STUDIES ON THE DEVELOPMENT OF AN ACQUIRED RESISTANCE TO *FASCIOLA HEPATICA* INFECTIONS IN CATTLE.

by

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GENERAL INTRODUCTION

The object of the work described in this thesis was to investigate the manner in which cattle respond to infections with the trematode parasite Fasciola hepatica commonly designated the common liver fluke.

It is perhaps apt to begin with a description of the life cycle of this parasite F. hepatica. The existence of worms termed 'flokes' had been recorded in the liver of diseased sheep in the 16th century (Fitzherbert 1534). The complex life cycle involving its intermediate host, a snail, was pieced together by various investigators over the next 4 centuries and was finally completed by the investigation of Krull and Jackson (1943). The sequence of discoveries which elucidated the life cycle of F. hepatica have been comprehensively reviewed by Pantelouris (1965) and Reinhard (1967). The life cycle of F. hepatica develops as follows.

Eggs are produced by mature parasites in the bile ducts of the final host. These eggs are passed out of the host in the faeces and under suitable conditions hatch to release a free swimming form, the miracidium. The function of the miracidium is to find and infect the intermediate host. A mud snail, Lymnaea truncatula is the intermediate host in Great Britain. In the intermediate host 3 stages develop, the miracidium develops into the next stage a sporocyst which is virtually a sac lined with germinal cells. The next stage, the rediae develop from the sporocyst and migrate from the pulmonary chamber to the liver of the snail. The rediae can develop in two ways, into a further generation of rediae or into cercariae. The cercariae emerge from the intermediate host, find a suitable site such as a blade of grass, and there encyst to form metacercariae. The metacercariae are ingested by a suitable host, excyst in the alimentary tract liberating a juvenile fluke. This then migrates through the wall of the alimentary tract across the peritoneum and through the liver to a final location in the bile ducts of the host. Here the parasites become mature and produce eggs.

The conditions necessary for the successful development of each stage of the parasite have been reviewed by Pantelouris (1965) but under optimum conditions in the laboratory it takes 7-9 weeks from the shedding of an egg to the liberation of cercariae from an infected snail. It requires 6-8 weeks after infection of a suitable host for the mature parasite to migrate to their final location in the bile ducts and a large proportion of this period is spent migrating in the parenchyma of the liver.

The earliest available description of a disease associated with liver fluke was recorded in England by Fitzherbert (1534) who described the clinical signs of a wasting disease in sheep associated with the finding of flukes in the liver. A similar disease was described in sheep in France by De Brie (1541). Liver flukes were not recorded as being associated with a disease of cattle until Willus (1674) described finding flukes in the livers of cattle in Seelund.

Davaine (1877) reviewed the literature concerning reported outbreaks of fascioliasis over the previous three centuries, described the clinical signs associated with F. hepatica infections in sheep and cattle, including the observation that the disease occurred much less frequently in cattle than in sheep and was rarely seen in cattle over 2 years old. The clinical signs as described by Davaine (1877) were dullness, inappetance, pallor of the mucous membrane, and subcutaneous oedema, a description of chronic fascioliasis which applied to the sheep could not be improved upon in the present day.

In Britain however, at the end of the 19th century, the only clinical signs attributable to fluke infection in the bovine as described in a text book of cattle disease (Steel 1895) were those of a transient jaundice due it was thought to the occlusion of the common bile duct by flukes.

A clinical description of fascioliasis in cattle did not become generally available in Britain till the publication of an English translation of a French parasitology text book (Neuman 1905). It noted that the disease in cattle was rarely fatal but the fatal disease was associated with marked wasting and diarrhoea. It also stated that severe liver damage associated with liver flukes was found at slaughter in the livers of cattle which did not show clinical signs of the disease during life.

Little was added to these clinical descriptions of the disease over the next 50 years and the clinical signs were recorded as loss of weight, anaemia and chronic diarrhoea by a standard veterinary text book in 1963 (Blood and Henderson). It was only recently that Reid, Armour, Jennings Kirkpatrick, Urquhart (1967) showed that the diarrhoea described in cases of cattle fascioliasis was in actual fact due to a concurrent infection with the gastro-intestinal nematode Ostertagi ostertagi. The clinical signs attributable solely in infection with F. hepatica in cattle were finally defined by Ross (1966a) Reid (1968) as progressive loss of weight, anaemia and hypoalbuminaemia. Diarrhoea was not recorded in any of these cattle nor was subcutaneous oedema. The clinical signs were associated with the presence of adult flukes in the bile ducts and became apparent from 12 weeks after infection onwards. The relationship between the development of clinical signs, the animal's acquired fluke burden and its nutritional status has been discussed by Reid, Doyle, Armour, Jennings (1972) where it was proposed that moderate burdens (100 - 300 flukes) may only result in clinical signs of fascioliasis if the animal is also on low plane of nutrition.

The clinical disease in cattle is predominantly chronic in nature and only sporadic cases of acute or subacute fascioliasis have been reported where heavily infected experimental calves (1,500 - 20,000 metacercariae) have died 3-10 weeks after infection with severe liver damage attributable to large numbers of migratory immature flukes (Boray 1967, Ross 1967a).

The many experiments concerning proposed mechanisms for the pathogenic effect of F. hepatica in chronic infections of animals have been extensively reviewed during the last ten years by Taylor (1964) Dawes and Huges (1964) Pantelouris (1965) Sinclair (1967) Boray (1969) Dawes and Hughes (1970) but only over the last three years has conclusive evidence become available to define several aspects of the pathogenesis of F. hepatica infections in sheep and rabbits.

The principal factor in the aetiology of the anaemia of chronic fascioliasis in sheep and rabbits has been shown using ^{51}Cr labelled erythrocytes to be a marked loss of erythrocytes from the circulation via the bile into the gut presumably due to the feeding activities of the flukes (Holmes, Dargie, Maclean, Mulligan 1968, Sewell, Hammond, Dinning 1968, Sinclair 1971c). It had previously been shown by Todd and Ross (1966) that the caecal contents of flukes contained such large amounts of breakdown products of haemoglobin that they could only result from the ingestion of whole blood by the flukes. This hypothesis received further substantiation when it was found that caecal material in flukes taken from sheep with ^{59}Fe in their plasma contained large amounts of the radioactive iron in the form of oxyhaemoglobin. Studies of the metabolism of ^{59}Fe (Symons and Boray 1968, Dargie and Mulligan 1970) showed the rate of erythropoesis in clinically infected sheep and rabbits had reached a maximum before the anaemia developed and that there was no evidence to

support the view that dyshaemopoesis is the major factor in the aetiology of the anaemia of chronic fascioliasis as proposed by Sinclair (1964) and Sewell (1966).

This loss of blood into the bile ducts has also been shown to be the principal factor in the aetiology of the hypoalbuminaemia associated with chronic fascioliasis and infected animals are markedly hypercatabolic with regards to albumin metabolsim. (Dargie, Holmes, MacLean, Mulligan 1968a). The hypoalbuminaemia of chronic fascioliasis therefore is due to excessive loss by leakage into the bile ducts associated with hyperproduction of albumin rather than a failure of production due to liver damage as previously supposed (Sinclair 1967). The simultaneous use of radioactively labelled erythrocytes and albumin in fluke infected rabbits (Dargie, Holmes, MacLean, Mulligan 1968b, Dargie and Mulligan 1971) has shown conclusively that hypercatabolism of these constituents was due to their loss into the gut and thus only became apparent after the adult flukes had entered the bile ducts.

Nansen (1970) in a study of the metabolism of the various subclasses of bovine IgG described the accelerated catabolism of the 'slow' subclass of IgG in 9 heifers with chronic fascioliasis due it was thought to the loss of this immunoglobulin along with other blood constituents into the bile as large quantities of the radioactively labelled protein were found in the bile of these animals at slaughter.

The weight of evidence for the haematophagic nature of the adult fluke is now so great as to render the hypothesis that the adult fluke feed by browsing on the biliary epithelium (Dawes 1963a, Dawes and Hughes 1964) highly unlikely.

The clinical disease caused by infections with F. hepatica in cattle then would appear to be chronic in nature, rarely fatal, associated with the haematophagic nature of adult flukes and confined to young cattle on a poor nutritional plane.

Economic losses due to fascioliasis in cattle however are not related to the clinical but rather to the subclinical infections in apparently healthy cattle. There are two aspects of the economic loss, the first is due to the effect of F. hepatica infection lowering production during economic life of the animal and the second is the loss of revenue due to condemnation of affected livers at slaughter.

There are few detailed experiments into the effect of F. hepatica infection in cattle upon the subsequent productivity of these cattle whether producing milk or beef. The effects of F. hepatica infection on milk production has been estimated to be in the region of 14-35% loss in heavy infections (Gebaur 1939), 11.5% loss in moderate infections (Doekson, Heringa, Swierstra 1949), 6-8% loss in light infections (Leinati, Carrara, Recalcati 1961, Vink 1961, Ross 1970). The effects of F. hepatica infections on beef production has been expressed in several ways. A difference of approximately .01 kg./day between the weight gains of infected and uninfected cattle, has been shown by Mikacic and Krsto (1960) and Ross (1970). Infected cattle has been reported as being an average of 40 kgs. lighter than comparable uninfected cattle at slaughter (Neuhaus and Six 1965). Hope, Cawdrey and Conway (1970) reported that bullocks with experimental chronic fascioliasis required 11% more food to achieve the same weight of gain as uninfected bullocks kept under the same conditions.

The overall infection rate in various countries as judged by the condemnation rates of infected livers at slaughter has been recorded and in some cases an estimation of the consequent financial loss has been provided.

In the Netherlandsin 1959, 70% of all bovine livers were totally or partially condemned due to fascioliasis with an estimated total consequent loss of 200 million guilders (23 million pounds) (Vink 1961).

Ten years later total losses attributable to bovine fascioliasis in the Netherlands was estimated at 150 million guilders (17 million pounds) (Koopman 1969).

In Switzerland approximately 66% of bovine livers in 1959 were condemned due to fascioliasis with a consequent loss of 500,000 S.F./year (£50,000) (Fuhriman 1961).

Losses in the German Federal Republic in meat production attributable to F. hepatica infections in cattle were estimated at an annual loss of 170 million D.M. (£16,400,000) (Neuhaus and Six 1965).

In the United States of America the losses due to condemnations of bovine liver due to fascioliasis were estimated in 1942 to be $2\frac{1}{2}$ million dollars (Frederick 1942) and in 1953 to be 3 million dollars with a total economic loss of 10 million dollars (Price 1953).

The incidence of bovine fascioliasis in abattoirs in Japan has been recently reported to be 20-30% (Watanabe 1967).

In Great Britain a survey of abattoir records in 1942 showed an average condemnation of 17.7% of bovine livers due to fascioliasis and this was estimated to result in a loss of £200,000 per annum (Peters and Clapham 1942). In 1964 a similar survey showed 21.7% of bovine livers condemned due to fascioliasis at an estimated value of £1 million pounds (Blamire 1964). This survey was extended over 1960-1968 and it was then shown that an average of 21.7 - 30.8% of all bovine livers were condemnded due to fascioliasis (Blamire, Crowley, Goodhand 1970).

The condemnation figures from 56 abattoirs in England and Wales for the years 1961-1970 are shown in table 1. It can be seen that an average 25.8% of bovine livers were condemned due to fascioliasis and if this figure is applied to the 3-5 million cattle slaughtered annually in Great Britain it represents an economic loss due to condemnations alone of £3-5 million pounds. The condemnation figures from 4 Scottish abattoirs for the years 1962-1970 are shown in table 2 and it can be seen that the average condemnation rate of bovine livers due to fascioliasis (26%) was the same as the average condemnation rate in the 56 abattoirs in England and Wales.

These average condemnation rates for bovine livers due to fascioliasis doe not however reflect the incidence of fascioliasis in the wetter western areas of the country where conditions favour the development of the parasite. In Northern Ireland surveys of abattoir records show that in 1963, 63% of all bovine livers were condemned due to fascioliasis and in 1965 the condemnation rate had risen to 80% of all bovine livers at slaughter (Ross 1966b, Thornton 1968). The condemnation returns for bovine livers due to fascioliasis from 11 abattoirs in the South West Region of England for the years 1969-1971 are shown in table 3 and can be seen to be 10-14% higher than the national condemnation rates in these years. Individual abattoirs had condemnation rates of affected livers as high as 73%. The average condemnation rate of Glasgow abattoir for the years 1962-1970 is 32.2% whereas the average condemnation rate for three abattoirs in the East of Scotland over the same period is 20.4%.

It can be seen therefore that approximately a quarter of the cattle population of Great Britain are infected with F. hepatica at some point during their lives and this must result in a severe economic loss due both to interference with production and loss of revenue at slaughter.

TABLE 1

Incidence of fascioliasis in cattle at 56 slaughterhouses in England and Wales for the years 1961 - 1970

Year	Number of cattle slaughtered	No. of offals condemned	% of offals condemned	Approx. cost at £2.50/liver
1961	675,562	156,062	23.1	£266,852.00
1962	677,215	165,212	24.4	£319,837.00
1963	690,734	152,731	22.1	£430,467.00
1964	642,639	139,216	21.7	£489,230.00
1965	588,929	141,316	24.0	£381,475.00
1966	583,291	152,590	26.2	£353,290.00
1967	636,050	195,692	30.8	£348,040.00
1968	612,031	172,187	28.1	£381,827.00
1969	361,989	127,935	35.3	£413,030.00
1970	373,077	106,741	28.6	£390,155.00
Average	584,152	150,968	25.8	£377,420.00

TABLE 2

Incidence of fascioliasis in cattle in 4 slaughterhouses in Scotland for the years 1962 - 1970

Year	No. of cattle slaughtered	No. of offals condemned	% of offals condemned	Approx. cost at £2.50 per liver
1962	302,975	70,938	23.4	£177,345
1963	323,609	83,711	25.9	£209,277
1964	315,291	76,669	24.3	£191,672
1965	302,405	68,466	22.6	£171,165
1966	296,751	83,303	28.1	£247,202
1967	306,361	99,158	32.4	£247,895
1968	287,509	83,685	29.1	£209,212
1969	254,107	73,676	29.0	£184,190
1970	265,563	49,841	19.1	£124,602
Average	294,952	76,605	26%	£191,512

TABLE 3

Incidence of fascioliasis in cattle at 10 slaughterhouses in the South West Region of England from 1st January, 1969

Year	Number of cattle slaughtered	No. of offals condemned	% of offals condemned
1969	65,111	31,951	49.1
1970	52,202	20,198	38.7
1971	13,086	4,409	33.7

Bovine fascioliasis can thus be seen to be an important disease of cattle and its economic effect is the result of subclinical rather than clinical infections.

This apparent discrepancy between the number of cattle with evident damage due to fascioliasis in their livers at slaughter and the lack of any corresponding clinical disease during life has been noted by various authors since the 19th century (Davaine 1877, Montgomerie 1931). It was also noted that these bovine livers contained fewer flukes than did sheep livers affected with fascioliasis and from both these observations it was deduced that cattle were more resistant than sheep to infection with F. hepatica. A detailed review of the relevant literature will be found in the introduction to the first part of this thesis.

The purpose of the experiments described in this thesis was to investigate and define this apparent resistance of cattle to infections with F. hepatica under controlled experimental conditions. It was necessary therefore to consider what criteria would be acceptable in the definition of any 'acquired resistance' of calves to experimental infections with F. hepatica.

Stirewalt (1963) listed the criteria used in assessing acquired resistance to schistosome infections in man and animals, the most important and widely adopted of these are as follows:-

1) The ability of a host to mount a 'self-cure' response.

2) A reduction in the expected worm burden from a given infecting dose.

3) A reduced level of disease in the host.

These criteria can thus be applied to the results of any experimental infection of cattle with F. hepatica to define the presence or absence of any such resistance.

While these criteria can be used to assess the presence or absence of an acquired resistance to infections with F. hepatica they do not however indicate the mechanism of such a resistance. A further set of criteria are required to define whether such a resistance is dependent on the expression of a specific immune response by the host or if it is the result of previous infections damaging host tissue thus rendering the environment unsuitable for the development of any subsequent infection. An acquired immunity is dependent on the expression of a limited number of allergic reactions as defined by Coombs and Smith (1968) and Coombs (1968). The possible allergic reactions were arranged into 4 groups or modes as follows:-

Mode A - involves the action of serum antibody with or without soluble molecular co-factors

Mode B - involves the action of serum antibody, soluble co-factors and non-allergized cells.

Mode C - involves serum antibody which passively allergizes cells such as mast cells or macrophages

Mode D - involves actively allergized cells such as lymphocytes, immune macrophages etc.

The involvement of one or more of these allergic reactions in the acquired resistance of cattle to infections with F. hepatica would show the acquisition of a specific protective immune response on the part of the host.

These two sets of criteria can be used therefore to assess the presence of an acquired resistance and any involvement of the immune response in such an acquired resistance.

The work described in this thesis is divided into 4 parts. The first part describes the experimental investigation into the acquired resistance of parasite-free calves to experimental infections with F. hepatica.

The second and third parts of the thesis describe immunologic developments associated with these infections and the fourth part describes some aspects of the gross and histopathological changes associated with F. hepatica infections in the calves.

PART 1

EXPERIMENTAL INVESTIGATION OF THE RESPONSE OF SUSCEPTIBLE CALVES TO PRIMARY AND SECONDARY INFECTIONS WITH *FASCIOLA HEPATICA*

Introduction and Review of the Literature

The literature currently available on fascioliasis is extensive and has been the subject of several recent review articles, (Dawes and Hughes 1964, Sinclair 1967, Boray 1969, Dawes and Hughes 1970) of which the most comprehensive is Boray (1969).

This review of the literature will therefore be confined to the literature concerning acquired resistance to infections with F. hepatica in domestic and laboratory animals. Investigations into acquired resistance to F. hepatica infections have been described in cattle, sheep, mice, rabbits and rats and the available literature for each species will be reviewed in turn. The literature on aspects of acquired immunity to Schistosome, Nippostrongylus brasiliensis and Trichinella spiralis infections which are considered relevant to this thesis will also be reviewed.

Cattle

It has been accepted for some considerable time that cattle are resistant to infections with Fasciola hepatica. This observation has been made by many authors with little or no experimental evidence cited to support this premise.

Hutyra and Marek (1926) considered that F. hepatica has a life span in various hosts including cattle of from 3-5 years but that the majority of the flukes lived for only 9 months to one year.

Montgomerie (1931) referring specifically to cattle livers in abattoirs observed that the livers of two and a half year old cattle commonly showed evidence of severe fluke infestation in the absence of any flukes in these livers.

Morrill and Shaw (1942) infected 2 cattle each with 1000 metaceracriae of F. hepatica and 5 months later reinfected one with a further 48 metacercariae. Both cattle developed only a mild anaemia and at slaughter

9 months after the initial infection 48 and 544 flukes were recovered from their livers. The authors concluded on the basis of the lack of any clinical disease that the pathogenic effect of F. hepatica in cattle is small and also that calcification of the bile ducts may be an important defensive reaction on the part of the host.

Dixon (1964) suggested on the basis of comparative stunting of flukes from infected cattle as compared to those from infected sheep that conditions in the bovine host were less favourable than in sheep.

Taylor (1964) stated that cattle are able to eliminate an infection with F. hepatica over a period of time unless the infection has been unusually severe but does not provide any evidence for this statement or any indication of what is considered to be an unusually severe infection.

Ross (1966c) stated that a limited life span is a feature of patent F. hepatica infections in cattle but that this could be influenced by a number of factors including the number and size of previous infections and the immune status of the host. No experimental evidence was put forward to support these suggestions. Ross, Todd, Dow (1966) had however previously reported an experiment where parasite free calves were infected with either 200, 300 or 1300 metacercariae of F. hepatica and no loss of adult parasites had occurred between eight to twenty three weeks after infection.

Keck and Supperer (1967) in a radiographic study of bile duct calcification in fluke infected bovine livers stated that the calcification of the bile ducts had become severe eight to nine months after infection and at this time only a few isolated flukes could be found. No further experimental evidence was reported.

More recently experiments have been described which would appear to support the premise that cattle can over a period of time eliminate at least part of the fluke population derived from a single infection of F. hepatica.

Boray (1967) infected four 6-8½ month old parasite free steers each with 1000 metacercariae of F. hepatica. A single calf became anaemic 21 weeks after infection, the faeces egg counts reached a maximum 20 weeks after infection, then declined and the calves were described as having made a spontaneous recovery. The calves were then used for further experiments so no details of acquired fluke burdens were given nor was any explanation put forward to explain this apparent spontaneous recovery.

Reid (1968) infected four parasite free calves each with 2000 metacercariae of F. hepatica. All four calves developed anaemia and hypoalbuminaemia. Two calves died twelve weeks after infection, the remaining two calves were killed twenty five weeks after infection. Three hundered and fifty three and six hundred and fifty four flukes were recovered from the livers of the calves dying 12 weeks after infection as compared to 15 and 93 flukes recovered from the livers of the calves killed 25 weeks after infection. Reid considered the possibility that an acquired self-cure reaction could have occurred due to bile duct calcification.

Ross (1968) using a mixture of field and artificial infections in calves described the loss of 75% of the acquired fluke burden between the 5th and 21st month after infection associated with decreasing faeces egg counts after 5 months of infection. This percentage reduction was derived from the comparison of the number of flukes recovered from a single calf with the mean burden of 4 calves killed 2-5 months after artificial infection with F. hepatica.

There was therefore in the literature no statistically significant experimental evidence based on the number of flukes surviving at given times after a single infection of cattle with F. hepatica to justify the widely held view that cattle could over a period of time eliminate their acquired fluke burden.

The response of previously infected calves to reinfection with F. hepatica had not been considered until the recent studies of Ross (1966a, 1967b) Boray (1967), Horchner (1969).

Ross (1966a) infected each of 6 calves with 200 metacercariae of F. hepatica and after 21 days challenged three of these calves and three previously uninfected control calves with 300 metacercariae of F. hepatica. The reinfected calves were killed 10, 14 and 19 weeks later and the control calves 7, 11 and 16 weeks later. No significant difference was found between the acquired fluke burdens of each goup of calves. The remaining three previously infected calves were reinfected with 300 metacercariae of F. hepatica 18 weeks after the initial infection and killed 6, 8 and 9 weeks later. Ross found a reduction in the numbers of flukes recovered from the livers of these calves (155 flukes) as compared to the number of flukes previously recovered from the control calves (306 flukes). Ross also described finding an apparent 'spontaneous self-cure' proposing that the flukes of the reinfection initiated the expulsion of the fluke population derived from the initial infection.

Ross (1967b) infected 2 calves each with 1,500 metacercariae of F. hepatica. Thirty weeks later these calves and two previously uninfected control calves were each infected with 200 metacercariae of F. hepatica and all four calves were killed 6 weeks later. Differentiating both fluke populations in the reinfected calves on the basis of size he found that 5.6% of the challenge dose was recovered from the reinfected calves as compared to an 18% recovery from the control calves. This would represent a 60% reduction in the acquired fluke burden of the reinfected calves.

Ross concluded from these experiments that any acquired resistance demonstrated involved some expression of host immunity.

Boray (1967) infected four, 17 month old steers each with 1000 metacercariae of F. hepatica and 14-19 weeks later terminated this infection with an anthelmintic. Three weeks later these four steers and four previously uninfected steers were each infected with 5000 metacercariae of F. hepatica and slaughtered 22 weeks later. A mean of 135 flukes were recovered from each liver of the reinfected compared to a mean of 620 flukes recovered from each liver of the infected control calves. This represents a 78% reduction in the acquired burden of the reinfected steers.

Boray (1967) also attempted immunisation of 3 calves using 3 doses at 6 weeks intervals of 3,000 metacercariae of F. hepatica irradiated with 20 kilorads (Kr.) Three weeks after receiving the last vaccinating dose these calves and 3 non-vaccinated control calves were each infected with 5000 metacercariae of F. hepatica and killed 16 weeks later. No significant difference was found between the acquired fluke burden of each group of calves.

Boray concludes that resistance to reinfection in cattle was simply a manifestation of the degree of tissue damage caused by the previous infection and did not involve an expression of the host's immune response.

Hörchner (1969) initially infected calves with either 150, 250 or 500 metacercariae of F. hepatica then reinfected these calves and a group of previously uninfected calves 16 weeks later with a further 200 metacercariae of F. hepatica. There was a 50% reduction in the number of flukes derived from the reinfection in previously infected calves as compared to the numbers of flukes recovered from the control calves.

This conclusion was based again on the differentiation of each fluke population in the reinfected calves on a basis of fluke size. Hörchner considered the possibility that either an immune response or a non-specific tissue reaction could have been responsible for this manifestation of acquired resistance.

Another aspect of the bovine response to infections with F. hepatica was suggested by Ross (1965, 1967a) that cattle are more resistant to challenge with a large single infection of metacercariae of F. hepatica than they are to challenge with a small single infection of F. hepatica. In a comparison of infection levels of 1,500, 2,500, 5,000, 15,000 metacercariae he found an inverse relationship between dose, percentage recoveries and the severity of the disease produced. Only calves receiving a single infection of 1,500 metacercariae of F. hepatica developed clinical signs of fascioliasis. Ross proposed that in a high level single infection with F. hepatica that the immature flukes are trapped and eliminated by the intense tissue reaction they provoke in the ventral lobe. Boray (1967) however, produced subacute fascioliasis and death in a calf infected with 5000 metacercariae of F. hepatica and all four calves receiving a single infection of 10,000 metacercariae showed clinical signs of fascioliasis and died within 5 months after infection.

In summary it can be said that there is no statistically significant experimental evidence described at present in the literature to validate the statement that cattle can over a period of time eliminate part of their fluke burden derived from a single infection with F. hepatica. There is also unequivocal evidence from only one experiment using four calves (Boray 1967) that cattle can become resistant to reinfection with F. hepatica.

It is only very recently that there has been any evidence to suggest that a specific immune mechanism may be implicated in this resistance. Corba, Armour, Roberts, Urquhart (1971) infected one of a pair of monozygous calves on two occasions with 1000 metacercariae of F. hepatica. Fourteen weeks after the second infection the calf was slaughtered and its sera and hepatic lymph nodes removed. The hepatic lymph node cells were then injected into the other twin calf and the sera injected into an unrelated calf. Both of these calves and an untreated control calf were then each infected with 1000 metacercariae of F. hepatica. At slaughter 23 flukes were recovered from the liver of the calf receiving the lymphoid cells, 100 flukes were recovered from the calf which received the sera and 113 flukes were recovered from the untreated control calf.

This represented an 80% reduction in the fluke burden of the calf receiving the lymphoid cells and while the results are by no means conclusive since so few calves were used they may provide the first indication that immunity plays some part in the acquired resistance of cattle to infections with F. hepatica.

Sheep

While cattle have been described as being resistant to infection with F. hepatica sheep have been described (Taylor 1964) as having little or no resistance to infection with F. hepatica.

Durbin (1952) infected sheep each with 100 metacercariae of what was presumed to be F. hepatica and recovered 21 and 19% of the initial dose from the livers of sheep dying 7 and 11 years after infection in the absence of any reinfection.

Ershov (1959) attempted to protect lambs by vaccination with a fluke polysaccaride-albumin complex. He reported finding a 10-25% of the lambs to be immune to infection but that the induced immunity did not

persist longer than 45 days after vaccination. No experimental evidence was cited to support these statements.

Hughes (1963) vaccinated 10 sheep with a series of nine intraperitoneal infections of a fluke antigen over a period of three weeks. The vaccinated sheep and 10 untreated control sheep were each infected with 200 metacercariae of F. hepatica some five weeks later. At post-mortem four months after reinfection no significant reduction was found in the numbers of flukes recovered from the vaccinated sheep as compared to the number recovered from the control sheep. Hughes (1963) also attempted vaccination of sheep by administring 2 doses at a 14 day interval of 500 metacercariae of F. hepatica x-irradiated with 3 Kr. Seven weeks after the second vaccinating dose 10 of these sheep and 10 unvaccinated sheep were each infected with 200 metacercariae of F. hepatica. At slaughter 16 weeks later no significant difference was found between the fluke burdens of both groups of sheep.

Ross (1967b) attempted to vaccinate lambs using implants of 6 week old viable F. hepatica into the intercostal muscles. Three lambs together with uninfected lambs were each infected four weeks later with 100 metacercariae of F. hepatica. Ten weeks later 11% of the infecting dose was recovered from the vaccinated lambs as compared to 22% recovered from the controls. This difference is only statistically significant at the 10% level.

Boray (1967) repeatedly infected sheep with large doses of metacercariae of F. hepatica x-irradiated with 20 Kr. Another group of sheep were given three doses each of 1000 metacercariae of F. hepatica and were treated with an anthelmintic 5 weeks after each infection. Each group of sheep together with non-vaccinated control sheep were then each infected with 4000 metacercariae of F. hepatica. All the sheep in the experiment died of acute or subacute fascioliasis and at post-mortem there was no significant difference between the acquired fluke burdens of any of the groups of sheep. The reinfected sheep however lived longer,

their anaemia developed later and less severe tissue reaction was present than in the control sheep. Boray (1968) infected nine sheep each with 500 metacercariae of F. hepatica and then terminated this infection with an anthelmintic twelve weeks later. Five of these sheep were each reinfected with 1000 metacercariae and four were each reinfected with 200 metacercariae of F. hepatica as were previously uninfected control sheep. No significant difference was found in the survival times or percentage of the reinfecting dose recovered from the reinfected sheep or the controls.

Sinclair (1968, 1970) investigated the existence of a resistance to F. hepatica infections in sheep by using various methods of immunosuppression in an attempt to exacerbate the disease. A group of sheep were treated with corticosteroid and a second group of sheep were splenectomised prior to infection with F. hepatica. The former sheep received 500 metacercariae of F. hepatica and the latter 3000 metacercariae of F. hepatica. The pathogenic effects of these infections as indicated by weight loss and anaemia were more severe in the immunosupressed sheep and the flukes recovered from these sheep were significantly bigger than those recovered from the normal infected control sheep.

Sinclair (1971a) infected 10 sheep each with 300 metacercariae of F. hepatica and 4 weeks later terminated the infection with an anthelmintic. Five weeks later these and 10 previously uninfected control sheep were each infected with 600 metacercariae of F. hepatica. Pairs of sheep were killed 6, 8, 10, 12 and 14 weeks after infection. Sinclair proposed that there was some evidence of an acquired resistance in the reinfected sheep on the basis of a reduction in the growth rate of flukes in reinfected sheep at about 8 weeks after infection and also the earlier appearance of lymphocytic and fibroblastic activity in the livers. There was no

significant difference however in the acquired fluke burden of both groups of sheep.

Sinclair (1971b) then attempted to transfer this proposed resistance to previously uninfected sheep by using the cell in spleen or lymph node homogenates from sheep previously infected with 500 metacercariae of F. hepatica. The sheep receiving the lymphoid cells and a group of control sheep were then each infected with 500 metacercariae of F. hepatica. Only minor differences in the weight gains, packed cell volumes, eosenophil counts and faecal loss of radioactive labelled plasma were found between both groups. At post-mortem eight weeks later no significant differences were found in the acquired fluke burdens of both groups of sheep.

The evidence available at present in the literature indicates that while some immunological response occurs during infection of sheep with F. hepatica and that suppression of this response results in an exacerbation of the disease no method of vaccination has been discovered which can confer significant protection in sheep against infection with F. hepatica.

Mice

A considerable amount of experimental investigation into the stimulation of an acquired immunity of F. hepatica in mice has been reported in the literature. The mouse is highly susceptible to infection with F. hepatica and develops a disease similar to that described in sheep and cattle. No evidence has been put forward to show that mice can acquire any resistance to a single infection with F. hepatica.

Hughes (1962, 1963) attempted to immunise mice using 2 vaccinating doses of 20 metacercariae of F. hepatica x-irradiated with 4 Kr. The vaccincated mice and a group of unvaccinated control mice were infected 3 weeks later with 10 metacercariae of F. hepatica. No evidence of the

development of an acquired immunity was found in this experiment. The experiment was then repeated using 2 vaccinating doses of 40 metacercariae of F. hepatica irradiated with 3 Kr. and subsequent challenge with 10 normal metacercariae. Again no significant difference was found between the acquired fluke burdens of vaccinated and control mice. Hughes then attempted to passively immunise mice with 1.5 ml. of heterologous serum from rabbits infected with F. hepatica. The treated and untreated control mice were then each infected with 5 metacercariae of F. hepatica. At post-mortem there was no significant difference in the fluke burdens of each group of mice.

Dawes (1964) infected fourteen mice each with 40 x-irradiated (3 Kr.) metacercariae of F. hepatica. Three weeks later he infected nine of these mice with 10 metacercariae of F. hepatica and the remaining five mice each with 5 metacercariae of F. Hepatica. The mice were killed serially over the next 8 - 53 days. Dawes reported finding no immunity to the challenge infection but this conclusion was based on comparison of the numbers of flukes recovered from the vaccinated mice with the numbers of flukes recovered from a group of control mice in an experiment previously described by Hughes (1963).

Lang (1966, 1967, 1968) Lang, Larsh, Weatherly, Goulson (1967) carried out extensive studies on the immunisation of white mice against infections with F. hepatica.

Lang (1967) immunised 96 mice on two occasions 60 days apart with 2 metacercariae of F. hepatica. These and previously uninfected control mice were then infected forty days later with a further 2 metacercariae of F. hepatica. At 20 and 40 days after challenge selected mice from both groups were killed to estimate their acquired fluke burdens. The vaccinated mice were found to have significantly fewer flukes 40 days after infection than did the control mice. The different fluke populations

of the immunising and challenge doses were decided on a basis of fluke size, a procedure however which is open to a substantial margin of error. Lang also found differences in the body weights, total and differential leucocyte counts, spleen weights, haemaglutinating antibody titres and liver histopathology between the groups of mice. It was postulated on the basis of the histopathology that a delayed type hypersensitivety reaction may have played some part in the immune response.

Lang (1966) then investigated the possibility that flukes were damaged by this immune response in mice. Seventeen day old flukes were transferred from the livers of previously immunised or previously uninfected mice into the peritoneal cavities of two groups of mice. A mouse died in each group after the transfer of these flukes. At the end of the experiment however no flukes were recovered from the remainder of the mice in Group 1 which had received the flukes from the immunised mice whereas nine out of twelve flukes transferred from previously noninfected mice were recovered from the mice in Group 2. This result was taken to indicate that the flukes taken from the previously immunised mice were damaged in such a way as to prevent their maturation in a normal mouse.

Lang et al (1967) attempted to transfer the immunity from previously infected mice using peritoneal exudate cells from these mice. A group of 10 normal mice were given 2.75×10^6 peritoneal exudate cells from previously infected mice by the intraperitoneal route and challenged 21 days later with 2 metacercariae of F. hepatica as were a group of untreated control mice. All mice were killed 40 days later when a total of 8 flukes were recovered from the 10 treated mice and 12 flukes were recovered from the 8 untreated control mice. Lang found that this difference was significant at the .001% level in the Student's 't' test. No details were given about the compatability of the mice used for the cell transfer

nor were any control groups used to eliminate the possibility that the reduction of the fluke burden found was due to involvement of the flukes in a graft-versus host type of reaction in the recipient mice. It is also unclear exactly what type of cell was being transferred in the peritoneal exudate, they may have been sensitised macrophages, immunologically competent lymphoid cells or most probably a mixture of both cell types.

Lang (1968) repeated his previous reinfection experiment using larger groups of mice and described finding that a significant reduction in the acquired fluke burden of the reinfected mice could be found by 25 days after challenge. Again the differentiation of the fluke populations derived from the initial and challenge infections was on the basis of fluke size.

Lang considered on the basis of all these experiments that specific antigens are released by the immature flukes from 8-17 days after infection which are responsible for the induction of an acquired immunity in the white mouse to infection with F. hepatica. This immunity he considered manifests itself between 20-25 days after challenge and involves a delayed type hypersensitivity reaction.

It is difficult to reconcile the different results obtained by Hughes and Lang especially as at the level of x-irradiation used by Hughes allowed development of part of the vaccinating dose to maturity (Hughes 1963). Hughes' vaccination schedule would therefore fulfill Lang's criteria for the induction of an acquired immunity in mice. In view of the recent advances in the understanding of the cellular mechanisms involved in both humoral and cellular immunity the evidence presented by Lang to implicate a delayed type hypersensitivity response would not be considered sufficient.

The work on acquired immunity to F. hepatica infections in mice can perhaps best be summarised by concluding that it is perhaps possible to induce such an immunity in the mouse but that this urgently requires some further confirmation.

Rabbits

The rabbit has also been widely used as an experimental model for investigating acquired immunity to infection with F. hepatica.

Kerr and Petkovitch (1935) immunised rabbits with intraperitoneal injection of a suspension of dried flukes. Seven immunised and three control rabbits were then each infected with 13 metacercariae of F. hepatica. At autposy a mean of 2.14 flukes were recovered from each vaccinated rabbit as compared to a mean of 8.35 flukes from the control rabbits. At no point were eggs found in the faeces of the vaccinated rabbits and at autopsy some of the flukes recovered were calcified. A normal patent period and undamaged flukes were found in the control rabbits.

Urquhart, Mulligan, Jennings (1954) vaccinated rabbits with an alum precipitated saline extract of flukes. Seven rabbits received 3 injections and a further seven rabbits received 6 injections of this extract. The immunised and control rabbits were each infected with 50 metacercariae of F. hepatica. The vaccination procedure produced high levels of precipitating antibody in the sera of the rabbits. It did not however reduce the number of flukes recovered from the vaccinated rabbits at atuposy as compared to the fluke burden of the controls although there was some inhibition of development of the flukes in the vaccinated animals as judged on their size and total protein nitrogen content.

Healy (1965) also vaccinated rabbits with extracts of F. hepatica and subsequently challenged these and control rabbits each with 50 metacercariae of F. hepatica. No significant difference was found between the fluke burdens of both groups of rabbits 60 days later. It was suggested however that there was some evidence of retardation of development of the flukes in the vaccinated rabbits up to their entry into the bile ducts at approximately 35 days after infection. No evidence was provided to support this observation.

Hughes (1963) vaccinated 4 rabbits with 2 doses of 500 irradiated (4 Kr.) metacercariae fourteen days apart. The vaccinated and control rabbits were each infected four weeks later with 15 metacercariae of F. hepatica. No differences were found between the required fluke burdens of both groups of rabbits at autopsy 10 weeks after challenge.

Hughes (1963) also attempted vaccination of rabbits with nine intraperitoneal injections over a 3 week period with 1 ml. of a 1% fluke extract. In the first experiment 9 vaccinated and 5 control rabbits were each infected with 13 metacercariae of F. hepatica and subsequently a mean of 1.7 flukes were recovered from the vaccinated rabbits as compared to a mean of 5.6 flukes from the control rabbits. The experiment was then repeated using vaccinating doses of 2, 1 or .5 ml. in the same vaccination procedure and the challenge dose was 15 metacercariae of F. hepatica. In this experiment however no significant difference was found between the numbers of flukes recovered from any group of vaccinated rabbits and the group of control rabbits.

Kendall, Herbert, Parfitt, Pierce (1967) initially infected rabbits each with 10 metacercariae of F. hepatica and 12 weeks later reinfected these rabbits and appropriate control rabbits each with 200 metacercariae of F. hepatica. At autopsy 20 weeks after the initial infection the number of flukes recovered from the reinfected rabbits was less than the expected fluke burden as judged by summation of the fluke recoveries from the control

rabbits for infections with 10 and 200 metacercariae of F. hepatica. The authors noted that it was impossible to decide if the flukes of the challenge infection in the reinfected rabbits had been destroyed or simply inhibited in their development thus were more difficult to recover and count.

Ross (1967b) vaccinated twelve rabbits with 2 subcutaneous injections fourteen days apart of 1 ml. of a 6 weeks old fluke homogenate. Three weeks later vaccinated and control rabbits were each infected with 20 metacercariae of F. hepatica. The mean percentage take in both groups of rabbits was not significantly different but there was some evidence of retardation of the development of the flukes in the vaccinated rabbits between 6-7 weeks after infection. Ross then repeated the experiment using eleven rabbits given 5 subcutaneous injections of a homogenate of adult flukes and also implanted a 6 week old viable fluke into the gluteal muscle of six of these rabbits. The vaccinated and control rabbits were each infected with 50 metacercariae of F. hepatica and killed 8 weeks later. No significant difference was found in the fluke burden of the rabbits vaccinated with homogenate alone and the fluke burdens of the control rabbits. There was however a reduction statistically significant at the 20% level between the acquired fluke burdens of the vaccinated and implanted rabbits as compared to the controls.

Kendall and Sinclair (1971) infected 41 rabbits each with 10 metacercariae of F. hepatica and then treated them with hexachlorophene 84 or 86 days later to remove the flukes present. Eighty-eight days after their initial infection these and control rabbits were each infected with 20 metacercariae of F. hepatica. At autopsy the anthelmintic treatment was found to have been only partially effective and the mean

number of flukes recovered from the rabbits which received on the first infection and hexachlorophene was subtracted from the mean numbers of flukes recovered from the reinfected rabbits. After this adjustment a significant reduction was found between the mean numbers of flukes recovered from the reinfected rabbits as compared to the previously uninfected control rabbits.

There appears therefore to be little consistency in the results of attempted immunisation of rabbits against F. hepatica. The only conclusion that can be drawn is the rabbits might on some occasions have been resistant to infections with F. hepatica but that much more confirmatory evidence must be provided before this conclusion could be accepted without reservations. The description of increased liver damage in rabbits immunosuppressed using anti-lymphocytic serum and infected with F. hepatica (Dodd and O'Nuallain 1970) may indicate that an immunological mechanism may be responsible for limiting the migratory activities of the flukes in the rabbit.

Rats

The rat has also been used as an experimental model for investigation of the immune response to infections with F. hepatica.

Thorpe and Broome (1962), Thorpe (1963) irradiated metacercariae of F. hepatica with either 1, 2.5, 5, 7.5 or 10 Kr. These metacercariae were then administered at a dose level of 10 per rat to give 5 groups of thirty rats corresponding to each level of irradiation. Seven weeks after vaccination 15 rats of each group and 10 normal rats were each infected with 20 metacercariae of F. hepatica. Eleven weeks after vaccination the remaining 15 rats in each group and a further 10 normal rats were each infected with 20 metacercariae of F. hepatica. The rats were killed ten weeks after the challenge infection was given. A similar number of flukes were recovered from the rats which had received the

metacercariae irradiated at 1 Kr. prior to challenge and a group of rats which had received only the irradiated metacercariae and were not subsequently challenged. This was interpreted as showing that no flukes from the challenge infection had developed in the vaccinated mice. At an irradiation level of 2.5 Kr. means of .6 and .4 flukes were recovered from the rats only receiving the vaccinating metacercariae. The vaccinated and challenged rats had mean recoveries of 2.7 and 1.1 flukes as compared to mean recoveries of 4.5 and 2.0 flukes from the infected control rats. Thorpe considered that this represented a 50% reduction in the acquired fluke burden of the vaccinated rats. Macroscopic liver damage was also much reduced in the vaccinated rats as compared to the challenge control rats. No evidence of acquired immunity was found in the groups of rats receiving metacercariae irradiated at 5, 7.5 or 10 Kr.

Recently Corba et al (1971) have described the experimental transfer of an induced immunity to F. hepatica to rats using a lymphoid cell suspension from previously infected rats. Cells were taken from rats with a pre-bile duct infection of F. hepatica, from rats with a patent infection of F. hepatica and from rats which had received 3 immunising doses of irradiated (2.5 Kr) metacercariae of F. hepatica. Following the cell transfer the rats were challenged with either 20 or 30 metacercariae of F. hepatica as were untreated control rats. It was found that lymphoid cells transferred from rats 18-20 weeks after infection with F. hepatica into syngeneic recipients conferred a statistically highly significant protection as judged by the fluke numbers recovered from the control rats (9.3) as compared to the treated rats (3.1). Lymphoid cells however failed to confer protection in rats when transferred from rats with a pre-bile duct infection.

Cells tranferred from the rats receiving the irradiated metacercariae conferred complete protection on the recipient rats and no flukes were recovered from these rats at post-mortem while a mean of 2-6 flukes were recovered from the control rats. The rats receiving the irradiated metacercariae were themselves markedly immune to a challenge infection with 20 metacercariae of F. hepatica as a mean of .5 flukes were found in these rats as against 2.6 flukes found in the controls.

There appears to be conclusive evidence therefore that rats can develop a significant immunity to F. hepatica infections under defined conditions and that this immunity is transferrable by lymphoid cell suspensions to previously uninfected rats.

The results of Corba et al (1971) are important in two respects. In the first instance they represent the first description of the induction of an acquired resistance to F. hepatica in animals which have not previously been exposed to the parasite and consequently suffered some degree of liver damage. The acquired resistance demonstrated in these experiments must therefore be immune in nature as it cannot be attributed to previous liver damage rendering the environment unsuitable for further infections.

The failure of lymphoid cell suspensions from rats with a pre-bile duct infection to confer an immunity to recipient rats represents the first significant evidence that antigens released by the mature parasite may be responsible for the induction of a successfull immunity.

The successful development of an acquired resistance to infections with F. hepatica has been demonstrated therefore only in a very limited number of experiments in the rat, mouse and bovine and only in the rat and perhaps the mouse has an immunological response been implicated in this resistance. The oral administration of viable metacercariae which then develop for at least a limited period in the liver would appear to be necessary for the successful induction of an acquired resistance. This observation is consistent with what has already been discovered about helminth immunity in that, with one important exception which will be discussed later, attempted immunisation of animals with extracts or homogenates of helminth parasites has proved uniformly unsuccessful. The only successful helminth vaccines currently available use irradiated attenuated larvae which migrate for a limited period in the host. It is perhaps only in this way that the appropriate parasite antigens are made available to the relevant lymphoid tissue in the host in order to successfully stimulate an immune response either cellular, humoral or both. There is only a limited amount known about the mechamisms involved in the induction and expression of an immune response against helminth parasites and a brief review of the relevant literature may serve to define possible mechanisms of the host response to infections with F. hepatica.

Schistosomes are trematode parasites with water snails as the intermediate hosts. The cercariae penetrate the skin of the final host and then migrate with the circulation to a final location in the portal veins. The host-parasite relationship between S. mansoni and the rhesus monkey Macaca mulatta has been the most intensively studied with

regards to the mechanisms of the immune responses of the host. In this system a large proportion of the infective dose will develop to maturity. The faeces egg counts reach a maximum between 8-12 weeks after infection then it falls sharply but persists at a low level for up to a year. Rhesus monkey can develop a complete immunity to reinfections with S. mansoni following a single infection with this parasite (Smithers and Terry 1969). The initial infection will become patent and persist through the destruction of the challenge infections, a phenomenon termed 'concomitant immunity'.

In this host parasite system some of the possible immunological responses of the host have been studied using a sophisticated system involving transfer of adult worms from the portal veins of an infected host to the portal system of a previously uninfected host. It has been shown in this way that the adult worm is responsible for the induction of a protective immunity to S. mansoni in the rhesus monkey (Smithers and Terry 1967).

Another finding using this host transfer system is that worms transferred from hosts such as mice into rhesus monkeys previously immunised against the original hosts cells can be shown using electron microscopy to suffer very severe tegumental damage within hours of the transfer taking place. The damage has been shown to be antibody mediated by the location of ferritin labelled antibodies at the sites of tegumental damage. (Smithers and Terry, 1969, Smithers, Terry Hockley 1969).

Since the antiserum was raised against tissue antigens of the original host, these antigens or similar parasitic antigens must be adherent to or incorporated into the tegument of these transferred worms. This somewhat

artificial system has shown that antibodies stimulated in monkeys by means other than a previous infection would destroy transferred worms and provided evidence that schistosomes were susceptible to the action of circulating antibody. It has now recently been shown (Hockley and Smithers 1970) that by infecting a monkey and a short while later transferring these worms into a hyperimmune monkey that similar tegumental damage occurs but it is less severe and more focal in nature than demonstrated in the previous system.

This observation of the small numbers of antigens recognised when worms are transferred from one monkey to a hyperimmune monkey as compared to the large number recognised when worms were transferred from a different host species into a monkey immune to the original host would appear to suggest that in a natural infection the monkey can recognise only a few of the antigenic sites on the tegument of the worm. The apparent inability of a host to recognise and mount a significant immune response against some parasitic antigenic determinants has been discussed at some length in the literature.

Dineen (1963) considered that in any host-parasite relationship the immunological response of the host provided an environment for the selection and evolution of genetic variants of the parasite physiologically and pathologically best suited to the host-parasite relationship. This evolution would eliminate the parasitic antigens which stimulated an effective immune response in the host and the parasite would eventually appear in the form which provided minimal antigenic stimulation of the host.

Damian (1964) after an extensive review of host-parasite relationships developed a premise similar to that proposed by Dineen (1963) Damian however considered that some parasites may have evolved a system for selection and formation of antigens similar to those of their host from some basic pool of genetic information. These antigens were termed 'eclipsed antigens' and were defined as antigenic determinants of parasitic origin which resemble antigenic determinants of their hosts to such a degree as not to stimulate antibody formation. Damian also warned against the possibility of confusing contaminating host antigens with true parasitic antigens.

Capron, Biguet, Vernes, Afchain (1968) reported an extensive investigation of cross reactions between host and parasite antigens using immunoelectrophoretic analysis. It was proposed that the common antigenic determinants which were found between various hosts and their parasites were so few in number and occurred with such regularity that they could not be due to chance contamination from a large possible number of host contaminating antigens. Capron et al disagreed with the previous hypothesis of parasitic adaptation and proposed instead that an inductive process is responsible in the parasites for the formation of latent protein and/or enzyme systems providing the parasite with antigenic determinants similar to its newly acquired host.

Smithers, Terry, Hockley (1969) in the series of experiments using worms transferred from one host to another noted that if worms were transferred from mice into normal monkeys they would eventually develop normally, if however, the worms were transferred into monkeys immune to mouse antigens then the transferred worms died within hours of transfer.

It was also found that if worms from mice were transferred into normal monkeys and a week later transferred into anti-mouse monkeys no damage occurs and the worms develop normally. This was taken as evidence for the loss of acquired mouse antigens and the adoption of monkey antigens within the period of a week. Smithers et al considered that this finding would support the 'inductive' hypothesis of Capron et al rather than the genetic selection theory of Dineen and Damion. Smithers et al then proposed another alternative explanation of their finding termed a 'selective contaminative hypothesis'. This proposes that the host antigens found in schistosomes were in actual fact synthesised by the host and then firmly bound to the surface or incorporated into the tegument of the parasite.

The possibility of a parasite adapting its antigenic structure to make it 'antigenically invisible' to its host is one of the most intriguing questions in parasitic immunity but at the moment no conclusive evidence is available to suggest how and when it may occur. The genetic adaptation of a parasite to its host is one of the most intriguing questions in parasitic immunity but at the moment no conclusive evidence is available to suggest how and when it may occur. The genetic adaptation of a parasite to its host could explain the apparent differences between F. hepatica infections in the ovine and bovine. It could be proposed that genetically the parasite is best adapated to the ovine and consequently less well adapted to the bovine so allowing a sufficient antigenic disparity to occur which can stimulate an effective immune response.

One of the most significant advances in the immunology of parasitic disease in recent years has been the successful induction of a functional immune response in a host by immunisation with purified antigenic material from a parasite. As has been discussed before the many previous attempts to immunise hosts with extracts or homogenates of parasites had been

uniformly unsuccessful. Recently however isolated parasitic antigens have been used successfully to induce an immunity against Trichinella spiralis infections in mice. The gravid female T. spiralis worm penetrates through the mucosa of the intestine into the lymph spaces where the female sheds viable larvae which have developed from the egg in utero. These larvae are distributed throughout the body via the lymphatics and bloodstream. The larvae which reach the voluntary muscles develop further and eventually encyst. The life cycle is completed by the ingestion of affected muscle by another host and the larvae encyst or grow to maturity in the intestine.

Despommier and Wostmann (1968, 1969) described an experimental system where mature T. spiralis muscle larvae were placed in diffusion chambers which were then implanted in the peritoneal cavity of mice. The mice were found, after 7 days of implantation, to have become immune to challenge infections with T. spiralis and showed a significant reduction in both developing muscle larvae and adults. The immunity persisted for at least 6 months after the removal of the chambers. Exposure to diffusion chambers for 5 days allowed maximal development of immunity against muscle larval stages while further increasing the exposure time reduced the number of adults developing. Despommier and Muller, (1970a, b) then located the functional antigens of the T. spiralis muscle larvae to the granule fraction of the stichocyte cell of the stichosome. Two types of granules were isolated an alpha and beta granule, each distinguished on size. Immunisation of mice with the alpha granule alone resulted in an 80% reduction in their acquired infection and immunisation of mice with both granule fractions resulted in an 90-95% reduction in their acquired infection. Further purification of these granules located the functional antigen in a B type of granule. Despommier (1971) then went on to show that only the mature muscle larvae and the adult worm possessed this antigen and that only these stages of the parasite were affected by the immune response.

This isolation and successful use of a functional antigen is perhaps the most encouraging result in the recent investigations of the immune response to parasites as it provides a positive indication that only a few antigens from the complex antigenic mosaic of any parasite may eventually be responsible for the induction of a successful immune response in the host species.

Both the schistosomes and T. spiralis have a final location within the host tissue easily accessible to circulating antibody or immunologically committed cells. F. hepatica has 2 stages in its migration in the final host which are functionally outside the host on the external surface of a mucous membrane. The first is an excysting metacercariae in the intestine and the second and most important is as an adult fluke in the bile ducts. The accessibility of the parasite at these stages to an immune response is consequently much more limited than that of a parasite whose final location is within host tissue. There has been an extensive study using Nippostrongylus brasiliensis infections in the rat as a laboratory model into the mechanisms of the immune response at mucous surfaces. The third stage larvae of this nematode parasite penetrate the skin of the host then migrate via the bloodstream and lungs to a final location in the small intestine of the rat.

The pattern of expulsion of N. brasiliensis from rats with a primary infection with this parasite was desdribed by Mulligan, Urquhart, Jennings, Neilson (1965). Rats infected with N. brasiliensis will expell the majority of their acquired worm burden between 10-20 days after infection and this expulsion is preceded by a sharp fall in the egg output of the worms. It was also shown that this immunity could be passively transferred using serum from previously infected rats. Ogilvie and Hockley (1968)

confirmed the immune nature of the expulsion by showing that when worms were repeatedly transferred at 4 days intervals into normal rats after 20 days no significant reduction in egg output or worm burden could be demonstrated.

Various aspects of the damage suffered by these worms have also been described. Ogilvie and Hockley (1968) using a system in which worms were transferred at various intervals after infection into the small intestine of normal rats described several aspects of this immunologic damage. It was found that worms taken from rats 10 days after a primary infection with N. brasiliensis had suffered irreversible damage and could not re-establish themselves in normal rats. The damaged worm showed vaccuolation in their gut cells both on light and electron microscopy. These studies were extended by Lee (1969) who noted structural changes in the cytology of the gut cells, in the male reproductive tract and an increase in the lipoid vacuoles in the cytoplasm of the hypodermis, muscles, excretory gland, reproductive system and intestine. Lee (1971) found that damaged worms had a significantly higher neutral fat content on chemical examination than did undamaged worms and discussed the possibility of this being the result of a move of the parasite away from the gut wall to a position where anaerobic respiration was necessary resulting in an increase in the amount of lipid which could not be metabolised. Henney, MacLean, Mulligan (1971) showed that damaged worms had a reduced uptake of radioactively labelled inorganic phosphate as compared to normal worms and that this difference became apparent as early as 7 days after infection. Edwards, Burt, Ogilvie (1971) then described changes in the iso-enzymes of acetylcholinesterase in irreversibly damaged worms from immune rats and proposed that this was due to immune damage either to the enzyme or its biosynthesis pathway. It was proposed that this enzyme may be the antigen responsible for the induction of immunity in the rats infected with N. brasiliensis..

There is then a considerable amount of evidence to show that immune damage can occur to parasites on the external surface of a mucous membrane but unfortunately little or no evidence available to define the means by which this damage occurs.

In conclusion it can be noted that there is evidence to suggest that F. hepatica as a trematode should be vulnerable to immune damage and that immune mechanisms exist which can involve parasites on the external surface of a mucous membrane. It is uncertain which stage of the parasite could be considered responsible for the induction of a successful acquired resistance but there is some evidence to suggest that the adult trematode may be the main antigenic stimulus to the host.

A series of experiments were devised to investigate the response of parasite free calves to infection with F. hepatica and their subsequent resistance to reinfection at various times after their initial infection in the presence or absence of the fluke population derived from the initial infection. The level of disease produced in the calves by these infections was assessed on the basis of an interference with weight gains, development of an anaemia, hypoalbuminaemia, hypergammaglobulinaemia and the serum glutamic oxaloacetic transminase level was used as an indication of hepatic cell necrosis. (Cornelius and Kaneko 1963).

MATERIALS AND METHODS

A Experimental Animals

(1) The rearing and maintenance of parasite free animals

Dairy calves aged three to seven days old were selected from groups of market calves on a basis of high serum immunoglobulin levels as determined by the zinc sulphate turbidity test (McEwan, Fisher, Selman, Penhale 1968). The level of 20 Z.S.T. units was taken as the minimum level required by the calf to give good protection against the lethal effects of calf diarrhoea (Gay, Anderson et al, 1965). The calves were housed individually in galvanised iron huts and fed warm whole milk at the rate of one pint of milk per 10 lb. bodyweight per day for the first two months of life. Hay, water and calf weaner pellets (British Oil and Cake Mills Ltd., Renfrew, Scotland) were provided from the beginning of the third week onwards. Milk was withdrawn over the eightweek and the amount of hay and concentrates increased, the calves being allowed concentrate feeding ad lib. At ten weeks of age the calves were housed together in calf-pens and remained housed in this accommodation till increasing size necessitated moving some calves into loose boxes where they stayed till the end of the experiment. The concentrate fed was changed gradually from calf weaner pellets (British Oil and Cake Milles Ltd., Renfrew, Scotland) to calf rearer pellets (B.O.C.M. Ltd., Renfrew, Scotland) from the fourteenth week onwards. The calves were given free access to concentrates and hay till five months of age when concentrate feeding was restricted to 8 - 10 lbs. per day.

(2) Weighing Procedures

The calves were weighed at the same time on the same day each week to give as consistent recordings as possible during the experiments. Young calves weighing up to 400 lbs. were weighed on a Gascoinge calf weigh balance (Gascoigne, Reading, England) but when over this weight they were

weighed on a Gascoigne 4 Point Weight Crush (Gascoigne, Reading, England) which was accurate to 10 lbs.

B. Haematological Estimations

(1) Collection of Samples

Blood samples were always collected from the jugular vein. Samples for routine haematology were collected into a bijou bottle (5 ml.) containing .1 ml. of 11% solution of ethylenediamine-tetracetate (E.D.T.A.) which had been allowed to evaporate to dryness at room temperature. Two to three mls. of blood were collected in the bottle which was then stoppered and shaked gently to dissolve the anticoagulant. Haematological examinations were carried out within four hours of the sample being taken.

Samples from which serum was to be collected were collected into 25 ml. Universal bottles which contained no anticoagulant. About 20 ml. of blood was collected into each bottle and the samples were allowed to clot and the clot contract by leaving them standing overnight at room temperature. The samples were then centrifuged for 10 minutes at 2,000 r.p.m. in a M.S.E. centrifuge. (Measuring Scientific Equipment, London, England). The serum was then drawn off using a pipette into 5 ml. bottles and stored at -20°C.

(2) Packed Cell Volume (P.C.V.)

The packed cell volume percentage was obtained using the micro-haematocrit method as described by Fisher (1962).

(3) Haemoglobin Concentration (Hb)

The haemoglobin concentration in grams per 100 ml. was determined using the oxyhaemoglobin method described by Dacie and Lewis (1966). The oxyhaemoglobin content was estimated by using an E.E.L. colorimeter (Evans Electro-selenium Ltd., Harlow, England) with a yellow-green filter

(Ilford, No. 625). The colorimeter was calibrated using the standard cyanmethaemoglobin solution (cyanmethaemoglobin standard solution to B.S. 3985 B.D.H., Poole,Dorset, England). The standard solution was used before each set of samples were read to check for error in the colorimeter.

(4) Total Red Cell Counts

Total cell counts were made on an electric particle counter (Coulter Model D, Coulter Industrial Sales Co., Elmhurst, Illinois, U.S.A.) which had been calibrated for bovine blood cells by the method described by Crighton (1965).

(5) a) Mean Corpuscular Volume (M.C.V.)

The mean corpuscular volume was derived from the following formula

$$\frac{\text{Packed Cell Volume \%} \quad \times 10}{\text{Total red cell count (millions/cu.mm.)}}$$

and expresssed in units of cubic microns (c.u)

b) Mean Corpuscular Haemoglobin Concentration (M.C.H.C.)

The mean corpuscular haemoglobin concentration was derived from the following formula

$$\frac{\text{Haemoglobin conc. (grams./100 ml.)} \times 100}{\text{Packed cell volume \%}}$$

and expressed as a percentage

C. Biochemical Estimations

(1) Total Serum Protein Concentration

The total protein concentration was estimated by the biuret method of Wiechselbaum (1946) using an E.E.L. Spectra colorimeter (Evans Electroselenium Ltd., Harlow, England) at a wavelength of 540 mu. The calibration was carried out using a standard solution of known protein concentration ("Versatol" W.R. Warner & Co. Ltd., Eastleigh, Hampshire, England), and the results expressed as gms. of protein/100 ml. of serum.

(2) Serum Protein Electrophoresis

The serum proteins were separated by electrophoresis using cellulose acetate strips (Oxoid Ltd., London, England) with n-octoate barbitone buffer (pH 8.6) in horizontal electrophoresis tanks (Shandon, London, England). Serum was applied to the strip in amounts of .0025 - .004 ml. using graduated pipettes (Shandon, London, England) and a current of 1 m.A./strip was applied for two hours using a Vokam power pack (Shandon, London, England). The strips were then removed from the tanks, fixed in 5% aqueous trichloracetic acid for 10 minutes then stained with .2% Ponceau S. electrophoresis stain (G.T. Gurr, Ltd., London, England) in aqueous trichoracetic acid for 5 minutes. The strips were washed clean of excess stain in 3 baths of 5% aqueous acetic acid. They were then evaluated automatically as described by Neill (1963) using a Chromoscan recording densitometer constituents. The quantitative values for albumin and total globulins were derived from the product of these proportions and the value for the total serum protein concentration. The values for serum albumin and total globulin are expressed in grams per 100 ml.

(3) Serum Glutamic-Oxalecetic transaminase (SGOT) Estimation

The SGOT levels were estimated using the colorometric method described in the Sigma Technical Bulletin No. 505 (Sigma, London Chemical Co. Ltd., London, England). The SGOT catalyses a reaction resulting in the formation of oxaloacetic acid. This then decomposes to pyruvic acid and a reaction with 2-4 dinitrophenylhydrozine forms the hydrazone which itself forms an intense brown colour on the addition of sodium hydroxide. The optical density of the reaction was measured in a Unicam S.P. 600 spectrophotometer (Pye Unicam, Cambridge, England) at 505 mu. and the results expressed in Sigma Frankel (S-F) units.

D. PARASITOLOGICAL TECHNIQUES

1. Production of metacercariae

The metacercariae used in Experiment 1, 2 and 3 were produced by the method described in detail by Reid (1968). Lymnaea truncatula were reared on mud slopes in polythene lined boxes and each box was constantly irrigated by a stream of deionised water. The snails were fed once a day and the food was made up of 4 parts milk powder (Cow and Gate, Guildford, England), 3 parts Farex (Glaxo Laboratories, Greenford, England), 1 part Bemax (Vitamins Ltd., London, England) and powdered sodium carbonate to about one quarter of the total volume. The ingredients were mixed and ground in a mortar and pestle.

Miracidiae were obtained by hatching F. hepatica eggs recovered from sheep bile under stimulus of light. When the snails were 3-5 mms. long they were each infected with 4-5 miracidiae then replaced on a freshly prepared mud slope. It took an average of 8 weeks for cercariae to develop in the snails. The infected snails were stimulated to shed the cercariae by placing them on melting deionised ice standing in a cellophane lined box. As the cercariae leave the snail they encyst as metacercariae on the cellophane and the cellophane and metacercariae were stored at $4^{o}C$. in deionised water.

The number of metacercariae produced by each snail kept under these conditions was low and their infectivity in the first three experiments was never greater than 16% although takes of 51% had been described in experimental infections of calves previously (Dixon, 1964).

2) Counting and Administration of Metacercariae

The metacercariae adhering to the cellophane strip were counted using a dissecting microscope and the doses were made up of strips of cellophane with their adherent metacercariae. These cellophane strips were dried on filter paper just prior to administration and were then folded into a piece of filter paper for the purposes of administration. The doses were administered to the calf using a dosing gun which was simply a 2 foot length

of $\frac{1}{2}$" diameter rubber hose with a thin cane as a plunger. The dose in its filter paper was pushed into the end of the hose, the calf's mouth opened, its tongue depressed and the end of the gun pushed down into the pharynx. The dose was then dislodged from the gun into the pharynx. The calf's head was then held up till it was seen to swallow several times.

3) Faeces Egg Counts

Faeces was always collected directly from the rectum of each calf.

In Experiment 1 the faeces egg counts were examined by the zinc sulphate flotation method and the number of eggs present estimated using a modified McMaster technique (Gordon & Whitlock, 1939). The results were expressed as the number of eggs per gram of faeces. The main disadvantage of this counting technique is that only a very small proportion of the eggs actually present in the original quantity of faeces are seen and counted. Multiplication by a factor of 100 is required to express the results as numbers of eggs/gm. of faeces. This renders the method insensitive and open to a wide margin of error.

In order to achieve greater sensitivity and reliability in Experiments 2 and 3 a water sedimentation technique was used as described by Happich and Boray, (1969). In this technique all the eggs present in the original 3 gms. of faeces were counted and the results expressed as number of eggs/10 gms of faeces.

4) Recovery of Flukes

All the calves were slaughtered using a captive bolt pistol, bled out and the livers removed. The livers were removed as quickly as possible after death, photographed, and details of the gross pathology noted as described in Part 4 of this thesis. The liver was then cut into slices about 1/4 - $\frac{1}{2}$" thick and each slice squeezed by hand and any flukes present removed and placed in a jar of water. After this initial squeezing the liver slices were placed

in a bucket containing warm .85% saline solution and left for an hour before being squeezed again. The saline was then drained off and the sediment collected and examined for flukes. The flukes recovered were counted in a petri dish and their length measured against a millimetre scale. Flukes greater than 12 mm. in length were termed adult flukes. Flukes smaller than this were considered to be immature flukes. As some flukes were broken in recovery a number of flukes found had only an enterior or posterior section present. These portion of flukes were counted as follows; as each anterior portion was counted a posterior portion was discarded till all the anterior portions had been counted, than any remaining portions were counted as individual flukes.

E. STATISTICS

The statistical methods used were as described by Bishop (1966) and Snedecor (1956). Figures quoted in this text are written as the mean and the standard error (S.E.) of the mean. When the variance ratio was too great to allow use of the Students t test in determination of significance the Behrens-Fisher test (Cochran, 1964) was used to determine the level of significance.

SECTION 1

INTRODUCTION

The first experiment of the series was designed to investigate the possibility that calves in which an initial infection of F. hepatica had been allowed to develop to maturity could then be shown to have developed a significant resistance to subsequent reinfection with this parasite.

The relationship between the development of any such acquired resistance and the continued presence of the original fluke population during the reinfection was also investigated by treating a group of infected calves with an anthelmintic to remove the flukes derived from the initial infection. The design of the experiment is shown below.

Group

1 C_1 —— 17 weeks —— C_2 —— 13 weeks —— K

2 C_1 —— 16 weeks —— T —— 1 week —— C_2 —— 13 weeks —— K

3 C_1 —— 16 weeks —— K

4 C_1 —— 30 weeks —— K

5 C_2 —— 13 weeks —— K

C_1 = initial infection with 750 metacercariae of F. hepatica

C_2 = infection with 1,650 metacercariae of F. hepatica

T = treatment with nitroxynil*

K = calves killed

The experiment consisted of 20, 3-4 month old parasite free dairy calves randomly allocated into 5 groups each of 4 calves. The 16 calves in Groups 1 - 4 were each infected with 750 metacercariae of F. hepatica at the start of the experiment. Sixteen weeks later the four calves in Group 3 were killed to estimate the number of flukes which developed from the initial infection and the four calves in group 2 were treated with anthelmintic to remove this infection. One week later the eight calves in Groups 1 and 2 along with the four previously uninfected calves in Group 5 were each infected

* 'Trodax' May & Baker Ltd., Dagenham, England.

with 1,650 metacercariae of F. hepatica. The experiment was terminated 13 weeks later. The calves were examined, bled, weighed and faecal samples taken at weekly intervals throughout the experiment.

RESULTS

Weight

The 16 calves in Groups 1-4 gained an average of 1.89 lbs/day over the 16 weeks after their infection with 750 metacercariae of F. heptica. The four uninfected calves in Group 5 gained 2.29 lbs/day over the same period. The eight calves in Groups 1 and 2 then gained an average of 2.04 lbs/day during the 13 weeks after their reinfection with 1,650 metacercariae of F. hepatica while the four previously uninfected calves of Group 5 gained only 1.68 lbs/day during the 13 weeks following their infection with 1,650 metacercariae of F. hepatica. The mean weekly weights of the groups of calves and their individual weekly weights are given in Appendix 1 Tables 1, 10, 21 and 31.

Haematology

There were no significant differences in any of the haematological indices studied between the 16 calves in Groups 1-4 each infected with 750 metacercariae of F. hepatica and the haematological indices of the 4 uninfected calves in Group 5 over the first 16 weeks of the experiment. Neither were there any significant differences between any of the haematological indices of the 8 calves in Groups 1 and 2 each reinfected with 1,650 metacercariae of F. hepatica and the haematological indices of the 4 previously uninfected calves in Group 5 each infected with 1,650 metacercariae of F.hepatica during the 13 weeks following infection. The mean weekly haematological indices of each group of calves and their individual weekly values are given in Appendix 1, tables 2-6, 11-16, 19, 22-26, 32-36.

The Mean Serum Albumin Levels

The significant changes in the mean serum albumin levels are shown in figures 1 and 2 and can be summarised as follows:

The mean serum albumin level of the 16 calves in Groups 1 - 4 reached a minimum (2.11 ± .09 gms/100 ml.) 16 weeks after their infection with 750 metacercariae of F. hepatica and this was significantly lower ($p<.001$) than the mean serum albumin level of the 4 uninfected calves (2.3 ± .04 gms/100 ml.) in Group 5. The mean serum albumin level of the eight calves in Groups 1 and 2 (1.87 ± .04 gms/100 ml.) then began to rise despite their reinfection with 1,650 metacercariae of F. hepatica and was significantly higher ($p < .01$) at the end of the experiment (2.33 ± .04 gms/100 ml.). Infection of the 4 previously uninfected calves in Group 5 with a similar dose of 1,650 metacercariae of F. hepatica however, resulted in a significant ($p < .02$) fall in their mean serum albumin levels by 13 weeks after infection (2.09 ± .02 gms/100 ml.) compared to the level prior to infection (2.33 ± .07 gms/100 ml.). There were no significant differences in the mean serum albumin levels after reinfection of the reinfected calves in Group 1 and the treated and reinfected calves in Group 2.

The weekly individual and mean group values are given in Appendix 1 tables 7, 16, 20, 27 and 37.

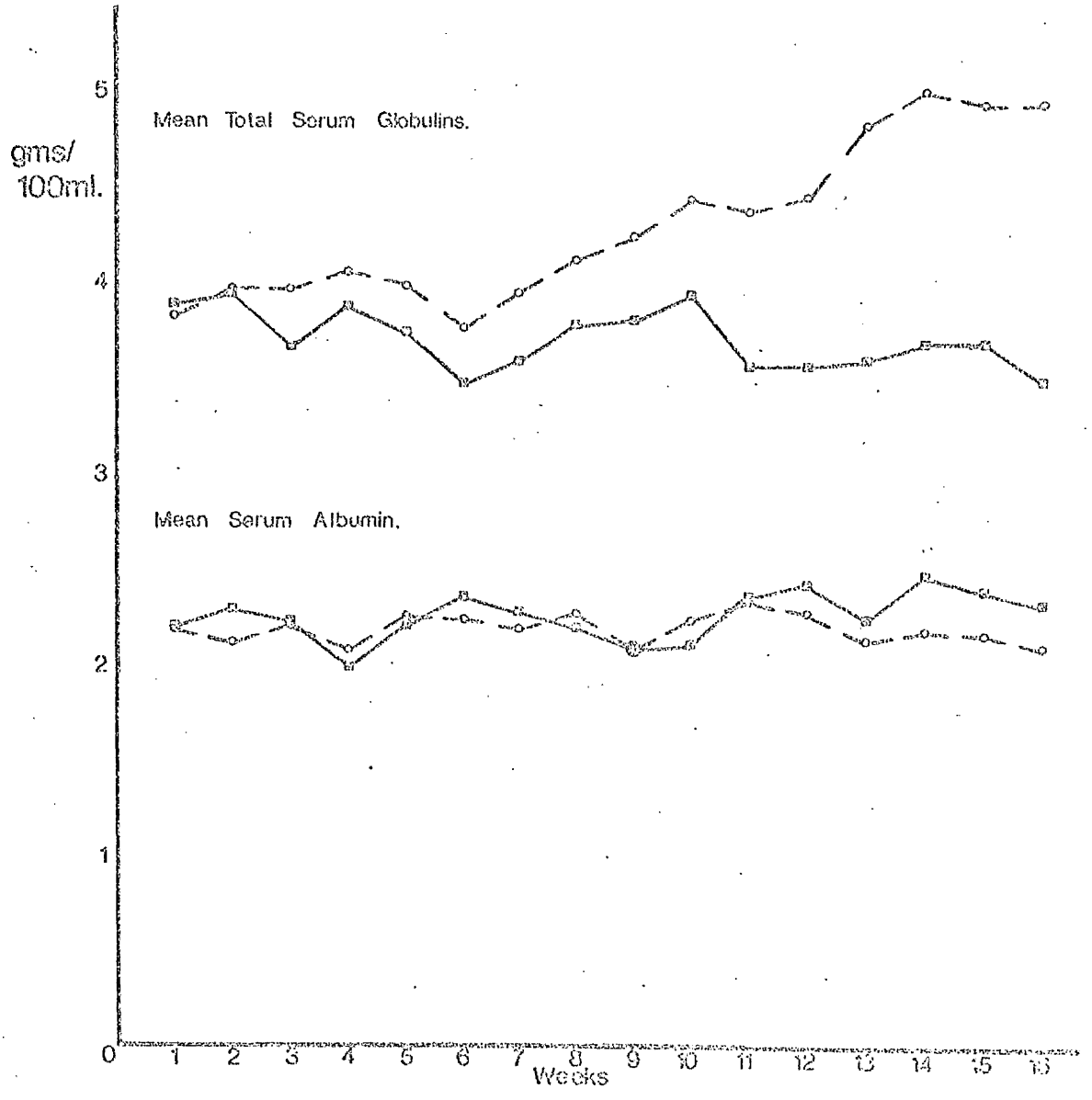
The Mean Total Serum Globulin Levels

The significant changes in the mean total serum globulin levels are shown in figures 1 and 2 and can be summarised as follows:-

There was a rise in the mean total serum globulins of the 16 calves in Groups 1-4 after their infection with 750 metacercariae of F. hepatica and at 11 weeks after infection the level (4.37 ± .09 gms/100 ml.) was significantly higher ($p < .001$) than the mean level of the four uninfected calves in Group 5 (3.57 ± .19 gms/100 ml.). The mean total serum globulin level of the 12 calves in Groups 1, 2 and 4 remained elevated from 11-24 weeks

Fig. 1

The mean serum albumin and total serum globulins of 16 calves following their infection with 750 metacercariae of F. hepatica and the mean serum albumin and total serum globulins of 4 uninfected control calves over the same period.

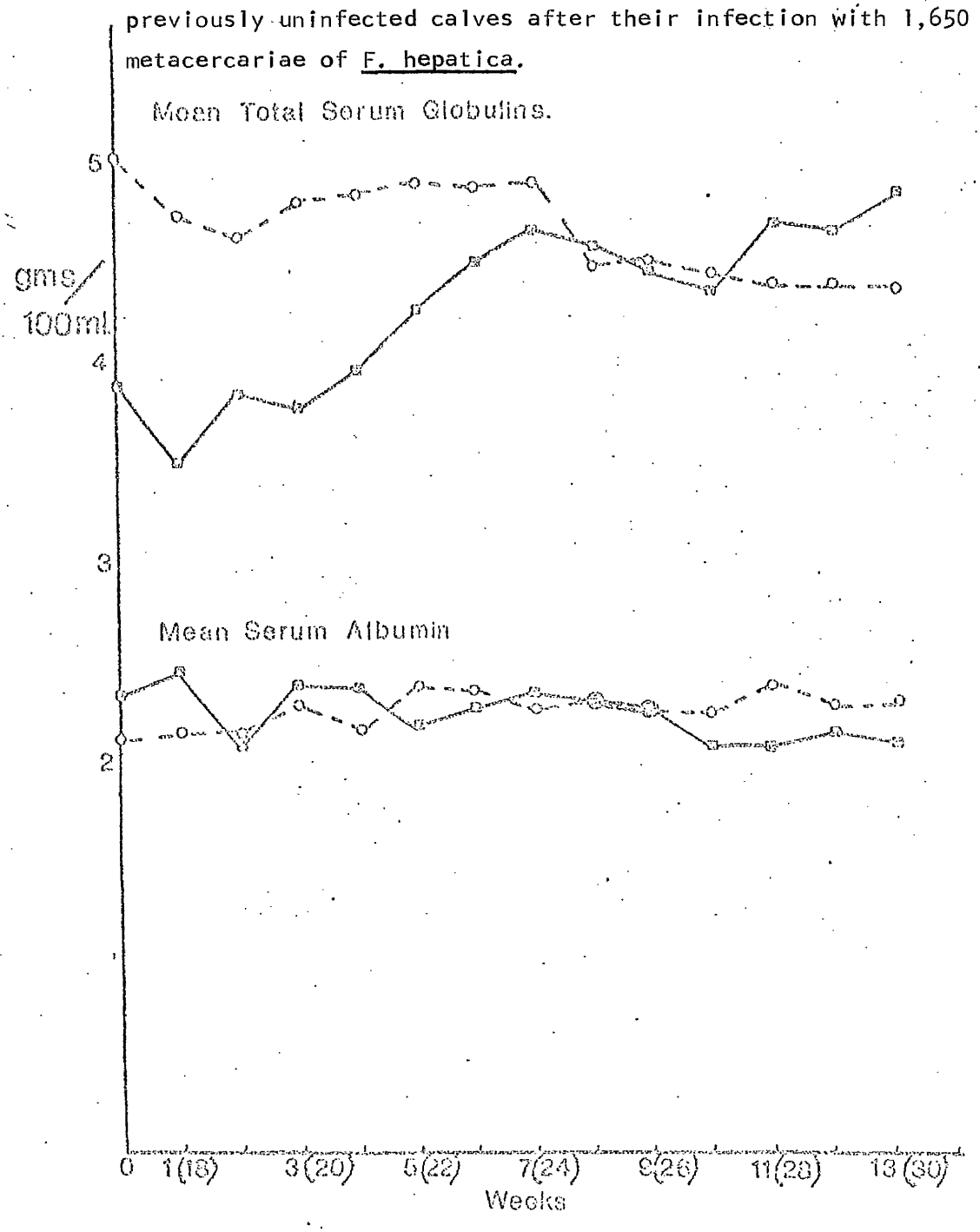


O--- --- --- O Mean values of 16 calves after infection with 750 metacercariae of F. hepatica

□——— ——□ Mean values of 4 uninfected control calves

Fig. 2

The mean serum albumin and total serum globulins of 8 calves following reinfection with 1,650 metacercariae of F. hepatica and the mean serum albumin and total serum globulins of 4 previously uninfected calves after their infection with 1,650 metacercariae of F. hepatica.



