction was inactivated by heating and dessication. The titres of the sera of various animals varied within wide limits. Precipitates occurring around the cercariae of schistosomes of man when the cercariae are placed in the sera from infested humans were noted by Papirmeister and Bang (1948). Heating of the serum destroyed this activity but restoration of activity

was produced by the addition of complement. Liu and Bang (1950) reported an agglutinin for the cercariae of schistosomes (S.mansoni) in the blood of rhesus monkeys which had been infested with the parasite. This agglutinin reached its highest point when eggs of the schistosome appeared in the faeces. It was heat-stable.

With the exception of the serum agglutinin described by Liu and Bang (1950) the action of sera on cercariae appears to be analagous to the reaction with miracidia described in this examination.

A reaction has been described by Sato (1933) with the filariform larvae of Strongyloides stercoralis and serum. Sato reported an "agglomeration" action on these larvae with normal serum, but serum from a human infested with S.stercoralis had a slightly greater effect than normal serum.

Ledoux-Lebard (1902) has described an action of serum on free swimming ciliates (Paramoecia) very similar to the action of serum on miracidia noted in this examination. When Paramoecia were placed in different dilutions of various species' sera the Paramoecia suffered a slowing and immobilisation. At the posterior end of the organism there appeared a flocculent irregular mass in the form of a "tail" which later encroached along the sides of the Paramoecium. After sedimentation to the bottom of

the tube the Paramoecia tended to "agglomerate" in a rosette fashion. This action occurred in from 15 minutes to 3 hours in an incubator (temperature not stated). The serum component responsible was inactivated by heating at 55°C for 30 minutes, but was replenished when unheated serum was added.

The action of serum on Paramoecium and miracidia is very similar and may represent a natural serum action on lower free swimming forms of life. A lethal action of normal serum on trypanosomes has been recorded by Culbertson and Strong (1935). This trypanocidal effect, however, was only slightly reduced by the inactivation of complement by heating but was completely inactivated when the fourth component of complement was destroyed.

The normal serum reactions on Paramoecia, miracidia and trypanosomes are probably similar in nature to the natural antibodies present in serum responsible for the lysis of many species of bacteria. Such antibodies have been described by Mackie and Finkelstein (1931).

The Intradermal Reaction.

The results of the intradermal tests indicated that there was a hypersensitivity which develops during infestation of animals with F.hepatica and persists for a long period after initial infestation.

This hypersensitivity did not appear to be

so well developed in sheep as in cattle (68.75% of infested sheep gave marked skin reactions, whereas 90.25% of infested cattle gave marked reactions).

Mazzotti (1951) was of the opinion that hypersensitivity to F.hepatica infestation was caused by damage and shock to the reticulo-endothelial tissues of the liver. F.hepatica infestation in cattle differs from that in sheep in that marked fibrosis of the bile ducts regularly occurs in cattle. Such a reaction in cattle indicates a more marked cellular response to F.hepatica than occurs in sheep. Consequently a greater stimulation of the reticulo-endothelial system will occur in cattle and thus according to Mazzotti (1951) a greater degree of hypersensitivity will be evident.

Hypersensitive reactions in infested cattle were notably stronger than the reactions which occurred in non-infested animals. No animal which was free from liver fluke displayed the remarkable increase in skin thickness which was evident in many infested animals.

This hypersensitivity could be passively transferred by means of serum to a non reacting animal (Prausnitz Küstner, 1921, reaction) indicating that the hypersensitivity existed, in some animals, in a circulatory or "reagin" form, (Coca and Grove, 1925). Hypersensitivity to F.hepatica infestation in humans was capable of passive transfer, as

indicated by Guerra, Mayer and Prisco (1945). Hypersensitivity to A.lumbricoides infestation and hydatid disease in humans has been shown to possess similar properties (Rachman and Stevens, 1927).

Desensitisation of a local area of skin could be accomplished to a marked degree by repeated injection of antigen at the same site. However, general desensitisation could not be brought about to any great degree by the intradermal injection of large quantities of antigen. Possibly larger quantities of antigen than were used in this examination would have produced a more complete desensitisation.

Animals which did not show evidence of liver fluke infestation but which gave marked skin reactions were mainly older animals. Such animals, owing to their age, would have had more opportunity to come into contact with F.hepatica, which, although not causing actual lesions, may have sensitised the animals to the F.hepatica antigen.

It is of interest to note that 75% of sheep which showed evidence of calcified nodules on the surface of the liver reacted to a marked extent to the F.hepatica extract. Though it was not possible to determine whether these lesions were caused by F.hepatica, the positive skin reactions suggested that the lesions were possibly evidence of abortive forms of F.hepatica infestation.

The dilution of the saline extract to 1-1,000 did not prove satisfactory for the demonstration of hypersensitivity. Mazzotti (1948, 1951) has stated that high dilutions of F.hepatica extract (up to 1-10,000) were as satisfactory as lower dilutions when used in humans. This did not apply to the test in cattle. Sobiech (1951) reported that dilutions of 1-1,000 and over produced an antigen which was unsuitable for use in the intradermal test in fascioliasis. He also stated that a 1-100 dilution of F.hepatica antigen prepared according to Trawinski (1937) produced a reaction which was too marked in nature. However the quantity of antigen injected by Sobiech was 0.3 to 0.5cc as compared with 0.1cc in this examination. Rich (1944, pp.352) mentions that humans are capable of developing a much higher degree of hypersensitivity to tuberculosis infection than animals. It is possible that this may also apply to parasitic diseases which produce hypersensitivity.

Attempts to produce a more purified antigen for the skin test by precipitating the active protein in F.hepatica with trichloracetic acid were unsuccessful.

The intradermal skin reaction in F.hepatica infestation has been shown to be specific by several authors (Sievers and Oyarzun, 1932; Wagner, 1935; Aygün and Baskaya, 1939; Mazzotti, 1942; 1948; 1951;

Morenas, 1943a; Lavier and Stephanopoulo, 1944; Szaflarski, 1950 and Sobiech, 1951). In this examination intradermal tests were carried out with various helminth antigens in conjunction with the F.hepatica extract. The results have indicated that the reaction to F.hepatica was specific to a great degree. Intradermal tests in cattle using extracts of hydatid cysts proved negative in animals infested with F.hepatica. Reactions occurring to the intradermal injection of Dictyocaulus antigen occurred independent of reactions caused by the F.hepatica extract. However extracts of Cysticercus bovis showed similar reactions to those caused by the F.hepatica extract, (see Part III) the animals showing no evidence of C.bovis infestation. It therefore appears that a common antigen existed between C.bovis and F.hepatica. A common antigen between F.hepatica and the adult form of C.bovis (Taenia saginata)has been demonstrated by Kellaway (1928). Kellaway found that the uterus of a guinea pig sensitised to T.saginata responded anaphylactically when treated with extracts of F.hepatica.

Two animals infested with Dicrocoelium dendriticum reacted with the F.hepatica extract indicating a cross reaction between these two liver flukes. Wagner (1935) has shown that sheep infested with D. dendriticum react in a hypersensitive manner to the F.hepatica extract. Sobiech (1951), however,

stated that the F.hepatica antigen was specific and failed to cause a hypersensitive reaction in sheep infested with D.dendriticum alone. The results of this examination did not confirm the findings of Sobiech.

Several (32) cattle which were examined were affected with tuberculosis. This infection did not influence the development of the reactions or cause reactions to develop in non-infested animals. Horne and Harrell (1944), Harrell and Horne (1945) and Harrell, Horne, Aikawa and Helsabeck (1947) have reported that patients in tuberculosis sanitoria showed a greater percentage of positive skin reactions to T.spiralis antigens than patients in other institutions and hospitals. Kochnev (1950) reported that cattle showing pulmonary fascioliasis frequently reacted to the tuberculin test in the absence of tuberculosis infection.

No evidence was found in this examination to suspect that tuberculosis infection in animals sensitised the animals to the F.hepatica extract.

The results of this examination confirmed the work of previous workers that a hypersensitive state exists during infestation with F.hepatica and that F.hepatica infestation can be diagnosed by an intradermal reaction, which is relatively specific.

Summary.

The precipitin reaction was a satisfactory method for the determination of circulating antibody in animals infested with F.hepatica. The greatest response occurred when the immature parasites invaded the liver substance; this stage probably being the origin of the circulating antibodies which were maintained after initial invasion by the absorption of antigenic material to a varying degree from the adult liver flukes in the bile ducts. There was no correlation between the number of adult flukes and the degree of precipitating power of the serum.

Specific complement fixation was not evident in this examination. The fixation which did occur was of a very low degree and most probably due to natural complement fixation antibodies in the sera.

A lethal action of sheep serum on the miracidia of F.hepatica was demonstrated. Complement was necessary for this reaction which occurred to almost the same degree with sera from sheep infested with F.hepatica as with sera from sheep not infested with the parasite. The serum component responsible for this reaction was probably analagous to the natural bactericidal antibodies.

Hypersensitivity to F.hepatica infestation was demonstrated successfully by means of an intradermal test. This proved accurate in 90% of cases in diagnosing F.hepatica infestation in cattle. The percentage of positive reactions in sheep infested

with F.hepatica was lower, (68.75%). The hypersensitivity persisted for a long time after infestation. It was capable of being passively transferred in some animals by means of serum to a non-reacting animal. Desensitisation could be accomplished to a marked extent on a local area of skin but only a slight general skin desensitisation occurred with the injection of large quantities of antigen.

Part III.

Cysticercus bovis.

Materials and Methods.

The Precipitin Reaction.

As far as can be ascertained there is no available literature concerning the use of the precipitin test in cattle infested with C.bovis. However this test has been used for the diagnosis of Cysticercus cellulosae infestation in humans and swine. Thus Trawinski and Rothfeld (1935) found the precipitin reaction of use for the diagnosis of cerebral cysticercosis in humans. The preparation used for the test was a saline extract of the dried and pulverised scolices of the cysticercus. Later Trawinski (1936) successfully used the precipitin test for the diagnosis of C.cellulosae infestation in pigs. The preparation used was a saline extract of scolices. Trawinski (1936) and Soltys (1936) reported that extracts of the flesh of pigs infested with C.cellulosae were satisfactory for use in the precipitin test. The above authors stated that the reactions occurring with the saline extract of the scolices were specific. However Chung and T'ung (1939) and Gaehtens (1941; 1943) reported that extracts of C.cellulosae produced positive reactions with the sera of patients infested with hydatid cysts.

In this examination a saline extract of C.bovis was used initially in the precipitin test.

This was prepared according to the methods described by Trawinski (1936) for the preparation of the saline extract of C.cellulosae, as follows:

The cysts of C.bovis were collected from infested cattle. As the number of cysts which occurred in an infested animal was generally small (1 or 2) it was necessary to collect the cysts over a period of time (8 weeks). In the interval collected cysts were stored in a frozen state.

After collection the cysts were washed well in water and the scolices were dissected away from the rest of the bladder. The scolices were washed, placed in a sterile petri dish and dried in an incubator at 37°C for 48 hours. The dried material was reduced to a powder in a mortar. It was then weighed and extracted with saline in the proportion of 1 gram of powdered scolices to 100cc of saline. The extraction was maintained for 8 days at 4°C, the suspension being agitated at frequent intervals. After extraction the suspension was centrifuged (3,000r.p.m. for 30 minutes) and the insoluble material discarded. The supernatant fluid was sterilised at 56°C for 30 minutes, passed through a "Pyrex" microfilter and was subsequently stored in the frozen state at -10°C. This solution represented a 1-100 diluted extract of C.bovis scolices. It was diluted by doubling dilutions in saline from the 1-100 dilution to 1-6,400. These

doubled dilutions were used in the precipitin test.

From the preliminary precipitin tests it was found that the saline extract of scolices was not specific and consequently it was decided to use a purified polysaccharide preparation for the test. Since sufficient numbers of C.bovis cysts were not available for the preparation of such a polysaccharide it was necessary to use the adult tapeworm form of C.bovis, Taenia saginata.

Adult tapeworm materials are capable of immunising animals against the intermediate stage of the parasite. Antibodies produced by infestation with the cysticercus stage of parasites can be absorbed by the adult tapeworm materials. Thus Miller (1931c) and Campbell (1936a) showed that immunising injections of adult tapeworm materials were capable of stimulating resistance in animals to infestation with the eggs of Taenia taeniaeformis. Campbell (1938b) demonstrated the absorption of protective antibodies from the sera of animals infested with Cysticercus fasciolaris with the adult tapeworm material of T.taeniaeformis.

