THE EFFECTS OF DRUGS ON EXPERIMENTAL HEPATIC CAPILLARIASIS IN MICE

Rosalind Frances Cheetham

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in fulfillment of the requirements for the Degree of Master of Science.

Johannesburg 1984

ABSTRACT

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CHEETHAM, Rosalind Frances, M.Sc.: University of the Witwatersrand, 1984.

In this study fourteen anthelmintic compounds were tested in white mice for their action against Capillaria hepatica (Nematoda). Mice, inoculated with 150 embryonated C. hepatica ova, were dosed with the drugs on days fourteen to eighteen post inoculation. The mice were sacrificed on day twenty-eight of the experiment. The livers, where the C. hepatica ova are deposited, were deep-frozen at -70°C to kill the eggs, and the number of eggs per gram of liver was subsequently determined by means of the McMaster chamber counting technique.

Of the anthelmintics tested, four prevented the deposition of C. hepatica ova in mouse liver at low dose-levels. These were: albendazole, 30.0 mg/kg; febantel, 30.0 mg/kg; mebendazole, 3.13 mg/kg; and oxfendazole, 12.5 mg/kg. Of these, mebendazole is the only one at present marketed locally for human use, and the total dose administered is within the recommended dose range.

Scanning electron microscopy was used to study the effects of these drugs on C. hepatica worms.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Rosalind Cheetham

Rosalind Frances Cheetham

1st day of November 1984.

ACKNOWLEDGEMENTS

I gratefully acknowledge financial assistance for this study from the South African Council for Scientific and Industrial Research, the South African Medical Research Council and the University of the Witwatersrand. I wish to thank the following pharmaceutical companies for their donations of anthelmintic drugs: Bayer, Ciba Geigy, Coopers, ICI, MSD, Panvet, Salsbury and Smith-Kline.

I have received help and encouragement from many people during the course of this work, and I am particularly indebted to Ms W. Meyer for her photographic assistance and to Ms D. Els who typed the first and second drafts of the manuscript. The final draft of this dissertation was produced by Express Word Processing Services, Benoni.

I am extremely grateful to Dr. A.N. Markus and Mr. W. Steinke who translated Spanish and German publications, respectively, for me.

Finally I wish to thank my supervisor, Prof. M.B. Markus, for his valuable assistance during the course of this work and his meticulous help in the preparation of this dissertation.

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INTRODUCTION

Capillaria hepatica (Bancroft, 1893) Travossos, 1915 is synonymic with both Trichocephalus hepaticus and Hepaticola hepatica. It is in the same family as the well known whipworm Trichuris trichiura. The classification of C. hepatica is as follows (Chitwood and Chitwood, 1974) :-

Phylum	:	Nematoda
Class	:	Aphasmidia
Order	:	Enoplida
Suborder	:	Dorylaimina
Superfamily	:	Trichuroidea
Family	:	Trichuridae
Subfamily	:	Capillarinae
Genus	:	Capillaria
Species	:	Capillaria hepatica

C. hepatica is parasitic in the liver of a wide range of animals, including rats, mice, dogs, muskrats, beavers, rabbits, peccaries, monkeys, squirrels, pigs and cats (Baylis, 1931; Luttermoser, 1938b; Faust, 1949). It is cosmopolitan in its distribution and there are several reports of C. hepatica from mammals in the Republic of South Africa and elsewhere in Africa. The infection rate varies but it is undoubtedly highest in rodents. For example, Shorb (1931) records an infection rate of 48 per cent amongst brown rats Rattus norvegicus in Baltimore, United States of America. In the same area, Luttermoser (1936) found C. hepatica in 86 per cent of adult R. norvegicus and 22 per cent of juveniles. Cochrane et al. (1957) recorded the parasite in 28 per cent of a random group of rats caught in the vicinity of Johannesburg, Republic of South Africa. Waddell (1969) found 79 per cent of a group of R. norvegicus from Brisbane, Australia to be infected with C. hepatica. More recently a survey in Connecticut, United States of

America by Conlogue et al. (1979), revealed that 81 per cent of the R. norvegicus rats examined were parasitised by C. hepatica. Amongst other host species the infection rate would appear to be lower. For example, Wright (1930) records C. hepatica infestation in only one per cent of the dogs he examined and McQuown (1954) reported it from 4 per cent of squirrels trapped in Louisiana, United States of America.