Mean values of 8 previously infected calves following reinfection with 1,650 metacercariae of F. hepatica

Mean values of 4 previously uninfected calves following infection with 1,650 metacercariae of F. hepatica

after infection then declined significantly ($p \approx .001$) between week 24 (4.87 ± .18 gms/100 ml.) and the end of the experiment (4.31 ± .09 gms/100 ml.) despite the reinfection of the 8 calves in Groups 1 and 2 with 1,650 metacercariae of F. hepatica, 17 weeks after their initial infection with 750 metacercariae of F. hepatica. The infection of the four previously uninfected calves in Group 5 with a similar dose of 1,650 metacercariae did however cause a rise in their mean total serum globulins and the level after 13 weeks at the end of the experiment (4.80 ± .24 gms/100 ml.) was significantly higher ($p < .01$) than the level prior to infection (3.89 ± .04 gms/100 ml.). There were no significant differences between the mean total serum globulin levels of the reinfected calves in Group 1 and the treated and reinfected calves in Group 2.

The weekly individual and mean group values are given in Appendix 1, tables 8, 17, 20, 28 and 38.

The Serum Glutamic Oxaloacetic Transaminase Levels

The significant changes in the mean SGOT levels are shown in figures 3 and 4 and can be summarised as follows:

The mean SGOT level of the 16 calves in Groups 1-4 began to rise two weeks after their infection with 750 metacercariae of F. hepatica and the mean level a weeks later (90.2 ± 2.9 S-F units) was significantly higher ($p < .01$) than the mean SGOT level (76 ± 3.7 S-F units) of the four uninfected calves in Group 5. The mean level of the infected calves reached a maximum (138.9 ± 8.5 S-F units) 9 weeks after infection. Then followed a significant fall ($p < .001$) till 16 weeks after infection, this level however (61.1 ± 3.7 S-F units) was still significantly higher than that of the 4 uninfected calves (45.5 ± 1.95 S-F units).

Fig. 3

The mean serum glutamic-oxaloacetic transaminase levels of 16 calves following their infection with 750 metacercariae of F. hepatica and of a group of 4 uninfected control calves over the same period.

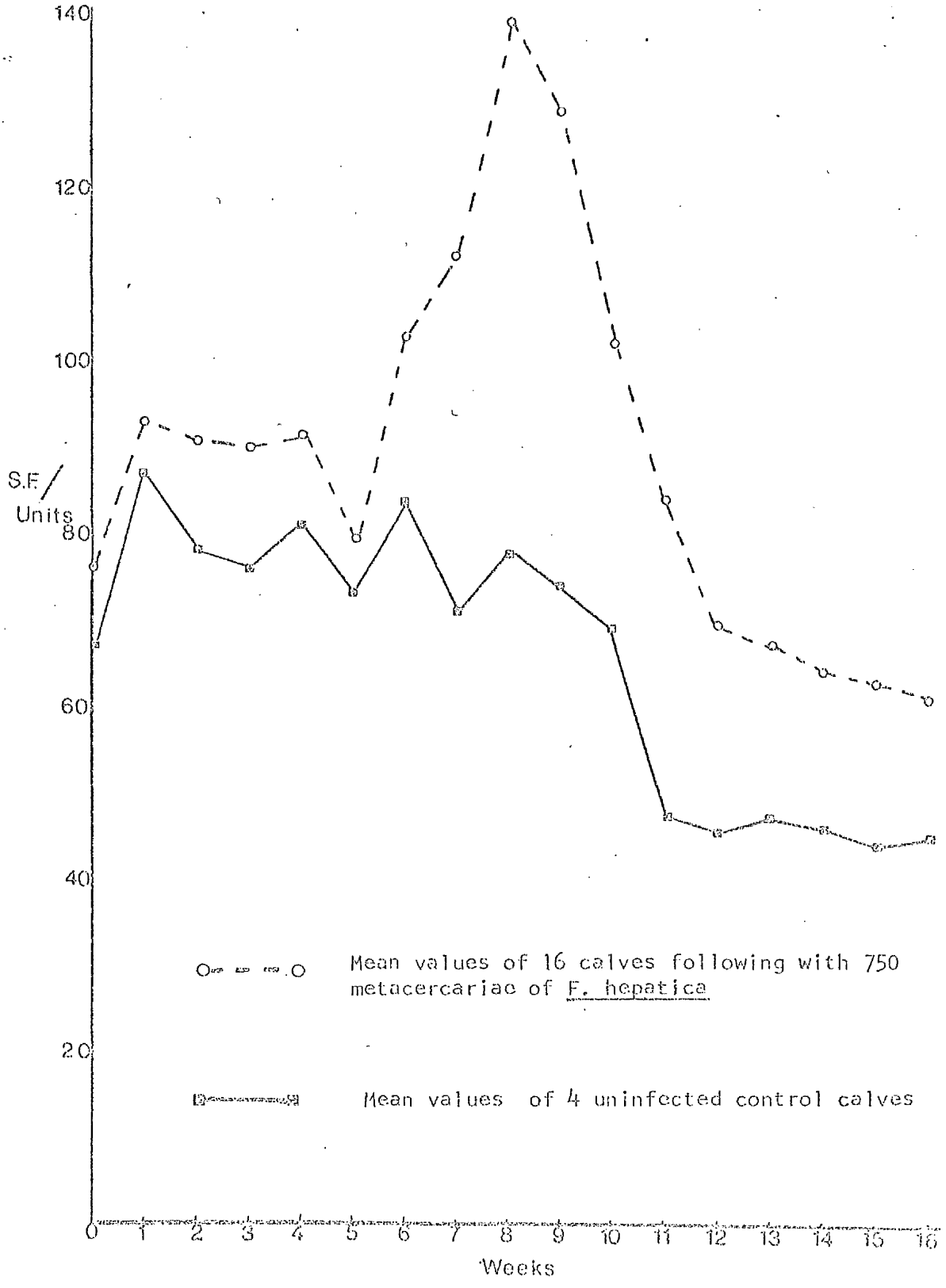
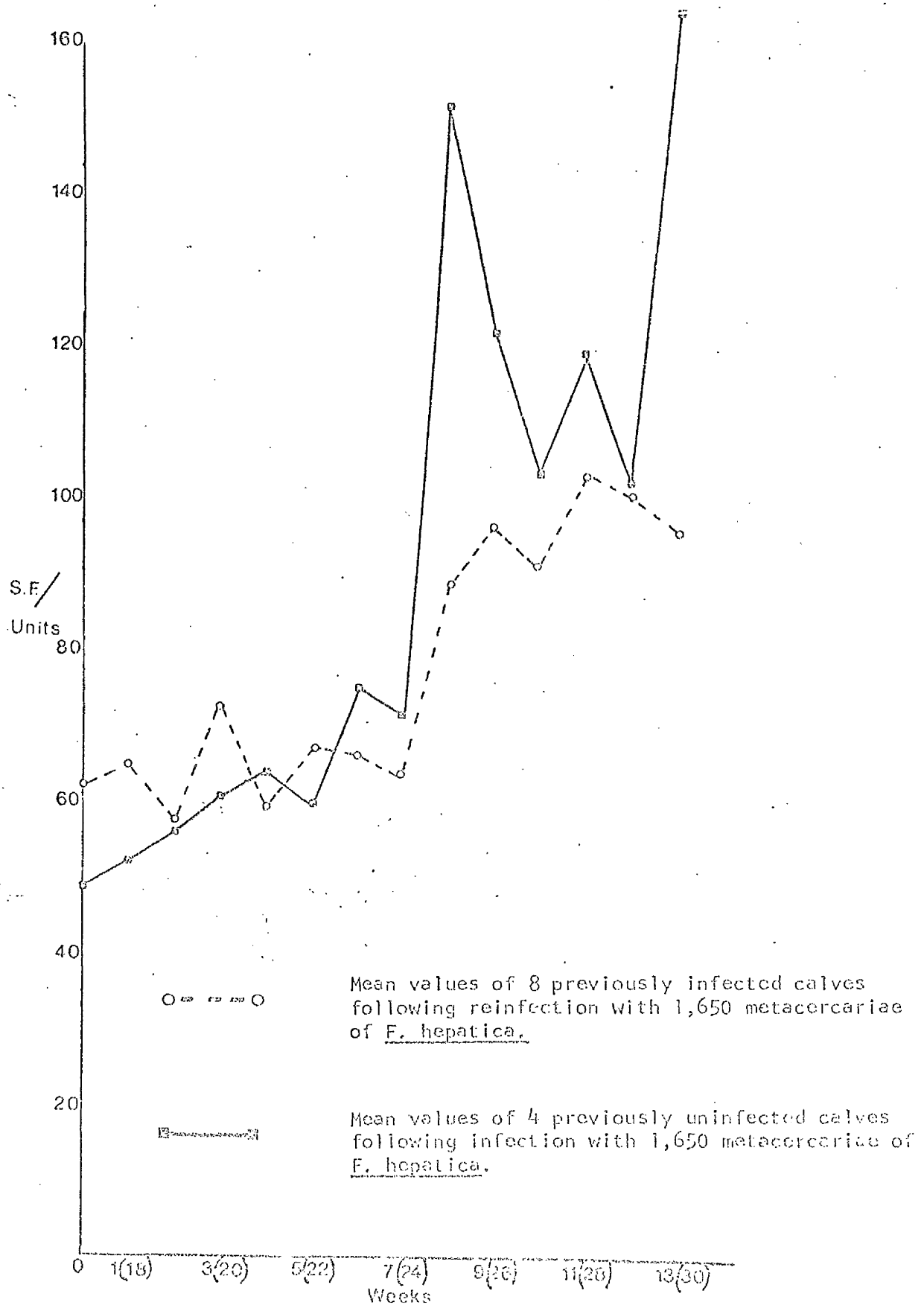


Fig. 4

The mean serum glutamic-oxatoacetic transaminase levels of a group of 8 calves following their reinfection with 1,650 metacercariae of F. hepatica and of a group of 4 previously uninfected calves following their infection with 1,650 metacercariae of F. hepatica.



Reinfection of the 8 calves in Groups 1 and 2 did not significantly alter their mean SGOT levels when compared to the mean level of the 4 infected but not reinfected calves in Group 4. Again there were no significant differences between the mean SGOT values of the reinfected calves in Group 1 and the treated and reinfected calves in Group 2. The mean SGOT level of the four previously uninfected calves in Group 5 was however significantly raised ($p < .01$) 3 weeks after their infection with 1,650 metacercariae of F. hepatica (60.2 ± 1.8 S-F units) when compared to the level prior to infection (48.7 ± 2.0 S-F units). This rise continued and at the end of the experiment the mean SGOT level of the calves receiving the single infection of 1,650 metacercariae of F. hepatica (167 ±19.9 S-F units) was significantly higher than that of the 8 calves reinfected with 1,650 metacercariae of F.hepatica (94.6 ± 2.4 S-F units).

The weekly individual and mean group values are given in Appendix 1 tables 9, 18, 20 29 and 39.

Faeces egg counts

The mean egg counts of the five groups of calves are shown in Table 4 and individual counts are given in Appendix 1 table 40.

Fluke eggs were first found in the treated and reinfected calves in Group 2, 11 weeks after their reinfection with 1,650 metacercariae of F. hepatica. A week later eggs were found in the faeces of the calves in Group 5 which had only received a single infection of 1,650 metacercariae of F. hepatica. No eggs derived from the reinfection were found in the faeces of the reinfected calves in Group 1 although eggs from the initial infection were found for 9 weeks after their reinfection.

TABLE 4

Mean faeces egg counts after infection with *F. hepatica* (eggs/gram)

Group	No. of metacercariae of *F. hepatica* administered.		Weeks after infection with 750 metacercariae of *F. hepatica*. (Weeks after infection with 1,650 metacercariae of *F. hepatica*.)																				
			10	11	12	13	14	15	16	17	18 (1)	19 (2)	20 (3)	21 (4)	22 (5)	23 (6)	24 (7)	25 (8)	26 (9)	27 (10)	28 (11)	29 (12)	30 (13)
1	750	1,650	-	37	112	162	112	75	225	137	62	75	50	25	50	12	-	12	12	-	-	-	-
2	750	1,650	-	75	125	62	75	112	162	T	-	-	-	-	-	-	-	-	-	-	12	37	-
3	750	-	-	50	-	112	137	112	150	X													
4	750	-	-	50	12	50	75	100	100	50	75	75	25	12	12	12	-	-	37	12	-	-	-
5	-	1,650	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	-

T = Calves treated with nitroxynil 16 weeks after infection.

X = Calves killed.

Fluke Counts

The mean numbers of flukes recovered from each group of calves are given in table 5 and individual recoveries are given in Appendix 1 table 30.

The response of calves to a single infection of F. hepatica is shown by comparison of the mean fluke burdens of the calves in Group 3 (126.75 ± 22.2) which were killed 16 weeks after infection with 750 metacercariae of F. hepatica and the mean fluke burden of the calves in Group 4 (19.75 ± 6.06) which were killed 30 weeks after a similar infection of 750 metacercariae of F. hepatica. This represents a significant reduction ($p < .01$) of 84% in the fluke population occurring between 16-30 weeks after the infection of calves with 750 metacercariae of F. hepatica.

The mean number of flukes recovered from the eight reinfected calves in Groups 1 and 2 (22 ± 4.4) when compared by analysis of variance with the mean number of flukes recovered from the four calves in Group 5 which only received the single infection of 1,650 metacercariae of F. hepatica (135.75 ± 42.7) showed a significant ($p < .01$) reduction in the acquired fluke burden of the reinfected calves. This represents an 84% reduction in the acquired fluke burden of the reinfected calves as compared to the previously uninfected control calves.

There was no significant difference between the mean numbers of flukes recovered from the reinfected calves in Group 1 (25.25 ± 6.04) and the treated and reinfected calves in Group 2 (18.75 ± 5.01). There was, however, a difference in the proportion of adult to immature flukes recovered from each group. The fluke population recovered from the calves of Group 1 which had retained the original infection during reinfection had a high adult to immature ratio (4.6/1) as compared to the ratio in the flukes recovered from the treated and reinfected calves in Group 2 (1/1), whose initial fluke population had been removed by the anthelmintic.

TABLE 5

Total and mean numbers of flukes recovered from 5 groups of calves following infection or infection and reinfection with *F. hepatica*.

Group	Number of metacercariae of *F. hepatica* administered per calf		Time of death after initial infection (wks).	Total number of *F. hepatica*	Mean number of *F. hepatica*	Ratio of adult/ immature flukes
1	750	1,650	30	101	25.2 ± 6.0	4.6/1
2	750	1,650	30	75	18.7 ± 5.0	1/1
3	750	-	16	507	126.7 ± 22.2	10.0/1
4	750	-	30	79	19.7 ± 6.1	14/1
5	-	1,650	13	544	135.7 ± 42.7	1.7/1

The ratio of adult to immune flukes (1.7/1) in the previously uninfected control calves which only received a single infection of 1,650 metacercariae of F. hepatica was similar to that found in the treated and reinfected calves of Group 2 (1/1). It would appear therefore that the high adult/immature ratio of the fluke population recovered from the reinfected calves in Group 1 is due to the continued presence of adult flukes derived from the initial infection of 750 metacercariae of F. hepatica. This premise is supported by the fact that these calves continued to pass eggs in their faeces until 9 weeks after reinfection and also similar numbers of flukes (19.75 ± 6.06) with a high adult/immature fluke ratio (14/1) were recovered 30 weeks after the initial infection of 750 metacercariae of F. hepatica from the calves of Group 5.

Pathology

The detailed pathology will be described in part 4 of this thesis but a brief mention will be made at this point of some important gross changes in the livers of the reinfected calves. These changes were most obvious in the livers of the calves in group 1, those reinfected in the presence of the original infection. There was a marked reduction in the size of the ventral lobe due to replacement of most of the parenchyma by fibrous tissue in the distended and calcified bile ducts in its substance. The shape of the liver was so altered by this fibrous of the ventral lobe and a marked hypertrophy of the dorsal lobe that the long axis of the liver was now in a transverse plane. Haemorrhagic lesions indicating the presence of migrating flukes were only found in one of the livers of the calves in Group 1. The gross changes in the livers of the treated and reinfected calves in Group 2 were not as severe as those in Group 1 but there were many more haemorrhagic lesions due to migratory fluke both in the dorsal and ventral lobes of the livers of the calves in Group 2.

Discussion

The results of this experiment showed that calves previously exposed to an infection with F. hepatica which had developed to the adult stage were subsequently highly resistant to reinfection with this parasite. They also show that in calves which receive only a single infection of F. hepatica there is a significant decrease in the established fluke population between 16 and 30 weeks after infection. It would appear therefore that cattle can develop a significant acquired resistance to both primary and secondary infections with F. hepatica.

The development of an acquired resistance to a primary infection with the resultant elimination of 84% of the population derived from a single infection of 750 metacercariae of F. hepatica between 16-30 weeks after infection is reflected in the changes observed in the serum proteins and faeces egg counts over this period. The rising serum level is consistent with a loss of flukes resulting in a reduction of the albumin leak into the bile. The decreasing faeces egg count seen in the latter half of the experiment in these infected calves is also consistent with the elimination of at least a large part of the egg producing fluke population.

These results are the first to provide significant evidence of the development of an acquired immunity to a primary infection with F. hepatica. Previous work by Boray (1967) had described a fall in the faeces egg counts of calves beginning 20 weeks after their infection with F. hepatica but the calves were not killed to demonstrate any reduction occurring in their acquired fluke burdens. The results of the experiment described in this section and those of Boray (1967) are completely at variance with those described by Ross (1968). Ross (1968) described a fall in the faeces egg counts of calves beginning 8 months after their infection with F. hepatica associated with a 75% reduction in their acquired fluke burden between the 5th and 21st month after infection. The discrepancy between the results obtained by Ross (1968)

and the results obtained in this experiment can be explained by the variability both in the time of infection and in the infective doses of F. hepatica used by Ross since a mixture of artificially infected calves and naturally infected calves were used in this series. The percentage reduction in acquired fluke burden described by Ross was based on the comparison of the number of flukes recovered from a single calf killed 21 months after infection with the mean number of flukes recovered from 4 calves killed 2-5 months after infection. A figure derived on this basis is obviously statistically meaningless and explains the discrepancy between the results of both sets of experiments.

The development on an acquired resistance to reinfection with F. hepatica resulted in the recovery of only 16% of the total fluke burden found in the infected control calves in Group 5 from the reinfected calves in Groups 1 and 2. This acquired resistance was also reflected in the higher weight gains, the rising serum albumin and the lower SGOT levels of the 8 reinfected calves as compared to the 4 previously uninfected calves. It would also appear that the fluke population recovered from the liver of the calves in Group 1 was still largely derived from the initial infection. The persistence of the original population would appear to have enabled calves to resist the establishment of the new population of flukes more successfully than the treated and reinfected calves in Group 2. Larger numbers of flukes derived from the reinfection were found in these calves and there was a much greater amount of fresh liver damage than in the livers of the reinfected calves of Group 1. These results confirm those of Boray (1967) who reported a 78% reduction in the fluke burden of four steers which were treated with an anthelmintic prior to reinfection as compared to the fluke burden of previously uninfected steers.

These results and those of Boray (1967) again differ from those described by Ross (1966 a,d). Ross (1966a,d) described a 51% reduction in the acquired fluke burden of 3 calves reinfected 18 weeks after their initial

infection with F. hepatica. He also described a 'spontaneous self-cure' proposing that the flukes developing from the reinfection initiated the expulsion of the flukes derived from the original infection, because few flukes which he considered attributable to the control infection were found in the reinfected livers at the end of the experiment. The control calves for the initial infection were killed 10, 14, 19 weeks after infection whereas the reinfected calves were killed 24, 26, 27 weeks after their initial infection. Therefore due to lack of suitable control calves, any reduction of the initial fluke population over the latter part of the experiment similar to that described in this experiment would not be detected to account for the low numbers of the initial population remaining in the reinfected calves. There was no evidence of a 'spontaneous self-cure' having occurred in the reinfected calves of Group 1 in this experiment, rather the reverse had occurred with a similar proportion of the initial population persisting through the reinfection as remained in the calves of Group 3 which only received the initial infection.

The results of this experiment demonstrates clearly that calves can develop an acquired resistance to a primary or secondary infection with F. hepatica as shown by a reduction in the expected fluke burden from the reinfecting dose, a reduced level of disease in the reinfected calves and the ability of calves receiving a single infection to mount a self-cure response, thus fulfilling the widely accepted criteria for assessing acquired resistance in animals (Stirewalt, 1963).

SUMMARY

Two groups of 4 calves were infected with 750 metacercariae of F. hepatica and a group killed at 16 and 30 weeks after infection. It was found that 84% of fluke population recovered at 16 weeks after infection had been eliminated by 30 weeks after infection. Eight calves which were each infected with 750 metacercariae of F. hepatica, 17 weeks previously and a group of 4 previously uninfected control calves were each infected with 1,650 metacercariae of F. hepatica and all calves killed 13 weeks later. The reinfected calves showed a high resistance to reinfection as only 16% of the fluke burden of the infected control calves was recovered from the reinfected calves. Treatment of one group of calves with an anthelmintic prior to reinfection did not significantly alter their resistance to reinfection.

SECTION 2

INTRODUCTION

In view of the results of the first experiment a second experiment was designed to investigate the response of previously uninfected calves to a single infection with F. hepatica.

It had been shown by the first experiment that there was now significant experimental evidence to support the premise that F. hepatica survives for a much shorter period in infected cattle as compared to infected sheep. It had been proposed that the majority of flukes may only survive 9-26 months in the bile ducts of infected cattle (Keck and Supperer, 1967, Ross 1968) whereas it had been shown experimentally that flukes can survive in the bile ducts of infected sheep for 6-11 years (Pecheur, 1967, Durbin, 1952).

The previous experiment had shown that a loss of 84% of the fluke population in calves derived from a single infection of 750 metacercariae of F. hepatica occurred between 16 and 30 weeks after infection but did not provide any indication of either the time of onset of this loss or the subsequent rate of elimination of the flukes. A second experiment was then devised to determine the time after infection when cattle begin to eliminate the fluke burden derived from a single infection of F. hepatica and to provide some information on the subsequent rate of elimination of the parasite.

The experiment consisted of 24, three month old parasite free dairy calves randomly allocated into 6 groups each of 4 calves. The calves were then each infected with 500 metacercariae of F. hepatica and a group of calves killed at monthly intervals from 1-6 months after infection. It was hoped that this experimental design would show whether a gradual loss of flukes occurred over a considerable time or if there was a rapid loss of flukes over a short period of time similar to the expulsion of N. brasiliensis from immune rats as described by Mulligan et al (1965).

The calves were clinically examined, bled, weighed and faeces samples taken at weekly intervals throughout the experiment. Weekly estimations were made of the haematological indices, serum glutamic dehydrogenase levels, and faeces egg counts. Estimations of the serum protein levels were carried out every fortnight.

RESULTS

Weight

The four calves which were killed 24 weeks after infection with 500 metacercariae of F. hepatica gained an average of 2.31 lbs/day over this time. Mean and individual values are given in Appendix 1, tables 43, 45 and 55.

Haematology

The only significant changes in the haematological indices were a significant decline ($p < .001$) in the mean erythrocyte levels from the mean level of the 24 calves prior to the experiment (8.59 ± .07 millions/cu.mm.) to the mean level of the 4 remaining calves 24 weeks later (7.07 ± .15 millions/cu.mm) and a significant ($p < .01$) rise in the mean corpuscular volumes over the same period from week 0 (39.3 ± .59 cu.microns) till week 24 (46.1 ± 2.00 cu.microns). The mean and individual values are given in Appendix 1 tables 41, 46-50.

Mean Serum Albumin Level

The significant changes in the mean serum levels are shown in figure 5 and group mean individual calf values are given in tables 42, 51 and 61. Appendix 1. The significant changes can be summarised as follows:-

There was a progressive decline in the mean serum albumin level of the infected calves during the first 18 weeks of the experiment. The mean serum albumin level of the 8 calves remaining 18 weeks after infection (2.11 ± .04 gms/100 ml.) was significantly lower ($p < .001$) than the mean serum albumin level of the 24 calves prior to infection (2.41 ± .04 gms/100 ml.)

Fig. 5

The mean serum albumin and total serum globulins of calves following infection with 500 metacercariae of F. hepatica.

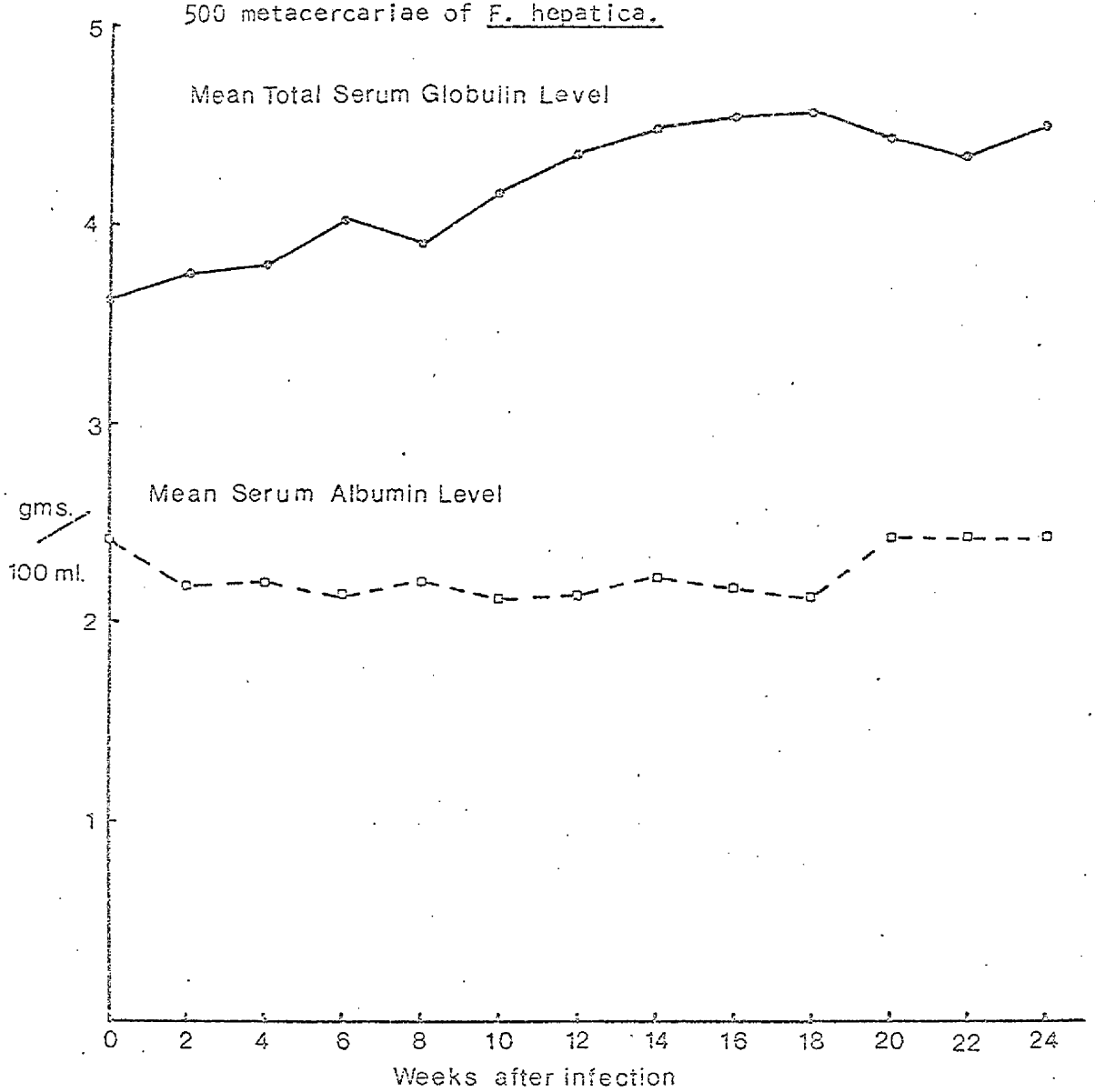
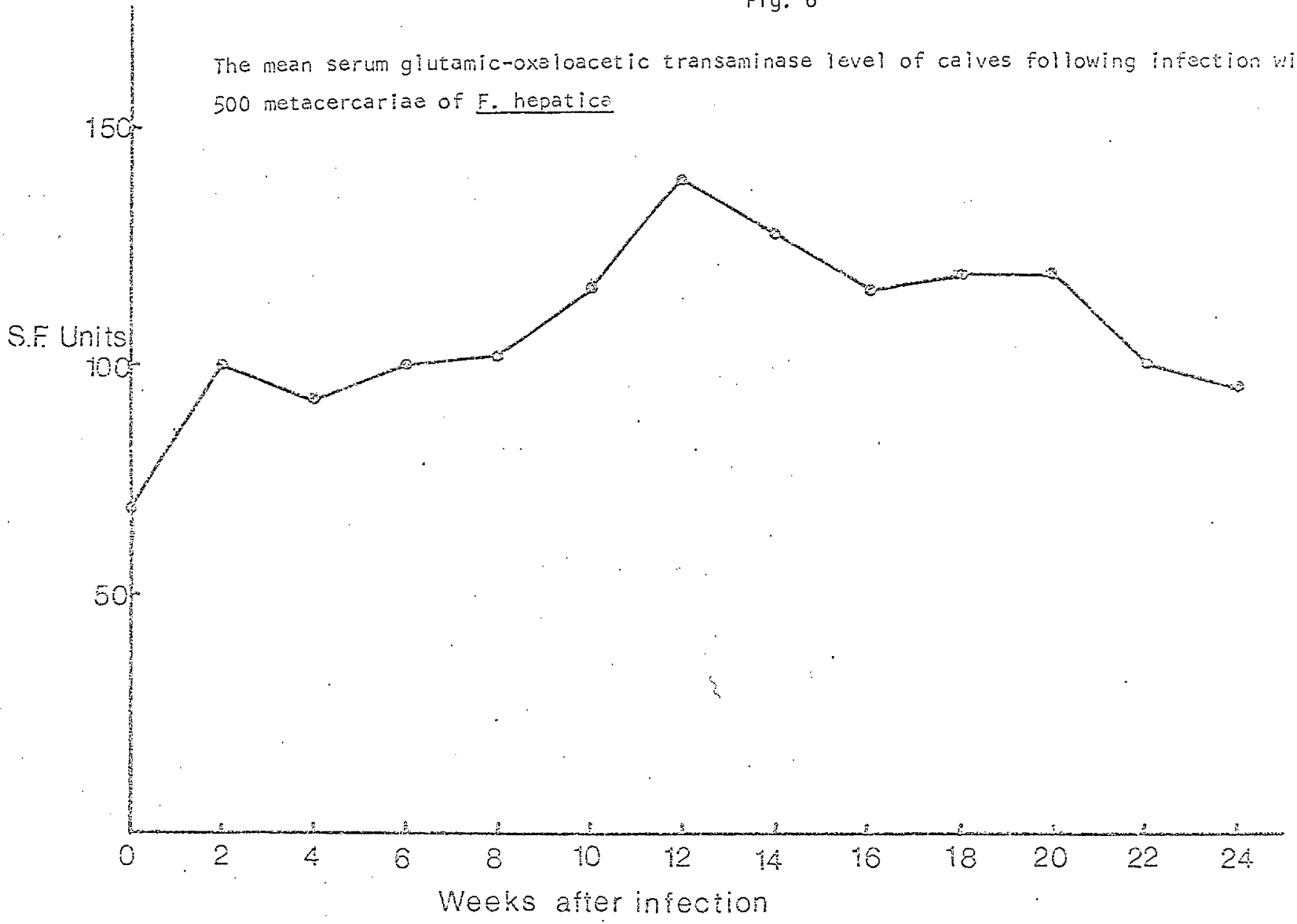


Fig. 6

The mean serum glutamic-oxaloacetic transaminase level of calves following infection with 500 metacercariae of F. hepatica



The mean serum albumin level of the calves remaining in the experiment began to rise between 18-24 weeks after infection. At the end of the experiment the mean serum albumin level of the 4 remaining calves (2.42 ± .03 gms/100 ml.) was significantly higher ($p < .001$) than the mean serum albumin level of the eight calves at 18 weeks after infection (2.11 ± .11 gms/100 ml.)

Mean Total Serum Globulin

The significant changes in the mean total serum globulin levels are shown in figure 5 and the group mean and individual values are given in tables 42, 52 and 62 Appendix 1.

The significant changes can be summarised as follows:-

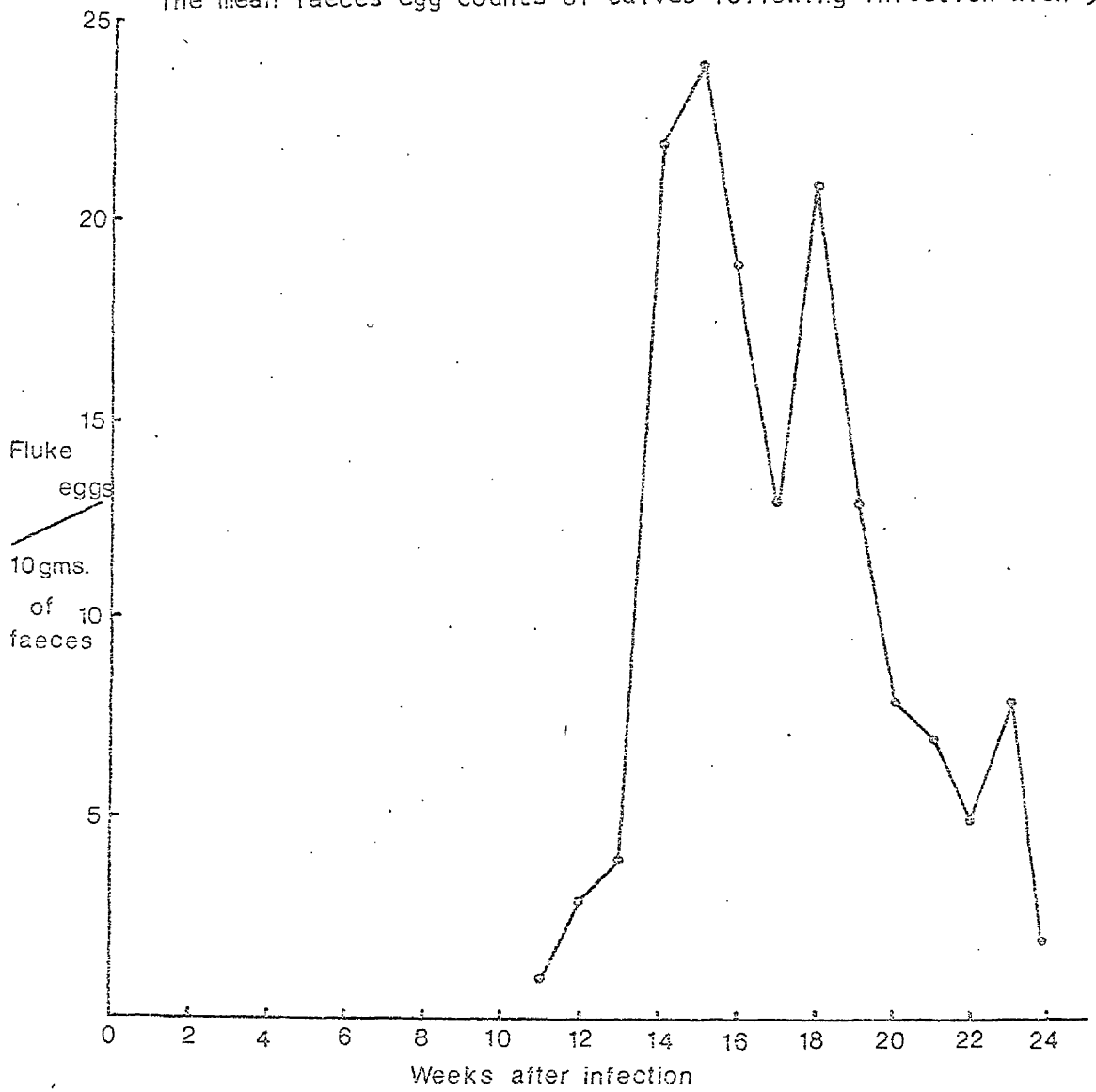
The mean total serum globulin level of the infected calves showed a progressive rise from the start of the experiment and at 6 weeks after infection the mean total serum globulin of the 20 calves (4.06 ± .07 gms/100 ml.) was significantly higher ($p < .001$) than the mean total serum globulin level of the 24 calves prior to infection (3.63 ± .07 gm/100 ml.). The mean total serum globulin level of the infected calves reached maximum levels between 16 weeks (4.54 ± .07 gms/100 ml.) and 24 weeks (4.53 ± .19 gms/100 ml.) after infection.

Mean Serum Glutamic-Oxaloacetic Transaminase Levels

The mean SGOT levels of the infected calves during the experiment are shown in figure 6 and group mean and individual values are given in tables 42, 53 and 63 in Appendix 1. At 2 weeks after infection the mean SGOT level of the 24 infected calves (100.2 ± .92 S-F units) was significantly higher ($p < .001$) than their mean SGOT level prior to infection (68.9 ± 1.59 S-F units).

Fig. 7

The mean faeces egg counts of calves following infection with 500 metacercariae of F. hepatica



Faeces Egg Counts

The mean egg counts of the infected calves during the experiment are shown in figure 7 and the mean and individual faeces egg counts are shown in tables 44 and 64 in Appendix 1.

Fluke eggs were first found in the faeces of infected calves 11 weeks after their infection with 500 metacercariae of F. hepatica. The mean faeces egg count of the infected calves rose rapidly from 11 weeks (11 egg/10 gms. faeces) to a maximum level at 15 weeks after infection (24 eggs/10 gms. faeces). The mean faeces egg count then declined over the remaining 9 weeks of the experiment to a mean level of 2 eggs/10 gms of faeces at 24 weeks after infection.

Fluke counts

The number of flukes recovered from each group of calves at monthly intervals after infection are shown in Table 6 and the individual fluke recovery from each calf is shown in table 54 Appendix 1.

The relative inefficiency of the recovery technique in detecting small numbers of very small flukes in a large bovine liver is shown by the fact that no flukes were recovered from the livers of the calves killed 4 weeks after infection although the livers showed pathological lesions attributable to migrating flukes. Flukes were recovered from the livers eight weeks after infection though the number recovered (130) was lower than the mean recovery over the next 3 months (252) again due to the very small size of a proportion of the fluke population at this time. The numbers of flukes recovered at 12, 16 and 20 weeks after infection were then remarkably constant with a mean recovery of 252 ± 13.4 flukes per group of calves. This gives a mean recovery of 13% of the infecting dose of 500 metacercariae of F. hepatica. At 24 weeks after infection, however, only 92 flukes were recovered from the livers of the 4 calves and this results in a mean of 24.2 ± 5 flukes per calf and a recovery of only 5% of the infecting dose

TABLE 6

The numbers of *F. hepatica* recovered at post-mortem from groups of 4 calves killed 4, 8, 12, 16 20 and 24 weeks after infection with 500 metacercariae.

Week after infection with 500 metacercariae of *F. hepatica*	4	8	12	16	20	24
Total number of *F.hepatica* recovered from groups of 4 calves	0	130	229	237	290	97
Mean number of *F. hepatica* recovered per calf	0	32.5 $8^{\pm}$	57.2 $14^{\pm}$	59.2 $10^{\pm}$	72.5 $16^{\pm}$	24.2 $5^{\pm}$
Percentage of infecting dose recovered	0	6.5%	11.4%	11.8%	16%	4.8%

of 500 metacercariae of F. hepatica. The mean number of flukes recovered per calf 24 weeks after infection (24.2 ± 5) is significantly less ($p < .01$) than the mean number of flukes recovered per calf between 12 and 20 weeks after infection (62.9 ± 17.1). It is also significantly less ($p < .05$) than the mean number of flukes recovered from each of the 4 calves killed only 4 weeks previously (72.5 ± 16).

It is evident that 64% of the fluke population existing between 12-20 weeks after infection with 500 metacercariae of F. hepatica had been eliminated by 24 weeks after infection.

Pathology

The details of gross pathology and histology will be described in part 4 of this thesis.

DISCUSSION

The results of this experiment show that following a single experimental infection with 500 metacercariae of F. hepatica an average of 13% (252 ± 13.4 flukes) of the infecting dose was recovered from the livers of groups of 4 calves killed 12, 16 and 20 weeks after infection. Only 4.8% (97 flukes) of the infecting dose was recovered from the livers of 4 calves killed 24 weeks after infection. This represents a significant reduction in the acquired fluke burdens of the calves occurring between 20-24 weeks after infection with the consequent elimination of 64% of fluke population found between 12.20 weeks after infection. The fall in the mean faeces egg counts which began 15 weeks after infection and the rise in the mean serum albumin level of the calves over the last 6 weeks of the experiment would seem to indicate a period of reduced biological activity of the flukes prior to their elimination by the calf. This sharp decrease in faeces egg counts followed by the expulsion of a large proportion of the acquired fluke burden is remarkably similar to the situation in the immune expulsion of N. brasiliensis from infected rats which is also preceded by a sharp decrease in the faeces egg count of the rats (Mulligan et al., 1965). These expulsion patterns differ only in their time scale in that infection with N. brasiliensis in the rat becomes patent 6 days after infection, faeces egg counts reach a maximum 10-12 days after infection and the worm expulsion takes place between 12-20 days after infection whereas patency in F. hepatica infections in calves does not occur till 11 weeks after infection, maximum counts are reached 13-15 weeks after infection and the expulsion begins 20-24 weeks after infection. Since the expulsion of N. brasiliensis has been clearly shown to be immune in nature (Mulligan et al. 1965) it is interesting to speculate that similar immune mechanism may play a part in the eventual expulsion of flukes from infected calves.

The SGOT level provide some indication of liver cell damage and the mean SGOT level also shows a marked decrease over the latter half of the experiment. The progressive decline in the mean erythrocyte level and corresponding rise in the mean corpuscular volumes over the entire experiment are unlikely to be caused by the acquired fluke burdens since the mean fluke burdens were not large. The anaemia attributable to the feeding activities of adult flukes in the bile ducts does not become evident till 14 weeks after infection and then becomes progressively more severe (Ross et al., 1966). Since the changes in mean erythrocyte levels and mean corpuscular volumes were not associated with changes in the mean packed cell volume, mean haemoglobin concentration or mean corpuscular haemoglobin concentration ,it is most likely that these changes are attributable to normal physiological changes occurring in the growing calf as similar findings have been reported in haematological studies of the calf from 1-10 months old (Schalm 1961).

The results of this experiment confirm and extend the findings of the first experiment where 83% of the fluke population derived from a single infection of 750 metacercariae of F. hepatica were eliminated between 16-30 weeks after infection associated with a rapidly decreasing faeces egg counts over the same period.

It would appear therefore that calves infected with a single infection of F. hepatica will eliminate a large population of their acquired fluke burden between 20-24 weeks after infection and that this loss is preceded by a decreasing biological activity of the flukes as evidenced by their decreasing egg production.

Summary

Twenty-four parasite free dairy calves were each experimentally infected with 500 metacercariae of F. hepatica. Groups of 4 calves were killed 4, 8, 12, 16, 20 and 24 weeks after infection. While 13% of the infecting dose was recovered from the calves killed at 12, 16 and 20 weeks after infection only 4.8% of the infecting dose was recovered from the livers of the calves killed 24 weeks after infection. This loss of 64% of the mean acquired fluke population between 20-24 weeks after infection was preceded by decreasing faeces egg counts and rising serum albumin levels of infected calves indicating a reduced biological activity of the flukes prior to their expulsion from the infected calves.

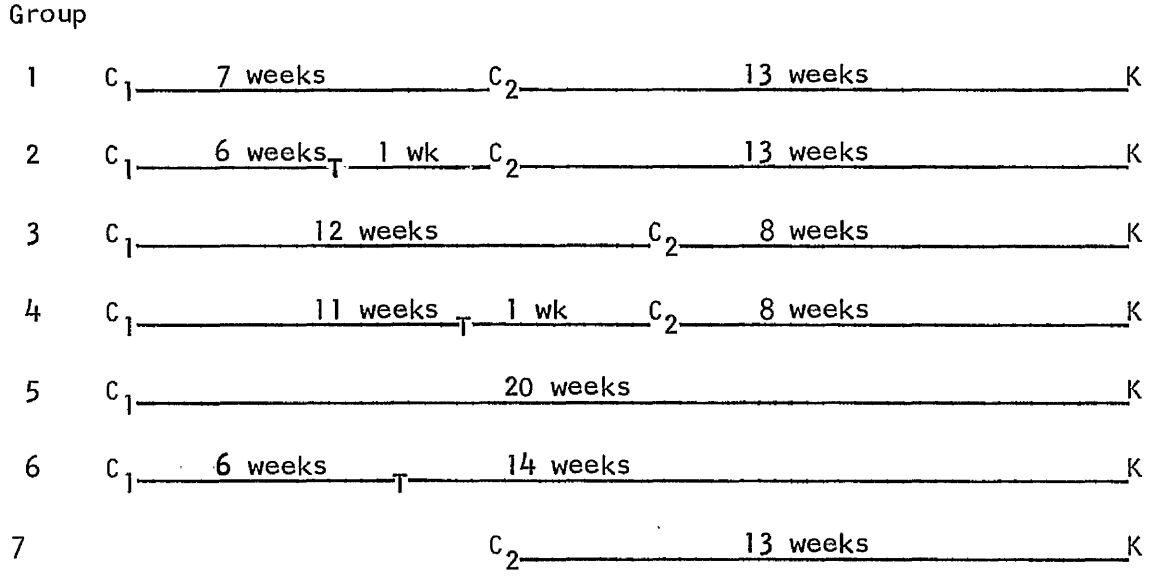
SECTION 3

INTRODUCTION

The results of the first experiment described in this thesis showed that under well defined experimental conditions cattle infected 16 weeks previously with F. hepatica showed a high degree of resistance when reinfected with this parasite. This resistance was apparent even when calves were treated prior to reinfection to remove the fluke population derived from the initial infection although the presence of the original infection may have enhanced the resistance of the untreated reinfected calves. The relationship between the duration of an initial infection with F. hepatica prior to reinfection with this parasite and the subsequent degree of the resultant acquired resistance is however uncertain. The reports of successful demonstrations of the development of an acquired resistance in cattle to reinfection with F. hepatica (Boray, 1967, 1969, Horchner 1969, Ross 1966a, d, 1967b) had a common factor in that in each experiment the initial infection was allowed to develop to maturity, reinfection occurring 12-30 weeks after the original infection. The failure of cattle reinfected 3 weeks after their original infection to develop any acquired resistance has also been described (Ross 1966a, d). This apparent relationship between the duration of the initial infection and the subsequent degree of acquired resistance produced is all the more interesting in view of the report of Corba et al (1971) that a minimum development of 8 weeks was necessary for F. hepatica infections in rats before lymphoid cell suspensions from infected rats could be used to successfully transfer immunity to previously non-infected isogenic recipients.

The experiment described in this section was designed to investigate the degree of resistance obtained when calves were reinfected just prior to the time when flukes derived from the initial infection reached the bile ducts or were reinfected when the majority of the population derived from the

initial infection had just reached the bile ducts and became sexually mature. The experimental design is shown below:-



C_1 = infection with 750 metacercariae of F. hepatica

C_2 = infection with 1,300 metacercariae of F. hepatica

T = treatment with rafoxanide *

K = calves killed

The experiment consisted of twenty-eight 3-4 month old parasite free dairy calves randomly allocated into 7 groups each of four calves and infected with F. hepatica as shown in the experimental design. The experiment was designed to investigate the effects of reinfection of the eight calves in Groups 1 and 2, 7 weeks after their initial infection when the flukes derived from the initial infection were still in the hepatic parenchyma and the effects of reinfection of the eight calves in groups 3 and 4, 12 weeks after their initial infection when the flukes derived from the initial infection were just developing to maturity in the bile ducts.

* Flukanide - Merck, Sharp & Dohme Ltd., Herts, England.

The effect of the removal of the flukes derived from the initial infection prior to reinfection was also investigated by the treatment, of the four calves in Group 2, 6 weeks after their initial infection, 1 week prior to their reinfection and the treatment of the four calves in Group 4, 11 weeks after their initial infection and 1 week prior to their reinfection. The anthelmintic used on each occasion was rafoxanide at a dose rate of 10 mg/kg., which was reported to be effective against the 6 week old and adult flukes (Riek, Butler, West 1971). The experiment was terminated 20 weeks after the initial infection as the results of the second experiment showed that there would not be any significant loss of flukes over this period. At weekly intervals throughout the experiment the calves were examined, bled, weighed and faecal samples taken. Weekly estimations were made of the haematological indices, serum glutamic-oxaloacetic transaminase levels and faeces egg counts. The serum albumin and total serum globulin levels were estimated at fortnightly intervals.

RESULTS

Weight

The 28 calves gained an average of 2.01 lbs/day during the 20 weeks of the experiment. There were no significant differences in the weight gains of any of the groups of calves after either infection or reinfection. The mean and individual values are given in tables 69 and 88 in Appendix 1.

Haematology

The only significant changes in all the haematological indices throughout the experiment were confined to the changes in total erythrocyte counts and mean corpuscular volumes of the eight calves in Groups 1 and 2, and the four calves in Group 7 following their reinfection or infection with 1,300 metacercariae of *F. hepatica.*

The mean erythrocyte count of the eight calves in Groups 1 and 2 was significantly ($p < .02$) lower at the end of the experiment (7.77 ± .41 millions/cu.mm.) 13 weeks after their reinfection with 1,300 metacercariae of F. hepatica as compared to the level prior to reinfection (8.53 ± .23 millions/cu.mm.) This decrease in erythrocyte level was accompanied by a significant rise ($p < .001$) in the mean corpuscular volume when the level prior to reinfection (38.2 ± .68 cu.microns), is compared to the level at the end of the experiment (43.3 ± .84 cu. microns). A similar change occurred in the mean erythrocyte level of the 4 previously uninfected calves in Group 7 and the mean erythrocyte level 13 weeks after infection (7.10 ± .10 millions/cu.mm.) was significantly lower ($p < .02$) than the level before infection (8.21 ± .38 millions/cu.mm.) The mean corpuscular volume of these calves was also significantly higher ($p < .05$) 13 weeks after infection (46.2 ± 1.35 cu.microns) as compared to the size prior to reinfection (40.3 ± 1.75 cu.microns).

There were no other significant changes in the haematological indices of any other group of calves during the experiment. The mean and individual values are given in appendix 1. tables 65, 66, 70-74, 79-83.

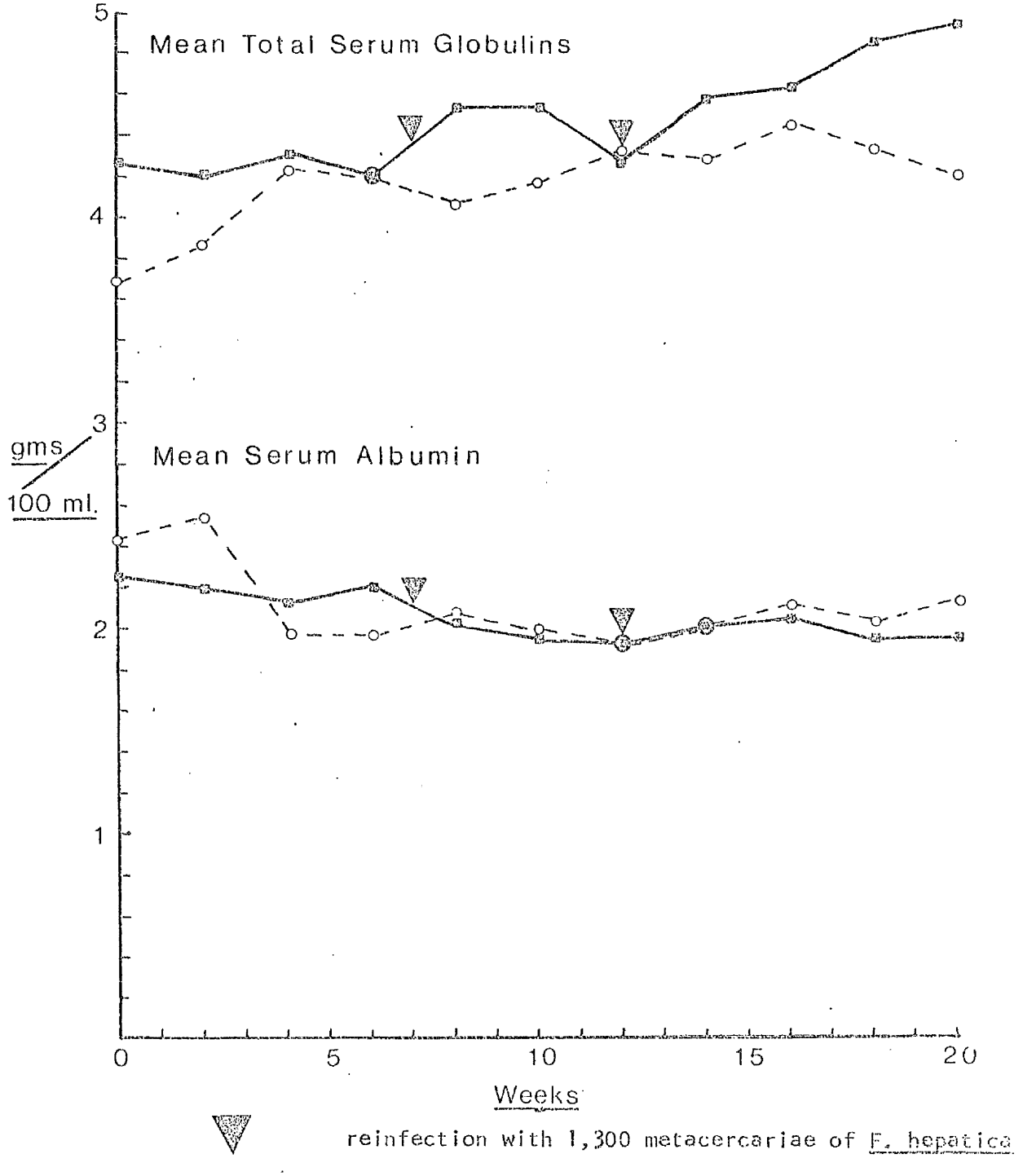
Mean Serum Albumin Level

The significant changes in the mean serum albumin levels are shown in figure 8, the group means and individual values are given in tables 67,68, 75 and 84 in Appendix 1.

The mean serum albumin level of the eight calves in Groups 1 and 2 which were reinfected with 1,300 metacercariae of F. hepatica, 7 weeks after their initial infection of 750 metacercariae of F. hepatica was significantly ($p < .02$) lower at the end of the experiment (1.98 ± .03 gms/100 ml.) as compared to the level prior to reinfection (2.19 ± .08 gms/100 ml.)

Fig. 8

The mean serum albumin and total serum globulins of 2 groups of 8 calves, all initially infected with 750 metacercariae of *F. hepatica*. One group was reinfected 7 weeks and the other group 12 weeks later with 1,300 metacercariae of *F. hepatica*.



The mean serum albumin level of the 8 calves in Groups 3 and 4 was significantly ($p < .001$) lower at 12 weeks after their initial infection with 750 metacercariae of F. hepatica (1.93 ± .03 gms/100 ml) as compared to the level at the start of the experiment (2.24 ± .03 gms/100 ml.). The mean serum albumin level of these eight calves in Groups 3 and 4 then rose over the latter part of the experiment despite their reinfection with 1,300 metacercariae of F. hepatica. At the end of the experiment it was significantly ($p < .01$) higher (2.16 ± .05 gms/100 ml.) than it had been prior to reinfection, 8 weeks previously (1.93 ± .03 gms/100 ml.).

At the end of the experiment the mean serum albumin level of the 8 calves in Groups 3 and 4 which were reinfected, 12 weeks after their initial infection (2.16 ± .05 gms/100 ml.) was also significantly higher ($p < .01$) than that of the 8 calves in Groups 1 and 2 reinfected 7 weeks after their initial infection (1.98 ± .03 gms/100 ml.).

There were no other significant changes in the serum albumin level in or between the seven groups of calves during the experiment.

Mean Total Serum Globulin Levels

The significant changes in the mean total serum globulins are shown in figure 8, the group means and individual values are given in Tables 67, 68, 76 and 85 in Appendix 1.

The mean total serum globulin level of the eight calves in Groups 1 and 2 reinfected with 1,300 metacercariae of F. hepatica, 7 weeks after their initial infection with 750 metacercariae of F. hepatica rose progressively throughout the experiment. At the end of the experiment the level (4.93 ± .13 gms/100 ml.) was significantly higher ($p < .001$) than at the start of the experiment (3.89 ± .17 gms/100 ml.). The mean total serum globulin level of the eight calves in Groups 3 and 4 rose significantly during the 12 weeks after their initial infection with 750 metacercariae of F. hepatica and the level at 12 weeks after infection (4.37 ± .11 gms/100 ml.)

just prior to their reinfection was significantly higher ($p < .001$) than the level at the start of the experiment (3.63 ±.12 gms/100 ml.) The mean total serum globulin level of the eight calves in Groups 3 and 4 did not increase significantly after their reinfection with 1,300 metacercariae of F. hepatica. The mean total serum globulin level of the eight calves in Groups 3 and 4 was in fact significantly lower ($p < .01$) at the end of the experiment (4.32 ± .10 gms/100 ml.) than the level of the eight calves in groups 1 and 2 (4.93 ± .13 gms/100 ml.)

The mean total serum globulin level of the 4 calves in Group 6 was significantly)$p < .02$) higher at the end of the experiment (4.38 ± .14 gms/100 ml.) compared to the level at the start of the experiment (3.73 ± .17 gms/100 ml.).

There were no other significant changes in the mean total serum globulin level in or between the 7 groups of calves during the experiment.

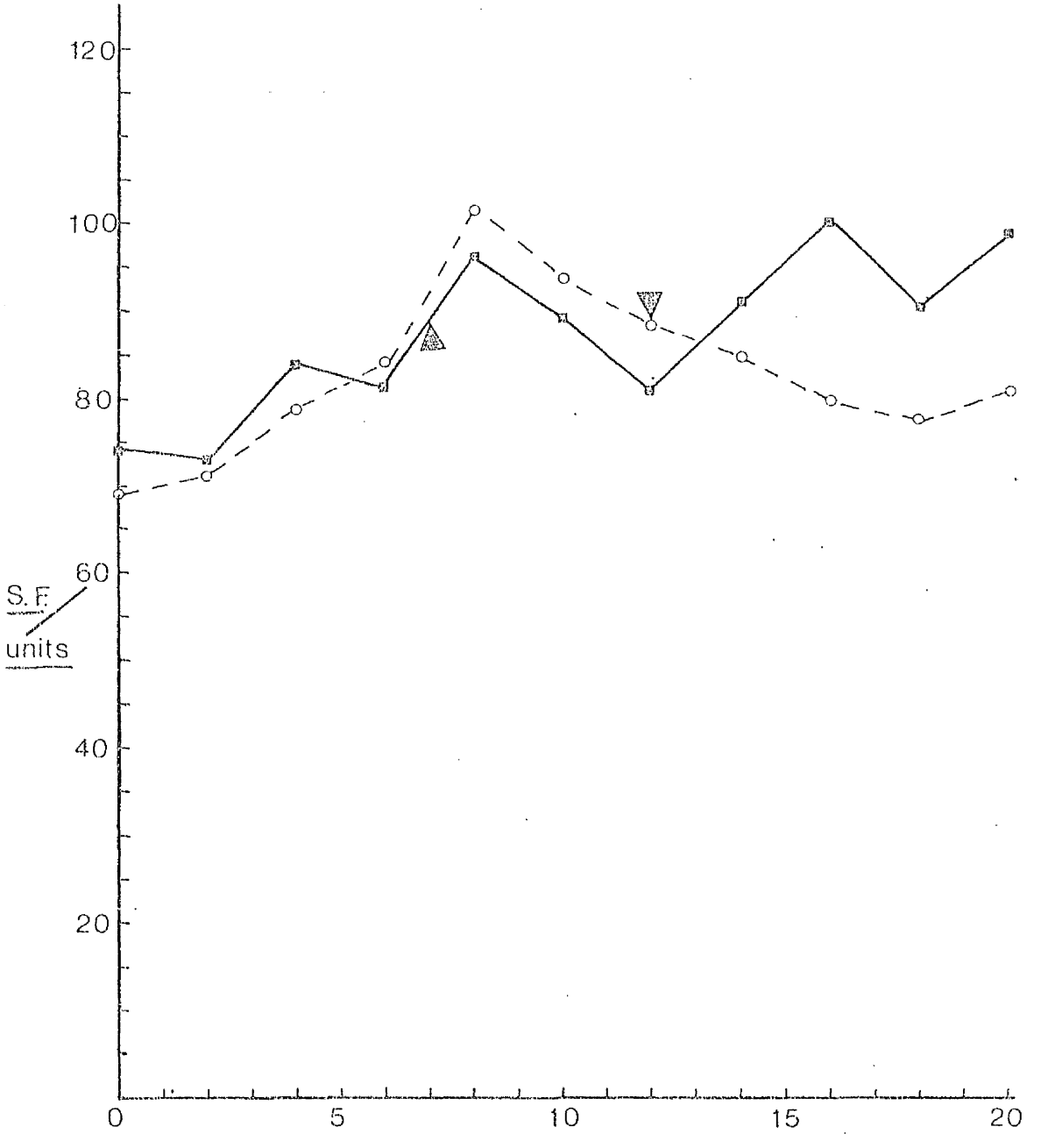
Mean serum glutamic-oxaloacetic transaminase levels

The significant changes in SGOT levels are shown in figure 9 and the group means and individual values are shown in tables 67, 68, 77 and 86 Appendix 1.

Three weeks after their initial infection of 750 metacercariae of F. hepatica the 24 calves in Groups 1 - 6 had a significantly higher ($p < .05$) mean SGOT level (104.9 ± 6.4 S-F units) than did the four uninfected calves in Group 7. (68.7 ± 3.9 S-F units). The mean SGOT levels of the 8 calves in Groups 1 and 2 showed a further significant rise following their reinfection with 1,300 metacercariae of F. hepatica, 7 weeks after their initial infection with 750 metacercariae of F. hepatica. The mean SGOT levels 8 weeks after the reinfection (121.4 ± 15.7 S-F units) was significantly higher ($p < .02$) than the level prior to reinfection (6%.9 ± 1.9 S-F units). The mean SGOT level of the eight calves in Groups 1 and 2 then remained elevated till the end of the experiment (98.2 ± 8.85 S-F units).

Fig. 9

The mean serum glutamic-oxaloacetic transaminase levels of 2 groups of 8 calves all initially infected with 750 metacercariae of F. hepatica. One group was reinfected 7 weeks and the other 12 weeks later with 1,300 metacercariae of F. hepatica.



Reinfection with 1,300 metacercariae of F. hepatica

Mean values of 8 calves reinfected 7 weeks after initial infection

Mean values of 8 calves reinfected 12 weeks after initial infection

The mean SGOT level of the 8 calves in Groups 3 and 4 did not however show any further significant rise following their reinfection with 1,300 metacercariae of F. hepatica, 12 weeks after their initial infection with 750 metacercariae of F. hepatica. The mean SGOT level at the end of the experiment (84.6 ± 4.75 S-F units) was not significantly different from the level prior to reinfection 12 weeks after their initial infection (88.9 ± 5.2 S-F units).

There were no other significant changes in the serum glutamic-oxaloacetic transaminase levels in or between the seven groups of calves during the experiment.

Faeces Egg Counts

The mean faeces egg counts of each group of calves are shown in Table 7. The individual faeces counts are shown in Table 87 Appendix 1.

Patency occurred 12 weeks after the initial infection of 750 metacercariae of F. hepatica. Fluke eggs were found in the faeces of the calves in Groups 2 and 6 which had been treated with rafoxanide 11 weeks after the initial infection. Patency occurred at 11 weeks after infection of the 4 previously uninfected calves in Group 1 with 1,300 metacercariae of F. hepatica.

Fluke Counts

The mean numbers of flukes recovered from each group of calves are shown in Table 8 and individual fluke recovery from each calf are shown in Table 78 Appendix 1.

The eight calves in Groups 1 and 2 reinfected with 1,300 metacercariae of F. hepatica 7 weeks after an initial infection with 750 metacercariae of F. hepatica showed no apparent resistance to reinfection as the mean number of flukes recovered from these 8 calves (52 ± 8.6) is not significantly different from the mean number of flukes recovered from

Table 7

Mean faeces egg counts of 7 Groups of 4 calves after infection or infection and reinfection with *F. hepatica* (eggs/10 gms. faeces)

Group No.	No. of metacercariae of *F. hepatica* administered		Time of administration of metacercariae after initial infection (Weeks)	Weeks after initial infection with 750 metacercariae of *F. hepatica* (Weeks after initial infection with 1,300 metacercariae of *F. hepatica*)											
				10	11	12	13	14	15	16	17	18	19	20
1	750*	1,300	7	-	-	-	-	5	3	3	8	12	22	13
2	750	1,300	7	-	-	1	1	1	2	-	2	4	21	16
3	750	1,300	12	-	-	-	1	-	7	2	14	10	8	8
4	750+	1,300	12	-	-	-	-	-	-	-	-	-	-	-
5	750*		-	-	-	1	-	2	2	4	16	8	12	7
6	750	-	-	-	-	-	-	2	1	2	2	8	2	3
				(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
7	-	1,300	-	-	-	-	-	-	-	-	-	3	24	18

* Treated with rafoxanide 6 weeks after infection infection + treated with rafoxanide 11 weeks after infection

the 4 previously uninfected calves in Group 7 which only received a single infection of 1,300 metacercariae of F. hepatica (75 ± 11.4). The anthelmintic at the dose rate used had very little effect on the immature flukes 6 weeks after the initial infection as the number of flukes recovered from the four calves of Group 6 (32) which were treated with rafoxanide 6 weeks after the initial infection is not significantly difference from the number of flukes (42) recovered from the livers of the 4 untreated calves in Group 5 which only received the initial infection.

There was, however, evidence of a significant resistance to reinfection in the 8 calves of groups 3 and 4 which were reinfected with 1,300 metacercariae of F. hepatica, 12 weeks after their initial infection with 750 metacercariae of F. hepatica. The mean number of flukes recovered from the livers of the 8 calves in Groups 3 and 4 (20.6 ± 2.6) is significantly lower ($p < .01$) than the mean number of flukes recovered from the 4 previously uninfected calves in Group 7 which only received a single infection of 1,300 metacercariae of F. hepatica (75 ± 11.4). This represents a reduction of 72.5% in the acquired fluke burden of the reinfected calves in Groups 3 and 4 as compared to the acquired fluke burdens of the infected control calves in Group 7.

There is no significant difference between the numbers of flukes recovered from the infected and reinfected calves in Group 3 (23.2 ± 3.4) and from the infected, treated and reinfected calves in Group 4 (18 ± 3.4). As can be seen in table 8 however, the proportion of mature/immature flukes in the fluke population recovered from the infected and reinfected calves in Group 4 (1.5/1) is quite different from that of the infected, treated and reinfected calves in Group 3 (1/5). This can be explained on the basis of the majority of flukes recovered from the livers of Group 4 are derived

Table 8

Mean and total fluke burdens of calves reinfected with 1,300 metacercariae of *F. hepatica* either 7 or 12 weeks after their initial infection with 750 metacercariae of *F. hepatica*.

Group No.	No. of Calves	Number of metacercariae of *F. hepatica* administered per calf		Type of reinfection after initial infection (weeks)	Total numbers of flukes recovered per group	Mean number of flukes recovered per calf	Ratio of adult/immature flukes
1	4	750	1,300	7	202	50.5± 6.3	1/1.8
2	4	750*	1,300	7	217	54.2 ± 15.8	1/2.2
1 & 2	8	750	1,300	7	419	52.4 ± 8.6	-
3	4	750	1,300	12	93	23.2 ± 3.5	1.45/1
4	4	750 +	1,300	12	72	18.0 ± 3.4	1/5
3 & 4	8	750	1,300	12	165	20.6 ± 2.6	-
5	4	750	-	-	42	10.5 ± 4.4	3.2/1
6	4	750*	-	-	32	8.0 ± 2.4	4.5/1
7	4	-	1,300	-	300	75 ± 11.4	1/22

* treated with rafoxanide 6 weeks after initial infection + treated with rafoxanide 11 weeks after the initial infection.

from the reinfection as these calves were treated with rafoxanide to remove the flukes from the initial infection. It would appear therefore that the presence of the original fluke population during the reinfection of the calves in Group 3 may have enhanced the degree of resistance obtained.

Pathology

The gross pathological findings in the calves receiving a single infection were similar to those to be described in the calves of the second experiment. The gross pathological findings of the reinfected calves were similar to those described in the reinfected calves of the first experiment.

Discussion

It would appear from the results of this experiment that the degree of resistance obtained to reinfection with F. hepatica is related to the duration of the initial infection.

The calves reinfected with 1,300 metacercariae of F. hepatica, 12 weeks after their initial infection with 750 metacercariae of F. hepatica had a reduction of 72.5% in their acquired fluke burdens as compared to the numbers of flukes recovered from previously uninfected calves which received a single infection of 1,300 metacercariae of F. hepatica. The resistance was reflected in the lack of changes in the haematological indices, mean serum glutamic-oxaloacetic transaminase levels, mean total serum globulins and the rise in their mean serum albumin levels over the period of their reinfection.

The degree of resistance obtained in those calves reinfected 12 weeks after their initial infection (72.5%) is approximately the same as that obtained by Boray (1967) in 4 calves treated and reinfected with F. hepatica 14-19 weeks after their initial infection (78%), but is greater than that found by Hochner (1969) and Ross (1966a,d, 1967b) who

described a reduction of 50-66% in calves reinfected 12, 18 and 30 weeks after their initial infection. In the experiments of Hochner (1969) and Ross (1966a, d, 1967b) the degree of resistance was calculated on the basis of distinguishing both fluke populations derived from the initial and reinfecting infections on the basis of fluke size and as this method is open to some degree of error the results are less satisfactory and more difficult to compare with the results described by Boray (1967) and those described in this thesis.

An 'acquired self-cure" as described by Ross (1966) did not occur in this experiment. The population of flukes recovered from the livers of the calves in Group 3 which were reinfected in the presence of the initial fluke population can be seen in Table 8 to have a considerably higher ratio of adult/immature flukes (1.5/1) than either the calves in Group 4 which were treated with anthelmintic prior to reinfection (1/5) or the previously uninfected control calves in Group 7 (1/2). This can only be explained by the persistence of the adult flukes resulting from the initial infection during the reinfection and this premise is supported by the fact that these adult flukes derived from the initial infection continued to pass eggs into the faeces during the period of reinfection. The calves in Group 3 may thus have been more resistant to reinfection than the calves in Group 4 since the mean numbers of flukes recovered from both Group 3 (23.2 ± 3.5) and Group 5 (18.0 ± 3.4) are similar but a large proportion of the flukes recovered from Group 3 would appear to have originated from the initial infection whereas in the case of the treated and reinfected calves in Group 4 the flukes recovered must have been derived from the reinfection.

The calves reinfected 7 weeks after their initial infection did not show any significant reduction in numbers of flukes which became established in these calves (52.4 ± 8.6) as compared to the previously uninfected control calves (75 ± 11.4). This was reflected in the fall in their mean erythrocyte count and consequent rise in mean corpuscular volume, the continued fall in their mean serum albumin level, the continued rise in the mean total serum globulins and mean serum glutamic-oxaloacetic transaminase levels similar to the changes in these values occurring in the previously uninfected calves in Group 7 following their infection with 1,300 metacercariae of F. hepatica.

It would appear therefore that although no significant degree of acquired resistance can be demonstrated when calves are reinfected 3 weeks (Ross 1966) or 7 weeks after their initial infection a substantial degree of resistance can be demonstrated when calves are reinfected 12 weeks after their initial infection. This acquired resistance would appear to increase if larger intervals are allowed before reinfection as an 84% reduction was obtained in the first experiment of this thesis.

The relationship between an acquired resistance and the duration of the initial infection has two possible explanations. The first of these is that proposed by Boray (1967, 1969) that the degree of resistance is dependent on physical factors such as the degree of fibrosis or cholangitis which result from the original infection and does not depend on any immune response by the host.

The second possible explanation is that the resistance is dependent on an expression of acquired immunity in the host. The recent preliminary results of Corba et al., (1971) using F. hepatica infections in rats and calves

would support this explanation. Corba et al (1971) described the transfer of immunity to isogenic recipients receiving lymphoid cell suspensions from infected rats. They also showed a tranfer of immunity using a similar system in identical twin calves. It was found that a minimum of 8 weeks exposure to F. hepatica infections in the rat was necessary before the acquired immunity could be successfully transferred. A similar immune response may occur in cattle. The time factor may have two possible explanations, the first that the antigens necessary for the stimulation of an effective immune response are only available from the mature flukes or the second is that prolonged antigenic stimulation over this time induces an immune response sufficiently great to eliminate the parasite.

Summary

No significant resistance to reinfection was found when two groups of 4 calves were reinfected with 1,300 metacercariae of F. hepatica, 7 weeks after their initial infection with 750 metacercariae of F. hepatica. A significant resistance was however demonstrated when two groups of 4 calves were reinfected with 1,300 metacercariae of F. hepatica 12 weeks after their initial infection with 750 metacercariae of F. hepatica. Only 27.5% of the fluke burden found in the previously uninfected control calves was recovered from these reinfected calves. The four calves in which the initial infection was allowed to persist during the reinfection were possibly more resistant to reinfection than the four calves in which the initial infection was removed using an anthelmintic prior to their reinfection.

GENERAL DISCUSSION

GENERAL DISCUSSION

The results of the experiments described in this part of the thesis show clearly that calves develop an acquired resistance to infections with Fasciola hepatica and that this resistance is manifest against both a single infection and reinfection with this parasite.

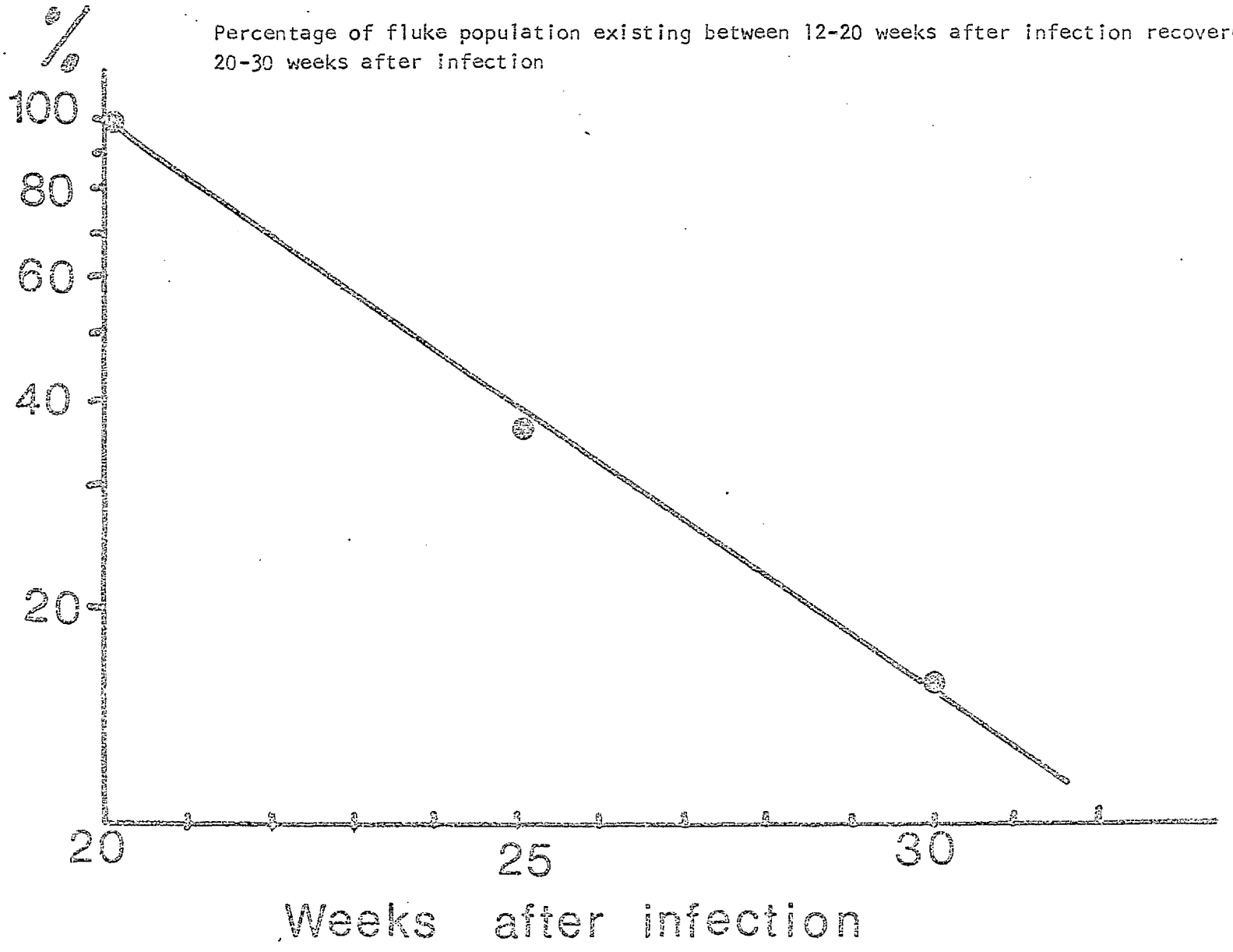
The use of the term 'acquired resistance' can be justified by comparison of the findings of these experiments with the criteria for assessing 'acquired resistance' as defined by Stirewalt (1963) and listed in the introduction to this thesis.

The response of calves to a single infection with F. hepatica will be considered first. It was found that calves receiving a single infection of 500-750 metacercariae of F. hepatica will begin to eliminate the flukes derived from this infection between 20-24 weeks after infection and that this expulsion is preceded by a period of 4-8 weeks of reduced biological activity of the flukes as evidenced by the sharp decrease in the faeces egg counts just prior to the expulsion of the flukes. It was found that only 36% of the fluke population existing from 12-20 weeks after infection was recovered at 24 weeks after infection and at 30 weeks after infection only 16% of the fluke population present 16 weeks after infection was recovered. When this rate of loss is plotted logarithmetically against time after infection assuming the loss of flukes begins 20 weeks after infection, it can be seen in figure 10 that a straight line results showing the exponential loss in the acquired fluke burden of these calves.

'Self-cure' has recently been defined (Herbert and Wilkinson 1971) with regards to intestinal nematodes as 'the suddenly initiated and thereafter exponential expulsion of the majority of the population of worms'.and so calves infected with a single infection of F. hepatica can be said to have developed a 'self-cure' which begins some 20 weeks after their infection.

Fig. 10

Percentage of fluke population existing between 12-20 weeks after infection recovered between 20-30 weeks after infection



This result then fulfils the first criteria of Stirewalt (1963) which is cited in the introduction to this thesis viz, "the ability of a host to mount a 'self-cure' response". Thus calves receiving a single infection of F. hepatica can thus be said to develop an acquired resistance to this parasite. These experiments represent the first significant experimental proof of the development of such an acquired resistance to a single infection with F. hepatica in cattle.

The response of calves to reinfection with F. hepatica can be summarsied as follows. Calves reinfected 16 weeks after an initial infection of F. hepatica resisted the establishment of all but 16% of the fluke burden which developed in previously uninfected calves. Calves reinfected 12 weeks after an initial infection with F. hepatica resisted the establishment of all but 27.5% of the fluke burden which became established in previously uninfected calves. This resistance to reinfection was also reflected in the failure of the reinfecting population to further significantly alter either the haematological or biochemical parameters studied in these experiments. The results of these experiments therefore fulfil the remaining 2 criteria of Stirewalt (1963) cited in the introduction to this thesis viz, "A reduction in the expected worm burden from a given infecting dose 'and' A reduced level of disease in the host". The calves reinfected with F. hepatica can therefore be considered to have developed an 'acquired resistance' to reinfection with this parasite.