The polysaccharide component of T.saginata was prepared following the technique of Campbell (1937) who described the preparation of polysaccharides from helminth parasites.

An adult T.saginata was obtained from a human carrier. The worm was washed well in running

water and was then frozen at -23°C until it was hard and brittle. The frozen worm was then ground to a pulp in a mortar, thus producing approximately 50 grams of material. This pulp was suspended in 150cc of distilled water and heated for 30 minutes in a boiling water bath. This procedure not only coagulated most of the soluble protein material but also destroyed the activity of the autolytic enzymes which under normal circumstances cause the rapid disappearance of the polysaccharide (Campbell, 1937). The suspension was vigorously stirred until cool and was then acidulated to approximately pH4.5 with glacial acetic acid. Twenty cubic centimetres of lead acetate (20%) were added and the mixture placed in the refrigerator overnight. The insoluble material was removed by centrifuge and discarded and the remaining solution precipitated with 1.2 volumes of 95% ethyl alcohol. The resulting precipitate, which consisted chiefly of carbohydrate (Campbell, 1937), was removed and dissolved in 0.1% NaCl solution. This solution was centrifuged for 45 minutes to remove any insoluble material. Further precipitation was carried out by fractional precipitation with 95% ethyl alcohol. The alcohol was initially added until a faint white precipitate was formed. This precipitate was discarded because, although it consisted chiefly of carbohydrate, Campbell has stated that the portion may be biuret positive. The

remaining solution from this partial precipitation was then precipitated with one volume of 95% alcohol. The resulting precipitate was collected and dissolved in saline which was then centrifuged to remove insoluble material. This procedure was repeated until a sample of the resulting saline solution showed no evidence of precipitation when the pH was lowered to approximately 3.0 with a solution of 10% phosphotungstic acid and 3% sulphuric acid. The polysaccharide was precipitated from the saline solution by the addition of one volume of 95% alcohol and the precipitate was washed with distilled water and finally dried at 37°C. By this method 50 milligrams of purified polysaccharide were obtained from 50 grams of fresh T.saginata.

A 0.1% solution of polysaccharide in saline (0.85% NaCl) was prepared from this powder and after centrifuging was stored in the frozen state at -10°C.

For the precipitin test the 0.1% solution of polysaccharide was diluted in saline by doubling dilutions to give three solutions of the following concentration, 1-1000, 1-2000 and 1-4000. These concentrations were used as test solutions in the precipitin test.

Blood was obtained from the ventricles of the heart of selected cattle after they had been slaughtered. The serum was allowed to separate off

overnight. Before use the serum was centrifuged for half an hour at 3,000 r.p.m. in order to produce a clear product. The animals were selected as follows:

Cattle infested with C.bovis but not with F.hepatica.
Cattle infested with C.bovis and F.hepatica.
Cattle not infested with either C.bovis or F.hepatica.
Cattle not infested with C.bovis but infested with F.hepatica.

The test was carried out in narrow tubes (5x32mm). The serum was carefully overlaid with the test solution (saline extract of scolices or polysaccharide) so as to produce a clear line of demarcation between the two fluids. In all the series of tests two control tubes were used, one consisting of serum overlaid with saline and the other consisting of a known positive serum. The tubes were incubated at constant room temperature for 1 hour after which they were examined for evidence of precipitation. A positive result consisted of the formation of a fine white ring or plane of precipitate at the junction of the two fluids, the saline control being negative.

The degree of precipitation was estimated from the density and the time of development of the ring of precipitate, being designated as follows:-

? doubtful

\+ slight precipitation

++ moderate precipitation

+++ marked precipitation

Intradermal Test.

Intradermal tests have been used only occasionally in cysticercus infestation.

Maternowska (1933) used an intradermal allergic test in two cases of epilepsy which were thought to be due to C.cellulosae. Saline extracts of the cysticercus were used for the test, which was positive in both cases.

Chung and T'ung (1939), who used C.cellulosae bladder fluid for intradermal allergic tests, found strong reactions not only in cases of cysticercosis, but also in Echinococcus and intestinal Taenia infestations.

Podyapolskaya and Kamalova (1942) used skin tests for the diagnosis of cysticercus infestations in man and animals. Intradermal tests with C.bovis extracts in humans were non-specific for T.saginata infestation and similarly were non-specific for C.bovis infestation in cattle.

As the method of preparing the antigen used for the skin tests by Podyapolskaya and Kamalova was not available, the extract used for the intradermal tests in cattle in this examination was a saline extract of fresh whole cysts of C.bovis, which was prepared as follows:

C.bovis cysts were collected from the

musculature of an infested animal. These were washed well in water and were then ground in a sterile mortar, sterile glass wool being added to assist pulverisation. After the cysts had been reduced to a fine emulsion the resulting fluid was made up to 100 times its volume with saline. Following thorough mixing of the diluted emulsion the mixture was centrifuged at 3,000 r.p.m. for 30 minutes, the insoluble material being discarded. The clear supernatant fluid was then passed through a "Pyrex" microfilter and placed in 1cc bottles fitted with a rubber stopper. These bottles were stored in the refrigerator at -10°C.

The selected sites were those chosen for the intradermal test in F.hepatica infestation (Part II) being the skin on the middle third of the neck on a line parallel to the spine of the scapula. The area was clipped free of hair and the thickness of the fold of skin at the site measured by means of callipers graduated in millimetres. 0.1cc of the saline extract of the cysticercus was injected intradermally using a "Record" type of dental syringe. The reaction was observed after four hours when the fold of skin at the site of the reaction was measured.

All the intradermal tests with C.bovis extract were carried out in conjunction with intradermal injections of F.hepatica extract (as

used for the skin tests in F.hepatica infestation).

The reactions observed at the site of the C.bovis injection were essentially similar as the intradermal reactions observed in F.hepatica infestation. Thus an early developing wheal was followed by an oedematous infiltration of the dermis. This reached its maximum height in 4 hours, being maintained for a further 2 to 4 hours, but having disappeared by 24 hours.

Post Mortem Examination.

All animals were numbered before the intradermal test was performed, being subsequently slaughtered separately when the carcase and organs of each animal were examined for C.bovis.

The external and internal masseter muscles were incised and the cut surfaces were examined for the C.bovis bladder. The heart muscle was incised through the interventricular wall and the muscle surfaces examined. The cut surface of the carcase (e.g. neck, sternum and pelvis) were examined for C.bovis.

The liver was examined for evidence of liver fluke, being incised across the main bile ducts and into the substance. Where no microscopic evidence of F.hepatica was present the contents of the gall bladder were centrifuged and the deposit examined for evidence of the eggs of F.hepatica.

An examination was made for the cysticerci

of animal cestodes such as Cysticercus tenuicollis.

Results.

The sera of the following animals (28 animals infested with C.bovis and 8 not infested) were examined by means of the precipitin test. In addition the sera of two sheep, which were infested with F.hepatica, were examined. The sera of these two sheep reacted strongly to the precipitin test when a 1-800 dilution of a saline extract of F.hepatica was used.

Table I.

Frequency Distribution of the sera of animals, infested and not infested with C.bovis, which gave positive precipitin reactions at different dilutions of the antigen, (1-100 saline extract of C.bovis scolices).

Dilution of Antigen	State and Degree of Infestation*.						
	Live Cysticerci.		Calcified Cysticerci.		Not infested.		Sheep.
	Liver Fluke	No Liver Fluke	Liver Fluke	No Liver Fluke	Liver Fluke	No Liver Fluke	Liver Fluke
1-100	8	4	6	2	6	2	2(2)
1-200	8	4	6	2	6	2	1(2)
1-400	8	4	6	2	6	2	1(2)
1-800	6	4	6	2	6	2	-(2)
1-1600	6	3	5	1	3	-	-
1-3200	-	2	2	-	-	-	-
1-6400	-	1	-	-	-	-	-
Total Number of Sera in each Group	8	4	6	2	6	2	2

*The degree of infestation in all cases, with the exception of one animal, consisted of one or two cysts. The exception was that of a multiple infestation of calcified cysticerci. The sera of this animal reacted up to a 1-800 dilution.

The figures in parenthesis indicate the dilutions at which the sheep sera gave positive reactions when tested with the saline extract of F.hepatica.

Table I shows that with the saline extract of C.bovis scolices precipitin reactions occurred indiscriminately with the sera of animals showing all the stages of infestation and with the sera of animals showing no evidence of infestation. As the sera of animals infested with F.hepatica gave positive precipitin reactions with the cysticercus antigen, cross reactions between C.bovis antigen and antibodies against F.hepatica would appear to be a factor in the occurrence of many of the reactions. However animals not infested with F.hepatica gave positive reactions and it is concluded that the saline extract of the cysticercus scolices was unsatisfactory for the demonstration of antibodies in C.bovis infestation.

As the saline extract of scolices proved unsatisfactory for the precipitin test further tests were carried out with the polysaccharide solution isolated from T.saginata. In addition to the bovine sera examined, the sera of 12 sheep, which were infested to varying degrees with F.hepatica, were examined to determine whether the polysaccharide solution caused cross reactions with antibodies against F.hepatica. The results of the tests are presented in Table II.

Table II.

Frequency Distribution of the sera of animals, infested and not infested with C.bovis which gave positive precipitin reactions at different dilutions of the antigen (1-1000 solution of T.saginata polysaccharide).

Dilution of Antigen	State and Degree of Infestation.*						
	Live Cysticerci		Calcified Cysticerci.		Not Infested.		Sheep.
	Liver Fluke	No Liver Fluke	Liver Fluke	No Liver Fluke	Liver Fluke	No Liver Fluke	Liver Fluke
1-1,000	4	1	5	1	4	1	-
1-2,000	3	-	2	-	-	1	-
1-4,000	1	-	-	-	-	-	-
Negative in all Dilutions.	5	2	11	8	13	14	12
Total number of sera in each group	9	3	16	9	17	15	12

Control tests with saline were negative in all cases.

*With the exception of 2 animals, the infestation of C.bovis consisted of one or two cysts. Of the two exceptions one animal showed a multiple infestation with calcified cysticerci in the muscles of the head and heart. The serum from this animal showed a positive (+) reaction with the 1-1,000 dilution of polysaccharide. The other exception showed a heavy infestation of the head, heart and tongue muscles. The serum from this animal gave strong precipitin reactions (+++) with the 1-1,000 and 1-2,000 solution of polysaccharide and a moderate (++) reaction with the 1-4,000 solution of polysaccharide. This serum was used as a positive control serum in all further tests.

Table II indicates that while precipitin reactions occurred with the sera of cattle not showing infestation with C.bovis, the sera of 12 sheep infested with F.hepatica failed to give precipitin reactions. It would appear therefore that antibodies to F.hepatica infestation, in themselves, were not the cause of the reaction in cattle not infested with C.bovis.

Intradermal Test.

The following 32 animals were subjected to intradermal tests with saline extracts of fresh C.bovis bladders. Injections were also made with a saline extract of F.hepatica. The results of the tests are presented in Table III.

Table III.

Frequency Distribution of the skin reactions occurring in cattle subjected to the intradermal injection of 1-100 saline extract of C.bovis.

Parasitic Infestation	Increase in Skin Thickness in millimetres. 4	5	6	7	8	9	10	11	12	13	14	15
C.bovis alone	(1)					1*						
C.bovis and F.hepatica	(1)					1*						
F.hepatica alone.		5 (2)	7 (3)	2 (9)	2 (1)	7 (7)	3 (2)	2 (1)	2 (1)			

The figures in parenthesis denote reactions occurring with a saline extract of F.hepatica which was used in conjunction with the C.bovis extract.

*The animal which had C.bovis infestation alone had one live cyst in the external masseter muscle. The animal which had F.hepatica and C.bovis infestation had one calcified cyst in the heart.

Table III indicates that animals infested with F.hepatica displayed a hypersensitive skin reaction when they are subjected to an intradermal test using an extract of C.bovis as antigen. Similarly an animal infested with C.bovis alone possessed hypersensitivity to a F.hepatica extract.

The degree of reaction to the C.bovis extract in animals infested with F.hepatica is similar to that produced by the homologous antigen.

Discussion.

Precipitin Test.