1.1 The Life Cycle of C. hepatica, including Comment on Spurious Infections

The life cycle of C. hepatica is summarised in Figure 1. The adult worms are found in the liver, where the females lay their eggs. C. hepatica worms are of typical trichurid appearance, having a thicker posterior portion containing the intestine and reproductive organs, and a whip-like anterior portion containing the stichosome, oesophagus and bacillary bands. According to Neafie et al. (1976) there is considerable variation in body size of the females, their length varying from 52 mm to 104 mm and width from 78 µm to 184 µm. Males are reported to be more uniform in size, namely in the region of 22 mm long (Neafie et al., 1976). However, Luttermoser (1938b) states that he found males which measured up to 37 mm in length and between 26 µm and 78 µm in width. It must be stated, however, that it is extremely difficult to measure the length of C. hepatica worms as they lie tightly coiled within the liver tissue and are very difficult to dissect out.

After mating, the female worms produce many thousands of eggs. These are not excreted from the host's body but remain within the liver until the animal dies and the eggs are released into the soil as a result of decomposition. Alternatively, if the host is eaten, the eggs pass unaltered and unharmed through the digestive tract, to be excreted with the faeces (Shorb, 1931). A good example of the latter method of egg release is cannibalism amongst rats, which is

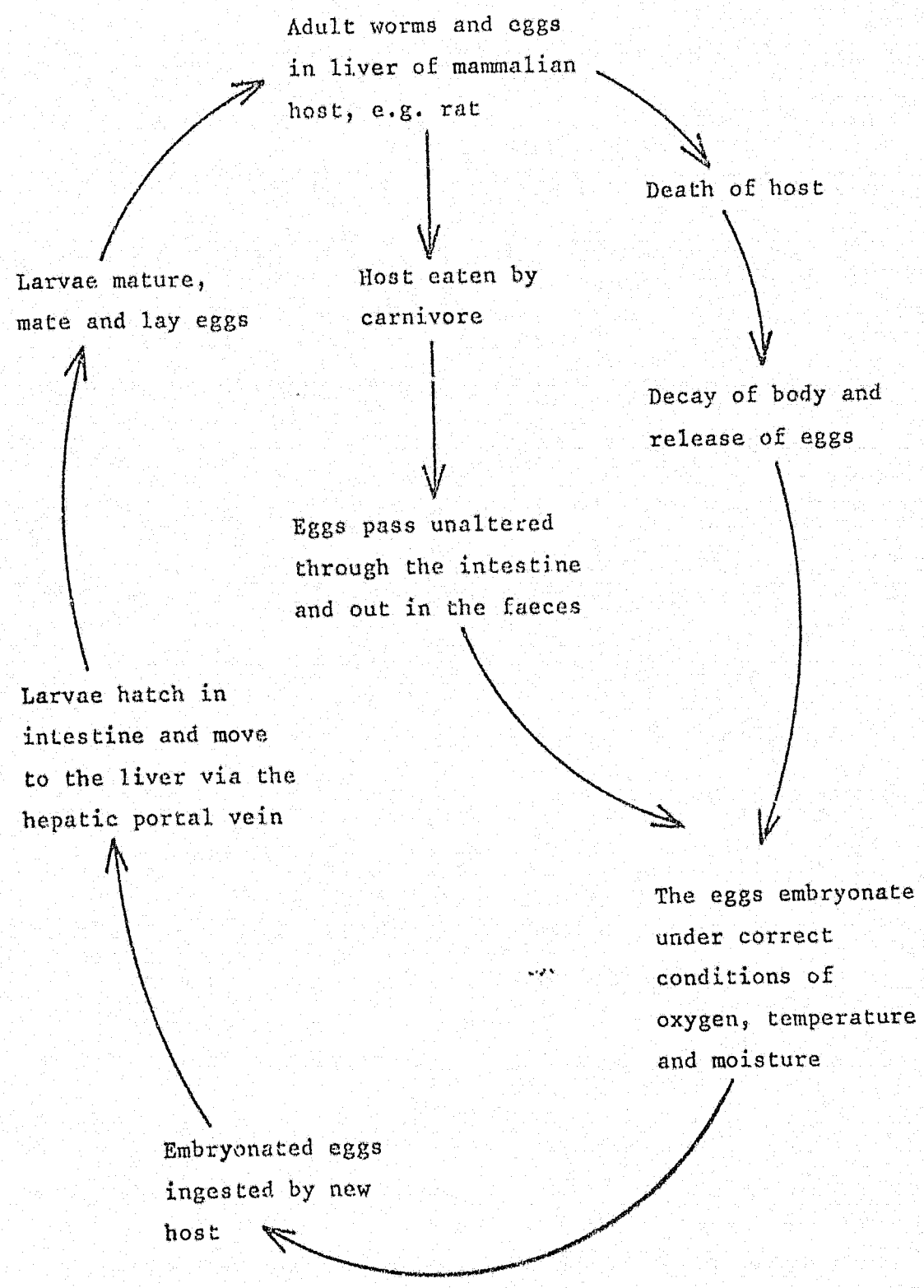

FIGURE I Life Cycle of C. hepatica