The relationship between the development of this acquired resistance and the duration of the initial infection prior to reinfection has also been demonstrated. Calves reinfected 7 weeks after their initial infection did not develop an acquired resistance to reinfection with F. hepatica while calves reinfected 12 weeks after their initial infection showed a 73% resistance and calves reinfected 16 weeks after their initial infection showed an 84% resistance to reinfection.

The results of these experiments confirm the preliminary results of Boray (1967) in 4 calves treated and reinfected 14-19 weeks after their initial infection and shows for the first time the relationship between the time of development of the initial infection and the magnitude of the acquired resistance induced. The results of these experiments also indicated that the persistance of the flukes derived from the initial infection through the subsequent reinfection may have increased the degree of resistance obtained as judged by the degree of hepatic damage observed in the treated and reinfected calves and the lower numbers of the reinfecting populations recovered from the livers of the untreated and reinfected calves. The latter observation, however, is based on distinguishing the populations on the basis of fluke size and is therefore somewhat unsatisfactory.

The appearance of an 'acquired self-cure' as defined by Ross (1966a, d) in calves reinfected with F. hepatica was not demonstrated in any of the 4 groups of calves reinfected with F. hepatica in the presence of the initial fluke population. The lack of suitable controls in the experiments of Ross (1966a, d) as discussed previously make the experimental results invalid and the term 'acquired self-cure' if used at all should be used to describe the response of calves to a single infection of F. hepatica as defined in this thesis.

The relationship between the successful development of an acquired resistance to F. hepatica infections and the previous administration of viable metacercariae by the oral route with the subsequent development of the parasites for a minimum period of time in the liver has been noted in the limited number of experiments successfully demonstrating the acquisition of some degree of resistance to reinfection with F. hepatica in various laboratory animal systems. The details of these experiments have already been discussed in the introduction to this part of the thesis. The results

from the experiments described in this part of the thesis would appear to support this premise with regards to the development of an acquired resistance to F. hepatica infections in calves in that the calves reinfected before the initial infection reached maturity did not develop an acquired resistance to F. hepatica, while calves reinfected at longer intervals after their initial infection showed increasing resistance to reinfection with this parasite.

As has been discussed at the end of the previous section of this thesis, the development of this acquired resistance in cattle has several possible explanations. It has been proposed by Boray (1967, 1969) that this resistance is purely a reflection of the fibrosis and cholangitis caused by the previous infection. This theory would then explain the increasing resistance with increasing duration of the initial infection as being simply related to increasing fibrosis and cholangitis making the environment unsuitable for the maintainance of the reinfecting fluke population. It would also explain the 'self-cure' of calves with a single infection of F. hepatica as being due to increasing cholangitis and subsequent calcification of the bile ducts rendering the bile ducts unsuitable for the feeding activities of the flukes.

The possibility that immune mechanisms are involved in this acquired resistance of cattle to infection and reinfection with F. hepatica can also provide possible explanations for the apparent dependence of the degree of acquired immunity obtained to the duration of the initial infection. The first of these possible explanations is that if a system of apparent antigenic similarity or rather minimal antigenic disparity occurs between F. hepatica, and cattle as suggested by Capron et al. (1968) similar to that described for Schistosoma mansoni and the Rhesus monkey (Smithers et al 1969) then it may simply require time for the immune response induced by a relatively small

number of weak antigens to reach sufficient magnitude to affect the parasite. This concept of a time related response to weak antigens has been investigated on a histocompatible skin graft system and it appears that when a weakly histocompatible skin graft is introduced into a recipient it requires much longer to destroy this graft than it would one with a strong histocompatibility antigen. (McKhann, 1970). The second possible explanation is that it is only after a certain point in the parasite's development that one or more specific antigens are released which are responsible for the induction of a specific immune response capable of affecting the parasite similar to the system previously described in Trichinella spiralis infections (Despommier 1971).

The results of the experiments described in this part of the thesis do not provide conclusive evidence to support or deny the implication of specific acquired immune responses in the development of an acquired resistance in cattle to infection or reinfection with F. hepatica. The description of the 'self-cure' of calves infected with a single infection associated with evidence of decreasing biological activity of the flukes prior to expulsion is however remarkably similar in pattern though on a much longer time scale to the immune expulsion of Nippostrongylus brasiliensis from infected rats. Thus 'self-cure' is more difficult to explain on the basis of purely physical factors since severe calcification is not found in the bile ducts of infected cattle till 23 weeks after infection (Dow, Ross, Todd, 1967). The flukes have been showing reduced biological activity for 4-8 weeks before this time and a large proportion of the fluke population has been expelled prior to this point as shown in the second experiment in this thesis. As an immunological mechanism may therefore have a part to play in the expulsion of the flukes from calves receiving only a single infection of F. hepatica so further investigation of the immune response of these calves was carried out and will be described in subsequent parts of this thesis.

GENERAL SUMMARY

GENERAL SUMMARY

An investigation into the response of susceptible calves to infections with F. hepatica revealed that calves could acquire a resistance to both primary and secondary infections with this parasite.

Calves infected with 500 or 750 metacercariae acquired a significant resistance to their infection as was manifest by the eventual expulsion of the majority of their acquired fluke burdens between 20-30 weeks after infection. A period of 4-8 weeks of reduced biological activity of the parasites evidenced primarily by a sharp decrease in egg output preceded their expulsion from their hosts.

An acquired resistance to reinfection with F. hepatica was also demonstrated in calves reinfected with 1,750 or 1,300 metacercariae after a primary infection with 750 metacercariae of F. hepatica. The development and magnitude of this acquired resistance was related to the degree of development of the primary infection prior to reinfection. Calves reinfected 12 or 16 weeks after the primary infection developed a significant resistance to this reinfection whereas calved reinfected 7 weeks after their initial infection did not develop a significant resistance to reinfection. The calves reinfected 16 weeks after their primary infection were more resistant to reinfection than were those calves reinfected 12 weeks after their primary infection. This resistance to reinfection was reflected in the stability of the haematological and biochemical parameters of the reinfected calves as compared to previously uninfected control calves.

PART 2

THE PRECIPITIN RESPONSE OF CALVES TO A PRIMARY INFECTION OF *F. HEPATICA*

INTRODUCTION AND REVIEW OF THE LITERATURE

The literature available on the occurrence of precipitating antibodies in the sera of animals affected with fascioliasis is not extensive. It is largely concerned with the detection of precipitating antibodies in the sera of animals suspected of being affected with fascioliasis or with the use of precipitating antibodies in investigating the antigenic structure of the fluke itself.

Precipitating antibodies to F. hepatica or in one instance F. gigantica have been used to diagnose infection in cattle, sheep, rabbits and man.

Ichihara, Susumi, Kuramoto (1956) used a complex antigen of F. hepatica prepared by mixing a purified polysaccharide and lipid antigen extracted from F. hepatica. This antigen was tested against the sera of 301 cattle using the Ouchterlony double diffusion plate technique. All the cattle were found to contain F. hepatica eggs in their bile or faeces at slaughter and precipitins were detected in approximately 90% of the sera.

Spuhler, Moosbrugger, Meyer (1958) used an acetone precipitated extract of F. hepatica again in an Ouchterlony double diffusion plate test against the sera of 87 cattle, 62 of which were found at slaughter to have liver damage attributable to infection with F. hepatica. The precipitin test gave positive results with the sera of 63% of the infected cattle.

Capron et al, (1968) used a saline extract of F. hepatica in an immunoelectrophoresis analysis developed against sera from infected cattle. A maximum of four precipitin lines occurred and these precipitins occurred in only 55% of the infected sera. No details were given of the criteria used to confirm the presence of infection in these cattle.

Gajos (1969) using extracts or suspensions of adult F. hepatica detected precipitins in the bile of cattle infected with F. hepatica but rarely detected precipitins in the sera of the same cattle. Little detailed experimental evidence was provided but it was noted that the globulin fraction of the bile was concentrated by ammonium sulphate precipitation. No such concentration was carried out on the serum globulins.

Nansen (1970) used a saline extract of adult F. hepatica in a double diffusion system against the sera of 9 heifers with chronic fascioliasis. Two to five precipitins were found in each of the nine sera.

There is, therefore, a wide variation in the reports of the percentage detection of precipitins in the sera of infected cattle.

Hughes (1963) investigated the serological response of sheep and rabbits infected with F. hepatica against various antigens of F. hepatica including a 4% suspension of ground adult flukes in saline and a metabolic product antigen derived from a physiological solution in which living flukes had been incubated for 48 hours at 37°C. Precipitins could first be detected 4 weeks after infection in rabbits and 2 weeks after infection in sheep. A maximum of four precipitins were formed by both sheep and rabbits against an extract of adult flukes and three of these precipitins could also be found in the metabolic products antigen.

Sewell (1964) investigated the precipitin response of mice and rabbits infected with F. hepatica and also of 2 cattle infected with F. gigantica. The rabbit antisera first reacted with a crude saline extract of F. hepatica four weeks after infection and the immune response reached a maximum 6-7 weeks after. The sera from infected cattle did not react with any protein antigen from either F. hepatica

or F. gigantica. Sera from infected mice gave a faint precipitin reaction against protein antigens on one occasion only. The mice and cattle sera, however, developed strong precipitin lines at 2-5 weeks after infection with a non-protein fraction isolated from the metabolic products of F. gigantica. Sewell (1966) found precipitins in sheep sera fourteen days after infection with F. hepatica which reacted with a metabolic product antigen of F. hepatica.

Capron, Rose, Luffau, Biguet, Rose (1965) investigated the precipitin response of rabbits each infected with either 10, 15 or 60 metacercariae of F. hepatica. The rabbits which each received 60 metacercariae had produced detectable levels of precipitating antibody fourteen days after infection and the maximum number of detectable precipitins occurred about 12 weeks after infection. The rabbits receiving the lower doses of metacercariae developed detectable precipitins four weeks after infection and these levels again reached a maximum at about 12 weeks after infection. Five to eight separate precipitins were found in the sera of each rabbit at this time.

The use of immunoelectrophoresis in diagnosing infections with F. hepatica in man has been described by Capron, Biguet, Vernes (1967). Sera from 180 cases of human fascioliasis were examined and 2-11 precipitins were found in 98% of these sera.

It would appear from the work into the precipitin response in laboratory animals that precipitins appear early in the course of an infection and reach a peak within 6-12 weeks of infection.

The antigenic composition of various extracts and homogenates of F. hepatica have also been reported.

Sewell (1964) found that rabbits immunised with a suspension of a freeze dried preparation of washed, chopped adult flukes produced antisera to 38 antigenic components both protein and non-protein in nature.

Korach and Benex (1966a, b) described the isolation of a lipoprotein antigen of F. hepatica which gave a single precipitin line with antisera raised against it in the rabbit and also gave a precipitin response with sera from rabbits infected with F. hepatica. This antigen was derived from adult flukes but could not be isolated from bile of infected rabbits.

Taillez and Korach (1970a, b) then described the isolation from F. hepatica of a genus specific antigen containing 14% nitrogen and 2% carbohydrate but no lipids. Cross reactions were observed between this antigen and antisera against F. gigantica but no other nematode parasite. The antigen was shown by autoradiography to be located near the cuticle of the fluke.

Tran Van Ky, Vaucelle, Capron, Biguet (1967) described immuno-electrophoresis of homogenates from F. hepatica and the identification of 15 of the resultant precipitin lines as sites of enzyme activity. The enzymes identified included dehydrogenases, carboxylases, alkaline phosphotase, trypsin and chymotrypsin.

Capron et al. (1968) undertook an extensive investigation into the antigenic composition and precipitin response elicited by many parasites including F. hepatica in various hosts. It was found that a saline extract of F. hepatica contained 25 different antigens on immunoelectrophoresis against sera from rabbits vaccinated with a homogenate of F. hepatica. F. hepatica shared 6 of these antigens with a similar extract of Dicrocoelium dendriticum five antigens with extracts of Paragonimus westermani., Clonorchis sinensis,

Schistosoma mansoni and S. haematobium and four antigens with S. japonicum. One of these 25 antigens was found to be specific for F. hepatica and precipitating antibody to this antigen could always be found in the serum from any animal infected with F. hepatica.

The number of precipitating antibodies found in each species after infection with F. hepatica was studied and the results are as shown in the table below:-

Table 9

The Maximum number of precipitins found in F. hepatica infections of man and animals (Capron et al, 1968)

Species	Man	Rabbit	Hamster	Cattle	Sheep
Precipitins	11	10	6	4	2
% of positive sera	92	100	100	55	55

It was concluded that this gradation of the precipitin response in these hosts indicated the degree of adaption of the parasite to each host. F. hepatica was considered to have developed as a specific parasite of sheep and perhaps cattle and only as an incidental parasite of the remaining species.

F. hepatica was found to have 6 antigens identical with antigens present in bovine liver, 4 of these antigens were common both to F. hepatica and D. dendriticum both considered as primary parasites of the ovine and bovine liver. On the basis of this apparent selectivity of the common antigens found between host and parasite it was decided that these antigens could not be due to chance contamination from host tissue ingested by the parasites but were in actual fact true parasite antigens. It was on the basis of these and similar findings in nematode and cestode parasites that Capron et al., proposed the theory of 'immuno-adoption' discussed in the introduction to the first part of this thesis.

Capron et al, (1965, 1967) discussed the occurrence of C substance and C reactive protein in the precipitin test for fascioliasis. C substance is a polysaccharide first isolated from the somata of the bacterium Pneumococcus. Since then it has been found in various fungi (Longbottom and Pepys, 1964) and in the extracts of various helminth parasites (Capron et al, 1965). C substance has the property of combining to form a precipitate with C reactive protein but it can also act as a specific antigen and induce the production of specific antibody.

C reactive protein is a 7.55S protein thought to be composed of identical subunits and has a molecular weight of 21,500. It is not an immunoglobulin but migrates in the B globulin position on electrophoresis. This protein is not detected in normal sera but appears very early, before the appearance of specific antibodies, in acute inflammatory conditions. It is used as a diagnostic marker in medicine for such conditions as acute rheumatism and myocardial infarctions. The combination of C substance and C reactive protein is dependent on the presence of Ca ++ ions (Abernethy and Avery, 1941). The precipitate so found is then soluble in solutions of sodium citrate or phosphate of high ionic strength at pH8 which remove the available Ca ++ ions.

Capron et al, (1965) detected a C reactive protein in rabbits infected with F. hepatica. Precipitin bands were found on double diffusion in agar which were soluble in a solution of sodium citrate and it was concluded that C substance in the extract had reacted with C reactive protein in the infected rabbit sera. Capron et al, then proposed that the precipitates obtained by Sewell (1964) using sera from animals shortly after infection and the non protein fraction of the antigen were probably due to a reaction of C substance with

C reactive protein.

In view of the wide variation in the percentage of infected cattle reported as having precipitins to F. hepatica and the information available on this response in other animals it was decided to investigate the precipitin response of parasite free calves experimentally infected with F. hepatica. The variations in the previous reports on the precipitin response in cattle could be accounted for by the fact that they are based on the results from single serum samples from animals of varying ages whose actual state of infection with F. hepatica was not clearly defined. The precipitin response in experimentally infected calves was investigated with a view to defining the pattern of this response including the time of appearance of precipitins, the antigens involved, the possibility of C substance reactions, and if possible attempt some quantitation of the response.

MATERIALS AND METHODS

Antigens

Four types of fluke antigen were used in the investigation of the precipitin response in cattle to single infections with *F. hepatica*. All the antigens were from adult *F. hepatica* recovered alive from the bile ducts of infected cattle. The antigens prepared were a crude homogenate of adult flukes, an extract of homogenised flukes in phosphate-buffered saline, a metabolic antigen prepared from the metabolic products of adult flukes and a lipid free extract of adult flukes. The homogenate or saline extract were prepared from either freshly recovered flukes or from flukes which had been freeze dried immediately after recovery. Prior to lypholisation the flukes were washed once in phosphate buffered saline, blotted gently on Green 904 filter paper (J. Green, Maidstone, England) and then snap frozen in liquid nitrogen (-196^{o}C). The frozen flukes were then transferred to a phosphorus pentoxide freeze dryer (Model 5P.S. Edwards, Sussex, England) and dried *in vacuo* overnight. The lypholised flukes were stored under nitrogen in sealed bottles at -50^{o}C. The various antigens were prepared as follows:-

a) Crude homogenate of flukes was prepared by homogenising living or lypholised fluke in 25 ml. of cold phosphate buffered saline in an ice bath at 2-4^{o}C using a Silverson mixer-emulsifier (Silverson Machines Ltd., London).

b) A saline extract of flukes (SFE) was prepared by centrifuging a crude homogenate of flukes at 4^{o}C in a Mistral 4L centrifuge (Measuring & Scientific Equipment Ltd., London, England) for 15 minutes and a

relative centrifugal force of 4,000 g. The supernatant was decanted and stored for immediate use at 4°C otherwise it was stored in 1 ml. amounts at 50°C. The frozen antigen retained most of its activity from 3-4 months under these conditions but there was a definite loss of antigenicity in antigen kept for up to 1 year.

c) A metabolic products antigen (M.A.) was prepared by maintaining adult flukes in 250 ml. of a physiological solution the constitution of which is shown below,

NaCl	- 7 gms/litre	$MgSO_4$	- .30 gms/litre
KCl	- .30 gms/litre	$Na_2HPO_4 2H_2O$	- .50 gms/litre
$CaCl_2$	- .10 gms/litre	Glucose	- 1 gm/litre

The flukes were maintained in this solution for 8 hours at 37°C. Under these conditions the flukes became motile, some climbing the sides of the beaker. After 8 hours the supernatant was decanted and the sediment removed by centrifugation. The supernatant was then equilibrated by dialysis against repeated changes of phosphate-buffered saline for 24 hours. The supernatant was reduced to a final volume of 10 ml. by dialysis against polyethylene glycol (Carbowax 4,000, Edward Gurr, Ltd., London) at 4°C. The metabolic antigen was stored under similar conditions to the saline extract antigen.

d) A lipid free antigen was prepared by homogenising lypholised flukes in 10 ml. of chilled anhydrous ether in a calcium chloride ice bath at -15°C. The homogenate was then transferred to pre-cooled centrifuge tubes and centrifuged at -20°C at a relative centrifugal force of 1,000 g. The

supernatant was then decanted and the sediment dried by suction from a vacuum pump. The dried sediment was homogenised with cold phosphate buffered saline in an ice bath at 2-4°C. The homogenate was left for 12 hours at 4°C and was then centrifuged at 4°C at a relative centrifugal force of 1,000 g. for 30 minutes. The supernatant was transferred in 1 ml. amounts to chilled bottles and stored at 4°C till used.

The protein content of these antigens was too low to be estimated using the Buiret technique previously described, so the Lowry method was used (Lowry, Rosenborough, Farr, Randall 1951). This method is based on a colometric change in Folin-Ciocalteu's reagent proportional to the amount of protein or more correctly the amount of tyrosene and tryptophan added. A standard graph was derived from a solution of know protein concentration (Versatol A., W.R. Warner & Co. Ltd., Hampshire, England), and the results expressed in mgs/100 ml.

Antisera to F. hepatica

a) Rabbit - antisera to the antigenic components in the crude fluke homogenate was raised in a pair of adult New Zealand White rabbits. The rabbits were given on 3 occasions at monthly intervals an intramuscular injection containing 1 mg. of crude fluke homogenate emulsified in an equal volume of complete Freund's adjuvant (Difco Laboratories, Michigan, U.S.A.).

The rabbits were bled out eight weeks after the last injection of antigen, their sera pooled and stored in 1 ml. amounts at -20°C.

b) Bovine - The sera used were from 24 calves each given a single experimental infection of 500 metacercariae of F. hepatica

and 8 calves each of which received a single experimental infection of 750 metacercariae of F. hepatica.

Immunodiffusion techniques (I/D)

Immunodiffusion was carried out using a combined double diffusion system in agar plates.

The plates were made up using disposable perspex petri dishes (Oxoid Ltd., London, England) of either 5 or 8.5 cms. diameter filled to an even depth of 2 mm. with agar. The agar was made up as a 1% solution of Special Agar-Noble (Difco Laboratories, Detroit, U.S.A.) in 8% saline solution with sodium azide added to a final concentration of .02% as a preservative. Wells were punched with a modified pipette and the agar removed by suction. Patterns with wells of 2 mm. diameter and a distance of 6 mm. between well centres were commonly used although a distance of 8 or 10 mm. between wells was used on occasions. The wells were filled with reactants using a capillary tube. The plates were incubated in a humidity chamber at room temperature till the precipitation pattern had developed.

Immunoelectrophoresis (I/E)

Immunoelectrophoresis was carried out using a microplate technique in apparatus designed by Shandon Scientific Co. Ltd., London, England.

Microscope slides (76 x 26 mm.) were used as carriers for the agar gel. The slides were washed in chromic acid, carefully rinsed in distilled water, dried and eight slides placed in the slide tray. This slide tray was then placed on a previously levelled surface and a thin layer of hot 1% agar in .05 glycine solution poured over the slides and allowed to dry. The tray was then filled with a 2% agar solution in barbitone-acetate buffer of pH 8.2 and ionic strength of .05. Sodium azide was again added to the gel in a final

concentration of .02% as a preservative. The agar was levelled while still hot and the plate allowed to cool. Patterns were cut in the agar using a cutter which gave 2 or3 wells of approximately 1 mm. diameter and one or two lateral troughs 60 mm. long by 1.5 mm. wide and placed 4 mm. from the wells. The agar was removed from the wells by suction and the wells filled using a micro-pipette. The agar in the lateral troughs was removed after electrophoresis of the sample. The tank buffer was a barbitone-acetate buffer of pH 8.2 and ionic strength of 0.1. Electrophoresis usually required 90-120 minutes with a potential drop across the slides of 5 volts/cm. After electrophoresis the appropriate antiserum was added to the lateral troughs and the plate incubated in a humidity chamber at room temperature till the precipitin pattern had developed. The slides were then washed for 24 hours in several changes of phosphate-buffered saline to remove excess reactants and allowed to dry out at room temperature under filter paper to avoid cracking of the gels as they dried.

Stains

After immunoelectrophoresis the dried gels were routinely stained with Amido black or Ponceau S. which stain the protein components of the I/E pattern.

1) Amido black - this was made up as a 1% solution of Napthalene Black 10B (G.T. Gurr Ltd., London) in 5% acetic acid. Slides were stained for 2-4 hours, washed in several changes of 5% acetic acid to remove excess stain, washed finally in distilled water and allowed to dry.

2) Ponceau S - a 2% solution of Ponceau S (B.D.H., Poole, England) in 5% acetic acid was used. The slides were

stained for 1 hour, then were washed and dried as before.

Amido black was found to give the best definition of the precipitin lines in the I/E plates.

On occasions special staining techniques were employed to investigate the existence of lipids, lipoproteins polysaccharides or glycoproteins in the extracts of F. hepatica used.

3) Sudan black - a Sudan black stain was used as a stain for lipids and lipoproteins using the technique described by Grabar and Burtin (1964).

4) Periodic-formazan - the periodic-formazan reaction was used as the basis for staining of polysaccharides and glycoproteins because of its specificity for aldehyde groupings. The technique was as described by Grabar and Burtin (1964).

C substance and C reactive protein

In the investigation of the existence of C substance and C reactive protein in the precipitin test the following techniques were employed.

a) The solubility of precipitin lines in 2 M sodium citrate. The precipitin patterns were noted then the plates were filled with the sodium citrate solution and left for 24 hours. The sodium citrate solution was then poured off, the plates washed in saline and the precipitin pattern re-examined.

b) Development of precipitin lines after double diffusion in 1% agar made up in McIlvaney's citric acid buffer at pH 7.2.

c) Possible cross reaction between bovine sera and a commercially produced anti C reactive protein sera for use in human diagnosis (Hoechst Pharmaceuticals, Brentford, England).

Quantitative estimation

Quantitation of the precipitin response was attempted using one dimensional double diffusion system in agar gel. A column, 8 ± .02 mm. long, of a 8% solution of agar in a .85% saline solution was formed in the middle of a glass tube 3 cm. long and of 3 mm. internal diameter. A measured amount of agar was introduced into each tube using a tuberculin syringe, the agar column centered while still fluid and then allowed to set on a level surface. A standard antigen solution and the appropriate antiserum were added to the opposite ends of the tube, the ends sealed with adhesive tape and the tubes placed in a humidity chamber at room temperature. After 120 hours the tubes were removed and the distance of the leading precipitin band from the antigen interface and the total length of the gel were measured using a micrometer. The distance of the leading precipitin band from the antibody interface was then calculated and expressed as a fraction of the total length of the gel column. This figure was taken as being proportional to the original concentration of antibody.

The theoretical considerations on which this conclusion is based are as follows. It has been found empirically that the distance from the antigen meniscus to the point where a precipitin band is formed was a linear function of the log antigen concentration when gel column lengths of 1 cm. length were used (Polson, 1971) and conversely the distance to the antibody interface must then be a function of the log antibody concentration. This can be developed from the equation cited below.

It has been shown that the concentration of the reactants at the position of the precipitin band is given by the equation.

$$\log \frac{Cog}{Cob} = \log \frac{Cg}{Cb} + \frac{Xg}{\sqrt{\pi Dgt}} - \frac{Xb}{\sqrt{\pi Dbt}} \qquad (1)$$

when the total gel length is small i.e. under 1.5 cms. (Polson, 1971). In the equation the subscripts g and b refer to antigen and antibody respectively and Co = original concentration, C = concentration at the precipitin line, X = distance from appropriate interface to precipitin band, D = the diffusion coefficient.

In the test system described above the time t, is constant, and the antigen/antibody system under study is constant the equation can be written as

$$\log \frac{Cog}{Cob} = \log \frac{Cg}{Cb} + \frac{Xg}{C_1} - \frac{Xb}{C_2} \qquad (2)$$

where C_1 and C_2 are constants.

The relationship between the original concentration of the reactants Cob and Cog and their concentration after diffusion through a distance X during time t is given by the equation.

$$C(X,t) = Co\left[1 - \frac{2}{\pi^{\frac{1}{2}}} \int_0^y e^{-y^2}\, dy\right] \qquad (3)$$

$$\text{where } y = \frac{X}{\sqrt{\pi Dt}}$$

It can be seen therefore that at any point the concentration of either reactant is dependent on its original concentration, time of diffusion and its diffusion coefficient. In the system under discussion where Cog, Dg, Db, and t are constant it follows that Cg is also a constant and that Cb is dependent on Cob.

Equation (2) can now be written.

$$K_1 \log Cob = K_2 \log Cob + \frac{Xg}{C_1} - \frac{Xb}{C_2} \qquad (4)$$

when K_1, K_2, C_1, and C_2 are constants.

Since Cb is directly related to Cob the only variables in equation (4) are Cob, Xg and Xb. The relationship between Xg and Xb however, can be given as $\frac{Xg}{L} + \frac{Xb}{L} = 1$ when L is the total gel length. This then allows the equation to be stated in its most simple form that there is a logarithmic relationship between the original concentration of the antibody (Cob) and the distance between the precipitin band and the antibody interface (Xb) expressed as a fraction of the total gel length (L).

This fraction of the total gel length was taken as representing the logarithm of the initial concentration of the precipitating antibody in the serum. The results were then arbitrarily expressed as precipitin units (P.U.) by conversion to antilog_{10} using conventional common logarithm tables.

RESULTS

1) Antigenic structure of F. hepatica

(a) Precipitin response of artificially immunised rabbits

Immune electrophoresis of a saline extract of F. hepatica developed against the pooled sera of rabbits immunised with crude homogenate of F. hepatica revealed the existence of 12 antigenic components in this complex antigen. The resultant I/E pattern is shown in figure 11 and the photograph of the pattern in appendix 2 plate 1.

Immune electrophoresis of the metabolic antigen of F. hepatica developed with the same rabbit antisera revealed the evidence of 6 antigenic components, 4 of which had electrophoretic mobilites similar to antigens present in the saline extract. The I/E pattern of this antigen is also shown in figure 11 and in appendix 2 plate 1.

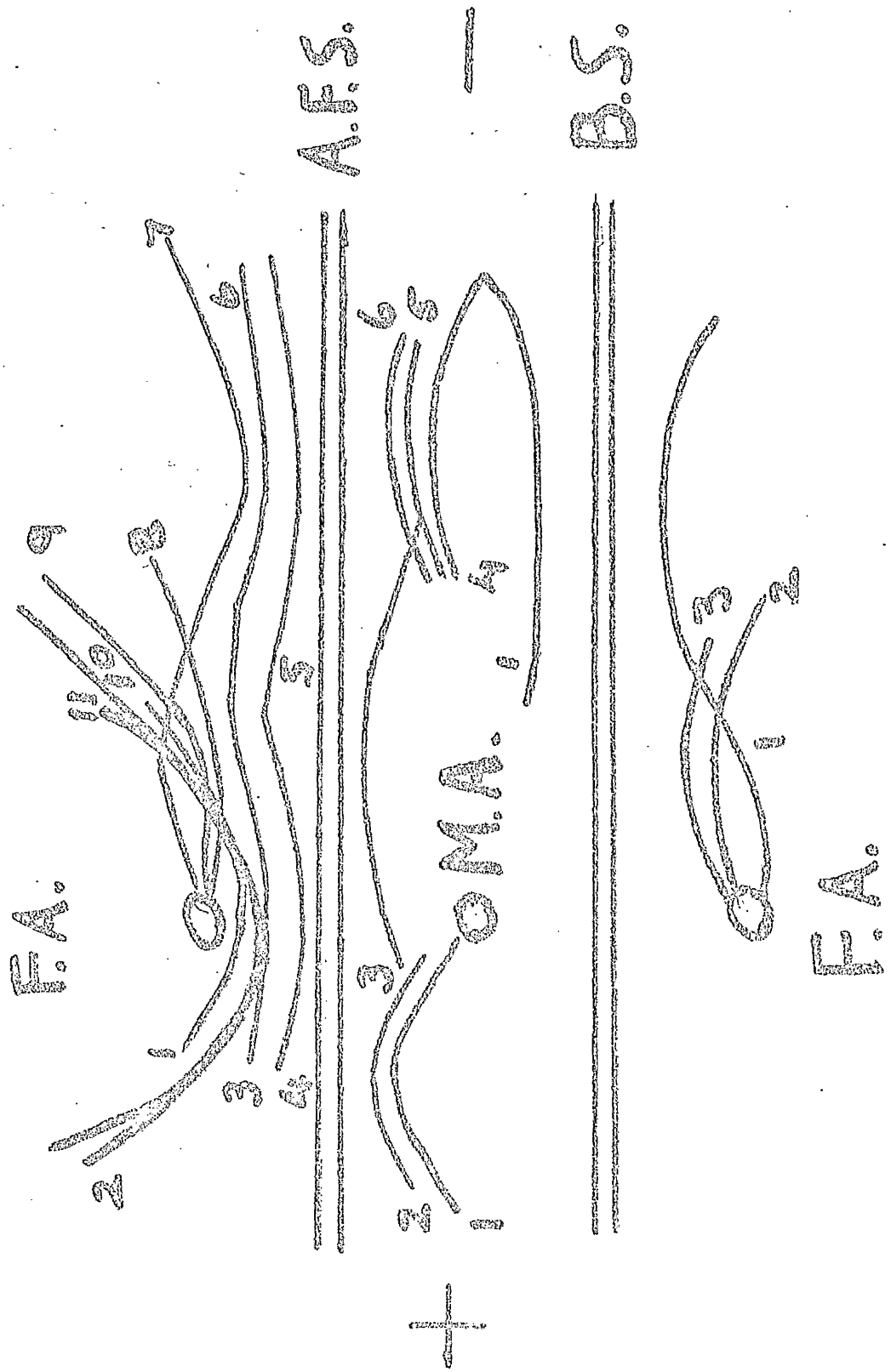
(b) Precipitin response of experimentally infected cattle

Precipitating antibody could be detected in the sera of most cattle by four weeks after infection with F. hepatica. Sera from infected cattle would develop a single precipitin line against either a saline extract or the metabolic antigen when tested in a combined double diffusion system in agar plates. When infected cattle serum was reacted against both antigens in a combined double diffusion test the precipitin line to each antigen was found to fuse with the other in a reaction of identity as shown in plate 2 in appendix 2. The antigen responsible for the precipitin response detectable in a double diffusion system is therefore present in both in the saline extract and the metabolic antigen of F. hepatica.

The relationship between antigens of F. hepatica and the precipitin response induced in infected cattle was further investigated using immunoelectrophorectic techniques. The I/E pattern of the

Fig. 11

I/E analysis of saline extract (F.A.) and metabolic products (M.A.) of F. hepatica developed against rabbit antisera (A.F.S.) and infected bovine sera (B.S.)



saline extract and metabolic antigen as developed by immune cattle sera is shown in figure 10 and plate 1 appendix 2. It can be seen that in this system the sera contained precipitins to three antigens in the saline extract and precipitins to one antigen in the metabolic antigen which had a similar electrophorectic mobility to one of the antigens revealed in the saline extract. Infected cattle therefore appear to mount a precipitin response to only a few of the antigenic determinants present in the extracts of F. hepatica and one of these determinants is present in a metabolic antigen of F. hepatica.

(c) Chemical composition of the antigens of F. hepatica

After electrophoresis in agar the antigens were stained specifically to identify proteins, lipids, lipoproteins, polysaccharides and glycoproteins. All the antigenic components stained specifically with protein stains but there was no evidence of the presence of any of the other chemical groupings using these techniques.

2) The possible occurrence of C substance and a C reactive protein

The possibility of the occurrence of C substance in the extracts of F. hepatica reacting with an acute phase C reactive protein if this were present during the early weeks after infection with F. hepatica to give a precipitin line which could be mistaken for a specific response as proposed by Capron et al. (1965, 1967) was investigated as follows:

The precipitin lines which were found in a double diffusion system against cattle sera taken from 3-8 weeks after their infection were tested for their solubility in a solution of sodium citrate of high ionic strength. The precipitin pattern was unaltered by this treatment on every occasion. This observation was confirmed when

similar sera were found to react with fluke antigens to give a detectable precipitin response in a double diffusion system carried out in agar buffered with citric acid. The citric acid would bind the available Ca++ ions and so prevent the combination of C substance and C reactive protein, a reaction dependent on the presence of free Ca++ ions.

One further attempt was made to define the presence of a C reactive protein in infected cattle sera using immunoelectrophoresis of cattle sera developed against a commercially available anti-human C reactive protein. This serum failed to react with any cattle sera taken from 3-8 weeks after infection with F. hepatica.

It can be stated therefore that with the antigens and sera used in these experiments the precipitin response detected in calves as early as 3 weeks after their infection with F. hepatica is the result of a specific immunological reaction and not the combination of C substance with an acute phase C reactive protein.

3) The occurrence of precipitins in cattle infected with Fasciola hepatica

In order to characterise the precipitin response of cattle infected with F. hepatica serum was taken from calves at 0-4, 8, 12, 16, 20 and 24 weeks after their infection with 500 metacercariae of F. hepatica. These sera were tested in a double diffusion system in agar plates against a standard solution of a lipid free fluke antigen. The plates were examined for the presence of precipitin lines after 24 hours incubation at room temperature. All the calves were found at autopsy to be infected with F. hepatica. The results of these precipitin tests are summarised in table 10 and the detailed results are given in table 1, appendix 2.

TABLE 10

Precipitin response of calves infected with 500 metacercariae of *F. hepatica*

Weeks after infection	0	1	2	3	4	8	12	16	20	24
No. of sera examined	24	24	24	24	24	20	16	12	8	4
No. of sera found to contain precipitins	0	0	1	18	22	17	8	11	6	4
Percentage of sera containing precipitins	0	0	4	75	92	85	50	92	75	100

Precipitating antibodies were detected in the serum of one calf as early as 14 days after infection with *F. hepatica* and precipitins had been detected in 92% of the sera tested four weeks after infection with *F. hepatica*. Precipitins were found in 80% of the 108 serum samples taken between 3-24 weeks after infection of the calves with *F. hepatica*. Each calf had detectable precipiting antibodies in its serum on at least one occasion.

The experiment was repeated using serum samples taken from four calves at 0-4, 8, 12, 16, 20, 24 and 28 weeks after their infection with 750 metacercariae of *F. hepatica*. All the calves were found to be infected with *F. hepatica* at autopsy. The detailed results of this experiment are shown in table 2, appendix 2 and can be summarised as follows:

Precipitins were first detected in serum samples taken 4 weeks after infection and 82% of the thirty two samples taken between 3-28 weeks after infection with *F. hepatica* contained detectable precipitins. Each calf had detectable precipitating antibodies in its sera on at least one occasion after infection.

Further investigation of the precipitin response was carried

TABLE 11

Sequential development of precipitins in the sera of calves infected with 500 metacercariae of F. hepatica

Calf No.	Time after infection (weeks)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
21	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+
23	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
24	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+

+ = present - = absent

out using an immuno-electrophoretic technique. In this method a similar antigen to that used in the immuno-diffusion test was, after electrophoresis in agar, reacted with sera from calves infected with F. hepatica. Serum samples were taken at weekly intervals over a 6 month period from 4 calves each infected with 500 metacercariae of F. hepatica. The results are shown in table 11.

It can be seen from the table that using this technique precipitins were detected most consistently in the early stages of the infection. Precipitins were detected in 55% of the eighty eight samples examined between 3-24 weeks after infection. When the same sera were examined for the presence of preciptins using both immuno-diffusion and immuno-electrophoretic techniques, the immuno-diffusion technique detected precipitins in 87% of the 24 samples tested while the immuno-electrophoretic technique detected precipitins in only 58% of the 24 samples tested. On no occasion did the immuno-electrophoretic technique detect precipitins which could not be detected using the immuno-diffusion technique.

The precipitin test was specific in that a positive reaction was never recorded with sera from uninfected calves and all calves infected with F. hepatica had detectable precipitins in their sera at some point after infection.

4) Quantitation of the precipitin response in calves infected F. hepatica

Quantitation of the precipitin response of calves infected with F. hepatica was carried out on serum samples taken at monthly intervals from 12 calves, eight of which were each infected with 750 metacercariae of F. hepatica and four of which were each infected with 500 metacercariae of F. hepatica. Eleven of the twelve calves were found to be infected with F. hepatica at autopsy. All eighty-four sera were tested against the same

standard solution of a lipid free fluke antigen which had a protein concentration of 6 mgs/ml. The individual results are given in table 3, appendix 2. The mean precipitating antibody level of these calves at monthly intervals is shown in table 12.

TABLE 12

Precipitin levels of calves following infection with 500 metacercariae of F. hepatica

Weeks after infection	0	4	8	12	16	20	24	28
Mean precipitating antibody level (P.U.)	0	4.14 ± .42	4.73 ± .39	3.99 ± .25	3.67 ± .19	3.83 ± .39	4.87 ± .52	5.39 ± .44
No. of sera examined	11	11	11	11	11	11	8	4

It can be seen that the precipitin response of these calves which had received a single experimental infection of F. hepatica tended to be biphasic with levels reaching a peak at 8 weeks after infection, then declining before rising again from 16 weeks after infection onwards. The mean precipitating antibody levels of the 11 calves at 16 weeks after infection (3.67 ± 1.9 P.U.) is lower than the level at 8 weeks after infection (4.73 ± .39 P.U.) though this difference is statistically significant only at the 2% level. However, the mean precipitin level of the remaining four calves 28 weeks after infection (5.39 ± .44 P.U.) is significantly higher ($p < .01$) than the level of the 11 calves, 16 weeks after infection (3.67 ± .19 P.U.)

The use of a double diffusion system in a single dimension as in the tube technique used to quantitate the precipitin response also revealed several other aspects of the precipitin response. The tube technique was the most sensitive of all methods used to detect precipitins in the sera from infected calves. Precipitins were

detected in 100% of the serum samples taken between 1-7 months after infection including those in which precipitins could not be demonstrated by either immuno-diffusion in agar plates or immuno-electrophoretic techniques. The tube precipitation test was also specific in that precipitins were never detected in sera from uninfected calves. Sera from infected calves developed one to three precipitin bands in the gel using this technique confirming the finding of the immuno-electrophoretic technique.

DISCUSSION

The results of these experiments illustrate the nature and extent of the precipitin response of calves induced by infection with F. hepatica.

Precipitins were first detected in the sera of calves 2-4 weeks after their infection with F. hepatica and were still present 7 months after infection. Precipitins are first found in the sera of infected cattle at a similar time after infection with F. hepatica as they appear in sheep, rabbits and mice infected with F. hepatica (Hughes 1963, Sewell 1964, 1966, Capron et al. 1965) and in cattle infected with F. gigantica although only two sera were studied in these infections (Sewell 1964).

The possibility that these precipitin lines detected in the early stages of the infection were caused by a reaction of C substance in the fluke extract with an acute phase C reactive protein in the cattle sera as suggested by Capron et al. (1965, 1967) was considered but no experimental evidence could be found to support this hypothesis. These precipitins detected in the early stages of the infection were therefore due to a specific immune response of the calves to their infection with F. hepatica.

The quantitative precipitin response of calves to infection with F. hepatica appeared to be biphasic in nature with a peak occurring about 8 weeks after infection at a time when the immature flukes are still in the liver parenchyma just prior to their entry into the bile ducts. There was a slight but significant decline in the precipitin response between 8-16 weeks after infection and thereafter there was a progressive rise in the response over the period when the adult flukes were known to be eliminated from the

bile ducts.

The precipitin response in rabbits infected with F. hepatica also appears to reach a maximum between 6-12 weeks after infection (Sewell 1964, Capron 1965).

There were wide variations in the sensitivities of the three different methods used to detect precipitins in the sera of infected cattle. The one dimensional double diffusion system in tubes as used in the quantitative technique was the most sensitive method used and it detected precipitins in 100% of all serum samples taken from 1-7 months after calves were infected with F. hepatica including samples in which precipitins could not be detected by any other method. The combined double diffusion system on plates was less sensitive than the tube system but detected precipitins in approximately 80% of all the serum samples taken from 1-7 months after infection. The immunoelectrophoretic technique was the least sensitive of the methods used and detected precipitins in only 58% of serum samples taken between 1-6 months after infection. The precipitin reaction was always specific no matter which of the methods was used as no precipitins were ever detected in the sera of uninfected calves. The differing sensitivities of these methods can be related to their ability to establish, in the agar gel, concentration gradients of both antigens and antibodies which allow their combination at or near their equivalence levels in such amounts as to allow formation of a visible precipitin line, even where the system is originally unbalanced with one of the reactants being present in marked excess. The tube technique allows the establishment of concentration gradients over a greater distance than the plate technique as the reactants can only diffuse towards each other in the gel and there are no losses due to radial

diffusion as occur in the plate technique. The failure of the immuno-diffusion method in agar plates and the immuno-electrophoresis method to detect precipitins known to be present in the sera of infected calves is that when one of the reagents has been present in marked excess these systems have been unable to reach equivalent concentration levels within the gel with the result that antigen/antibody complexes soluble in the excess reactant have been formed which do not aggregate to form visible precipitin lines.

This limitation of a combined immuno-diffusion system in agar plates may explain the wide differences reported in the occurrence of precipitins in the sera of cattle infected with F. hepatica (Ichihara et al. 1956, Spuhler et al. 1958, Gajos 1969, Nansen 1970). It is interesting to note that the use of a relatively simply prepared fluke extract in a combined immuno-diffusion system in these experiments detected precipitins in approximately the same percentage of sera from infected cattle (80-90%) as did the highly purified antigens used in a similar mthod by Ichihara et al. (1956). The immuno-electrophoretic method used in these experiments also gave remarkably similar results to those previously reported by Capron et al. (1968) in that the same technique and antigens in both sets of experiments detected precipitins in 55-58% of the sera from infected cattle.

Cattle infected with F. hepatica produce detectable precipitating antibodies to remarkably few of the large number of antigens present in the highly complex antigenic structure of F. hepatica. Precipitins could only be detected to three of the 12 antigenic components known to be present in the extract of F. hepatica and to only 1 of the 6 antigens known to be present in the

metabolic products of F. hepatica. This confirms the report of Capron et al. (1968) who detected precipitins in the sera of infected cattle to a maximum of 4 of the 25 antigens known to be present in a similar extract of F. hepatica as was used in these experiments. Nansen (1970) also found a maximum of 2-5 precipitins to a saline extract of F. hepatica in the sera from naturally infected heifers. The apparent limitation of the precipitin response of calves to infection with what is undoubtedly an antigenically highly complex parasite may indicate that the antigens in the fluke extract which stimulate a precipitin response in artificially immunised rabbits are not accessible to the cells responsible for mounting an immune response or, if they are, they are not capable of inducing a precipitin response in these calves. It is generally recognised that rabbits readily produce large quantities of precipitating antibodies in response to many antigens and so are used in the commercial production of many specific precipitating antisera. Cattle, however, may produce proportinately less precipitating antibody in response to these antigens and produce greater quantities of other types of non precipitating antibodies such as reaginic antibodies.

Fluke antigens would appear to be readily accessible to the reticulo-endothelial system of infected calves since during the fluke's digestive process enzymes are secreted into the gut (Dawes 1962, Pantelouris 1965, Thorsell and Bjorkman 1965) These gut contents are later regurgitated through the oral pore into the area surrounding the parasite in the liver, an organ with a highly organised reticulo-endothelial system. The tegumental cells of the fluke are also involved in the absorption and excretion of metabolites

(Bjorkman and Thorsell 1964, Lee 1966) so releasing further antigens outside the parasite. It would seem therefore that there would be a number of parasitic antigens available to the host and that many of these would be enzymes. In this context it is to be remembered that the majority of the antigens present in the extract of F. hepatica are enzymes (Tran Van Ky et al. 1967) and that a genus specific antigen of F. hepatica has been localised by autoradiography to the tegumental layer (Taillez and Korach 1970 a, b).

A genuine failure of cattle infected with F. hepatica to mount an immune response to the particular antigens, enzymes or otherwise available to their lymphoid system would indicate that the parasite has in some way become adapted to its host in an attempt to avoid stimulating an effective immune response. The possibility of parasitic adaptation of its antigenic structure to match its host's in an attempt to avoid the consequences of an induced immune response has been considered by various authors and various theories have been proposed to explain the mechanism by which this adaptation takes place (Dineen 1963, Damian 1964, Capron et al. 1968, Smithers et al. 1969 a). As of yet however, though the premise of parasitic adaptation may be generally accepted, no mechanism for this adaptation has been successfully demonstrated.

Since cattle are eventually able to eliminate their acquired fluke burden at a time when their precipitin response is showing a marked rise it may be that the adaptation of the parasite to cattle is not as complete as it is in the sheep and antibodies are formed to enzymes which eventually result in the elimination of the parasite in a similar manner to that proposed by Edwards et al. (1971) for the expulsion of N. brasiliensis from immune rats. The importance of even a single functional antigen in the development of

a successful acquired immunity has been clearly demonstrated by Despommier and Wostmann (1970 a, b) in T. spiralis infections in mice. The apparently limited precipitin response of cattle to infection with F. hepatica, may therefore still have an important part to play in the eventual elimination of the parasite and the subsequent resistance to reinfection with this parasite.

As an incidental finding of these experiments concerning the precipitin response of cattle to infections with F. hepatica is that precipitins can be detected in the sera of previously uninfected cattle within 1 months after infection allowing confirmation of infection at least 2 months before it becomes patent. This test may be useful in confirming experimental infections of previously uninfected cattle. It may also have a limited application in the field since using it will detect the presence of infection in susceptible young cattle 8 weeks before it would be detected using conventional faeces egg counting techniques and so allow earlier treatment of the infection with one of the newer anthelmintics effective against immature flukes.

SUMMARY

Precipitating antibodies to antigens present in a saline extract of adult F. hepatica could first be detected in the sera of calves 2-4 weeks after a primary infection with 500 or 750 metacercariae of F. hepatica and were still detectable 28 weeks after infection. Quantitation of this precipitin response using a one dimensional double diffusion technique revealed that the level of precipitating antibodies to F. hepatica rose from 4-8 weeks after infection then declined slightly before rising again from 16-28 weeks after infection. This latter rise in the level of precipitating antibodies coincided with the period of expulsion of the adult parasites from the bile ducts of their hosts.

The technique of double diffusion in a single dimension in tubes was the most successful of the three methods used to detect precipitins in the sera of infected calves. This technique detected precipitins in all of the samples examined between 4-28 weeks after infection including those in which precipitins were not detected by other techniques. Two other techniques, a combined double diffusion system on plates and an immunoelectrophoretic system were also used but only detected precipitins in 80% and 58% respectively of the samples examined between 4-28 weeks after infection.

Precipitins to only 3 of the 12 antigenic components known to be present in the saline extract of adult F. hepatica and to only 1 of the 6 antigens known to be present in the metabolic products of F. hepatica were produced by these calves in response to their infection with this parasite.

PART 3

HYPERSENSITIVITY REACTIONS INDUCED BY INFECTION OF SUSCEPTIBLE CALVES WITH *F. HEPATICA*

INTRODUCTION AND REVIEW OF THE LITERATURE

This part of the thesis is concerned with the investigation of hypersensitivity reactions, their appearance and function in cattle infected with F. hepatica.

Hypersensitivity reactions in man and animals have been classified into four types (Pepys 1963, Turner-Warwick 1969) and these can briefly be described as follows.

Type I or 'immediate hypersensitivity' is defined as a reaction initiated by the allergen reacting with tissue cells that have already been passively sensitised by antibody, or reagin, produced elsewhere in the body leading to the release of pharmacologically active substances such as histamine. The intradermal injection of minute quantities of antigen in a sensitised animal results in the appearance of an urticarial weal and erythematous flare which reaches a maximum size 10-20 minutes later and has resolved within $1\frac{1}{2}$ - 2 hours.

Type II reactions are those in which the antibody reacts with an antigen present on or attached to cell surfaces as occurs for example in mismatched blood transfusions.

Type III reactions or "Arthus type" reactions are initiated by antigen reacting with antibody and complement in the tissue spaces. The resulting microprecipitates after ingestion by phagocyte cells liberate lysosomes causing damage to cells and blood vessels. Intradermal injection of antigen again results in an erythematous swelling but the reaction develops slowly and appears some hours after injection of antigen.

Type IV reactions involve the reaction between antigen and sensitised cells, and is generally termed 'delayed type hypersensitivity' since a reaction to intradermal injection of antigen does not reach maximum size till 48-72 hours later. It can be seen that Type I and

and Type IV reactions correspond to Modes C and D of the classification of allergic reactions as defined by Coombs (1968) and detailed in the introduction to this thesis.

The demonstration of skin hypersensitivity in cattle, sheep, carabaos and man has been used on several occasions as a diagnostic test for fascioliasis.

Sobiech (1951) described the use of the intradermal test for F. hepatica injections in cattle and recorded a 61% correlation between positive skin tests and F. hepatica infections.

Gonzalez, Rivera Anaya, De Jesus (1951) reported the use of a saline extract of dried F. hepatica to test for F. hepatica infections in 40 cattle. All 30 cattle which were judged to be infected by the finding of F. hepatica eggs in their faeces gave a positive skin test. The skin reaction resulted in an erythematous weal reaching a maximum size 15-20 mm. in diameter within 30 minutes of the antigen reaction. The non-infected cattle only gave small skin reactions 4-5 mm. in diameter.

Soulsby (1954) also used a saline extract of dried F. hepatica in an intradermal test on 212 cattle just prior to slaughter. Ninety per cent of cattle found at slaughter to have evidence of F. hepatica infections were judged to have shown a positive skin test as defined by an increase in skin thickness of 6 mm. or more. The skin reaction was described as reaching a maximum size 4 hours after injection of the antigen. Sixteen per cent of cattle not found to be infected with F. hepatica at slaughter all gave skin reactions of 6 mm. or greater. Passive transfer of hypersensitivity could only be demonstrated with 2 out of 16 serum samples taken from cattle which had given a positive skin test prior to slaughter.

Favati and Della Croce (1965) used a lipid free extract of

F. hepatica in saline as the antigen in an intradermal test in 55 cattle prior to slaughter. The test was reported to be 100% accurate in diagnosing infection with F. hepatica alone or combined with Dicrocoelium dendriticum. A positive skin test was taken to be the appearance of an erthymatous indurated swelling at the injection site which reached a maximum size within 30 minutes after the injection of antigen.

Frick (1968) using a filtered extract of F. hepatica homogenised in Coca's solution (NaCl .85%, $NaHCO_3$ .275%, Thiomersal .02%) found the intradermal test gave a positive reaction in 67% of cattle subsequently found to be infected with F. hepatica.

Patnaik and Das (1961) used a saline extract of dried F. gigantica in the intradermal test of 40 cattle prior to slaughter and all 21 cattle found to be infected at slaughter gave a positive skin test as judged by the appearance of an indurated weal within 18-31 minutes of the antigen injection and whether resolved within 59-86 minutes after the injection.

Abdou, El Sherif, El Sawi (1966) used a similar extract of F. gigantica in the intradermal diagnosis of F. gigantica infections in sheep. Ninety-four per cent of the sheep passing F. gigantica eggs in their faeces gave positive skin testsas judged by the appearance of a weal within 30 minutes of the antigen injection. No reading was observed in sheep not passing F. gigantica eggs in their faeces.

Topacio and Martin (1963) used a saline extract of dried F. hepatica in the intradermal test of 150 carabaos prior to slaughter and all 86 carabaos found to be infected at slaughter gave a positive reaction as judged by the appearance of an indurated weal 26-63 mm. in diameter between 9-24 minutes after antigen injections and this

resolved within 46-129 mins. of the injection.

Pautrizel, Bailenger, Duret, Tribouley (1962a) used a lipid free saline extract of F. hepatica in the intradermal diagnosis of F. hepatica infections in humans. A total of 50 ug of antigen protein was injected and the size of the subsequent weal measured 15 minutes later. The test was positive in 58% of females and 69% of males subsequently found to be infected with F. hepatica.

The results of all these experiments with the exception of those of Soulsby (1954) would appear to indicate that animals and man infected with either of the Fasciola spps. develop at some point a Type I or immediate hypersensitivity to antigens in these parasites as intradermal injection of these antigens results in an indurated weal reaching maximum size within 30 minutes of the antigen injection. The results of Soulsby (1954) would however appear to describe the occurrence of a Type III or Arthus type reaction since the intradermal reaction did not reach a maximum till 4 hours after injection of the antigen.

It would appear therefore that cattle infected with F. hepatica produce reagin-like antibodies which mediate a Type I or immediate type hypersensitivity reaction following intradermal injection of the appropriate antigen in sensitised cattle. The results of these experiments however do not allow any conclusions to be drawn as to the relationship between the stages of infection of cattle with F. hepatica and the presence of reagin-like antibodies since the criteria for establishing the presence or absence of F. hepatica infections in these cattle did not relate to the degree or stage of development of any infection present.

The antigenic components in homogenates of F. hepatica responsible for the induction of a Type I response have been

studied by Kellawy (1928), Maekawa, Kitzawa, Kushibe 1954, Maekawa and Kushibe 1956, 1961a, b, 1964, Ono and Watanabe (1954).

Kellaway (1928) found 2 antigenic substances are present in extracts of flukes, one soluble in saline which was capable of actively sensitising and discharging isolated guinea pig uterus, the other soluble in absolute alcohol which could sensitise but not discharge guinea pig uterus.

Maekawa and his colleagues in a series of investigations (Maekawa et al 1954, Maekawa and Kushibe 1956, 1961a, b, 1964) isolated and identified 3 allergens present in extracts of F.hepatica. The first antigen was protein in nature, the second and third were both largely comprised of ribonucleic acid with some added peptide. All three components were found to be highly active when used in the intradermal test in cattle infected with F. hepatica.

Ono and Watanabe (1956) isolated a specific polysaccharide which was also reported to be active in the intradermal test for F. hepatica infections in cattle.

It would seem therefore that there is a number of antigens of varying chemical composition which are capable of inducing and eliciting a Type I response in animals infected with F. hepatica.

The mechanisms involved in the induction and expression of Type I responses in man and animals have been the subject of intensive study and as a result some of the processes in this reaction have recently been elucidated. The mechanisms of immediate allergic reactions have been reviewed (Osler 1971, Stanworth 1971) and the sequence of events leading to the allergic release of vasoactive compounds can be summarised as having 4 stages. The first involves the fixation of bivalent immunoglobulins to a metabolically active target cell. The second is the induction of a

conformational change, probably in the hinge region of the cell bound immunoglobulin, following its interaction with multivalent antigen. The third is the activation of one or more temperature sensitive, energy requiring enzymatic system(s) as a consequence of the conformational change. The fourth and final stage is the enhancement of a secretory response leading to the release of vasoactive amines. The metabolically active cells involved in this reaction are in most cases mast cells or basophills.

The antibodies involved in this reaction can be divided into 2 types (Sadun, Duxbury, Gore, Stechschulte 1967). There are those which belong to the IgG class of immunoglobulins and are both heat stable and insensitive to reduction with mercaptoethanol but can be detected by passive cutaneous anaphylylactic reactions for only four hours after passive transfer. The second and most important type of antibodies are those which are both heat labile, sensitive to reduction with mercaptoethanol and persist for long periods as detected by homologous passive cutaneous anaphylactic reactions following passive transfer. In man the latter type of antibody has now been isolated and demonstrated to belong to a new class of immunoglobulins which possess specific heavy chain antigenic determinants (Ishizaka, Ishizaka, Hornbrook 1966, Johansson 1968) which has been designated IgE (Bennich et al 1968). This antibody is only found in very small amounts (100-200 ngs/ml.) in the serum of normal people but in atopic individuals who exhibit severe Type I hypersensitive reactions to various antigens the serum IgE level may be 30-60 times as high as those of normal individuals. The binding of the IgE molecule to the surface of mast cells is dependent on the confirmational integrity of the Fc. part of the immunoglobulin molecule and factors altering this integrity destroy the biological activity of the

molecule. Mild heating is sufficient to alter the confirmation of the molecule as is the reduction cleavage of the accessible disulphide bonds in the molecule by mercapatonethanol so giving the IgE molecule its characteristic heat and mercaptan sensitivity (Stanworth, Housley, Bennich, Johansson 1970).

Skin sensitising antibodies capable of eliciting a Type I reaction 24-72 hours after passive transfer to a homologous recipient have been produced in cattle by vaccination with certain antigens, ovalbumin, horse serum, ragweed pollen and ferritin (Dungworth 1965, Pierce 1967, Weil and Reddin 1943, Wells and Eyre 1970) and they also occur in naturally occurring milk allergy in cattle (Campbell 1970). These antibodies would appear to be heat labile in that heating for 30 minutes at 56°C diminishes the activity of these sera and heating for 2-4 hours completely abolishes the skin sensitising activity of these sera. There has been no demonstration of identity between the heavy chain determinants of these homocytotropic antibodies in cattle and the IgE class of immunoglobulin in man, despite their apparently similar function and heat sensitivity, although cross reactions between the specific heavy chain determinants of human IgG, IgM and IgA have been used to identify the corresponding classes of bovine immunoglobulins (Butler et al 1971). Pierce (1967) however, reported that the bovine homocytotropic antibodies belonged to the IgG class of immunoglobulins.

Homocytotropic or 'reaginic' antibodies have been associated with parasitic infections in man, (Pautrizel and Bailenger 1961, Pautrizel et al 1962b, Zvaifler 1966), sheep (Hogarth-Scott 1969), dogs (Williams and Perez Esandi 1971), rabbits (Sadun et al 1967), rats(Ogilvie 1964) and in both rabbit and dog immunoglobulins analogous to human IgE have been isolated and described (Zvaifler 1969,

Rockey and Schwartzman 1967).

The role of these reaginic antibodies in the expression of a protective immunity in parasitic infections has been studied using N. brasiliensis infections in the rat as a model system. In this system as described in the introduction to the first part of this thesis the worms of a primary infection show evidence of immune damage by 10 days after infection and the majority of worms are expelled from the intestines by 16 days after infection. Reaginic antibodies can be detected as early as 10 days after infection by eliciting a Type I skin response on the intradermal injection of N. brasiliensis antigen in infected rats (Jarrett and Stewart 1972). Circulating reaginic antibody can be detected by passive transfer techniques from about 16 days after infection and the level or serum reagins rises between 16-25 days after infection (Ogilvie 1967). The presence of a local anaphylactic reaction with increased vascular permeability in the wall of the small intestine of infected rats induced by N. brasiliensis antigens (Urquhart, Mulligan, Eadie, Jennings 1965), the occurrence of reaginic antibodies and the immune expulsion of the worms from the intestine prompted an intensive investigation of the population kinetics of the metabolically active target cells for reaginic antibodies, the mast cells, in the intestine and their relationship to the immune expulsion of the parasite. A dramatic increase in the number of mast cells per unit area of intestine was found to occur 10 days after infection. The presence of large numbers of discharging mast cells was found to coincide with a marked permeability of the intestinal epithelium to macromolecules and the expulsion of worms in the self-cure reaction, (Jarrett, Jarrett, Miller, Urquhart 1967,

Murray, Jennings, Jarrett, Miller 1969, Miller and Jarrett 1971). Various techniques have been used to depress the number of mast cells in the intestine of infected rats, (Jarrett et al 1967), to interfere with their storage of vasoactive amines (Craig-Sharp and Jarrett 1968, Keller 1970) or specifically antagonise these amines (Murray, Smith, Wadell, Jarrett 1971). All these methods of interference with mast cell function can prevent the expulsive phase of the self-cure reaction. The function of reaginic antibodies and their metabolically active target cells in N. brasiliensis infections in the rat would appear to be primarily concerned in the expulsion of previously damaged worms from the small intestine of the rat, possibly by facilitating the passage of further large amounts of antibody into the gut, (Jones and Ogilvie 1971, Miller and Jarrett 1971).

Mast cell discharge does not always require this previous sensitisation with homocytotropic antibody and subsequent interaction of antibody with allergen. Pharmacological analysis of extracts of Ascaris suis (Uvnas and Wold 1967), N. brasiliensis (Jarrett et al 1967) and F. hepatica (Baglioni and Locatelli 1969, Baglioni, Locatelli, Pholetti 1969) have shown the existence of substances in these extracts which can discharge mast cells without the involvement of homocytotropic antibodies. It has been postulated that secretion of these substances by N. brasiliensis and F. hepatica with release of vasoactive amines from mast cells resulting in an increase in endothelial and epithelial permeabilities may be involved in the supply of nutrient to the parasites. (Jarrett et al 1967, Baglioni and Locatella 1969).

The experiments described in this part of the thesis were therefore devised to investigate the appearance of a hypersensitive

state in parasite free calves following their experimental infection with F. hepatica. It was also decided to investigate the nature of the antibodies responsible for the induction of this hypersensitivity and if possible attempt some quantitation of this antibody response.

MATERIALS AND METHODS

1. Antigen

The antigen used in these experiments was a lipid free extract of *F. hepatica* prepared as described in the previous part of this thesis. Care was taken to reduce bacteriological contamination of the extract during the preparation, sterile glassware was used throughout and the antigen was subsequently sterilised by heating to 56°C for 2 hours on 3 separate occasions.

2. Skin Tests

(a) Direct skin tests

The intradermal test in infected calves was carried out as follows.

Two or three areas of skin over the ribs of infected calves were shaved and .1ml. of fluke antigen containing 50 ug of protein were injected intradermally into 2 or 3 separate sites using a disposable tuberculin syringe (Becton, Dickinson and Co., Rutherford, N.J.). Similar amounts of sterile diluent (Phosphate-buffered saline) were also injected in 2 or 3 sites as controls for the specificity of the antigen. The skin thickness at each site was measured before and immediately after the injection of antigen or saline. Skin thickness at each site was then measured at 15 minutes, 30 minutes, 45 minutes, 1 hour, 3 hours and 6 hours later. On certain occasions skin thickness was also measured 24, 48 and 72 hours after injection.

(b) Cutaneous transfer tests

The passive sensitisation of the skin of parasite free calves using sera from infected calves was carried out as follows.

White or nearly all white calves 100-140 lbs. bodyweight of the Ayrshire and Friesian breeds were used in these tests. The

calves were first clipped down to the skin over an area extending over the last ribs and the lateral abdominal wall. The test serum and its dilutions in .2 ml. amounts were injected using disposable tuberculin syringes at sites 2-3 cm. apart in a checkerboard pattern previously outlined on the skin using a fibre tip pen (Staedler, Germany). The test sera were diluted 1/4, 1/16, 1/64, 1/256 in sterile phosphate buffered saline immediately prior to injection. Control injection of .2 ml. amounts of the diluent were included on each test. Seventy-two hours was allowed to elapse prior to the attempted induction of a Type $\underline{\underline{I}}$ hypersensitivity response at these sites.

The Type $\underline{\underline{I}}$ response was elicited in one of two ways. In the early experiments .1 ml. amounts of the antigen containing 50 ug of protein were injected intradermally at the sites of the previous serum or diluent injections. The thickness and diameter of the resultant weal was measured using calipers and a vernier micrometer. This method of injection of antigen gave anomalous results at the site of injection of the sterile diluent in some calves so rendering the test invalid and this method of testing was discontinued.

The intravenous injection of the antigen at a dose rate of 50 ug/lb. bodyweight was found to give the most consistent results in eliciting the Type $\underline{\underline{I}}$ response at sites where homocytotropic antibodies were present. This method of antigen administration was then used in all the subsequent test calves and the weals were outlined by the intravenous administration of 10-15 ml. of 1% Evans blue solution (G.T. Gurr Ltd., London) which allowed accurate measurement of the diameter of the resulting weals using a vernier micrometer. The weal sizes were measured 15 minutes

after administration of the antigen.

(c) Biopsies

Biopsies were taken from sites of direct skin tests 15 minutes, 6 and 72 hours after the intradermal injection of antigen. The skin pieces were fixed in Bouin's solution for 18 hours, trimmed, dehydrated in an alcohol-amyl acetate - chloroform series, embedded in paraffin wax, sections 5 μ. thick cut and subsequently stained with haematoxylen counterstained with eosin.

3) Bovine homocytotropic antibody

Blood was taken from 4 calves 20 weeks after their infection with 750 metacercariae of F. hepatica and the sera pooled to provide a standard serum for the investigation of the characteristics of the bovine homocytotropic antibody. The standard serum was kept in 1 ml. aliquots at -50°C till used. The following characteristics of the bovine homocytotropic antibody were investigated; sensitivity to heat and reduction with mercaptoethanol, persistence in homologous skin following passive transfer, the fraction of the bovine sera containing homocytotropic activity and the possibility of a cross reaction with sera prepared against heavy chain determinants of human IgE.

(a) Heat sensitivity

The sensitivity of the homototropic antibody to heat was tested by heating .5 ml. amounts of the standard serum in a water bath at 56°C for $\frac{1}{2}$ hour, 1 hour, 2 hours and 4 hours. The heated sera were diluted and used in the passive transfer test within 2-4 hours of heating.

(b) Reduction with mercaptoethanol

The sensitivity of the homocytotropic antibody to reduction with 2-mercaptoethanol and subsequent alkylation with iodoacetamide

was carried out as described by Sadun et al (1967). In the passive transfer tests control sera were included for all the stages of reduction and alkylation.

(c) Salt fractionation of serum

The serum was fractionated using saturated ammonium sulphate at pH 6.5 as described by Stelos (1967). Saturated ammonium sulphate was added to the standard serum to final concentrations of $33^{1}/3\%$ and 50%. The former concentration precipitates largely gamma globulins while the latter concentration precipitates most of the globulins present in the sera. The precipitates were re-suspended in sterile phosphate buffered saline, dialysed to remove the ammonium sulphate and made up to their original concentrations in the standard serum before use in passive transfer tests. The supernatants of both precipitates were also dialysed free of ammonium sulphate, made up to their original concentrations and used in the passive transfer test.

(d) Antigenic similarity to human IgE

Demonstration of a possible antigenic similarity between the heavy chain determinants of human IgE and the bovine homocytotropic antibody was investigated in the following manner. Various quantities of a specific anti-human IgE sera (Miles-Yeda Ltd., Rhovot, Israel) were added to the standard bovine serum to see if it interfered with the ability of the serum to sensitise bovine skin in the passive transfer test. Anti-human IgE serum containing 5 ug. of antibody/ul was added in 10 µl., 5 µl., and 2.5 µl. amounts to 1 ml. amounts of the standard bovine serum which contained 54,000 µg. of total globulins/ml. resulting in dilutions of 1 µg. of antisera total globulins to 1,080, 2,160, 4,320 µgs. of total bovine globulins at each dilution.

RESULTS

1) Direct skin tests

The response of calves at intervals after a single infection of 750 metacercariae of F. hepatica to the intradermal injection of F. hepatica antigen was studied in individual calves at 2, 3, 4, 5, 6, 8, 10, 12, 16 and 18 weeks after infection during Experiment 3. All 28 calves used in Experiment 3 were tested for their response to the intradermal injection of F. hepatica antigen prior to slaughter at the end of the experiment. Six uninfected parasite free calves were also used as controls for the specificity of the antigen at various times during these skin tests. The maximum increase in skin thickness produced by the intradermal injection of antigen in the uninfected control calves or the injection of saline in control and infected calves was found to be 4 mm. and therefore the first 4 mm. increase in skin thickness at any test site was discounted as being non-specific.

The results of the 10 calves tested between 2-18 weeks after infection are summarised in Table 13, the net increase in skin thickness after subtraction of 4 mm. for the non-specific reaction is shown at 30 minutes, 1 hour and 6 hours after injection of the antigen. Individual test results for each calf, both infected and control are given in Tables 1-14 in Appendix 3.

As can be seen from Table 13 a Type I or immediate hypersensitivity response was first detected 3 weeks after infection and was still present 18 weeks after infection. This response was characterised by the formation of a raised indurated area as shown in Plate 4, Appendix 3 and in unpigmented areas the weal and surrounding areas were markedly erythematous. The weal reached a maximum size about

30 minutes after the injection of antigen and in the early stages of the infection it had largely resolved within 1-2 hours later. In the later stages of the infection the initial weal became larger and persisted for longer periods before it regressed. At 12 weeks after infection it can be seen that an increase in the skin thickness at the injection site could still be detected 6 hours after injection of the antigen. Similar reactions were also detected at 16 and 18 weeks after infection. It would appear that the early weal characteristic of the Type I response is prolonged in the later stages of the infection by a Type III or 'Arthus' reaction. No reactions were detectable at any test sites 24-72 hours after injection of the antigen.

Histological examination of the biopsy taken 15 minutes after the injection of antigen showed marked oedema of the connective tissue of the dermis with a sparse cellular infiltrate of the dermis mainly composed of eosinophills with a few neutrophils present. Details of the histology are shown in Plate 1, Appendix 3. Histological examination of the biopsy taken 6 hours after injection of antigen again showed some oedema of the connective tissue of the dermis but associated with a marked cellular infiltrate both of the connective tissue of the dermis and the perimysium and endomysium of the subcutaneous muscle. The cellular infiltrate consisted largely of eosinophils and neutrophils with occasional plasma cells. There was also marked perivascular cuffing associated in some cases with vascular damage and thrombosis. Details of the histological changes are shown in Plates 2 and 3 in Appendix 3.

Histological examination of biopsy material taken from the site of antigen infection 72 hours later revealed changes similar

TABLE 13

The net increase in skin thickness at sites of intradermal injection of *F. hepatica* antigen in calves at 2, 3, 4, 5, 6, 8, 10, 12, 16 and 18 weeks after their infection with 750 metacercariae of *F. hepatica*

Time after injection	Net increase in skin thickness (mm)									
	2	3	4	5	6	8	10	12	16	18 weeks
30 mins.	-	4	6	6	10	8	10	12	10	12
1 hour	-	2	6	2	6	8	6	12	8	10
6 hours	-	-	-	-	-	-	-	2	4	4

to that described at 6 hours after infection, the cellular infiltrate was now composed of eosinophils, neutrophils and a large proportion of lymphocytes and plasma cells. Vascular damage was still evident associated with marked perivascular cuffing by the cellular infiltrate but the interstitial oedema associated with the earlier lesions had disappeared.

Histological examination of the skin biopsies would appear to confirm that a Type I response is succeeded by changes which merit classification as a Type III or 'Arthus' reaction and that the infiltration and vascular damage associated with this reaction are still present 72 hours after the injection of antigen.

The results of the intradermal test in all 28 calves used in Experiment 3 together with 2 uninfected parasite free control calves are summarised in Table 14 and individual results are given in Table 15, in Appendix 3. All thirty calves were tested just prior to the slaughter of the 28 experiment calves and the weal sizes were measured at 30 minutes after intradermal injection of the antigen. It can be seen from the Table that after discounting the first 4 mm. of any increase in skin thickness as being due to a non-specific reaction that 25 of the 27 calves found to be infected with F. hepatica at slaughter had net increases in skin thickness of 4 mm. and greater at the injection site and 21 of the 27 calves had net increases of 8 mm. or greater at the injection site 30 minutes after intradermal injection of the antigen. The 2 uninfected control calves and calf No. 17 which was found to be uninfected at slaughter did not show any increase in skin thickness greater than the 4 mm. discounted as being due to the non specific reaction. Two experimental calves found to

TABLE 14

Net increase in skin thickness of 30 calves infected with F. hepatica 30 minutes after the intradermal injection of F. hepatica antigen.

	Net increase in skin thickness (mm)							
	0	2	4	6	8	10	12	14
No. of calves	3	2	1	3	5	11	2	3
No. of calves found to be infected	-	2	1	3	5	11	2	3
Number of flukes recovered per calf	-	19 80	70	5, 6 9	18, 21 31, 44, 44	2, 7, 8 11,17,18 27,29,36 52,86	4, 5	4, 14 15

be infected with F. hepatica at slaughter only showed a further increase of 2 mm. in skin thickness at the injection site 30 minutes after antigen injection.

It would appear that in these 30 calves tested a net increase skin thickness of 4 mm. or over could be regarded as a positive indication of infection allowing correct diagnosis of the presence of F. hepatica infection in 93% of the infected calves tested. The intradermal reaction gave negative results in the 3 uninfected calves tested and gave doubtful results in 7% of the infected calves tested. There was no significant correlation between the acquired fluke burdens of the calves and the magnitude of their hypersensitivity response.

2) Quantitation of the homocytotropic antibody response

The results of the direct skin test had shown that antibodies capable of mediating a Type I hypersensitivity response to F. hepatica antigens could be detected in the skin of calves from 3 weeks after their infection with F. hepatica. These results however, apart from an apparent increase in the size of the resultant weal as the infection progressed gave no indication of the pattern and magnitude of this antibody response.

In an attempt to quantitate this response sera from 6 calves were studied following their experimental infection with F. hepatica. Serum samples were taken prior to and at 4, 8, 12, 16, 20 and 24 weeks after the infection of 3 calves each with 500 metacercariae of F. hepatica. Serum samples were also taken prior to and at 4, 6, 8, 12, 16, 20, 24, 28 and 30 weeks after the infection of 3 calves each with 750 metacercariae of F. hepatica. Each serum sample was used in the passive cutaneous transfer test undiluted and at dilutions of 1/4, 1/16, 1/64, 1/256 along with the

requisite phosphate-buffered saline controls. In all 288 samples were tested using 6 uninfected parasite free calves as homologenous recipients for the test sera, antigen was given intravenously as was Evan's blue to delineate the resultant weals. The highest dilution of the test serum giving a measurable blue weal was taken as a measure of the homocytotropic antibody content of that serum. No reaction was ever detected at the sites of injection of preinfection sera or of the control saline. The results of the individual calf serum samples are given in Tables 16-21, Appendix 3 and the results are summarised in Table 15. Reagins were detected in small amounts on several occasions in the sera of individual calves taken between 4 and 16 weeks after infection. At 20 weeks after infection, however, reagins were detected in all the sera tested. Thereafter, there was a marked rise in the amount of circulating homocytotropic antibody, the level reaching a peak about 28 weeks after infection. The level of the circulating homocytotropic antibody then declined sharply over the next 2 weeks.

3) Characteristics of the bovine homocytotropic antibody

a) Heat sensitivity

The standard bovine serum consistently gave detectable weals up to a dilution of 1/64 in the passive cutaneous transfer test. The results of the passive transfer test using this serum after heating at 56°C for $\frac{1}{2}$, 1, 2 and 4 hours are summarised in Table 16 and the individual results are given in Table 24 Appendix 3. It can be seen that heating at 56°C for 30 minutes reduced the skin sensitising activity of the standard serum by over 60%, the highest dilution still giving a detectable weal was reduced to $\frac{1}{4}$, and heating for a further $1\frac{1}{2}$ hours at 56°C abolished the skin

TABLE 15

Reciprocal of the highest serum dilution giving a detectable response in the passive cutaneous transfer test. Serum samples were taken at intervals from calves 21, 22, 24 following their infection of 500 metacercariae of F. hepatica and from calves 13, 14, 15 following their infection with 750 metacercariae of F. hepatica

Calf No.	No. of metacercariae administered	Reciprocal of highest detectable serum dilution								
		0	4	8	12	16	20	24	28	30 weeks
21	500	-	1	-	-	1	4	256		
22	500	-	1	1	-	1	64	4		
24	500	-	-	-	1	1	16	64		
13	750	-	-	-	-	1	1	1	16	4
14	750	-	-	-	-	-	1	4	256	1
15	750	-	-	-	-	-	1	1	256	16

TABLE 16

Reciprocal of highest serum dilution giving detectable results in the passive cutaneous transfer test following heating of the standard bovine serum for 1/2, 1, 2 and 4 hours at 56°C.

	Time of heating (hrs.)				
	0	.5	1	2	4
Reciprocal of highest detectable serum dilution	64	4	1	-	-

sensitising activity of the serum completely.

b) Sensitivity to reduction with mercaptoethanol

Two samples of the standard bovine serum were reduced for 3 hours by dialysis again .1 M. 2-mercaptoethanol, then the terminal residues alkylated by dialysis again .02 M. iodoacetamide and finally dialysed against several changes of phosphate buffer. Similar samples of the standard sera were dialysed against only iodacetamide and phosphate buffered saline or only against phosphate buffered saline. The reduced sera and the control sera were tested in the passive transfer test and the results are shown in Table 22, Appendix 3. It can be seen that this mild degree of reduction with mercaptoethanol was sufficient to abolish the skin sensitising activity of the standard serum.

c) Salt fractionation

Fractionation of the standard serum with ammonium sulphate located the skin sensitising activity to the fraction precipitated at 50% and 33 1/3% saturation with ammonium sulphate thus precipitating the homocytotropic antibody in the same serum fraction as the gamma-globulins.as shown in Table 23, Appendix 3.

d) Antigenic similarity to human IgE class

A method of investigating a possible antigenic similarity between the specific heavy chain determinants of the human IgE immunoglobulin and the bovine homocytotropic antibody was the use of a monospecific antisera to human IgE antibodies in the inhibition of the skin sensitising activity of the bovine homocytotropic antibody. The results of adding very small quantities of the specific antiserum to the standard bovine serum are shown in Table 17. It can be seen that 25 µg. of the total antibody

TABLE 17

Diameter of weals (mm.) in passive cutaneous transfer test using the standard bovine serum to which anti-human IgE serum had been added to final dilutions of 1:100, 1:200, 1:400.

Dilution of anti-IgE serum	Ratio of amount of total antibody added to total antibody present in standard serum	Weal size (mm.) at reciprocal of dilutions				
		1	4	16	64	256
-	-	14	14	18	10	-
1/400	1:4,320	18	12	11	-	-
1/200	1:2,160	18	-	-	-	-
1/100	1:1,080	17	-	-	-	-

present in the antisera was sufficient to abolish the activity contained in the 13,500 μg. of total bovine globulins contained in the 1/4 dilution of the standard bovine serum.

DISCUSSION

The results of the experiments demonstrate the existence of antibodies capable of mediating a Type I or immediate hypersensitivity response in the skin of calves during infection with F. hepatica. This response can be elicited by the intradermal injection of small amounts of a complex lipid free fluke antigen and consists of an indurated erythematous weal which reaches its maximum size about 30 minutes after injection of the antigen and resolves within a further 1½-2 hours. A Type I response can be elicited as early as 3 weeks after infection of calves with F. hepatica and is still present 20 weeks after infection.