As indicated in Table I the saline extract of C.bovis scolices was an unsatisfactory preparation for the precipitin test. The sera of sheep which were infested with F.hepatica gave positive precipitin reactions with this antigen, thus indicating that antibodies produced during F.hepatica infestation have a cross reaction with the C.bovis antigen. This cross reaction, however, was probably not the only reason for the marked reactions noted in all the sera examined. Many of the reactions observed were probably non-specific in nature, being analogous to the non-specific reactions noted by Bachman, Rodriguez-Molina and Oliver-Gonzalez (1934) in human sera when tested with extracts of T.spiralis. These non-specific reactions, similar to the reactions occurring in this examination, did not

appear in the saline control tubes, thus being in contrast to the "anomalous" reactions reported by the above authors and in Part II of this examination. Bachman et al (1934) were of the opinion that the administration of quinine induced such non-specific reactions. In this examination no such conclusion could be made, as the history of any therapeutic treatment could not be ascertained.

The use of the polysaccharide component of T.saginata proved much more successful than the saline extract of Cysticercus scolices. The serum of one animal with a heavy infestation of C.bovis gave a moderately strong precipitin reaction with the 1-4000 dilution of polysaccharide solution. Such a strong reaction was not evident in any of the other sera from animals with a lighter infestation.

Animals with only a slight infestation, one or two cysts, frequently did not show precipitin antibodies. Where the parasitic cyst was calcified it is probable that any antibody which had been present had disappeared after calcification. A disappearance of antibody was evident in Hydatid disease when calcification took place (Fairley, 1922). In the cases where the cysticercus was still alive the degree of infestation appeared to be an important factor in the occurrence of demonstrable antibody, since the serum of one animal with a marked

infestation showed a strong precipitin reaction. The invasion of the bovine organism with small numbers of C.bovis may be insufficient to induce a demonstrable precipitin response. That is, there may be a minimal threshold value in numbers of cysticerci below which no serological response is evident. Penfold and Penfold (1937) reported that as few as five cysticerci in the muscles served to give immunity to an animal. It is possible that five cysts may represent a minimal threshold value for the establishment of immunity. All the animals in this examination, with the exception of two, were infested with only 1 or 2 cysts at the predilection sites and so the animals may have been unable to elaborate antibodies demonstrable by the precipitin test.

The exact number of cysticerci in a carcase could not be accurately determined, since this would have entailed slicing the whole of the musculature into portions with a maximum thickness of ¼". This procedure was economically impossible in this examination, and only the predilection sites (masseters, heart and tongue) were examined. Consequently the animals examined may have been infested with a greater number of cysticerci than was evident at the predilection sites. This, also, may be the reason why the sera of animals, which showed no evidence of cysticerci in the sites of

predilection but gave positive precipitin reactions.

Though the sera of sheep, infested with F.hepatica, failed to give precipitin reactions with the polysaccharide of T.saginata, the infestation of cattle with F.hepatica may produce non-specific antibodies which cause a precipitin reaction with unrelated polysaccharides. Thus, Oliver-Gonzalez (1946b) has indicated that the polysaccharides from various helminths, including F.hepatica and T.saginata, showed a close immunological relationship. He suggested that an isoagglutinin-like producing antigen may have been the cause of this cross reactivity. When the isoagglutinin-like portion was checked in the individual sera the precipitin antibodies were still present and reacted specifically with their own homologous polysaccharide.

Graña (1942, 1944) has recorded an increase in isoagglutinins for human erythrocytes (Type A and B) in humans following repeated injections of hydatid material into persons infested with hydatid cysts.

Antigens which cause the production of isoagglutinins for the erythrocytes of man have been demonstrated in the pneumococcus (type XIV) (Levine, Bullova and Katzin, 1939; Finland and Curnen, 1940). The portion of the pneumococcus responsible was the capsular polysaccharide. This has been shown to be similar chemically to the group A erythrocyte

factor polysaccharide (Goebel, Beeson and Hoagland, 1939).

Since this isoagglutinin-producing antigen was present in both F.hepatica and T.saginata, infestation of cattle with F.hepatica might cause an increase in isoagglutinins which would react with the T.saginata polysaccharide in the precipitin test. As is the case with the Forssman antigen, only animals which did not possess this isoagglutinin-producing antigen in their erythrocytes and tissue cells would develop isoagglutinins as the result of F.hepatica infestation.

The definition of the blood groups of cattle is not yet clearly explained. Little (1929a,b,c.d) has indicated that at least three blood groups exist in cattle, being dependent on a single erythrocyte factor. Ferguson (1941, 1947) claims to have identified seven different isoagglutinin-producing antigens in cattle. The influence of the isoagglutinin-producing antigen in F.hepatica in animals with different blood groups may explain the irregular appearance of precipitin reactions to the T.saginata polysaccharide.

Intradermal Tests.

The results of the intradermal test indicated that infestation of animals with F.hepatica influenced the hypersensitivity of the skin to extracts of C.bovis. The homologous antigen in

each infestation elicited the more marked reaction. It was evident that the hypersensitivity demonstrated was of a group nature.

Kellaway (1928) demonstrated that a common anaphylactic substance existed in F.hepatica and T.saginata, the adult parasite of C.bovis. Kellaway found that the uterus of a guinea pig sensitised to T.saginata would react anaphylactically when treated with extracts of F.hepatica.

The isoagglutinin-producing antigen, present in F.hepatica and T.saginata (Oliver-Gonzalez, 1946b) might be responsible for such cross reactions noted in the hypersensitivity tests. This cross reaction was demonstrated in all animals infested with F.hepatica in the intradermal test, but only a percentage of animals infested with F.hepatica showed precipitins in their sera which reacted with the T.saginata polysaccharide. It is more probable that the cross reactions are due to a common allergen which is distinct from the isoagglutinin-producing antigen.

Summary.

Saline extracts of C.bovis scolices proved unsatisfactory for the demonstration of precipitin antibodies in animals infested with the cysticercus.

A polysaccharide solution of the parent parasite, T.saginata, demonstrated precipitin

antibodies in the serum of a heavily infested animal. Many animals infested to only a slight degree with C.bovis failed to show antibodies. It was thought that the number of cysts in these animals was insufficient to establish demonstrable antibodies. Precipitin reactions to the T.saginata polysaccharide occurred in animals infested with F.hepatica, C.bovis infestation being absent. An isoagglutinin-like antigen in both of the parasites was thought to be the cause of this cross reaction.

Skin hypersensitivity to a saline extract of C.bovis occurred in animals infested with F.hepatica, thus indicating a group hypersensitive reaction between the two parasites.

Part IV.

Ascaris lumbricoides.

Materials and Methods.

Precipitin Reaction.

Precipitin tests and cutaneous tests were used by Coventry and Taliaferro (1928) for the determination of skin hypersensitivity and precipitin antibody production to A.lumbricoides infestation in Hondurean patients. The preparation used in the tests was a saline extract of the dried and pulverised parasites. Positive results were obtained in 80% and 61% of cases with the cutaneous and precipitin tests respectively. These reactions however could not be correlated with the presence of Ascaris eggs in the stools. Coventry (1929), using a saline extract of Ascaris, was able to detect precipitin antibodies in the sera of rabbits and guinea pigs infested or immunised with A.lumbricoides.

Campbell (1936b) described the preparation of a purified polysaccharide component of A.lumbricoides which reacted specifically with anti sera artificially produced against the whole worm. Later Campbell (1937) improved the method of preparation of the polysaccharide and reported that this new preparation was highly specific being capable of differentiating, in the precipitin reaction, between antisera produced by the pig Ascaris and the human Ascaris worms.

Canning (1929) demonstrated that antibodies against the various individual tissues of A.lumbricoides possessed a partial specificity of their own, while Oliver-Gonzalez (1946a) has reported that many antibodies are present in Ascaris infested animals. These antibodies reacted specifically with the various antigenic portions of the Ascaris worm mentioned by Canning (1929). With the exception of the antibody produced by the egg tissue the antibodies possessed no functional (antiparasitic) value. Though the anti-egg serum proved to be the only functional antibody it was the anti-intestine serum which displayed most species specificity. Oliver-Gonzalez further demonstrated that polysaccharide preparations of the Ascaris worm could be used to detect antibodies in the sera of animals infested with Ascaris worms. Since Campbell (1936b; 1937) demonstrated that such polysaccharide portions were extremely specific it was decided to use that component of the Ascaris worm for the precipitin tests in the present work.

The preparation of the polysaccharide was carried out according to the method described by Campbell (1937), as follows:

Mature A.lumbricoides worms were obtained fresh from the intestines of pigs which had newly been slaughtered. The worms were washed well in

running water and were then frozen at -10°C until they were hard and brittle. The worms were then ground in a mortar until a fine pulp was formed. Fifty grams of this pulp were suspended in one litre distilled water and heated for thirty minutes in a boiling water bath. The suspension was then vigorously stirred until cool and was then acidulated to approximately pH 4.5 with glacial acetic acid. Twenty cubic centimetres of lead acetate (20%) were added and the mixture placed in the refrigerator overnight, after which the insoluble material was centrifuged out and discarded. The remaining solution was added to 1.2 volumes of 95% ethyl alcohol. The resulting precipitate, which consisted chiefly of carbohydrate was removed by centrifuging and was dissolved in 0.1% NaCl solution. This solution was centrifuged for 45 minutes to remove any insoluble material. Further purification was carried out by fractional precipitation with 95% ethyl alcohol. The alcohol was added until a faint but definite precipitate was formed. This precipitate was discarded because, although it consisted chiefly of carbohydrate Campbell (1937) has stated that this portion was frequently biuret positive. The remaining solution from this partial precipitation was then precipitated by the addition of one volume of 95% ethyl alcohol. The resulting precipitate was collected and redissolved in 0.1%

saline. The solution was centrifuged to remove any insoluble material. This procedure was repeated until a sample of the resulting solution showed no evidence of precipitation when the pH was lowered to approximately 3.0 with a solution of 10% phosphotungstic acid and 3% sulphuric acid. The final solution was precipitated with an equal volume of alcohol and collected by centrifuge. The precipitate was washed with distilled water and was then dried and weighed. The yield of polysaccharide from 50 grams of fresh Ascaris was 70 milligrams. The polysaccharide was then dissolved in saline to give a concentration of 1 part polysaccharide to 1000 parts saline. This solution was then centrifuged and stored in the frozen state at -10°C. For the precipitin test the polysaccharide solution was diluted in saline by two doubling dilutions to give three dilutions of 1:1000, 1:2000 and 1:4000.

Blood was obtained from pigs as they were slaughtered. The sera were allowed to separate off from the blood overnight and were centrifuged for 30 minutes before use in order to produce clear products.

The precipitin test was carried out in narrow tubes (5 x 32 m.m.). The various antigenic dilutions were carefully layered over the samples of sera so that a clear line of demarcation was produced.

The tubes were then incubated at constant room temperature for one hour and they were then examined. A positive reaction consisted of a fine ring or plane of precipitate at the interface of the two fluids. The degree of reaction was estimated from the density of the ring of precipitate and designated as follows:-

?	doubtful
+	slight precipitation
++	moderate precipitation
+++	strong precipitation

A control tube consisting of serum and saline was made up for each set of precipitin tests. In addition a positive serum was tested with each set of sera in order to determine the effectiveness of the antigen.

Intradermal Test.

Wilkening (1937) carried out skin tests on pigs for the determination of hypersensitivity to A.lumbricoides infestation. The preparation used for the test was the saline extract of the dried and powdered uterus and intestine of the Ascaris worm. The results of the examination indicated that there was no correlation between the skin reactions and helminth infestation as determined by faeces examination.

Coventry and Taliaferro (1928) obtained similar results with the skin test when they used

a saline extract of Ascaris to determine hypersensitivity to Ascaris in Hondurean patients.

Canning (1929) has demonstrated that the Ascaris intestinal tissue is the most species specific tissue of the worm. Oliver-Gonzalez (1946a) showed that anaphylactic reactions could be provoked in guinea pigs, sensitised to Ascaris, by means of the coelomic fluid and saline extracts of the intestine of the Ascaris worm. No anaphylactic reactions were noted with the saline extracts of other tissues of the worm (cuticle, muscle, egg and sperm tissues). In this examination it was decided to utilise the Ascaris intestine for intradermal hypersensitivity tests and a saline extract was prepared as follows:-

The gut of the Ascaris worm was removed from both male and female worms of the species and as much as possible of the intestinal contents removed by gently squeezing the gut on a tile with a glass rod. It was then washed in several changes of water and subsequently dried for two days at 37°C. The dried material was reduced to a powder in a mortar and the powder extracted with saline in the proportion of 1 gram of powder to 100cc of saline. Extraction was carried out for 10 days at 4°C the mixture being shaken at frequent intervals. After extraction the suspension was centrifuged and the insoluble material discarded. The clear remaining

fluid was recovered and phenol was added to give a concentration of 0.5%. The material was placed in 1cc bottles fitted with a rubber stopper and stored in the refrigerator at -10°C.