thought to account for the high prevalence of this parasite within rat populations (Calle, 1961; Farhang-Azad, 1977). It is also possible for humans to ingest unembryonated ova of C. hepatica, for example, by eating the liver of infected wild game (Brosius et al. 1948; McQuown, 1950). The occurrence of C. hepatica eggs within the host as a result is termed a spurious infection and, although it may cause transient symptoms of pain and diarrhoea, it is not a long-term infection of the liver. It is probable that the prevalence of such spurious infections is greater than presently thought, because of the similarity between the eggs of C. hepatica and T. trichiura (McQuown, 1950). Both these species have the same basic nematode eggshell structure, that is, there is an outer vitelline layer, a middle chitinous layer and an inner lipid layer. In C. hepatica, the inner portion of the chitinous layer is helicoidal whilst the outer portion forms a "pillar and beam-like" network which is not present in T. trichiura (Grigonis and Solomon, 1976; Warton, 1980). This unusual structure in the chitinous layer of the ova of C. hepatica serves to give the outer layer, of what appears to be a double shell, a striated appearance not seen in T. trichiura. The shape of the eggs of C. hepatica and T. trichiura are similar but closer inspection reveals that while both ova are elliptical, those of C. hepatica are larger, measuring 54 µm to 65 µm by 29 µm to 33 µm that 49 µm to 54 µm by 21 µm to 23 µm as in T. trichiura. The ova of both species have polar plugs but whereas in C. hepatica they do not protrude beyond the outer shell layer (see Figures 2a, 2b, 2c and 2d), they do in T. trichiura. It should also be noted that the C. hepatica ova are passed in the faeces when in the morula or immature two-cell stage. T. trichiura ova, on the other hand, are always passed while still unsegmented (Calle, 1961).

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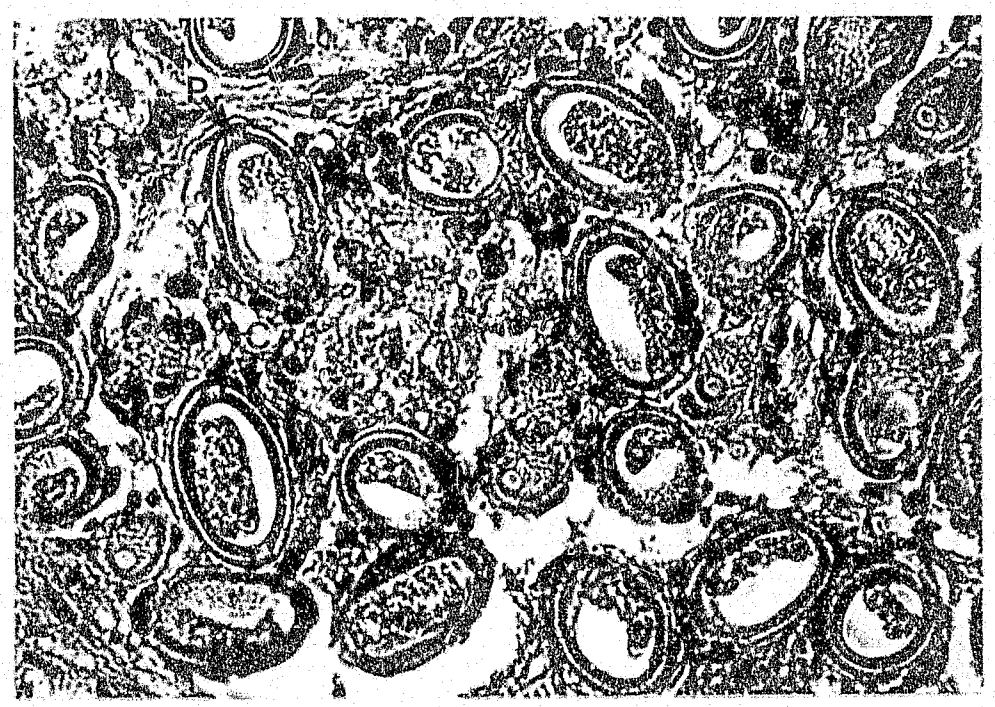

FIGURE 2a Light Micrograph of Sectional C. hepatica Eggs in Mouse Liver (x 400)
(P - Polar Plug, C - Chitinous layer)