As the infection progressess the weal initiated by the Type I response persists for longer periods prolonged by the appearance of a Type III or 'Arthus' reaction after the Type I reaction. This Type III response results in weals still detectable 4-6 hours after injection and is present between 16-20 weeks after infection. The rise in the level of precipitating antibodies which occurs at this time as shown in Part 2 of this thesis may be the factor responsible for the appearance of the Type III hypersensitivity response. It has previously been shown (Siqueira and Bier 1961, Bier, Passos, Siqueira 1968) that microprecipitates of immune complexes capable of fixing complement can cause vascular damage similar to that found in Type III reactions at the site of passive cutaneous anaphylaxis elicited in the skin of guinea pigs. The appearance of a Type III response in cattle infected with F. hepatica may therefore depend on the simultaneous presence of both homocytotropic and precipitating antibodies with their specific antigens at the

injection site in the dermis.

The results of these experiments confirm the descriptions of a Type I response in cattle naturally infected with F. hepatica (Gonzalez et al 1951, Favati and Della Croce 1965, Frick 1968) and provide confirmation and a possible explanation for the hitherto unconfirmed report of a Type III response to the intradermal injection of F. hepatica antigens in cattle (Soulsby 1954).

The appearance of a Type I reaction induced by the intraderaml injection of a fixed amount (50 ug) of a standard F. hepatica antigen was used as a diagnostic test for the presence of infection in the 28 calves used in Experiment 3 just prior to slaughter and 2 uninfected parasite free control calves. A net increase of 4 mm. or more in skin thickness at the injection site was regarded as a positive response and 25 of the 27 calves subsequently found to be infected at slaughter gave positive responses, the 2 remaining infected calves gave a doubtful response and the 3 parasite free calves gave negative results.

Under controlled conditions, therefore, this intradermal test for F. hepatica allowed diagnosis of infection as early as 3 weeks after infection and was 93% accurate in the diagnosis of infected calves.

The antibodies responsible for mediating the Type I response could be detected in the skin of infected calves from 3-4 weeks after infection onward but they only appeared sporadically and in small amounts in the sera of these calves during the first 16 weeks after infection. The amount of homocytotropic antibody in the sera then began to rise between 16-20 weeks after infection, reached a peak between 24-28 weeks after infection and then declined.

This pattern of the homocytotropic response induced by a single infection of F. hepatica in calves is again similar albeit on a much longer time scale, to the homocytotropic antibody levels in rats infected with N. brasiliensis in that in both cases the level of homocytotropic antibody in the serum starts to rise about the time when the parasites are being expelled by the host. In the case of the single infection with F. hepatica in calves it can be seen that this rise begins between 16-20 weeks after infection and so coincides with the period of reduced bilogical activity of the flukes as discussed in Part 1 of this thesis.

This rise in the amount of homocytotropic antibody present in the serum over this period has two possible explanations. The first is that it is the results of an increased production of this antibody over this period due perhaps to the appearance of a specific allergen liberated from the flukes at this time. The second and more complex explanation involves the possible secretion from the flukes in their feeding activities of the mast cell degranulating substance (M.C.D.) described by Baglioni and Locatelli (1969) and Baglioni et al (1969). Secretion of such a substance with the consequent release of vasoactive amines from mast cells in the biliary epithelium would ensure increased vascular and epithelial permeability thus facilitating the haemotophagic activities of the flukes. Continued secretion of M.C.D. substance would involve the discharge of these mast cells and their continual replacement by new populations of mast cells. This rapid turnover in the mast cell population would necessitate the formation and consequent fixation of large amounts of homocytotropic antibody to sensitise each new mast cell

population with the result that there would be little excess homocytotropic antibody left in the circulation. A marked diminution or cessation in the secretion of M.S.D. substance would allow some stabilisation of the biliary mast cell population and result in the relative excess of homocytotropic antibody appearing in the serum. Some measure of support for this hypothesis derives from the fact that this rise in the level of serum homocytotropic antibody level begins during the period when there is evidence for reduced biological activity of the flukes prior to their expulsion and continues over the period of expulsion of the flukes. This stabilisation of the mast cell population may allow the expression of a homocytotropic antibody response if this is responsible for the expulsion of the damaged flukes by a similar mechanism to that proposed for the expulsion of N. brasiliensis from immune rats as discussed in the introduction to this part of the thesis.

The bovine homocytotropic antibody responsible for mediating the Type I hypersensitivity reaction has many characteristics similar to those described for the human IgE class of antibodies in that it is sensitive to heat and mild reduction with mercaptoethanol, it is capable of passive transfer to a homologous recipient and persists in the skin of this recipient for at least 72 hours after transfer. It would also appear to share common specific heavy chain determinants with the human IgE class of immunoglobulins since anti-human IgE serum was capable of inhibiting the action of a standard serum containing bovine homocytotropic antibody, the degree of inhibition being related to the amount of anti-IgE serum added. This inhibitory effect of the anti-IgE serum can be explained by a combination

of the anti- serum with common antigenic determinants on the heavy chains of the bovine homocytotropic immunoglobulin molecule occluding the binding site of the molecule so preventing its attachment to the target cell. It would appear therefore that there may be a class of bovine immunoglobulins responsible for mediating Type I hypersensitive reactions which share common antigenic determinants with the IgE class of immunoglobulins in man.

SUMMARY

An immediate or Type I hypersensitivity response could be elicited in calves as early as 3 weeks after their infection with 750 metacercariae of F. hepatica by the intradermal injection of small amounts of a complex lipid-free antigen of this parasite. A Type I response could be elicited till the end of the experiment at 20 weeks after infection but as the infection progressed the weal initiated by this response persisted for longer periods prolonged by the appearance of a Type III or 'Arthus' reaction. This Type III response resulted in weals detectable 4-6 hours after antigen injection and was present between 16-20 weeks after infection.

The antibodies responsible for mediating the Type I response could only be detected sporadically and in small amounts in the sera of infected calves during the first 16 weeks after infection as detected by a passive cutaneous transfer test. The amount of circulating homocytotropic antibody detected then rose to a peak between 24-28 weeks after infection so coinciding with the period of expulsion of the parasites from their hosts.

Bovine homocytotropic antibody has many characteristics similar to those described for the human IgE class of antibodies in that it is sensitive to heat and mild reduction with mercaptoethanol, it has some heavy chain antigenic determinants similar to the human IgE molecule and it is capable of passive transfer to a homologous recipient persisting in the skin of this recipient for at least 72 hours after transfer.

PART 4

THE PATHOLOGICAL CHANGES INDUCED IN CALVES BY INFECTION OR REINFECTION WITH *F. HEPATICA*

INTRODUCTION AND REVIEW OF THE LITERATURE

The pathological changes induced by F. hepatica in ruminants have been reviewed by Taylor (1964), Pantelouris (1965), Sinclair (1967) and included in the standard textbooks of the pathology of domestic animals (Nieberle and Cohrs 1967, Jubb and Kennedy 1970). These descriptions have, however, been derived from livers recovered at the abattoirs from sheep and cattle whose stage of infection or reinfection with F. hepatica could not be determined.

The descriptions of the sequential pathological changes induced by F. hepatica included in these textbooks have however largely been derived from experimental infection of the rabbit (Urquhart 1954), rat (Thorpe 1965) and mouse (Dawes 1963 a, b, 1966, Dawes and Hughes 1964), where animals given standard infections with F. hepatica were killed at regular intervals after this infection

The principal pathological changes induced by F. hepatica in the laboratory animals can be summarised in two phases. In the first or parenchymal phase of the infection the migration of the immature flukes through the hepatic parenchyma result in haemorrhagic tunnels which are eventually replaced by fibrous tissue. There is also disruption or thrombosis of blood vessels involved in or near these tracks with subsequent haemorrhage into, or infarction of areas of the parenchyma which are then replaced by fibrous tissue. The entry of flukes into the bile ducts marks the second phase of the infection characterised by a severe cholangitis of the parasitised bile ducts with proliferation of both duct epithelium and the fibrous tissue of the bile duct wall. The parenchymal lesions attributable to migrating flukes become much less numerous during this period. During the bile duct phase of the infection a diffuse periportal and peri-

lobular fibrosis of unknown etiology becomes superimposed on the fibrosis which had resulted from migratory damage in the initial phase of the infection.

Descriptions of the sequential pathological changes induced by a standard infection of F. hepatica in previously uninfected cattle killed at regular intervals after infection have not been recorded in the literature though recently Dow et al (1967) described the pathological changes seen in the livers of experimental calves killed at various intervals between 3-30 weeks after their infection with 200, 300, 1300, 2500 or 25,000 metacercariae of F. hepatica. Dow et al (1967) considered that many of the lesions in calves were similar to those previously observed in the laboratory animals. The ventral lobe of the ruminant liver appeared to be the prime site for the penetration, migration and subsequent localisation in the bile ducts of the flukes. Dow et al (1967) described an arteritis occurring during the migratory phase of the infection which although it occurred in areas of fluke damage was not apparently induced by direct traumatic damage and had not been previously described in infections of the laboratory animals.

The presence of flukes in the bile ducts of infected cattle results in marked cholangitis which eventually progresses to calcification of the walls of these parasitised ducts. Dow et al (1967) noted that calcification of the bile ducts was obvious at 23 weeks after infection. Keck and Supperer (1967) reported that calcification of the bile ducts in fluke infected cattle had developed by 8-9 months after infection and had fully resolved by 18 months after infection with the shedding and disintegration of the calcaereous plaques and their execretion in the biliary system.

The recent descriptions of Rahko (1969, 1970) of an increase in

the number of mast cells and globule leucocytes in the bile ducts of naturally infected cattle as compared to uninfected cattle is interesting in view of the implication that the discharge of sensitised mast cells is involved in the immune expulsion of N. brasiliensis from infected rats as reviewed in the introduction to Part 3 of this thesis. In this context Murray, Miller, Jarrett, (1968) showed the globule leucocyte to be a partially discharged mast cell. Miller and Jarrett (1971) reported the migration of numerous sub-epithelial mast cells into the intestinal epithelium where they became globule leucocytes during the period of the expulsion of the worms from rats infected with N. brasiliensis.

In view of the lack of detailed information on the sequential pathological changes both in the parenchyma and bile ducts induced by a standard infection of F. hepatica as seen in previously uninfected calves killed at regular intervals after infection, it was decided to study the pathological changes seen in the livers of the calves in Experiment 2. These calves received a single infection of 500 metacercariae of F. hepatica and were killed at 4, 8, 12, 16, 20 and 24 weeks after infection.

Details of the pathological changes associated with the experimental reinfection with F. hepatica of previously experimentally infected animals are confined to the report of experimentatl reinfections in cattle (Ross 1966a) and the white mouse (Lang 1967).

Ross (1966a) provided a brief description of the pathological changes in calves reinfected with 300 metacercariae of F. hepatica 3 or 18 weeks after their initial infection with 200 metacercariae of F. hepatica. Ross considered that the changes seen in calves reinfected 3 weeks after their infection were similar to those seen in the control calves which received only the initial or second

infections. The pathological changes seen in the calves reinfected 18 weeks after their initial infection were considered similar to those of an initial infection but that the 'cellular reaction' was more rapid and the fibrosis marginally more severe. The histological descriptions are vague and it is difficult to see how comparisons could be made with single infections of similar duration because as previously described in the introduction to Part 1 of this thesis there were no specific control calves for either the initial or second infections killed at the same intervals as the reinfected calves in the experiments of Ross (1966a).

Lang (1967) described the histopathological changes in mice reinfected with F. hepatica at 60 and 100 days after a primary infection and killed 2-40 days later. The significant difference in histopathology between the reinfected and control mice was the rapid onset of a lymphocytic infiltration of the reinfected livers in areas associated with parasitic damage as compared to the initially infected control livers.

In view of the lack of detailed information of the pathological changes induced by reinfection of calves with F. hepatica under controlled conditions it was decided to study the pathological changes induced by the reinfection of the calves in Experiment 1 with 1650 metacercariae of F. hepatica 17 weeks after their initial infection of 750 metacercariae of F. hepatica and the reinfection of the calves in Experiment 3 with 1300 metacercariae of F. hepatica 7 or 12 weeks after their initial infection with 750 metacercariae of F. hepatica.

MATERIALS AND METHODS

The calves were stunned with a captive bolt pistol, bled out and the organs removed for gross examination. Details of the gross pathology were noted and photographed. The liver was then isolated and sectioned at 4 standard sites to provide a consistent method of examination of all the livers. The first section was made along a line joining the umbilical fissure and the ventral end of the oesophogeal notch. The area below this line was considered to be the ventral lobe and was further sub-divided along a line midway between the first incision and the tip of the ventral lobe thus dividing the ventral lobe into 2 areas. The caudate lobe was then removed and the area remaining was considered to be the dorsal lobe. This was then sectioned along a line 2 cms. dorsal and parallel to the common bile duct to divide the dorsal lobe also into two areas. Tissue for histology was taken from each area before the flukes were recovered as described in Materials and Methods of Part 1 of this thesis. Representative tissues were also taken from other organs.

Histopathology

Tissues were routinely fixed for 24-48 hours in either formal-sublimate or Carnoy's fluid both prepared according to Baker, Silverton, Luckcock (1966).

Tissue blocks were then trimmed, dehydrated, cleared in an alcohol-amyl acetate-chloroform series and embedded in paraffin wax. Sections 5 microns thick were cut and routinely stained with haemo-toxylin and eosin as described by Culling (1963).

Selected sections were stained for collagen using the picro Mallory modification of the Mallory tri-chrome stains as described by Culling (1963). Mast cells were stained in sections from Carnoy's

fixed tissue using Astra blue counterstained with safranin O as described by Murray et al (1968).

RESULTS

1) The pathological changes seen at 4, 8, 12, 16, 20 and 24 weeks after the infection of parasite free calves with 500 metacercariae of F. hepatica.

Four weeks after infection

a) Gross Pathology

A mild peritonitis was seen over the rumen, spleen and greater omentum. The mesenteric lymph nodes were enlarged and on section occasional small (5-10mm) greenish foci were found in these nodes. There was, on the serosal surface of the intestines, multiple small (1-2cm) greenish raised nodules some with haemorrhagic centres. These nodules were fairly evenly distributed throughout the length of the small intestines and occasional nodules were found on the wall of the rumen and caecum. The peritonitis and nodules are illustrated in plates 1 and 2, Appendix 4.

The liver was removed and the majority of lesions were visible on the surface of the caudate lobe and on the surface of the ventral lobe below the line of reflection of the peritoneum. Three types of lesions were present. The most numerous were white tracks approximately 2mm broad running for 1-2cm along the surface. There was no haemorrhage evident at the margins of these tracts and they had a well defined boundary with the hepatic parenchyma. The second type was also a white track but with a haemorrhagic border merging into a diffuse greyish area 5-10mm broad in the surrounding parenchyma. In contrast to the previous lesions the third type was a small (2-5mm) raised honeycombed haemorrhagic area. The visceral surface of a liver four weeks after infection is shown in plate 3, Appendix 4. On section of the liver the tracks on the surface were seen to continue through

the hepatic parenchyma. Numerous small haemorrhagic lesions were also found in the substance of the liver. The lesions were most numerous in the distal end of the ventral lobe. More lesions were found on section of the caudate lobe than in any equivalent area of the dorsal lobe.

Distention of the bile ducts was not apparent in any lobe.

b) Histopathology

The white tracks found on the surface and in the substance of the liver appeared histologically as areas of parenchymal necrosis which had largely been replaced by fibrous tissue. The parenchyma at the margin of these tracks was sparsely infiltrated with eosinophils and lymphocytes. The second type of track with the haemorrhagic border was seen on histology to consist of a central area of degenerating hepatocytes infiltrated with eosinophils, macrophages and lymphocytes. There was an area of haemorrhage at the edge of this track and the surrounding parenchyma was heavily infiltrated with eosinophils. Recent fluke tracks were only seen histologically and had a central area of haemorrhage and disrupted parenchymal cells which contained a few eosinophils. This central area was surrounded by degenerating parenchymal cells which were not disrupted. The perimeter of the track was defined by an area of infiltration of the parenchyma by eosinophils and a few lymphocytes. The small flukes found in the parenchyma were surrounded by an area of haemorrhage and hepatic cell necrosis. The parenchyma at the periphery of the lesions was infiltrated with eosinophils. The tracks seen on the surface and in the substance of the liver therefore represent fluke tracks at various stages of repair.

The haemorrhagic lesions seen on the surface and in the substance of the liver appeared histologically as an area of

haemorrhage and infarction resulting from the rupture of blood vessels, predominately branches of the portal and hepatic veins involved in fluke tracks. Thrombosis of branches of the portal and hepatic veins closely associated with but not directly involved in fluke tracks was also a common finding.

A marked feature of the histopathology at 4 weeks after infection was the appearance of an eosinophilic phlebitis and thrombosis of the smaller conducting and axial distributing branches of the portal veins which were not directly involved in areas of traumatic fluke damage. The phlebitis began with the accumulation of numerous eosinophils in the intima of the vein accompanied by oedema and an eosinophil infiltration of the media and adventitia of the vein. The eosinophil infiltration of the intima eventually became so great that the lumen of the vein became occluded and the endothelium disrupted. The stages in the development of this phlebitis are shown in plates 9 - 13, Appendix 4.

The connective tissue of the portal canals containing such thrombosed vessels was also heavily infiltrated with eosinophils and lymphatic distention was obvious. In areas where the portal veins were infiltrated or thrombosed the marginal distributing venules in the peri-portal area became more obvious due to the infiltration of the perivascular area with eosinophils and a few small lymphocytes and plasma cells. In these areas there was necrosis of cells in the limiting plate and adjacent parenchymal cells.

On occasions there was an eosinophil infiltrate confined to the media and adventitia of the hepatic artery involved in areas of fluke damage.

Eight weeks after infection

a) Gross pathology

After the abdomen was opened a diffuse peritonitis was only found over some areas of the greater omentum. The mesenteric lymph nodes were still enlarged and the occasional small greenish focus was found on section of the nodes.

Raised semi-translucent nodules, 2-3cm in diameter were found on the serosal surface of the intestines in the same regions as the nodules described at four weeks after infection.

The majority of the lesions visible were on the surface of the ventral lobe. These were haemorrhagic lesions 3-4mm in diameter surrounded by a pale fibrous area 1-2cm wide. A few white tracks 2-3mm broad were also visible on the surfaces of the ventral and caudate lobes.

The visceral surface of a liver 8 weeks after infection is shown in plate 4, Appendix 4.

On section of the liver haemorrhagic lesions 3-4mm in diameter were most numerous in the substance of the ventral and caudate lobes. Small flukes were recovered from some of the haemorrhagic lesions.

Some major bile ducts were visible at the visceral surface of the ventral lobe and distention and fibrosis of the major ducts was apparent on section of the ventral lobe. There was also a diffuse fibrosis of the area immediately surrounding the major bile ducts at the distal end of the lobe. The major bile ducts leading from the caudate lobe through the dorsal lobe were also on section seen to be enlarged and fibrosed.

b) Histopathology

Recent and healing fluke tracks were present as well as haemorrhagic lesions some of which were being replaced by areas

of fibrous tissue.

The eosinophilic phlebitis and thrombosis of the smaller branches of the portal vein described at 4 weeks after infection had largely resolved. The cellular infiltrate of the intima had been replaced by connective tissue, the veins had become recannulated though with a much narrowed lumen. The cellular infiltrate of the portal tracts seen 4 weeks after infection had also been replaced by connective tissue containing some eosinophils and mast cells.

In areas of fluke damage, predominately at the distal end of the ventral lobe there was obvious periportal fibrosis.

The fibrosis extended outward from the connective tissue of the portal canal into the periportal area as a sequel to necrosis of the hepatocytes adjacent to the marginal distributing branches of the portal vein. The hepatocytes showed degenerative changes in their nuclei and cytoplasm, lost their chord formation and were finally encircled by strands of connective tissue. On occasions groups of hepatocytes were incorporated in the connective tissue to form structures resembling bile ductules. Numerous eosinophils, some lymphocytes and plasma cells were present in these areas of hepatic cell necrosis. The progression of this periportal fibrosis is illustrated in plates 14 - 20, Appendix 4.

The epithelium of the larger bile ducts in the ventral and caudate lobes had begun to proliferate and was thrown into short villi. The 'lamina propria' of these villi consisted of connective tissue with a few eosinophils, lymphocytes and mast cells present. The increase in size of the bile duct walls was the result of a severe fibrosis of the adventitia of these ducts.

Twelve weeks after infection

a) Gross Pathology

There was no peritonitis evident on examination of the abdominal cavity nor were any lesions found on the serosal surface of the intestines.

The visceral surface of a liver 12 weeks after infection is shown in plate 5, Appendix 4.

Fewer haemorrhagic lesions were visible on the surface of the ventral lobe.

The major bile ducts were visible at the visceral surface of the ventral lobe. The major bile ducts had an external diameter of 4-5mm with walls 2-3mm thick. Flukes were recovered from the bile ducts. There was a diffuse fibrosis of the area immediately surrounding the major bile ducts and extending into the surrounding parenchyma of the ventral lobe.

b) Histopathology

The predominant histopathological changes seen at 12 weeks after infection concern the extension of the process of periportal fibrosis and the progressive cholangitis of the major bile ducts.

The process of periportal fibrosis described at 8 weeks after infection continued and was severest in the distal end of the ventral lobe where a diffuse fibrosis extended throughout the parenchyma. The periportal fibrosis in the remainder of the ventral lobe was severest in the area immediately surrounding a parasitised bile duct. There was little diffuse periportal fibrosis found in the dorsal lobe except in the areas surrounding the major bile ducts leading from the caudate lobe. There was diffuse periportal fibrosis of the caudate lobe.

The major bile ducts of the ventral and caudate lobe showed marked epithelial hypertrophy and villus formation. Adult fluke

were found in the large bile ducts and in these parasitised ducts the lining epithelium was stretched and eroded in areas. Eosinophils, lymphoid cells and globule leucocytes were found in the lamina propria and epithelium of these ducts. Mast cells were found in the epithelium and lamina propria of small bile ducts but not in the epithelium and lamina propria of parasitised ducts.

There were occasional areas of haemorrhage, thrombosis and infarction containing a small fluke in the parenchyma of the ventral lobe. The margin of these lesions was infiltrated by numerous eosinophils and some lymphoid cells.

Sixteen weeks after infection

a) Gross Pathology

The overall shape of the liver was altered by a reduction in size of the ventral lobe. Distended bile ducts were obvious at the visceral surface of the ventral lobe. Very few haemorrhagic lesions were visible either on the surface or on section of the liver. The visceral surface of a liver at 16 weeks after infection is shown in plate 6, Appendix 4.

The major bile ducts in the ventral lobe and those leading from the caudate lobe were now 5-10mm in diameter with walls 3-4mm thick. Adult flukes were found in these ducts. There was a diffuse fibrosis of the ventral lobe which was most severe at the distal end of the ventral lobe.

The bile ducts in the dorsal lobe with the exception of those leading from the caudate lobe were not as distended or fibrosed as those in the ventral lobe. There was no diffuse fibrosis of the dorsal lobe apart from the areas associated with the ducts leading from the caudate lobe.

The gall bladder was distended and the bile a dark green colour.

b) Histopathology

The histopathological changes seen at 16 weeks after infection were very similar to those described at 12 weeks after infection.

The perilobular fibrosis now extended throughout most of the ventral lobe and was severest at the distal end and in the areas surrounding the major bile ducts. Perilobular fibrosis was also extensive in the caudate lobe but was only seen in the dorsal lobe in areas associated with parasitised bile ducts. There were accumulations of lymphoid cells in the connective tissue of the smaller branches of the portal canal in both dorsal and ventral lobes.

The larger bile ducts again showed epithelial hypertrophy with the formation of long villi. The lamina propria of these villi contained some eosinophils, lymphocytes and an occasional globule leucocyte. Adult flukes were found in the major bile ducts predominately in the ventral lobe and in some areas were responsible for the complete erosion of the biliary epithelium.

Mast cells were again found in the epithelium of small and medium sized bile ducts but not in the parasitised ducts.

Twenty weeks after infection.

a) Gross Pathology

The shape of the liver was altered by a further reduction in size of the ventral lobe and the dorsal lobe was slightly enlarged with rounded edges.

Fibrin tags were found on the parietal surface of the ventral

lobe. Large distended bile ducts were obvious on the visceral surface of the lobe. The visceral surface of a liver 20 weeks after infection is shown in plate 7, Appendix 4.

The ventral lobe was firm to the touch and there was a diffuse fibrosis of the entire lobe which increased in severity towards its distal end. The major bile ducts in the ventral lobe were now 10-20mm in diameter with walls 6-8mm thick and offered considerable resistance to section with a scalpel. The larger bile ducts had areas of calcification in their walls and on section black gritty material was found free in the lumen as were adult flukes.

On section of the dorsal lobe the bile ducts leading from the caudate lobe were found to be distended with areas of calcification in their walls. The other major bile ducts in the dorsal lobe were distended with a diameter of 3-4 mm and walls 2-3mm thick but no calcification and few flukes were found in these ducts. Diffuse fibrosis of the dorsal lobe was only apparent in the areas surrounding the major bile ducts leading from the caudate lobe.

The caudate lobe however showed a marked resistance to section and had a severe diffuse fibrosis with distended and calcified bile ducts in its substance.

The gall bladder was enlarged and the bile a greenish-black colour.

b) Histopathology

The perilobular fibrosis is similar in extent to that described at 16 weeks after infection. The process of hepatic cell necrosis and subsequent fibrosis was however much less obvious and confined to the badly damaged areas of the ventral and caudate lobes. Discrete accumulations of lymphoid cells were found in the smaller

portal canals adjacent to the small bile ducts.

The most outstanding histopathological changes evident at this time were seen in the medium and large bile ducts. The lamina propria of these ducts contained large numbers of lymphocytes and eosinophils. Numerous globule leucocytes were present both in the lamina propria and between the epithelial cells of these ducts. This cellular reaction occurred not only in visibly parasitised ducts but also in medium sized apparently uninfected ducts. The changes in bile duct mast cell/globule leucocyte populations are shown in plates 21 - 28, Appendix 4.

There was fibrosis and calcification on areas of the lamina propria of the larger parasitised bile ducts.

Twenty four weeks after infection

a) Gross Pathology

The liver was similar in appearance to that described at 20 weeks after infection with a severe diffuse fibrosis of the ventral and caudate lobes. There was marked calcification of the major bile ducts in these lobes. The visceral surface of a liver 24 weeks after infection is shown in plate 8, Appendix 4.

Fibrosis of the dorsal lobe was confined to the area surrounding the major parasitised ducts leading from the caudate lobe.

The gall bladder was distended with greenish-black bile and occasionally contained disintegrating flukes.

b) Histopathology

The histopathological changes were in general similar to those seen in previous months.

There was sever diffuse periportal fibrosis of the ventral

and caudate lobes. At this stage of the infection however, the active process of hepatic cell necrosis and fibrosis had all but ceased except in badly damaged areas of the ventral lobe.

Large areas of the lamina propria of the major bile ducts in the ventral and caudate lobes were fibrosed and calcified. The lamina propria remaining was heavily infiltrated with lymphocytes and eosinophils. Numerous globule leucocytes were found in the epithelium and lamina propria of the parasitised bile ducts.

2) The pathological changes resulting from reinfection of calves with F. hepatica.

a) Gross Pathology

The livers of the reinfected calves were more severely fibrosed than the livers of the control calves at equivalent stages of either the first or second infections. The fibrosis of the ventral lobe of the reinfected livers was accompanied by some hypertrophy of the dorsal lobe. These changes were most evident in the livers of the calves in Experiment 1 reinfected with 1650 metacercariae of F. hepatica 17 weeks after their initial infection of 750 metacercariae of F. hepatica and killed 13 weeks later. In these livers the ventral lobe was very small and was largely composed of fibrous tissue with calcified bile ducts in its substance while the dorsal lobe had hypertrophied to the extent that the long axis of the liver was in a transverse plane instead of the usual dorso-ventral plane. These changes as compared to control livers are shown in plates 29 - 32, Appendix 4. There was also a diffuse fibrosis evident in the dorsal lobe of these livers which was not present in the livers of the control calves.

The livers of the calves in Experiment 3 reinfected with 1300 metacercariae of F. hepatica 7 or 12 weeks after their initial infection with 750 metacercariae of F. hepatica and killed 8 or 13 weeks later were also more severely fibrosed especially in the ventral lobe than the livers of the equivalent control calves but were not as severely fibrosed as the livers from the calves in Experiment 1.

Honeycombed haemorrhagic lesions were found on the surface and in the substance of the livers of both groups of calves in Experiment 3 reinfected 7 weeks after their initial infection and in the livers of calves successfully treated with anthelmintic prior to reinfection in Experiment 2 at 12 weeks after their initial infection. Haemorrhagic lesions were also found in the livers of the calves in Experiment 1 which were successfully treated with anthelmintic prior to their reinfection 17 weeks after their initial infection. These honeycombed haemorrhagic lesions were larger (15-25mm) than those seen in the primary infections, contained live or disintegrating flukes and were found predominately in the ventral lobe. Haemorrhagic lesions were found on two occasions only in the livers of the 8 calves in Experiment 1 and 3 which were reinfected in the presence of the original infection.

b) Histopathological changes

The histopathological changes observed in the livers of the control calves which received only the initial infection of 750 metacercariae of F. hepatica and were killed 20 or 30 weeks later were similar to those already described in the previous results of calves killed 20 or 24 weeks after their infection with 500 metacercariae of F. hepatica. There was a diffuse periportal fibrosis of the ventral and caudate lobes and bile duct distention with

calcification of areas of the lamina propria of these ducts. There was little periportal fibrosis in the dorsal lobe except in the area surrounding the bile ducts leading from the caudate lobe. The process of periportal fibrosis was continuing only in badly damaged areas of the ventral and caudate lobes.

The histopathological changes in the livers of the control calves which received either 1300 or 1750 metacercariae and were killed 8-13 weeks later were more extensive but of the same nature as those previously described in the calves killed 8 and 12 weeks after infection with 500 metacercariae of F. hepatica. There was fibrosis of the ventral and caudate lobes as the result of both healing of fluke tracks and haemorrhagic lesions periportal fibrosis of areas surrounding the distended and fibrosed bile ducts in these lobes. There was a predominately eosinophil infiltrate in the areas of periportal hepatic cell necrosis and fibrosis. There was little fibrosis in the dorsal lobe apart from the area surrounding the fibrosed and enlarged bile ducts leading from the caudate lobe.

A severe diffuse fibrosis was found in the ventral and caudate lobes of the reinfected calves. The diffuse fibrosis had largely replaced the parenchyma in the ventral lobe of the calves in Experiment 1 reinfected 17 weeks after their infection and large areas of the ventral lobe consisted of fibrous tissue infiltrated with eosinophils, lymphocytes and mast cells. Areas of periportal fibrosis were found in the dorsallobes of the livers of the reinfected calves in areas apparently unrelated to fluke damage or parasitised bile ducts. This fibrosis was the result of the reactivation and extension of the periportal fibrosis induced by the initial infection. The process of periportal hepatic cell necrosis and fibrosis in the reinfected livers was however associated with a

predominately lymphocyte infiltration although some eosinophils and plasma cells were also present in these areas. This process of periportal fibrosis in the reinfected livers as compared to the control livers is shown in plates 33 - 37, Appendix 4.

Focal accumulation of lymphocytes were commonly found in the portal canals in areas of fibrosis and isolated accumulations of lymphoid cells were occasionally found in the parenchyma of the livers of the reinfected calves.

The large haemorrhagic lesions found on gross examination of the livers of the calves whose initial infection had been removed with anthelmintic prior to reinfection at either 12 or 17 weeks after infection and in the livers of calves reinfected 7 weeks after their initial infection appeared histologically as areas of hepatic cell necrosis, thrombosis and haemorrhage surrounding a fluke. There was a marked lymphoid infiltration of the hepatic parenchyma at the margin of these lesions.

The histopathological changes in the bile ducts of the infected calves were similar to those described in the control calves killed at 20 and 30 weeks after their infection with 750 metacercariae of F. hepatica.

DISCUSSION

The sequence of pathological changes induced by a standard single challenge of 500 metacercariae of F. hepatica can now be summarised in relation to the stage of infection with the parasite.

The lesions associated with the migrations of the immature flukes in the hepatic parenchyma appeared predominantly in the ventral and to a lesser extent caudate lobes and were at a maximum between 4 and 8 weeks after infection.

The fluke tracts were characterised by areas of haemorrhage, necrosis, infarction. There was also a proliferative phlebitis, with marked eosinophil infiltration in the intima, media and lamina propria, of branches of the portal vein. The repair of the traumatic damage involved replacement fibrosis and some periportal fibrosis was also seen to develop at this time. This periportal fibrosis did not appear to be directly related to traumatic damage but was in some areas associated with the proliferative phlebitis of radicles of the portal vein.

The periportal fibrosis of the caudate and ventral lobes then increased between 8-20 weeks after infection. This fibrosis resulted in a reduction in size of the caudate and ventral lobe whereas little pathological change was seen in the dorsal lobe except for a slight hypertrophy between 16-20 weeks after infection. The process of periportal necrosis and fibrosis had ceased in all but the badly damaged areas of the ventral lobe by 24 weeks after infection.

At 8 weeks after infection the first flukes were found in the bile ducts. The presence of the flukes in the bile ducts was associated with marked epithelial hypersplasia and a sparse infiltration of the epithelium and adventitia with eosinophils,

lymphocytes and globule leucocytes. At 20 and 24 weeks after infection large numbers of lymphocytes and globule leucocytes were found in the epithelium, lamina propria and adventitia of these ducts. Areas of calcification were also evident in the lamina propria of the major bile ducts in the ventral and caudate lobes both on gross and histological examination at 20 weeks after infection. This calcification had become much more extensive by 24 weeks after infection.

The sequential pathological changes described in the livers of calves infected with F. hepatica with the exception of the proliferative phlebitis and calcification of the bile ducts were in general similar to those seen in experimental fascioliasis of the laboratory animals (Urquhart 1956, Thorpe 1965, Dawes 1963 a, b, 1966, Dawes and Hughes 1964). Dow et al (1967) did not describe the occurrence of a proliferative phlebitis in the livers of calves experimentally infected with F. hepatica but illustrated however what was termed an endarteritis with eosinophil infiltration and intimal fibrosis which bears a remarkable similarity to the proliferative lesions seen in the portal veins in this experiment.

In this context it is interesting to note that Hussein (1971) has also described and illustrated a proliferative phlebitis of the smaller branches of the portal vein in the livers of calves experimentally infected with another trematode parasite Schistosoma bovis. The etiology of this proliferative phlebitis with eosinophil infiltration associated with infection by F. hepatica and S. bovis is however unclear. Hussein (1971) considered that the presence of large numbers of eosinophils in the walls of the affected veins may have indicated that the lesion was due to an allergic reaction to foreign proteins released by the parasite but did not

develop this hypothesis further.

Eosinophils have long been associated with mast cell disruption and histamine release (Archer and Feldberg 1963) but it has recently been shown that in both the guinea pig (Kay, Stenschulte, Austen 1971) and man (Kay and Austen 1971) the reaction of antigen with reaginic antibody in sensitised tissue mediated the reaction of a specific eosinophil chemotactic factor distinct from the vasoactive amines. Eosinophils have also been shown to be attracted to and responsible for the phagocytosis of both soluble and insoluble antigen-antibody complexes with subsequent lysis of the eosinophil granules (Archer 1969, Archer, Nelson, Johnston 1969). The presence of the eosinophils in the intima, media and adventitia of these veins at a time when precipitins and reagins to fluke antigens have been shown to be present in these calves may therefore indicate the involvement of some immunological mechanisms in the development of this phlebitis.

One of the most striking findings in this study was the increase in the numbers of globule leucocytes found in the parasitised and non-parasitised bile ducts at 20 and 24 weeks after infection. This confirms the finding by Rahko (1971) of large numbers of mast cell/globule leucocytes in the bile ducts of naturally infected cattle. The rise in the mast cell/ globule leucocyte population which occurred between 16-24 weeks after infection coinciding with the period of reduced biological activity of the flukes and their eventual expulsion from the bile ducts is remarkably similar to that seen during the immune expulsion of N. brasiliensis from infected rats (Miller and Jarrett 1971).

The most significant change induced by the reinfection of calves was an increase in the amount of periportal fibrosis present

in these livers. The pathological changes associated with periportal fibrosis of bovine fascioliasis are similar to those described in certain human hepatic diseases which are thought to have an autoimmune basis. These include active chronic hepatitis, primary biliary cirrhosis, and possibly cryptogenic cirrhosis. There is considerable overlap in the histological findings in each of these conditions (deGroote, Desmet, Gedigk et al 1968, Scheure 1967 1971, Popper 1971). It has been hypothesised (Doniach and Walker 1969) that a single process related to autoimmunity may damage hepatocytes or bile ductules and depending on which is the more severely affected the condition will progress as a hepatitis or biliary cirrhosis.

These 'autoimmune' cirrhotic conditions in man are accompanied by the appearance of circulating antibodies against tissue antigens derived from mitochondria, bile ductules or smooth muscle cells. Despite intensive investigations into the pathogenesis of these conditions (Gadjuseck 1958, Glynn and Holbrow 1965, Doniach 1970, Smith and Williams 1971) there is as yet little agreement on the mechanisms involved in the pathogenesis of these conditions. The role of the tissue antibodies in the pathogenesis of the fibrosis is uncertain and there are only two reports (Dodd, Bigley, Geyer et al 1962, Dobias and Balzas 1967) of the induction of cirrhotic changes by the passive administration of homologous or heterologous antisera raised against liver constituents. More recently, however, experimental evidence has been reported (Smith, Eddleston, Williams, 1971, Smith, Golding, Miller et al 1971, MacLaurin 1971) which would appear to implicate cell mediated immunity in the pathogenesis of these diseases.

It is possible that the traumatic damage inflicted by the flukes during their sojourn in the liver may release sequestered or modified

hepatic antigens capable of inducing an immunological response directed against liver tissues. It may even be that if a system of minimal antigenic disparity occurs between the parasite and the host as discussed earlier in this thesis that an immunological response directed against some parasitic antigens may cross react with some hepatic antigens. The pathogenesis of the periportal fibrosis found in fluke infected animals is therefore uncertain and investigation of a possible 'autoimmune' mechanism may provide some explanation for this process.

The results of this investigation of the pathological changes induced by F. hepatica infections in calves show that there is considerable evidence of an immunological reaction to the presence of the parasites in the bile ducts over the period when the flukes show evidence of reduced biological activity and their subsequent elimination from the bile ducts.

The diffuse periportal fibrosis resulting from a primary infection of F. hepatica is markedly exacerbated by reinfection with this parasite and this process of periportal fibrosis appears to have characteristics in common with the so called 'autoimmune' cirrhotic conditions in man.

SUMMARY

The sequence of pathological changes induced by a single infection of 500 metacercariae of F. hepatica was studied in the livers of calves killed 4, 8, 12, 16, 20 and 24 weeks after infection.

The traumatic lesions resulting from the migratory activities of the immature flukes were found predominantly in the parenchyma of the ventral and caudate lobes and were most numerous 4-8 weeks after infection. A proliferative phlebitis of radicles of the portal vein was also observed at this time apparently unrelated to traumatic damage and an immunological mechanism may be implicated.

Flukes were first found in the bile ducts at 8 weeks after infection. The presence of the parasites in the bile ducts resulted in a marked biliary epithelial hyperplasia. At 20 and 24 weeks after infection during the period when the flukes were being expelled from the bile ducts numerous eosinophils, lymphocytes and globule leucocytes were found in the epithelium and lamina propria of the ducts. Calcified areas were apparent in the walls of parasitised bile ducts at 24 weeks after infection.

The period between 8-20 weeks after infection was characterised by the development of a diffuse periportal fibrosis found predominantly in the parenchyma of the ventral and caudate lobes. This process of periportal fibrosis was markedly exacerbated by reinfection as was seen in the livers of calves reinfected 7, 12 or 17 weeks after an initial infection of 750 metacercariae of F. hepatica.

The histopathological changes associated with this periportal fibrosis of bovine fascioliasis were similar to those described in

certain human hepatic diseases thought to have an autoimmune basis and a similar immunological mechanism may be responsible for the cirrhosis of bovine fascioliasis.

CONCLUSIONS

CONCLUSIONS

The object of this thesis as defined in the introduction was to investigate and define the possible occurrence of an acquired resistance in parasite free calves to experimental infection with F. hepatica and then if such a resistance existed to investigate the mechanisms involved in its expression.

The results of the experiments described in the first part of this thesis provide the first significant experimental evidence that calves acquired a resistance to infections with F. hepatica and that this resistance is manifest against both primary and secondary infections with this parasite.

The acquired resistance which calves developed against a primary infection with F. hepatica was manifest as a 'self-cure' reaction with the subsequent loss of the majority of their acquired fluke population between 20-30 weeks after infection. This period of expulsion was preceded by a period of reduced biological activity of the flukes as evidenced by a sharp decrease in their egg output.

The degree of resistance to reinfection was shown to be related to the duration of the initial infection. Calves reinfected 7 weeks after their initial infection did not develop any resistance to the reinfection. Calves reinfected 12 weeks after their initial infection resisted the establishment of all but 27% of the fluke population which developed in previously uninfected control calves. Calves reinfected 17 weeks after their initial infection resisted the establishment of all but 16% of the fluke population which developed in the previously uninfected control calves.

As the results obtained in the first part of this thesis clearly demonstrated that calves would acquire a resistance to

infections with F. hepatica the experiments in Parts 2, 3 and 4 of this thesis were concerned with the investigation of possible mechanisms involved in the expression of this acquired resistance.

The two possible mechanisms responsible for this acquired resistance previously discussed in Part 1 of this thesis were that this resistance was either simply due to the physical damage caused by the flukes rendered the environment unsuitable for their development or it was due to the expression of a specific immunologic response on the part of the host. Each of these mechanisms will now be considered in relation to the development of an acquired resistance to a primary or secondary infection with F. hepatica.

The process of calcification of the lamina propria of parasitised bile ducts can be related to the acquired 'self-cure' found in primary infections as the period of calcification of these ducts, 20-30 weeks after infection, coincided with the period of expulsion of the flukes from these ducts. It could be postulated that this calcification of the lamina propria of parasitised ducts prevents the feeding activities of the flukes and hence leads to their eventual death and expulsion. Calcification of the bile ducts however, cannot explain the period of reduced biological activity of the flukes prior to their expulsion as this occurs between 16-20 weeks after infection before calcification of the ducts occurs. Physical damage cannot therefore be held entirely responsible for the 'self-cure' reaction as it is seen in primary infections with F. hepatica.

The results described in Parts 2 and 3 of this thesis have shown that a primary infection with F. hepatica can induce the

the formation of both circulating and cell bound antibodies directed against various parasitic antigens. The period of reduced biological activity and expulsion of the flukes coincides with a rise in the level of precipitating antibodies present in the sera of infected calves and also with the appearance of increasing amounts of circulating reaginic antibody. The results of Part 4 of this thesis provide histopathological evidence of increasing immunological activity in the parasitised bile ducts from 16-20 weeks after infection culminating 20 weeks after infection with the appearance of large numbers of immunologically competent cells and globule leucocytes in the lamina propria of these ducts.

In view of the fact that certain of the trematode parasites, the schistosomes, have been shown to be susceptible to immune damage it is conceivable that F. hepatica may also be susceptible to immune damage and that the 'self-cure' reactions may have an immunological basis. The feeding activities of the adult F. hepatica would ensure its accessibility to circulating antibody. In this context it can be seen that the rise in the level of precipitating antibodies in the sera of infected calves coincides with the period from 16 weeks after infection onwards when the flukes show reduced biological activity and are expelled from the bile ducts.

The presence of large numbers of globule leucocytes in the epithelium and lamina propria of the major bile ducts at 20-24 weeks after infection coinciding with the expulsion of the flukes from the bile ducts may also indicate the existence of a similar mechanism to the reaginic antibody mediated mast cell discharge associated with the immune expulsion of N. brasiliensis from

infected rats.

The resistance to reinfection and its apparent relationship to the duration of the initial infection could be related to the degree of development of the fibrosis and cholangitis which results from the initial infection rendering the environment unsuitable for the migration and maturation of any subsequent fluke population. This concept does not, however, explain the presence of numerous lesions due to migrating flukes found in the livers of the calves treated with anthelmintic prior to reinfection at 12 or 17 weeks after their intial infection as compared to the few lesions found in the livers of calves reinfected in the presence of the original population since the degree of fibrosis and cholangitis in both groups of livers on each occasion would be similar. The difference in susceptibility to migratory fluke damage in livers with similar degree of cholangitis and fibrosis could, however, be explained if the resistance to reinfection represented the expression of an acquired immunity to this parasite. The calves retaining their original fluke population could become more immune than those calves in which the original fluke population was removed either because the antigenic stimulus is more prolonged in these calves or more antigens involved in a protective immune response become available as the parasite develops further.

The development of a significant immunity to F. hepatica infections in rats has already been shown to be dependent on the duration of the initial infection (Corba et al 1971) and so it is also possible that the acquired resistance of cattle with its relationship to the duration of the primary infection may also be immunological in nature.

The results of this thesis then provide significant evidence for the development of an acquired immunity to infections with F. hepatica in previously parasite free calves. The mechanism of this acquired resistance is still uncertain but it would appear that it cannot satisfactorily be explained as the eventual result of traumatic damage caused by the flukes rendering their environment unsuitable for them. The results of investigations described in this thesis show for the first time that specific immunological responses are associated with the infection of calves with F. hepatica and indicate the possibility that these rather than physical factors may be responsible for the acquired resistance exhibited by calves to infections with F. hepatica.

The eventual separation of the role of immunological and physical factors in the mechanism of acquired resistance to F. hepatica infections in calves will depend on the successful demonstration of an acquired resistance in calves rendered immune to F. hepatica in a manner which does not result in hepatic fibrosis and cholangitis. This could be done in several ways. Attenuation of the metacercariae by irradiation to a point where juvenile flukes, only migrate through the intestinal wall and die before causing any hepatic damage may provide sufficient antigenic stimulus at the appropriate site to induce a successful immune response in a manner similar to that of the successful irradiated vaccine for Dictyocaulus viviparus and Ancylostomum caninum. The transfer of lymphoid cell suspension from infected calves to genetically identical recipients may render these calves immune to infection with F. hepatica as this has already been successfully demonstrated in isogenic rats

(Corba et al 1971). The isolation of specific fluke antigens capable of inducing a successful immunity when used to vaccinate calves may also be possible in view of the success of this technique in inducing a successful acquired immunity to T. spiralis (Despommier and Muller, 1970 a, b).

It would appear that of these three techniques the use of irradiation to limit the degree of development of the fluke to a point where it provides sufficient antigenic stimulus to induce a successful immune response without causing hepatic damage would appear at the present time to be the most practical method of investigating the role of a protective immunity in the acquired resistance of calves to infections with F. hepatica.

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STUDIES ON THE DEVELOPMENT OF AN ACQUIRED RESISTANCE TO *FASCIOLA HEPATICA* INFECTIONS IN CATTLE.

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Volume II - Appendices

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APPENDIX 1

Table 1

Mean weekly weights of a group of 16 calves infected with 750 metacercariae of *F. hepatica* and mean weekly weights of a group of 4 uninfected control calves.

Weeks after infection	Mean weekly weights of 16 calves infected with *F. hepatica* (lbs)	Mean weight of 4 infected control calves (lbs)
0	210 ± 6	233 ± 24
1	214 ± 6	233 ± 26
2	223 ± 6	249 ± 28
3	244 ± 6	271 ± 31
4	252 ± 7	277 ± 30
5	267 ± 7	289 ± 30
6	281 ± 7	308 ± 30
7	300 ± 13	330 ± 32
8	323 ± 7	350 ± 34
9	333 ± 9	358 ± 38
10	346 ± 9	367 ± 31
11	364 ± 10	397 ± 36
12	373 ± 9	415 ± 37
13	394 ± 11	440 ± 36
14	406 ± 11	452 ± 36
15	411 ± 15	472 ± 35
16	422 ± 15	490 ± 36

Table 2

Mean weekly erythrocyte counts of a group of 16 calves each infected with 750 metacercariae of *F. hepatica* and the mean weekly erythrocyte count of a group of 4 uninfected control calves.

Weeks after infection	Mean R.b.c. counts of 16 calves infected with *F. hepatica* (millions/cu.mm.)	Mean R.b.c. counts of 4 uninfected control calves (millions/cu.mm.)
0	7.47 ± .16	7.20 ± .36
1	7.35 ± .15	7.10 ± .19
2	7.20 ± .14	6.97 ± .25
3	7.48 ± .15	6.99 ± .38
4	7.20 ± .17	6.89 ± .22
5	7.31 ± .13	7.26 ± .33
6	7.17 ± .15	7.04 ± .20
7	7.41 ± .16	7.14 ± .20
8	7.68 ± .17	7.22 ± .23
9	7.44 ± .20	7.14 ± .25
10	7.39 ± .17	7.18 ± .28
11	7.37 ± .22	6.75 ± .27
12	7.34 ± .23	7.41 ± .11
13	7.45 ± .17	7.10 ± .54
14	7.20 ± .13	6.97 ± .25
15	6.90 ± .12	6.67 ± .17
16	7.17 ± .19	6.87 ± .20

Table 3

Mean weekly packed cell volume percentage of a group of 16 calves each infected with 750 metacercariae of *F. hepatica* and the mean weekly packed volume percentages of a group of 4 uninfected control calves.

Weeks after infection	Mean p.c.v. of 16 calves infected with *F. hepatica* (%)	Mean p.c.v. of 4 uninfected control calves (%)
0	33.4 ± .46	32.5 ± 1.08
1	32.4 ± .40	32.5 ± .43
2	32.2 ± .40	32.5 ± .75
3	31.5 ± .34	30.2 ± .96
4	31.3 ± .36	30.5 ± .43
5	32.6 ± .30	32.2 ± 1.19
6	30.5 ± .37	31.2 ± .75
7	32.1 ± .49	31.7 ± .82
8	33.1 ± .49	33.0 ± .35
9	32.4 ± .47	31.5 ± .82
10	32.7 ± .38	31.5 ± 1.14
11	32.2 ± .56	30.2 ± 1.03
12	31.5 ± .66	30.5 ± .62
13	32.2 ± .46	31.0 ± 1.17
14	32.7 ± .70	32.0 ± 1.12
15	33.2 ± .55	31.2 ± .43
16	33.3 ± .68	31.0 ± .61

Table 4

Mean weekly haemoglobin levels of a group of 16 calves each infected with 750 metacercariae of F. hepatica and the mean weekly haemoglobin level of 4 uninfected control calves.

Weeks after infection	Mean haemoglobin level of 16 calves infected with F. hepatica gms/100 ml.	Mean haemoglobin level of 4 uninfected control calves gms/100 ml.
0	11.2 ± .16	11.3 ± .32
1	10.8 ± .16	11.2 ± .26
2	10.6 ± .15	10.8 ± .24
3	11.1 ± .12	11.0 ± .09
4	10.8 ± .18	11.0 ± .18
5	11.2 ± .12	11.4 ± .19
6	10.9 ± .15	11.2 ± .16
7	11.6 ± .21	11.8 ± .31
8	11.6 ± .15	11.8 ± .15
9	11.6 ± .18	11.5 ± .25
10	11.4 ± .16	11.4 ± .31
11	11.1 ± .35	10.8 ± .32
12	11.4 ± .23	11.1 ± .39
13	11.9 ± .50	11.9 ± .32
14	11.6 ± .20	11.1 ± .22
15	11.5 ± .16	11.2 ± .20
16	11.6 ± .20	10.7 ± .23

Table 5

Mean weekly mean corpuscular volume of a group of 16 calves each infected with 750 metacercariae of F. hepatica and the mean weekly mean corpuscular volume of a group of 4 uninfected control calves.

Weeks after infection	Mean M.C.V. of 16 calves infected with F. hepatica (cu. microns)	Mean M.C.V. of 4 uninfected control calves (cu. microns)
0	44.9 ± .69	45.4 ± .72
1	44.2 ± .61	45.8 ± .75
2	44.8 ± .72	46.7 ± 1.43
3	45.3 ± .39	43.3 ± .76
4	43.7 ± .72	44.3 ± 1.13
5	44.5 ± .75	44.5 ± 1.50
6	45.2 ± .36	44.6 ± 2.0
7	43.5 ± .88	44.5 ± 1.46
8	43.4 ± .81	45.1 ± 1.37
9	43.9 ± .82	44.2 ± 1.11
10	44.5 ± .83	44.0 ± 1.98
11	44.1 ± .85	44.9 ± 1.42
12	43.2 ± .92	41.1 ± .61
13	43.4 ± .85	43.8 ± 1.11
14	45.6 ± .90	46.0 ± 1.90
15	48.2 ± .61	46.9 ± .98
16	46.8 ± 1.10	45.3 ± 1.88

Table 6

Mean weekly mean corpuscular haemoglohin concentration of a group of 16 calves each infected with 750 metacerariae of F. hepatica and the mean weekly mean corpuscular haemoglobin concentration of a group of 4 uninfected control calves.

Weeks after infection	Mean M.C.H.C. of 16 calves infected with F. hepatica (%)	Mean M.C.H.C. of 4 uninfected control calves (%)
0	33.5 ± .22	34.8 ± .25
1	33.3 ± .27	34.4 ± .39
2	33.0 ± .32	33.4 ± .75
3	35.3 ± .29	35.9 ± 1.66
4	34.6 ± .36	36.0 ± .31
5	33.5 ± .27	34.8 ± 1.11
6	35.6 ± .46	35.9 ± .56
7	35.9 ± .29	37.1 ± .38
8	35.2 ± .34	35.8 ± .47
9	35.6 ± .38	35.9 ± .89
10	35.0 ± .32	36.3 ± .57
11	34.6 ± .55	35.6 ± .37
12	36.1 ± .27	36.4 ± .86
13	36.8 ± .31	38.3 ± .46
14	35.4 ± .35	34.7 ± .24
15	34.6 ± .29	35.8 ± .25
16	34.8 ± .40	34.5 ± .40

Table 7

Mean weekly serum albumin levels of a group of 16 calves each infected with 750 metacerariae of F. hepatica and the mean weekly serum albumin levels of a group of 4 uninfected control calves.

Weeks after inspection	Mean serum albumin level of 16 calves infected with F. hepatica (gms/100 ml.)	Mean serum albumin level of 4 uninfected control calves (gms/100 ml.)
0	2.19 ± .03	2.28 ± .06
1	2.19 ± .06	2.21 ± .08
2	2.13 ± .04	2.30 ± .02
3	2.22 ± .05	2.24 ± .06
4	2.09 ± .04	2.00 ± .03
5	2.27 ± .04	2.22 ± .06
6	2.25 ± .04	2.37 ± .08
7	2.20 ± .04	2.29 ± .02
8	2.28 ± .03	2.21 ± .04
9	2.10 ± .02	2.10 ± .05
10	2.24 ± .03	2.12 ± .10
11	2.34 ± .03	2.37 ± .08
12	2.29 ± .04	2.43 ± .03
13	2.14 ± .03	2.25 ± .05
14	2.19 ± .03	2.48 ± .03
15	2.17 ± .04	2.40 ± .06
16	2.11 ± .03	2.33 ± .04

Table 8

Mean weekly total serum globulins of a group of 16 calves each infected with 750 metacercariae of F. hepatica and the mean weekly total serum globulins of a group of 4 uninfected control calves.

Weeks after infection	Mean total serum globulins of 16 calves infected with F. hepatica (gms/100 ml.)	Mean total serum globulins of 4 uninfected control calves (gms/100 ml.)
0	3.88 ± .10	3.69 ± .13
1	3.83 ± .07	3.89 ± .25
2	3.97 ± .13	3.94 ± .21
3	3.97 ± .07	3.67 ± .16
4	4.06 ± .08	3.88 ± .09
5	3.99 ± .08	3.75 ± .19
6	3.77 ± .06	3.48 ± .12
7	3.95 ± .05	3.60 ± .15
8	4.12 ± .06	3.79 ± .08
9	4.24 ± .08	3.81 ± .19
10	4.43 ± .09	3.94 ± .21
11	4.37 ± .09	3.57 ± .19
12	4.44 ± .09	3.57 ± .16
13	4.81 ± .10	3.60 ± .16
14	4.99 ± .10	3.69 ± .08
15	4.92 ± .09	3.69 ± .08
16	4.92 ± .10	3.49 ± .06

Table 9

Mean weekly serum glutamic-oxaloacetic transamimase levels of a group of 16 calves each infected with 750 metacercariae of F. hepatica and the mean serum glutamate-oxaloacetic dehydrogenase levels of a group of 4 uninfected control calves.

Weeks after infection	Mean S.G.O.T. levels of 16 calves infected with F. hepatica (S.-F. units)	Mean S.G.O.T. levels of 4 uninfected control calves (S.-F. units)
0	75.6 ± 3.8	66.7 ± 2.7
1	93.2 ± 3.0	87.7 ± 4.4
2	91.4 ± 2.4	78.2 ± 3.5
3	90.2 ± 2.5	76.0 ± 3.7
4	91.6 ± 1.3	81.2 ± 2.9
5	78.9 ± 2.1	73.2 ± 5.0
6	103.1 ± 4.4	84.5 ± 3.1
7	112.4 ± 7.5	70.7 ± 2.7
8	138.9 ± 7.8	77.2 ± 3.5
9	129.3 ± 9.4	73.7 ± 4.8
10	102.4 ± 5.3	68.5 ± 2.6
11	83.9 ± 7.8	47.0 ± 1.1
12	69.4 ± 5.2	44.7 ± 2.0
13	67.7 ± 3.6	47.0 ± 3.2
14	63.9 ± 3.1	46.2 ± 3.0
15	63.2 ± 3.9	44.0 ± 2.3
16	61.1 ± 3.4	45.5 ± 1.9

Table 10

Mean weekly weights of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacerariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly weights of the 4 previously uninfected calves in Group 5 following their infection with 1,650 metacerariae of F. hepatica.

Weeks after infection	Mean weights of 8 calves reinfected with 1,650 metacercariae of F. hepatica (lbs.)	Mean weight of 4 calves infected with 1,650 metacercariae of F. hepatica (lbs.)
0	435 ± 16	503 ± 34
1	466 ± 11	517 ± 32
2	481 ± 11	532 ± 29
3	481 ± 13	532 ± 33
4	510 ± 12	563 ± 27
5	527 ± 13	572 ± 31
6	538 ± 13	574 ± 35
7	558 ± 14	593 ± 27
8	582 ± 12	616 ± 39
9	574 ± 13	608 ± 37
10	590 ± 14	617 ± 35
11	606 ± 16	630 ± 39
12	602 ± 12	642 ± 35
13	622 ± 14	656 ± 32

Table 11

Mean weekly erythrocyte counts of 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly erythrocyte counts of the four previously uninfected calves in Group 5 following their infection with 1,650 metacercariae of F. hepatica.

Weeks after infection	Mean R.b.c. count of 8 calves reinfected with 1,650 metacercariae of F. hepatica (millions/cu.mm.)	Mean R.b.c. count of 4 calves infected with 1,650 metacercariae of F. hepatica (millionsEcu.mm.)
0	7.16 ± .20	7.01 ± .16
1	7.27 ± .20	6.78 ± .21
2	6.46 ± .34	6.47 ± .14
3	7.14 ± .26	6.58 ± .28
4	6.68 ± .22	6.69 ± .36
5	6.85 ± .24	6.00 ± .55
6	6.77 ± .23	6.33 ± .49
7	6.75 ± .16	6.29 ± .35
8	6.60 ± .19	6.25 ± .35
9	6.76 ± .22	6.30 ± .39
10	6.42 ± .15	5.98 ± .30
11	6.41 ± .14	5.89 ± .37
12	6.04 ± .18	5.52 ± .27
13	6.15 ± .19	5.85 ± .24

Table 12

Mean weekly packed cell volume percentage of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae and the mean weekly packed cell volume percentage of the four previously uninfected calves in Group 5 following their infection with 1,650 metacercariae of F. hepatica.

Weeks after infection	Mean p.c.v. of 8 calves reinfected with 1,650 metacercariae of F. hepatica (%)	Mean p.c.v. of 4 calves infected with 1,650 metacercariae of F. hepatica (%)
0	11.7 ± .18	11.2 ± .30
1	11.7 ± .05	11.3 ± .38
2	11.1 ± .15	9.5 ± 1.0
3	12.2 ± .14	10.5 ± .39
4	11.1 ± .18	10.4 ± .36
5	11.0 ± .23	10.7 ± .30
6	11.3 ± .23	10.8 ± .23
7	11.2 ± .17	10.3 ± .24
8	10.2 ± .21	10.0 ± .38
9	10.9 ± .30	11.0 ± .13
10	10.3 ± .23	10.1 ± .40
11	10.7 ± .33	10.9 ± .42
12	10.9 ± .25	10.6 ± .36
13	10.9 ± .32	11.0 ± .59

Table 13

Mean weekly haemoglobin concentrations of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly haemoglobin concentration of the 4 previously uninfected calves in Group 5 following their infection with 1,650 metacercariae of F. hepatica.

Weeks after infection	Mean haemoglobin concentration of the 8 calves reinfected with 1,650 metacercariae of F. hepatica (%)	Mean haemoglobin concentration of the 4 calves infected with 1,650 metacercariae of F. hepatic (%)
0	11.5 ± .17	10.8 ± .37
1	11.5 ± .15	10.5 ± .30
2	10.3 ± .44	10.2 ± .25
3	11.4 ± .26	10.5 ± .30
4	10.8 ± .20	10.7 ± .47
5	10.8 ± .20	9.8 ± .74
6	11.0 ± .20	10.5 ± .42
7	10.8 ± .19	10.0 ± .47
8	10.1 ± .20	10.1 ± .34
9	11.0 ± .22	10.4 ± .25
10	10.2 ± .22	10.0 ± .48
11	10.8 ± .28	10.3 ± .50
12	10.8 ± .22	11.0 ± .35
13	11.0 ± .31	11.3 ± .50

TABLE 14

Mean weekly mean corpuscular volumes of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly mean corpuscular volumes of the four previously uninfected calves in Group 5 following their infection with 1,650 metacercariae of F. hepatica.

Weeks after infection	Mean M.C.V. of 8 calves reinfected with 1,650 metacercariae of F. hepatica (cu. microns)	Mean M.C.V. of 4 calves infected with 1,650 metacercariae of F. hepatica (cu. microns)
0	48.1 ± .98	44.9 ± 1.39
1	48.1 ± .84	46.1 ± 1.59
2	48.9 ± .99	47.1 ± 1.19
3	47.8 ± 1.18	47.6 ± 1.61
4	47.8 ± .96	46.1 ± 1.41
5	48.2 ± 1.07	46.9 ± 2.17
6	49.3 ± 1.14	49.8 ± 2.23
7	47.7 ± .81	47.8 ± 1.15
8	47.2 ± .92	50.3 ± 2.17
9	49.0 ± 1.00	47.9 ± 2.33
10	48.3 ± 1.01	48.4 ± 2.08
11	48.5 ± .88	50.0 ± 1.31
12	51.4 ± 1.30	57.3 ± 1.53
13	47.6 ± 1.21	51.3 ± .65

Table 15

Mean weekly mean corpuscular haemoglobin concentration of the 8 calves in Group 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly mean corpuscular haemoglobin concentrations of the four previously uninfected calves in Group 5 following their infection with 1,650 metacercariae of F. hepatica.

Weeks after infection	Mean M.C.H.C. of 8 calves reinfected with 1,650 metacercariae of F. hepatica (%)	Mean M.C.H.C. of 4 calves infected with 1,650 metacercariae of F. hepatica (%)
0	33.5 ± .22	34.0 ± .45
1	33.0 ± .24	33.7 ± .54
2	33.0 ± .25	33.4 ± .17
3	33.9 ± .34	33.5 ± .32
4	34.1 ± .23	34.7 ± .13
5	33.3 ± .32	34.2 ± .85
6	33.5 ± .53	33.9 ± .63
7	33.6 ± .28	33.5 ± 1.39
8	32.7 ± .19	32.4 ± .73
9	33.4 ± .34	34.8 ± .85
10	32.9 ± .26	34.7 ± .93
11	34.6 ± .28	35.1 ± .76
12	35.0 ± .27	35.1 ± .94
13	37.6 ± .55	37.5 ± .83

Table 16

Mean weekly serum albumin levels of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly serum albumin level of the 4 previous uninfected calves in Group 5 following their infection with 1,650 metacercariae of F. hepatica.

Weeks after infection	Mean serum albumin level of 8 calves reinfected with 1,650 metacercariae of F. hepatica (gms./100 ml.)	Mean serum albumin level of 4 calves infected with 1,650 metacercariae of F. hepatica (gms./100 ml.)
0	1.87 ± .04	2.33 ± .07
1	2.14 ± .03	2.43 ± .15
2	2.14 ± .04	2.05 ± .04
3	2.25 ± .03	2.39 ± .07
4	2.16 ± .03	2.36 ± .08
5	2.37 ± .03	2.16 ± .08
6	2.34 ± .04	2.28 ± .12
7	2.24 ± .04	2.32 ± .11
8	2.29 ± .03	2.20 ± .10
9	2.22 ± .04	2.22 ± .09
10	2.21 ± .02	2.06 ± .08
11	2.28 ± .03	2.07 ± .10
12	2.24 ± .02	2.24 ± .08
13	2.23 ± .04	2.09 ± .02

Table 17

Mean weekly total serum globulin levels of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of *F. hepatica* 17 weeks after their initial infection with 750 metacercariae of *F. hepatica* and the mean weekly total serum globulin levels of the 4 previously uninfected calves in Group 5 following their infection with 1,650 metacercariae of *F. hepatica*.

Weeks after infection	Mean total serum globulin level of 8 calves reinfected with 1,650 metacercariae of *F. hepatica* (gms./100 ml.)	Mean total serum globulin levels of 4 calves infected with 1,650 metacercariae of *F. hepatica* (gms./100 ml.)
0	5.01 ± .08	3.89 ± .04
1	4.72 ± .09	3.50 ± .07
2	4.61 ± .10	3.83 ± .06
3	4.80 ± .12	3.76 ± .16
4	4.83 ± .08	3.94 ± .08
5	4.87 ± .09	4.24 ± .07
6	4.86 ± .09	4.48 ± .25
7	4.87 ± .09	4.63 ± .23
8	4.46 ± .09	4.55 ± .24
9	4.49 ± .11	4.41 ± .30
10	4.43 ± .12	4.33 ± .28
11	4.39 ± .13	4.65 ± .32
12	4.39 ± .06	4.70 ± .30
13	4.37 ± .09	4.80 ± .24

Table 18

Mean weekly serum glutamic-oxaloacetic transaninase levels of the 8 calves in Groups 1 and 2 following their reinfection with 1,650 metacercariae of F. hepatica 17 weeks after their initial infection with 750 metacercariae of F. hepatica and the mean weekly serum glutamic-oxaloacetic dehydrogenase levels of the 4 previously uninfected calves in Group 5 following their infection with 1,650 metacercariae F. hepatica.

Weeks after infection	Mean S.G.O.T. level of 8 calves reinfected with 1,650 metacercariae of F. hepatica (S.-F. units)	Mean S.G.O.T. level of 4 calves infected with 1,650 metacercariae of F. hepatica (S.-F. units)
0	62.0 ± 3.6	48.7 ± 2.0
1	64.5 ± 3.1	50.2 ± 2.8
2	57.4 ± 2.8	55.7 ± 2.3
3	73.6 ± 6.5	60.2 ± 1.3
4	58.6 ± 2.3	63.7 ± 4.3
5	67.0 ± 4.4	59.5 ± 3.7
6	66.0 ± 4.7	75.0 ± 10.0
7	63.9 ± 2.7	71.2 ± 6.6
8	88.5 ± 3.3	151 ± 26.2
9	96.1 ± 4.1	120.7 ± 9.2
10	90.0 ± 5.0	103.2 ± 9.5
11	102.9 ± 3.1	119 ± 18.1
12	99.9 ± 3.6	101 ± 15.3
13	94.6 ± 2.4	167 ± 19.7

Table 19

Mean weekly haematological indices during the latter part of the experiment of the calves in Group 4 which only received the initial infection of 750 metacercariae of F. hepatica.

Weeks after infection	Mean erythrocyte count (million/cu.mm.)	Mean packed cell volume (%)	Mean haemoglobin concentration (gms./100 ml)	Mean corpuscular volume (cu. microns)	Mean corpuscular haemoglobin concentration (%)
17	7.51 ± .33	32.2 ± 1.14	10.8 ± .41	43.0 ± 1.04	33.6 ± .48
18	6.99 ± .36	30.7 ± 1.30	10.6 ± .49	44.2 ± 2.13	34.4 ± .24
19	7.33 ± .28	31.2 ± .75	10.5 ± .24	42.7 ± 1.19	33.8 ± .24
20	7.32 ± .37	31.5 ± 1.67	10.7 ± .48	43.0 ± 0.71	34.2 ± .50
21	7.57 ± .12	32.7 ± 1.09	10.9 ± .32	43.2 ± 1.55	33.5 ± .58
22	7.03 ± .27	30.7 ± .90	10.3 ± .26	43.8 ± 1.25	33.8 ± .29
23	7.09 ± .38	32.5 ± 1.64	11.1 ± .32	46.0 ± 1.92	34.2 ± .90
24	7.18 ± .30	31.5 ± 1.14	10.6 ± .51	43.9 ± 0.73	34.2 ± .41
25	6.78 ± .31	30.5 ± 1.34	10.2 ± .37	45.7 ± 1.11	33.6 ± .43
26	6.99 ± .30	30.7 ± 1.64	10.5 ± .47	44.0 ± 1.31	34.1 ± .48
27	6.89 ± .25	30.5 ± 0.56	10.3 ± .10	44.4 ± 1.49	33.8 ± .75
28	6.52 ± .25	29.7 ± .75	10.6 ± .19	45.7 ± 1.22	36.3 ± .71
29	6.26 ± .27	30.2 ± .90	10.8 ± .22	48.5 ± 1.33	35.7 ± .72
30	6.65 ± .30	29.7 ± .90	10.9 ± .37	45.0 ± 2.35	36.6 ± .61

Table 20

Mean weekly values of serum biochemistry during the latter part of the experiment of the four calves in Group four which only received the initial infection of 750 metacercariae of *F. hepatica*.

Weeks after infection	Mean serum albumin level (gms/100 ml)	Mean total serum globuline (gms./100 ml)	Mean S.G.O.T. levels (S.-F. units)
17	2.10 ± .09	4.42 ± .15	61.2 ± 6.8
18	2.26 ± .09	4.38 ± .14	55.5 ± 3.9
19	2.16 ± .02	4.47 ± .16	60.5 ± 3.9
20	2.38 ± .05	4.55 ± .26	65.5 ± 6.7
21	2.35 ± .04	4.92 ± .27	60.5 ± 3.9
22	2.23 ± .02	4.75 ± .18	65.0 ± 4.4
23	2.16 ± .07	4.96 ± .28	54.0 ± 2.9
24	2.19 ± .11	4.88 ± .21	72.5 ± 5.9
25	2.29 ± .05	4.53 ± .21	77.2 ± 2.0
26	2.32 ± .03	4.30 ± .15	76.7 ± 4.8
27	2.15 ± .05	4.33 ± .17	78.7 ± 5.1
28	2.28 ± .05	4.36 ± .13	84.5 ± 5.8
29	2.31 ± .03	4.29 ± .14	105.2 ± 12.1
30	2.25 ± .05	4.18 ± .14	103.0 ± 15.2

Table 21

Mean weekly weights of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly weights of the four calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean weight of 4 reinfected calves (lbs.)	Mean weight of 4 treated and reinfected calves (lbs.)
0	460 ± 26	410 ± 34
1	481 ± 22	451 ± 18
2	497 ± 24	465 ± 17
3	501 ± 26	461 ± 22
4	529 ± 24	491 ± 17
5	550 ± 24	503 ± 20
6	556 ± 30	519 ± 19
7	579 ± 30	537 ± 19
8	600 ± 26	563 ± 16
9	599 ± 26	549 ± 17
10	616 ± 32	564 ± 17
11	633 ± 32	580 ± 23
12	626 ± 26	577 ± 15
13	652 ± 22	591 ± 11

Table 22

Mean weekly erythrocyte counts of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly erythrocyte counts of the four calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean R.b.c. count of 4 reinfected calves (millions/cu.mm.)	Mean R.b.c. count of 4 treated and reinfected calves (millions/cu.mm.)
0	6.98 ± .21	7.34 ± .51
1	7.30 ± .19	7.24 ± .52
2	6.85 ± .32	6.07 ± .86
3	7.30 ± .32	6.98 ± .64
4	6.78 ± .31	6.58 ± .52
5	6.84 ± .33	6.86 ± .59
6.	6.78 ± .48	6.75 ± .44
7	6.73 ± .28	6.77 ± .35
8	6.63 ± .35	6.56 ± .42
9	6.76 ± .47	6.76 ± .38
10	6.53 ± .32	6.31 ± .28
11	6.51 ± .38	6.31 ± .11
12	6.33 ± .38	5.74 ± .28
13	6.31 ± .34	5.98 ± .17

Table 23

Mean weekly packed cell volumes of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly erythrocyte count of the four calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean p.c.v. of 4 reinfected calves (%)	Mean p.c.v. of 4 treated and reinfected calves (%)
0	34.5 ± 1.34	34.0 ± 1.22
1	35.2 ± .66	34.2 ± 1.25
2	33.7 ± 1.25	28.7 ± 3.15
3	35.5 ± .02	32.0 ± 1.50
4	32.2 ± .83	31.0 ± 1.2
5	33.2 ± .90	32.0 ± 1.27
6	34.0 ± 2.0	32.2 ± 1.20
7	33.7 ± 1.30	30.5 ± 1.09
8	31.2 ± 1.48	30.7 ± 1.30
9	33.0 ± 1.71	32.5 ± .56
10	31.5 ± 1.41	29.5 ± 1.14
11	31.2 ± 1.98	31.0 ± 1.50
12	31.7 ± 1.48	29.7 ± .83
13	29.7 ± 1.63	29.7 ± 1.56

Table 24

Mean weekly haemoglobin concentration of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly haemoglobin concentration of the four calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean haemoglobin concentration of 4 reinfected calves (gms./100 ml.)	Mean haemoglobin concentration of 4 treated and reinfected calves (gms./100 ml.)
0	11.7 ± .36	11.2 ± .30
1	11.7 ± .10	11.3 ± .38
2	11.1 ± .30	9.5 ± 1.05
3	12.2 ± .28	10.7 ± .38
4	11.1 ± .36	10.4 ± .36
5	11.0 ± .47	10.7 ± .30
6	11.3 ± .45	10.8 ± .23
7	11.2 ± .35	10.3 ± .24
8	10.2 ± .41	10.0 ± .38
9	10.9 ± .60	11.0 ± .13
10	10.3 ± .46	10.1 ± .40
11	10.7 ± .67	10.9 ± .42
12	10.9 ± .50	10.6 ± .36
13	10.9 ±.63	11.0 ± .59

Table 25

Mean weekly mean corpuscular volumes of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly mean corpuscular volumes of the 4 calves in Group 5 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean M.C.V of 4 reinfected calves (cu. microns)	Mean M.C.V. of 4 treated and reinfected calves (cu. microns)
0	49.4 ± 1.52	46.8 ± 2.11
1	48.4 ± 1.87	47.9 ± 1.35
2	49.5 ± 1.99	48.2 ± 1.92
3	49.0 ± 2.00	46.6 ± 2.53
4	47.8 ± 1.60	47.7 ± 2.19
5	48.9 ± 2.07	47.4 ± 2.14
6	50.4 ± 2.32	48.2 ± 2.12
7	50.2 ± 1.18	45.2 ± .88
8	47.3 ± 1.77	47.2 ± 1.91
9	49.5 ± 1.87	48.5 ± 2.08
10	48.4 ± 1.63	48.2 ± 2.35
11	48.0 ± 1.54	49.0 ± 1.93
12	50.3 ± .82	52.4 ± 3.51
13	45.5 ± 1.71	49.7 ± 2.55

Table 26

Mean weekly mean corpuscular haemoglobin concentration of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly mean corpuscular haemoglobin concentration of the 4 calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean M.C.H.C. of 4 reinfected calves (%)	Mean M.C.H.C. of 4 treated and reinfected calves (%)
0	33.9 ± .43	33.1 ± .38
1	33.1 ± .51	32.9 ± .46
2	33.1 ± .50	33.0 ± .52
3	34.4 ± .56	33.4 ± .67
4	34.4 ± .53	33.7 ± .28
5	33.0 ± .77	33.5 ± .42
6	33.5 ± 1.28	33.5 ± .78
7	33.3 ± .42	33.9 ± .64
8	32.7 ± .49	32.7 ± .20
9	32.8 ± .42	33.9 ± .77
10	32.8 ± .29	32.9 ± .70
11	34.2 ± .40	34.9 ± .63
12	34.3 ± .30	35.8 ± .35
13	38.2 ± 1.49	37.0 ± .75

Table 27

Mean weekly serum albumin level of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly serum albumin level of the 4 calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean serum albumin level of 4 reinfected calves (gms./100 ml.)	Mean serum albumin level of 4 treated and reinfected calves (gms./100 ml.)
0	1.83 ± .06	1.90 ± .09
1	2.16 ± .05	2.13 ± .07
2	2.16 ± .04	2.13 ± .09
3	2.20 ± .07	2.31 ± .04
4	2.10 ± .04	2.22 ± .06
5	2.42 ± .06	2.32 ± .03
6	2.28 ± .09	2.40 ± .07
7	2.10 ± .04	2.39 ± .02
8	2.26 ± .05	2.33 ± .07
9	2.16 ± .07	2.27 ± .07
10	2.16 ± .02	2.26 ± .06
11	2.39 ± .03	2.39 ± .08
12	2.24 ± .03	2.25 ± .05
13	2.20 ± .09	2.25 ± .05

Table 28

Mean weekly total serum globulins of the 4 calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly total serum globulins of the four calves in Group 2 following their treatment with nitroxynil and subsequent reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean total serum globulins of 4 reinfected calves (gms./100 ml.)	Mean total serum globulins of 4 treated and reinfected calves (gms./100 ml.)
0	4.87 ± .11	5.14 ± .16
1	4.74 ± .19	4.69 ± .15
2	4.57 ± .19	4.64 ± .20
3	4.77 ± .25	4.80 ± .23
4	4.74 ± .18	4.93 ± .12
5	4.88 ± .22	4.83 ± .11
6	4.89 ± .22	4.83 ± .11
7	5.07 ± .19	4.66 ± .07
8	4.56 ± .24	4.36 ± .11
9	4.56 ± .26	4.42 ± .13
10	4.51 ± .22	4.34 ± .26
11	4.25 ± .24	4.50 ± .25
12	4.38 ± .13	4.40 ± .09
13	4.42 ± .19	4.32 ± .17

Table 29

Mean weekly serum glutamic-oxaloacetic transaninase levels of the four calves in Group 1 following their reinfection with 1,650 metacercariae of F. hepatica and the mean weekly serum glutamic-dehydrogenase levels of the four calves in Group 2 following their treatment with nitroxnil and subsequnet reinfection with 1,650 metacercariae of F. hepatica.