Two control solutions were used, one of 0.5% phenol in saline and one of a 1% peptone solution with 0.5% phenol added. These were stored in the same way as the saline extract of the Ascaris intestine.

The injections were made with a "Record" type dental syringe with a graduated plunger shaft so that a regulated amount of fluid could be delivered. After thorough cleansing of the site, 0.1cc of the specific antigen was injected intradermally into the loose fold of skin at the base of the left ear, the right ear being similarly used for the control solution.

The reaction was observed at 15 and 45 minutes and again at 18 hours after the injection. Measurements were made by means of callipers graduated in millimetres.

Reactions.

The immediate response to the injection of both solutions (test antigen and control solution) was the production of a wheal 3-4mm in diameter. Within a few seconds this had increased in area and become somewhat flatter; in addition a zone of erythema had begun to develop, increasing the

diameter of the affected area to 6-8mm. In the case of the non-specific solution this reaction disappeared within 10 minutes. When the pig showed hypersensitivity to the Ascaris extract the early reaction with the specific antigen was usually replaced within 45 minutes by an area of discoloration varying from light red to deep purple. At the point of the injection the skin sometimes became black. This abnormal colour, which varied in diameter, usually disappeared within 6 hours, but where the reaction was strong discoloration was still obvious at 18 hours.

In a few cases an atypical reaction developed in which the original wheal increased in size, being surrounded by a zone of erythema. This increase in wheal area was maintained for approximately one hour.

Where no hypersensitivity to Ascaris was displayed the specific antigen produced only a transitory wheal with a small area of erythema.

In a few cases no reaction to the specific antigen was apparent at one hour but the reaction followed several hours later, being a "delayed type" of reaction. In this case a reaction was evident at 18 hours consisting of central dark red or purple point surrounded by a wide zone of erythema. The colour of this erythema varied from light red to light purple.

Initially intradermal tests were carried out with a 1-1000 dilution of the Ascaris extract. However this dilution was not so satisfactory as the original 1-100 dilution in producing the skin reactions and consequently all further work was carried out with the 1-100 saline extract of Ascaris intestine.

As Fülleborn (1929) has reported that the substance responsible for the skin reactions in hookworm and Ascaris infestations is heat stable, a portion of the 1-100 Ascaris antigen was heated for 30 minutes at 100°C in a water bath. This heated portion was used for the intradermal test, in conjunction with the unheated extract, on 25 pigs.

In addition to the control injections of phenolised saline and peptone solution a series of intradermal injections were carried out with a 1-1000 solution of Ascaris polysaccharide as prepared for the precipitin test. The polysaccharide was used in order to determine its ability to produce skin reactions.

Intradermal tests were also carried out with a 1-100 saline extract of F.hepatica, as prepared for the intradermal tests in F.hepatica infestation in cattle. The F.hepatica extract was used to determine the relative specificity of the reactions occurring with the Ascaris extract.

Post Mortem Examination.

The pigs which had been subjected to the intradermal test were numbered during the test and subsequently were slaughtered separately. The carcase and viscera of these animals and of those from which blood had been taken for the precipitin test were examined.

The liver of each pig was examined for signs of chronic focal interstitial hepatitis (white spot) which Oldham and White (1944) ascribe to the migration of the larvae of A.lumbriccides in the liver. The lungs were palpated and incised to detect Metastrongylus spp. infestation. The small intestine of each pig was palpated throughout its length to detect the adult A.lumbricoides. A rectal sample of faeces was taken from each pig and examined by the smear method for Ascaris eggs and the eggs of other bowel helminths.

The rest of the viscera and the carcase was examined for other helminth and bacterial diseases. The age of the animal was estimated from an examination of the canine and incisor teeth. The state of nutrition was noted.

Table I.

Frequency Distribution of reactions occurring at different dilutions of Ascaris polysaccharide solution with the sera of pigs infested to varying degrees with A.lumbricoides. (93 animals examined).

	Degree of Chronic Focal Interstitial Hepatitis.															
Number of Animals in each group	Slight. 60				Moderate 19				Marked 11				No evidence of infestation 3			
Type of Reaction	?	+	++	+++	?	+	++	+++	?	+	++	+++	?	+	++	+++
Dilution of antigen 1-1,000	1	2	3 (3)	4 (2)	-	3 3"	3	3 (1)	-	3 (1)	1	4 (1)				
1-2,000	2 (2)		1 (1)			1	2		1		1	1				
1-4,000										1 (1)						
Negative in all Dilutions			50 (12)	2* 6"			10	1* 2"			3				3	

The figures in parenthesis indicate the animals which had mature A.lumbricoides in the intestine.

*Pigs which showed M.apri infestation of the lungs.

" Denotes pigs which showed eggs of Oesophagostomum in the faeces.

Table II.

Percentage of sera from animals with different degrees of chronic focal interstitial hepatitis which gave positive precipitin reactions.

	Chronic Focal Interstitial Hepatitis		
	Slight.	Moderate	Marked.
Percentage.	16.6%	47.36%	72.72%
Number of Animals Examined.	60	19	11

Tables I and II indicate that the sera of animals which showed chronic focal interstitial hepatitis only occasionally showed the presence of precipitin antibodies. The presence of adult A.lumbricoides worms in the intestine did not appear to influence the reactions. The percentage of sera showing positive precipitin reactions tended to rise as the degree of interstitial hepatitis increased.

Other helminth infestations (M.apri and Oesophagostomum dentatum) which were present in a few animals did not appear to influence the reactions.

Table III.

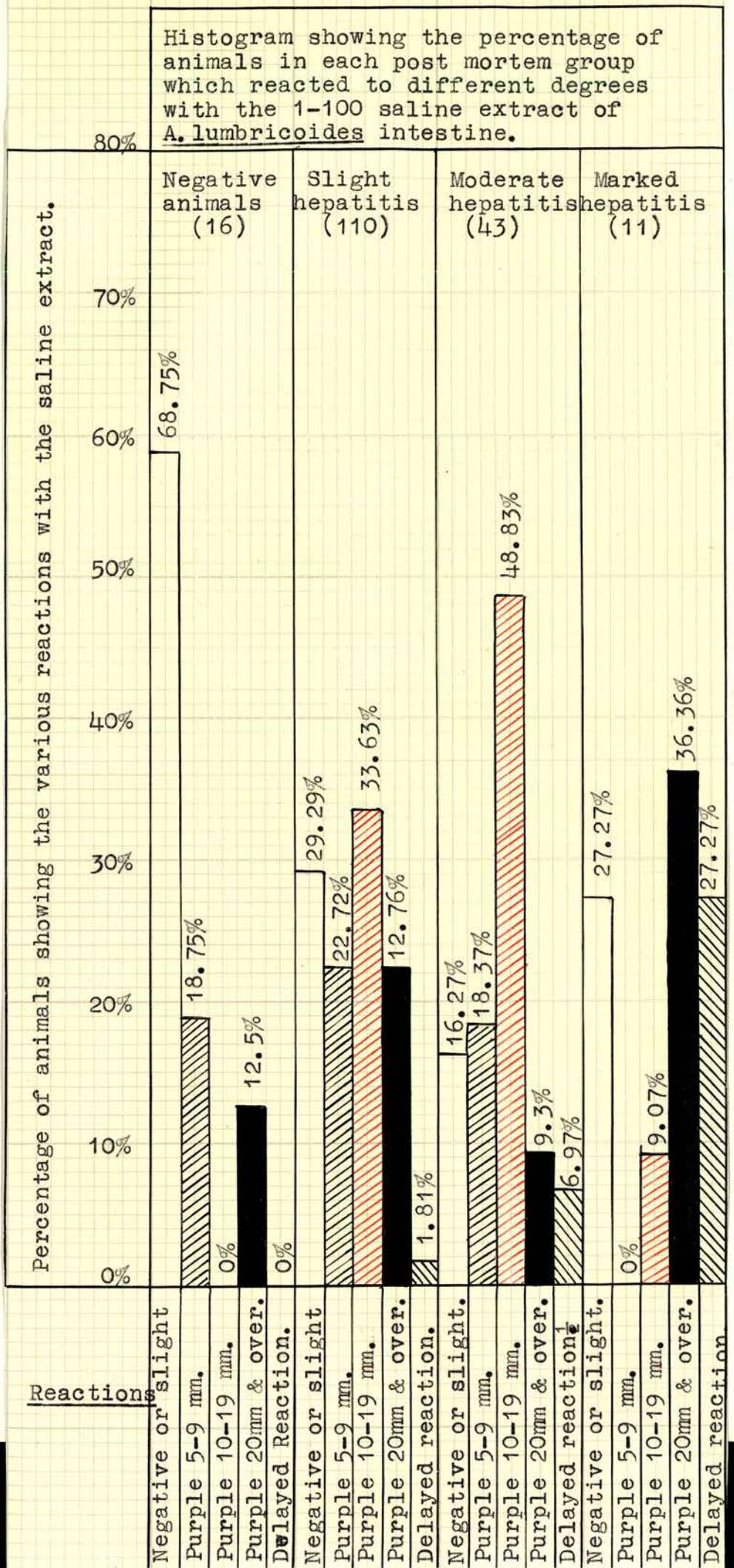
Frequency Distribution of reactions occurring in pigs to the intradermal injection of 1-100 saline extract of A.lumbricoides intestine (180 pigs examined).

	Post Mortem Findings. Chronic Focal Interstitial Hepatitis.			No Evidence of Infestation.
	Slight.	Moderate.	Marked.	
Type of Reaction.				
Negative or very Slight.	32 (9) 13* 4"	7 (2)	3 (1)	11 2*
Purple area or wheal 5-9mm.	25 (3)	8 (1) 3*	-	3
Purple area or wheal 10-19mm.	37 (14)	21 (9)	1 (1)	-
Purple area or wheal 20mm & over.	14 (2)	4 (1)	4 1*	2
Delayed Reaction. Purple area 10mm and over.	2	3	3	
Total.	110 (28)	43 (13)	11 (2)	16

The figures in parenthesis indicate the animals which had mature A.lumbricoides in the intestine.

" Denotes pigs which were infested with M.apri.
* Denotes pigs which showed Oesophagostomum eggs in their faeces.

Histogram showing the percentage of animals in each post mortem group which reacted to different degrees with the 1-100 saline extract of A. lumbricoides intestine.



The heated saline extract was used in conjunction with the unheated extract of A.lumbricoides intestine. The reactions occurring with the heated extract were identical with those produced by the unheated extract. Consequently the heating of the saline extract at 100°C for 30 minutes did not reduce in any way its ability to induce hypersensitive reactions.

In order to determine the relative specificity of the reaction produced by the A.lumbricoides extract, the following 57 animals were subjected to the simultaneous intradermal injection at different sites of Ascaris extract and a 1-100 saline extract of F.hepatica (as used for the intradermal test in F.hepatica infestation).

Table IV.

Comparison of the Reactions occurring in pigs following the intradermal injection of:-

a. Saline extract of A.lumbricoides intestine
b. Saline extract of F.hepatica.

	Antigen. a.	b.
Negative or Slight Reaction.	11	44
Purple area or wheal 5-9mm.	8	10
Purple area or wheal 10-19mm.	20	3
Purple area or wheal 20mm and over.	18	-
Total.	57	57

None of the above pigs was infested with F.hepatica.

The results presented in Table IV indicate that, with the exception of 3 animals, thirty eight pigs which gave marked hypersensitive reactions to the Ascaris extract failed to show such reactions to the intradermal injection of F.hepatica.

In order to compare the ability of the protein and polysaccharide components of A.lumbricoides to produce hypersensitive skin reactions in pigs the following eleven pigs were subjected to the simultaneous intradermal injection of these two components. The resulting reactions are presented in the following table V.

Table V.

Comparison of the reactions occurring in pigs following the intradermal injection of:-

a. 1-100 saline extract of A.lumbricoides intestine.

b. 1-1000 solution in saline of A.lumbricoides polysaccharide.