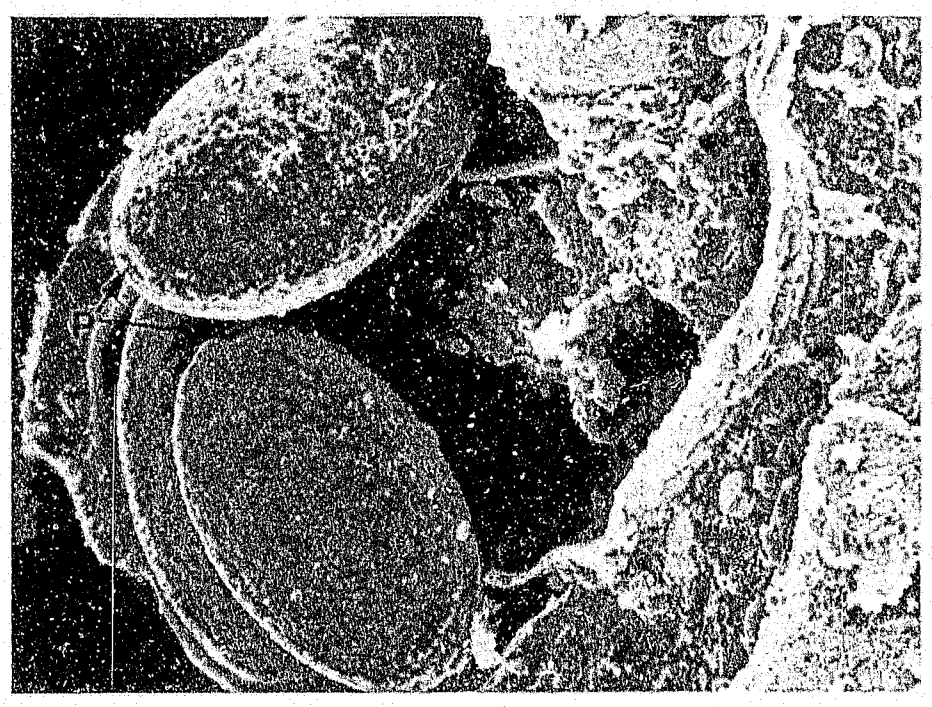

FIGURE 2b Scanning Electron Micrograph of C. hepatica Eggs (x 1 000)
(P - Position of Polar Plug)

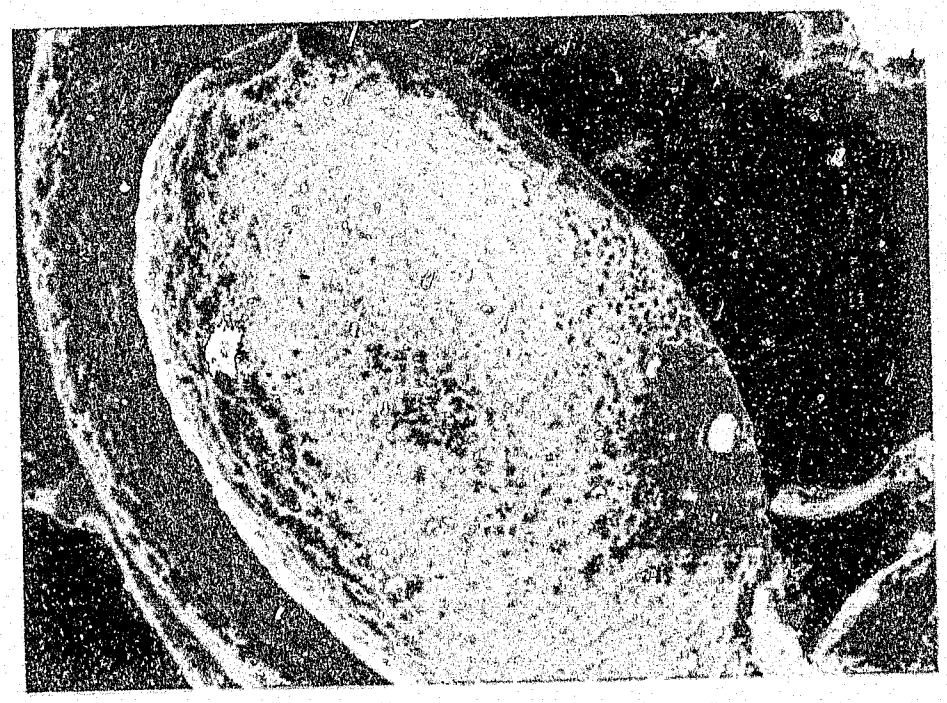

FIGURE 2c Scanning Electron Micrograph of C. hepatica Eggs (x 2 000)
(P - Position of Polar Plug)