Weeks after reinfection	Mean S.G.O.T. levels of 4 reinfected calves (S.-F. units)	Mean S.G.O.T. levels of 4 treated and reinfected calves (S.-F. units)
0	70.5 ± 7.3	53.5 ± 4.1
1	71 ± 7.0	58.0 ± 2.6
2	65 ± 5.7	49.7 ± 1.6
3	73 ± 8.2	74.2 ± 16.0
4	61.5 ± 5.4	55.7 ± 2.9
5	74.7 ± 11.0	59.2 ± 2.4
6	63.2 ± 6.5	68.7 ± 11.5
7	66.2 ± 5.9	61.5 ± 4.7
8	83.2 ± 2.7	93.7 ± 8.1
9	95.0 ± 7.2	97.2 ± 8.9
10	85.7 ± 4.1	94.2 ± 13.2
11	106.0 ± 6.6	99.7 ± 5.5
12	91.7 ± 4.6	108.0 ± 7.0
13	93.2 ± 3.2	96 ± 5.3

Table 30

Number and size of Fluke recovered from individual calves in Experiment 1.

Calf No.	Flukes recovered			Total
	> 12 m.	6-12 m.	< 6 m.	
1	10	7	-	17
2	8	8	1	17
3	45	1	-	46
4				
5	5	3	4	12
6	19	8	-	27
7	12	14	3	29
8	2	4	-	6
9	60	2	6	68
10	105	6	5	116
11	175	7	6	188
12	89	4	7	100
13	37	-	-	37
14	6	1	-	7
15	6	3	1	10
16	21	-	-	21
17	45	28	2	75
18	79	30	22	131
19	35	21	4	60
20	185	89	4	278

Table 31

Individual weekly weights (lbs.) of calves during Experiment 1.

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	257	253	261	281	294	305	317	337	361	386	412	413	432	452	460
2	218	221	220	243	250	270	284	294	325	330	346	355	385	412	416
3	208	208	228	249	257	282	290	312	333	342	344	365	375	414	418
4	180	188	196	218	224	252	263	274	296	304	312	320	336	340	356
5	231	249	249	268	282	291	308	315	345	348	347	362	368	392	409
6	230	242	250	268	274	291	311	334	353	367	394	404	408	431	452
7	206	211	214	244	244	262	270	283	310	322	342	358	370	372	379
8	169	173	186	200	206	220	225	245	269	269	286	300	312	322	338
9	206	204	221	234	240	256	276	309	340	338	358	389	385	412	420
10	237	233	242	262	276	290	310	335	351	359	365	390	383	404	426
11	200	200	212	235	242	260	277	292	317	334	340	359	383	408	416
12	172	172	176	200	200	210	224	247	263	266	278	297	296	312	305
13	232	245	225	271	284	291	312	331	357	376	398	418	430	458	478
14	228	233	236	261	271	276	283	303	315	335	341	398	356	378	389
15	200	200	205	229	234	250	264	281	300	300	315	323	348	364	389
16	192	198	211	240	250	264	288	312	337	348	360	375	400	426	440
17	272	272	297	316	324	336	357	388	409	419	401	457	478	500	513
18	214	206	223	244	244	253	276	300	316	324	347	364	380	406	412
19	174	174	182	200	211	220	240	254	272	269	290	310	328	354	372
20	272	282	295	325	330	342	358	380	404	420	430	458	474	500	512

Group 1 = calves 1-4

Group 2 = calves 5-8

Group 3 = calves 9-12

Group 4 = calves 13-16

Group 5 = calves 17-20

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
480	491	538	544	572	575	600	622	640	665	670	672	692	724	674	732
446	465	468	504	505	500	540	570	575	590	620	620	645	650	670	686
413	416	440	452	470	500	512	520	530	560	586	576	608	618	612	624
372	387	396	426	440	430	465	490	480	500	525	530	520	540	550	566
420	428	442	466	470	450	490	490	510	520	530	540	555	560	570	558
468	479	496	504	520	534	545	568	581	598	618	606	617	650	620	655
319	323	312	416	440	450	478	495	510	534	554	540	566	584	582	606
353	364	392	418	430	410	450	460	475	495	550	510	520	525	535	546
441	450														
449	454														
426	454														
322	330														
500	518	532	542	562	589	602	614	624	658	664	669	700	705	696	710
405	415	450	438	460	455	500	525	535	545	555	545	565	565	575	591
406	423	426	446	453	450	500	510	520	545	540	535	540	555	565	576
456	462	496	496	520	517	548	566	576	595	622	608	639	654	662	674
528	549	556	572	570	583	602	628	634	650	678	664	684	698	700	700
432	448	462	476	510	490	550	520	510	510	525	525	540	545	565	595
394	411	414	434	450	445	480	500	498	520	555	545	555	560	580	590
534	552	580	586	600	610	620	642	656	682	708	700	688	716	724	740

Table 32

Individual weekly erythrocyte counts (millions/cu. mm.) of calves during

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	8.33	6.58	6.77	7.14	6.72	7.03	6.92	7.35	7.62	7.89	7.83	7.14	7.46	7.75	8.36
2	7.16	7.07	6.59	7.12	6.61	6.99	6.76	6.88	7.04	6.25	6.59	6.29	6.80	6.50	6.15
3	7.59	8.19	7.66	8.01	7.84	8.03	8.54	8.73	8.46	7.86	8.01	7.85	8.41	7.90	7.73
4	7.31	7.46	7.10	7.65	7.13	7.00	7.24	7.64	7.99	7.50	7.44	6.90	6.90	7.45	6.90
5	7.80	7.68	7.47	7.70	7.38	7.82	7.31	7.59	7.73	7.62	7.30	7.75	7.25	7.57	7.53
6	7.01	7.41	7.09	7.68	7.03	7.17	7.01	7.24	7.10	7.23	7.15	6.89	7.08	7.21	6.51
7	7.18	7.52	7.32	7.96	8.02	7.57	7.19	7.86	7.83	8.05	8.55	8.67	9.08	8.46	7.49
8	6.32	6.67	6.15	5.99	6.05	6.20	5.92	5.99	6.09	6.32	6.06	5.91	5.44	6.23	7.91
9	7.45	7.92	7.86	8.00	8.18	7.33	7.42	7.78	8.15	8.67	8.05	8.12	8.27	7.95	6.93
10	7.67	6.57	6.51	6.67	6.34	6.52	6.35	6.34	6.87	6.53	6.35	6.10	6.22	6.60	6.93
11	7.67	6.61	7.19	7.31	7.01	7.60	7.41	7.64	7.88	7.52	7.91	7.48	7.27	7.65	7.01
12	7.01	7.10	6.62	6.83	6.44	6.74	6.54	6.91	7.64	6.49	6.57	6.40	6.56	6.74	7.52
13	7.35	7.04	7.30	7.38	7.31	7.43	6.89	7.05	6.92	6.78	6.98	7.06	7.21	7.20	7.32
14	6.49	7.30	7.77	7.96	6.98	7.61	7.45	7.42	8.05	7.29	7.46	8.51	6.80	7.22	7.31
15	8.40	8.45	7.98	8.15	8.29	7.92	7.78	8.21	8.89	9.06	8.11	8.52	8.52	8.58	6.94
16	8.76	7.98	7.86	8.12	7.91	8.06	7.92	7.88	8.57	8.06	7.83	8.30	8.17	8.31	6.63
17	8.29	7.48	7.69	7.82	7.15	7.83	7.69	7.74	7.91	7.06	7.94	7.35	7.40	7.43	7.42
18	6.82	6.88	6.60	6.22	6.25	6.37	6.42	6.69	6.63	6.67	6.78	6.51	7.40	6.24	6.70
19	6.85	7.37	6.88	7.07	6.94	7.16	6.79	6.59	7.21	7.03	6.50	5.99	7.11	6.71	6.31
20	6.85	6.69	6.71	6.87	7.22	7.67	7.26	7.56	7.13	7.79	7.50	7.16	7.75	8.01	7.46

Group 1 = calves 1-4 Group 4 = calves 13-16

Group 2 = calves 5-8 Group 5 = calves 17-20

Group 3 = calves 9-12

Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
7.73	8.08	7.58	7.86	7.46	7.17	6.74	7.10	7.32	6.51	6.56	6.27	6.17	5.70	5.10	5.71
6.45	6.44	6.39	6.78	5.78	6.31	5.84	5.88	5.38	6.16	5.89	5.71	5.94	6.38	6.31	6.07
6.08	7.00	6.91	7.22	7.14	8.03	7.61	7.71	7.95	7.66	7.78	8.28	7.63	7.76	7.14	7.47
7.14	7.35	7.05	7.34	7.03	7.69	6.93	6.68	6.49	6.59	6.31	6.78	6.39	6.20	6.76	5.99
7.05	7.76	7.70	7.28	3.78	6.54	5.77	5.76	5.74	6.57	6.28	6.60	6.16	6.13	6.55	6.41
6.78	6.85	6.98	7.01	6.81	6.56	6.45	7.19	7.06	6.92	7.49	6.51	5.67	6.08	5.52	5.75
6.84	8.11	8.75	8.87	8.43	9.12	8.33	8.65	8.05	7.77	7.17	8.01	7.21	6.43	6.66	5.94
5.73	5.33	5.93	5.79	5.28	5.70	5.77	5.83	6.17	6.84	5.32	5.94	6.21	6.62	5.14	5.84
7.04	7.11														
6.52	6.82														
7.21	8.08														
6.92	8.02														
6.97	6.53	6.95	6.20	6.64	6.27	7.47	6.46	6.67	6.49	6.28	6.45	6.74	5.82	5.34	5.68
7.22	7.33	7.41	6.53	7.01	7.30	7.53	6.54	6.06	6.71	6.45	6.33	6.14	6.31	6.46	6.63
7.59	7.56	8.62	8.13	8.14	8.39	7.99	7.50	7.75	7.96	7.34	7.70	7.39	7.19	6.56	7.16
7.06	6.36	7.07	7.09	7.54	7.32	7.31	7.64	7.89	7.58	7.07	7.50	7.30	6.78	6.68	7.15
6.72	7.34	7.45	6.93	6.81	7.43	7.87	7.56	7.42	7.40	7.29	7.56	6.84	6.48	5.87	6.40
6.43	6.51	7.13	6.81	6.53	6.58	6.59	4.41	5.21	5.59	5.69	5.73	6.22	6.71	6.23	6.25
6.34	6.41	6.58	6.10	6.06	5.86	5.92	5.85	5.50	5.53	5.53	5.59	5.32	4.85	5.00	5.33
7.20	7.22	6.90	7.27	6.50	6.47	6.4	6.17	7.19	6.64	6.49	6.33	5.55	5.51	5.00	5.42

Table 33

Individual weekly packed cell volume (%) of calves during Experiment 1.

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	35	30	32	30	30.5	33	30	33	34	34	33	32	32	34	40
2	35	34	31.5	33	32.5	34	32	33	35	31	32	30	33	33	29
3	34	34	33	33	32.5	33	35	35	37	35	33	34	35	35	32
4	35.5	34	31.5	33	30.5	31	30	36	34	33	32	29	29	32	29
5	34.5	33	32	32	31.5	33	31	32	33	32	33	34	30	31	32
6	34	34.5	35	33.5	33	33	32	32	31	33	34	32	31	30	33
7	30	30	30.5	30.5	32	32	31	31	32	34	35	36	36	34	34
8	30.5	31	30	29	29	31	29	30	31	32	30	29	27	30	31
9	32	33	34.5	32	34	33	29	33	33	35	34	34	34	32	34
10	32	31	33	32	31	33	30	32	32	31	33	32	32	33	36
11	33	30.5	31	30.5	30	32	29	31	33	32	33	30	28	31	28
12	33	33	30.5	30	29	30	30	29	30	29	29	29	29	30	32
13	33	32	33.5	32	30.5	33	30	30	31	31	33	32	33	32	34
14	32.5	31	33	30.5	31	32	29	29	33	29	31	35	28	29	33
15	35	35	33.5	32.5	34	34	30	34	35	35	35	35	33	34	34
16	37	33	35	33.5	33	35	31	33	36	33	33	33	34	35	33
17	36	33	34	32	30	34	31	32	34	30	31	30	30	30	33
18	32	33	33	27.5	30	31	33	33	33	32	34	32	32	29	35
19	30	33	30.5	31	30	29	29	29	32	30	28	27	29	30	29
20	32.5	31.5	33	31.5	32	35	32	33	33	34	33	32	31	35	31

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
40	40	39	37	38	35	32	33	37	33	28	30	29	26	27	27
33	36	34	36	32	35	31	33	31	33	31	32	32	34	32	30
30	31	33	34	33	36	35	36	39	38	36	39	36	36	35	35
33	34	32	34	32	36	31	31	29	31	30	32	29	29	33	27
33	35	34	34	19	29	30	29	31	31	32	33	32	30	31	32
32	32	32	34	32	31	29	34	35	31	34	31	26	30	31	29
33	36	38	38	36	37	35	35	34	33	30	34	29	28	27	25
30	30	32	31	28	31	30	30	29	27	27	32	31	36	30	33
31	29														
34	35														
33	34														
34	34														
33	30	32	32	31	27	34	31	35	30	31	30	32	28	28	30
33	31	30	27	29	30	29	28	27	29	26	26	29	29	30	27
35	34	36	34	33	36	34	31	33	35	33	32	30	30	30	32
34	32	31	30	32	33	34	33	35	32	32	35	31	32	33	30
31	30	31	30	30	32	34	33	35	34	33	33	32	30	32	32
32	33	35	35	33	34	33	24	30	29	33	32	34	36	34	33
30	30	30	28	29	29	28	26	26	26	26	26	26	24	29	27
32	31	30	32	30	30	28	28	34	31	33	29	24	28	31	29

Table 34

Individual weekly haemoglobin levels (gms./100 ml.) of calves during

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	11.7	9.3	9.8	11.2	9.8	10.9	10.3	11.2	11.2	11.7	11.2	10.7	11.8	12.0	13.3
2	11.4	10.9	10.1	10.9	10.1	11.5	11.0	11.5	12.0	11.3	12.0	10.7	12.2	11.6	10.4
3	11.5	11.3	11.2	12.2	11.1	11.9	12.3	12.5	12.9	12.2	12.1	11.9	12.6	12.4	11.9
4	12.1	11.5	11.0	12.0	11.0	10.9	10.5	13.0	12.4	12.3	11.7	12.0	10.6	11.9	10.9
5	11.4	11.1	10.6	11.5	11.3	11.7	11.2	12.0	11.4	11.5	11.4	11.4	10.0	11.8	11.0
6	11.2	11.6	11.2	11.2	11.4	11.4	11.2	11.6	11.4	12.1	11.4	11.0	11.5	11.7	11.6
7	9.7	10.1	9.9	10.5	10.6	10.5	10.1	10.9	10.7	11.2	11.7	11.6	13.0	12.0	11.3
8	9.8	10.3	9.9	10.2	9.8	10.7	10.2	10.9	10.7	11.3	10.9	10.4	10	11.3	10.6
9	11.0	11.6	12.0	11.45	12.6	11.6	11.4	12.3	12.3	13.4	12.6	11.9	12.6	12.3	12.2
10	11.3	10.7	11.0	11.2	10.9	11.1	11.0	11.7	11.9	11.4	11.4	10.7	11.3	12.6	12.1
11	11.1	10.0	10.2	10.9	10.6	11.4	10.6	11.2	11.6	11.3	11.5	11.1	10.4	11.9	10.4
12	10.8	10.2	10.1	10.7	9.8	10.6	9.8	10.6	11.0	10.3	9.7	9.4	10.7	10.9	11.1
13	11.6	10.9	11.2	10.8	10.7	11.0	10.7	11.1	11.0	11.3	11.4	11.2	11.6	11.7	12.0
14	10.7	10.5	10.4	10.6	10.6	10.8	11.0	10.9	11.9	10.9	11.0	11.9	12.3	10.9	11.5
15	11.5	11.7	10.7	11.45	11.6	12.0	11.2	12.0	11.9	12.5	12.2	11.8	11.4	12.2	12.6
16	12.1	11.1	11.0	11.1	11.3	11.9	11.4	11.9	11.9	11.3	11.0	10.5	11.9	12.6	12.1
17	12.3	11.6	11.6	11.1	10.9	11.9	11.5	11.5	11.7	10.9	11.7	10.4	10.1	11.6	11.6
18	11.4	11.6	10.9	11.2	10.5	11.1	11.5	12.5	12.2	11.8	12.1	11.5	11.9	11.5	12.3
19	10.4	11.3	10.6	10.8	11.0	11.0	10.7	10.9	11.4	11.3	10.4	9.9	10.6	11.4	10.1
20	11.1	10.3	10.3	10.8	11.5	11.7	11.1	12.2	11.9	12.2	11.5	11.3	11.9	13.0	10.5

Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
13.1	13	12.9	12.0	12.1	11.6	10.9	10.7	11.6	10.7	9	10.2	9.4	9	9.4	9.3
11.3	11.6	11.5	11.5	10.4	11.8	10.2	10.2	10.1	11.1	10.5	10.3	10.8	11.2	11	11.2
10.2	10.9	11.0	11.7	11.1	12.5	12.2	12.6	12.6	12.4	11.3	13.0	11.6	12.6	12.2	12.8
11.8	12.0	11.3	11.5	11.0	13	11.1	10.5	11	10.7	10.0	10.2	9.5	10.0	11	10.4
11.6	12.0	11.5	11.7	6.1	9.5	9.9	10.1	10.2	10.7	10.3	11.1	10.1	11.0	11.2	11.9
11.5	11.1	10.7	11.2	11.0	10.6	10	11.2	11.4	10.6	11.1	10.8	9.7	11	11	10.7
10.6	11.5	12.1	12.3	11.5	11.7	11.7	11.4	11.0	10.5	9.8	10.7	10.2	7.6	9.4	9.2
10.7	10.1	10.7	10.2	9.4	10.9	10.2	10.1	10.5	9.5	9	11.4	11.4	12	11	12.2
11.1	11														
12.0	12.7														
11.8	12.4														
11.6	12.4														
11.7	10.3	10.5	11.1	10.2	9.7	11.6	10.2	11.8	10	10.1	10.0	10.5	10	10.0	10.9
11.6	11	10.5	9.3	9.9	10.1	10	9.6	10	9.5	9.1	9.2	10.5	10.8	11.1	9.7
11.6	12.1	12.2	11.9	11.1	12.2	11.5	10.6	11.3	12.2	11	11.1	10.2	10.8	11.0	11.4
11.6	11	10.1	10.1	10.9	11	10.7	11	11.2	10.7	10.8	11.6	10	11.0	11.0	11.6
10.9	10	10.6	9.8	10.2	11	11.7	12.2	11.4	11.5	10.5	11.0	10.8	10.4	11.0	11.4
11.5	11.3	12.1	11.4	11	11.1	11.6	8.1	10.3	10.1	10.8	10.8	11	12	12.0	12.8
10.7	10.6	10.2	9.7	9.6	9.7	9.7	9.6	9.3	9.5	9	9.8	9.8	9	10.9	10.3
11.7	10.9	10.5	11.2	10	10.1	9.7	9.3	11.2	9.0	10.1	10	8.6	9.8	10	10.6

Table 35

Individual mean corpuscular volumes (cu. microns) of calves during

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	42	45.6	47.3	42.0	44.6	46.9	43.3	44.9	44.6	43.1	42.1	44.8	42.9	43.9	47.8
2	48.9	48.1	47	46.3	48.4	48.6	47.3	48	49.7	49.6	48.6	47.7	48.5	50.8	47.1
3	44.8	41.5	43.1	41.2	40.8	41.1	41	40.1	43.7	44.5	41.2	43.3	41.6	44.3	41.4
4	47.9	45.6	43.7	43.1	42.1	44.3	41.4	47.1	42.5	44	43	42	42	42.9	42
5	43.6	43	41.5	41.6	42	42.2	42.4	42.2	42.7	42.0	45.2	43.9	41.4	40.9	42.5
6	48.5	45.9	49.4	43	46.9	46	45.6	44.2	43.7	45.6	47.5	46.4	43.8	41.6	50.7
7	41.8	39.9	41	37.7	39.9	42.3	43.1	39.4	40.9	42.2	40.9	41.5	39.6	40.2	45.4
8	47.5	46.5	48.8	48.4	47.9	50	49	50.1	50.9	50.6	49.5	49.1	49.6	48.1	39.2
9	42.9	41.7	43.2	40	41.6	40.9	39.1	42.4	40.4	40.4	42.2	41.9	41.4	40.2	49.1
10	41.7	47.2	40.7	48	48.9	50.6	47.2	50.5	46.6	47.5	52	52.4	51.4	50	51.6
11	43	45.4	33.1	41	42.8	42.1	39.1	40.6	41.9	42.5	41.7	40.1	38.5	40.5	39.9
12	47.1	46.5	45.3	43.9	45	44.5	45.9	42	39.3	44.7	44.1	45.3	44.2	44.5	42.5
13	44.9	45.4	45.2	43.4	41.0	44.4	43.5	42.5	44.8	45.7	47.3	45.3	45.8	44.4	46.4
14	49.3	42.5	42.5	37.2	44.4	42	38.9	39.1	41	39.8	41.5	41.1	41.2	40.2	45.1
15	41.7	41.4	41.3	39.3	41.0	42.9	38.6	41.4	39.4	38.6	43.1	41.4	38.5	40.3	49
16	42.2	41.3	44.5	40.6	41.7	43.4	39.1	41.9	42	40.9	42.1	39.8	41.6	42.1	49.7
17	43.4	44.1	44.2	40.9	41.9	43.4	40.3	41.3	43	42.5	39.0	40.8	40.5	40.4	44.5
18	46.9	48	50	43.4	48	48.7	51.4	49.3	49.8	48.	50.1	49.1	43.2	46.5	52.2
19	44.8	44.8	43.6	43.8	43.2	40.5	42.7	44	44.4	42.7	43.1	45.1	40.8	44.7	46
20	46.7	46.3	49.2	45.1	44.3	45.6	44.1	43.6	43.3	43.6	44	44.7	40.0	43.7	41.5

Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
51.7	49.5	51.4	47	50.9	48.8	47.5	46.5	50.5	50.6	42.7	47.8	47	45.6	52.9	47.3
51.2	55.9	53.2	53.1	55.4	55.5	53.1	56.1	57.6	53.6	52.6	56	53.9	53.3	50.7	49.4
49.3	44.3	47.7	47.1	46.2	44.8	46	46.7	49	49.6	46.3	47.1	47.2	46.4	49	46.8
46.2	46.3	45.4	46.3	45.5	46.8	44.7	46.4	44.7	47	47.5	47.2	45.4	46.8	48.8	45.1
46.8	45.1	44.1	46.7	50.3	44.3	52	50.3	54	47.2	50.9	50	51.9	48.9	54.8	49.9
47.2	46.7	45.8	48.5	47	47.3	45	47.3	49.6	44.8	45.4	47.6	51	49.3	56.1	56.4
48.2	44.4	43.4	42.8	42.7	40.6	42	40.5	42.5	42.5	41.8	42.4	40.2	43.5	40.5	42.1
52.3	56.3	54	53.5	53	54.4	52	51.5	47	46.2	50.7	53.9	49.9	54.4	58.4	56.5
44	40.8														
52.1	51.3														
45.8	42.1														
49.1	42.4														
47.3	45.9	46	51.5	46.7	43.1	45.5	48	52.5	46.2	49.4	46.5	47.5	48.1	52.4	52.8
45.7	42.3	40.5	41.3	41.4	41.1	38.5	42.8	44.5	43.2	43.4	41.1	47.2	45.9	46.4	40.7
46.1	45	41.8	41.8	40.5	42.8	42.5	41.3	42.6	44	44.9	41.6	40.6	41.7	45.7	44.7
48.1	50.3	43.8	42.3	42.4	45.1	46.5	43.2	44.3	42.2	45.3	46.7	42.5	57.2	49.4	42
46.1	40.9	41.6	43.3	44	43.1	43.2	47.2	45.9	45.9	45.6	43.6	46.8	46.3	54.5	50
49.1	50.7	49.1	51.4	50.5	51.7	50.1	54.4	57.6	57.8	50	55.8	54.7	53.6	54.6	61.2
47.3	46.8	45.6	45.9	47.8	49.4	47.3	44.4	47.3	47	47.0	46.5	48.9	49.5	58	50.7
44.4	42.9	43.4	44	46.1	46.4	43.7	45.4	47.3	46.7	50.8	45.8	43.2	50.8	62	53.5

Table 36

Individual mean corpuscular haemoglobin levels (%) of calves during

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	33.4	34.1	30.6	37.3	32.7	33.0	34.3	33.9	32.9	34.4	33.9	33.4	36.9	35.2	33.2
2	32.6	32.1	32.6	33.3	31.6	33.8	34.4	34.8	34.3	36.4	36.6	35.7	37.0	35.1	35.9
3	33.8	33.2	33.9	37.0	34.7	36.1	35.1	35.7	34.9	34.9	36.7	35.0	36.0	35.4	37.2
4	34.6	33.8	35.5	36.3	36.7	35.2	35.0	36.1	36.5	37.3	36.6	41.4	36.5	37.2	37.6
5	33.5	33.6	33.1	35.9	36.4	33.9	36.1	37.5	34.5	35.9	34.5	33.5	33.3	38.1	34.4
6	32.9	34.1	32.0	33.9	34.5	33.9	35.0	36.2	36.8	36.7	33.5	34.4	37.1	39	35.1
7	32.3	33.7	33	35.0	33.1	31.6	31.6	35.2	33.4	32.9	33.4	32.2	36.1	35.3	33.2
8	32.7	33.2	33	35.2	33.8	32.9	35.1	36.3	34.5	35.3	36.3	35.9	37.0	37.7	34.2
9	34.4	35.1	35.3	35.6	37.1	34.5	39.3	37.3	37.3	38.2	37.1	35.0	37.1	37.3	35.9
10	35.3	34.5	33.3	35.0	35.2	33.3	36.7	36.6	37.2	32.6	34.5	33.4	35.3	38.2	33.6
11	33.6	33.3	32.9	36.3	35.3	33.1	36.5	36.1	35.1	35.3	34.8	37.0	37.1	38.4	37.1
12	32.7	30.9	33.7	35.7	33.8	32.7	32.7	36.5	36.7	35.5	33.4	32.4	36.9	36.3	34.7
13	35.1	34.1	33.9	33.7	35.7	32.4	35.7	33.3	35.5	36.4	34.5	35.0	35.1	36.6	35.3
14	33.4	33.9	31.5	35.3	34.2	34.4	37.9	37.6	36.1	37.5	35.5	34.0	36.8	37.5	34.8
15	32.9	33.4	32.4	35.6	34.1	32.9	37.3	35.3	35.0	35.7	34.8	33.7	34.5	35.9	37.0
16	32.7	33.6	31.4	33.6	34.2	32.6	36.8	36.1	33.0	34.2	33.3	31.8	35.0	36.0	36.7
17	34.2	36.1	34.1	34.7	36.3	33.8	37.1	35.9	34.4	33.0	37.7	34.7	33.7	38.7	35.1
18	35.6	35.1	33.0	41.5	35	37.1	34.8	37.9	37.0	36.9	35.6	35.9	37.2	39.6	35.1
19	34.7	34.2	35.3	34.8	36.7	36.9	36.9	37.6	35.6	37.7	37.1	36.7	36.5	38.0	34.8
20	34.7	33.2	31.2	32.7	35.9	31.7	34.7	37.0	36.1	35.9	34.8	35.3	38.4	37.1	33.9

Group 1 = calves 1-4 Group 4 = calves 13-16

Group 2 = calves 5-8 Group 5 = calves 17-20

Group 3 = calves 9-12

Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
32.7	32.5	33.1	32.4	31.8	33.1	34.1	32.4	31.3	32.4	32.1	34.0	32.4	34.6	34.8	34.4
34.2	32.2	33.8	31.9	32.5	33.7	32.9	30.9	32.6	33.6	33.9	32.2	33.7	32.9	34.4	37.3
34	35.2	33.3	34.4	33.6	34.7	34.9	35.0	32.3	32.6	31.4	33.3	32.2	35.0	34.8	36.6
35.7	35.3	35.3	33.8	34.4	36.1	35.8	33.9	37.9	34.5	33.3	31.9	32.8	34.5	33.3	38.5
35.1	34.3	33.8	34.4	32.1	32.7	33.0	34.8	32.9	34.5	32.2	33.6	31.6	35.4	36.1	37.2
35.9	34.7	33.4	32.9	34.4	34.2	34.5	32.9	32.6	34.2	32.6	34.8	33.5	36.7	35.5	36.9
32.1	31.9	31.8	32.4	31.9	31.6	33.4	32.6	32.3	31.8	32.7	31.5	31.7	34.3	34.8	36.8
35.7	33.7	33.4	31.9	33.6	35.2	34.0	33.7	36.2	35.2	33.3	35.6	35.0	33.3	36.7	37.0
35.8	37.9														
35.3	36.3														
35.7	36.5														
34.1	36.5														
35.4	34.3	32.8	34.7	32.9	35.9	34.1	32.9	33.7	33.3	32.6	33.3	32.8	35.7	35.7	36.3
35.1	35.5	35.0	34.4	34.1	33.7	34.5	34.3	37.0	35.1	35.0	35.4	36.2	38.3	37.0	35.9
33.1	35.6	33.9	35.0	33.6	33.9	33.8	34.2	34.2	34.9	33.3	34.7	34.0	36.7	36.7	35.6
34.1	34.4	32.6	33.7	34.1	33.3	31.5	33.3	32.0	33.4	33.7	33.1	32.3	34.4	33.3	38.7
35.2	33.3	32.6	32.7	34.	34.4	34.4	33.9	32.6	33.8	31.8	33.3	33.7	34.6	35.4	35.6
35.9	34.2	34.6	32.6	33.3	32.6	35.1	33.7	34.3	34.8	32.7	33.7	32.3	33.3	35.3	40.0
35.7	35.3	34.0	34.6	33.1	33.4	34.6	36.9	35.8	36.5	34.6	37.7	37.1	37.5	37.6	38.1
36.6	35.2	35.0	35.0	33.3	33.7	34.6	32.2	32.9	29.0	30.6	34.5	35.8	35.0	32.3	36.5

Table 37

Individual serum albumin levels (gms./100 ml.) of calves during

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	2.10	2.13	1.94	2.05	2.11	2.26	2.19	1.91	2.23	2.13	2.39	2.38	2.32	2.11	2.33
2	2.18	2.13	2.08	2.30	2.08	2.21	2.20	1.96	2.27	2.00	2.34	2.16	2.05	2.12	1.94
3	2.32	2.78	2.23	2.38	2.36	2.62	2.40	2.38	2.40	2.17	2.37	2.41	2.37	2.31	2.39
4	2.10	2.15	2.05	2.17	2.09	2.52	2.23	2.63	2.30	2.25	2.41	2.29	2.43	2.22	2.25
5	2.06	2.01	1.95	2.02	1.91	2.32	2.00	2.18	1.85	2.04	1.96	2.31	2.00	1.99	2.05
6	2.20	2.00	1.91	2.02	1.82	2.11	2.00	2.06	2.17	1.98	2.15	2.20	2.07	2.05	2.23
7	1.96	2.20	2.15	2.58	2.21	2.19	2.24	2.11	2.24	1.96	2.16	2.26	2.28	1.86	2.03
8	2.16	2.16	2.12	2.53	2.09	2.36	2.30	2.27	2.28	2.01	2.23	2.36	2.32	2.20	2.25
9	2.15	2.34	2.34	2.16	1.96	2.05	2.19	2.24	2.28	1.99	2.20	2.51	2.48	2.32	2.11
10	2.20	1.96	2.44	2.21	1.96	2.35	2.21	2.33	2.45	2.26	2.14	2.31	2.25	2.02	2.29
11	2.00	1.79	1.84	1.96	1.91	2.19	2.13	2.15	2.13	2.18	2.20	2.31	2.23	2.03	2.12
12	2.30	2.53	2.22	1.99	2.42	2.17	2.58	2.32	2.41	2.13	2.62	2.49	2.61	2.41	2.21
13	2.27	2.01	2.30	2.25	1.91	2.01	2.25	2.07	2.35	2.06	2.14	2.54	2.43	2.19	2.17
14	2.45	2.10	2.34	2.27	1.99	2.20	2.61	2.03	2.47	2.13	2.08	2.15	2.20	2.24	2.01
15	2.32	2.62	2.15	2.45	2.31	2.60	2.35	2.35	2.31	2.29	2.36	2.45	2.19	2.08	2.26
16	2.19	2.09	2.03	2.12	2.25	2.11	2.21	2.26	2.32	2.09	2.12	2.37	2.33	2.17	2.33
17	2.42	2.51	2.30	2.33	1.93	2.41	2.38	2.30	2.27	1.96	2.20	2.51	2.36	2.25	2.48
18	2.10	2.12	2.23	2.13	1.97	2.22	2.50	2.31	2.08	2.09	2.12	2.30	2.38	2.09	2.42
19	2.25	2.12	2.35	2.10	2.09	2.22	2.11	2.33	2.18	2.10	1.83	2.15	2.49	2.26	2.44
20	2.36	2.11	2.34	2.41	2.02	2.04	2.51	2.23	2.33	2.27	2.34	2.54	2.51	2.40	2.60

Group 1 = calves 1-4

Group 2 = calves 5-8

Group 3 = calves 9-12

Group 4 = calves 13-16

Group 5 = calves 17-20

Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2.10	2.08	2.07	2.18	2.26	2.24	2.10	2.36	2.60	2.23	2.41	2.09	2.17	2.42	2.25	2.34
2.03	1.91	1.74	2.11	2.14	1.97	1.99	2.31	2.08	2.00	2.10	1.98	2.10	2.29	2.15	2.02
2.15	2.10	1.78	2.04	2.22	2.26	2.21	2.39	2.18	2.08	2.20	2.31	2.22	2.46	2.22	2.03
2.13	2.25	1.76	2.31	2.02	2.33	2.12	2.64	2.26	2.10	2.24	2.28	2.16	2.40	2.34	2.42
1.80	1.82	1.74	1.94	1.80	2.17	2.12	2.26	2.36	2.44	2.45	2.45	2.09	2.19	2.19	2.13
2.09	1.97	2.13	2.07	2.26	2.38	2.13	2.42	2.49	2.43	2.51	2.40	2.19	2.39	2.26	2.30
2.03	2.06	1.68	2.33	2.25	2.35	2.17	2.30	2.57	2.34	2.20	2.10	2.40	2.33	2.13	2.18
1.90	2.04	2.06	2.17	2.21	2.33	2.45	2.30	2.19	2.34	2.18	2.15	2.35	2.67	2.42	2.40
2.40	2.07														
2.27	2.19														
2.29	2.10														
2.38	2.41														
2.17	2.07	1.92	2.05	2.10	2.22	2.28	2.20	2.01	2.05	2.24	2.38	2.33	2.22	2.26	2.16
2.24	2.21	2.12	2.21	2.13	2.53	2.29	2.18	2.22	2.47	2.43	2.41	2.12	2.47	2.26	2.36
2.29	2.27	1.95	2.26	2.23	2.37	2.36	2.22	2.39	2.32	2.13	2.25	2.08	2.24	2.42	2.35
2.41	2.20	2.41	2.54	2.18	2.39	2.49	2.31	2.04	1.92	2.36	2.26	2.08	2.21	2.31	2.12
2.50	2.36	2.23	2.70	2.01	2.61	2.58	2.09	2.15	2.44	2.50	2.39	1.99	2.26	2.06	2.08
2.30	2.31	2.15	1.91	1.95	2.34	2.19	1.93	1.95	2.02	2.12	1.96	1.84	1.87	2.26	2.15
2.26	2.21	2.39	2.55	2.09	2.22	2.26	2.32	2.38	2.26	2.03	2.13	2.28	2.29	2.18	2.04
2.54	2.45	2.55	2.56	2.16	2.38	2.42	2.30	2.63	2.57	2.46	2.39	2.15	1.88	2.15	2.10

Table 38

Individual total serum globulin levels (gms./100 ml.) of calves during

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	4.20	3.65	4.05	4.15	3.96	3.93	3.95	3.88	3.79	4.17	4.21	4.30	4.27	4.98	5.17
2	3.62	3.76	3.71	3.49	3.51	3.52	3.39	4.05	3.83	3.90	3.75	4.13	4.11	4.19	4.66
3	3.63	3.20	3.87	3.72	3.74	3.37	3.69	3.81	4.17	4.27	4.52	4.59	4.93	4.90	5.11
4	3.90	3.54	3.63	3.81	3.81	3.48	4.04	4.19	4.29	3.95	4.58	4.20	5.16	5.14	5.54
5	3.94	3.99	4.27	3.98	4.05	4.27	3.36	4.22	3.84	4.55	5.14	4.68	5.00	5.31	5.25
6	4.60	4.49	4.08	4.07	4.27	3.98	3.72	3.94	4.03	3.92	4.04	4.09	3.93	4.81	4.40
7	3.04	3.59	3.95	4.36	3.80	3.81	3.98	4.08	4.25	4.73	4.73	4.94	4.92	5.04	5.27
8	3.14	4.13	4.28	3.66	4.35	4.05	3.99	3.93	4.23	4.59	4.36	4.24	4.18	4.80	4.66
9	3.85	3.83	4.25	4.17	4.84	4.44	4.06	3.99	4.50	4.06	4.49	4.36	4.44	4.77	4.99
10	4.30	4.07	4.35	4.48	4.32	4.45	4.31	4.26	4.45	4.43	4.65	4.78	5.25	5.38	5.51
11	4.20	3.71	3.94	3.48	4.28	3.88	3.63	3.54	4.36	4.50	4.79	4.58	4.45	4.67	4.67
12	3.70	3.77	3.77	4.20	3.77	4.02	3.76	3.94	4.08	4.25	3.97	4.01	4.28	5.19	5.78
13	3.93	3.96	3.98	4.00	4.08	4.33	3.65	3.92	3.84	3.84	4.16	4.15	4.39	4.70	4.81
14	3.94	4.09	3.75	4.22	4.20	4.08	3.65	3.61	3.72	3.76	4.06	3.55	4.00	4.05	4.68
15	3.88	3.57	3.45	3.79	3.98	3.90	3.65	3.94	4.39	4.81	4.73	5.14	4.40	5.02	5.04
16	4.21	3.90	4.26	3.87	3.94	4.29	3.57	3.83	4.18	4.10	4.67	4.21	4.27	4.02	4.29
17	3.37	3.08	3.28	3.17	3.56	3.18	3.07	3.13	3.55	3.19	3.39	2.98	3.14	3.26	3.42
18	4.10	4.47	4.46	3.65	3.96	3.79	3.60	3.98	3.72	4.00	3.97	3.70	3.41	3.51	3.70
19	3.75	3.93	4.00	4.09	3.91	3.78	3.53	3.52	4.01	4.24	4.54	4.04	4.01	3.84	3.85
20	3.54	4.07	4.05	3.77	4.08	4.25	3.72	3.76	3.87	3.82	3.85	3.55	3.72	3.81	3.79

Group 1 = calves 1-4

Group 2 = calves 5-8

Group 3 = calves 9-12

Group 4 = calves 13-16

Group 5 = calves 17-20

Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
5.00	4.62	4.52	4.41	4.14	4.16	4.28	4.45	4.81	4.77	3.99	3.91	4.12	3.68	3.95	3.86
4.67	4.88	5.01	4.80	4.46	4.73	4.62	4.79	4.71	4.90	5.00	4.82	4.80	4.21	4.65	4.89
4.65	5.10	5.12	5.36	5.20	5.54	5.30	5.62	5.62	5.73	5.11	5.29	5.08	5.03	4.57	4.67
5.27	5.65	4.84	4.39	4.49	4.67	4.76	4.66	4.43	4.89	4.16	4.22	4.04	4.10	4.36	4.28
5.66	5.08	5.25	4.85	5.32	5.48	5.28	5.17	4.75	4.86	4.45	4.05	4.31	4.40	4.21	4.17
4.71	4.62	4.87	4.64	4.54	4.40	4.67	4.87	4.91	4.67	4.49	4.29	4.21	4.31	4.44	4.20
5.50	4.93	5.62	5.07	4.23	4.34	4.73	4.60	5.14	4.45	4.20	4.60	3.70	3.97	4.27	4.01
4.93	5.06	4.83	4.22	4.48	4.97	5.04	4.80	4.52	4.67	4.32	4.75	5.15	5.33	4.68	4.90
4.48	4.63														
5.02	5.31														
4.77	4.89														
5.72	5.85														
4.92	4.77	4.29	4.76	4.69	4.88	5.72	5.00	5.40	5.09	5.06	4.52	4.77	4.68	4.44	4.34
4.46	4.59	4.58	4.18	4.08	4.06	4.31	4.33	4.58	4.62	4.07	3.89	3.88	4.12	4.14	3.84
4.70	4.82	4.83	4.54	4.88	5.22	5.14	5.18	5.61	5.47	4.86	4.64	4.52	4.55	4.68	4.55
4.39	3.99	3.99	4.06	4.22	4.03	4.51	4.49	4.26	4.36	4.14	4.14	4.14	4.09	4.09	3.98
3.50	3.54	3.77	3.40	3.69	3.29	3.70	4.13	3.75	3.96	4.00	3.71	3.81	3.84	4.05	4.22
3.59	3.60	3.85	3.44	3.85	3.85	4.01	4.27	5.14	5.27	5.28	5.36	5.27	5.63	5.64	5.55
3.94	3.29	3.99	3.44	4.01	4.21	4.16	4.47	4.42	4.74	4.67	4.27	4.31	4.71	4.68	4.85
3.75	3.55	3.96	3.74	3.76	3.71	3.89	4.10	4.62	4.54	4.24	4.31	3.95	4.41	4.44	4.60

Table 39

Individual weekly serum glutamatic-oxaloacetic transaminase levels

Calf No.	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	100	107	93	86	85	124	136	169	204	138	170	74	86	68
2	94	81	85	85	80	101	127	102	123	97	62	48	51	55
3	87	99	83	86	77	93	143	162	125	103	74	67	63	70
4	119	113	105	103	95	135	123	187	207	147	100	116	85	94
5	94	85	83	96	77	129	123	136	147	107	107	60	82	53
6	89	85	95	95	82	112	89	121	95	89	51	43	51	62
7	102	91	99	97	93	120	183	173	170	135	122	84	61	83
8	100	91	105	91	84	98	83	167	100	87	56	52	81	60
9	78	83	85	89	72	79	80	97	111	84	51	44	62	39
10	74	91	67	84	64	79	114	109	87	91	80	55	46	56
11	102	88	93	95	73	102	142	190	87	88	99	77	66	67
12	98	91	97	93	77	111	102	104	147	97	100	112	94	67
13	87	97	93	93	79	101	111	114	151	123	78	67	48	65
14	91	99	77	86	74	74	74	133	87	79	58	67	62	74
15	70	71	85	93	85	93	93	148	116	97	85	81	65	61
16	107	91	99	95	65	98	75	111	112	76	50	64	80	59
17	78	80	64	74	64	75	62	68	66	63	44	38	53	39
18	80	74	76	77	64	90	70	73	72	77	46	46	38	43
19	99	70	81	86	77	83	75	83	67	67	48	48	44	48
20	94	89	83	88	88	90	76	85	90	67	50	47	53	55

Group 1 = calves 1-4

Group 2 = calves 5-8

Group 3 = calves 9-12

Group 4 = calves 13-16

Group 5 = calves 17-20

(S.-F. Units) of calves during Experiment 1.

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
85	63	70	86	78	72	62	74	64	56	90	82	88	122	89	87
68	52	52	55	58	58	51	56	43	53	87	79	76	93	82	97
59	55	67	59	50	62	54	58	67	76	76	110	81	93	89	87
98	95	93	84	74	100	79	111	79	80	80	109	98	116	102	102
76	58	63	60	47	131	60	62	108	71	117	123	82	97	122	112
52	46	43	49	46	49	46	52	50	52	93	73	64	85	95	93
83	60	60	60	53	58	61	65	63	71	94	100	95	116	93	97
63	57	48	63	53	59	56	58	54	52	71	93	136	101	122	87
44	52														
38	51														
63	83														
68	67														
58	46	82	58	62	62	73	64	61	67	83	83	82	80	89	82
56	52	45	50	49	52	61	52	46	56	78	65	67	71	75	75
51	79	63	67	60	88	56	67	58	87	76	89	72	103	135	153
49	62	55	47	71	60	52	77	51	80	72	70	94	84	122	102
38	44	51	48	49	60	59	67	67	93	136	136	129	111	153	164
42	42	44	46	54	56	78	47	108	63	218	129	100	182	93	104
45	46	46	47	60	63	55	62	54	59	76	89	76	90	79	193
51	50	54	60	60	62	63	62	71	70	177	129	108	93	79	207

Table 40

Individual weekly faeces egg counts (eggs/gm.) of calves during Experiment 1.

Calf No.	Weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0	0	0	0	0	0	0	0	0	0	50	50	50	50
2	0	0	0	0	0	0	0	0	0	0	0	100	150	350	200
3	0	0	0	0	0	0	0	0	0	0	0	0	200	200	150
4	0	0	0	0	0	0	0	0	0	0	0	0	50	50	50
5	0	0	0	0	0	0	0	0	0	0	0	50	50	50	50
6	0	0	0	0	0	0	0	0	0	0	0	150	0	50	0
7	0	0	0	0	0	0	0	0	0	0	0	0	300	50	150
8	0	0	0	0	0	0	0	0	0	0	0	100	150	100	100
9	0	0	0	0	0	0	0	0	0	0	0	0	0	150	150
10	0	0	0	0	0	0	0	0	0	0	0	0	0	50	50
11	0	0	0	0	0	0	0	0	0	0	0	200	0	250	200
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150
13	0	0	0	0	0	0	0	0	0	0	0	50	50	50	50
14	0	0	0	0	0	0	0	0	0	0	0	100	0	50	100
15	0	0	0	0	0	0	0	0	0	0	0	0	0	150	100
16	0	0	0	0	0	0	0	0	0	0	0	50	0	0	50
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Group 1 = calves 1-4

Group 2 = calves 5-8

Group 3 = calves 9-12

Group 4 = calves 13-16

Group 5 = calves 17-20

15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0	150	100	0	0	0	0	0	0	0	0	0	0	0	0	0
200	300	200	50	100	50	0	0	0	0	0	0	0	0	0	0
50	150	150	50	100	100	50	100	50	0	50	0	0	0	0	0
50	300	100	150	100	50	50	100	0	0	0	50	0	0	0	0
150	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	50	0	0	0	0	0	0	0	0	0	0	0	50	100	0
50	250	0	0	0	0	0	0	0	0	0	0	0	0	50	0
150	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	100														
0	50														
150	200														
200	250														
0	50	0	50	50	0	0	0	0	0	0	0	0	0	0	0
0	0	50	50	0	50	50	0	50	0	0	50	0	0	0	0
300	300	0	100	150	0	0	50	0	0	0	0	0	0	0	0
100	50	150	100	100	50	0	0	0	0	0	100	50	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0

Table 41

Mean erythrocyte counts (R.b.c.), packed cell volumes (P.C.V.) haemoglobin concentrations (Hb) mean corpuscular volumes (M.C.V.) and mean corpuscular haemoglobin concentrations (M.C.H.C.) of calves following a single infection of 500 metacercariae of F. hepatica.

Weeks after infection	Number of calves	R.b.c. (million/cu.mm.)	P.C.V. (%)	Hb (gms/100 ml.)	M.C.V. (cu.microns)	M.C.H.C. (%)
0	24	8.59 ± .07	33.7 ± .37	12.2 ± .85	39.3 ± .59	36.2 ± .27
1	24	8.60 ± .14	33.0 ± .39	12.5 ± .11	38.5 ± .61	37.8 ± .31
2	24	8.18 ± .11	32.8 ± .44	12.1 ± .10	40.1 ± .39	36.9 ± .30
3	24	8.26 ± .13	31.9 ± .45	12.0 ± .11	38.2 ± .55	37.9 ± .42
4	24	8.56 ± .20	32.0 ± .61	12.0 ± .13	37.5 ± .55	37.7 ± .43
5	20	8.38 ± .18	32.7 ± .05	12.6 ± .22	39.3 ± .69	38.4 ± .40
6	20	8.23 ± .15	33.0 ± .41	12.5 ± .18	40.3 ± .80	38.0 ± .34
7	20	7.81 ± .15	31.2 ± .29	12.2 ± .17	40.2 ± .76	39.1 ± .31
8	20	8.01 ± .15	32.7 ± .35	12.7 ± .17	41.1 ± .68	38.8 ± .35
9	16	7.60 ± .14	31.4 ± .33	12.2 ± .12	41.5 ± .78	38.8 ± .31
10	16	7.60 ± .12	30.4 ± .37	12.1 ± .18	40.1 ± .61	39.7 ± .40
11	16	7.43 ± .12	30.2 ± .48	12.0 ± .20	40.8 ± .71	39.9 ± .37
12	16	7.23 ± .16	30.6 ± .50	11.6 ± .26	40.9 ± .80	38.0 ± .37
13	12	7.56 ± .07	31.6 ± .94	12.0 ± .27	41.7 ± .92	38.0 ± .49
14	12	7.77 ± .05	31.7 ± .66	12.4 ± .18	40.8 ± .88	39.3 ± .67
15	12	7.67 ± .06	30.5 ± .62	12.3 ± .26	39.8 ± .88	40.3 ± .54
16	12	7.71 ± .06	31.7 ± .69	12.0 ± .21	41.2 ± .83	37.9 ± .67
17	8	7.67 ± .13	32.2 ± .46	11.6 ± .11	41.5 ± .91	36.1 ± .62
18	8	7.49 ± .18	31.2 ± .34	11.8 ± .28	42.1 ± 1.05	37.8 ± .79
19	8	7.14 ± .19	30.2 ± .70	11.5 ± .22	42.5 ± 1.18	38.1 ± .60
20	8	7.28 ± .11	31.9 ± .37	12.1 ± .21	43.8 ± .80	38.3 ± .65
21	4	7.29 ± .18	32.7 ± 1.24	12.3 ± .60	44.8 ± .64	37.5 ± .45
22	4	7.09 ± .32	32.2 ± .54	11.8 ± .31	45.9 ± 1.61	36.9 ± .41
23	4	7.10 ± .29	32.2 ± .54	12.3 ± .34	45.6 ± 1.38	38.0 ± .83

Table 42

Mean serum albumin, total serum globulins, serum glutamatic-oxaloacetic transaminase (SGOT) levels of calves following their infection with 500 metacercariae of F. hepatica.

Weeks after infection	Number of calves	Serum albumin level (gms/100 ml.)	Total serum globulins level (gms/100 ml.)	SGOT level (S-F units)
0	24	2.41 ± .04	3.63 ± .07	68.9 ± 1.6
1	24	-	-	75.5 ± 1.9
2	24	2.18 ± .02	3.77 ± .07	100.2 ± 0.9
3	24	-	-	103.2 ± 3.3
4	24	2.20 ± .03	3.82 ± .07	92.9 ± 2.9
5	20	-	-	89.5 ± 2.9
6	20	2.15 ± .03	4.06 ± .07	100 ± 4.0
7	20	-	-	81.3 ± 2.4
8	20	2.22 ± .03	3.90 ± .05	102.1 ± 4.4
9	16	-	-	109.9 ± 5.8
10	16	2.11 ± .04	4.16 ± .05	115.6 ± 6.1
11	16	-	-	128.6 ± 7.2
12	16	2.15 ± .03	4.36 ± .06	138.7 ± 6.4
13	12	-	-	141.1 ± 4.60
14	12	2.23 ± .03	4.46 ± .08	128.2 ± 14.0
15	12	-	-	94.5 ± 6.3
16	12	2.17 ± .04	4.54 ± .07	117.6 ± 7.0
17	8	-	-	120.1 ± 4.7
18	8	2.11 ± .04	4.55 ± .07	119.7 ± 7.4
19	8	-	-	124.4 ± 7.8
20	8	2.46 ± .05	4.42 ± .08	120.0 ± 6.0
21	4	-	-	88 ± 11.6
22	4	2.41 ± .10	4.36 ± .07	101 ± 5.9
23	4	-	-	101 ± 12.8
24	4	2.42 ± .03	4.53 ± .19	94.5 ± 13.2

Table 43

Mean weekly weights of calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Number of calves	Mean bodyweight (lbs.)
0	24	285 ± 10
1	24	295 ± 10
2	24	309 ± 11
3	24	324 ± 11
4	24	340 ± 11
5	20	351 ± 12
6	20	265 ± 12
7	20	386 ± 12
8	20	411 ± 11
9	16	427 ± 15
10	16	460 ± 16
11	16	487 ± 16
12	16	497 ± 17
13	12	516 ± 20
14	12	531 ± 20
15	12	549 ± 19
16	12	561 ± 20
17	8	570 ± 80
18	8	593 ± 70
19	8	619 ± 25
20	8	626 ± 25
21	4	637 ± 41
22	4	645 ± 43
23	4	660 ± 40
24	4	676 ± 42

Table 44

Mean faeces egg counts of calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Number of calves	Number of calves passing fluke eggs	Mean faeces egg count (eggs/10gms faeces)
0	24	0	0
1	24	0	0
2	24	0	0
3	24	0	0
4	24	0	0
5	20	0	0
6	20	0	0
7	20	0	0
8	20	0	0
9	16	0	0
10	16	0	0
11	16	6	1.5
12	16	8	2.9
13	12	7	3.9
14	12	12	22.0
15	12	10	24.3
16	12	12	18.6
17	8	7	13.3
18	8	8	20.6
19	8	8	13.3
20	8	6	8.0
21	4	4	7.5
22	4	2	4.2
23	4	3	7.5
24	4	2	1.7

Table 45

Mean bodyweight (lbs.) of groups of 6 calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Group. 1	2	3	4	5	6
0	302 ± 30	295 ± 19	280 ± 19	280 ± 23	268 ± 22	287 ± 35
1	314 ± 31	299 ± 16	288 ± 22	296 ± 21	279 ± 23	295 ± 32
2	326 ± 34	316 ± 23	301 ± 25	313 ± 20	291 ± 24	309 ± 38
3	340 ± 33	327 ± 12	313 ± 22	327 ± 20	308 ± 25	327 ± 38
4	357 ± 34	347 ± 11	333 ± 26	342 ± 19	325 ± 24	335 ± 40
5		362 ± 12	346 ± 25	361 ± 19	338 ± 25	349 ± 40
6		375 ± 8	356 ± 26	378 ± 17	351 ± 25	364 ± 39
7		400 ± 11	380 ± 27	395 ± 18	372 ± 25	383 ± 40
8		419 ± 14	405 ± 24	429 ± 15	400 ± 22	405 ± 36
9			424 ± 29	447 ± 17	412 ± 27	426 ± 42
10			459 ± 33	477 ± 18	447 ± 27	459 ± 43
11			484 ± 82	504 ± 18	479 ± 30	484 ± 43
12			492 ± 34	515 ± 21	486 ± 27	495 ± 46
13				540 ± 18	504 ± 28	504 ± 45
14				552 ± 18	521 ± 30	520 ± 46
15				564 ± 16	541 ± 30	541 ± 45
16				572 ± 15	557 ± 28	552 ± 49
17					569 ± 32	569 ± 48
18					596 ± 25	590 ± 44
19					614 ± 26	607 ± 44
20					617 ± 25	620 ± 44
21						637 ± 41
22						645 ± 43
23						660 ± 40
24						676 ± 42

Table 46

Mean erythrocyte levels (millions/cu.mm.) of groups of 4 calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Group 1	2	3	4	5	6
0	8.10 ± .12	8.89 ± .66	8.91 ± .38	9.02 ± .23	8.29 ± .15	8.36 ± .20
1	8.68 ± .22	8.65 ± .60	8.47 ± .19	8.69 ± .23	8.41 ± .16	8.69 ± .46
2	8.07 ± .05	8.50 ± .30	8.11 ± .25	8.21 ± .12	7.66 ± .22	8.53 ± .23
3	7.87 ± .16	8.69 ± .32	8.22 ± .22	8.89 ± .21	7.93 ± .27	7.94 ± .27
4	7.79 ± .61	9.34 ± .51	8.30 ± .35	9.07 ± .24	8.56 ± .22	8.28 ± .40
5		9.15 ± .40	7.90 ± .39	8.74 ± .16	8.10 ± .28	8.01 ± .27
6		8.89 ± .41	7.86 ± .36	8.36 ± .12	8.17 ± .08	7.87 ± .26
7		8.13 ± .54	7.55 ± .32	8.13 ± .19	7.69 ± .02	7.56 ± .21
8		8.53 ± .42	7.65 ± .12	8.39 ± .16	7.67 ± .29	7.33 ± .31
9			7.55 ± .17	7.91 ± .25	7.45 ± .25	7.50 ± .35
10			7.46 ± .44	7.85 ± .23	7.54 ± .28	7.53 ± .18
11			7.26 ± .20	7.68 ± .18	7.40 ± .22	7.37 ± .32
12			7.04 ± .27	7.64 ± .15	7.04 ± .13	7.18 ± .17
13				8.04 ± .21	7.35 ± .22	7.29 ± .22
14				8.10 ± .06	7.70 ± .17	7.50 ± .25
15				8.02 ± .24	7.49 ± .20	7.49 ± .17
16				8.11 ± .22	7.66 ± .09	7.36 ± .25
17					7.68 ± .14	7.66 ± .23
18					7.51 ± .29	7.39 ± .31
19					7.19 ± .23	7.09 ± .29
20					7.50 ± .11	7.06 ± .12
21						7.29 ± .18
22						7.09 ± .32
23						7.10 ± .29
24						7.07 ± .15

Table 47

Mean packed cell volume (%) of groups of 4 calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Group 1	2	3	4	5	6
0	32.2 ± .55	35.0 ± .07	33.7 ±1.20	35.0 ± .35	32.7 ± .97	33.2 ± .41
1	32.2 ± .85	33.7 ± .85	33.2 ± .66	33.7 ± .43	32.5 ± .25	32.5 ± 1.3
2	32.0 ± 1.0	34.2 ± .75	33.2 ±1.24	33.7 ± .21	30.5 ±1.03	33.0 ± .70
3	30.2 ± .41	33.0 ±1.25	33.0 ±1.27	32.8 ± .95	31.0 ± .70	31.2 ± .89
4	28.5 ±1.60	33 ±1.37	31.0 ±1.43	34.5 ± .56	32.5 ±1.03	32.2 ±1.30
5		34.2 ±1.14	31.0 ±1.05	34.0 ± .79	31.5 ± .83	33.0 ± .61
6		34.0 ±1.27	33.0 ± 1.0	33.0 ± .35	32.2 ± .89	32.7 ± .41
7		31.7 ±1.24	30.5 ± .75	32.0 ± .35	30.5 ± .25	31.5 ± .56
8		33.2 ± .54	32.5 ± .56	33.5 ± .43	31.0 ± .93	33.5 ± .56
9			31.0 ± .61	31.7 ± .41	30.7 ± .65	32.2 ± .67
10			30.0 ± .93	30.7 ± .21	29.5 ± .43	31.5 ± .75
11			29.2 ± .82	30.7 ± .21	29.0 ± .35	32.0 ±1.25
12			30.5 ±1.09	31.7 ± .74	28.7 ± .65	31.2 ± .74
13				33.5 ±1.60	29.7 ± .74	31.5 ±1.75
14				32.5 ± .90	30.5 ± .43	32.0 ±1.04
15				31.5 ±1.03	29.3 ± .89	30.7 ± .96
16				33.5 ±1.34	30.2 ± .75	31.5 ± .75
17					31.5 ± .43	33.0 ± .61
18					30.5 ± .25	32.0 ± .35
19					29.0 ± .35	31.5 ±1.03
20					32.0 ± .61	31.7 ± .41
21						32.7 ±1.24
22						32.2 ± .54
23						32.2 ± .54
24						32.5 ± .75

Table 48

Mean haemoglobin level (gms/100 ml.) of groups of 4 calves following their infection with 500 metacercariae of *F. hepatica*

Weeks after infection	Group 1	2	3	4	5	6
0	11.2 ± .08	13.1 ± .30	12.4 ± .39	12.7 ± .20	11.8 ± .40	12.0 ± .27
1	11.9 ± .28	12.8 ± .44	12.4 ± .37	12.8 ± .27	12.2 ± .36	12.7 ± .59
2	11.5 ± .20	13.0 ± .35	12.3 ± .40	12.5 ± .30	11.5 ± .33	12.0 ± .44
3	11.1 ± .10	13.2 ± .54	11.9 ± .38	12.4 ± .21	11.8 ± .18	11.5 ± .33
4	11.1 ± .60	13.0 ± .44	11.8 ± .37	12.5 ± .11	11.7 ± .33	11.9 ± .52
5		13.4 ± .64	12.1 ± .41	13.0 ± .37	12.2 ± .15	12.1 ± .29
6		13.1 ± .69	12.2 ± .23	12.6 ± .12	12.5 ± .30	12.3 ± .22
7		12.4 ± .43	11.8 ± .30	12.8 ± .26	11.8 ± .20	12.3 ± .32
8		13.2 ± .38	12.8 ± .31	13.0 ± .20	11.8 ± .24	12.7 ± .31
9			12.2 ±1.20	12.2 ± .08	12.0 ± .26	12.2 ± .34
10			12.2 ± .36	12.4 ± .19	11.7 ± .27	12.1 ± .39
11			11.7 ± .29	12.6 ± .26	11.4 ± .08	12.4 ± .44
12			11.4 ± .32	12.0 ± .14	11.1 ± .36	11.8 ± .32
13				12.6 ± .45	11.4 ± .27	12.0 ± .47
14				12.9 ± .28	12.1 ± .31	12.2 ± .14
15				12.7 ± .32	11.8 ± .52	12.3 ± .36
16				12.6 ± .37	11.8 ± .31	11.6 ± .14
17					11.5 ± .17	11.7 ± .09
18					11.7 ± .39	11.9 ± .40
19					11.2 ± .31	11.8 ± .23
20					12.4 ± .21	11.8 ± .28
21						12.3 ± .60
22						11.9 ± .31
23						12.3 ± .34
24						12.2 ± .20

Table 49

Mean corpuscular volume (cu.microns) of groups of 4 calves following their infection with 500 metacercariae of F. hepatica.

Weeks after infection	Group 1	2	3	4	5	6
0	39.8 ± .08	39.7 ±2.08	38.2 ±2.33	38.9 ± .87	39.5 ± .46	39.9 ±1.40
1	37.2 ±1.42	39.5 ±2.17	39.3 ± .86	38.9 ± .89	38.6 ±1.03	37.6 ±1.73
2	39.8 ± .89	40.4 ±1.40	40.9 ± .92	41.1 ± .79	39.7 ± .18	38.6 ± .49
3	38.4 ± .73	38.1 ±1.75	40.1 ±1.07	36.9 ±1.81	37.6 ± .88	39.3 ± .79
4	36.9 ±1.20	35.6 ± 2.0	37.5 ±1.24	38.1 ± .65	38.0 ± .92	39.1 ± .93
5		37.8 ±1.05	39.4 ±1.05	38.9 ± .93	39.0 ±1.07	41.3 ±1.34
6		38.7 ±2.77	42.1 ±1.12	39.5 ± .89	39.5 ±1.41	41.8 ±1.37
7		39.7 ±2.68	40.6 ±1.63	39.4 ± .91	39.7 ± .35	41.8 ±1.55
8		39.4 ±2.44	42.5 ± .80	39.9 ± .65	40.4 ± .41	43.0 ±1.36
9			41.4 ±1.12	40.3 ±1.56	41.3 ± .91	43.3 ±1.97
10			40.2 ± .81	39.2 ±1.15	39.2 ±1.05	41.9 ±1.29
11			40.3 ±1.42	40.1 ±1.01	39.3 ± .73	43.6 ±1.35
12			43.3 ± .90	41.6 ±1.62	40.8 ±1.19	37.7 ±1.10
13				41.5 ±1.06	40.5 ±1.25	43.1 ±1.99
14				40.1 ±1.35	39.7 ±1.23	42.7 ±1.82
15				39.3 ± .05	39.0 ± .68	41.0 ±1.07
16				41.3 ±1.20	39.5 ±1.32	42.9 ±1.23
17					41.1 ±1.25	41.9 ±1.28
18					40.6 ± .65	43.5 ±1.73
19					40.5 ±1.43	44.5 ±1.18
20					42.6 ± .84	45.0 ±1.06
21						44.8 ± .64
22						45.9 ±1.61
23						45.6 ±1.38
24						46.1 ±2.00

Table 50

Mean corpuscular haemoglobin concentration (%) of groups of 4 calves following their infection with 500 metacercariae of F. hepatica.

Weeks after infection	Group 1	2	3	4	5	6
0	34.8 ± .33	37.4 ± .62	36.7 ± .33	36.2 ± .45	36.2 ± .64	36.2 ± .19
1	37.1 ± .41	37.8 ± .72	37.3 ± .77	37.9 ± .77	37.6 ± .44	38.9 ± .56
2	36.1 ± .71	37.0 ± .66	37.2 ± .66	37.1 ± .84	37.8 ± .27	36.3 ± .81
3	36.7 ± .29	39.9 ± .89	36.2 ± .35	38.0 ±1.05	38.3 ± .39	36.9 ± .85
4	39.0 ± .79	39.8 ±1.44	38.0 ± .36	36.3 ± .30	36.1 ± .45	37.0 ±1.01
5		39.1 ± .80	39.2 ± .47	38.1 ± .41	38.7 ± .53	36.9 ±1.31
6		38.3 ± .68	36.9 ± .56	38.1 ± .20	38.9 ± .80	37.5 ± .91
7		39.2 ± .95	38.6 ± .19	40.1 ± .51	38.7 ± .72	38.9 ± .50
8		39.7 ±1.00	39.3 ± .36	38.9 ± .25	38.2 ± .91	37.8 ± .69
9			39.4 ± .46	38.6 ± .58	39.0 ± .64	37.9 ± .49
10			40.7 ± .52	40.2 ± .91	39.6 ± .56	38.4 ± .60
11			40.1 ± .66	41.0 ± .64	39.6 ± .57	38.8 ± .63
12			37.6 ± .31	38.0 ± .69	38.5 ± .49	27.7 ± 1.1
13				37.5 ± .95	38.2 ± .35	38.3 ±1.02
14				39.7 ± .70	39.8 ±1.01	38.3 ±1.46
15				40.4 ± .80	40.4 ±1.20	40.1 ± .68
16				37.7 ± .59	39.2 ±1.54	37.0 ± .80
17					36.5 ± .99	35.6 ± .64
18					38.5 ±1.11	37.2 ±1.00
19					38.7 ± .82	37.5 ± .78
20					39.5 ± .88	37.1 ± .48
21						37.5 ± .45
22						36.9 ± .41
23						38.0 ± .83
24						37.7 ± .47

Table 51

Mean serum albumin level of groups of 4 calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Group 1	2	3	4	5	6
0	2.41 ± .02	2.30 ± .09	2.23 ± .05	2.54 ± .05	2.41 ± .06	2.55 ± .10
2	2.15 ± .03	2.20 ± .04	2.13 ± .07	2.17 ± .06	2.20 ± .06	2.21 ± .08
4	2.30 ± .04	2.16 ± .05	2.03 ± .10	2.23 ± .01	2.13 ± .05	2.32 ± .04
6		2.06 ± .04	2.26 ± .05	2.25 ± .10	2.04 ± .04	2.13 ± .04
8		2.03 ± .07	2.30 ± .06	2.25 ± .09	2.28 ± .03	2.21 ± .05
10			2.08 ± .12	2.19 ± .07	2.11 ± .04	2.05 ± .03
12			2.10 ± .04	2.14 ± .08	2.15 ± .05	2.19 ± .08
14				2.21 ± .05	2.28 ± .03	2.20 ± .04
16				2.19 ± .09	2.27 ± .10	2.05 ± .03
18					2.15 ± .07	2.07 ± .03
20					2.48 ± .03	2.43 ± .08
22						2.41 ± .10
24						2.42 ± .03

Table 52

Mean total serum globulin level of groups of 4 calves following their infection with 500 metacercariae of *F. hepatica.*

Weeks after infection	Group 1	2	3	4	5	6
0	3.63 ± .09	3.63 ± .13	3.84 ± .07	3.51 ± .10	3.35 ± .10	3.82 ± .26
2	3.75 ± .11	3.60 ± .14	3.97 ± .14	3.81 ± .15	3.55 ± .07	3.96 ± .14
4	3.75 ± .16	3.86 ± .14	4.10 ± .12	3.62 ± .11	3.74 ± .13	3.86 ± .21
6		4.29 ± .27	4.16 ± .09	3.82 ± .16	4.01 ± .12	4.00 ± .15
8		3.94 ± .09	3.97 ± .18	3.90 ± .08	3.76 ± .02	3.91 ± .10
10			4.42 ± .03	4.06 ± .12	4.04 ± .06	4.12 ± .13
12			4.42 ± .13	4.26 ± .08	4.34 ± .04	4.41 ± .14
14				4.47 ± .08	4.61 ± .17	4.30 ± .06
16				4.60 ± .10	4.51 ± .07	4.52 ± .15
18					4.48 ± .02	4.63 ± .16
20					4.39 ± .13	4.44 ± .10
22						4.36 ± .07
24						4.53 ± .19

Table 53

Mean serum glutamate-oxaloacetic transaminase levels (S-F units) of groups of 4 calves following their infection with 500 metacercariae of *F. hepatica*.