	Antigen.	
Reactions.	a.	b.
Negative or Slight Reaction.	1	6
Purple area or Wheal 5-9mm.	-	1
Purple area or Wheal 10-19mm.	5	3
Purple area or Wheal 20mm & over.	5	1
Total.	11	11

Table V indicates that the polysaccharide component of A.lumbricoides is much less satisfactory than the saline extract in eliciting hypersensitive reactions.

Discussion.

Precipitin Reaction.

The results of the precipitin test in pigs indicate that of 93 animals examined only 27 animals showed precipitin antibodies in their sera. The occurrence of these antibodies was independent of the presence of mature A.lumbricoides in the intestine. This finding was in agreement with that of Coventry and Taliaferro (1928) who found that precipitin reactions occurring with the sera of Hondurean natives were independent of the presence of Ascaris eggs in the stools.

As Coventry and Taliaferro (1928) were limited to stool examination for the determination of infestation by Ascaris no correlation could be attempted between the serological findings and post mortem evidence of Ascaris larval migration. In this examination it was possible to determine the degree of chronic focal interstitial hepatitis, which Oldham and White (1944) ascribe to the migration of Ascaris larvae in the liver. It was found that as the degree of interstitial hepatitis increased the percentage of animals which showed precipitin antibodies in their sera also increased.

It appears probable therefore that the migration of the Ascaris larvae through the host, prior to their establishment as adults in the intestine, was largely, if not wholly, responsible for the productions of antibodies.

Antibody formation to A.lumbricoides infestation has been demonstrated in experimental animals in which the adult parasite does not become established. Thus Coventry (1929) reported that precipitin antibodies can be demonstrated in rabbits and guinea pigs infested with Ascaris. The precipitin content of the sera varied greatly from time to time, but precipitins were observed to persist for at least 8 months in rabbits.

Blackie (1931) has demonstrated, by means of the complement fixation test, that rabbits infested with Ascaris megalocephala showed a definite immune response during the migratory stage of the parasite. This response faded after the migratory phase had been completed.

Oliver-González (1943) found that the sera of rabbits infested with Ascaris caused circumlarval precipitates around the larvae of Ascaris when these were incubated in sera from infested animals. This circumlarval precipitate reached its maximum on eighteenth to twenty first day after infestation (i.e. when the larvae were undergoing migration). Some evidence of the precipitate was demonstrable in

a few cases on the one hundred and twenty sixth day after infestation.

In another study Oliver-González (1946a) demonstrated that the precipitin reaction occurring during the infestation of rabbits with Ascaris showed an increase in titre after the seventh day. The titre reached its highest point on the thirty fifth day, thereafter falling to a low titre on the forty second day after infestation. During reinfestation the titre again rose but later fell.

The infestation of experimental animals (rabbits and guinea pigs) with A.lumbricoides results in the migration of the larvae through the tissues of the animal but no adult parasites become established in the intestine. Consequently, as such animals produce antibodies during Ascaris infestation, the presence of the adult parasite is not necessary for the production of antibodies.

The results of this examination also indicated that the presence of precipitin antibodies was independent of mature parasites but that the degree of chronic focal interstitial hepatitis bore a relationship to the antibody production.

The antibody will tend to vary quantitatively according to the degree of infestation and migration. The persistence of this antibody so produced will be influenced by the period of time which has elapsed since it was formed. The persistence of the

antibody in naturally infested animals may however be influenced by the natural reinfestation of the animal. This reinfestation will have the effect of increasing the antibody level and adding to the duration of the antibody in the blood. Such an effect was demonstrated by Oliver-González (1946a) when rabbits were reinfested with Ascaris.

A slight quantitative difference in antibody was noted in this examination, since animals markedly affected with interstitial hepatitis showed a greater degree of strong precipitin reactions. The majority of the animals in the examination were between 9 and 12 months of age. The age at which infestation occurred was unknown; consequently the time which antibodies persisted in the blood could not be determined. However two animals which showed precipitin antibodies in their sera were aged 1½ years. Both pigs which showed marked interstitial hepatitis. The persistence of antibody in these two animals may have been due to a strong initial antigenic stimulus or to the stimulating effect of reinfestation.

The Intradermal Test.

The results of the examination indicate that the majority of the pigs examined showed evidence of skin hypersensitivity to A.lumbricoides. Only approximately 50% however showed a marked reaction (a purple area or wheal 10mm or greater in

diameter).

As was the case with the precipitin reaction the presence of mature A.lumbricoides in the intestine did not appear to influence the reactions. Wilkening (1937), who used a skin reaction to detect hypersensitivity to Ascaris in pigs, found that there was no correlation between skin reactions and the presence of Ascaris eggs in the faeces. Similar results were reported in humans by Coventry and Taliaferro (1928).

When the post mortem finding of chronic focal interstitial hepatitis was considered with the hypersensitive reactions it was evident that, within limits, animals with a more marked interstitial hepatitis possessed a greater degree of hypersensitivity.

Thus it appeared that the migration of the Ascaris larvae was responsible for the development of hypersensitivity. The presence of the adult Ascaris in the intestines did not appear to produce or maintain a skin hypersensitivity. Laurell (1927) has postulated that the migration of Ascaris larvae in the tissues of the host rendered the host hypersensitive. Hypersensitivity has been shown to occur in Ascaris infested animals where the adult worm did not become established. Thus Coventry (1929) demonstrated that rabbits and guinea pigs infested with Ascaris showed cutaneous reactions

(delayed type) following the intracutaneous injection of Ascaris extracts. The reactions varied in intensity but persisted for months after the disappearance of the parasites from the infested animals. Kerr (1938b) determined that guinea pigs which were subjected to a reinfestation with Ascaris showed marked cellular reactions in the liver and lungs. These reactions were attributed to a hypersensitivity phenomenon. They were not evident in animals undergoing initial infestation. Oliver-González (1946a) has reported that anaphylactic reactions were present in guinea pigs sensitised to A.lumbricoides, either by infestation or passive sensitisation, when injected with the coelomic fluids or extracts of the intestine of the Ascaris worm.

The persistence of hypersensitivity in Ascaris infestation appears to vary a great deal. Fülleborn (1926) has stated that sensitivity may persist for 4 years after loss of the adult parasite. Maternowska (1938) states that the skin reaction in Ascaris infestation may persist for 4 to 16 months after loss of the parasites. As the majority of the pigs in this examination were between 9 and 12 months of age it was not possible to determine how long hypersensitivity could persist.

The hypersensitive state will tend to vary quantitatively with the degree of the infestation

which induced it. This was demonstrated to a certain extent in this examination in that pigs with a greater degree of focal hepatitis showed a higher percentage of marked hypersensitive reactions.

Animals which had undergone only a light infestation of A.lumbricoides may have lost after a short time, any hypersensitivity which they might have developed. Taliaferro and Sarles (1939), who studied the humoural and cellular factors resulting from Nippostrongylus muris infestation, mentioned that the immunity expressed by various tissues was dependent on the degree of infestation. Thus the intestine was the first to become immune, followed by the lungs, and finally the skin became involved. This phenomenon of the production of generalised immunity in definite stages or steps might be evident in A.lumbricoides infestation. It is known that a hypersensitive type of reaction occurred in the liver and lungs of guinea pigs following reinfestation with Ascaris (Kerr 1938b). It is possible that in A.lumbricoides infestation sensitisation of the skin follows sensitisation of the liver and lungs. Where an animal has only suffered a light infestation skin sensitisation may be absent though the liver and lungs are sensitised to a varying degree.

It is interesting to note that in eight animals an immediate type of reaction was missing

but was replaced by a delayed (24 hours) type of reaction. Such delayed skin reactions have been reported in humans (Spink 1937). Spink demonstrated that in the early stages of Trichinella infestation the skin reaction was of the delayed type, while from the second week onwards the reaction became immediate in nature. Similar reports concerning the development of the skin reaction in T.spiralis infestation have been made by Maternowska (1933), MacNaught, Beard and Myres (1941) and Spaeth (1942).

Jones and Mote (1934) and Mote and Jones (1936) sensitised humans to the peritoneal fluid of rabbits. They noted that the skin sensitivity, as demonstrated by intradermal tests, was first evident as a delayed, 24 hour, reaction. Later this delayed reaction was replaced by an immediate type of reaction. The skin sensitivity was lost in the reverse order. The immediate type of reaction was replaced by the delayed reaction, this latter being finally lost. Dienes (1936) showed a similar effect when guinea pigs were immunised with white of egg or horse serum.

Franck (1940) has reported that a delayed type of skin reaction was evident in Ascaris infestation in humans. This delayed type of reaction was manifest when immature males or females alone were present, that is during the early stages of infestation. Coventry (1929) reported a delayed

type of reaction in rabbits and guinea pigs infested with Ascaris.

It seems probable, therefore, that skin hypersensitivity becomes established in a similar manner to that in T.spiralis, being first delayed in character and then becoming immediate.

The eight animals, in this examination, which demonstrated a delayed type of skin reaction, were possibly in the latter stages of hypersensitivity when the immediate reaction was replaced by the delayed type prior to the total disappearance of hypersensitivity. Maternowska (1938), however, has reported that the early (immediate) type of reaction in Ascaris and other infestations of bowel parasites may, after a period of time, become two phase (this being characterised by an immediate reaction which is followed in 18 to 24 hours by a delayed reaction). She postulated that this was due to the resorption of protein material from the helminths in the bowel.

In the eight cases mentioned a marked early reaction was not evident, merely the transitory erythema as noted in the control solutions. However it is possible that the migration of newly acquired larvae would provide the protein resorption required for the development of this reaction, if Maternowska's theory is accepted.

Reactions with the heated antigen were

identical with those induced by the unheated Ascaris antigen. This was indicative that the component of the worm responsible for the hypersensitive reactions was heat stable. This was in accordance with the views of Fülleborn (1929).

Reactions produced by the purified polysaccharide component of A.lumbricoides were not comparable with those evident at the site of the saline extract. Consequently the polysaccharide component of Ascaris was not satisfactory for eliciting hypersensitive reactions. Pirosky, Pirosky and Casiraghi (1942) demonstrated that polysaccharide preparations of hydatid material produced poor antigens for the skin test in hydatid disease, while protein preparations proved the most satisfactory antigens.

Reactions occurring to the intradermal injection of a saline extract of F.hepatica were, in the majority of animals, either of a negative or very slight character. A few animals (3) showed a marked reaction to the F.hepatica extract. These three reactions occurred in animals which gave a very marked reaction to the Ascaris extract (purple areas 30 x 40mm; 38 x 40mm and 20 x 12mm). It is possible that a phenomenon comparable to that reported by Lewis and Loomis (1928) may be responsible for this reaction with the F.hepatica extract. Lewis and Loomis reported an increase in reactivity

of the skin to non-specific irritants in animals which possessed a marked degree of skin sensitivity to tuberculoprotein.

Eisenbrandt (1936, 1938), who utilised serological tests for the determination of taxonomic relationships of various helminths, reported that A.lumbricoides var.suum and T.pisiformis possessed a relationship of 0.03% (when the titres of the precipitin tests were expressed as a percentage of the homologous titres). The relationship between T.pisiformis and F.hepatica was 0.65%. This is indicative that F.hepatica possesses very little serological similarity to A.lumbricoides.

Infestation of pigs with M.apri and Oesophagostomum spp. did not appear to influence the development of hypersensitive reactions. Fifteen of 21 pigs infested with O.dentatum gave no reaction to the skin test. Four pigs infested with M.apri also failed to react.

Summary.

Precipitin antibodies were present in the sera of only a few pigs. These antibodies occurred independent of the presence of mature worms in the intestine.

The sera of animals which showed marked chronic focal interstitial hepatitis showed a greater percentage of positive precipitin reactions than the sera of animals having a lighter degree of

focal hepatitis.

The majority of pigs examined possessed skin hypersensitivity to A.lumbricoides. This was more marked in the groups of animals which were affected with a moderate or marked degree of chronic focal interstitial hepatitis than in the groups which had a lighter or no degree of focal hepatitis.

Skin hypersensitivity was independent of the presence of adult worms in the intestine.

A polysaccharide component of A.lumbricoides, which was used for the precipitin tests, proved unserviceable for the demonstration of hypersensitive reactions.

Delayed reactions were evident in some animals and it was thought that these indicated the process of skin sensitivity being lost.