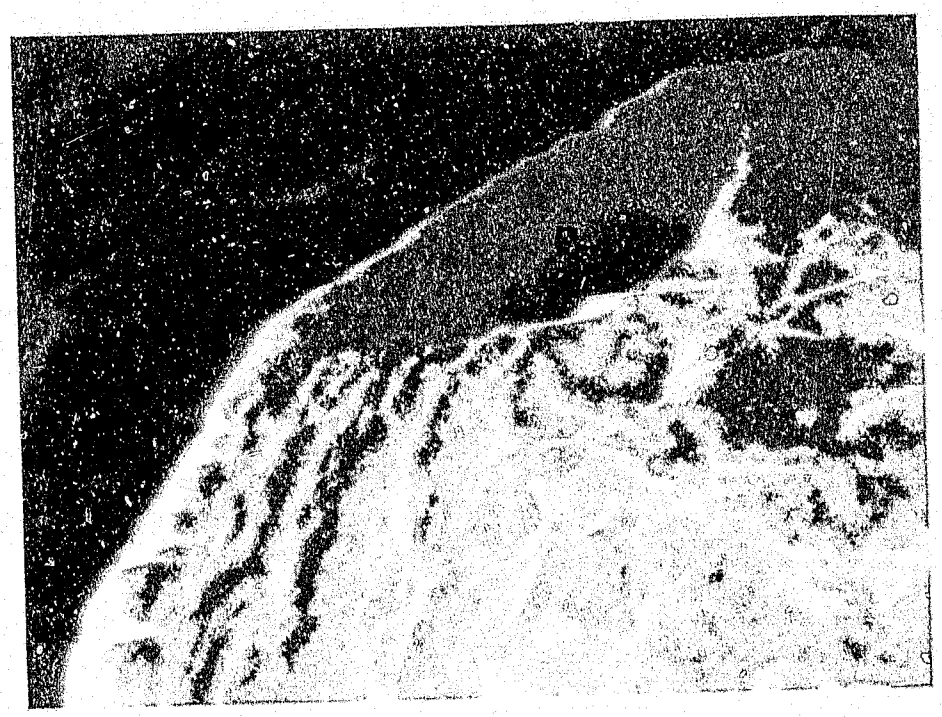

FIGURE 2d Scanning Electron Micrograph of C. hepatica Eggs - Polar Plug Region (x 10 000)
(P - Position of Polar Plug)

Taking account of the above features, differentiation between the ova of these two helminth species should be straightforward.

Once free in the soil, the eggs must embryonate before they are infective. The factors which determine the rate of embryonation are oxygen and moisture availability and the ambient temperature. As early as 1931, Shorb experimented with embryonating C. hepatica ova at temperatures of 22°C, 30°C and 37.5°C for periods of twenty-five and forty-two days. His results indicated that although many of the ova at 37.5°C had embryonated by day twenty-five, most had degenerated by day forty-two. Those at 22°C and 30°C, although slower to embryonate, did not degenerate. Wright (1961) confirmed these findings, determining the embryonation time at 20°C to 24°C to be a maximum of eight weeks. He also found that embryonation did not occur in eggs which had been previously subjected to a temperature of -40°C for a period of twenty-two hours.

Embryonated ova, when ingested by a new host, hatch in the intestine and penetrate the intestinal mucosa from six hours after hatching (Luttermoser, 1938b). The larvae enter the hepatic portal vein and are carried to the liver. Wright (1961) studied the development of C. hepatica larvae and found that second stage larvae were present in the liver three to seven days after infection, that third stage larvae were apparent from day five and that fourth stage larvae were present from day nine. The male larvae matured faster than the females, being considered fully developed at day eighteen, but females were not mature until two days later. Wright (1961) records first seeing female worms bearing eggs on day twenty-one, while Luttermoser (1938b) reports seeing female worms containing eggs on day eighteen in mice and on day twenty-one in rats.

It would appear that animals can withstand very large doses of C. hepatica ova, the number of eggs ingested being of more critical importance in juveniles. Luttermoser (1938b) found that adult mice could withstand doses of 1 000 eggs, although the development of the worms was slower than normal and they were smaller. Immature mice, however, were killed by doses of 500 eggs. Similarly, Luttermoser (1938b) demonstrated that a lethal dose in juvenile rats was between 2 500 and 5 000 ova, whereas adults could tolerate doses of 30 000 ova. In animals with massive infections, worms were found free in the body cavity, where they appeared to mature normally. In addition, worms were infrequently found in the lungs, but here the worms lived only eighteen days. Luttermoser (1938b) demonstrated that a heavy infestation with C. hepatica caused retarded growth of the host, and, especially in mice, emaciation. It would appear from his results that in rats a small initial infection of C. hepatica "immunised" the animal against a larger challenge infection given later. Zahner et al. (1980) pursued this idea in some work using the multimammate mouse Mastomys natalensis as the host animal. They determined that an initial infection of C. hepatica did suppress the reproductivity of a large, but sublethal, challenge infection, but if the challenge infection was only of moderate size then the existing infection had no effect on it.

Normal egg production increases rapidly from about day twenty of the infection to a peak at day forty, with no further egg production after about day seventy (Lämmler et al., 1974). After their reproductive functions have been completed, the C. hepatica worms die and slowly disintegrate.