Weeks after infection	Group 1	2	3	4	5	6
0	68.7 ± 3.0	73.2 ± 3.7	64.0 ± 3.6	70.2 ± 4.6	73.7 ± 1.8	63.5 ± 2.5
1	72.2 ± 3.9	76.7 ± 4.5	79.0 ± 1.6	72.2 ± 4.4	81.7 ± 4.9	71.2 ± 10.3
2	90.5 ± 5.6	99.0 ± 5.8	98.0 ± 4.2	104.7 ± 9.0	113.0 ± 6.3	95.7 ± 4.5
3	107 7 ± 7.1	111.7 ±10.6	93.4 ± 7.7	100.2 ± 4.4	113.2 ± 4.0	93.0 ± 5.7
4	94.2 ± 6.5	94.0 ± 3.9	99.7 ± 7.6	87.7 ± 4.3	101.7 ± 7.0	79.7 ± 5.2
5		90.7 ± 7.7	95.0 ± 5.0	86.7 ± 3.5	95.2 ± 7.9	79.7 ± 1.9
6		87.0 ± 1.7	124.0 ±11.7	95.5 ± 4.2	100.2 ± 4.5	93.0 ± 3.8
7		80.0 ± 3.3	82.0 ± 4.8	83.0 ± 3.8	92.5 ± 4.7	69.2 ± 3.1
8		117.0 ± 9.4	94.0 ± 8.0	108.0 ± 6.9	105.2 ±11.1	86.2 ± 3.4
9			117.0 ± 7.7	121.0 ± 4.3	114.2 ± 8.2	88.0 ± 6.7
10			127.0 ± 5.4	125.0 ±11.6	116.2 ±14.5	91.7 ± 5.4
11			133.0 ± 4.2	136.2 ±18.2	125.2 ±13.4	120.2 ±16.2
12			159.0 ± .35	152.0 ± 3.2	137.0 ± 6.0	121.0 ±13.9
13				130.0 ±10.0	145.0 ± 7.0	126.0 ± 6.6
14				113.0 ± 9.6	151.0 ±36.1	104.0 ± 8.6
15				87.0 ± 5.7	111.0 ±14.2	85.2 ± 4.4
16				111.5 ±10.8	138.0 ±12.7	103.0 ± 2.2
17					125.0 ± 4.2	115.5 ± 7.7
18					124.5 ±10.3	115.0 ± 9.8
19					117.0 ± 8.1	132.0 ±12.1
20					122.0 ± 9.3	119.0 ± 7.4
21						88.0 ±11.6
22						101.0 ± 5.9
23						101·0±128
24						94·5 ± 13·2

Table 54

Numbers of flukes recovered from each calf following their infection with 500 metacercariae of *F. hepatica.*

Calf No.	Number of flukes recovered	% of infecting dose recovered
1	0	0
2	0	0
3	0	0
4	0	0
5	16	3.2
6	40	8.0
7	18	3.6
8	56	11.2
9	23	4.6
10	103	20.6
11	56	11.2
12	47	9.4
13	44	8.8
14	92	18.4
15	57	11.4
16	44	8.8
17	68	13.6
18	97	19.4
19	103	20.6
20	22	4.4
21	26	5.2
22	39	7.8
23	24	4.8
24	8	1.6

Table 55

Individual weekly weights (lbs.) of calves during Experiment 2

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	262	270	280	290	308										
2	225	235	240	258	270										
3	350	363	385	398	420										
4	370	388	400	414	430										
5	250	260	283	300	320	332	350	365	385						
6	265	275	298	310	330	346	368	400	400						
7	330	330	350	360	372	395	403	425	460						
8	335	330	335	340	365	375	380	410	430						
9	254	268	273	292	308	320	336	360	395	405	435	455	470		
10	230	224	233	250	260	275	280	300	330	335	360	390	390		
11	320	330	358	362	390	400	405	430	445	485	520	550	560		
12	318	330	340	350	374	390	405	430	450	470	520	540	550		
13	280	293	307	320	390	360	372	390	440	445	470	505	515	540	560
14	212	240	262	278	288	310	330	350	385	400	435	450	455	485	495
15	290	290	308	320	345	355	380	390	420	444	470	505	515	545	560
16	340	360	376	390	395	420	430	450	470	500	535	555	515	590	595
17	265	285	295	316	330	335	358	380	390	420	450	480	495	510	520
18	225	230	240	258	270	282	296	315	350	340	380	410	415	425	445
19	244	250	260	272	300	310	322	345	390	400	430	460	470	490	505
20	340	350	370	388	400	420	430	450	470	490	530	565	565	590	615
21	345	360	380	398	410	430	445	466	480	510	540	580	595	600	610
22	360	360	390	410	420	430	440	460	475	510	550	560	580	590	615
23	218	230	220	240	245	260	280	295	330	335	380	395	400	410	420
24	224	232	245	260	265	277	292	310	335	350	365	400	405	415	425

15	16	17	18	19	20	21	22	23	24

585	590								
510	520								
570	580								
590	600								
545	560	570	590	610	615				
460	480	480	525	540	545				
550	550	565	600	615	620				
650	640	660	670	690	690				
630	650	670	680	700	705	708	710	730	750
635	650	660	670	700	710	730	750	750	770
450	460	485	510	530	545	565	370	600	610
450	450	460	500	500	520	545	550	560	575

Table 56

Individual weekly erythrocyte counts (millions/cu.mm.) of calves during Weeks

Calf No.	0	1	2	3	4	5	6	7	8	9	10	11	12
1	8.05	8.29	8.03	7.81	8.2								
2	8.05	8.88	8.21	8.37	8.84								
3	7.83	9.34	7.86	7.4	5.7								
4	8.47	8.23	8.2	7.92	8.44								
5	9.97	9.73	9.0	9.1	10.1	10.14	9.34	8.84	9.21				
6	7.96	6.89	7.71	7.73	8.1	8.15	7.9	6.76	7.75				
7	9.54	9.79	9.21	9.44	10.6	9.7	9.99	9.5	9.51				
8	8.1	8.19	8.1	8.49	8.58	8.62	8.32	7.44	7.64				
9	9.64	8.1	7.46	7.58	7.75	7.72	7.58	7.28	7.47	7.4	7.23	7.11	6.44
10	8.7	8.32	7.91	8.65	8.65	8.62	8.02	7.99	8.02	7.83	7.96	7.19	7.75
11	8.11	8.69	8.92	8.00	7.53	6.68	6.92	6.63	7.39	7.05	6.86	6.84	6.58
12	9.19	8.79	8.16	8.67	9.25	8.59	8.94	8.31	7.71	7.92	7.79	7.92	7.4
13	9.6	7.92	7.86	9.25	9.73	9.15	8.64	8.3	8.86	8.2	8.57	8.16	8.07
14	8.7	8.91	8.53	9.19	9.34	8.92	8.52	8.6	8.47	7.43	7.26	7.15	7.42
15	8.45	9.1	8.08	8.17	8.59	8.58	8.17	7.56	8.12	7.43	7.71	7.75	7.3
16	9.33	8.85	8.37	8.96	8.62	8.32	8.1	8.08	8.11	8.6	7.88	7.76	7.76
17	8.81	8.88	8.46	8.2	8.96	7.7	7.88	7.73	7.91	7.42	7.79	7.38	6.91
18	8.13	8.51	7.52	8.7	8.99	9.07	8.35	7.67	8.52	8.29	8.31	8.12	7.28
19	8.13	8.0	7.39	7.27	8.42	7.8	8.24	7.71	7.24	6.99	6.83	6.97	6.65
20	8.1	8.27	7.3	7.56	7.88	7.84	8.21	7.64	7.02	7.1	7.24	7.13	7.32
21	7.73	7.55	8.2	7.07	6.96	7.08	7.00	6.84	6.74	6.3	6.88	6.44	6.59
22	8.75	10.14	8.72	8.41	8.85	8.48	8.23	7.88	8.16	8.15	7.76	7.66	7.38
23	8.65	8.56	7.98	7.85	8.32	8.1	8.36	7.67	8.19	7.91	7.72	7.18	7.5
24	8.3	8.51	9.24	8.46	8.99	8.38	7.9	7.84	8.24	7.63	7.75	8.2	7.26

Experiment 2

13	14	15	16	17	18	19	20	21	22	23	24
8.4	8.16	8.62	8.3								
8.51	8.19	8.07	8.71								
7.81	8.17	8.12	7.9								
7.45	7.88	7.26	7.53								
7.32	7.42	7.85	7.63	7.81	7.43	6.87	7.27				
8.07	8.19	7.83	7.75	8.07	7.31	7.19	7.76				
6.91	7.84	6.83	7.88	7.31	7.31	6.76	7.27				
7.1	7.36	7.46	7.4	7.53	8.0	7.96	7.7				
6.73	6.77	6.94	6.53	6.95	6.39	6.14	6.65	6.77	6.39	6.65	6.67
7.41	7.7	7.49	7.63	8.05	7.95	7.2	7.24	7.65	8.12	7.88	7.28
7.06	7.38	7.68	7.83	8.07	7.92	7.72	7.16	7.16	7.15	7.44	7.42
7.96	8.16	7.87	7.47	7.56	7.32	7.32	7.2	7.59	6.69	6.44	6.91

Table 57

Individual weekly packed cell volume (%) of calves during Experiment 2

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
1	32	30	30	29	31								
2	32	34	34	31	30								
3	31	31	30	30	23								
4	34	34	34	31	30								
5	35	35	35	32	32	34	34	31	32				
6	33	31	32	30	29	33	33	30	33				
7	35	34	34	33	35	32	31	32	33				
8	37	35	36	37	36	38	38	34	35				
9	30	32	31	30	29	31	31	29	32	30	28	28	28
10	36	34	34	37	34	34	35	33	34	33	33	32	34
11	35	35	37	33	30	28	31	30	33	31	29	28	30
12	34	32	31	32	31	31	35	30	31	30	30	29	30
13	35	33	34	32	35	33	32	31	34	32	31	31	31
14	34	35	34	34	34	34	33	33	34	33	31	31	34
15	35	34	34	35	34	35	34	32	34	31	30	31	32
16	36	33	33	30	33	32	33	32	32	31	31	30	30
17	36	36	34	33	36	29	34	31	31	32	30	29	30
18	32	30	30	31	32	33	31	30	34	32	30	30	27
19	32	32	39	31	31	31	30	30	30	30	28	28	28
20	31	32	39	39	31	33	34	31	29	29	30	39	30
21	34	31	33	29	29	32	32	32	32	31	31	30	31
22	33	35	33	31	33	32	32	31	33	33	31	30	29
23	32	29	31	31	31	33	33	30	34	31	30	32	32
24	34	35	35	34	36	35	34	33	35	34	34	36	33

13	14	15	16	17	18	19	20	21	22	23	24

34	32	33	31								
38	34	33	38								
33	33	32	33								
29	30	28	32								
32	32	32	32	31	31	30	31				
30	30	29	30	31	30	28	31				
29	30	27	28	33	30	29	32				
28	30	29	31	31	31	29	34				
32	32	30	30	32	31	29	32	29	32	31	34
28	30	29	30	32	32	30	31	35	34	34	31
29	29	30	33	33	32	33	31	32	32	32	31
37	37	34	33	35	33	34	33	35	31	32	34

Table 58

Individual weekly haemoglobin levels (gms/100 ml.) of calves during

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
1	11.3	11.2	11.2	10.9	11.3								
2	11.1	12.7	12.2	11.4	11.8								
3	11.0	12.2	11.2	10.9	9.1								
4	11.5	11.7	11.5	11.2	12.2								
5	13.7	13.2	13.2	13.0	13.6	13.9	13.1	13.1	13.5				
6	12.5	11.7	12.5	12.4	12.1	12.9	12.9	11.2	13.0				
7	12.5	12.2	12.2	12.2	12.3	11.7	11.2	12.1	12.1				
8	13.7	14.0	14.0	15.0	14.2	15.2	15.1	13.4	14.2				
9	11.2	12.0	11.2	10.9	11.1	11.7	12.0	11.3	12.3	11.9	11.6	10.9	10.6
10	13.2	12.0	12.5	13.0	12.9	13.3	12.5	12.8	13.6	12.5	13.4	12.4	12.4
11	13.0	13.7	13.5	12.2	11.1	11.1	11.5	11.4	13.2	12.4	12.1	11.5	11.4
12	12.2	12.0	12.2	11.7	12.1	12.5	12.7	11.6	12.0	12.1	11.7	12.1	11.4
13	13.0	11.7	11.7	11.7	12.6	12.9	12.4	12.0	13.0	12.1	12.1	13.3	12.1
14	12.0	13.5	13.0	12.5	12.8	13.7	12.4	13.3	13.4	12.3	12.0	12.4	12.1
15	13.0	13.5	13.2	13.0	12.5	13.5	13.0	12.8	13.4	12.1	13.0	12.9	12.4
16	12.7	12.5	12.2	12.5	12.2	11.8	12.5	13.3	12.4	12.5	12.4	11.9	11.6
17	13.0	13.2	12.7	12.2	12.8	11.7	13.0	12.0	12.5	12.9	12.4	11.6	11.6
18	11.2	11.2	11.2	12.0	11.5	12.5	11.5	11.2	12.0	11.8	11.5	11.5	10.0
19	12.2	12.0	11.0	11.7	11.7	12.1	12.5	12.3	11.6	11.8	11.2	11.1	10.9
20	11.0	12.5	11.2	11.2	11.0	12.4	13.0	11.7	11.2	11.5	11.7	11.3	11.9
21	12.0	12.0	12.0	10.9	11.4	12.0	12.1	12.6	12.1	11.9	11.8	11.3	11.9
22	12.7	14.3	12.7	12.2	12.6	13.1	12.9	12.4	12.9	12.9	11.8	12.3	11.6
23	11.2	11.2	10.5	10.8	10.5	11.5	11.7	11.2	12.1	11.3	11.1	12.3	10.9
24	12.2	13.2	12.7	12.2	13.2	12.0	12.4	12.9	13.6	12.9	13.7	13.8	12.7

Experiment 2

13	14	15	16	17	18	19	20	21	22	23	24
12.2	12.4	12.8	11.9								
13.8	13.3	12.8	13.7								
13.0	13.6	13.5	12.9								
11.4	12.3	11.7	11.9								
12.2	12.5	13.5	12.4	11.5	12.7	11.5	12.3				
11.2	11.4	10.6	10.9	11.5	10.5	10.2	11.8				
11.4	13.0	11.5	12.4	11.0	11.9	11.3	12.7				
10.7	11.7	11.8	11.0	12.0	11.9	11.9	12.9				
12.0	12.1	12.1	11.4	11.7	11.1	11.1	12.1	10.5	11.6	12.4	12.7
11.7	11.8	12.2	11.6	11.5	12.7	11.9	11.6	13.3	13.0	13.3	12.2
10.9	12.2	11.5	11.4	12.0	11.1	11.8	11.0	11.9	11.6	11.4	11.6
13.5	12.6	13.5	12.1	11.7	12.7	12.4	12.5	13.5	11.5	12.0	12.5

Table 59

Individual mean corpuscular volumes (cu.microns) of calves during Experiment 2

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
1	39.7	36.2	37.6	37.1	37.8								
2	39.7	38.2	41.4	37.0	33.9								
3	39.6	33.2	38.2	40.5	40.3								
4	40.1	41.3	41.5	39.1	35.5								
5	35.1	35.9	38.9	35.1	31.7	33.7	36.4	35.1	34.7				
6	41.5	44.9	41.5	38.8	35.8	40.5	41.8	44.3	42.6				
7	36.7	34.7	36.9	34.9	33.0	33.0	31.0	33.6	34.7				
8	45.7	42.7	44.4	43.5	42.0	44.1	45.7	45.7	45.8				
9	31.1	39.5	41.5	39.5	37.4	40.1	40.8	39.8	42.8	40.5	38.7	39.4	43.4
10	41.4	40.9	42.9	42.7	39.3	39.4	43.6	41.3	42.4	42.1	41.4	44.5	43.8
11	43.2	40.3	41.5	41.2	39.8	41.9	44.8	45.2	44.7	43.9	42.2	40.9	45.5
12	37.0	36.4	37.9	36.9	33.5	36.1	39.1	36.1	40.2	37.8	38.5	36.6	40.5
13	36.5	41.7	43.2	34.5	36.0	36.1	37.0	37.4	38.3	39.0	36.1	38.0	38.3
14	39.1	39.3	39.8	36.9	38.5	40.4	38.7	38.4	40.1	44.4	42.6	43.4	45.8
15	41.4	37.4	42.1	42.8	39.6	40.8	41.6	42.3	41.9	41.7	38.9	40.0	43.8
16	38.6	37.3	39.4	33.4	38.3	38.5	40.7	39.6	39.5	36.0	39.3	39.1	38.6
17	40.9	40.5	40.2	40.2	40.2	37.7	43.1	40.1	39.2	43.1	38.5	39.3	43.4
18	39.4	35.2	39.9	35.6	35.6	36.4	37.1	39.1	39.9	38.6	36.1	36.9	37.0
19	39.4	40.0	39.2	36.5	36.8	39.7	36.4	38.9	41.4	42.9	40.9	40.2	42.1
20	38.3	38.7	39.7	38.3	39.3	42.1	41.4	40.6	41.3	40.8	41.4	40.7	40.9
21	44.0	41.1	40.2	41.1	41.7	45.2	45.7	46.8	47.5	49.2	45.0	46.6	38.4
22	37.7	34.5	37.8	36.8	37.3	37.7	38.9	39.3	40.4	40.4	39.9	39.2	40.0
23	37.0	33.9	38.8	39.4	37.3	40.7	39.5	39.1	41.5	39.1	38.8	44.6	34.1
24	41.0	41.1	37.8	40.1	40.0	41.8	43.0	42.1	42.5	44.5	43.8	43.9	38.5

13	14	15	16	17	18	19	20	21	22	23	24
40.4	39.2	38.3	37.3								
44.6	42.7	40.9	43.6								
42.2	40.4	39.4	41.8								
38.9	38.1	38.6	42.5								
43.7	43.1	40.8	41.9	39.7	41.7	43.7	42.6				
37.1	36.6	37.0	38.7	38.4	41.0	38.9	39.9				
41.9	38.3	39.5	35.5	45.1	41.0	42.9	44.0				
39.4	40.8	38.9	41.9	41.2	38.7	36.4	44.4				
47.5	37.3	43.2	45.9	39.7	48.5	47.2	48.1	42.8	50.8	46.6	51.0
37.7	39.0	38.7	39.3	40.9	40.2	41.6	42.8	45.7	41.9	43.1	42.6
41.0	39.3	39.1	42.1	40.9	40.4	42.7	43.3	44.7	44.7	43.0	41.8
46.4	45.3	43.2	44.2	46.3	45.1	46.4	45.8	46.1	46.3	49.7	49.2

Table 60

Individual mean corpuscular haemoglobin levels (%) of calves during

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
1	35.3	37.3	37.3	37.6	36.4								
2	34.7	37.4	35.9	36.8	39.3								
3	35.5	39.4	37.3	36.3	39.6								
4	33.8	36.4	33.8	36.1	40.7								
5	39.1	37.7	37.7	40.6	42.5	40.9	38.5	42.2	42.2				
6	37.9	37.7	35.7	41.7	41.7	39.1	39.1	37.3	39.4				
7	35.7	35.9	35.9	37.0	35.1	36.6	36.1	37.8	36.7				
8	37.0	40.0	38.9	40.5	39.4	40.0	39.7	39.4	40.6				
9	37.3	37.5	36.1	36.3	38.3	37.7	38.7	39.0	38.4	39.7	41.4	38.9	37.8
10	36.7	35.3	56.8	35.1	37.9	39.1	35.7	38.8	40.0	37.9	40.6	38.7	36.5
11	37.1	39.1	36.5	37.0	37.0	39.6	37.1	38.0	40.0	40.0	41.7	41.1	38.0
12	35.6	37.5	39.5	36.6	39.0	40.3	36.3	38.7	38.7	40.3	39.0	41.7	38.0
13	37.1	35.5	34.4	36.6	36.0	39.1	38.7	38.7	38.2	37.3	39.0	42.9	39.0
14	35.3	38.6	38.2	36.8	35.5	38.0	37.6	40.3	39.4	37.3	38.7	40.0	35.6
15	37.1	39.7	38.8	37.1	36.8	38.6	38.2	40.0	39.4	39.0	43.3	41.6	38.7
16	35.3	37.9	37.0	41.7	37.0	36.9	37.9	41.6	38.7	40.3	40.0	39.7	38.7
17	36.1	36.7	37.3	37.0	35.5	40.3	38.2	38.7	40.3	40.3	41.3	41.4	38.7
18	35.0	37.3	37.3	38.7	35.9	37.9	37.7	37.3	35.3	36.9	38.3	38.3	37.0
19	38.1	37.5	37.9	39.0	37.7	39.0	41.7	41.0	38.7	39.3	40.0	39.6	38.9
20	35.5	39.1	38.6	38.6	35.5	37.6	38.2	37.7	38.6	39.6	39.0	39.0	39.7
21	35.3	38.7	36.4	37.6	39.3	37.5	37.8	39.4	37.8	38.4	38.1	37.7	38.4
22	38.5	40.8	38.5	39.3	38.2	40.9	40.3	40.0	39.1	39.1	38.1	41.0	40.0
23	35.0	38.6	33.9	34.8	33.9	34.8	35.4	37.3	35.6	36.4	37.0	38.4	34.1
24	35.9	37.7	36.3	35.9	36.7	34.3	36.5	39.1	38.9	37.9	40.3	38.3	38.5

Experiment 2

13	14	15	16	17	18	19	20	21	22	23	24
35.0	38.7	38.8	38.4								
36.3	38.0	38.8	36.0								
39.4	41.2	42.2	39.1								
39.3	41.0	41.8	37.2								
38.1	39.1	42.2	38.7	37.1	41.0	38.3	39.7				
37.3	38.0	36.5	36.3	37.1	35.0	36.4	38.1				
39.3	43.3	42.6	44.3	33.3	39.7	39.0	42.3				
38.2	39.0	40.7	37.4	38.7	38.4	41.0	37.9				
37.5	37.8	40.3	38.0	36.6	35.8	38.3	37.8	36.2	36.2	40.0	37.3
41.8	39.3	42.1	38.7	35.9	39.7	39.7	37.4	38.0	38.2	39.1	39.3
37.6	42.1	38.3	39.5	36.4	34.7	35.7	35.5	37.2	36.2	35.6	37.4
36.5	34.0	39.7	36.7	33.4	38.5	36.5	37.9	38.6	37.1	37.5	36.8

Table 61

Individual serum albumin levels (gms/100 ml.) of calves during Experiment 2

Calf No.	Weeks 0	2	4	6	8	10	12	14	16	18	20	22	24
1	2.49	2.18	2.29										
2	2.40	2.07	2.27										
3	2.44	2.23	2.20										
4	2.34	2.12	2.45										
5	2.27	2.16	2.25	2.14	1.89								
6	2.38	2.34	2.23	2.10	1.99								
7	2.51	2.20	2.18	1.93	1.99								
8	2.03	2.09	2.00	2.06	2.26								
9	2.23	2.11	1.70	2.19	2.41	1.79	2.09						
10	2.07	2.11	2.09	2.15	2.10	1.88	1.95						
11	2.25	2.37	2.19	2.42	2.39	2.39	2.16						
12	2.37	1.94	2.16	2.28	2.32	2.27	2.20						
13	2.50	2.14	2.22	2.06	2.05	2.17	2.12	2.31	2.02				
14	2.40	2.03	2.20	2.05	2.07	1.95	1.96	2.04	2.10				
15	2.55	2.14	2.25	2.47	2.48	2.30	2.07	2.19	2.17				
16	2.70	2.36	2.26	2.43	2.39	2.35	2.42	2.29	2.49				
17	2.59	2.30	2.30	2.20	2.37	2.20	2.10	2.28	2.30	2.39	2.53		
18	2.48	2.02	2.06	2.01	2.29	1.98	2.02	2.18	2.11	2.01	2.37		
19	2.41	2.31	2.07	1.99	2.31	2.12	2.26	2.38	2.29	2.09	2.50		
20	2.21	2.16	2.10	1.96	2.17	2.14	2.24	2.30	2.37	2.10	2.53		
21	2.52	1.99	2.23	2.17	2.33	2.10	2.31	2.32	2.13	2.12	2.45	2.31	2.53
22	2.89	2.43	2.43	2.21	2.21	2.11	2.09	2.24	1.96	2.12	2.66	2.48	2.39
23	2.47	2.25	2.35	1.98	2.06	1.97	2.06	2.07	2.05	1.98	2.18	2.70	2.40
24	2.34	2.20	2.26	2.15	2.26	2.03	2.30	2.16	2.07	2.07	2.44	2.16	2.36

Table 62

Individual total serum globulin levels (gms/100 ml.) of calves during Experiment 2.

Calf No.	Weeks 0	2	4	6	8	10	12	14	16	18	20	22	24
1	3.51	3.52	3.41										
2	3.5	3.83	3.83										
3	3.56	3.57	3.50										
4	3.96	4.08	4.25										
5	4.03	4.04	4.15	4.06	3.81								
6	3.35	3.26	3.47	4.00	3.81								
7	3.69	3.50	3.72	4.47	3.91								
8	3.47	3.61	4.10	4.64	4.24								
9	3.67	3.79	4.30	3.91	3.49	4.41	4.11						
10	3.73	3.59	4.11	4.05	3.90	4.42	4.25						
11	3.95	4.33	4.31	4.38	4.51	4.51	4.54						
12	4.03	4.16	3.70	4.32	3.98	4.33	4.80						
13	3.50	3.56	3.38	3.54	3.65	4.13	3.98	4.29	4.28				
14	3.30	3.47	3.70	3.45	3.93	3.75	4.34	4.66	4.80				
15	3.85	4.26	3.95	4.13	3.92	4.40	4.43	4.61	4.73				
16	3.40	3.94	3.44	4.17	4.11	3.95	4.28	4.31	4.61				
17	3.01	3.40	3.70	3.90	3.73	3.90	4.40	4.42	4.30	4.51	4.17		
18	3.42	3.48	3.34	3.69	3.71	4.22	4.28	4.42	4.69	4.49	4.73		
19	3.59	3.79	3.83	4.11	3.79	3.98	4.24	5.22	4.61	4.41	4.10		
20	3.39	3.54	4.10	4.34	3.83	4.06	4.46	4.40	4.43	4.50	4.57		
21	2.78	4.11	3.57	3.83	3.77	4.10	4.39	4.08	4.77	4.98	4.75	4.59	4.87
22	3.71	3.97	4.07	4.19	4.09	4.09	4.01	4.36	4.14	4.28	4.24	4.22	4.11
23	4.63	4.25	4.45	4.42	4.14	4.33	4.84	4.33	4.85	4.92	4.52	4.40	4.90
24	3.16	3.50	3.34	3.55	3.64	3.97	4.40	4.44	4.33	4.33	4.26	4.24	4.24

Table 63

Individual weekly serum glutamic-oxaloacetic transaminase levels

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
1	65	83	87	119	99								
2	70	63	74	87	78								
3	78	66	104	123	87								
4	62	78	97	102	113								
5	70	71	99	97	89	83	89	90	121				
6	77	87	100	122	93	89	86	72	114				
7	63	65	82	87	87	75	82	77	90				
8	83	84	115	141	107	116	91	82	143				
9	71	80	84	93	78	95	98	77	122	93	136	121	135
10	53	84	101	69	102	93	112	82	85	122	127	134	155
11	62	76	100	101	98	82	125	97	83	116	137	145	150
12	70	76	107	111	121	110	161	71	87	136	110	131	195
13	75	78	93	87	74	87	83	71	87	136	110	104	115
14	69	77	97	99	87	97	97	91	126	160	161	182	195
15	81	77	136	104	93	77	107	87	107	90	131	161	136
16	56	57	93	111	97	86	95	83	111	97	106	98	107
17	80	85	96	104	119	82	111	107	136	122	110	108	144
18	73	90	107	126	110	119	104	93	117	136	161	170	152
19	71	65	119	111	82	80	86	89	83	92	114	101	120
20	71	87	130	112	96	100	100	81	85	107	80	122	133
21	65	71	93	107	93	77	82	63	80	91	110	82	133
22	60	71	82	76	87	83	91	75	87	87	83	112	101
23	58	57	104	90	69	75	98	63	81	68	85	172	89
24	71	86	104	99	70	84	102	76	97	106	89	115	160

(S-F units)

13	14	15	16	17	18	19	20	21	22	23	24

159	156	89	115								
157	136	102	145								
114	100	97	98								
147	127	70	88								
156	119	113	121	131	135	102	124				
162	275	157	172	129	149	144	152				
129	95	88	107	110	93	108	104				
134	115	87	151	129	121	113	109				
117	99	87	110	135	149	145	140	113	100	109	133
117	78	71	99	120	102	94	101	73	111	93	87
149	119	95	105	115	102	159	124	107	82	137	99
122	120	88	100	92	107	130	110	59	111	66	59

Table 64

Individual weekly faeces egg counts (egg/3 gms of faeces) of calves

Calf No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0	0	0	0	0												
2	0	0	0	0	0												
3	0	0	0	0	0												
4	0	0	0	0	0												
5	0	0	0	0	0	0	0	0	0								
6	0	0	0	0	0	0	0	0	0								
7	0	0	0	0	0	0	0	0	0								
8	0	0	0	0	0	0	0	0	0								
9	0	0	0	0	0	0	0	0	0	0	0	0	0				
10	0	0	0	0	0	0	0	0	0	0	0	1	2				
11	0	0	0	0	0	0	0	0	0	0	0	2	0				
12	0	0	0	0	0	0	0	0	0	0	0	1	0				
13	0	0	0	0	0	0	0	0	0	0	0	1	0	4	3	1	11
14	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	20	16
15	0	0	0	0	0	0	0	0	0	0	0	0	2	0	10	4	8
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	18	5
17	0	0	0	0	0	0	0	0	0	0	0	1	2	1	2	20	2
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	8	9
19	0	0	0	0	0	0	0	0	0	0	0	0	0	3	11	9	3
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	1
21	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	3
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1
23	0	0	0	0	0	0	0	0	0	0	0	1	1	1	10	1	2
24	0	0	0	0	0	0	0	0	0	0	0	0	3	1	24	6	6

during Experiment 2

17	18	19	20	21	22	23	24
5	4	1	0				
11	8	4	3				
3	11	10	1				
0	1	2	0				
3	8	9	2	1	3	3	1
1	3	1	1	2	2	6	1
1	4	6	11	4	0	0	0
8	11	3	1	2	0	1	0

Table 65

Mean erythrocyte counts (R.b.c.), packed cell volumes (P.C.V.) haemoglobin concentrations (Hb) mean corpuscular volumes (M.C.V.) and mean corpuscular haemoglobin concentrations (M.C.H.C.) of 8 calves following their initial infection with 750 metacercariae of F. hepatica and subsequent reinfection with 1,300 metacercariae of F. hepatica 7 weeks later.

Weeks after infection	R.b.c. (millions/cu.mm)	P.C.V. (%)	Hb (gm/100ml.)	M.C.V. (cu.microns)	M.C.H.C. (%)
0	8.41 ± .09	32.1 ± .74	12.4 ± .34	38.2 ± .64	38.6 ± .57
1	8.08 ± .12	33.7 ± .53	12.5 ± .12	41.8 ± .68	37.0 ± .52
2	8.31 ± .20	32.4 ± .53	12.2 ± .29	39.0 ± .45	37.5 ± .48
3	8.88 ± .11	34.5 ± .33	13.2 ± .41	38.9 ± .67	38.3 ± .49
4	8.11 ± .14	33.7 ± .31	12.7 ± .09	41.7 ± .44	37.5 ± .43
5	8.40 ± .14	32.9 ± .44	12.3 ± .18	39.2 ±1.13	37.6 ± .57
6	8.42 ± .19	31.7 ± .49	12.3 ± .15	37.8 ± .64	38.9 ± .45
7	8.53 ± .25	32.5 ± .53	12.6 ± .23	38.2 ± .68	38.8 ± .48
8	8.62 ± .26	34.2 ± .77	13.1 ± .24	39.8 ± .61	38.2 ± .54
9	8.31 ± .24	34.4 ± .60	12.8 ± .23	41.5 ± .85	37.1 ± .66
10	8.30 ± .28	32.7 ± .62	12.6 ± .24	39.6 ± 1.0	38.4 ± .36
11	8.35 ± .22	32.6 ± .82	12.7 ± .22	39.1 ± .49	39.1 ± .51
12	7.82 ± .20	33.5 ± .50	12.7 ± .27	43.0 ±1.07	37.9 ± .55
13	8.12 ± .25	32.4 ± .62	12.7 ± .20	40.1 ±1.24	39.4 ± .26
14	7.78 ± .23	30.9 ± .69	12.3 ± .13	39.8 ± .92	40.3 ± .52
15	8.03 ± .21	31.9 ± .58	12.7 ± .58	39.8 ± .88	40.0 ± .33
16	7.72 ± .21	31.4 ± .46	12.5 ± .11	40.7 ± .85	40.0 ± .30
17	7.82 ± .18	31.2 ± .59	12.3 ± .13	40.0 ± .90	39.6 ± .65
18	7.88 ± .20	33.4 ± .50	12.9 ± .20	42.5 ±2.93	38.6 ± .39
19	7.57 ± .22	30.6 ± .62	11.9 ± .25	40.9 ±1.12	38.8 ± .41
20	7.77 ± .16	33.6 ± .73	13.0 ± .24	43.3 ± .84	38.9 ± .57

Table 66

Mean erythrocyte counts (R.b.c.), packed cell volumes (P.C.V.),Haemoglobin concentrations (Hb), mean corpuscular volumes (M.C.V.) and mean corpuscular haemoglobin concentrations (M.C.H.C.) of 8 calves following their initial infection with 750 metacercariae of F. hepatica and subsequent reinfection with 1,300 metacercariae of F. hepatica 12 weeks later.

Weeks after infection	R.b.c. (millions/cu.mm)	P.C.V. (%)	Hb (gm/100ml)	M.C.V. (cu.microns)	M.C.H.C. (%)
0	7.96 ± .16	32.6 ±1.05	12.1 ± .36	41.0 ±1.47	37.3 ± .54
1	7.71 ± .29	33.2 ± .99	12.1 ± .40	43.3 ± .97	36.3 ± .62
2	8.22 ± .26	33.1 ± .69	12.5 ± .28	40.4 ± .84	37.7 ± .62
3	8.24 ± .31	34.9 ±1.00	12.9 ± .37	42.5 ± .97	37.0 ± .40
4	7.89 ± .23	33.1 ± .83	12.4 ± .29	42.2 ± .45	37.4 ± .37
5	7.79 ± .30	32.2 ± .86	11.8 ± .32	41.6 ± .77	36.6 ± .35
6	8.14 ± .27	31.7 ± .80	12.2 ± .29	39.2 ±1.15	38.3 ± .63
7	8.23 ± .32	32.6 ±1.05	12.5 ± .36	39.7 ± .67	38.5 ± .33
8	8.14 ± .23	34.7 ± .84	12.7 ± .23	42.9 ±1.14	36.7 ± .92
9	7.98 ± .26	33.9 ± .72	13.0 ± .28	42.6 ±1.22	38.3 ± .58
10	7.92 ± .32	32.6 ±1.11	12.3 ± .29	41.4 ±1.22	37.7 ± .59
11	8.05 ± .29	32.4 ± .84	12.8 ± .45	40.3 ± .85	39.5 ± .98
12	7.52 ± .27	32.6 ± .98	12.2 ± .42	43.5 ± .83	37.4 ± .71
13	7.86 ± .29	32.9 ± .81	12.5 ± .36	42.0 ± .95	38.0 ± .31
14	7.71 ± .26	32.7 ±1.19	12.3 ± .32	42.6 ±1.41	37.6 ± .87
15	7.86 ± .22	32.9 ± .77	12.9 ± .33	41.9 ± .85	39.3 ± .28
16	7.52 ± .23	31.4 ±1.01	12.2 ± .92	41.7 ± .70	39.1 ± .46
17	7.72 ± .20	32.1 ± .55	12.4 ± .70	41.7 ± .94	38.8 ± .33
18	7.57 ± .28	32.9 ± .91	12.7 ± .48	43.6 ±2.05	38.8 ± .52
19	7.36 ± .25	30.7 ± .94	12.0 ± .33	42.8 ± .98	38.4 ± .48
20	7.51 ± .26	3.30 ± .98	12.7 ± .41	44.0 ± .77	38.4 ± .36

Table 67

Mean serum albumin, total serum globulins, serum glutamic-oxaloacetic transaminase (SGOT) levels of 8 calves following their infection with 750 metacercariae of F. hepatica and their subsequent reinfection with 1,300 metacercariae of F. hepatica 7 weeks later.

Weeks after infection	Serum albumin levels (gm/100 ml.)	Total serum globulin level (gms/100 ml.)	SGOT levels (S-F units)
0	2.19 ± .08	3.89 ± .17	72.7 ± 4.5
1			82.0 ± 5.5
2	2.24 ± .03	3.89 ± .11	72.6 ± 4.0
3			119.0 ± 14.6
4	2.26 ± .07	4.19 ± .10	83.6 ± 7.0
5			107.5 ± 11.2
6	2.21 ± .09	4.57 ± .31	81.9 ± 5.1
7			66.9 ± 1.9
8	2.14 ± .04	4.21 ± .13	96.0 ± 5.0
9			83.9 ± 5.6
10	2.21 ± .03	4.56 ± .09	89.5 ± 7.2
11			88.2 ± 5.6
12	1.98 ± .05	4.55 ± .16	80.6 ± 3.7
13			86.7 ± 5.6
14	2.02 ± .05	4.27 ± .14	91.2 ± 7.4
15			121.4 ± 15.7
16	2.03 ± .04	4.60 ± .15	100.4 ± 9.8
17			102.5 ± 10.5
18	1.96 ± .04	4.86 ± .13	91.0 ± 5.8
19			106.0 ± 7.3
20	1.98 ± .03	4.93 ± .13	98.2 ± 8.8

Table 68

Mean serum albumin, total serum globulins, serum glutamic-oxaloacetic transaminase (SGOT) levels of 8 calves following their infection with 750 metacercariae of F. hepatica and their subsequent reinfection with 1,300 metacercariae of F. hepatica 12 weeks later.

Weeks after infection	Serum albulin levels (gms/100 ml.)	Total serum globulin level (gms/100 ml.)	SGOT levels (S-F units)
0	2.24 ± .03	3.63 ± .11	68.9 ± 3.1
1			75.5 ± 3.4
2	2.26 ± .07	3.65 ± .10	71.0 ± 3.2
3			109.5 ± 9.9
4	2.40 ± .07	3.86 ± .14	80.0 ± 4.0
5			88.4 ± 5.4
6	2.54 ± .19	4.25 ± .10	84.5 ± 6.8
7			81.7 ± 5.3
8	1.97 ± .08	4.21 ± .07	103.0 ± 12.5
9			82.6 ± 7.2
10	1.98 ± .08	4.00 ± .19	92.7 ± 8.1
11			104.4 ± 7.9
12	1.93 ± .03	4.37 ± .11	88.9 ± 5.2
13			73.4 ± 1.5
14	1.98 ± .08	3.77 ± .38	85.4 ± 4.2
15			98.0 ± 11.7
16	2.13 ± .04	4.31 ± .11	79.7 ± 2.9
17			82.5 ± 5.0
18	2.01 ± .08	4.40 ± .13	78.2 ± 4.5
19			82.9 ± 4.7
20	2.16 ± .05	4.32 ± .10	84.6 ± 4.7

Table 69

Mean weekly weights (lbs.) of the 7 groups of calves during Experiment 3

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	422 ± 25	441 ± 24	459 ± 22	466 ± 26	438 ± 28	482 ± 26	495 ± 27	516 ± 27	530 ± 29	546 ± 33	562 ± 34	582 ± 34	596 ± 35	607 ± 38	619 ± 34	637 ± 35	660 ± 36	662 ± 39	679 ± 42	685 ± 40	691 ± 38
2	321 ± 29	337 ± 29	351 ± 29	375 ± 30	380 ± 33	392 ± 33	397 ± 37	419 ± 40	431 ± 37	444 ± 40	450 ± 36	472 ± 37	492 ± 37	500 ± 42	507 ± 42	524 ± 43	552 ± 47	554 ± 51	575 ± 52	586 ± 52	590 ± 52
3	357 ± 39	391 ± 43	393 ± 35	411 ± 13	416 ± 28	430 ± 29	445 ± 30	472 ± 29	486 ± 31	506 ± 30	520 ± 37	532 ± 35	550 ± 35	560 ± 32	569 ± 35	582 ± 36	605 ± 35	616 ± 39	636 ± 38	637 ± 39	645 ± 38
4	410 ± 23	430 ± 21	441 ± 20	461 ± 20	469 ± 17	479 ± 13	491 ± 15	506 ± 16	522 ± 18	549 ± 16	566 ± 16	582 ± 19	604 ± 20	614 ± 17	624 ± 17	640 ± 19	655 ± 18	666 ± 18	686 ± 16	691 ± 11	700 ± 12
5	401 ± 23	412 ± 55	432 ± 52	449 ± 50	455 ± 48	464 ± 48	477 ± 44	500 ± 46	520 ± 48	452 ± 46	551 ± 50	570 ± 51	585 ± 53	602 ± 60	614 ± 53	632 ± 48	651 ± 46	657 ± 50	675 ± 48	682 ± 47	688 ± 46
6	324 ± 34	337 ± 33	348 ± 33	361 ± 30	372 ± 30	389 ± 29	387 ± 27	407 ± 27	420 ± 25	439 ± 23	447 ± 26	462 ± 21	481 ± 22	492 ± 20	506 ± 17	522 ± 16	540 ± 17	547 ± 18	563 ± 16	566 ± 18	575 ± 16
7	364 ± 28	377 ± 29	394 ± 31	417 ± 29	415 ± 29	426 ± 30	441 ± 33	455 ± 32	469 ± 32	489 ± 34	507 ± 38	516 ± 37	535 ± 39	542 ± 38	555 ± 40	575 ± 41	597 ± 40	599 ± 45	617 ± 43	630 ± 43	637 ± 44

Table 70

Mean weekly erythrocyte counts (millions/cu.mm.) of the 7 groups of

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	8.56 ± .12	8.14 ± .24	8.56 ± .33	9.05 ± .18	8.03 ± .27	8.43 ± .25	8.15 ± .26	8.62 ± .50	8.62 ± .49	8.25 ± .43	8.22 ± .42
2	8.25 ± .12	8.02 ± .11	8.05 ± .16	8.70 ± .08	8.18 ± .10	8.37 ± .17	8.68 ± .24	8.44 ± .19	8.62 ± .29	8.36 ± .28	8.38 ± .44
3	7.83 ± .26	7.50 ± .53	8.15 ± .44	8.07 ± .52	7.80 ± .27	7.63 ± .53	8.00 ± .44	7.90 ± .36	7.96 ± .40	7.69 ± .41	7.74 ± .62
4	8.09 ± .22	7.91 ± .29	8.29 ± .34	8.40 ± .40	7.98 ± .41	7.94 ± .35	8.28 ± .35	8.56 ± .52	8.32 ± .24	8.26 ± .63	8.09 ± .28
5	8.28 ± .29	7.40 ± .53	7.38 ± .42	7.53 ± .36	7.01 ± .25	7.28 ± .24	7.25 ± .24	7.32 ± .34	7.43 ± .38	7.08 ± .23	7.26 ± .36
6	8.29 ± .32	8.36 ± .31	8.38 ± .24	8.43 ± .34	8.03 ± .46	7.96 ± .48	7.90 ± .36	7.91 ± .27	8.31 ± .36	7.94 ± .46	7.88 ± .24
7	8.05 ± .26	7.85 ± .14	7.94 ± .19	7.70 ± .18	7.55 ± .17	7.60 ± .11	7.72 ± .16	8.14 ± .25	8.21 ± .29	7.92 ± .16	8.14 ± .30

calves during Experiment 3

11	12	13	14	15	16	17	18	19	20
8.29 ± .28	7.74 ± .32	7.98 ± .46	7.64 ± .33	8.00 ± .33	7.64 ± .24	7.80 ± .22	7.81 ± .38	7.44 ± .34	7.76 ± .12
8.40 ± .38	7.90 ± .30	8.25 ± .24	7.91 ± .36	8.06 ± .32	7.81 ± .38	7.84 ± .33	7.96 ± .21	7.71 ± .32	7.77 ± .31
8.06 ± .53	7.26 ± .47	7.71 ± .57	7.49 ± .34	7.91 ± .29	7.27 ± .33	7.74 ± .32	7.55 ± .39	7.17 ± .31	7.33 ± .43
8.03 ± .33	7.78 ± .25	8.02 ± .25	7.93 ± .41	7.80 ± .38	7.77 ± .30	7.70 ± .28	7.58 ± .46	7.56 ± .42	7.70 ± .38
7.13 ± .57	6.91 ± .56	7.26 ± .58	6.87 ± .44	7.31 ± .40	6.89 ± .27	6.94 ± .27	6.96 ± .32	6.93 ± .33	7.29 ± .35
8.28 ± .46	7.68 ± .32	7.76 ± .32	7.32 ± .36	7.83 ± .38	7.54 ± .33	7.50 ± .37	7.55 ± .39	7.48 ± .40	7.69 ± .48
7.63 ± .24	7.28 ± .14	7.55 ± .17	7.31 ± .26	7.44 ± .20	7.16 ± .13	7.16 ± .15	7.06 ± .16	7.04 ± .30	7.10 ± .10

Table 71

Mean weekly packed cell volumes (%) of the 7 groups of calves

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	32.5 ± .87	33.0 ± .81	32.7 ± .85	34.0 ± .41	33.2 ± .48	33.2 ± .63	30.7 ± .48	32.7 ± 1.03	33.7 ± .95	34.5 ± 1.04	32.7 ± .85
2	31.7 ± 1.31	34.5 ± .64	32.0 ± .71	35.0 ± .41	34.2 ± .25	33.5 ± .50	32.7 ± .48	32.2 ± .48	34.7 ± 1.31	34.2 ± .75	32.7 ± 1.03
3	32.5 ± 1.44	32.2 ± 1.55	33.0 ± 1.35	34.0 ± .71	32.2 ± .75	32.0 ± 1.29	30.7 ± .85	31.7 ± .48	33.0 ± 1.08	33.0 ± .41	31.2 ± 1.25
4	32.7 ± 1.75	34.2 ± 1.25	33.2 ± .63	35.7 ± 1.93	34.0 ± 1.47	32.5 ± 1.32	32.7 ± 1.25	33.5 ± 2.10	36.5 ± .29	34.7 ± 1.31	34.0 ± 1.73
5	30.7 ± 1.70	32.7 ± 2.50	33.2 ± 1.93	35.5 ± .64	32.5 ± 1.32	32.0 ± .71	32.0 ± 1.58	34.2 ± 1.31	35.2 ± .95	33.0 ± 1.08	32.5 ± .87
6	31.5 ± .64	33.0 ± .82	32.5 ± 1.19	32.0 ± 1.08	33.0 ± .82	31.2 ± .48	31.7 ± .48	31.5 ± .87	32.7 ± .48	32.5 ± .95	30.5 ± .64
7	31.7 ± 1.11	32.5 ± .64	32.2 ± .48	33.2 ± .85	31.0 ± .91	30.7 ± .48	31.2 ± .48	32.7 ± .86	32.7 ± 1.55	34.5 ± 1.88	32.5 ± 1.19

during Experiment 3

11	12	13	14	15	16	17	18	19	20
32.0 ± .41	32.7 ± .48	31.0 ± .00	30.0 ± .41	31.0 ± .41	31.0 ± .41	31.7 ± .48	32.7 ± .48	30.7 ± .26	33.7 ± .85
33.2 ± 1.65	34.2 ± .75	33.7 ± .75	31.7 ± 1.25	32.7 ± .95	31.7 ± .85	30.7 ± 1.11	34.0 ± .82	30.5 ± 1.32	33.5 ± 1.32
31.7 ± .75	31.2 ± 1.03	32.5 ± 1.32	32.2 ± 1.93	33.0 ± .41	30.0 ± .71	31.7 ± .48	32.5 ± .87	30.0 ± 1.29	32.5 ± 1.26
33.0 ± 1.58	34.0 ± 1.47	33.2 ± 1.11	33.2 ± 1.65	32.7 ± 1.60	32.7 ± 1.75	32.5 ± 1.04	33.2 ± 1.75	31.5 ± 1.44	33.5 ± 1.66
30.5 ± 1.19	33.5 ± 1.85	33.7 ± 2.14	32.7 ± 1.11	34.5 ± 1.87	30.5 ± .50	31.2 ± .25	32.0 ± .41	31.2 ± .25	34.5 ± 1.55
32.2 ± 1.25	32.2 ± .63	31.0 ± .41	2.90 ± .58	30.7 ± 1.11	30.2 ± 1.03	30.2 ± .48	31.0 ± 1.08	29.7 ± 1.44	32.0 ± 1.35
31.7 ± .25	30.2 ± .95	31.0 ± 1.08	30.5 ± .64	30.7 ± .48	30.7 ± 1.03	29.7 ± 1.11	30.2 ± 1.11	30.2 ± 1.70	31.5 ± .64

Table 72

Mean weekly haemoglobin concentrations (gms/100 ml.) of the 7 groups

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	12.8 ± .30	12.5 ± .07	12.3 ± .26	13.2 ± .19	12.6 ± .12	12.1 ± .19	12.1 ± .23	12.7 ± .43	13.1 ± .30	12.5 ± .20	12.4 8 .24
2	11.9 ± .56	12.4 ± .25	12.0 ± .56	13.2 ± .25	12.7 ± .13	12.5 ± .29	12.6 ± .11	12.5 ± .24	13.0 ± .42	13.1 ± .39	12.8 ± .43
3	11.8 ± .48	11.7 ± .72	12.4 ± .45	12.7 ± .43	12.1 ± .16	11.5 ± .50	11.7 ± .25	12.2 ± .54	12.6 ± .27	12.7 ± .11	12.0 ± .39
4	12.5 ± .52	12.4 ± .37	12.5 ± .41	13.0 ± .68	12.7 ± .56	12.1 ± .42	12.6 ± .45	12.8 ± .69	12.8 ± .40	13.2 ± .55	12.5 ± .44
5	11.9 ± .78	12.2 ± 1.06	11.6 ± .69	13.0 ± .67	12.3 ± .50	12.1 ± .57	12.4 ± .65	13.0 ± .59	13.0 ± .72	13.1 ± .41	12.8 ± .54
6	12.3 ± .53	12.7 ± .49	12.2 ± .39	12.9 ± .44	12.4 ± .27	11.7 ± .25	12.30 ± .45	11.9 ± .45	12.8 ± .32	12.6 ± .42	12.2 ± .38
7	12.4 ± .20	12.3 ± .12	12.2 ± .12	12.4 ± .26	12.0 ± .27	11.4 ± .23	11.9 ± .06	12.5 ± .23	12.9 ± .51	12.6 ± .46	12.8 ± .41

of calves during Experiment 3

11	12	13	14	15	16	17	18	19	20
12.6 ± .28	12.2 ± .16	12.3 ± .08	12.1 ± .06	12.5 ± .09	12.4 ± .07	12.4 ± .07	12.6 ± .10	11.7 ± .17	12.9 ± .44
12.9 ± .35	13.2 ± .36	13.2 ± .16	12.5 ± .20	12.9 ± .40	12.6 ± .21	12.2 ± .26	13.1 ± .35	12.1 ± .50	13.2 ± .23
13.1 ± .66	11.9 ± .74	12.3 ± .62	12.0 ± .32	13.0 ± .11	11.8 ± .27	12.4 ± .31	12.4 ± .54	11.8 ± .44	12.5 ± .64
12.5 ± .68	12.5 ± .45	12.7 ± .43	12.5 ± .58	12.9 ± .71	12.6 ± .57	12.5 ± .43	13.1 ± .82	12.3 ± .54	12.8 ± .61
13.3 ± .47	12.9 ± .78	12.9 ± .72	12.7 ± .51	13.9 ± .59	12.5 ± .35	12.4 ± .23	12.9 ± .41	12.2 ± .26	13.3 ± .26
12.7 ± .50	12.4 ± .32	12.2 ± .15	11.7 ± .36	13.1 ± .50	12.2 ± .54	12.2 ± .07	12.5 ± .64	12.1 ± .59	12.7 ± .49
12.2 ± .30	11.9 ± .37	12.1 ± .22	12.2 ± .04	12.5 ± .29	12.0 ± .38	11.7 ± .38	12.3 ± .30	12.2 ± .62	12.3 ± .21

Table 73

Mean weekly mean corpsucular volumes (cu.microns) of the 7 groups

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	37.9 ± .85	40.6 ± .53	38.3 ± .70	37.6 ± .52	41.4 ± .84	38.3 ± .83	37.7 ± 1.02	38.1 ± 1.12	39.4 ± 1.24	42.0 ± 1.46	40.0 ± 1.44
2	38.4 ± 1.10	43.0 ± .95	39.7 ± .34	40.2 ± .79	41.9 ± .41	40.0 ± .83	37.8 ± .94	38.2 ± .94	40.3 ± .31	41.0 ± 1.05	39.2 ± 1.58
3	41.5 ± 1.69	43.2 ± 1.35	40.6 ± .79	42.5 ± 2.00	41.4 ± .56	42.2 ± 1.42	38.7 ± 2.24	40.3 ± 1.27	41.9 ± 2.00	43.2 ± 2.03	40.8 ± 2.56
4	40.5 ± 2.66	43.4 ± 1.60	40.3 ± 1.64	42.5 ± .62	42.9 ± .50	40.9 ± .70	39.6 ± 1.00	39.1 ± .51	44.1 ± 1.29	42.1 ± 1.60	42.0 ± 1.27
5	37.0 ± .91	44.1 ± 1.56	45.0 ± .95	47.3 ± 1.69	46.3 ± .94	44.0 ± .69	44.2 ± 1.53	46.8 ± .63	47.6 ± 1.22	46.7 ± 2.14	45.3 ± 1.30
6	38.0 ± .87	39.6 ± 1.60	39.0 ± 2.24	38.1 ± 1.75	41.4 ± 1.97	39.6 ± 2.05	40.4 ± 1.89	40.0 ± 2.02	39.7 ± 2.14	41.0 ± 2.76	38.8 ± 1.51
7	39.5 ± 1.55	41.4 ± 1.12	40.7 ± 1.47	43.1 ± 1.93	41.1 ± 1.49	40.4 ± .74	40.5 ± 1.29	40.3 ± 1.76	39.9 ± 1.42	43.6 ± 1.99	40.0 ± 1.53

of calves during Experiment 3

11	12	13	14	15	16	17	18	19	20
38.6 ± .82	42.5 ± 1.83	39.2 ± 2.37	39.4 ± 1.33	38.8 ± 1.29	40.7 ± 1.31	40.8 ± 1.81	42.2 ± 1.71	41.9 ± 1.67	43.5 ± 1.74
39.5 ± .59	43.4 ± 1.36	40.9 ± 1.02	40.2 ± 1.44	40.7 ± 1.16	40.8 ± 1.29	39.2 ± .25	42.8 ± 1.36	39.8 ± 1.55	43.1 ± .43
39.7 ± 1.69	43.2 ± 1.72	42.5 ± 1.53	53.2 ± 2.89	41.8 ± 1.46	41.4 ± 1.04	41.2 ± 1.61	43.3 ± 1.68	42.8 ± .97	44.4 ± 1.11
41.0 ± .45	43.6 ± .50	41.5 ± 1.32	42.0 ± .84	42.0 ± 1.12	42.0 ± 1.07	42.2 ± 1.17	43.9 ± .70	43.5 ± 1.77	43.5 ± 1.18
43.3 ± 2.44	48.7 ± 1.32	46.6 ± .91	47.9 ± 1.74	47.4 ± 1.69	44.4 ± 1.13	45.2 ± 1.30	45.5 ± 2.05	45.7 ± 1.90	44.9 ± 2.04
39.3 ± 2.76	42.2 ± 2.16	40.1 ± 1.26	39.8 ± 1.89	39.6 ± 2.59	40.2 ± 1.63	39.9 ± 2.38	41.2 ± 1.26	39.7 ± 2.60	42.0 ± 2.74
41.7 ± 1.42	41.6 ± 1.30	41.1 ± 1.52	41.7 ± 1.46	41.4 ± 1.12	42.9 ± 1.01	41.5 ± 1.02	40.3 ± 2.35	43.1 ± 1.53	46.2 ± 1.36

Table 74

Mean weekly mean corpuscular haemoglobin concentrations (%) of

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	39.6 ± .86	38.0 ± .75	37.6 ± .38	39.0 ± .73	37.9 ± .84	37.7 ± .27	39.3 ± .62	38.8 ± .90	38.9 ± .85	36.0 ± .98	36.6 ± 1.49
2	37.6 ± .33	36.0 ± .30	37.4 ± .97	37.7 ± .58	37.2 ± .29	37.5 ± 1.20	38.5 ± .77	38.8 ± .53	37.5 ± .55	38.1 ± .59	39.0 ± .30
3	36.2 ± .40	36.2 ± 1.10	37.6 ± .64	37.5 ± .75	37.6 ± .57	36.1 ± .47	38.3 ± 1.17	38.6 ± .53	38.2 ± 1.03	38.5 ± .40	38.5 ± .60
4	38.3 ± .71	36.4 ± .76	37.8 ± 1.18	36.4 ± .15	37.3 ± .37	37.1 ± .39	38.4 ± .72	38.4 ± .47	35.2 ± 1.17	38.2 ± 1.19	36.9 ± .92
5	38.1 ± .27	37.2 ± .52	35.0 ± 1.28	36.5 ± 1.45	37.9 ± .31	37.7 ± .78	38.7 ± .77	37.8 ± .30	36.9 ± 1.06	39.9 ± 2.17	39.5 ± 1.15
6	39.0 ± 1.09	38.6 ± 1.29	38.0 ± .99	40.7 ± 2.69	37.7 ± .41	37.5 ± .37	38.7 ± .85	37.8 ± .47	39.0 ± 1.07	39.0 ± .60	39.9 ± .85
7	39.1 ± .90	37.9 ± .40	37.9 ± .76	37.3 ± .43	38.6 ± .59	37.0 ± .26	38.2 ± .45	38.1 ± .73	39.3 ± .69	36.6 ± 1.17	39.6 ± 1.02

the 7 groups of calves during Experiment 3

11	12	13	14	15	16	17	18	19	20
39.3 ± .73	37.2 ± .98	39.6 ± .28	40.2 ± .44	40.5 ± .31	40.1 ± .35	39.1 ± .49	38.6 ± 1.34	37.9 ± .59	38.2 ± .63
38.9 ± .81	38.5 ± .43	39.2 ± .47	40.3 ± 1.04	39.4 ± .47	39.9 ± .53	40.1 ± 1.26	38.7 ± .78	39.6 ± .48	39.5 ± .91
41.1 ± 1.52	38.0 ± 1.29	37.9 ± .42	37.5 ± 1.86	39.4 ± .35	39.5 ± .54	39.0 ± .54	38.2 ± .74	39.1 ± .36	38.3 ± .70
37.9 ± .69	36.8 ± .69	38.3 ± .53	37.7 ± .22	39.3 ± .49	38.6 ± .74	38.5 ± .42	39.4 ± .67	37.7 ± .80	38.4 ± .36
40.9 ± 2.07	38.6 ± .32	38.2 ± .49	38.7 ± .52	40.2 ± .75	41.0 ± .51	39.7 ± .99	40.4 ± 1.75	38.9 ± .48	38.7 ± 1.16
39.4 ± .24	37.3 ± 1.52	39.5 ± .47	40.4 ± .67	42.5 ± .63	40.2 ± .84	40.4 ± .92	40.4 ± 1.15	41.1 ± .27	39.7 ± .31
40.0 ± 1.62	39.4 ± .49	39.0 ± 1.16	40.0 ± .79	40.7 ± .83	39.0 ± .48	39.6 ± .58	40.6 ± .90	40.2 ± .58	39.2 ± .97

Table 75

Mean serum albumin level (gms/100 ml.) of the 7 groups of calves during Experiment 3.

	Weeks										
Group	0	2	4	6	8	10	12	14	16	18	20
1	2.26 ± .09	2.31 ± .06	2.25 ± .00	2.26 ± .03	2.08 ± .08	2.02 ± .09	1.93 ± .05	2.02 ± .50	1.99 ± .03	1.97 ± .04	1.94 ± .04
2	2.23 ± .12	2.11 ± .16	2.03 ± .04	2.16 ± .06	1.97 ± .07	1.95 ± .06	2.10 ± .05	2.05 ± .14	2.13 ± .11	1.98 ± .08	1.98 ± .04
3	2.42 ± .11	2.37 ± .16	1.86 ± .07	1.91 ± .09	2.06 ± .03	1.96 ± .06	1.93 ± .06	2.07 ± .07	2.13 ± .07	1.90 ± .05	2.14 ± .06
4	2.38 ± .10	2.70 ± .14	2.09 ± .09	2.06 ± .15	2.09 ± .16	2.03 ± .15	1.92 ± .03	1.98 ± .04	2.12 ± .08	2.13 ± .10	2.18 ± .19
5	2.21 ± .13	2.41 ± .14	2.17 ± .12	2.15 ± .11	2.06 ± .05	1.96 ± .09	1.83 ± .12	2.13 ± .03	2.31 ± .22	2.13 ± .04	2.22 ± .05
6	2.25 ± .17	2.54 ± .08	1.98 ± .07	1.99 ± .11	2.06 ± .13	2.02 ± .06	1.99 ± .11	1.91 ± .07	1.96 ± .03	2.03 ± .09	2.29 ± .18
7	2.17 ± .06	2.53 ± .20	2.09 ± .13	2.15 ± .09	2.20 ± .04	2.15 ± .13	2.04 ± .07	1.89 ± .05	2.07 ± .09	2.11 ± .05	2.02 ± .07

Table 76

Mean total serum globulin level (gms/100 ml.) of the 7 groups of calves during Experiment 3.

	Weeks										
Group	0	2	4	6	8	10	12	14	16	18	20
1	4.56 ± .80	4.24 ± .14	4.28 ± .22	3.97 ± .11	4.52 ± .14	4.58 ± .09	4.30 ± .21	4.88 ± .06	4.53 ± .14	4.91 ± .05	4.95 ± .15
2	3.98 ± .20	4.14 ± .17	4.36 ± .16	4.44 ± .16	4.60 ± .15	4.52 ± .33	4.25 ± .23	4.31 ± .20	4.59 ± .07	4.81 ± .28	4.92 ± .22
3	3.77 ± .12	4.16 ± .11	4.42 ± .16	4.36 ± .10	4.37 ± .17	4.45 ± .17	4.51 ± .20	4.37 ± .22	4.58 ± .19	3.66 ± .15	4.52 ± .13
4	3.53 ± .14	3.55 ± .14	4.08 ± .04	4.07 ± .04	3.62 ± .23	3.84 ± .22	4.24 ± .07	4.24 ± .09	4.11 ± .15	4.14 ± .09	4.13 ± .08
5	4.10 ± .24	4.11 ± .29	4.09 ± .17	4.36 ± .11	4.25 ± .14	4.22 ± .18	4.42 ± .19	4.30 ± .11	4.57 ± .22	4.42 ± .11	4.32 ± .19
6	3.73 ± .17	3.78 ± .27	4.27 ± .14	4.16 ± .15	4.07 ± .11	4.53 ± .07	4.26 ± .07	4.70 ± .15	4.71 ± .19	4.58 ± .18	4.38 ± .14
7	4.05 ± .23	3.79 ± .21	3.96 ± .12	4.22 ± .08	4.32 ± .05	4.26 ± .03	4.45 ± .04	4.26 ± .13	4.59 ± .19	4.53 ± .13	4.66 ± .14

Table 77

Mean weekly serum glutamate oxaloacetic transaminase levels

Group	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	74.2 ± 5.8	81.5 ± 5.4	68.7 ± 6.4	126.0 ± 21.7	88.0 ± 13.4	105.0 ± 17.4	77.7 ± 5.0	64.7 ± 1.9	101.0 ± 9.3	91.2 ± 9.9	96.2 ± 3.6
2	71.2 ± 7.73	82.5 ± 10.6	76.5 ± 4.8	112.0 ± 22.3	78.5 ± 5.7	110.0 ± 16.7	86.0 ± 9.3	69.0 ± .32	90.7 ± 3.6	76.5 ± 3.7	82.7 ± 14.0
3	62.7 ± .75	76.2 ± 1.9	72.0 ± 5.0	103.2 ± 9.8	82.2 ± 6.5	93.2 ± 8.6	75.5 ± 3.7	71.7 ± 1.9	99.2 ± 16.3	72.0 ± 5.8	87.7 ± 13.9
4	75 ± 4.49	74.7 ± 7.0	70.0 ± 5.0	115.7 ± 18.3	77.7 ± 5.4	83.5 ± 6.9	93.5 ± 12.1	91.7 ± 7.9	106.7 ± 21.5	93.2 ± 11.7	97.7 ± 9.7
5	84.2 ± 9.3	78.5 ± 4.5	70.0 ± 3.6	88.2 ± 6.6	81.7 ± 12.4	76.5 ± 5.2	78.7 ± 5.9	78.0 ± 5.0	81.5 ± 4.6	87.2 ± 7.8	89.5 ± 13.3
6	72.2 ± 4.5	66.2 ± 3.1	6.72 ± 7.5	84.2 ± 3.7	69.7 ± 3.8	80.2 ± 6.3	77.0 ± 6.5	84.7 ± 19.7	83.0 ± 7.5	83.5 ± 4.9	76.0 ± 6.7
7	66.7 ± 2.8	64.2 ± 2.6	69.0 ± 6.4	68.7 ± 3.9	67.7 ± 4.7	80.7 ± 14.6	77.5 ± 10.0	63.2 ± 3.5	72.0 ± 2.2	74.7 ± 6.3	72.2 ± 1.6

(S-F units) of the 7 groups of calves during Experiment 3.

11	12	13	14	15	16	17	18	19	20
88.7 ± 1.7	76.5 ± 5,2	91.5 ± 9.0	91.0 ± 13.2	120.0 ± 19.4	105.7 ± 18.9	116.0 ± 16.8	91.7 ± 4.8	113.7 ± 5.5	95.5 ± 12.2
87.7 ± 23.9	84.7 ± 5.1	82.0 ± 7.2	90.2 ± 7.3	122.7 ± 27.9	95.0 ± 8.6	89.0 ± 10.3	90.2 ± 11.5	93.2 ± 10.4	101.0 ± 14.5
103.0 ± 12.0	83.0 ± 8.1	74.5 ± 3.1	83.0 ± 6.9	107.5 ± 23.3	79.5 ± 3.4	87.5 ± 4.9	79.7 ± 7.2	87.0 ± 1.7	79.5 ± 1.8
105.0 ± 12.0	94.7 ± 6.1	72.2 ± .75	87.7 ± 5.8	88.5 ± 6.0	80.0 ± 5.2	83.5 ± 9.6	76.7 ± 6.3	78.7 ± 9.4	89.7 ± 9.1
8.15 ± 5.4	103.7 ± 8.7	84.0 ± 4.8	97.0 ± 7.0	83.0 ± .49	74.7 ± 7.6	73.7 ± 4.3	71.0 ± 3.5	78.0 ± 5.5	74.5 ± 10.5
81.2 ± 6.0	88.0 ± 16.0	77.0 ± 7.7	78.7 ± 10.8	76.0 ± 8.1	71.2 ± 5.1	73.0 ± 10.1	72.5 ± 7.4	78.0 ± 4.7	70.2 ± 4.3
74.0 ± 4.6	72.2 ± 3.6	78.0 ± 4.9	77.7 ± 4.3	86.0 ± 7.0	100.7 ± 7.3	98.2 ± 12.3	108.5 ± 10.2	93.7 ± 7.5	113.7 ± 27.5

Table 78

Individual fluke burdens recovered from each calf in Experiment 3.

Group	Calf No.	Flukes recovered >12	6-12	< 6	Total
1	1	16	26	2	44
	2	19	12	5	36
	3	20	29	3	52
	4	18	44	8	70
2	5	15	26	3	44
	6	3	3	1	7
	7	25	51	4	80
	8	19	57	11	86
3	9	13	2	-	15
	10	19	10	-	29
	11	2	14	2	18
	12	21	8	2	31
4	13	3	1	4	8
	14	7	12	8	27
	15	-	15	3	18
	16	2	8	9	19
5	17	-	-	-	-
	18	18	3	-	21
	19	11	6	-	17
	20	3	1	-	4
6	21	7	4	-	11
	22	4	2	-	5
	23	2	-	-	2
	24	14	-	-	14
7	25	26	58	4	88
	26	41	54	9	104
	27	17	22	5	44
	28	11	47	6	64

Table 79

Individual erythrocyte counts (millions/cu.mm.) of calves during

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	8.53	8.58	9.21	9.36	8.45	8.91	8.44	8.93	9.26	7.44	8.66
2	8.71	8.2	8.42	9.15	7.95	8.23	7.81	9.66	8.52	8.66	8.16
3	8.42	7.47	7.7	8.52	7.3	7.8	7.63	7.26	7.28	6.99	7.08
4	8.59	8.31	8.91	9.18	8.44	8.77	8.74	8.65	9.41	8.93	9
5	8.52	7.79	8.34	8.62	8.32	8.24	9.04	8.6	9.06	8.1	8.51
6	8.27	8.29	7.58	8.95	8.12	8.53	8.69	8.15	8.12	8.19	7.93
7	7.92	7.9	8.19	8.6	7.93	8.75	9.02	8.89	9.19	9.21	9.55
8	8.3	8.12	8.10	8.63	8.36	7.96	7.99	8.11	8.12	7.96	7.53
9	7.8	6.3	8.17	6.63	7.46	7.0	7.56	7.28	7.4	7.55	7.31
10	8.3	8.58	8.82	8.77	8.15	8.6	8.53	8.37	8.41	8.21	8.73
11	8.12	8.16	8.69	8.96	8.38	8.46	8.92	8.67	8.86	8.42	8.75
12	7.12	8.96	6.91	7.87	7.22	6.45	6.99	7.28	7.16	6.59	6.19
13	7.75	7.19	7.51	7.64	7.1	7.15	8.04	7.35	8.04	7.52	7.53
14	8.66	8.29	8.85	9.08	8.69	8.58	9.06	9.77	8.52	8.92	8.75
15	8.23	7.72	7.93	7.77	7.47	7.59	7.44	8.19	7.83	7.99	7.71
16	7.72	8.45	8.89	9.12	8.65	8.46	8.58	8.93	8.91	8.63	8.36
17	8.3	7.2	7.32	7.11	6.59	7.02	6.8	6.58	6.74	6.65	6.98
18	8.96	8.91	8.44	8.55	7.55	7.99	8.2	8.25	8.53	7.67	8.23
19	8.31	7.1	7.38	7.64	8.32	7.16	7.27	7.27	7.18	7.19	7.3
20	7.55	6.42	6.39	6.88	6.59	6.94	6.73	7.2	7.27	6.83	6.53
21	8.41	8.1	7.93	8.37	8.11	7.55	8.03	7.77	8.13	7.67	7.62
22	7.83	7.6	8.02	7.74	6.71	6.85	7.42	7.2	7.42	6.78	7.36
23	8.39	8.91	8.93	9.06	8.62	8.39	7.28	8.37	8.53	8.39	8.11
24	8.55	8.85	8.59	8.79	8.67	9.34	8.86	8.32	9.15	8.93	8.42
25	8.3	8.01	7.81	7.73	7.51	7.83	7.48	8.23	8.58	8.23	8.74
26	7.53	7.74	8.11	8.19	8.02	7.71	7.73	8.13	8.05	8.16	8.16
27	8.65	8.14	8.36	7.6	7.24	7.55	8.17	8.71	8.75	7.72	8.36
28	7.71	7.5	7.49	7.3	7.42	7.33	7.52	7.48	7.48	7.56	7.31

Experiment 3

11	12	13	14	15	16	17	18	19	20
8.38	8.19	9.06	8.17	8.56	7.97	8.05	8.63	8.11	8.08
8.19	7.56	7.91	7.65	8	7.28	7.68	7.87	7.79	7.68
7.63	6.91	6.8	6.72	7.08	7.19	7.23	6.8	6.54	7.49
8.98	8.29	8.16	8.04	8.38	8.12	8.25	7.93	7.31	7.81
8.15	7.81	7.8	8.56	7.81	7.43	7.36	7.96	7.77	7.42
8.2	7.46	8.16	7.83	7.53	7.65	7.53	7.8	6.91	7.31
9.51	8.77	8.93	8.93	8.97	8.93	8.81	8.53	8.46	8.69
7.75	7.58	8.11	7.31	7.92	7.22	7.68	7.56	7.71	7.68
2.52	6.69	7.43	7.43	8.15	7.39	7.91	7.42	7.15	7.6
8.63	8.02	8.48	7.62	7.81	7.24	8.12	8.16	7.6	8.02
9.22	8.1	8.7	8.24	8.51	8.03	8.15	8.11	7.63	7.62
6.89	6.22	6.22	6.63	7.16	6.42	6.78	6.5	6.3	6.08
7.48	7.38	7.28	6.71	6.84	7.24	7.04	6.46	6.68	6.72
8.78	8.25	8.38	8.48	8.19	8.27	8.25	8.69	8.1	8.6
7.49	7.31	8.15	8.21	7.6	7.26	7.45	7.47	7.02	7.68
8.39	8.19	8.27	8.32	8.59	8.34	8.08	7.72	8.44	7.81
7.05	6.41	6.31	6.32	6.54	6.36	6.35	6.28	6.27	6.78
8.58	8.6	8.93	8.15	8.42	7.64	7.63	7.83	7.73	8.23
7.11	6.25	7.18	6.25	7.31	6.66	6.76	6.83	6.52	6.72
8.77	6.2	6.64	6.77	6.98	6.91	7.04	6.91	7.2	7.43
8.37	7.52	7.36	6.83	7.72	7.55	6.85	7.63	7.35	7.77
6.99	6.85	7.08	6.64	6.83	6.65	6.88	6.44	6.38	6.41
8.66	8.24	8.2	8.21	8.59	8.21	8.78	8	8.07	8.73
9.12	8.1	8.42	7.62	8.2	7.76	8.11	8.15	8.11	7.84
7.33	7.42	7.6	7	7.26	7.35	7.44	7.07	7.81	6.99
7.8	7.0	7.24	7.44	8.03	9.42	7.33	7.48	6.34	7.36
8.23	7.6	8.0	8.59	7.31	6.88	7.11	6.68	6.95	7.27
7.16	7.11	7.36	7.22	7.15	6.98	6.77	7.02	7.05	6.95

Table 80

Individual packed cell volumes (%) of calves during

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11
1	32	34	35	34	34	32	30	33	35	34	32	32
2	35	34	32	34	33	32	31	35	34	37	33	32
3	32	31	31	33	32	31	30	30	31	32	31	31
4	31	33	33	35	34	34	32	33	35	35	35	33
5	34	35	30	35	34	33	34	33	37	33	33	33
6	32	34	30	34	34	35	33	31	32	33	30	31
7	28	33	32	35	34	33	32	32	37	36	35	38
8	33	36	30	36	35	33	32	33	33	35	33	31
9	29	38	34	32	31	31	33	31	30	33	32	31
10	36	34	34	35	33	35	30	32	35	34	34	33
11	33	35	35	35	34	33	31	33	33	33	31	33
12	32	32	29	34	31	29	29	31	34	32	28	30
13	31	34	33	33	31	30	34	29	37	35	33	30
14	34	37	32	38	37	36	34	38	36	37	39	37
15	29	31	33	32	32	31	29	31	36	31	31	31
16	37	35	35	40	36	33	34	36	37	36	33	34
17	31	34	35	35	32	32	33	32	34	32	32	32
18	34	38	37	36	35	34	36	38	38	31	35	31
19	32	33	33	37	34	31	30	34	34	36	32	32
20	26	26	28	34	29	31	29	33	35	33	31	27
21	33	33	33	29	35	32	33	34	32	35	32	35
22	31	33	34	32	31	30	32	31	34	31	30	32
23	30	32	29	33	33	31	31	30	32	32	29	29
24	32	34	34	34	33	32	31	31	33	31	31	33
25	31	34	33	34	31	32	32	35	37	39	36	32
26	29	33	31	35	29	30	30	31	30	34	31	31
27	33	31	32	31	33	30	31	32	33	30	31	32
28	34	32	33	33	34	31	32	33	31	35	32	32

Experiment 3

12	13	14	15	16	17	18	19	20
32	31	31	31	30	31	34	31	32
34	31	30	31	31	32	33	31	33
32	31	29	30	31	33	32	30	36
33	31	30	32	32	31	32	31	34
35	33	31	33	32	29	36	33	32
32	32	29	30	30	30	32	27	31
35	35	35	34	34	34	34	30	37
35	35	32	34	31	30	34	32	34
29	32	31	33	31	32	32	29	33
33	34	38	32	30	33	35	33	35
33	35	30	34	31	31	32	31	33
30	29	30	33	28	31	31	27	29
32	32	29	30	31	32	29	29	31
37	36	37	36	37	35	37	34	38
31	31	33	30	29	30	32	29	31
36	34	34	35	34	33	35	34	34
31	31	32	34	30	31	33	31	34
39	40	36	37	32	31	31	32	39
32	33	32	34	30	31	32	31	33
32	31	31	33	30	32	32	31	32
34	31	30	34	33	29	33	34	36
32	30	28	30	28	31	28	28	30
32	31	30	29	30	30	31	28	31
31	32	28	30	30	31	31	29	31
33	34	31	32	33	33	31	35	32
29	29	29	31	31	29	33	29	30
29	30	30	30	28	29	29	27	31
30	31	32	30	31	28	28	30	33

Table 81

Individual haemoglobin concentrations (gms/100ml.) of calves

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	12.9	12.7	13.1	12.7	12.4	12.3	11.8	12.4	12.9	12.0	12.3
2	13.2	12.4	12.0	13.3	12.4	12.0	11.7	14.0	13.0	12.9	12.1
3	13.3	12.4	12.0	13.5	12.9	11.7	12.2	12.2	12.6	12.4	12.1
4	12.0	12.6	12.2	13.5	12.6	12.6	12.7	12.2	14.0	12.7	13.1
5	12.7	12.3	12.7	12.6	12.9	12.0	12.6	12.4	13.8	12.9	12.7
6	12.2	12.4	10.5	13.1	12.4	12.2	12.3	11.9	11.9	12.2	11.8
7	10.3	11.9	11.8	13.3	12.7	12.7	12.7	12.8	13.5	14.1	13.9
8	12.6	13.1	13.0	13.8	13.0	13.3	12.8	13.0	12.9	13.1	12.7
9	10.8	9.9	12.9	11.7	11.8	11.6	11.6	12.2	12.3	12.5	12.1
10	13.0	13.4	13.1	13.8	12.5	12.6	12.2	12.6	13.4	13.0	12.7
11	12.0	12.0	12.5	12.6	12.2	11.8	12.1	12.7	12.3	12.6	12.4
12	11.3	11.5	11.1	12.9	11.9	10.2	11.1	11.5	12.3	12.7	10.9
13	12.0	11.8	11.8	12.2	11.35	11.1	12.4	11.4	12.4	12.2	11.9
14	13.4	13.4	13.1	13.8	13.6	13.0	13.5	14.5	13.8	14.6	13.6
15	11.3	11.9	11.9	11.6	12.2	11.7	11.4	12.1	12.0	12.5	11.7
16	13.4	12.7	13.4	14.5	13.6	12.5	13.0	13.4	13.2	13.7	12.9
17	11.9	12.4	12.2	12.9	12.4	11.9	12.1	12.1	12.3	12.5	12.9
18	13.5	14.7	12.0	14.4	13.1	13.8	14.2	14.7	15.2	14.3	13.8
19	12.4	12.3	12.7	13.5	12.9	11.9	12.1	12.8	12.4	13.0	13.4
20	9.8	9.5	9.6	11.2	10.9	11.1	11.1	12.3	12.3	12.6	11.3
21	13.4	13.9	12.9	13.8	13.2	12.3	13.6	13.2	13.5	13.8	12.7
22	12.7	12.7	12.9	13.6	12.0	11.1	12.2	11.9	13.0	12.3	12.7
23	10.9	11.5	11.3	12.1	12.1	11.7	11.8	11.2	12.0	11.9	11.1
24	12.2	12.9	11.9	12.3	12.4	11.8	11.6	11.4	12.6	12.3	12.2
25	12.5	12.6	12.3	12.8	12.4	12.0	12.1	13.0	14.0	13.7	13.9
26	11.8	12.3	12.2	12.9	12.1	11.2	11.8	12.1	12.0	13.0	12.9
27	12.7	12.0	12.5	11.9	11.2	10.9	11.9	12.7	13.4	11.7	12.7
28	12.5	12.3	11.9	12.0	12.2	11.5	11.9	12.1	12.0	12.0	11.9

during Experiment 3

11	12	13	14	15	16	17	18	19	20
12.1	12.1	12.2	12.1	12.7	12.2	12.4	12.9	11.7	11.7
12.3	11.8	12.5	12.2	12.5	12.5	12.3	12.6	12.2	13.1
12.6	12.6	12.3	11.9	12.3	12.5	12.6	12.6	11.4	13.8
13.4	12.2	12.1	12.1	12.7	12.5	12.3	12.4	11.7	13.0
12.9	13.2	12.9	12.6	13.2	12.9	12.4	14.1	13.0	13.1
12.3	12.2	13.0	12.3	11.8	12.3	11.5	12.9	10.7	12.6
13.9	13.9	13.5	13.1	13.7	13.1	12.7	13.1	12.3	13.7
12.5	13.5	13.5	12.2	13.0	12.3	12.4	12.4	12.5	13.4
13.4	10.5	12.0	11.6	13.2	11.8	12.1	12.1	11.5	12.6
14.6	13.8	13.2	12.5	12.8	12.2	13.3	13.8	12.9	14.1
12.9	12.3	13.4	12.6	13.2	12.3	12.2	12.6	11.9	12.2
11.4	11.0	10.7	11.3	12.8	11.1	11.9	11.2	10.8	11.0
11.8	12.2	11.9	11.0	11.4	11.6	12.0	11.2	11.4	11.7
14.3	12.9	13.9	13.8	14.1	14.0	13.7	14.5	13.4	14.5
11.2	11.4	12.3	12.6	11.9	11.8	11.8	12.3	11.3	12.2
12.8	13.5	12.9	12.7	14.1	13.1	12.6	14.5	13.0	13.0
12.2	12.1	11.7	12.0	13.2	12.3	11.9	12.1	12.0	13.5
14.5	15.3	14.9	14.2	15.6	13.5	12.9	13.8	13.0	13.9
13.1	12.3	13.0	12.2	13.8	12.3	12.7	13.4	12.2	12.7
13.3	12.1	12.0	12.3	13.0	11.9	12.2	12.3	11.8	13.1
13.9	13.4	12.5	12.7	14.5	13.8	12.4	14.3	13.8	14.1
12.4	12.3	12.1	11.1	13.0	11.6	12.1	11.3	11.1	11.9
11.5	12.1	11.9	11.8	12.6	11.5	12.2	12.2	11.6	12.6
13.0	12.0	12.5	11.3	12.2	11.8	12.1	12.2	11.8	12.2
13.1	13.0	12.6	12.2	12.6	12.7	12.7	12.6	14.0	12.5
11.8	11.4	12.1	12.2	13.2	12.5	11.9	12.9	11.5	12.6
12.2	11.8	12.1	12.1	11.8	11.0	11.5	11.5	11.3	11.7
11.9	11.5	11.5	12.3	12.5	11.8	10.9	12.1	12.0	12.5

Table 82

Individual mean corpuscular volumes (cu.microns) of calves

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10
1	37.5	39.6	38.0	36.3	40.2	35.9	35.5	37.0	37.7	40.3	37
2	40.2	41.5	38.0	37.2	41.5	38.9	39.7	36.2	40.0	42.7	40.4
3	38.0	41.5	40.3	38.7	43.8	39.7	39.3	41.3	42.6	45.8	43.8
4	36.1	39.7	37	38.1	40.3	38.8	36.6	38.1	37.2	39.2	38.9
5	39.9	44.9	39.6	40.6	40.9	40.0	37.6	38.4	40.8	40.7	38.8
6	38.6	41.0	39.6	38.0	41.9	41	38	38	39.4	40.3	37.8
7	35.3	41.8	39.1	40.7	42.9	37.7	35.5	36	40.3	39.1	36.6
8	39.8	44.3	40.7	41.7	41.9	41.4	40.1	40.6	40.6	44	43.8
9	37.2	44.4	41.6	47.9	41.6	44.3	43.6	42.5	40.5	43.7	43.8
10	43.4	39.6	38.5	39.9	40.5	40.7	35.2	38.2	41.6	41.4	38.9
11	40.6	42.9	40.3	39.1	40.6	39	34.7	38.1	38.1	39.1	35.4
12	44.9	45.9	42	43.2	42.9	44.9	41.5	42.6	47.5	48.6	45.2
13	39.8	47.3	43.9	43.2	43.7	41.9	42.3	39.5	46	46.5	43.8
14	39.3	44.6	36.2	41.8	43.7	42	37.5	38.9	42.3	41.4	44.6
15	35.2	40.2	41.6	41.2	42.8	40.8	39	37.9	46	38.8	40.2
16	47.9	41.4	39.4	43.9	41.6	39	39.6	40.3	41.5	41.7	39.5
17	37.3	47	47.8	49.2	48.6	45.6	48.5	48.6	50.4	48.1	47.6
18	37.9	42.6	43.8	42.3	46.4	42.5	43.9	46.1	44.5	40.4	42.5
19	38.5	46.5	44.7	48.4	46.4	43.3	41.3	46.8	47.4	50	43.8
20	34.4	40.5	43.8	49.4	44	44.7	43.1	45.8	48.1	48.3	47.5
21	39.2	40.7	41.6	34.6	43.2	42.4	41.1	43.8	39.4	45.6	42
22	39.6	43.4	42.3	42.7	46.2	43.8	43.7	43.1	45.8	45.7	40.8
23	35.8	35.9	32.5	36.4	38.3	36.9	42.6	35.8	37.5	38.1	35.8
24	37.4	38.4	39.6	38.7	38.1	35.4	34.9	37.3	36.1	34.7	36.8
25	37.3	42.4	42.2	43.9	43.9	40.9	42.8	42.5	43.1	47.4	41.2
26	38.5	42.6	38.2	42.7	37.4	38.9	38.6	38.1	37.3	41.7	38
27	38.1	38.1	38.3	40.8	40.1	39.7	37.9	36.7	37.7	38.9	37.1
28	44.1	42.7	44.1	45.2	43.1	42.3	42.6	44.1	41.4	46.3	43.8

during Experiment 3

11	12	13	14	15	16	17	18	19	20
38.2	39	34.2	37.9	36.2	37.6	38.5	39.4	38.2	39.6
39.1	45	39.2	39.2	38.6	42.6	41.7	41.9	40.4	43
40.6	46.3	45.6	43.2	42.4	43.1	45.6	47.1	45.9	48.1
36.7	39.8	38	37.3	38.2	39.4	37.6	40.4	43.1	43.5
40.5	44.8	42.3	41	42.3	43.1	39.4	45.2	42.5	43.1
37.8	42.9	39.2	37	39.8	39.2	39.8	41	39.8	42.4
39.6	39.9	39.2	39.2	37.9	38	38.6	39.9	35.5	42.6
40	46.2	43.1	43.8	42.9	42.9	39.1	45	41.5	44.3
41.2	43.3	43	41.7	40.5	41.9	40.5	43.1	40.6	43
38.2	41.1	40.1	49.9	41	41.4	40.6	42.9	43.7	43.6
35.8	40.7	40.2	36.2	39.6	38.6	38	39.5	40	43.3
43.5	48.2	46.6	45.2	46.1	43.6	45.7	47.7	43.6	47.7
40.1	43.4	44	43.2	43.9	42.8	45.5	44.9	43.4	46.1
42.1	44.8	43	43.6	44	44.7	42.4	42.6	48.6	44.2
41.4	42.4	38	40.2	39.6	39.9	40.3	42.8	41.3	40.4
40.5	43.9	41.1	40.9	40.7	40.8	40.8	45.3	40.9	43.5
45.4	48.5	49.1	50.6	52	47.2	48.8	49.1	50.2	50.1
36.1	45.3	44.8	44.2	43.9	41.9	40.6	39.6	42	47.4
45	49.6	46	51.2	46.5	45	45.9	46.9	47.5	49.1
46.8	51.6	46.7	45.8	47.3	43.4	45.5	36.3	43.1	43.1
41.8	45.2	42.1	43.9	44	43.7	42.3	43.3	46.3	46.3
45.8	46.7	42.4	42.2	43.9	42.1	45.1	43.5	41.5	46.8
33.5	38.8	37.8	36.5	33.8	36.5	34.2	38.8	35.3	35.5
36.2	38.3	38	36.7	36.6	38.7	38.2	39.3	45.8	39.5
43.7	44.5	44.7	44.3	44	44.9	44.4	33.8	44.8	45.8
39.7	41.4	40.1	39	38.6	41.8	39.6	44.1	45.7	48.9
38.9	38.2	37.5	39.5	41	40.7	40.8	43.4	38.8	42.6
44.6	42.2	42.1	44.3	42	44.4	41.4	39.9	43.3	37.5