Extracts of F.hepatica generally produced no hypersensitive reactions in pigs.

Part V.

Dictyocaulus viviparus.

Materials and Methods.

During this examination it was hoped to carry out serological tests (Complement fixation and Precipitin tests) on the sera of animals clinically and sub clinically infested with Dictyocaulus viviparus. However, such animals did not become available and consequently the work was confined to a study of the hypersensitivity produced by D.viviparus infestation.

The Intradermal Test.

Hudson (1951) utilised a skin test and a complement fixation test in cattle clinically infested with D.viviparus. These tests met with limited success, the period during which the animals reacted being very limited. Older animals which were infested with the parasite appeared to be more likely to react to the tests than animals undergoing their first infestation. The preparation used for the tests was a partially purified polysaccharide prepared from defatted dried worms.

Allergic phenomena have been noted in Trichostrongyloidea (Haemonchus contortus) infestation in sheep by Stoll and Nelson (1930) and Kholoshcanov (1950). These authors utilised saline extracts of the parasite for the skin tests.

As this work was initiated before the

report by Hudson was available the preparation used for the intradermal test was a saline extract of the dried and powdered parasites, prepared as follows:

Mature D.viviparus worms were obtained from the bronchi and bronchioles of an infested animal after death. The worms were washed well in running water and were then placed in a sterile petri dish and then dried at 37°C for 48 hours. The dried material was reduced to a powder in a mortar. The powder was then extracted with saline in the proportion of 1 gram of powdered worms to 100cc of saline. Extraction was carried out for 10 days at 4°C, the mixture being frequently shaken. Subsequently the suspension was filtered and centrifuged and the insoluble material discarded. The supernatant fluid was passed through a "Pyrex" microfilter and placed in 1cc bottles fitted with a rubber stopper. These bottles were stored in the refrigerator at -10°C.

A similar extract was prepared from D.filaria, the lung worm of sheep. This extract was used for intradermal tests in sheep and in some instances in cattle.

In order to determine whether the hypersensitive skin reactions occurring in cattle were specific and independent of sensitisation produced by infestation of stomach helminths, saline extracts of the common stomach parasites were

prepared as follows and used for intradermal tests in cattle. Members, male and female, of the species H.contortus, Trichostrongylus axei and Ostertagia ostertagi were collected from the abomasum of sheep and cattle. The helminths were separated from the stomach contents by means of sieves and by removal of the worms by hand from a suspension of the stomach contents. The worms thus isolated were washed well in running water and were then dried on filter paper. After weighing the worms were ground to a fine emulsion in a mortar, sterile glass wool being added to assist pulverisation. Sterile saline was then added to the emulsion in the proportion of 100cc of saline to 1 gram of the fresh worms. The diluted emulsion was then centrifuged (3,000 r.p.m. for 30 minutes) and the supernatant fluid was passed through a "Pyrex" microfilter and placed in 1cc bottles fitted with a rubber stopper. The bottles were stored in a refrigerator at -10°C.

A 1% solution of peptone was used as a control solution in all tests to ascertain the degree of skin sensitivity, if any, to simple trauma or the presence of a foreign substance in the tissues.

The intradermal injections were made with a "Record" type dental syringe fitted with a graduated plunger shaft so that a regulated amount of fluid could be delivered. Separate syringes were

used for the different antigen solutions and the syringes were cleaned and sterilised after use daily.

Injections of the antigenic material were made intradermally, in 0.1cc amounts, at the selected sites. These sites were the same as those selected for the intradermal tests in F.hepatica infestation; namely the inside of the right hind leg in sheep and the middle third of the neck in cattle. The method of injection was the same as for the intradermal tests in F.hepatica infestation.

Reactions.

The reactions occurring with the intradermal injections of D.viviparus and D.filaria extracts were essentially the same as those occurring with the F.hepatica extract. In sheep the reaction consisted of an elevated wheal surrounded by a zone of erythema which varied in diameter. The reaction in sheep was measured one hour after the injection of the fluid.

In cattle an oedematous infiltration of the skin developed at the site of injection after the initial wheal formation. This reaction reached its maximum in 4 hours, being maintained for a further 2 to 4 hours, but by 24 hours the reaction had disappeared. The site of the reaction at 24 hours was marked only by a slight thickness of the skin. The reaction in cattle was observed four hours after the injection of the extract, the fold of skin at

the site being measured by callipers.

Histological examination of the skin reaction was identical with that described for the intradermal reaction in F.hepatica infestation. This consisted of a marked perivascular infiltration of eosinophilic polymorphonuclear leucocytes together with a marked oedema of the dermis. This infiltration of eosinophils was noticeable as early as 20 minutes after injection of the antigenic material. Both reactions therefore appear to be hypersensitive reactions of the anaphylactic type.

The reactions with the peptone solution were similar to those recorded for the peptone control solution used in the intradermal tests in F.hepatica infestation. These were of a very insignificant nature.

Post Mortem Examination.

All animals were numbered before the intradermal injections were made. After slaughter the viscera and carcase of each animal were examined. The lungs were examined microscopically for D.viviparus infestation or in the case of sheep for D.filaria infestation. The main bronchi and bronchioles were incised and examined for adult worms. The surface of the lungs was examined for the presence of small wedge shaped areas of consolidation, especially along the diaphragmatic border. Such areas are frequently associated with

Dictyocaulus infestation.

Where no evidence of mature parasites was found a portion of the lung tissue was removed and together with the mediastinal and several mesenteric lymph glands was minced through a household mincer. The larvae of the lung worms were collected by the Baermann technique as follows: A glass funnel closed at the bottom by clamped rubber tubing was fixed in a metal water jacket. A circular piece of wire gauze was placed in the funnel and on it a piece of fine muslin. The minced lung tissue and lymph glands were spread out on the surface of the gauze and then water at 40°C was poured down the side of the funnel until the lung tissue floated. The whole apparatus was kept at 40°C for 4 hours. After this time a sample of the fluid was drawn off at the clamped tubing and examined for evidence of larvae.

In a number of animals (20 cattle and 30 sheep) an examination was made to determine the presence of stomach parasites. In these animals the abomasum was opened and the contents removed. The surface of the mucous membrane was scraped with a knife and the scrapings added to the stomach contents. The contents thus obtained were mixed with water to give a fluid consistency and they were then passed through a sieve (mesh 3mm) to remove coarse particles. The resulting finer suspension

was then passed through a finer sieve (mesh 1.5mm). Samples of the sieved suspension were placed in a large, flat, shallow dish, with a matt black surface; the suspension was allowed to settle and it was then examined for the presence of stomach worms. The sieve was rewashed in a dish of water and the washings examined for parasites. Worms collected by this method were identified under the microscope.

A note was made of other parasitic and bacterial diseases present in the organs and carcase. The state of nutrition of the animal was noted. The age of the animal was ascertained by an examination of the incisor teeth.

Results.

Table I.

Frequency Distribution of animals (cattle) showing the following increase in skin thickness (in mm's) after the intradermal injection of 0.1cc of 1-100 saline extract of D.viviparus. (134 animals examined).

Skin Increase in m.m.'s.	Age Group (In Years). 0-1½	1½-2½	2½-3½	3½-4½	4½-5½	5½ & Over.
1-2	1	1		1	3	1
3	5	2	2		1	
4		5	1	1	5	1
5	1	1	4	1	3	3
6	1	2	6	3	4	2
7		2	4		6	

Table I. - Continued.

Skin Increase. in m.m.'s	Age Group (In Years). 0-1½	1½-2½	2½-3½	3½-4½	4½-5½	5½ & Over
8		3	4		2	2
9		1	3		2	
10		1	3		7	
11			2	1	3	2
12		3	3	1	2	1
13			2			2
14		1	2	1		2
15 & over.		2	2	1		
Total.	8	24	38	10	38	16

The most frequently occurring observation is the increase in skin thickness of 6mm (Modal Value).

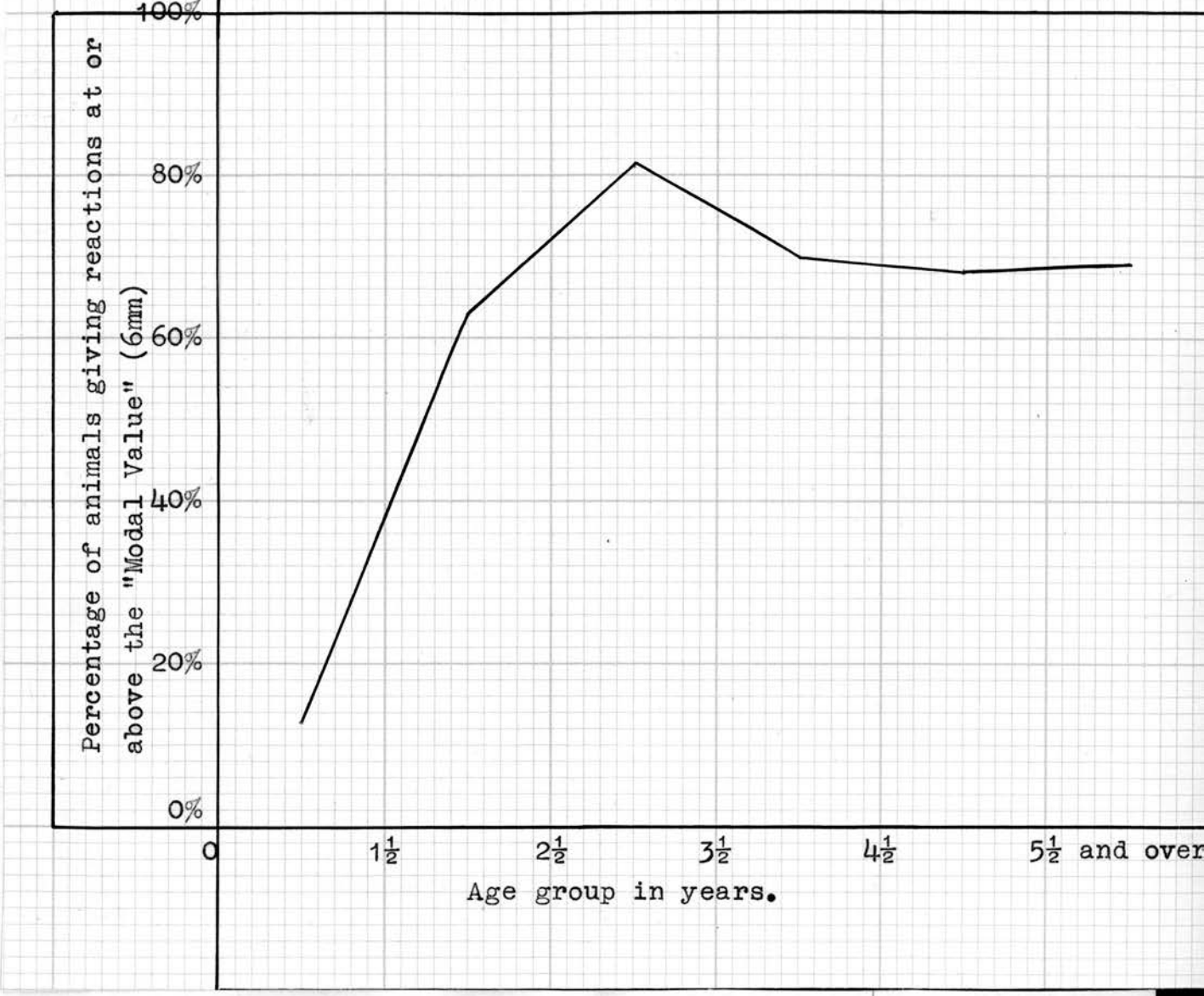
Table II.

Table showing the percentages of animals in the various age groups giving reactions at or above the Modal Value (6mm).

Age Group (In Years)					
0-1½	1½-2½	2½-3½	3½-4½	4½-5½	5½ and over.
12.5%	62.5%	81.57%	70.0%	68.5%	68.8%
(8)	(24)	(38)	(10)	(38)	(16)

Figures in parenthesis denote the number of animals examined in each age group.

Polygon comparing the percentage of animals in the different age groups which gave reactions at or above the "Modal Value" (6mm).



In Table I only two cattle showed evidence of infestation with D.viviparus, larvae being isolated from the lungs by means of the Baermann technique. The increase in skin thickness in these two animals was 10mm and 14mm.