1.2 Reasons for Study

The present investigation has been prompted by the fact that there are thirteen cases of C. hepatica infestation in humans documented in the literature to date (see Historical Review). Three of the cases occured in South Africa. Over half of the thirteen cases resulted in death, the infection either having been discovered at autopsy; or, even though the infection was diagnosed by liver biopsy, the patient died due to lack of suitable treatment.

There is no established cure for C. hepatica infection in man. Some German researchers have looked at the effects of a number of drugs on C. hepatica in M. natalensis (Lämmler and Grüner, 1976), but it was thought that there was scope for more work in view of the production of new anthelmintics.

2. HISTORICAL REVIEW

2.1 Drug Treatment for Experimental C. hepatica Infection

The first report of experimental chemotherapy of C. hepatica infection is given by Waddell (1969). He treated rats Rattus rattus subcutaneously and intraperitoneally with three 200 mg/kg doses of methyridine on days fourteen to sixteen of the infection. The results of this study indicated that oviposition had been almost totally prevented.

The second study was carried out using the multimammate rat Mastomys natalensis (Lämmler and Grüner, 1976). Their results are summarised in Table 1. These authors examined twenty-four compounds for activity against C. hepatica by giving five doses, one on each of days fourteen to eighteen of the infection. Fenbendazole, mebendazole and parbendazole were the most effective drugs at low dose levels, as indicated by the high percentage reduction in the egg counts as compared to the control groups. These three drugs were tested further against stage two and stage three larvae - by dosing on days five to nine of the infection. Mebendazole and parbendazole were proved to be most effective.

TABLE 1 Summary of Results of Lämmler and Grüner (1976) for Drug Treatment of C. hepatica Infection.

DRUG	DOSE mg/kg x 5[1]	% REDUCTION IN EGG COUNTS [1]
Bitoscanate p.o. [2]	20 40	0 0
Cambendazole p.o.	1.56 3.12 6.25 12.5 25 50 100	0 56.2 99.0 98.6 100 100 100
Diethylcarbamazine p.o.	200 400	0 32.3
Disophenol s.c. [3]	10 20	0 0
Dithiazanine p.o.	40	9.4
Fenbendazole p.o.	1.56 3.12 6.25 12.5 25 50 100	0 33.8 93.6 99.5 100 99.9 100
Fenthione p.o.	20	17.0
HOE 28637a p.o.	200 400	1.7 0
HOE 33258d s.c.	2.5 5 10 20 20 40	0 31.1 83.3 99.8 100 100
Hycanthone s.c.	10	19.1
Levamisole p.o.	10 15 20 40	0 43.6 98.8 99.3

TABLE 1 (Continued)

DRUG	DOSE mg/kg x 5[1]	% REDUCTION IN EGG COUNTS[1]
Mebendazole p.o.	0.78	0
	1.56	35.2
	3.12	89.5
	6.25	99.98
	12.5	100
	25	100
	100	100
Methyridine s.c.	50	0
	100	0
	200	84.2
	400	100
Metrifonate p.o.	200	15.1
	400	33.5
Morantel Tartrate p.o.	200	0
	400	0
Niridazole p.o.	100	0
Nitrofurantoine p.o.	100	14.9
Nitroxynil s.c.	5	5.3
	10	58.9
	20	62.1
	40	12.6
Parbendazole p.o.	1.56	3.7
	3.12	13.9
	6.25	56.1
	12.5	83.9
	25	98.2
	50	99.6
	100	100
	200	100
Piperazine Citrate p.o.	250	0
	500	0
Pyrantel Tartrate p.o.	100	0
	200	0
Suramine s.c.	20	0
	40	43.3
Tetramisole p.o.	20	0
	30	95.3
	40	99.7

TABLE 1 (Continued)

DRUG	DOSE mg/kg x 5[1]	% REDUCTION IN EGG COUNTS[1]
Thiabendazole p.o.	12.5	0
	25	61.7
	50	34.4
	100	77.5
	200	87.2
	300	98.1
	400	99.95
	500	99.97

1 See text
2 p.o. per oralis
3 s.c. subcutaneous

2.2 Review of True Human Infections with C. hepatica

The first report of a true case of C. hepatica infection in man was recorded by Dive et al. (1924). The case was that of a twenty-year-old man who had been serving in the army in India. He exhibited the symptoms of septic pneumonia, and after his death the autopsy revealed abcesses in the lungs and the liver. Microscopic examination of tissue in thevicinity of the liver abcess revealed masses of eggs which were identified as Hepaticola hepatica (a synonym for C. hepatica). Material from the periphery of the liver lesion revealed mature female worms. No such eggs or worms were recovered from the lung tissue. It was concluded that pyaemia, resulting from the nematode infection and the secondary lung infection, was the cause of death.