Table 83

Individual mean corpuscular haemoglobin concentrations (%) of

Calf No.	0	1	2	3	4	5	6	7	8	9	10
1	40.3	37.3	37.4	37.4	36.5	38.4	39.3	37.6	36.9	34.3	38.4
2	37.7	36.5	37.5	39.1	37.6	37.5	37.7	4o	38.2	34.9	36.7
3	41.6	40	38.7	40.9	40.3	37.7	40.7	40.7	40.6	38.7	39.0
4	38.7	38.2	36.9	38.6	37.1	37.1	39.7	37	40	36.3	32.4
5	37.3	35.2	38.5	36	37.9	36.4	37.1	37.6	37.3	39.1	38.5
6	38.1	36.5	35	38.5	36.5	34.8	37.3	38.4	37.2	36.9	39.3
7	36.8	36.1	36.9	38	37.3	38.5	39.7	40	36.5	39.2	39.7
8	38.2	36.4	39.4	38.3	37.1	40.3	4o	39.4	39.1	37.4	38.5
9	37.2	35.4	37.9	36.6	38.1	37.4	35.1	39.3	41	37.9	37.8
10	36	39.4	38.5	39.4	37.9	36	40.7	39.4	38.3	38.2	37.3
11	36.4	34.3	35.7	36	35.9	35.7	39	38.5	37.3	38.2	40
12	35.3	35.9	38.3	37.9	38.4	35.2	38.3	37.1	36.2	39.7	38.9
13	38.7	34.7	35.8	36.9	36.6	37	36.5	39.3	33.5	34.9	36.1
14	39.4	36.2	40.9	36.3	36.8	36.1	39.7	38.2	38.3	39.4	34.9
15	38.9	38.4	36.1	36.3	38.1	37.7	39.3	39.0	33.3	40.3	37.7
16	36.2	36.3	38.3	36.3	37.8	37.8	38.2	37.2	35.7	38.1	39.1
17	38.4	36.5	34.8	36.8	38.8	37.2	36.7	37.8	36.2	39.1	40.3
18	37.7	38.7	32.4	40	37.4	39.4	39.4	38.7	4o	46.1	39.4
19	38.8	37.3	38.5	36.5	37.9	38.4	40.3	37.6	36.5	36.1	41.9
20	37.7	36.5	34.3	32.9	37.6	35.8	38.3	37.3	35.1	38.2	36.5
21	40.6	42.1	39.1	47.6	37.7	38.5	41.2	38.8	42.2	39.4	39.7
22	40.9	38.4	38.9	42.5	38.7	37	38.1	38.4	38.2	39.7	42.3
23	36.3	35.9	38.9	36.7	36.7	37.7	38.1	37.3	37.5	37.2	38.3
24	38.1	37.9	35	36.2	37.6	36.9	37.4	36.8	38.2	39.7	39.4
25	40.3	37.1	37.3	37.6	37.6	37.5	37.8	37.1	37.8	35.1	38.7
26	40.7	37.3	39.3	36.9	40.3	37.3	39.3	39	40	38.2	41.6
27	38.5	38.7	39.1	38.4	38.6	36.3	38.4	39.7	40.9	39	41
28	36.8	38.4	36.1	36.4	38.1	37.1	37.2	36.7	38.7	34.2	37.2

calves during Experiment 3

11	12	13	14	15	16	17	18	19	20
37.8	37.8	39.3	39	41	40.7	40.2	37.9	37.7	36.7
38.4	34.7	40.3	40.7	40.3	40.3	38.4	38.2	38.7	39.8
40.6	39.4	39.7	41	41	40.3	38.2	39.4	38	38.3
40.6	37	39	40.3	39.7	39.1	39.7	38.9	37.3	38.2
39.1	37.7	39.1	40.7	40	40.3	42.9	39.2	39.4	41.1
39.7	38.1	40.6	42.4	39.3	41	38.5	40.3	38.9	40.6
36.6	39.7	38.6	37.4	40.3	38.5	37.5	38.7	41	37
40.3	38.6	38.6	40.7	38.2	39.7	41.5	36.6	39.1	39.4
43.2	36.2	37.5	37.4	40	38.1	37.8	37.8	4o	38.2
44.2	41.8	38.8	32.9	40	40.7	40.3	39.4	38.5	40.3
39.1	37.3	38.3	42	38.8	39.7	39.4	39.4	38.5	37
38	36.7	36.9	37.7	38.8	39.6	38.5	36.3	39.3	37.9
39.3	38.1	37.2	37.9	38	37.4	37.5	38.8	35.5	37.7
38.6	34.9	38.6	37.3	39.2	37.8	39.1	39.2	38.8	38.2
36.1	36.8	39.7	38.2	39.7	40.7	39.3	38.4	39	39.4
37.6	37.5	38.9	37.4	40.3	38.3	38.2	41.4	37.7	38.2
38.1	39	37.7	37.5	38.8	41	38.4	36.7	38.1	39.9
46.8	39.2	37.3	39.4	42.2	42.2	41.6	44.5	40	35.8
40.9	38.4	39.4	38.1	40.6	41	41.1	41.9	39.4	38
49.3	37.8	38.7	39.7	39.4	39.7	37.5	38.4	38.1	41.1
39.7	39.4	40.3	42.3	42.7	41.8	42.9	43.5	41.	39.2
38.8	38.4	40.3	39.6	43.3	41.4	39	40.5	41.9	39.8
39.7	37.8	38.4	39.3	43.5	38.3	40.7	39.4	40.9	40.6
39.4	38.7	39.1	40.4	40.7	39.3	39	38.1	40.7	39.4
40.9	39.4	37.1	39.4	39.4	38.5	38.6	40.6	40	39.1
44.5	39.3	41.7	42.1	42.6	40.3	41.2	39.1	39.7	42
38.1	40.7	40.3	40.3	39.3	39.3	39.8	39.7	41.9	37.9
37.2	38.3	37.1	38.4	41.7	38.1	38.9	43.2	39.3	37.9

Table 84

Individual albumin levels (gms/100 ml.) of calves during Experiment 3

Calf No.	Weeks 0	2	4	6	8	10	12	14	16	18	20
1	2.23	2.27	2.12	2.25	2.34	1.83	1.77	2.05	2.0	1.9	1.94
2	2.15	2.39	2.35	2.24	2.24	2.18	2.09	1.98	1.91	1.94	1.96
3	2.48	2.42	2.41	2.25	2.27	2.12	2.2	1.8	2.16	2.06	1.9
4	2.02	2.04	2.36	2.25	2.2	2.2	2.01	1.91	2	2.02	2.1
5	2.04	2.17	2.37	2.15	2.01	2.07	1.98	2.08	1.99	2.13	1.9
6	2	2.23	2.4	2.03	2.28	2.01	1.77	1.99	1.9	2.06	2.1
7	2.06	1.97	1.91	1.99	2.15	1.77	1.97	2.23	2.07	1.99	1.99
8	2.58	2.57	1.78	1.95	2.22	2.03	2.08	2.11	2.23	1.77	1.95
9	2.13	2.28	2.26	1.98	2.03	2.03	1.78	1.86	1.93	1.8	2.12
10	2.39	2.73	2.44	1.91	2.09	2.16	2.07	1.92	2.02	2.02	2.26
11	2.28	2.22	2.01	1.9	1.8	2.07	1.99	2.1	2.26	1.93	2.21
12	2.36	2.45	2.78	1.65	1.71	2	1.99	1.85	2.07	1.85	1.98
13	2.13	2.6	2.67	2.26	1.98	2.05	2.17	1.97	2.03	1.97	1.94
14	2.22	2.37	2.58	2.05	2.4	2.51	2.35	1.97	1.99	2.23	2.38
15	2.28	2.44	2.44	2.21	2.15	1.7	1.65	1.86	1.86	1.95	2.27
16	2.16	2.13	3.12	1.86	1.7	2.1	1.96	1.89	2.06	2.37	2.15
17	2.44	2.37	2.72	1.9	1.94	1.98	2.15	1.78	2.18	2.16	2.3
18	2.04	2.43	2.56	2.48	2.33	2.18	2.07	2.17	2.14	2.09	2.3
19	1.86	2.24	2.36	2.2	1.96	2.07	1.86	1.69	2.03	2.23	2.15
20	2.07	1.85	2.04	2.12	2.36	2	1.77	1.67	2.19	2.05	2.17
21	2.2	1.92	2.47	2.09	2.34	2.13	2.14	1.74	2.05	2.04	2
22	2.3	2.31	2.65	2.1	1.9	1.86	1.98	1.99	1.97	1.81	1.76
23	1.92	2.08	2.33	1.81	1.88	1.85	1.87	1.99	1.7	2.01	2.02
24	2.18	2.7	2.7	1.93	1.84	2.39	2.11	2.26	1.94	2.26	2.32
25	2.36	2.08	2.89	2.17	2.3	2.33	2.49	2.23	1.9	2.25	2.7
26	1.92	2.36	2.87	2.31	1.97	2.15	1.86	2.01	2.02	2.13	2.2
27	2.38	2.09	2.18	2.18	2.29	2.13	2.05	2.04	1.88	2	2.05
28	2.21	2.16	2.2	1.72	2.03	2.18	2.19	1.9	1.78	2.08	2.46

Table 85

Individual total serum globulins (gms/100 ml.) of calves during Experiment 3

Calf No.	Weeks 0	2	4	6	8	10	12	14	16	18	20
1	3.91	3.78	4.25	4.64	4.09	4.6	4.49	4.56	4.72	4.95	4.6
2	4.31	4.05	4.59	4.65	4.22	4.8	4.85	4.55	5.01	4.77	5.07
3	3.54	3.59	3.93	3.78	3.77	4.53	4.45	3.7	4.88	4.96	5.31
4	3.48	3.84	4.19	4.06	3.82	4.16	4.54	4.38	4.92	4.97	4.83
5	4.85	4.56	4.6	4.82	4.84	4.89	5.34	4.51	4.55	5.19	5.3
6	3.55	3.72	3.8	4.28	4.32	4.18	3.97	3.6	3.86	4.14	4.3
7	4.01	3.96	4.15	4.04	4.53	4.69	4.0	4.27	4.09	4.56	4.87
8	3.48	3.67	4.03	4.29	4.08	4.65	4.77	4.61	4.74	5.34	5.19
9	3.08	4.02	4.12	4.17	4.47	4.53	4.12	4.6	4.53	4.4	4.25
10	3.75	3.71	4.41	4.9	4.54	4.6	4.89	5.04	4.94	4.92	4.89
11	3.65	3.89	4.25	4.3	4.35	4.49	4.54	4.2	4.03	4.93	4.47
12	3.33	3.48	3.88	4.3	4.07	3.88	4.27	4.19	4.01	4.4	4.49
13	4.15	3.2	3.59	4.06	4.09	3.53	4.07	4.26	4.32	4.23	4.09
14	3.85	3.9	3.93	4.15	3.95	3.17	4.33	4.27	4.32	4.07	4.35
15	3.5	3.49	3.38	3.98	4.09	3.5	3.38	4.14	4.16	4.35	3.97
16	3.77	3.54	3.32	4.15	4.14	4.28	3.59	4.3	4.16	3.93	4.1
17	3.44	3.66	3.53	3.65	4.05	4.01	3.8	4.21	4.07	4.19	4.04
18	4.06	3.98	3.86	4.44	4.54	4.55	4.44	4.87	4.6	4.37	4.44
19	4.07	3.96	4.14	4.04	4.44	4.43	4.6	4.0	4.22	4.39	4.01
20	4.07	4.8	4.9	4.24	4.41	4.03	4.05	4.59	4.33	4.74	4.8
21	4.68	3.65	4.49	4.38	4.39	3.93	4.62	4.14	4.25	4.74	4.82
22	3.72	3.73	3.21	3.92	3.74	3.88	4.45	4.28	4.93	4.38	4.74
23	4	4.18	3.85	4.21	4.36	4.39	4.37	4.17	4.85	4.72	4.83
24	4.07	3.37	3.59	4.59	4.14	4.1	4.67	4.47	4.79	4.49	4.24
25	4.47	4.32	3.52	4.05	4.19	4.31	4.19	4.49	4.5	4.91	4.64
26	3.9	3.38	3.35	4.09	4.04	4.19	4.32	4.43	4.22	4.31	4.24
27	4.4	4.16	4.09	4.09	4.43	4.38	4.25	4.55	4.59	4.52	3.8
28	4.52	4.34	4.2	3.6	4.23	4.41	4.30	4.35	4.03	4.39	4.3

Table 86

Individual serum glutamatic-oxaloacetic transaminase levels

Calf No.	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
1	67	82	68	84	93	101	88	62	112	83	95	85	89
2	90	93	87	137	127	155	84	62	122	87	104	87	68
3	76	84	60	182	75	87	73	70	85	75	87	90	68
4	64	67	60	101	67	77	66	75	86	120	99	93	81
5	55	64	71	111	67	155	112	60	93	67	63	71	71
6	63	71	57	67	71	80	80	69	86	75	62	82	84
7	77	83	82	97	55	90	68	72	84	80	84	123	95
8	90	112	66	173	91	115	84	75	100	84	122	75	89
9	64	76	66	107	74	82	66	68	71	61	60	97	76
10	62	78	61	93	71	85	73	75	102	82	98	104	107
11	61	80	80	84	100	119	82	75	80	63	71	77	71
12	64	71	81	129	84	87	81	69	144	82	122	135	78
13	81	75	63	154	87	77	102	95	167	127	122	111	111
14	81	78	76	129	80	103	85	85	82	75	93	107	83
15	76	90	81	113	82	53	65	75	71	88	101	73	88
16	62	56	60	67	62	71	122	112	107	83	75	131	97
17	110	87	77	107	119	90	93	83	93	83	86	89	87
18	67	76	75	77	67	74	83	83	85	110	128	89	122
19	75	67	66	88	69	77	73	63	73	75	75	82	91
20	85	84	62	81	72	65	66	83	75	81	69	66	115
21	75	75	71	84	67	93	93	143	93	80	93	94	100
22	69	61	71	91	81	80	82	75	75	98	76	89	128
23	53	66	71	74	64	85	67	58	66	76	60	71	62
24	62	63	56	88	67	63	66	63	98	80	75	71	62
25	61	58	65	77	81	123	107	65	73	73	74	87	93
26	72	62	88	74	67	63	65	53	66	65	70	70	80
27	63	67	61	62	59	60	65	66	76	68	69	66	71
28	71	70	62	62	64	77	73	69	75	93	76	73	75

(S-F units) in calves during Experiment 3.

13	14	15	16	17	18	19	20
77	59	90	62	75	104	135	104
104	95	84	108	133	85	112	73
110	123	161	99	104	83	112	79
75	87	145	154	152	95	116	126
75	84	106	79	67	71	111	122
65	77	67	118	82	70	71	70
93	111	118	98	91	107	80	83
95	89	200	85	116	113	111	129
73	66	70	77	86	67	58	82
82	80	105	59	67	71	82	78
67	87	174	79	88	82	89	83
76	99	81	73	85	99	89	75
73	93	97	82	76	88	60	82
77	97	82	72	80	69	70	109
71	90	100	94	111	87	104	100
74	71	75	72	67	63	81	68
97	113	85	94	86	76	89	106
82	93	94	79	73	76	85	66
83	102	83	67	69	71	65	61
74	80	70	59	67	61	73	65
106	108	94	80	102	94	88	82
71	82	85	64	71	65	82	66
66	60	65	61	62	61	66	62
71	65	60	80	87	70	76	71
80	80	75	97	133	107	108	105
65	65	73	115	97	104	94	76
78	84	101	82	76	87	73	84
89	82	95	109	87	136	100	100

Table 87

Individual faeces egg counts (eggs/3 gms of faeces) of calves

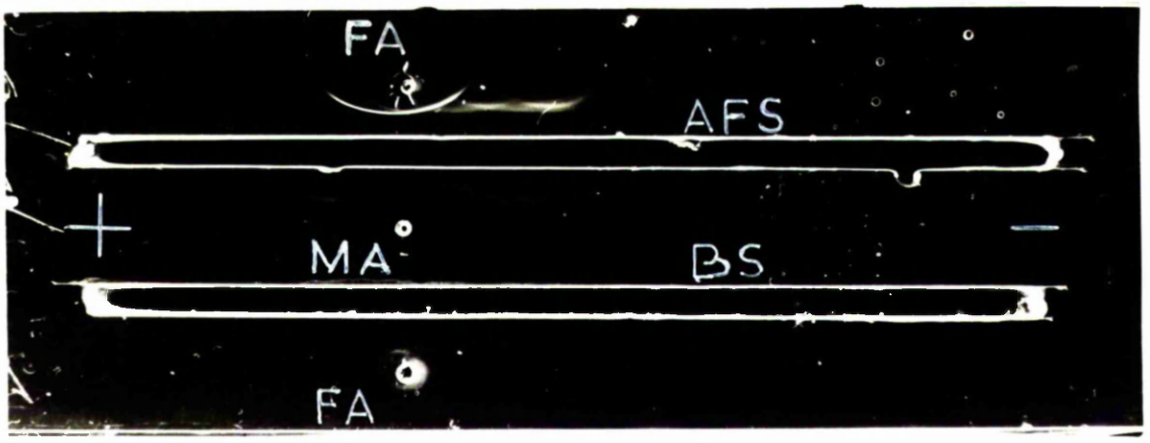
Calf No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	3
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
19	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

during Experiment 3

16	17	18	19	20
2	3	6	10	5
2	5	5	11	6
0	1	2	3	3
0	1	1	3	2
0	0	4	5	2
0	0	0	0	1
0	1	0	17	11
0	1	1	3	5
2	3	7	7	3
0	7	3	0	4
0	0	0	0	0
0	7	2	3	3
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
5	9	7	11	3
0	6	3	1	2
0	4	0	3	2
0	0	2	0	3
1	0	0	1	0
1	1	0	0	0
1	2	8	1	1
0	0	0	3	16
0	0	0	0	11
0	0	0	1	2
0	0	0	0	0

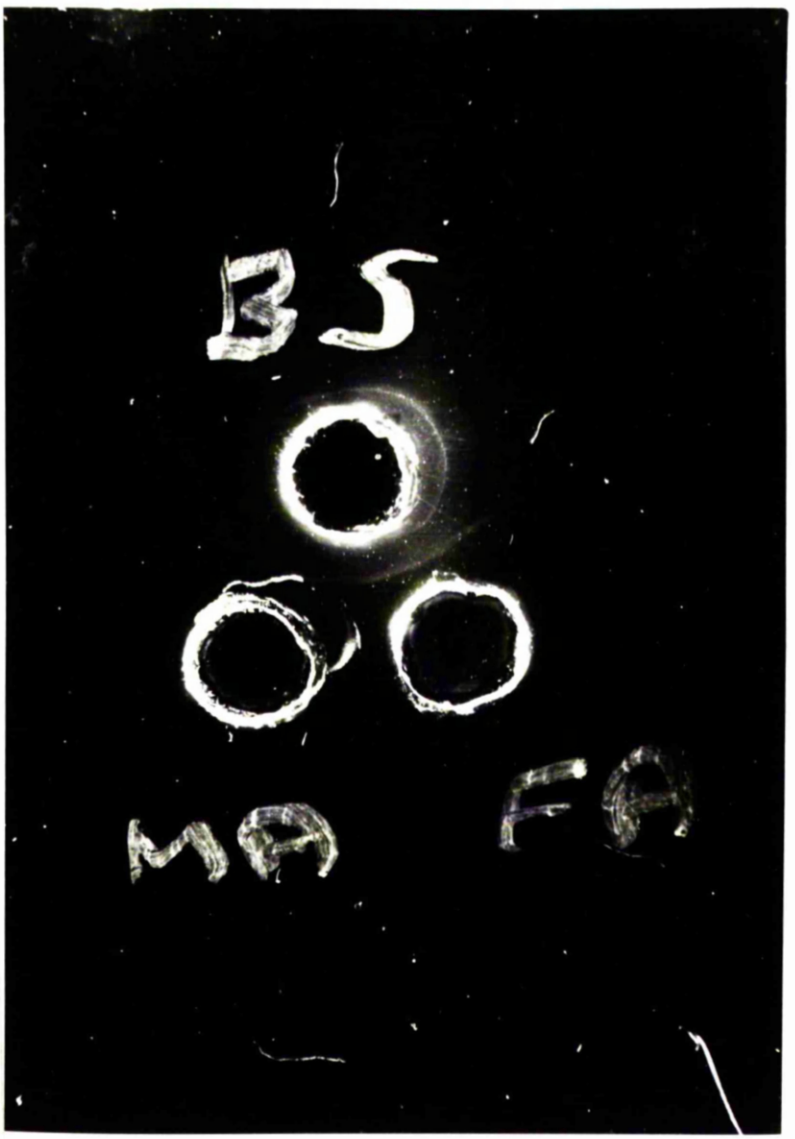
APPENDIX 2

Plate I



I/E analysis of a saline extract (F.A.) and the metabolic products (M.A.) of F. hepatica developed against an antiserum produced in rabbits (A.F.S.) and serum from a calf infected with F. hepatica (B.S.).

Plate 2



Double diffusion in agar showing a line of identity between an antigen in a saline extract (F.A.) and metabolic products from F. hepatica as revealed by serum from a calf infected with F. hepatica.

TABLE 1

Individual precipitin response of calves following infection with 500 metacercariae of F. hepatica as detected by combined immunodiffusion on plates.

	Weeks after infection									
Calf No.	0	1	2	3	4	8	12	16	20	24
1	-	-	-	-	+					
2	-	-	-	+	+					
3	-	-	-	-	+					
4	-	-	-	+	+					
5	-	-	-	+	+	+				
6	-	-	-	+	+	+				
7	-	-	-	+	+	+				
8	-	-	-	+	+	+				
9	-	-	-	+	+	+	+			
10	-	-	-	+	+	-	-			
11	-	-	-	+	+	+	-			
12	-	-	-	+	+	+	+			
13	-	-	-	+	+	+	+	+		
14	-	-	-	+	+	+	+	+		
15	-	-	-	-	-	-	-	+		
16	-	-	-	-	-	+	-	+		
17	-	-	-	-	-	+	+	+	+	
18	-	-	-	+	+	+	-	+	+	
19	-	-	-	+	+	-	-	-	-	
20	-	-	-	+	+	+	+	+	+	
21	-	-	-	+	+	+	+	+	+	+
22	-	-	-	+	+	+	+	+	+	+
23	-	-	-	-	+	+	+	+	+	+
24	-	-	+	+	+	+	-	+	-	+

+ = present - = absent

TABLE 2

Individual precipitin response of calves following infection with 750 metacercariae of *F. hepatica* as detected by combined immunodiffusion plates.

	Weeks after infection										
Calf No.	0	1	2	3	4	8	12	16	20	24	28
13	-	-	-	-	+	+	+	+	+	+	+
14	-	-	-	-	+	+	+	+	+	+	+
15	-	-	-	-	-	+	+	+	+	+	+
16	-	-	-	-	-	+	+	+	+	+	+

+ = present - = absent

TABLE 3

Precipitin levels (P.U.) of calves following infection with *F. hepatica*

Calf No.	No. of metacercariae administered.	No. of flukes recovered.	Weeks after infection							
			0	4	8	12	16	20	24	28
13	750	37	0	4.31	4.01	4.23	2.80	4.74	5.41	5.83
14	750	7	0	2.97	4.29	3.45	3.74	5.26	7.89	6.16
15	750	10	0	4.40	5.99	3.70	3.63	4.01	6.35	5.71
16	750	21	0	1.84	5.47	5.37	4.35	3.73	3.64	3.88
17	750	0	0	0	0	0	0	0	0	0
18	750	21	0	6.98	4.73	4.89	3.41	5.45	-	-
19	750	17	0	5.62	7.56	4.12	4.92	3.71	-	-
20	750	4	0	2.71	2.92	3.26	2.92	2.44	-	-
21	500	26	0	4.45	3.44	5.23	4.56	2.33	4.34	-
22	500	39	0	3.23	5.69	3.40	3.31	3.24	3.92	-
23	500	24	0	5.20	3.76	3.62	3.38	2.17	3.32	-
24	500	8	0	3.79	4.09	2.60	3.45	3.08	4.07	-

APPENDIX 3

Plate I.

Skin biopsy showing the epidermis and dermis at the site of the intradermal injection of *F. hepatica* antigen in a fluke infected calf. Biopsy taken 15 mins. after the antigen injection. Ther is marked oedema (oe) of the dermis with scanty cellular infiltration.

Stain: H&E. Magnification x 150.

1

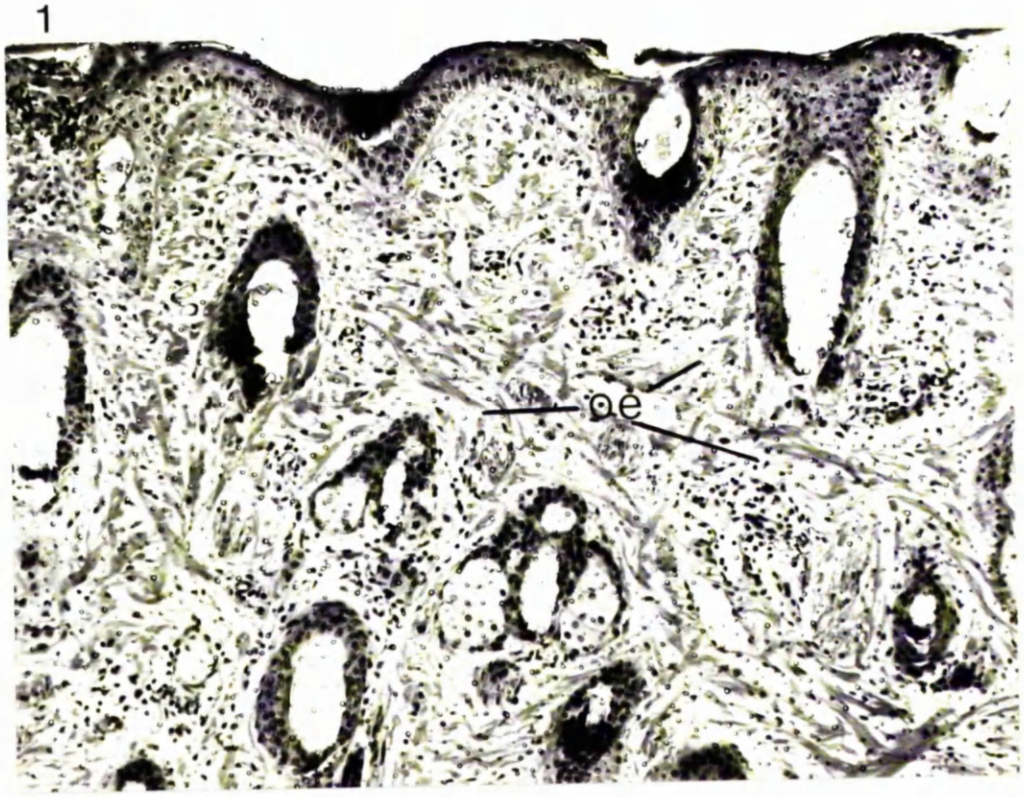


Plate 2.

Similar biopsy to that described in plate I but taken 6 hrs. after injection of antigen. There is oedema and cellular infiltration (ci) of the dermis.

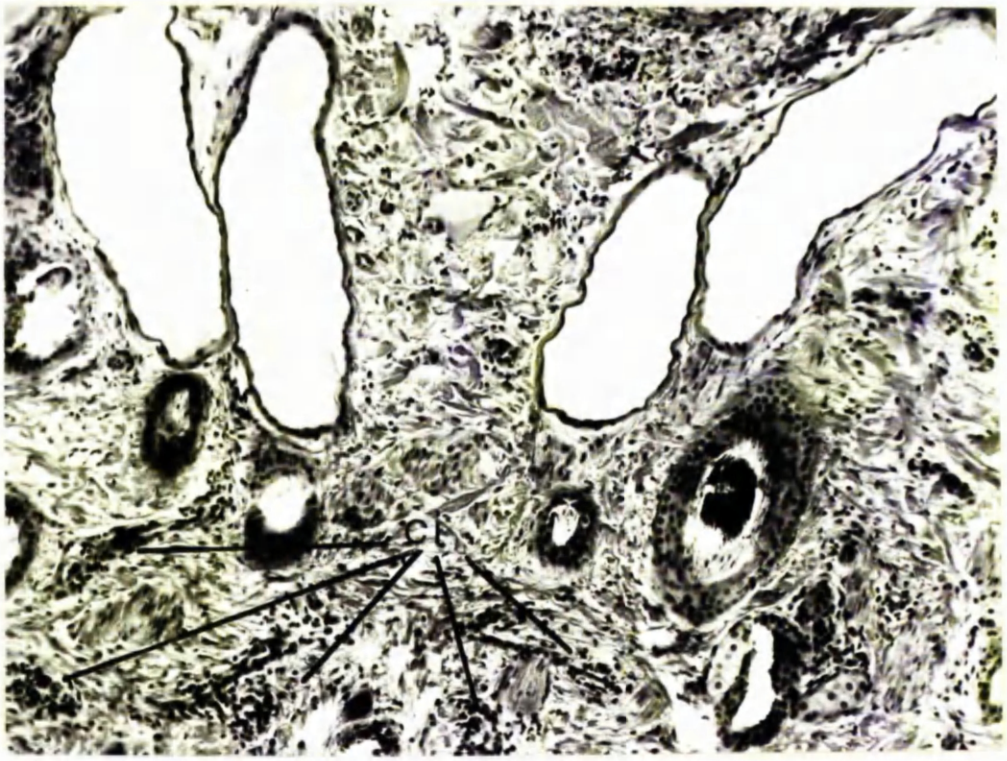
Stain: H&E. Magnification x 150.

Plate 3.

A blood vessel (bv) in the dermis of the biopsy taken 6 hrs. after the injection of the antigen. The vein is thrombosed (tr) and its wall infiltrated and disrupted by polymorphs and lymphocytes.

Stain: H&E. Magnification x 300.

2



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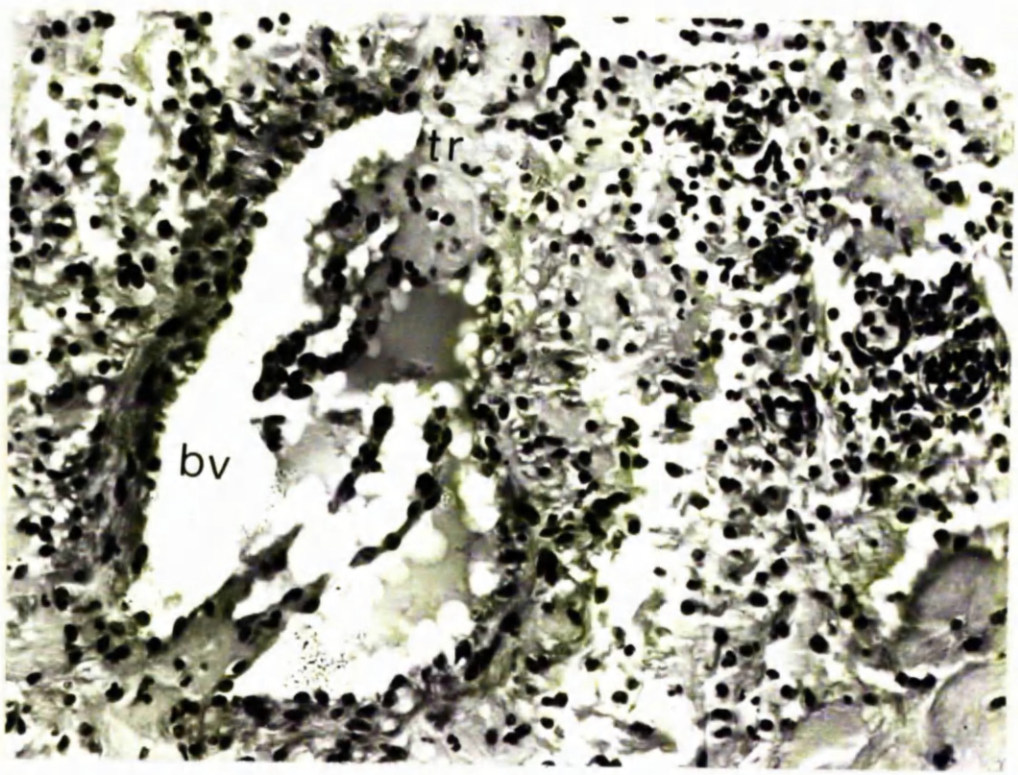


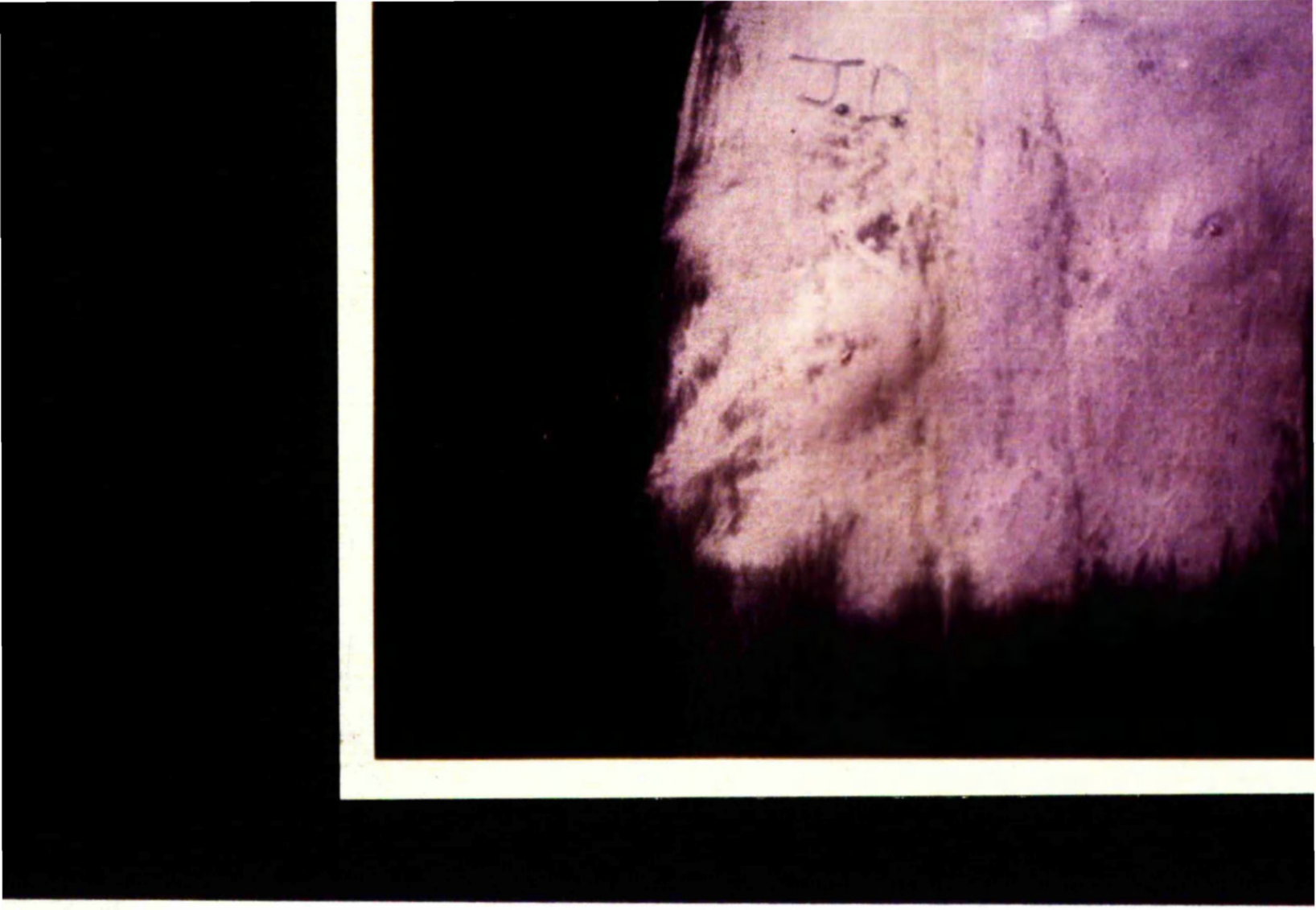


Plate 4

Skin reactions in calf infected with F. hepatica injection 20 mins. previously at 2 sites of the antigen.

Table 1

Skin thickness of calf 1 before and after the intradermal injection of *F. hepatica* antigen at 2 weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness.	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	6	4	6	4	6	6
Injection	10	6	8	6	8	10
After 15 mins.	10	10	12	8	6	8
30 mins.	4	8	10	6	6	6
45 mins.	6	10	12	4	6	6
1 hr.	8	8	12	4	6	6
3 hrs.	8	8	10	4	6	6
6 hrs.	6	4	6	4	6	6

Table 2

Skin thickness of calf 3 before and after the intradermal injection of *F. hepatica* antigen at three weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	4	4	4	4	4	4
Injection	6	6	6	6	6	6
After 15 mins.	8	8	8	6	6	6
30 mins.	12	12	12	6	6	6
45 mins.	10	14	12	6	6	6
1 hr.	10	12	10	4	4	4
3 hrs.	8	10	8	4	4	4
6 hrs.	4	4	4	4	4	4

Table 3

Skin thickness of calf 5 before and after the intradermal injection of *F. hepatica* antigen at 4 weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness.	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	6	8	8	6	6	6
Injection	8	10	10	8	8	8
After 15 mins.	10	12	12	8	10	10
30 mins.	18	18	18	8	8	8
45 mins.	18	18	18	6	6	6
1 hr.	18	18	18	6	6	6
3 hrs.	12	14	16	6	6	6
6 hrs.	6	8	8	6	6	6

Table 4

Skin thickness of calf 6 before and after the intradermal injection of *F. hepatica* antigen at 5 weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness.	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	6	6	6	6	6	6
Injection	8	8	8	8	8	8
After 15 mins.	14	16	18	8	8	8
30 mins.	12	16	18	6	6	6
45 mins.	10	14	14	6	6	6
1 hr.	10	12	12	6	6	6
3 hrs.	8	10	8	6	6	6
6 hrs.	6	6	6	6	6	6

Table 5

Skin thickness of calf 9 before and after the intradermal injection of F. hepatica antigen at 6 weeks after infection with 750 metacercariae of F. hepatica.

Skin thickness	Fluke antigen			saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	4	4	4	4	4	4
Injection	6	8	8	6	6	6
After 15 mins.	18	18	18	8	8	8
30 mins.	20	18	18	8	6	6
45 mins.	20	16	16	6	6	6
1hr.	16	14	14	4	4	4
3 hrs.	12	12	12	4	4	4
6 hrs.	8	8	10	4	4	4

Table 6

Skin thickness of calf 18 before and after the intradermal injection of F. hepatica antigen at 8 weeks after infection with 750 metacercariae of F. hepatica.

Skin thickness	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	6	6	6	6	6	6
Injection	8	8	8	8	10	8
After 15 mins.	18	20	18	6	8	6
30 mins.	18	22	18	6	6	6
45 mins.	16	24	20	6	6	6
1 hr.	18	24	18	6	6	6
3 hrs.	14	18	14	6	6	6
6 hrs.	8	12	10	6	6	6

Table 7

Skin thickness of calf 28 before and after the intradermal injection of *F. hepatica* antigen at 10 weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness.		Fluke antigen			Saline control		
(mm.)	Site	1	2	3	1	2	3
Pre-injection		10	10	10	10	10	10
Injection		14	14	14	12	12	12
After 15 mins.		20	20	22	14	14	14
30 mins.		24	24	20	12	12	12
45 mins.		20	20	20	12	12	12
1 hr.		20	20	20	10	10	10
3 hrs.		18	18	18	10	10	10
6 hrs.		10	10	10	10	10	10

Table 8

Skin thickness of calf 20 before and after the intradermal injection of *F. hepatica* antigen at 12 weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness		Fluke antigen			Saline control		
(mm.)	Site	1	2	3	1	2	3
Pre-injection		6	6	6	6	6	6
Injection		10	10	10	8	10	10
After 15 mins.		20	20	22	10	12	12
30 mins.		20	22	26	10	12	10
45 mins.		18	22	26	10	10	10
1 hr.		18	22	22	8	8	10
3 hrs.		20	20	20	6	6	6
6 hrs.		12	12	12	6	6	6

Table 9

Skin thickness of calf 19 before and after the intradermal injection of *F. hepatica* antigen at 16 weeks after infection with 750 metacercariae of *F. hepatica*.

Skin thickness	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	8	10	10	8	10	10
Injection	12	14	14	12	14	14
After 15 mins.	22	24	32	12	14	14
30 mins.	22	24	30	8	10	10
45 mins.	22	24	30	8	10	10
1hr.	22	22	28	8	10	10
3 hrs.	22	18	28	8	10	10
6 hrs.	14	18	18	8	10	10

Table 10

Skin thickness of calf 23 before and after the intradermal injection of *F. hepatica* antigen at 18 weeks after infection with 750 meyacercariae of *F. hepatica*.

Skin thickness	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	10	10	10	10	10	10
Injection	12	12	14	12	12	12
After 15 mins.	22	24	30	12	12	12
30 mins.	22	26	30	10	10	10
45 mins.	22	24	30	10	10	10
1 hr.	22	24	30	10	10	10
3 hrs.	20	20	24	10	10	10
6 hrs.	18	16	20	10	10	10

Table 11

Skin thickness of the first uninfected control calf before and after the intradermal injection of F. hepatica antigen

Skin thickness	Fluke antigen			Saline control		
(mm.)	Site 1	2	3	1	2	3
Pre-injection	6	6	6	6	6	6
Injection	10	10	10	8	8	8
After 15 mins.	8	8	8	8	8	8
30 mins.	10	10	10	6	6	6
45 mins.	6	6	6	6	6	6
1 hr.	6	6	6	6	6	6
3 hrs.	6	6	6	6	6	6
6 hrs.	6	6	6	6	6	6

Table 12

Skin thickness of the second uninfected control calf before and after the intradermal injection of F. hepatica antigen.

Skin thickness	Fluke antigen			Saline control		
(mm.)	Site 1	2	3	1	2	3
Pre-injection	4	6	6	4	4	4
Injection	6	8	8	6	6	6
After 15 mins.	8	10	10	8	8	8
30 mins.	8	8	12	6	6	6
45 mins.	8	8	10	6	6	6
1 hr.	8	8	8	6	6	6
3 hrs.	4	6	6	4	4	4
6 hrs.	4	6	6	4	4	4

Table 13

Skin thickness of the third uninfected control calf before and after the intradermal injection of *F. hepatica* antigen.

Skin thickness	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	6	6	6	6	6	6
Injection	8	8	8	8	8	8
After 15 mins.	6	8	8	8	8	8
30 mins.	6	6	8	6	6	6
45 mins.	6	6	6	6	6	6
1 hr.	6	6	6	6	6	6
3 hrs.	6	6	6	6	6	6
6 hrs.	6	6	6	6	6	6

Table 14

Skin thickness of the fourth uninfected control calf before and after the intradermal injection of *F. hepatica* antigen

Skin thickness	Fluke antigen			Saline control		
(mm.) Site	1	2	3	1	2	3
Pre-injection	8	8	8	8	8	8
Injection	10	10	10	10	10	10
After 15 mins.	8	10	8	10	8	10
30 mins.	8	10	8	8	8	8
45 mins.	8	10	8	8	8	8
1 hr.	8	8	8	8	8	8
3 hrs.	8	8	8	8	8	8
6 hrs.	8	8	8	8	8	8

Table 15

Skin thickness of 30 calves before and then 30 mins. after the intradermal injection of *F. hepatica* antigen.

Calf no.	Skin thickness prior to injection (mm.)	Skin thickness after injection (mm.)	Increase in in skin thickness (mm.)	Presence of *F. hepatica* at slaughter.
1	6	18	12	+
2	4	18	14	+
3	6	20	14	+
4	4	12	8	+
5	6	18	12	+
6	6	20	14	+
7	6	12	6	+
8	6	20	14	+
9	6	24	18	+
10	4	18	14	+
11	6	18	12	+
12	6	18	12	+
13	4	18	14	+
14	6	20	14	+
15	6	20	14	+
16	6	12	6	+
17	8	12	4	-
18	6	18	12	+
19	8	22	14	+
20	4	22	18	+
21	4	18	14	+
22	6	16	10	+
23	4	20	16	+
24	6	24	18	+
25	4	20	16	+
26	6	16	10	+
27	4	20	16	+
28	8	18	10	+
29	4	8	4	-
30	6	10	4	-

Table 16

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by serum taken from calf 21 at 0, 4, 8, 12, 16, 20 and 24 weeks after infection with 500 metacercariae of F. hepatica.

Weeks after infection	Dilution of serum				
	1/1	1/4	1/16	1/64	1/256
0	-	-	-	-	-
4	19	-	-	-	-
8	-	-	-	-	-
12	-	-	-	-	-
16	19	-	-	-	-
20	38	18	-	-	-
24	40	31	15	14	19

Table 17

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by serum taken from calf 22 at 0, 4, 8, 12, 16, 20 and 24 weeks after infection with 500 metacercariae of F. hepatica.

Weeks after infection	Dilution of serum				
	1/1	1/4	1/16	1/64	1/256
0	-	-	-	-	-
4	35	-	-	-	-
8	24	-	-	-	-
12	-	-	-	-	-
16	30	-	-	-	-
20	40	28	18	11	-
24	20	12	-	-	-

Table 18

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by serum taken from calf 24 at 0, 4, 8, 12, 16, 20 and 24 weeks after infection with 500 metacercariae of F. hepatica.

Weeks after infection	Dilution of serum				
	$\frac{1}{1}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$
0	-	-	-	-	-
4	-	-	-	-	-
8	-	-	-	-	-
12	17	-	-	-	-
16	22	-	-	-	-
20	34	28	36	-	-
24	70	35	31	20	-

Table 19

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by serum taken from calf 13 at 0, 4, 8, 12, 16, 20, 24, 28, and 30 weeks after infection with 750 metacercariae of F. hepatica.

Weeks after infection	Dilution of serum				
	$\frac{1}{1}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$
0	-	-	-	-	-
4	-	-	-	-	-
8	-	-	-	-	-
12	-	-	-	-	-
16	10	-	-	-	-
20	10	-	-	-	-
24	15	-	-	-	-
28	19	15	10	-	-
30	17	13	-	-	-

Table 20

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by serum taken from calf 14 at 0, 4, 8, 12, 16, 20, 24, 28 and 30 weeks after infection with 750 metacercariae of F. hepatica.

Weeks after infection	Dilution of serum $\frac{1}{1}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$
0	-	-	-	-	-
4	-	-	-	-	-
8	-	-	-	-	-
12	-	-	-	-	-
16	-	-	-	-	-
20	13	-	-	-	-
24	16	13	-	-	-
28	26	16	20	21	16
30	18	-	-	-	-

Table 21

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by serum taken from calf 15 at 0, 4, 8, 12, 16, 20, 24, 28 and 30 weeks after infection with 750 metacercariae of F. hepatica.

Weeks after infection	Dilution of serum				
	$\frac{1}{1}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$
0	-	-	-	-	-
4	-	-	-	-	-
8	-	-	-	-	-
12	-	-	-	-	-
16	-	-	-	-	-
20	10	-	-	-	-
24	12	-	-	-	-
28	20	15	15	12	10
30	18	14	13	-	-

Table 22

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by a standard bovine serum, standard serum reduced with mercaptoetanol (M.E.) and standard control sera dialysed against only iodoacetamide and phosphate buffered saline (P.B.S.).

	Dilution of serum				
	$\frac{1}{1}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$
Standard serum	24	20	15	11	-
M.E. treated serum	-	-	-	-	-
Iodoacetamide control	26	22	17	10	-
M.E. treated serum.	-	-	-	-	-
P.B.S. control	29	24	20	12	-

Table 23

Diameter of weals (mm.) in the passive cutaneous transfer test elicited by solutions of the standard serum fractionated by the addition of ammonium sulphate to final concentrations of $33\frac{1}{3}$ & 50%.

Serum fraction	Dilution of solution				
	1	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$
Precipitate at $33\frac{1}{3}$% saturation	30	27	20	10	-
Supernatant at $33\frac{1}{3}$% saturation	-	-	-	-	-
Precipitate at 50% saturation	30	25	18	18	-
Supernatant at 50% saturation	-	-	-	-	-

APPENDIX 4

The following is a list of the abbreviations used in describing the histology in this appendix. Each abbreviation will be used only once in the text and then will be omitted on subsequent occasions.

bd	-	bile duct
ct	-	connective tissue
dpbd	-	developing pseudo-bile duct
dpf	-	diffuse periportal fibrosis
eos	-	eosinophil
fl	-	fluke
gl	-	globule leucocyte
ha	-	hepatic artery
H & E	-	haematoxylin and eosin
ly	-	lymphocyte
mc	-	mast cell
mdv	-	marginal distributing vein
oe	-	oedema
pbd	-	pseudo-bile duct
pc	-	parenchymal cell
pp	-	periportal
pv	-	portal vein

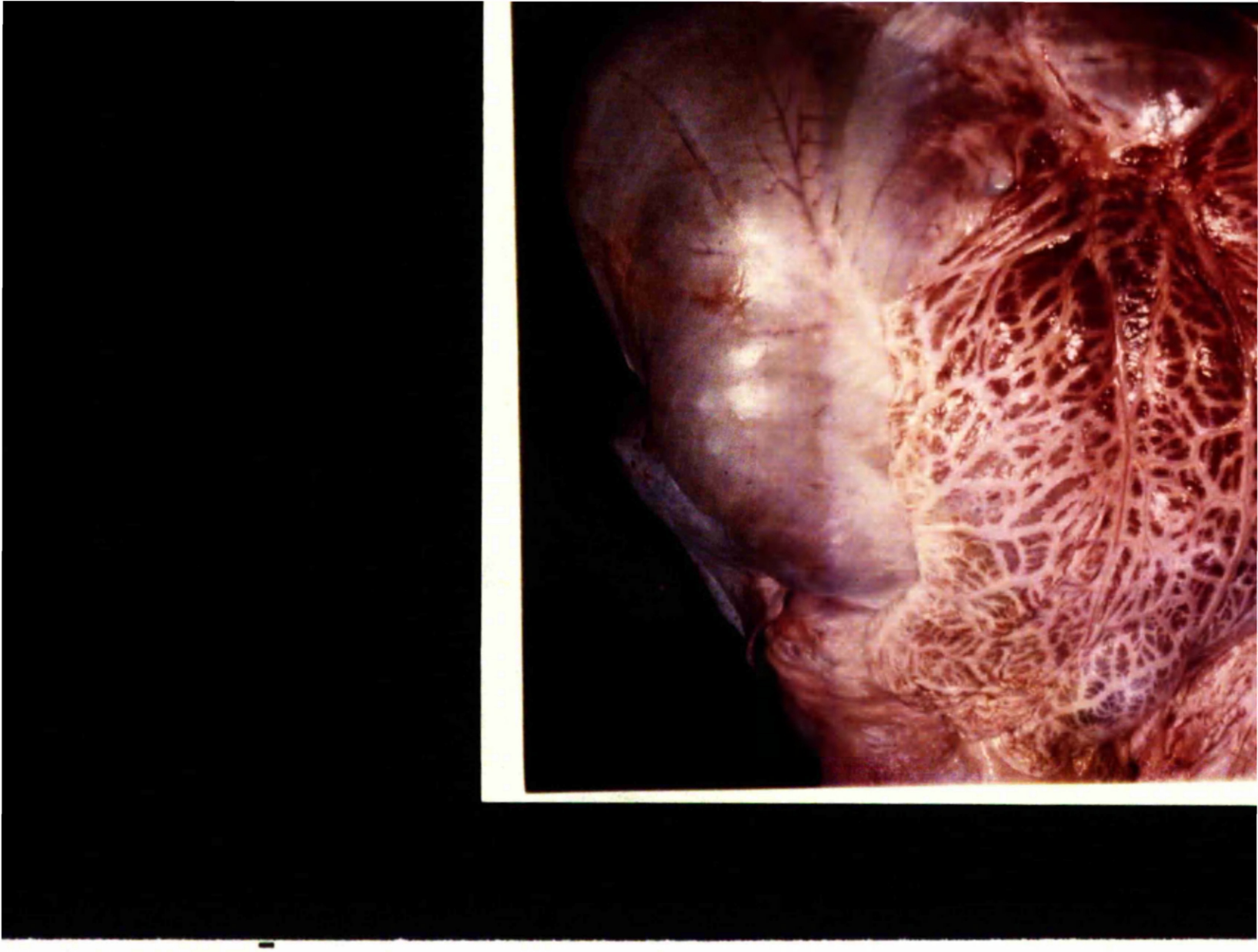


Plate I

Diffuse peritonitis of the greater omentum in

after infection with 500 metacercariae of F. h

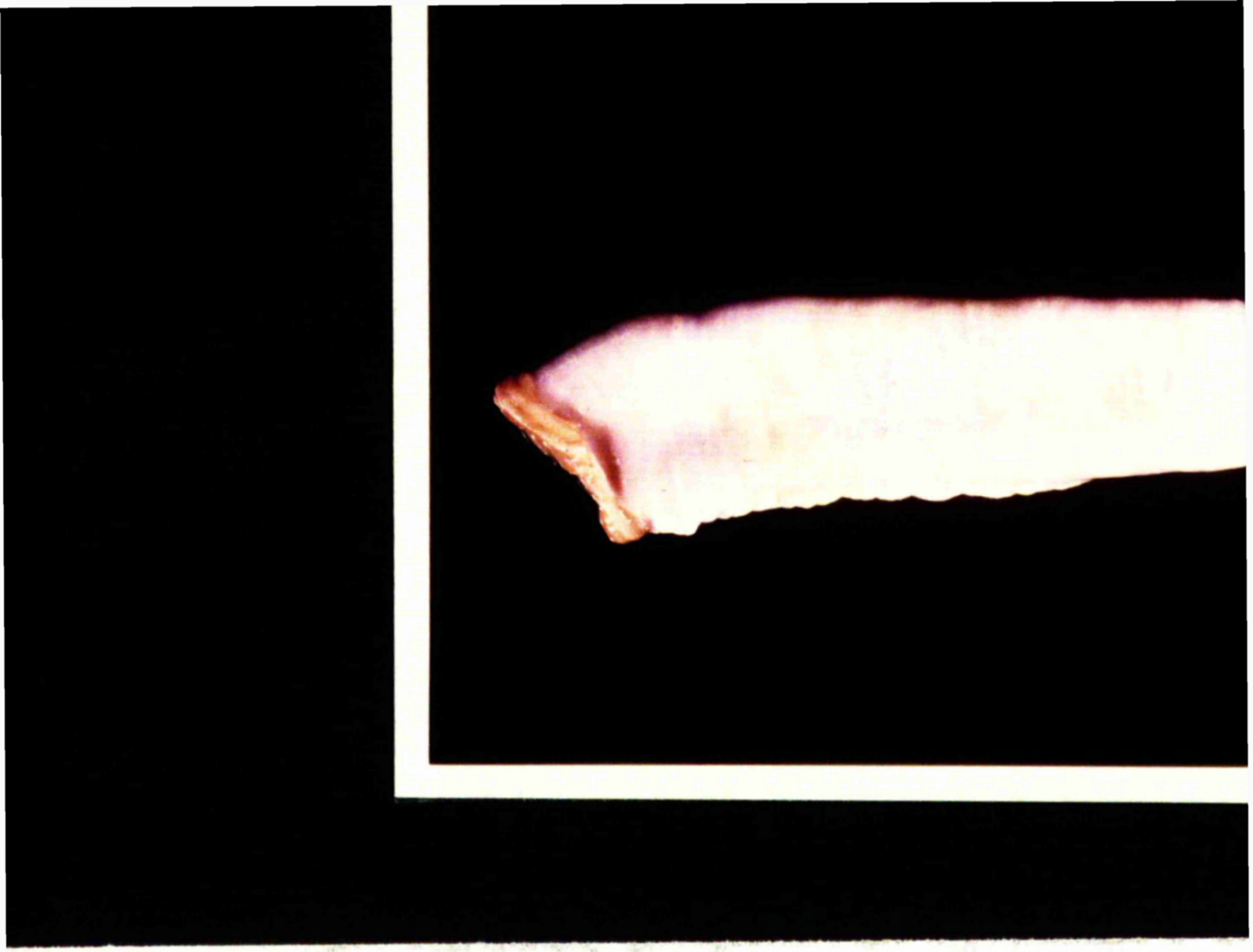


Plate 2

Section of the small intestine taken from a

after infection with 500 metacercariae of F

the raised nodules with haemorrhagic centre

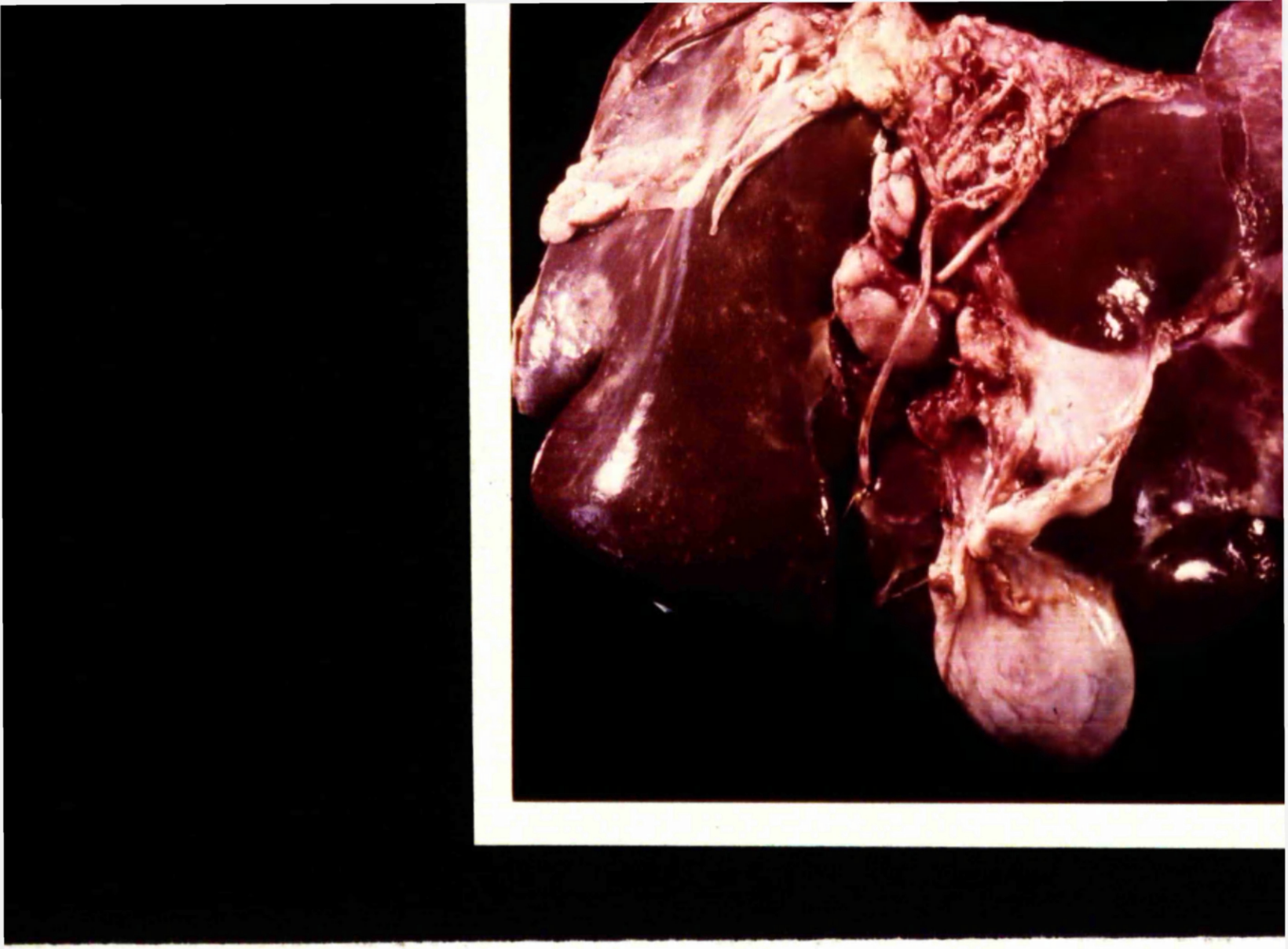


Plate 3

Visceral aspect of the liver from a calf kille

with 500 metacercariae of F. hepatica.

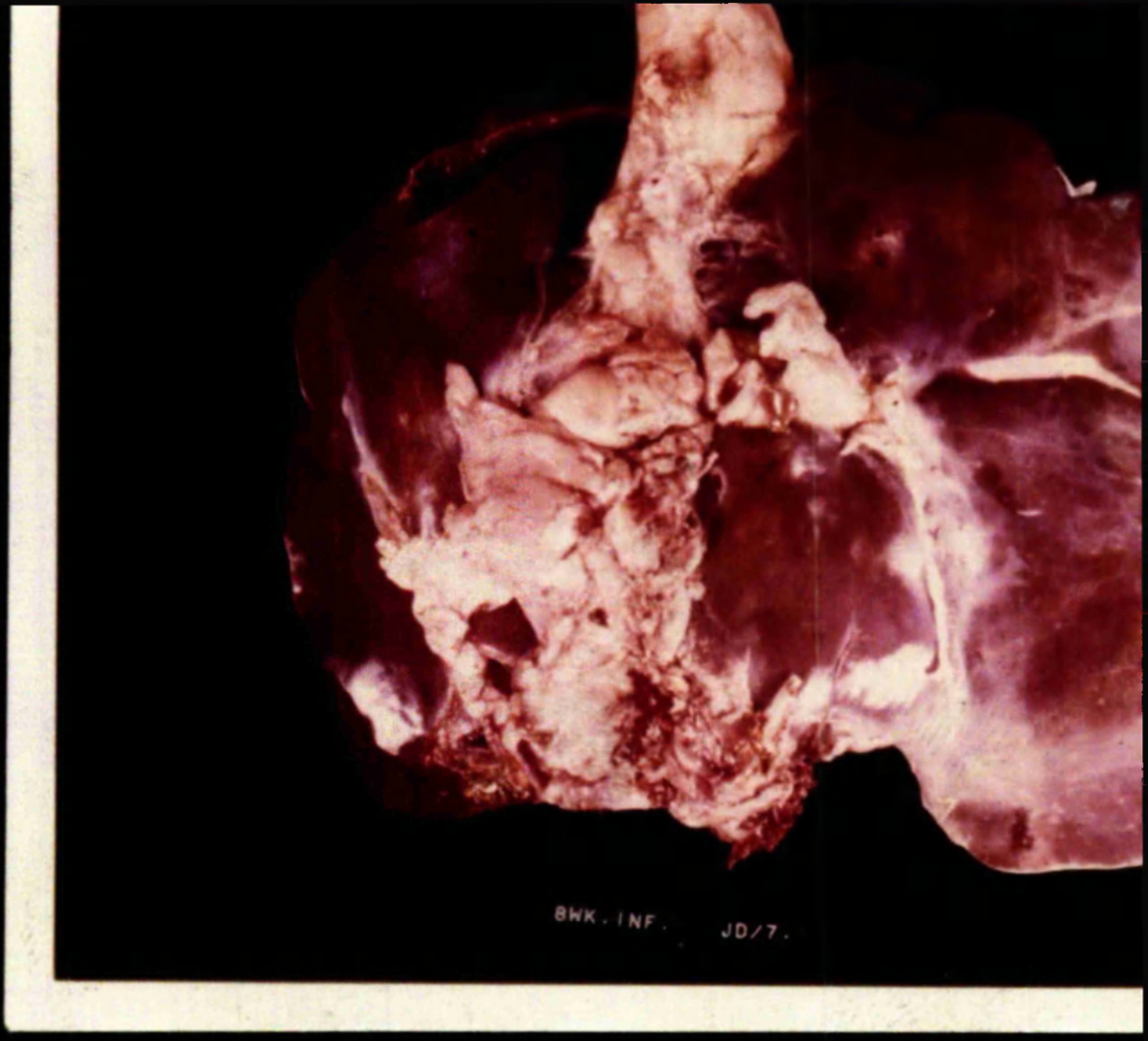


Plate 4

Visceral aspect of the liver from a calf kille

with 500 metacercariae of F. hepatica.

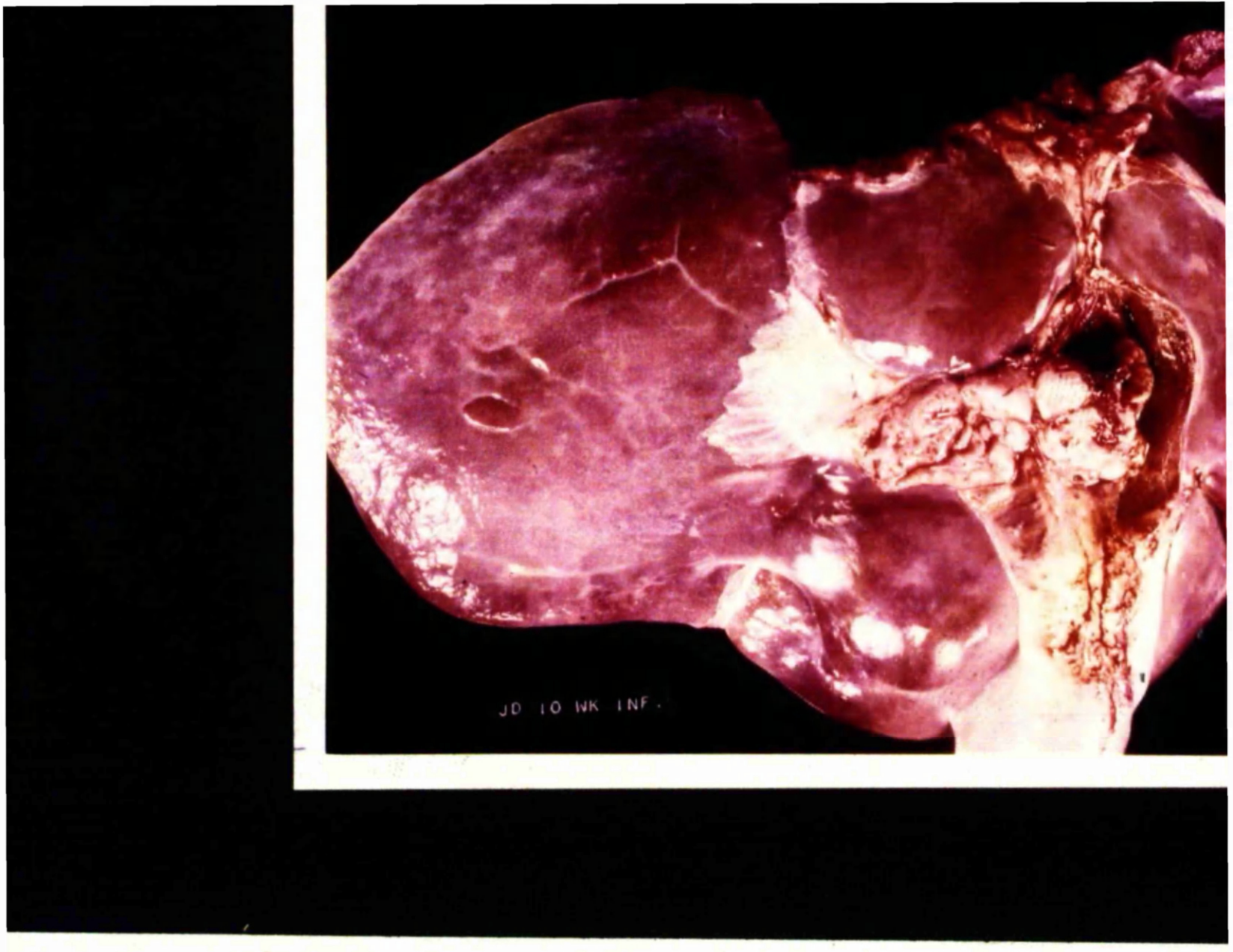


Plate 5

Visceral aspect of the liver from a calf killed

with 500 metacercariae of F. hepatica.

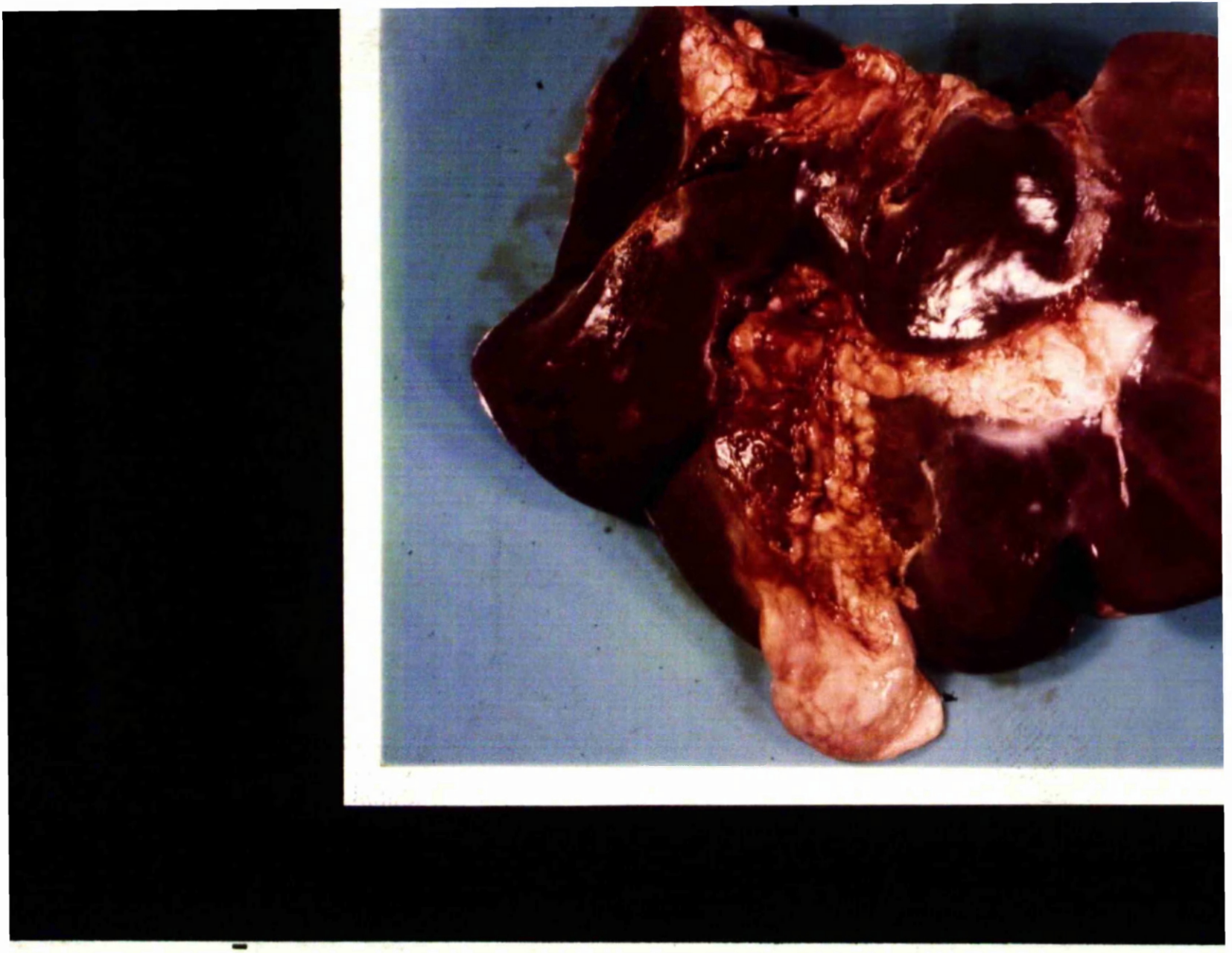


Plate 6

visceral aspect of the liver from a calf killed with 500 metacercariae of F.hepatica.

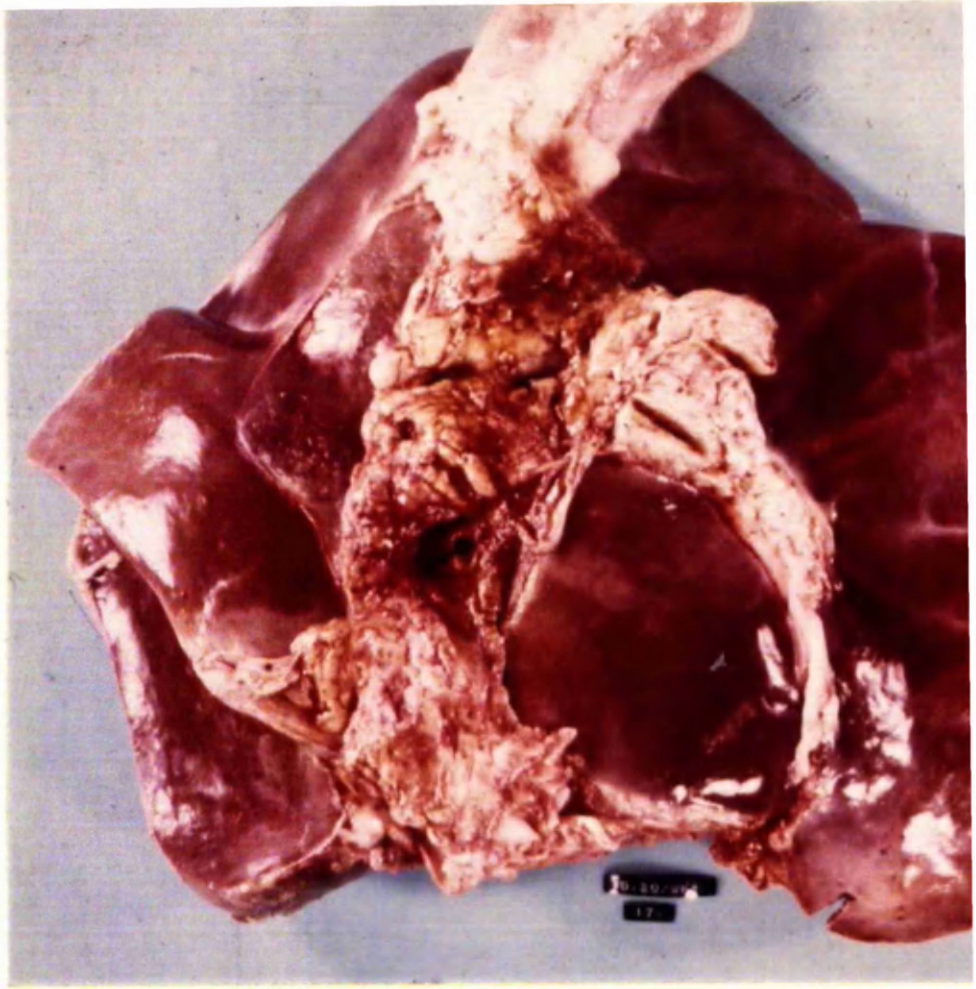


Plate 7

Visceral surface of the liver from a calf killed with 500 metacercariae of F. hepatica.

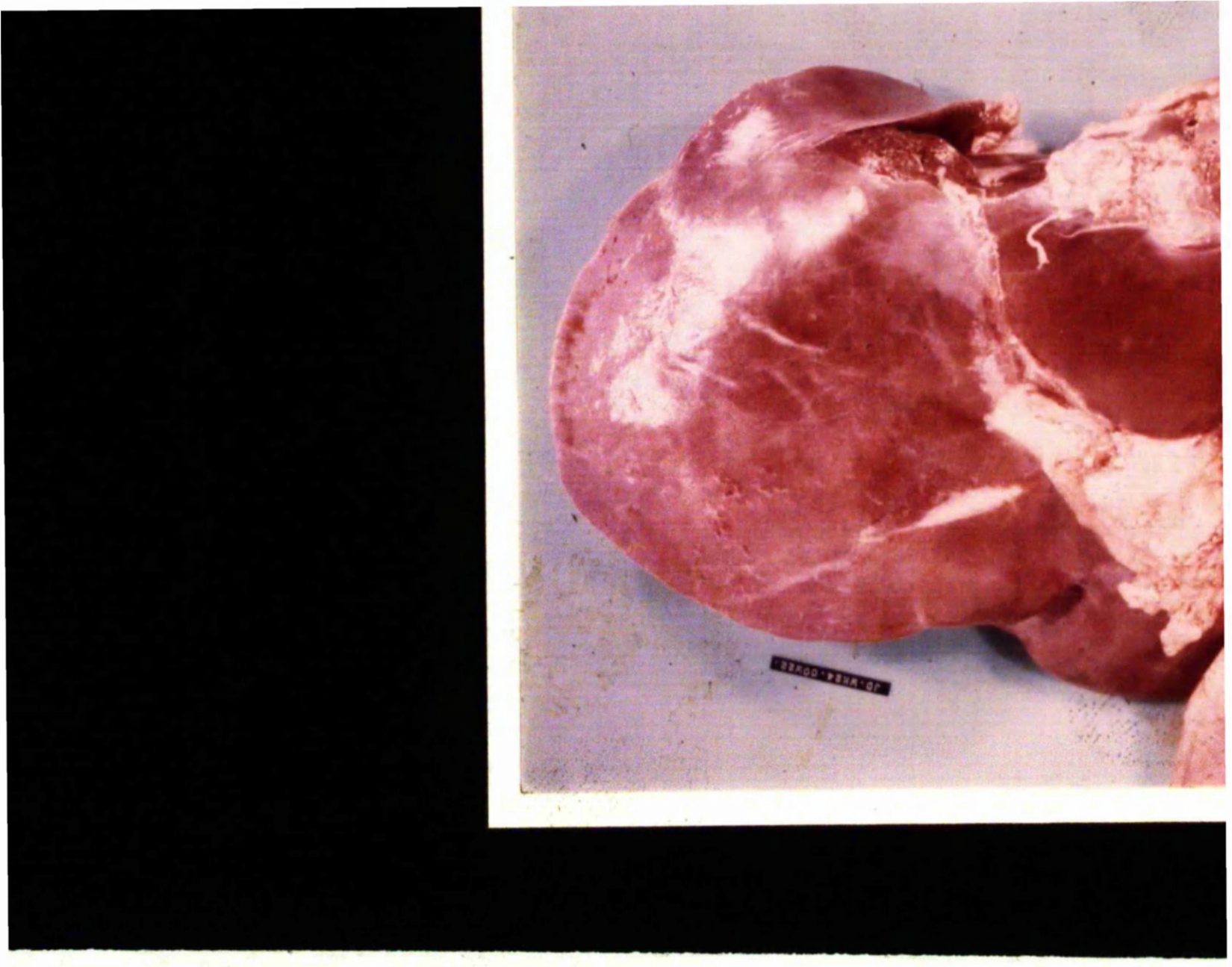


Plate 8

Visceral surface of the liver taken from a ca

infection with 500 metacercariae of F. hepati

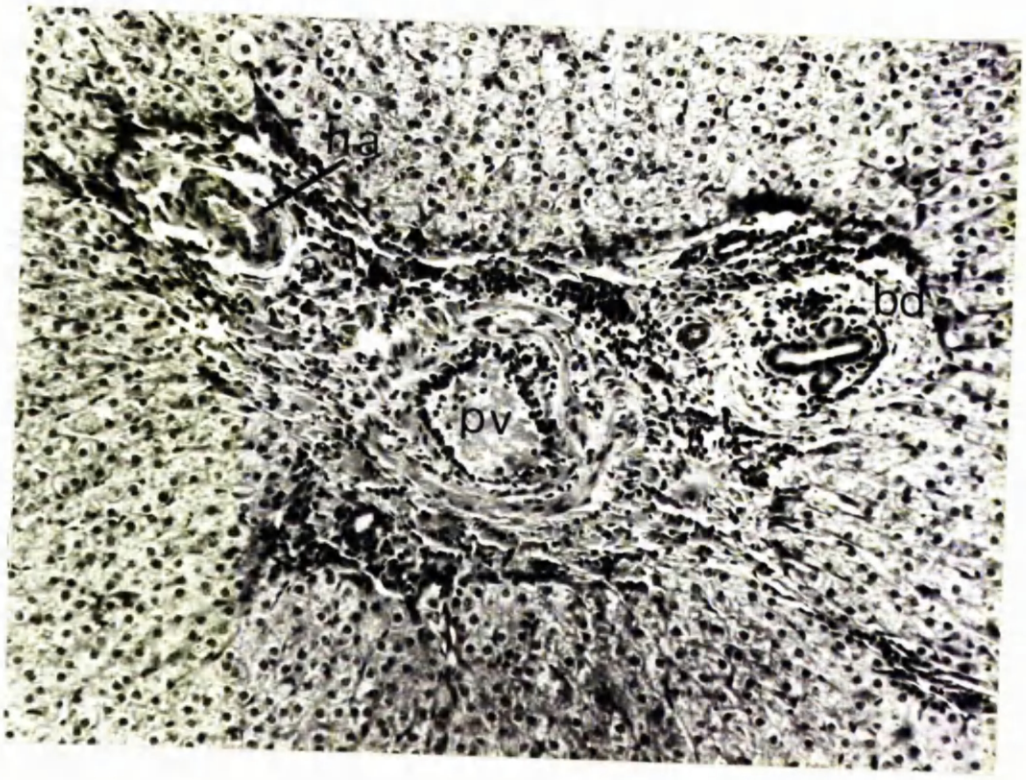
Plate 9.

Portal triad in the ventral lobe of liver from a calf 4 weeks after infection with 500 metacercariae of *F. hepatica* showing a branch of the hepatic artery (ha) bile duct (bd) and a branch of the portal vein (pv). The portal vein shows oedema of the media and adventitia with eosinophil infiltration of the intima. Stain: H. & E. Magnification x 150.

Plate 10.