Twenty-five cattle showed small areas of lung collapse on the borders of the lungs but no adult or larval worms could be isolated from these areas. Histological examination showed no evidence that these areas might have been due to lung worm infestation.

Six cattle were affected with tuberculosis. This infection did not appear to influence the development of the reactions.

Thirty cattle were subjected to intradermal tests with D.filaria extract at the same time as intradermal tests with the D.viviparus extract. The reactions produced by the two extracts were identical indicating that the hypersenstivie proteins of D.viviparus and D.filaria are closely related, if not identical.

The following twelve cattle were subjected to intradermal injections with Dictyocaulus extract and with an extract of abomasal parasites. The abomasal contents of each animal were examined for helminths after slaughter. The results are presented in table III.

Table III.

Comparison of the Reactions occurring with

a.) 1-100 saline extract of D.viviparus

and

b.) 1-100 saline extract of stomach helminths (H.contortus, T.axei and O.ostertagi).

	Skin Increase (in mm's.) to extract a.)	Skin Increase (in mm's.) to extract b.)
	11	2
	7	4
	11	4
Age group	9	0
$2\frac{1}{2}$-$3\frac{1}{2}$ yrs.	8	4
	14	6
	1	0
	12	4
Age group	9	3
$4\frac{1}{2}$-$5\frac{1}{2}$ yrs.	6	3
	4	3
	5	3
Average skin increase	8.08mm.	3.0mm.

None of the above animals showed the presence of helminths in the abomasum.

The above table indicates that the skin reactions produced by the Dictyocaulus extract were independent of any sensitisation produced by stomach parasites.

In addition to the above 12 animals the abomasal contents of a further 8 cattle, which had reacted to the D.viviparus extract, were examined for the presence of stomach helminths. None of these animals showed the presence of abomasal helminths.

Thirty sheep were examined by the intradermal test using the saline extract of D.filaria. All the sheep were examined for the presence of adult worms and larvae of the three species of lung worms (D.filaria, Muellerius capillaris and Protostrongylus rufescens). The abomasal contents of 15 of the sheep were examined for the presence of helminths.

The results of the examination are presented in the table IV.

Table IV.

Frequency Distribution of Reactions occurring in sheep to the intradermal injection of *Dictyocaulus filaria* extract (1-100)

Animals and Type of Infestation.		Reactions.		
		Wheal 10mm & over	Wheal 5-9mm	Neg.
Lambs. (27)	*D.filaria* only (4)	3		1
	M.capilleris only (6)	5	1	
	D.filaria and *M.capilleris* (3)	2	1	
	No lung worm infestation (14)	2	1	11
Ewes. (3)	*M.capilleris* only (2)	2		
	No lung worm infestation (1)			1
(15 animals)				
	Abomasal parasites present (10)	6		4
	No abomasal parasites present (5)	3		2

The figures in parenthesis indicate the numbers of animals examined in each group.

Discussion.

The results of the intradermal tests on cattle indicated that the majority of adult cattle were hypersensitive to extracts of D.viviparus. The incidence of animals showing strong positive reactions was highest in the age group $2\frac{1}{2}$ to $3\frac{1}{2}$ years. In only two animals was it possible to correlate the skin hypersensitivity with the presence of parasites. In other cases old standing lesions of pneumonia were found but these showed no evidence of being parasitic in origin.

As no clinically infested animals were available for examination it is impossible to state what degree of hypersensitivity occurs in such animals. Hudson (1951), however, has reported a hypersensitive skin reaction in clinically infested animals following the intradermal injection of an extract of D.viviparus. The period during which the animals reacted was limited. Animals tested several months after recovery from an attack of parasitic bronchitis did not react.

In contrast to Hudson's report it was found in this examination that lambs infested with D.filaria usually did react to an intradermal injection of a saline extract of the causal parasite (6 of 7 infested lambs gave a marked reaction, see Table IV.). Such lambs could only be undergoing their first season's infestation and it was apparent

that this initial infestation, even though sub-clinical in nature, was sufficient to induce sensitisation of the animals.

Since the incidence of hypersensitive reactions increases with age to a maximum at the age group 2½ to 3½ years, it is suggested that repeated seasonal reinfestation of animals at grazing may be a factor in maintaining or increasing hypersensitivity to D.viviparus.

Hudson (1951) and Taylor (1951a) have recorded outbreaks of parasitic bronchitis in adult cattle. Taylor (1951b) was of the opinion that D.viviparus infestation could sensitise the lung tissue of the bovine and that the invasion of such sensitised lung tissue by D.viviparus larvae would precipitate an allergic reaction resulting in the clinical manifestation of parasitic bronchitis. Kauzal (1934) has shown that the exposure of lambs to D.filaria infestation resulted in an extraordinarily high degree of eosinophilia (37%). Kauzal considered that this eosinophilic phenomenon was suggestive of an anaphylactic response due to the sensitising effects of the initial infestation to which the lambs were exposed.

The cattle presented in this examination were of varying ages and probably would have been previously exposed to infestation with D.viviparus. It appeared that this infestation or repeated

infestations sensitised the animals and that this sensitisation persisted even in the absence of parasites.

Taylor (1951b) has reported the presence of microscopic immature worms in the lungs of adult cattle which showed clinical signs of parasitic bronchitis. These immature worms could remain in the lungs for several weeks without further development. None of the cattle in this examination showed clinical signs of parasitic bronchitis and only two cattle showed evidence of larvae. Consequently it was indicated that the persistence of skin hypersensitivity in cattle to D.viviparus, demonstrated in this examination, was independent of the presence of immature worms in the lung tissue.

The reactions produced by the D.viviparus extract could be produced to the same degree by an extract of D.filaria. This indicated that there was a group reaction with the two species of lung worms. A further group reaction was noted with the intradermal tests in sheep. Animals infested with M.capilleris alone reacted to the extract of D.filaria. It appeared that infestation of an animal by one species of lung worm sensitised that animal to extracts of any of the four common lung worms. A similar group reaction has been mentioned by Fairley (1951a) in relation to Schistosomiasis.

The infestation of humans by one species of Schistosome sensitised the humans to extracts of several different species of human and animal Schistosomes.

Stewart (1950a) has reported that extracts of many intestinal parasites caused complement fixation with the sera of animals infested with H.contortus. An alcoholic extract of D.filaria was shown to cause complement fixation with H.contortus natural antisera; saline extracts were not examined.

In this examination the skin reactions caused by the Dictyocaulus extract were shown to be independent of sensitisation or infestation by abomasal parasites. The intradermal injection of a saline extract of abomasal helminths produced only a slight increase in skin thickness (Average 3mm). Hypersensitive reactions observed in sheep to intradermal injections of D.filaria extract occurred independent of infestation by abomasal parasites.

As indicated in the intradermal tests in F.hepatica infestation (Part II) the skin hypersensitive reactions occurring with F.hepatica extract were independent of reactions occurring with the D.viviparus extract.

Summary.

Adult cattle, of varying age, were found to

be hypersensitive to the intradermal injection of D.viviparus extract. The incidence of marked reactions increased with age reaching a maximum at the age group $2\frac{1}{2}$-$3\frac{1}{2}$ years.

With the exception of two animals, no parasites could be found in the lungs or lymph glands of such animals and it was considered that the hypersensitivity originated at initial infestation and persisted after the loss of the parasites.

Lambs infested with D.filaria gave a marked hypersensitive skin reaction to extracts of the causal organism indicating that a single sub-clinical infestation sufficed to establish skin hypersensitivity in sheep.

The skin hypersensitivity to lung worms was a group reaction for the three common species of lung worms. Intestinal parasites did not appear to sensitise animals to D.viviparus extract; reactions to the Dictyocaulus extract were evident in the absence of reactions to the intradermal injection of an extract of abomasal parasites.

Part VI.

Trichinella spiralis.

Materials and Methods.

Intradermal Test.

Schwartz and McIntosh (1929) determined that pigs experimentally infested with T.spiralis developed a marked skin hypersentivity to Trichinella extracts within 2 months after infestation. One of the pigs tested had developed the skin reaction within 15 days after infestation. The extract used was a saline extract of pulverised T.spiralis larvae. The reaction was characterised by a large oedematous swelling; in more striking reactions the skin usually showed evidence of haemorrhage in the reaction area. Several animals not infested with the parasite failed to react to the extract.

Schwartz, McIntosh and Mitchell (1930) reported that pigs not infested with T.spiralis not uncommonly gave marked skin reactions to the subcutaneous injection of Trichinella protein. Of a large series of pigs examined, (486), 39% of these showed marked skin reactions to the T.spiralis extract. Only one of the animals was infested with T.spiralis.

Augustine and Theiler (1932) determined that the intradermal test was specific in Trichinosis in man and swine. The use of a high dilution of antigen (1-10,000) eliminated reactions which frequently

occurred in non infested animals. The antigen consisted of a Coca saline extract of dried and pulverised T.spiralis larvae.

Lichterman and Kleeman (1939) used intradermal tests in swine which had been fed uncooked garbage. The antigen used was a saline extract of pulverised larvae in a dilution of 1-10,000. The test was found to be accurate in 97% of cases of Trichinosis. No animals which were infested with T.spiralis failed to react to the test, and only 5 of 190 which were not infested showed reactions to the intradermal injection of the T.spiralis antigen.

Spindler and Cross (1939) and Spindler, Cross and Avery (1941) carried out an extensive survey of the use of intradermal tests for the diagnosis of Trichinosis in swine. Using a saline extract of Trichinella larvae these authors found that a percentage (17.51%) of animals uninfested with T.spiralis gave positive skin reactions. Of infested animals 36.09% gave no reaction to the test antigen. The authors considered that the prolonged consumption of non viable T.spiralis larvae could cause sensitisation of the animal to Trichinella antigen. Infestation of the animal with helminths closely related to T.spiralis, such as Trichuris trichiura, were thought to be partially responsible for the occurrence of false positive

reactions.

McCoy, Miller and Friedlander (1933) believed that human infestation with T.trichiura caused sensitivity to T.spiralis extract, the skin reaction to T.spiralis therefore being a group reaction in nature.

In the present examination intradermal tests were carried out on pigs which were due for slaughter within 24 hours of the tests being carried out. The preparation used as antigen was a 1-1000 saline extract of dried and pulverised T.spiralis larvae prepared as follows:

Dried and pulverised larvae, as used for the skin test, were obtained from Canada. The powder was extracted with saline in the proportion of 1 part powder to 1000 parts saline. The suspension was extracted for 3 days at 4°C being agitated at frequent intervals. After extraction the suspension was centrifuged and the deposit discarded. The clear supernatant fluid was passed through a "Pyrex" microfilter and subsequently placed in 1cc bottles fitted with a rubber stopper. The bottles were stored in a refrigerator at -10°C.

Injections of the T.spiralis extract were made intradermally, in 0.1cc amounts, into the loose skin at the base of the left ear of the pigs to be tested. The right ear was similarly used for the intradermal injection of a 1-100 saline extract

of A.lumbricoides. The intradermal injections were made with a "Record" type of dental syringe, which was fitted with a graduated plunger shaft so that a regulated amount of fluid could be delivered.

The results of the test were observed at 20 minutes and again at 60 minutes after the injection had been made. The pigs were re-examined 18 hours after the injection of the antigen.

Reactions.

Of 109 pigs examined only 6 pigs showed a reaction to the T.spiralis extract. Five of these pigs reacted only to a slight degree, the reaction being of an insignificant nature. With the remaining 103 pigs the following reactions were seen. The immediate response to the injection of the antigen was the production of a wheal 3-4mm in diameter. Within a few seconds this had increased in area and had become somewhat flatter. In addition a zone of erythema had begun to develop increasing the diameter of the affected area to 6-8mm. In animals which showed no hypersensitivity to the T.spiralis antigen this reaction had disappeared within 10 minutes. In the 5 animals which showed a slight reaction, this early reaction was replaced within 45 minutes by an area of discoloration varying in colour from light red to purple. This discoloured area measured from 6mm

to 10mm in diameter. In the one pig which gave a marked reaction the discoloration was of a deep purple character measuring 23mm in diameter.

The reactions observed with the A.lumbricoides extract were similar to those recorded in Part IV of the present examination. Thus they consisted of a wheal or discoloration varying in diameter from 5-40mm and over. When the reaction was intense the discoloration was markedly blackish purple.