The next description of a true human infection of C. hepatica was given by McQuown (1950) after a girl aged seventeen months had been admitted to hospital in Louisiana in the United States of America, thought to be suffering from pneumonitis and possibly meningitis. When she was first seen as an outpatient she was suffering from a nasal infection, cough and dyspnoea with a raised temperature. She was treated with sulfadiazine but three days later she was

admitted to hospital acutely ill, apathetic, stuporous and with a grunting respiration. Her liver was enlarged. The patient was placed on routine critically ill care but died twenty-six hours after admission. At post-mortem the heart, lungs, spleen, kidneys and liver were seen to be enlarged. The appearance of the liver was abnormal, being yellowish-brown. When cut, the liver proved to be soft with numerous small haemorrhagic areas and greyish-tan areas 1 mm to 3 mm in diameter scattered throughout the parenchyma and resembling small focal areas of necrosis or abcesses. Microscopically, the liver was found to contain numerous granulomas filled with C. hepatica ova and located in the periportal spaces. The surrounding chronic inflammatory reaction encroached on, and in some places, destroyed, the liver lobules. No adult parasites were seen. The lungs did not contain parasites. Ascaris lumbricoides nematodes were found in the intestine. The final pathological diagnoseswere: C. hepatica infection of the liver, pulmonary oedema, interstitial pneumonitis, acute cardiac dilation with congestive failure, malnutrition, dehydration and ascariasis.

Otto et al. (1954) documented the case of a seven-year-old girl whose C. hepatica infection was diagnosed by liver biopsy, but who subsequently died. This girl suffered from sickle-cell anaemia and had been admitted three times previously for sickle-cell crises, before being admitted to hospital in Maryland in the United States of America in 1951 with fever, shortness of breath and nose bleeds of six days duration. In addition to these symptoms, anorexia, lethargy and severe malaise were evident after admission and she vomited clotted blood. She later developed headaches and epigastric, scapular and neck pains. The liver and spleen were found to be enlarged. On the forty-sixth hospital day a laparotomy was performed. The spleen was seen to be twice its normal size and the liver was enormous. The liver surface was smooth with purplish and white speckles but without gross scarring. A liver biopsy and lymph node were

taken. Grossly, the liver revealed tiny yellow foci 1 mm in diameter scattered evenly throughout. Microscopically, it could be seen that the normal liver architecture had been completely disrupted by the parasitic infection. Adult worms, often containing eggs within their body cavities, were usually at the centre of the necrotic foci, which were surrounded by an intense inflammatory response. It was thought that these lesions were probably located in the portal areas, but because of the disruption of the liver structure, the authors could not be certain. Eggs were present in the liver but no embryonated eggs were seen, which is characteristic of C. hepatica infection. The treatment of this patient was largely supportive and initially consisted of administration of vitamin D and calcium. The liver remained enlarged and two courses of chloroquine were given. Chloroquine was chosen because its hepatotoxicity is low; and it is known to be heavily deposited in the liver and is active against some helminths. This treatment was completed on the 107th hospital day but the liver continued to enlarge and on day 168 a second liver biopsy was performed. This second biopsy revealed tiny grey foci which were seen to be broad areas of scarring containing enormous numbers of eggs. Again the eggs were thought to be concentrated in the portal areas. The liver remained enlarged and the patient was discharged on day 203, to be readmitted twice more (on days 307 and 331 after the initial admission), in an unchanged condition. Nearly two years after the onset of the illness the patient was chronically ill and was readmitted for weakness, nosebleeds and anorexia. No treatment was given and two months later she was admitted to hospital with acute fever. She developed convulsions and suffered respiratory arrest and died five minutes after admission. At autopsy the heart, thymus, spleen and kidneys were enlarged, the liver being grossly enlarged at nearly four times the normal weight. Histological examination of the liver supported the previous observations which identified the parasite as C. hepatica. The authors state that one lung section showed

possible evidence of C. hepatica infection in the form of hyaline and calcified bodies in giant cells, although no actual eggs or worms were seen. The final diagnosis in this patient was C. hepatica infection of the liver with granulomatous lesions containing eggs and worms, cirrhosis of the liver, ascites, granulomatous lesion of the lung with a foreign body reaction, a history of sickle cell anaemia with repeated crises, sickle cell lesion of the spleen with splenemegaly, marked intravascular sickling of red cells, foci of encephalemalacia, brain, cardiac hypertrophy and dilatation; possible rheumatic myocarditis with Aschoff body formation; renal hypertrophy with glomerular engorgement, slight focal calcification of renal tubular ephithelium. In the final analysis it was admitted that the sickle cell disease contributed to the child's illness and hepatic dysfunction. The hepatic disruption was, however, wholly attributed to C. hepatica, but hepatic damage, known to occur in sickle cell anaemia, may have further aggrevated the symtoms of hepatic insufficiency.