Higher magnification of portal vein in plate 9 showing eosinophil (eo) infiltration of the intima and oedema (oe) of the media and adventitia. Stain: H. & E. Magnification x 500.

9



10

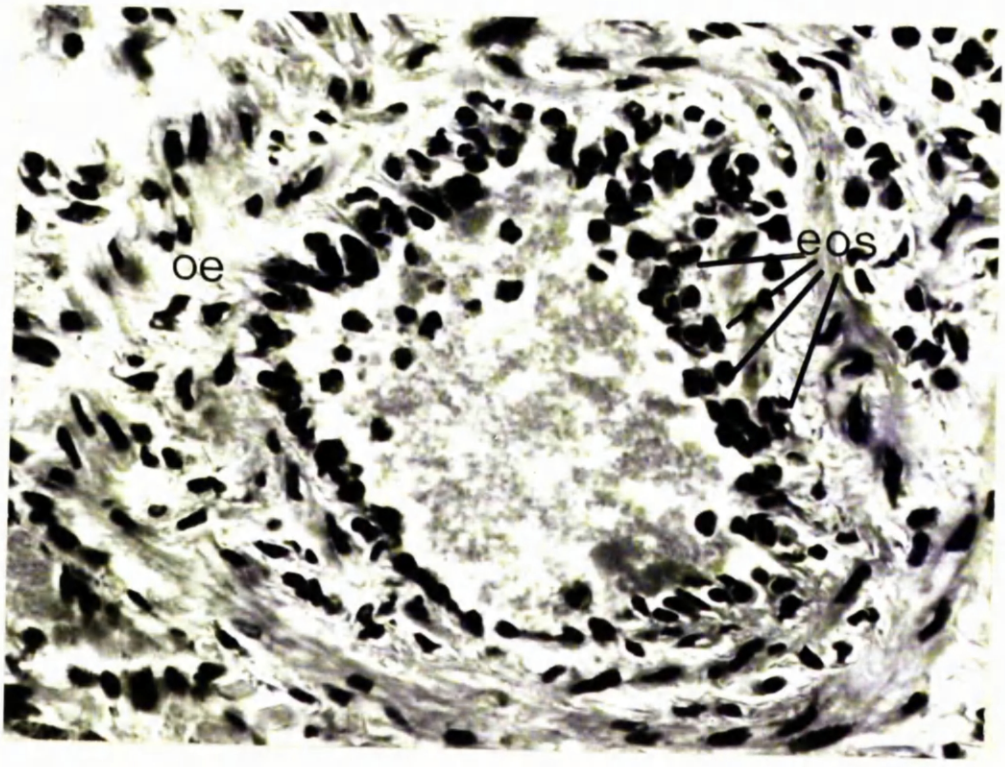


Plate 11.

Portal triad in the ventral lobe of the liver from a calf 4 weeks after infection with 500 metacercariae of F. hepatica showing branch of hepatic artery, bile duct and a branch of portal vein. The portal vein shows eosinophil infiltration and oedema of the intima, media and adventitia.

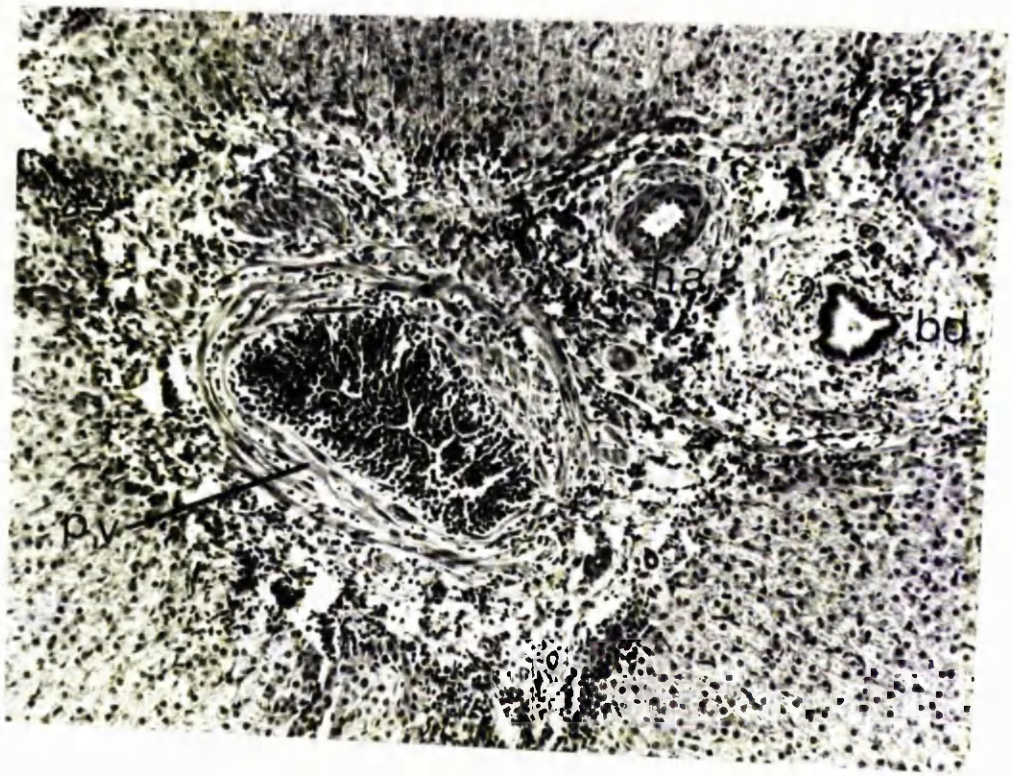
Stain:H. & E. Magnification x 150

Plate 12.

Higher magnification of portal vein in plate 11 showing intensive eosinophil infiltration of the intima with convolution and disruption of the endothelium.

Stain:H. & E. Magnification x 300.

11



12

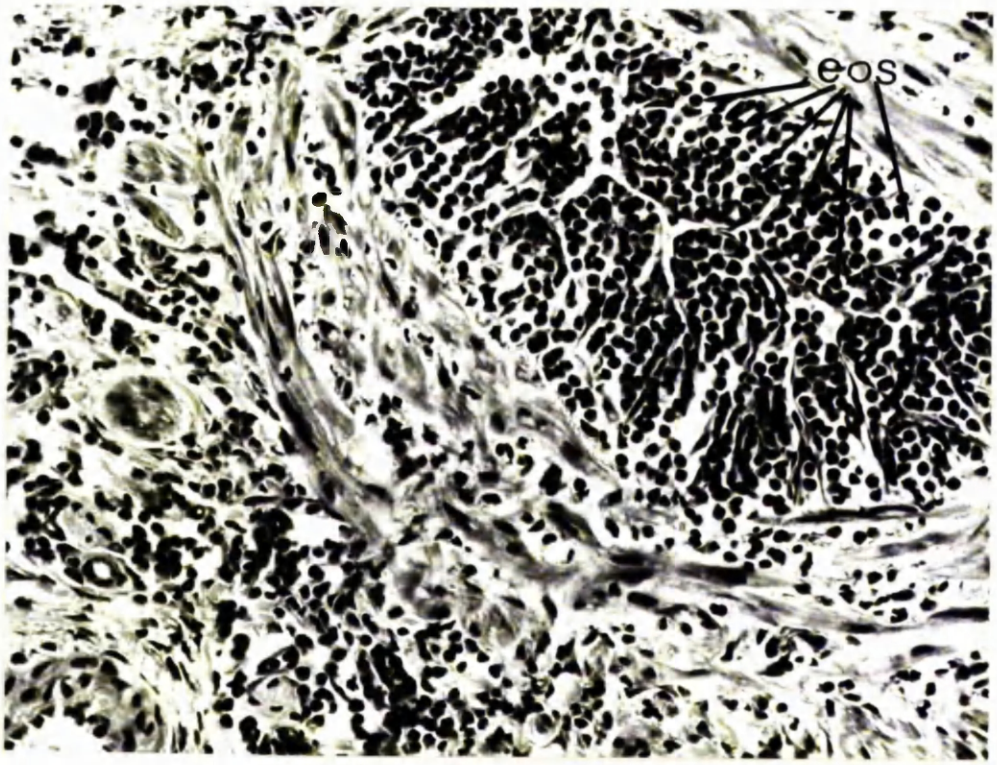


Plate 13.

Portal triad in the ventral lobe of the liver from a calf 8 weeks after infection with 500 metacercariae of *F. hepatica* showing branch of hepatic artery, bile duct and a branch of the portal vein. The intima of the portal vein is heavily fibrosed and recannulated.

Stain:H. & E. Magnification x 150.

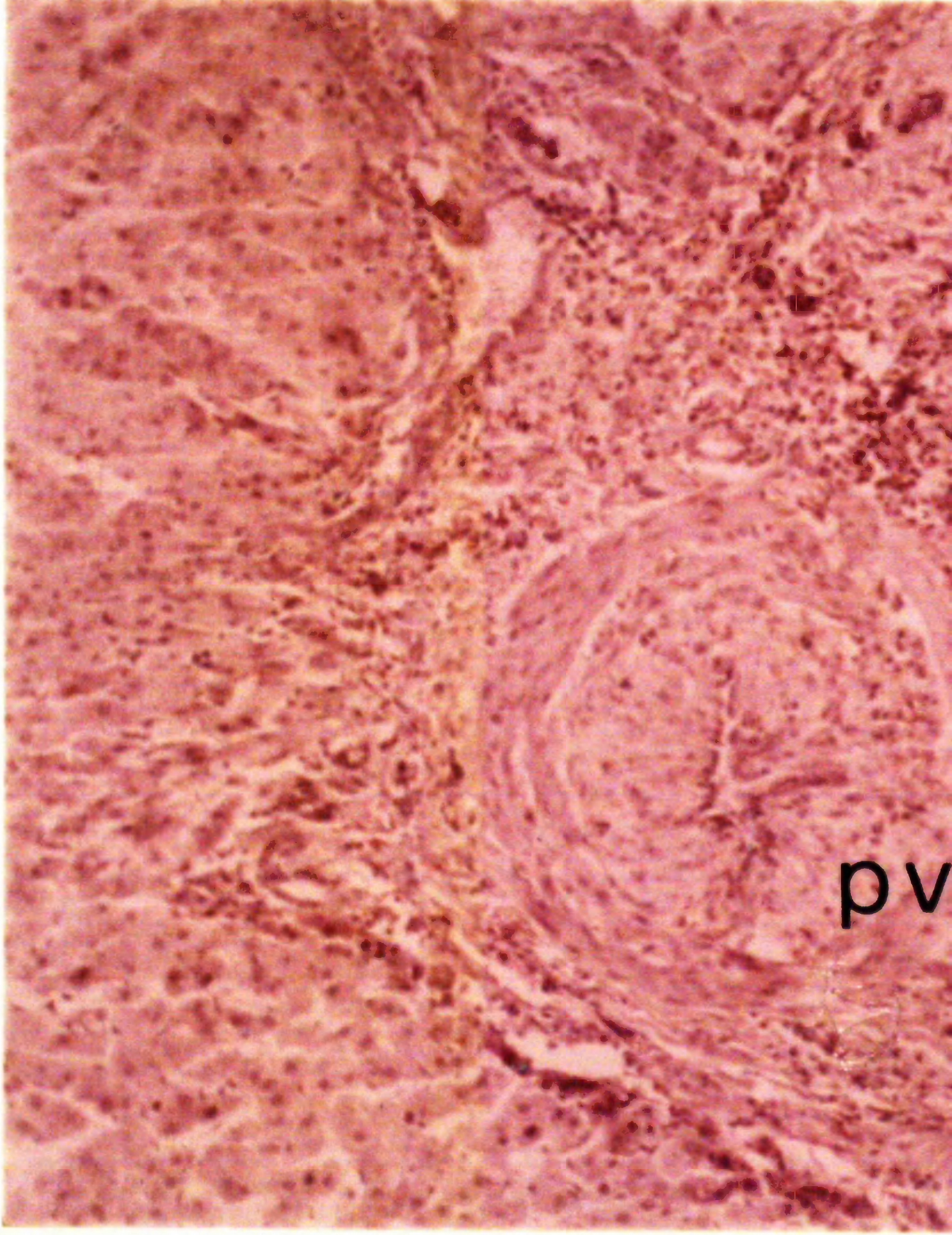

pv

Plate 14.

Area in the ventral lobe of the liver from a calf 8 weeks after infection with 500 metacercariae of F. hepatica. The periportal areas (pp) are obvious due to cellular infiltration and necrosis of adjacent parenchymal cells.

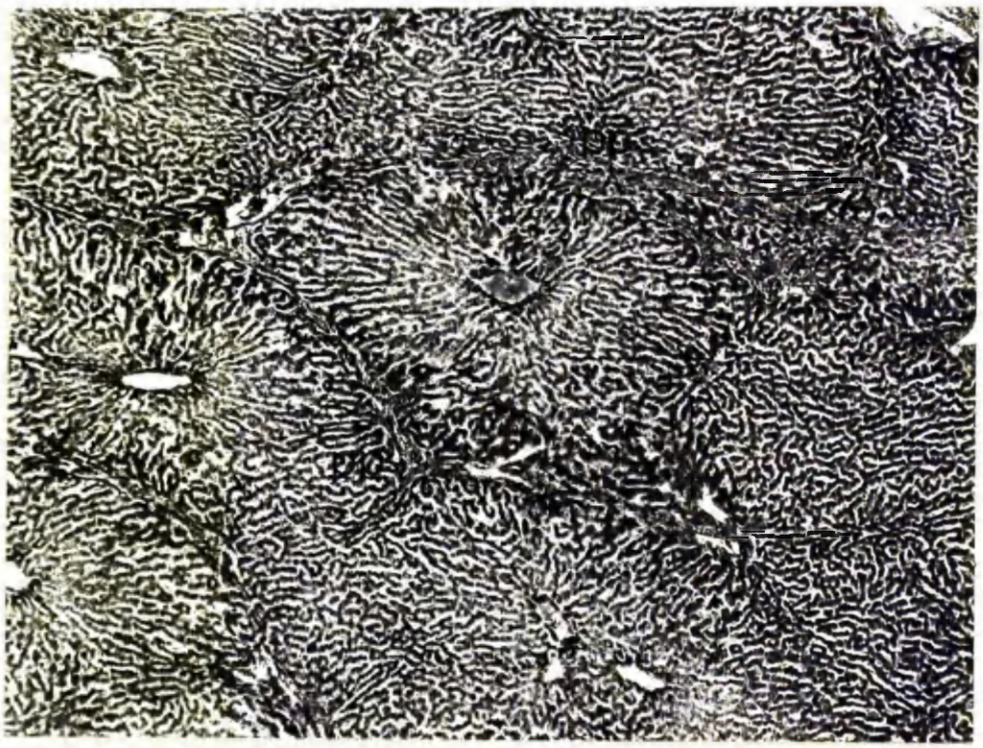
Stain: H. & E. Magnification x 50

Plate 15.

Higher magnification of a periportal area in the ventral lobe of the liver from a calf 8 weeks after infection with 500 metacercariae of F. hepatica. The marginal distributing vein (mdv) is surrounded by eosinophils and lymphocytes (ly) The adjacent parenchymal cells (pc) show degenerative changes.

Stain: H. & E. Magnification x 500.

14



15

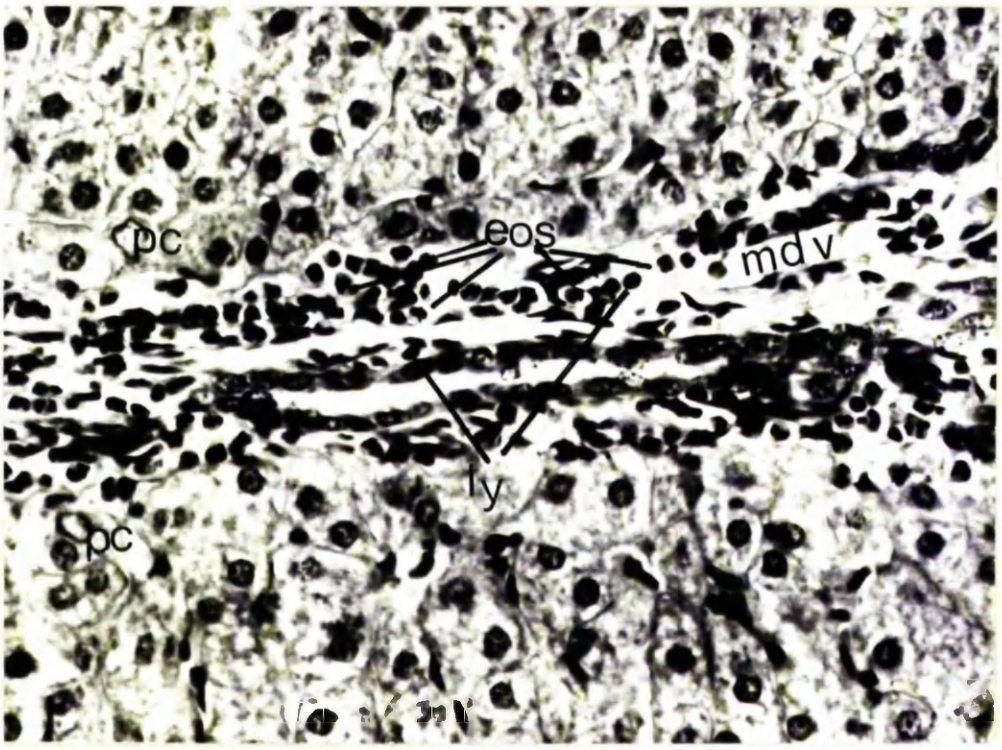


Plate 16.

Area in the ventral lobe of the liver from a calf 8 weeks after infection with 500 metacercariae of F. hepatica. Area surrounding branch of portal vein is heavily infiltrated with eosinophils and lymphocytes. The adjacent parenchymal cells show degenerative changes. In some areas these are 'pseudo-bile ducts' (pbd) and also columns of degenerating parenchymal cells can be seen to be developing the appearance of 'pseudo-bile ducts' (dpbd).

Stain: H. & E. Magnification x 150.

16

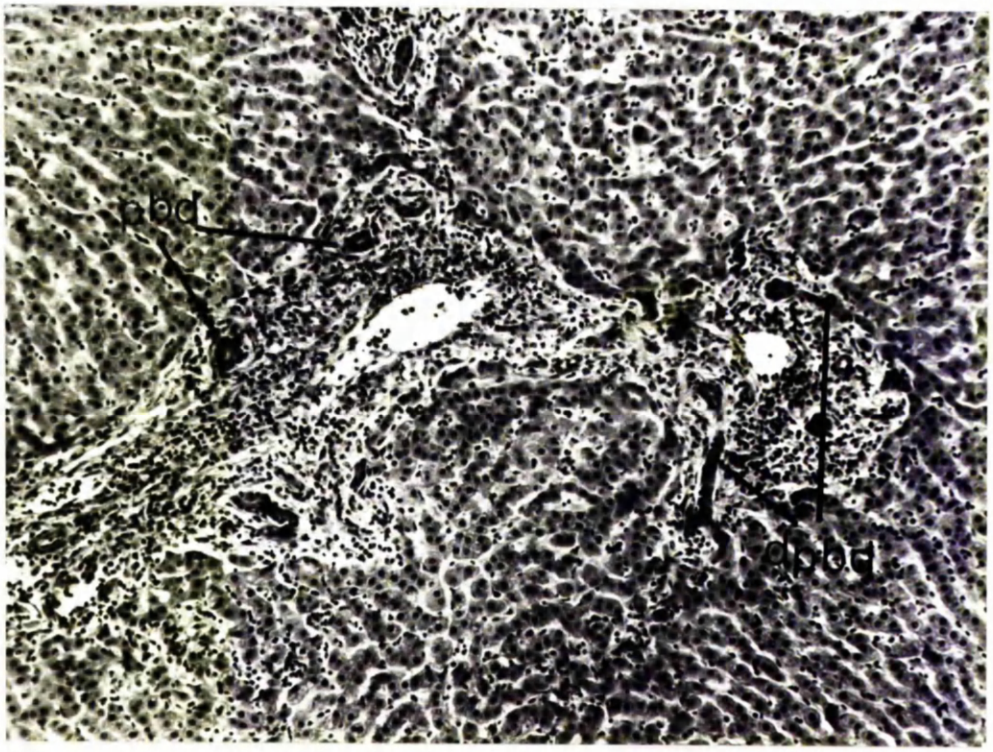


Plate 17.

Higher magnification of periportal area in the ventral lobe of the liver of a calf 8 weeks after infection with 500 metacercariae of *F. hepatica*. Connective tissue (ct) can be seen to encircle columns of degenerating parenchymal cells to form 'pseudo-bile ducts'.

Stain: Mallory's. Magnification x 300.

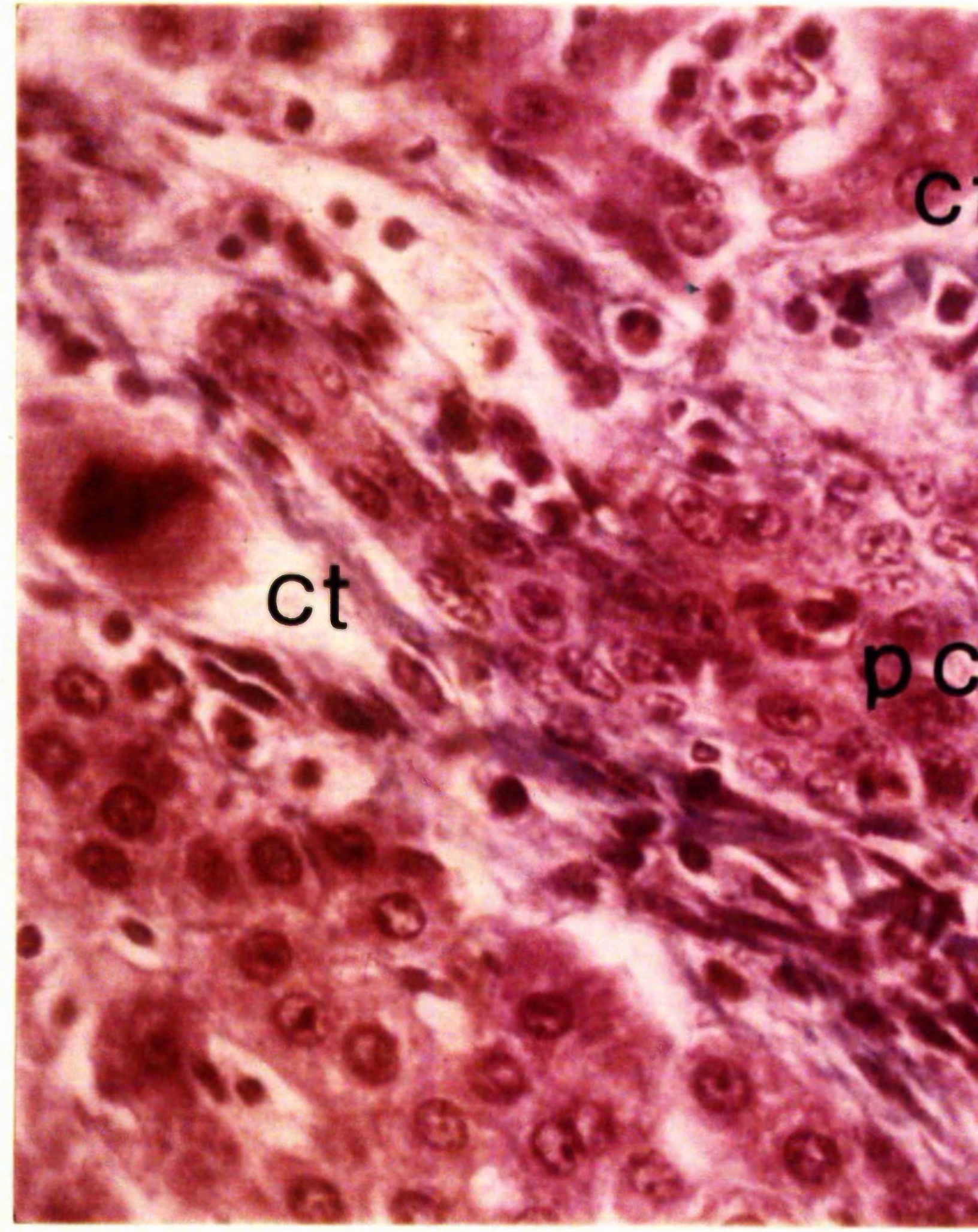

ct
pc

Plate 18.

Further magnification of periportal area in the ventral lobe of the liver from a calf 8 weeks after infection with 500 metacercariae of F. hepatica. The parenchymal cells in the periportal area show degenerative changes in their nuclei and cytoplasm prior to encirclement by connective tissue.

Stain: Mallory's. Magnification x 500.

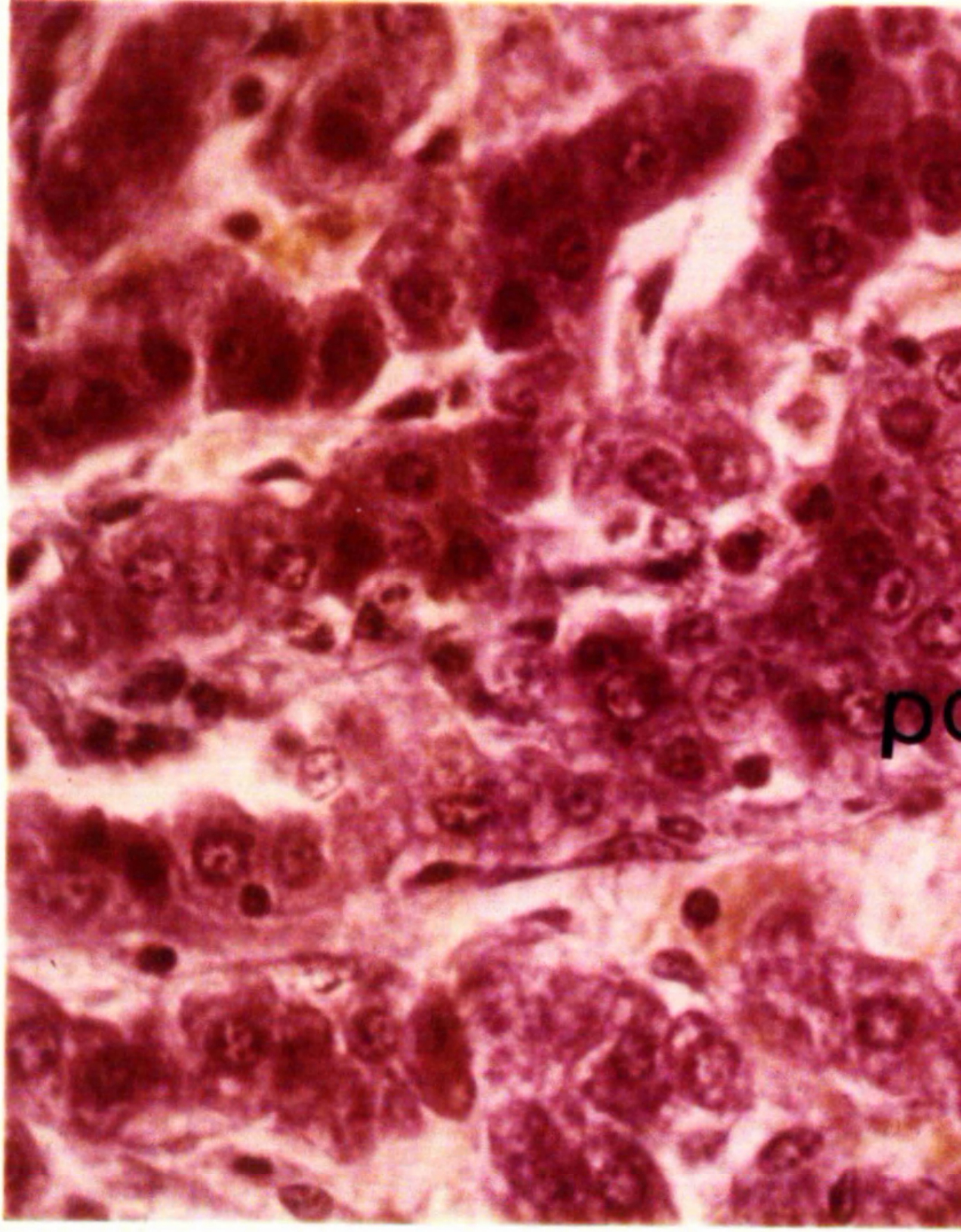


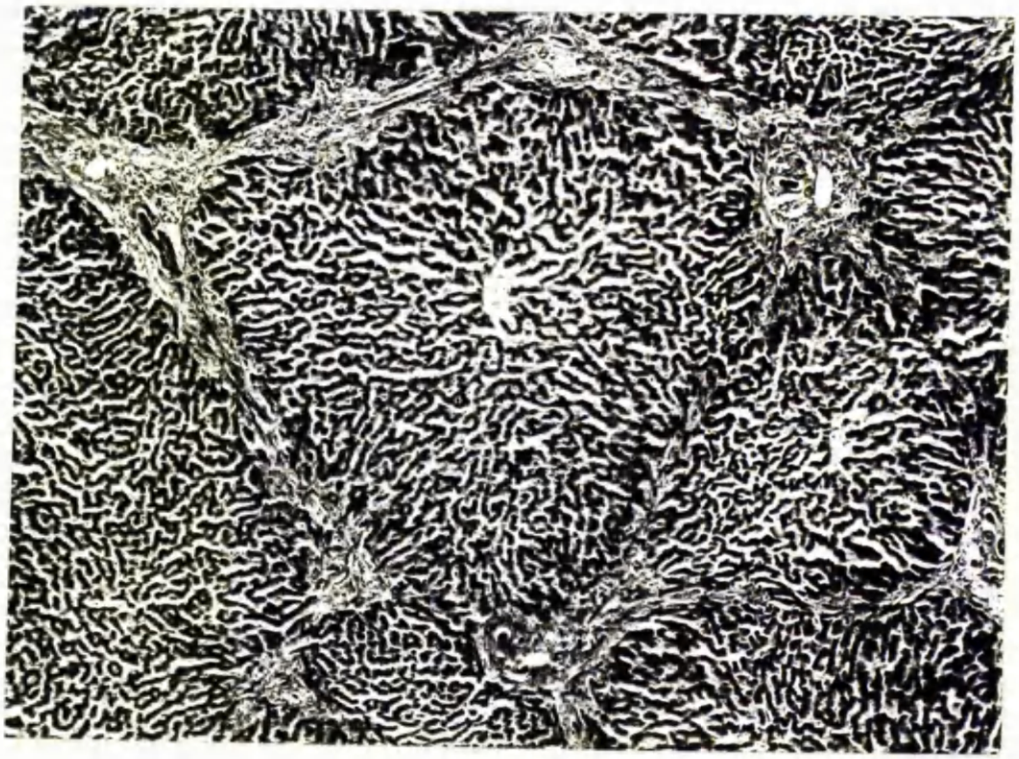
Plate 19.

Area in the ventral lobe of the liver from a calf 12 weeks after infection with 500 metacercariae of F. hepatica. The periportal fibrosis has become more severe and connects adjacent portal areas. Cellular infiltration and parenchymal cell necrosis are still obvious in these areas.
Stain: Mallory's. Magnification x 50.

Plate 20.

Area in the ventral lobe of the liver from a calf 16 weeks after infection with 500 metacercariae of F. hepatica. Severe diffuse periportal fibrosis (dpf) is present but the cellular infiltration and parenchymal cell necrosis in these areas is much less obvious than 4 weeks previously.
Stain: H. & E. Magnification x 50.

19



20

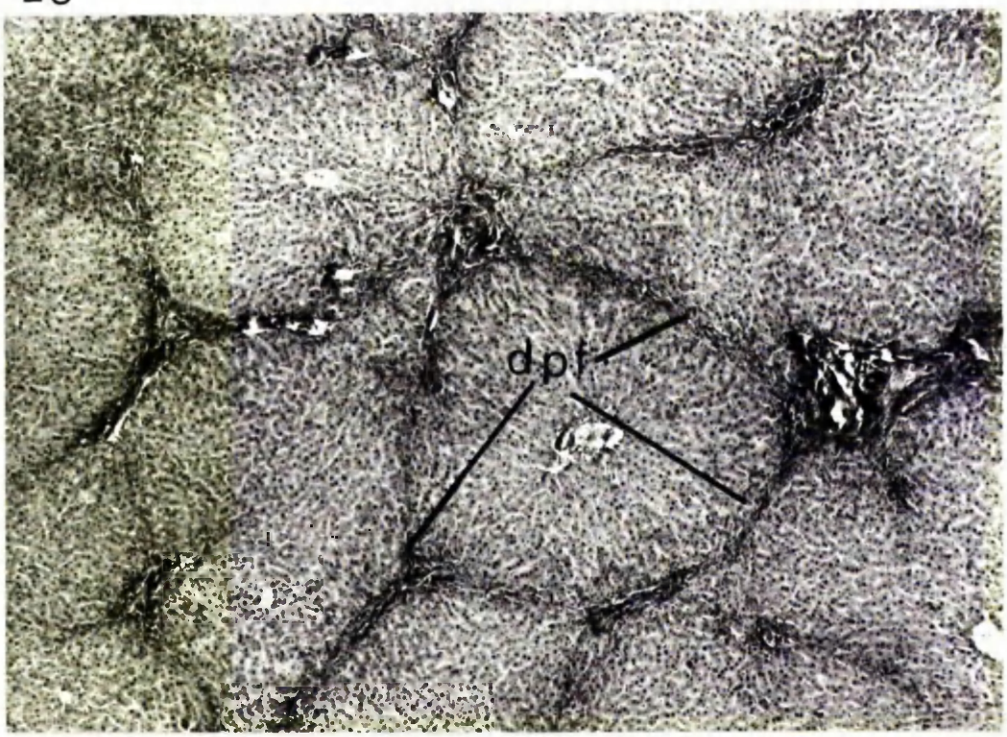


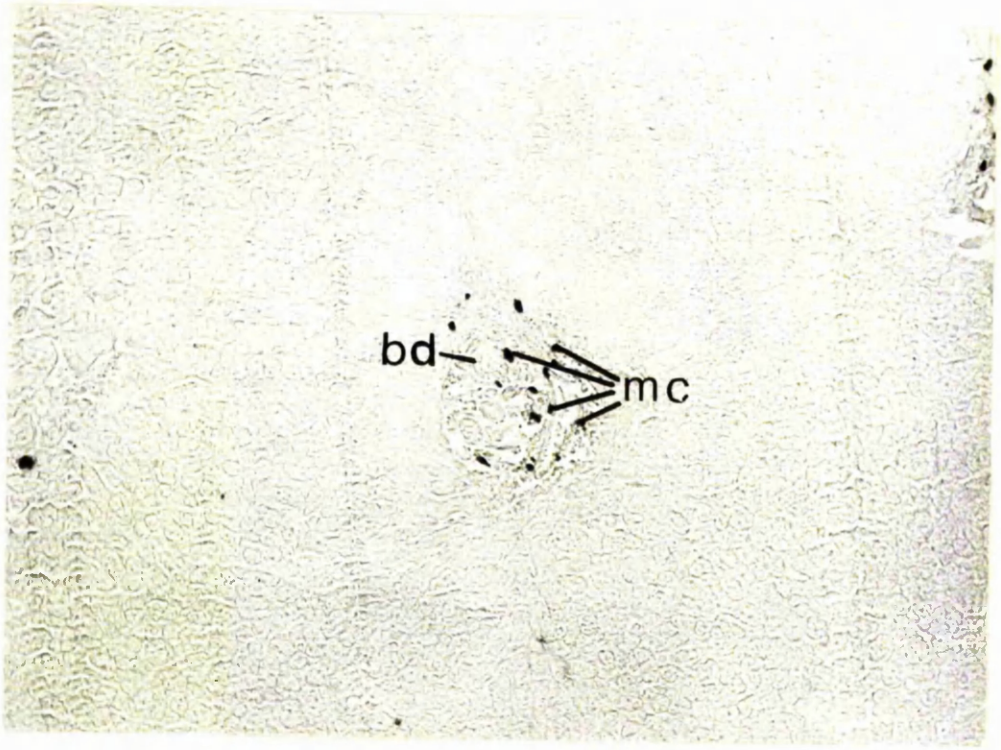
Plate 21.

A portal triad in the parenchyma of the ventral lobe of the liver from a calf 4 weeks after infection with 500 metacercariae of F. hepatica. There are numerous mast cells (mc) in the connective tissue of the triad and a single mast cell associated with the bile duct.
Stain: Astra blue/Safranin O.Magnification X 150.

Plate 22.

Amedium sized bile duct in the parenchyma of the ventral lobe of the liver from a calf 8 weeks after infection with 500 metacercariae of F. hepatica. Numerous sub-epithelial mast cells are present.
Stain:Astra blue / Safranin O. Magnification x 150.

21



22

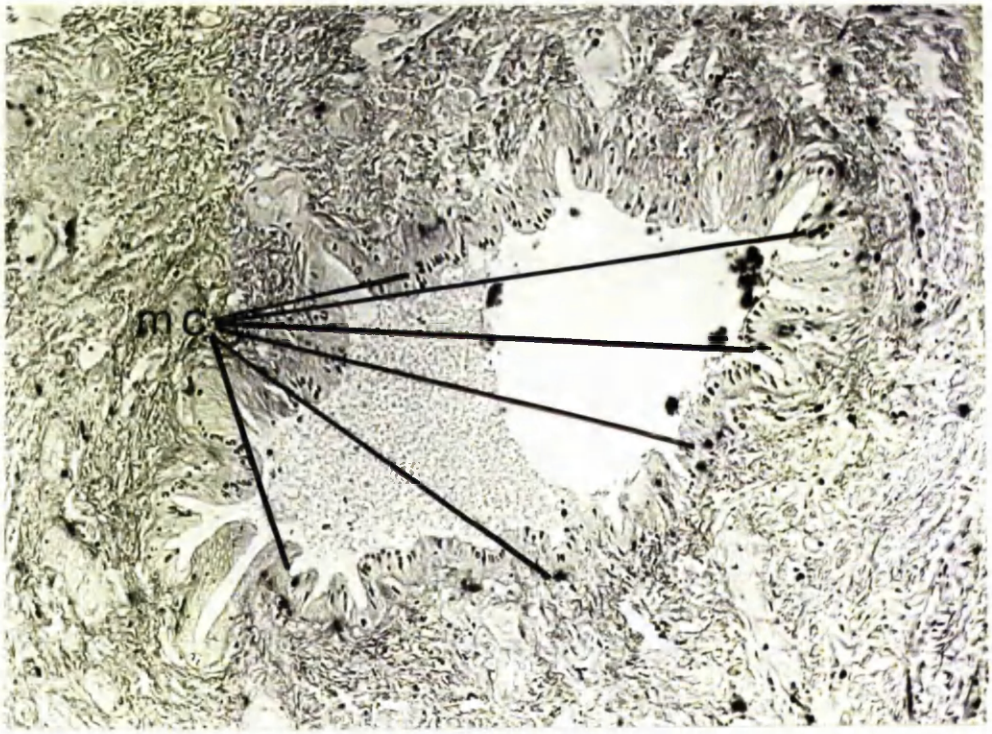


Plate 23.

Medium sized bile duct in the parenchyma of the ventral lobe of the liver from a calf 12 weeks after infection with 500 metacercariae of F. hepatica. Numerous sub-epithelial mast cells are present.

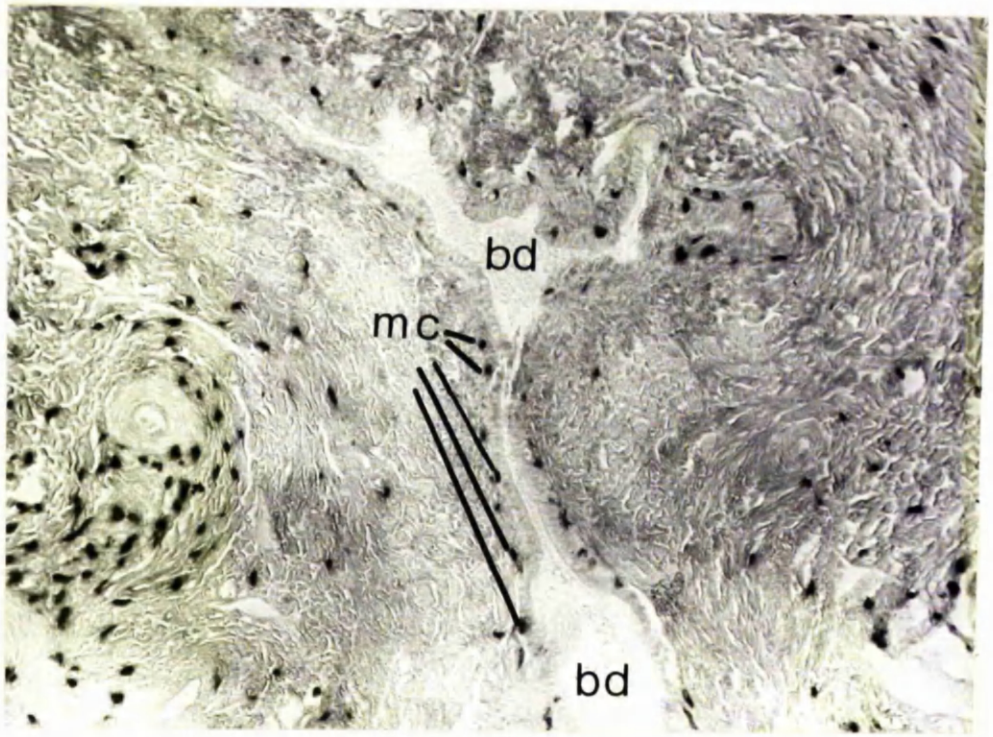
Stain: Astra blue / Safranin O. Magnification x 150.

Plate 24.

Parasitised medium sized bile duct in the parenchyma of the ventral lobe of the liver from a calf 16 weeks after infection with 500 metacercariae of F. hepatica. A part of a fluke (fl) can be seen in the lumen of the duct. Despite the epithelial hypertrophy present few mast cells are present in the epithelium.

Stain: Astra blue / Safranin O. Magnification x150.

23



24

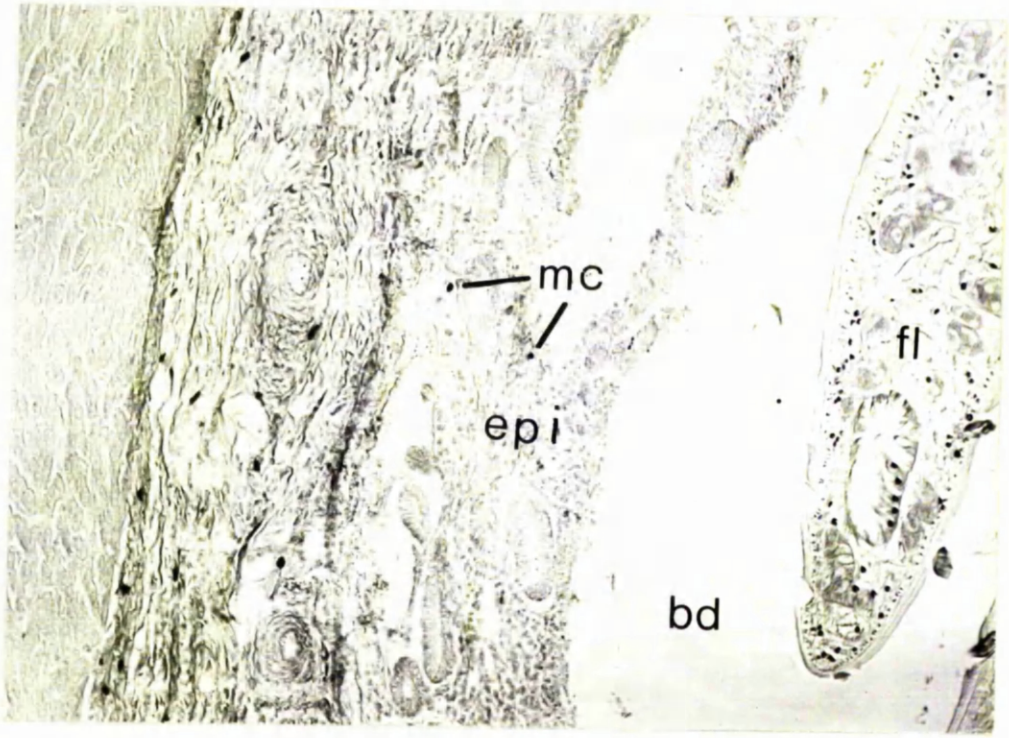


Plate 25.

A small uninfected bile duct in the parenchyma of the ventral lobe of the liver from a calf 20 weeks after infection with 500 metacercariae of F. hepatica. Mast cells though present in the connective tissue of the bile duct wall are not present in the epithelium or lamina propria of the duct.

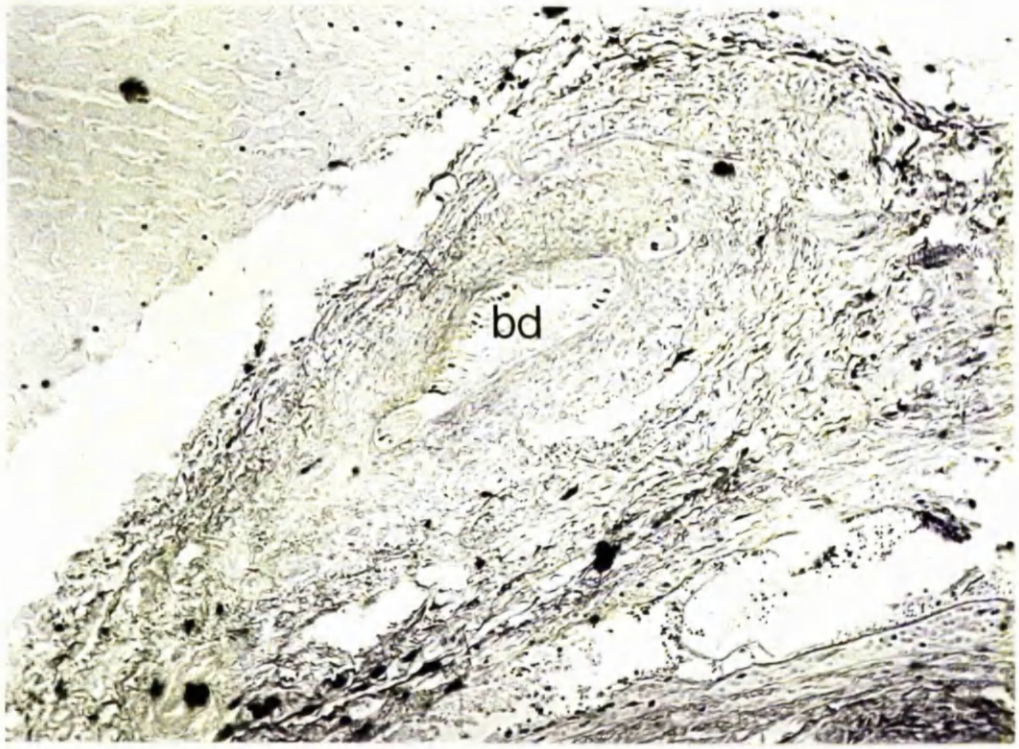
Stain: Astra blue / Safranin O. Magnification x 150.

Plate 26.

A section through the wall of a large bile duct in the parenchyma of the ventral lobe of the liver from a calf 24 weeks after infection with 500 metacercariae of F. hepatica. Despite the epithelial hypertrophy few sub-epithelial mast cells are present.

Stain: Astra blue/ Safranin O. Magnification x 150.

25



26

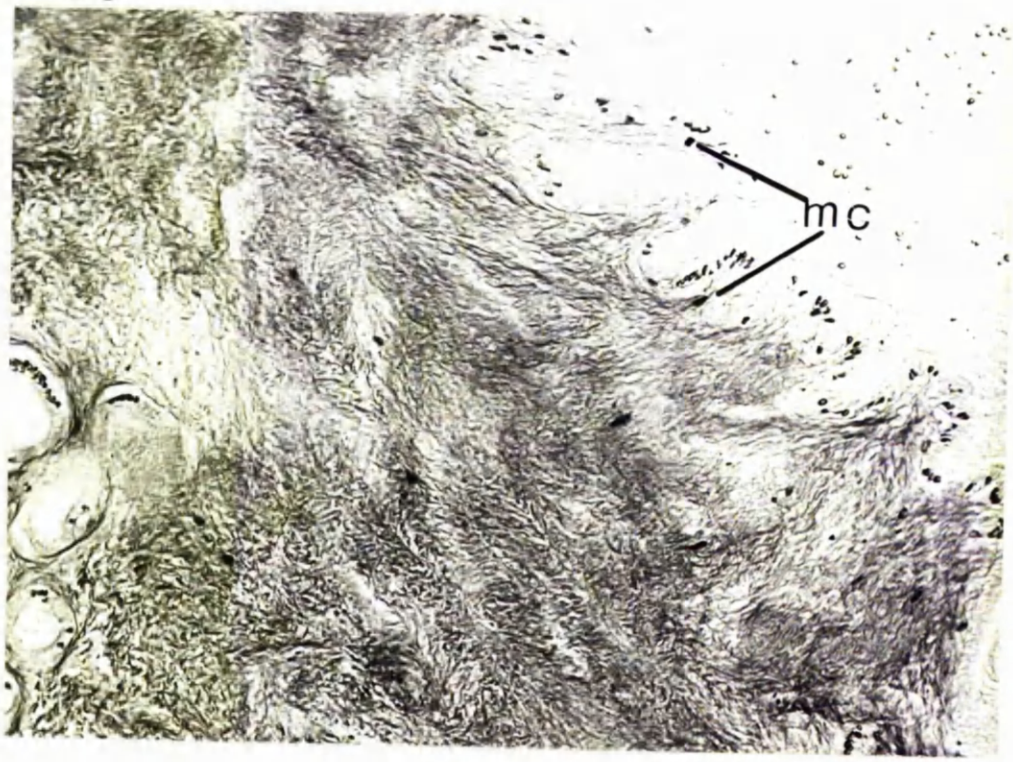


Plate 27.

An area in the epithelium and lamina propria of a parasitised bile duct in the parenchyma of the ventral lobe from a calf 20 weeks after infection with 500 metacercariae of F. hepatica. Numerous globule leucocytes (gl) can be seen in the epithelium of the duct. The surrounding lamina propria is heavily infiltrated with eosinophills, lymphocytes and plasma cells.

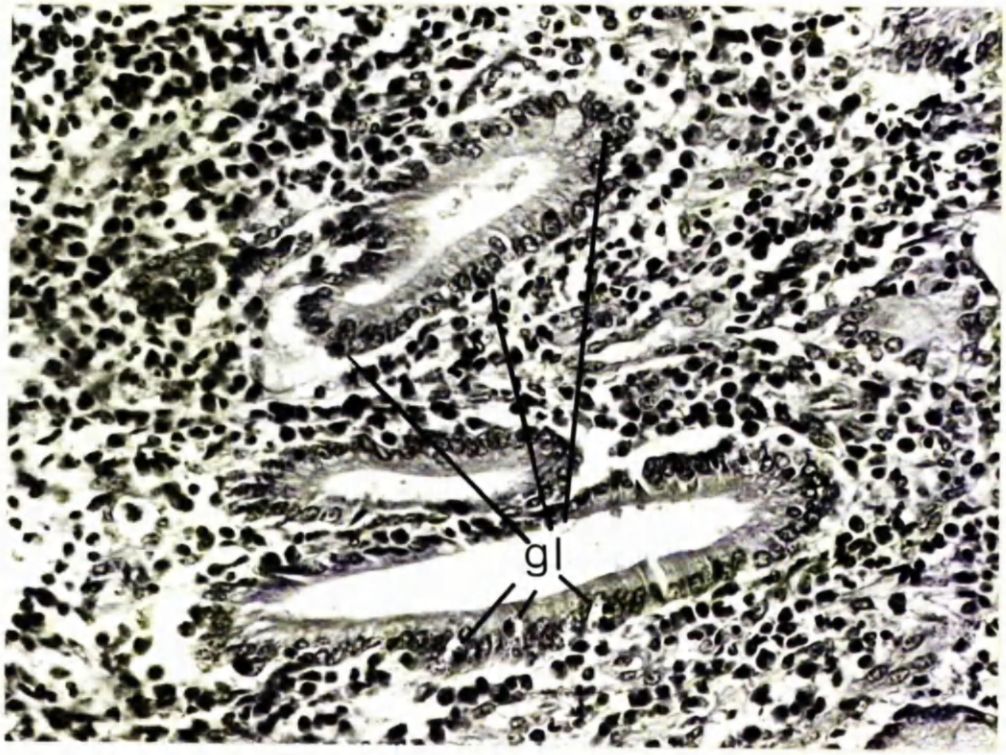
Stain: Astra blue / Safranin O. Magnification x 300.

Plate 28.

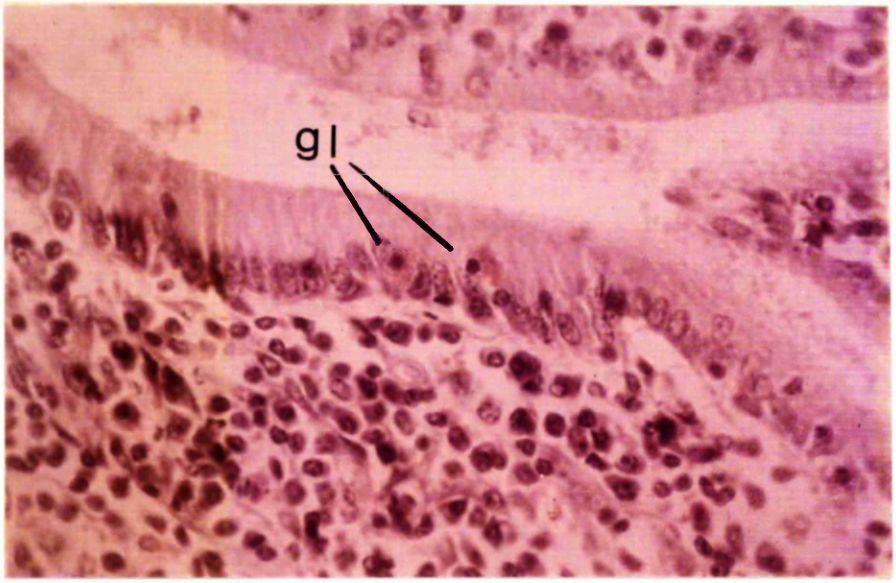
A higher magnification of the biliary epithelium shown in plate 27. Globule leucocytes are present in the epithelium of the duct.

Stain: H&E. Magnification x 500.

27



28



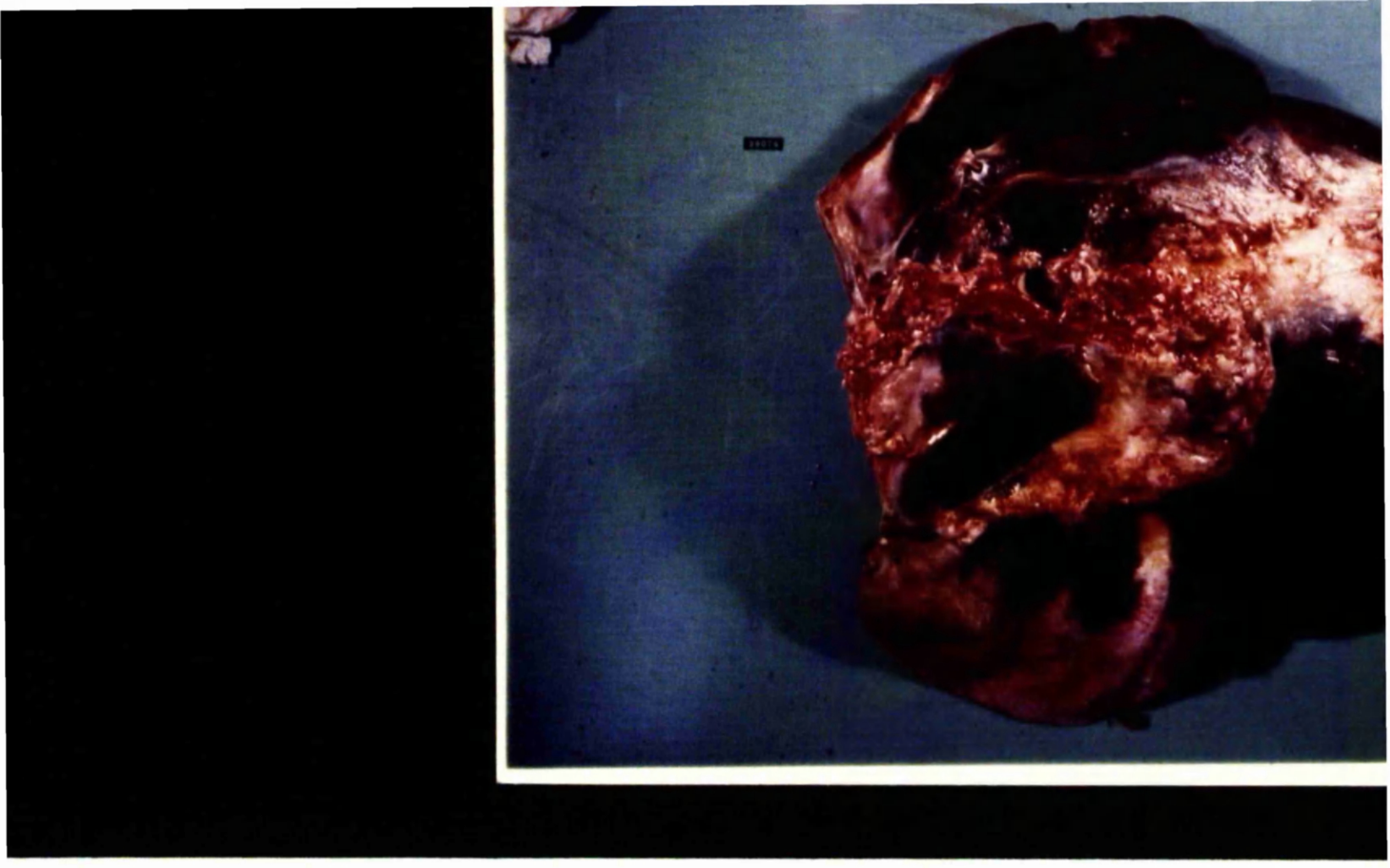


Plate 29

The visceral surface of the liver from a calf re

metacercariae of F. hepatica 17 weeks after an i

infection with 750 metacercariae of F. hepatica

after the initial infection.

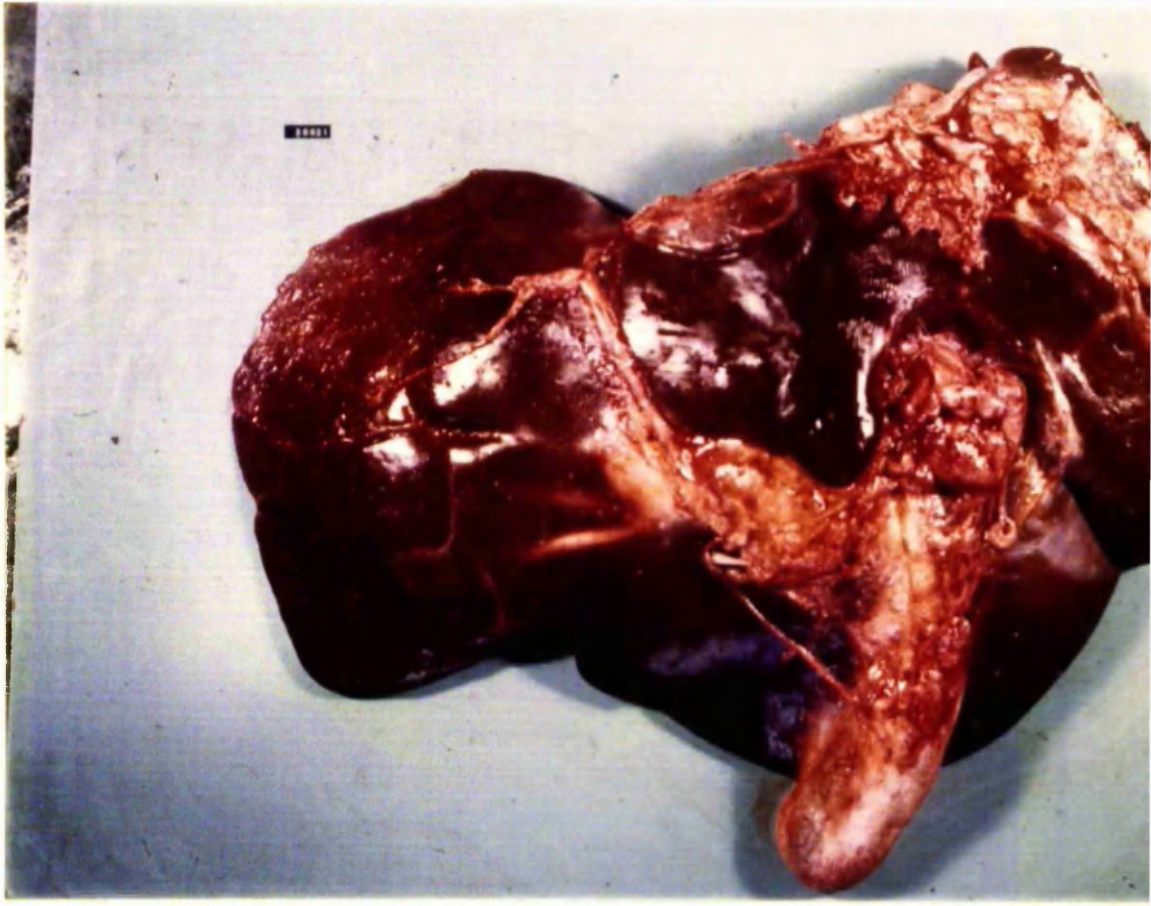


Plate 30

The visceral surface of the liver from a calf treated
anthelmintic 16 weeks after an initial infection with
metacercariae of F. hepatica, reinfected with 1,650 m
of F. hepatica one week later and killed 30 weeks aft
infection.

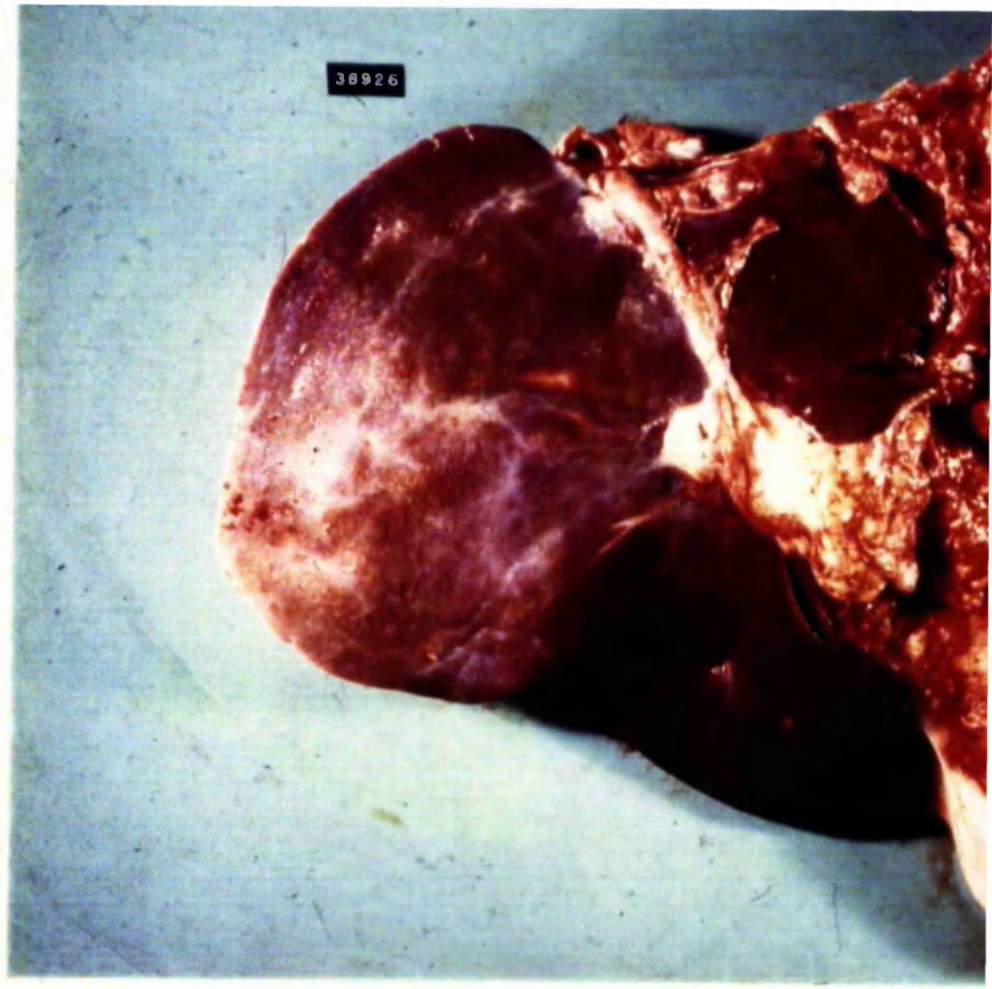


Plate 31

The visceral surface of the liver from a con

weeks after infection with 750 metacercariae

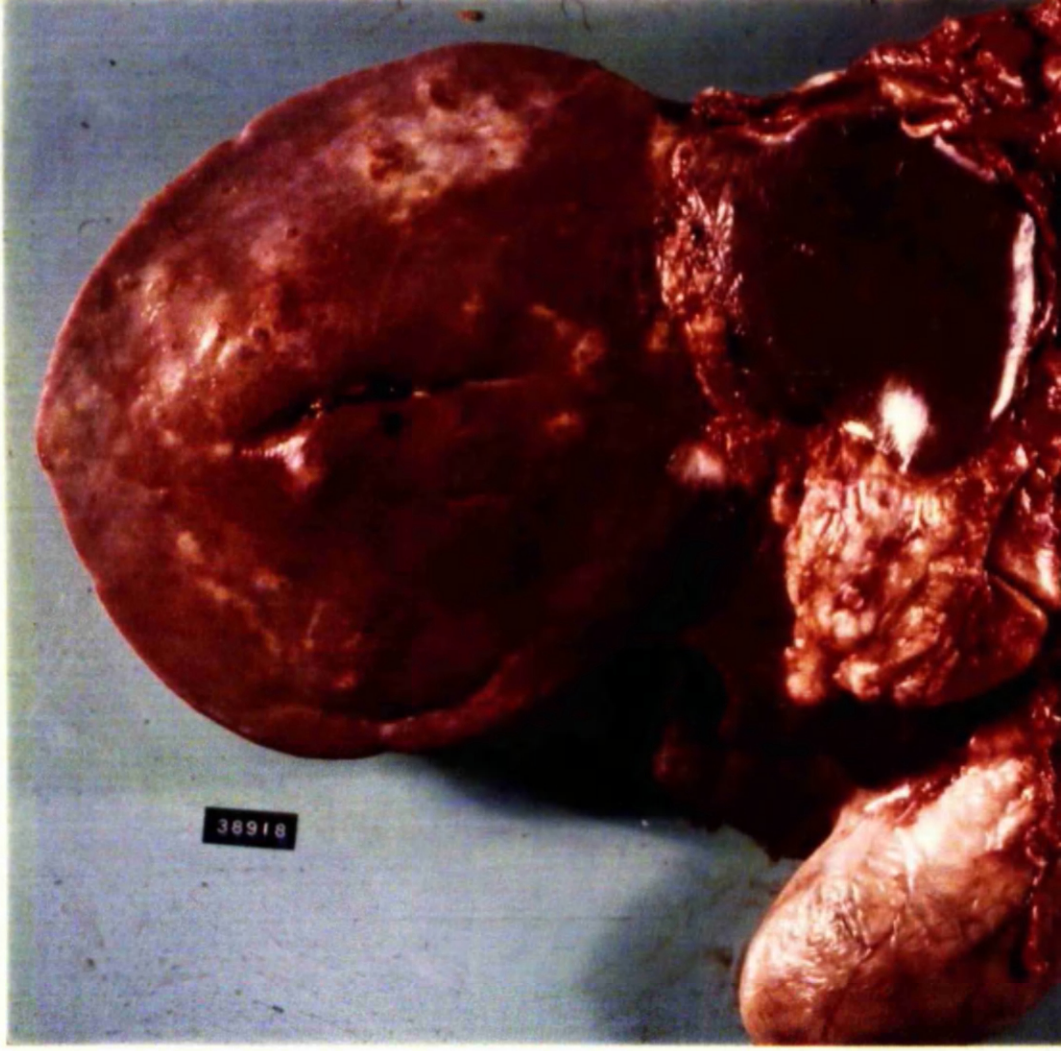


Plate 32

the visceral surface of the liver from a co

weeks after infection with 1,650 metacerca

Plate 33.

Section through a periportal area in the parenchyma of the liver from a calf reinfected with 1,300 metacercariae of F. hepatica 13 weeks after an initial infection with 750 metacercariae of F. hepatica and killed 7 weeks later. A marked cellular infiltrate is superimposed on the existing periportal fibrosis.

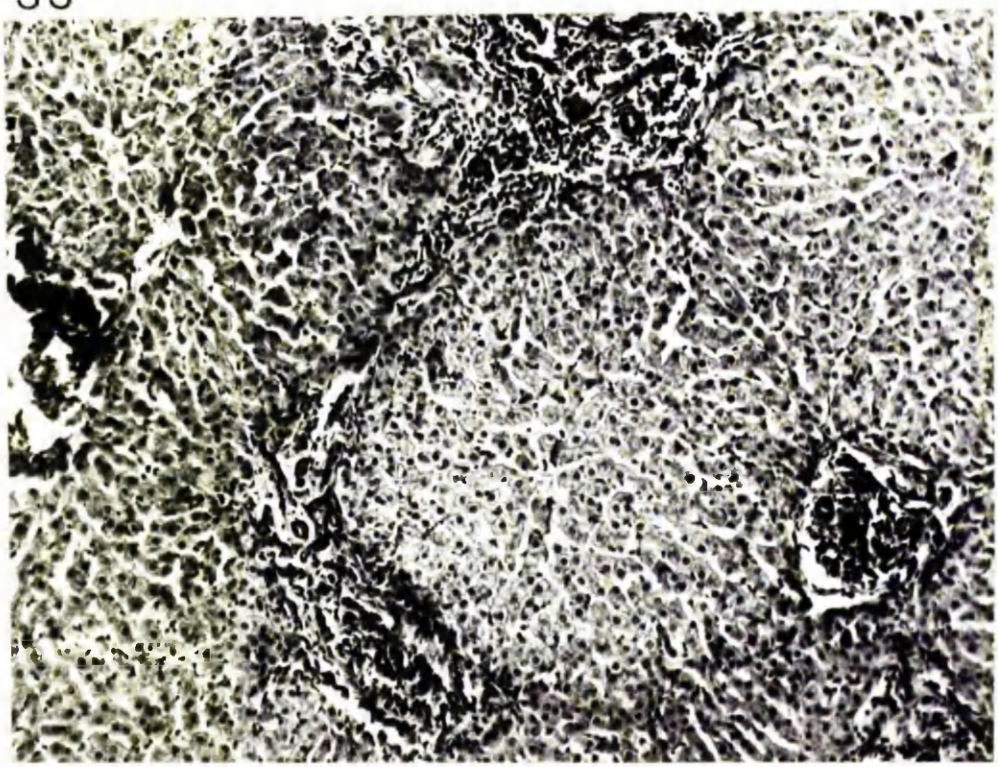
Stain: H&E. Magnification x 150.

Plate 34.

A portal area in the same section of liver as described in plate 33. The portal area is heavily infiltrated with lymphocytes surrounding columns of degenerating parenchymal cells.

Stain: H&E. Magnification x 150.

33



34

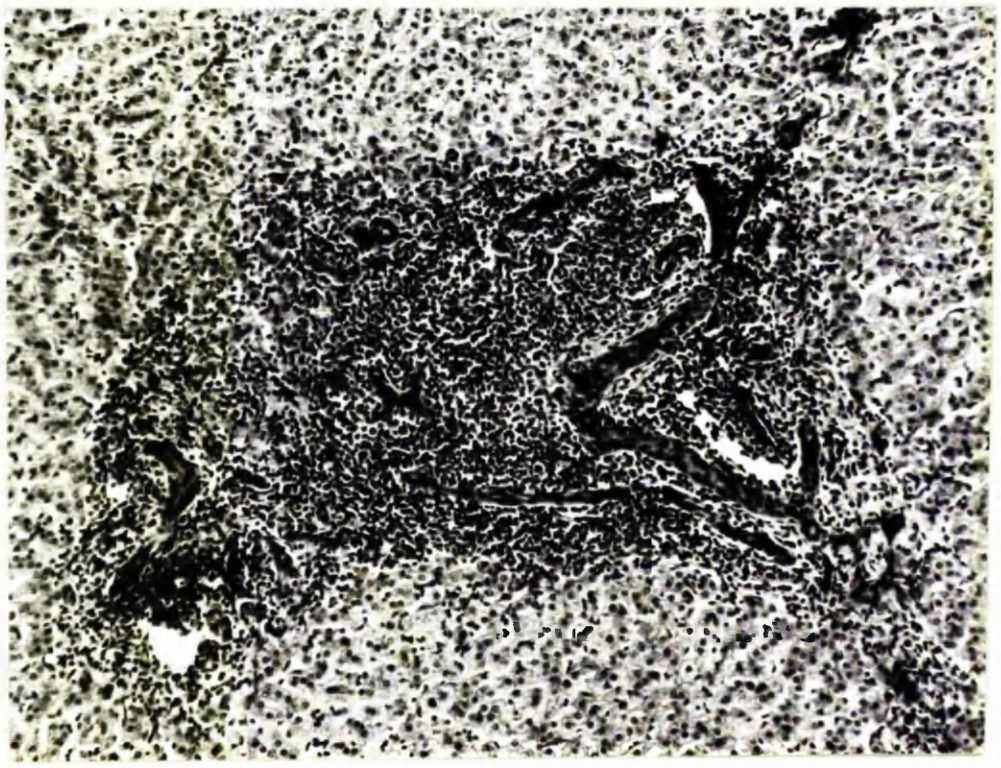


Plate 35.

Higher magnification of the periportal area in plate 33 showing the lymphocytic infiltration superimposed on the pre-existing fibrosis with the degeneration of adjacent parenchymal cells.

Stain: H&E. Magnification x 500.

35

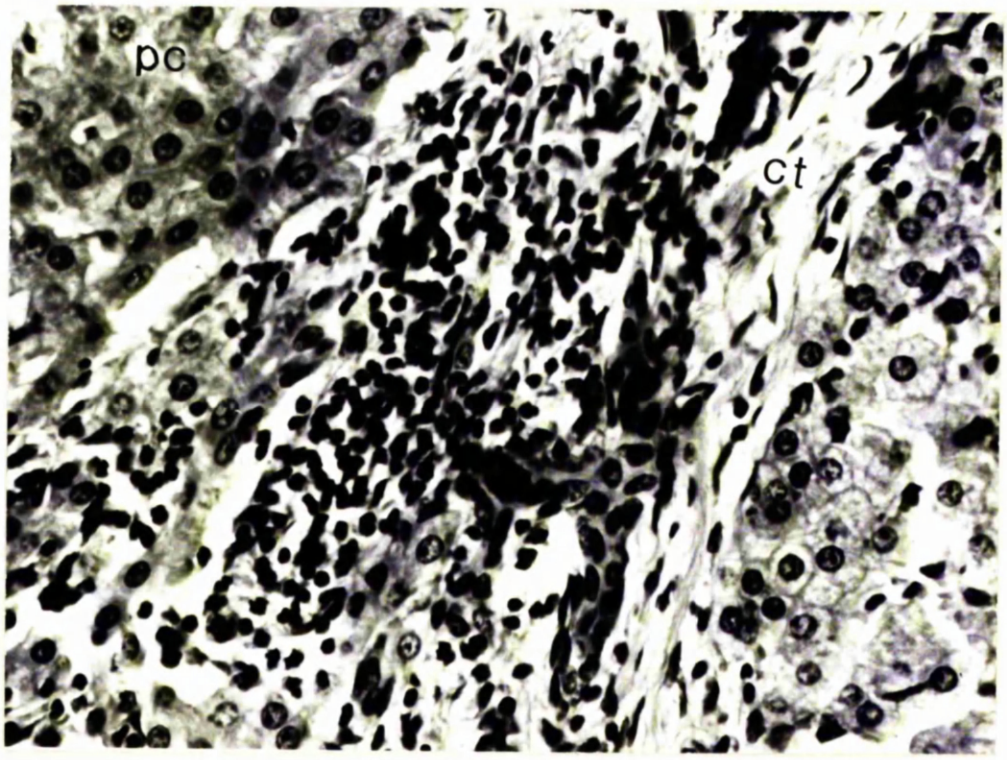


Plate 36.

A periportal area in the parenchyma of the ventral lobe of the liver from a control calf 20 weeks after a single infection with 750 metacercariae of F. hepatica. There is severe periportal fibrosis with little cellular infiltration or parenchymal cell necrosis .

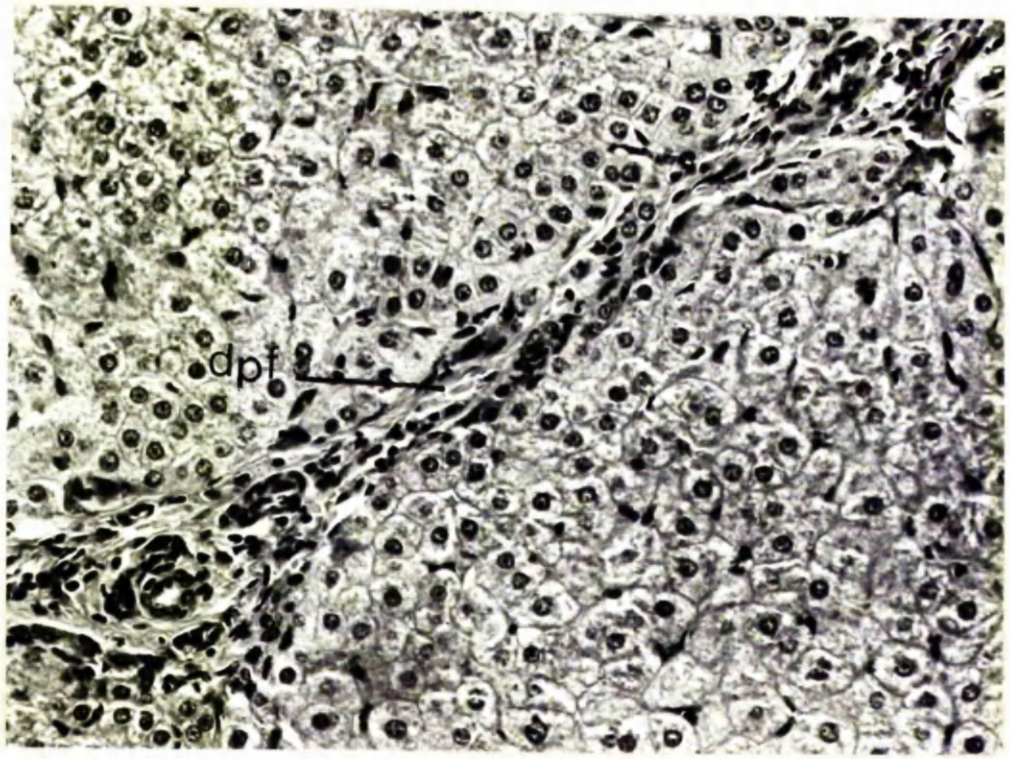
Stain: H&E. Magnification x 300.

Plate 37.

A periportal area in the parenchyma of the ventral lobe of a control calf 7 weeks after infection with 1,300 metacercariae of F. hepatica. There is parenchymal cell necrosis an eosinophill infiltration in this area. Little periportal fibrosis has developed.

Stain: H&E. Magnification x 150.

36



37