As only one pig showed a marked reaction to the Trichinella extract no clear indication could be obtained regarding the typical skin reaction in pigs to the intradermal injection of T.spiralis protein. Augustine and Theiler (1932) described the typical reaction in pigs as a wheal or a dark purple area with a clear cut margin which developed 30 minutes after the injection of the antigen.

Schmid and Schipull (1937) describe the reaction as consisting of an elevated wheal 1.5 - 1.8 c.m. in diameter surrounded by a zone of marked redness. Lichterman and Kleeman (1939) report that a positive reaction consisted of a wheal 1.5 to 2.5 c.m. in diameter, which assumed a purplish red hue and developed in from 25 to 45 minutes after the injection of the antigen.

Spindler and Cross (1939) state that positive reactions reach their maximum intensity 15 to 20 minutes after the injection of the antigen.

They consisted more or less of a blanched wheal surrounded by a bluish red zone. In some cases the bluish red area appeared without distinct wheal formation but with a central anaemic area; in other animals only a solid purple area was evident. Reactions consisting of either a pinkish or red discoloration were classed as doubtful.

The reactions reported by the above authors correspond closely to the various types of skin reactions which have been noted to A.lumbricoides extract in this examination. It appears, therefore, that the reactions to be expected to the Trichinella extract would be similar in character to those observed with the A.lumbricoides extract.

The intradermal tests with Trichinella extract were carried out simultaneously with A.lumbricoides extract in order to determine whether hypersensitivity to A.lumbricoides influenced the occurrence of skin reactions to T.spiralis. Baron and Brunner (1942) have indicated that a common allergic factor existed in the antigens of T.spiralis and A.lumbricoides. These antigens were to some extent interchangeable in persons sensitive to either of the parasites. With passive sensitivity tests the homologous antigen always produced more pronounced results than the heterologous antigen.

Since, as far as can be ascertained, no tests combining the T.spiralis antigen and the Ascaris antigen have been carried out in pigs it was thought advantageous to determine whether the false reactions reported by previous workers in America occurred in Great Britain and whether these could be correlated with hypersensitivity to A.lumbricoides.

Post Mortem Examination.

All the pigs tested were numbered with an indelible marker before the test was made. Subsequently the pigs were slaughtered separately and the viscera and carcase of each animal examined individually.

The liver was examined for evidence of chronic focal interstitial hepatitis which Oldham and White (1944) ascribe to the migration of Ascaris larvae.

The small intestine was palpated throughout its entire length to detect the adult A.lumbricoides. An incision was made into the wall of the caecum and the contents removed. These and the caecal wall were examined for the whip worm of pigs, T.trichiura. A rectal sample of faeces was taken and examined by the smear method for Ascaris and Trichuris eggs. No egg count was made.

The lungs were palpated and incised and examined for evidence of the pig lung worms, Metastrongylus spp.

Samples of muscle from the crura of the diaphragm, tongue and larynx were obtained from each carcase. These were examined for T.spiralis as follows. A small portion of muscle from each sample was pressed between two pieces of glass and examined under the low power of a microscope for evidence of encysted T.spiralis larvae. Three pieces of muscle from each sample were examined by this method. The remaining portion of muscle sample was divided into two, one of which was retained. The other portion, together with the half portions of the muscle samples from the other pigs was passed through a household mincer and subsequently digested for 24 hours at 37°C in a solution of 0.4% pepsin and 0.3%HCl. The digestion material was agitated at frequent intervals. After digestion the material was placed in a Baermann apparatus and allowed to sediment. A sample of fluid was drawn off at intervals from the funnel and examined under the low power of a microscope for T.spiralis larvae. Since no T.spiralis infestation was found it was unnecessary to carry out separate digestion of the muscle samples retained.

Table I.

Frequency Distribution of the reactions occurring in pigs to the intradermal injection of a. 1-1,000 saline extract of T.spiralis larvae
b. 1-100 saline extract of A.lumbricoides intestine.

Reactions.	Antigen a.	Antigen b. Chronic Focal Interstitial Hepatitis			
		Slight	Moderate.	Marked.	No Infestation
Negative or very slight.	103 (28)(6")(5+)	25 (7)	2 (2)	1	9 (4")
Wheal or purple 5mm to 9mm in area.	4 (1)	20 (11)(2")(3+)	2 (1)		2 (1)
Wheal or purple 10mm to 19mm in area.	1 (1")	32 (3)(1")	5 (2)(1+)		2 (1)
Wheal or purple 20mm and over in area.	1	6 (1)(1+)		2*	
Delayed Reaction; 10mm and over				1	
Total animals in each group.	109	83	9	4	13

The figures in parenthesis indicate the animals infested with T.trichiura
The figures thus " and in parenthesis indicate the animals infested with Oesophagostomum spp.
The figures thus + and in parenthesis indicate the animals infested with Metastrongylus spp.
* Two pigs which showed hypersensitivity of a generalised nature.

Two pigs presented in table I displayed hypersensitivity of a generalised nature to the A.lumbricoides extract. These two pigs, after the intradermal injection of the A.lumbricoides extract developed a diffuse light purple coloration over the whole body. The breathing was rapid, the pulse fast and weak and the cojunctiva engorged. The pigs were unable to rise for an hour after the injection. The effect of this hypersensitivity had disappeared after 18 hours. Post mortem examination showed congestion of the liver, lungs and intestine. In addition the large intestine was marked with diffuse haemorrhages in a zebra formation. Ransom, Harrison and Couch (1924) have recorded a similar occurrence in a pig following instillation of ascaris material into the cojunctiva sac.

Discussion.

Table I indicates that only 6 pigs out of 109 displayed any reaction to the T.spiralis extract. None of the pigs examined were infested with T.spiralis. Of the 6 reactions noted to the Trichinella extract only one was of a marked nature. One other reaction measured 10mm in diameter and was a red coloration at the site of the injection. The other 4 reactions were insignificant circumscribed areas of red erythema.

With the exception of the marked reaction, the reactions to the T.spiralis extract occurred in

pigs which gave a much more marked reaction to the A.lumbricoides extract (20mm and more). The pig which gave a marked reaction to the extract showed a similar reaction to the Ascaris extract.

Since no infestation by T.spiralis was found and since the reactions observed to the Trichinella extract occurred in animals giving marked reactions with the Ascaris extract, it was concluded that a marked hypersensitivity to A.lumbricoides could sensitise the skin of swine to extracts of T.spiralis. The homologous protein, however, gave the more marked reaction. This was in accordance with the statements of Barron and Brunner (1942).

Spindler and Cross (1939) have postulated that false positive skin reactions to T.spiralis antigen in non-Trichinous swine may have three explanations: firstly, that the muscle larvae had not progressed to such a point where they would withstand artificial digestion. Animals can develop skin sensitivity to Trichinella infestation as early as 10 days after infestation (Augustine and Theiler, 1932) and Spindler and Cross have observed that 10 day larvae could not withstand artificial digestion. Trawinski and Maternowska (1933) have reported that unencapsulated larvae would not withstand digestion (artificial or natural) but that the larvae could be detected by

microscopical examination. Since no such migrating unencapsulated larvae were observed in this examination it was unlikely that the reactions observed were due to sensitisation by this source.

Secondly, Spindler and Cross postulated that the consumption, over a long period, of non-viable Trichinella larvae may induce weak positive skin reactions. Avera, Yow, Harrell and Fowler (1946) were unsuccessful in their attempts to induce positive skin reactions in guinea pigs and rabbits by repeated feedings of dead T.spiralis larvae. However, Gould (1942) offered confirmation to the views of Spindler and Cross in that the incidence of non specific reactions to Trichinella extracts was much lower in Orthodox Jews than with the general population.

Spindler and Cross finally postulated that infestation with closely related parasites, such as Trichuris, might cause hypersensitivity to T.spiralis. McCoy and Miller (1931) have reported that 62% of persons infested with T.trichiura gave positive skin reactions to intradermal injections of T.spiralis protein. McCoy, Miller and Friedlander (1933) later observed many positive reactions to a Trichinella extract in children. These children showed infestation with T.trichiura only, T.spiralis infestation being absent.

In this examination, however, of 29 pigs which showed infestation with Trichuris only one gave a reaction with the T.spiralis extract. This reaction was of a slight nature only. All the other pigs failed to give a reaction. It is evident therefore, that Trichuris infestation does not influence the reactions to the T.spiralis antigen in pigs.

Summary.

Of 109 pigs given intradermal injections of T.spiralis antigen only six pigs showed a reaction. Only one marked reaction was seen. No pig was found to be infested with T.spiralis.

The reactions which did occur were found in animals which possessed a marked skin reaction to A.lumbricoides. It is concluded that a marked hypersensitivity to A.lumbricoides may sensitise pigs to a lesser degree to the T.spiralis extract.

The infestation of pigs with T.trichiura did not produce hypersensitivity to the T.spiralis extract.

Part VII.

General Summary.

A study has been made of the serological responses occurring in domestic animals as a result of natural infestation with helminth parasites. The helminth infestations studied were those of Fasciola hepatica, Cysticercus bovis, Ascaris lumbricoides, Dictyocaulus viviparus and Trichinella spiralis.

F.hepatica.

The serological response, as indicated by the precipitin test, in F.hepatica infestation was most marked when the immature parasites were in their migratory phase. Thereafter the precipitin antibody level became lower.

Specific complement fixation antibodies were demonstrated in the sera of only one infested animal. This was indicative that the complement fixation technique was unsuitable for the demonstration of antibodies in F.hepatica infestation. Alcoholic extracts of the parasite were found to give varying degrees of non specific complement fixation with sheep sera, irrespective of F.hepatica infestation.

The miracidia of F.hepatica were observed to suffer a lethal effect when placed in sheep serum. Complement was necessary for the reaction.

Hypersensitivity, demonstrable by an

intradermal test, was evident in a large percentage of animals infested with F.hepatica. In some cases this hypersensitivity was passively transferable, by means of serum, to a non sensitive animal. Local skin desensitisation could be produced to a marked degree but generalised skin desensitisation was less obvious when large quantities of antigenic material were administered.

C.bovis.

Precipitin antibodies were demonstrated in the sera of animals which showed multiple cysticerci in the musculature. When only a few (1 or 2) cysticerci were present a serological response was frequently absent. Polysaccharide solutions of the parent parasite, T.saginata, produced precipitin reactions with the sera of animals infested with F.hepatica only. This was thought to be due to an isoagglutinin like producing antigen present in both T.saginata and F.hepatica.

Animals infested with F.hepatica produced hypersensitive skin reactions to extracts of C.bovis. A common allergen in the two parasites was thought to be responsible.

A.lumbricoides.

Precipitin antibodies were demonstrated in the sera of pigs. These were found to bear no relationship to the presence of mature A.lumbricoides in the intestine. They did, however, show a

relationship to the degree of chronic focal interstitial hepatitis.

Hypersensitivity to extracts of A.lumbricoides was evident in the majority of swine. This was similar to the precipitin reaction, in that it was related to the degree of chronic focal interstitial hepatitis but not to the presence of intestinal forms of A.lumbricoides.

D.viviparus.

The majority of cattle examined showed skin hypersensitivity to D.viviparus extracts. This hypersensitivity was most evident in the age group 2½ to 3½ years. The hypersensitivity demonstrated occurred in the absence of parasites. Intestinal helminths did not influence the hypersensitive state.

Hypersensitivity was demonstrated in sheep undergoing a sub-clinical infestation with D.filaria. Infestations with other lung worms produced a hypersensitive state which could be demonstrated by D.filaria extracts. This was indicative of a group reaction.

T.spiralis.

Hypersensitivity to extracts of T.spiralis was demonstrated in only 5.4% of swine. Only 0.9% of swine showed marked reactions. Since these occurred in animals possessing marked hypersensitivity to A.lumbricoides it was concluded that a strong

hypersensitivity to A.lumbricoides could sensitise pigs to extracts of T.spiralis. Infestations with T.trichiura did not produce hypersensitivity to T.spiralis extracts.

The use of animals at an abattoir afforded the opportunity to correlate the serological findings with post mortem evidence of infestation. Since the lack of helminth eggs in the faeces does not necessarily indicate absence of helminth infestation the post mortem examinations carried out gave a more complete picture of the parasitic burden. As a result it was found that light infestations or pathological evidence of helminth infestation could be correlated with the serological findings (vide F.hepatica, C.bovis and A.lumbricoides). If faeces examination had been the sole criterion of evidence of infestation no correlation between serological findings and infestation would have been evident and false conclusions may have been drawn.

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