Turhan et al. (1954) published the fourth case of true C. hepatica infection. In this instance, C. hepatica was found incidentally at autopsy in a sixty-year-old man. The clinical findings were bronchopneumonia and senile dementia, and the pathological findings were bronchopneumonia and C. hepatica in the liver.

The fifth case of true human infestation with C. hepatica was diagnosed in Honolulu and reported by Ewing and Tilden (1956). This was the case of a fifteen-month-old female infant who was first seen by a physician because of fever, anorexia, cough, constipation and a reluctance to walk. The parents stated that their daughter had been known to eat dirt. An ascarid infection was diagnosed and treated with two courses of hexylresorcinal, which in turn required Benadryl ® (diphenhydramine HCL) to treat the side effects. Terramycin ® (oxytetracycline) was prescribed for the fever

but upon failing to respond to this treatment, and also in view of the pulmonary congestion, the child was hospitalised. Fourteen days after the patient was first examined a course of Atabrine ® (quinacrine) was administered. At about the same time the liver and spleen were found to be enlarged. Shortly after this a course of Hetrazan ® (diethylcarbamazine), an effective treatment against ascariasis and filariasis, was given together w.. Lederplex ®, a brand of vitamin B complex. Nearly one month after the girl was first examined a liver biopsy was performed. The liver was seen to be greatly enlarged and the surface was studded with pearly white granulomatous lesions up to 2 mm in diameter. Microscopically, the liver showed massive deposition of ova in the portal areas together with many worms, and an associated inflammatory reaction. The parasite was identified as C. hepatica. The patient was discharged, although the liver and spleen were still enlarged. Three months after the initial examination, the patient was readmitted to hospital with bronchopneumonia, cough and dyspnoea. The liver and spleen were further enlarged. Treatment consisted of continous oxygen, penicillin and streptomycin, blood transfusion, intravenous and subcutaneous fluids and parenteral vitamins. However, the child died a week later. At necropsy the spleen was enlarged and the liver greatly enlarged, microscopical examination of the liver revealing the ova previously identified as C. hepatica. However, the worms had by now degenerated. There was considerable liver cell destruction towards the periphery of the lobules and the resultant fibrosis had distorted the normal liver architecture considerably. Microscopic examination of both the lungs and kidneys revealed calcified material, which it was thought provided presumptive evidence of C. hepatica parasitism in these organs, but no eggs or worms were seen.

Cochrane et al. (1957) provide an account of the sixth case of C. hepatica in man to be reported in the world, and the

first case from South Africa. The patient in this instance was a fifteen-month-old girl who was reported to be an earth-eater. She was admitted to hospital in Vanderbijlpark, Transvaal, seriously ill with fever, umbilical hernia, enlarged glands and enlarged liver. A liver biopsy was performed approximately two months after the onset of the child's illness. The liver was found to be moderately enlarged and the surface studded with pinhead-sized yellow-grey nodules, in places aggregated to form an irregular lesion of 2 cm to 3 cm in diameter. The spleen was not enlarged. Microscopically, large numbers of ova were seen in the portal tracts. The ova were surrounded by areas of inflammation, which had resulted in distortion of the liver architecture. The parasite was identified as C. hepatica. Non-specific drugs which were given included Terramycin ® (oxytetracycline), Achromycin ® (tetracycline with sodium-metaphosphate), penicillin, Chloromycetin ® (chloramphenicol), Meticorten®(prednisone) and Methischol®(choline dihydrogen citrate). As specific therapy against the parasitic infection an antimonyl drug, Triostam ® (sodium antimonyl gluconate), was given. This controlled the temperature and the liver enlargement decreased. Approximately two-and-a-half years after the first liver biopsy, a second one was performed. The liver size and surface were deemed normal. No ova were seen on microscopic examination, although some periportal fibrosis was present. The haematological findings were also normal. Nearly four years after this child was first examined Cochrane and Skinstad (1960) published a follow-up report.

The seventh published case of human infestation with C. hepatica (Ward and Dent, 1959) describes the illness of a two-year-old girl. Her case history had included diarrhoea; and stool examination had revealed ova of A. lumbricoides and T. trichiura, for which infections she was treated. However, the diarrhoea persisted and she developed cough, fever and nausea with vomiting. Prior to hospital admission she had a

convulsion. On admission to hospital in New Orleans in the United States of America, she was lethargic, dehydrated and suffered from dyspnoea. The patient was treated with intravenous fluids, antibiotics, antipyretics and sedatives. About eight hours after admission she expired, having received treatment for respiratory distress. The clinical diagnoses were bronchopneumonia, intestinal parasitism and gastroenteritis due to Shigella sonnei, with anaemia and dehydration. Autopsy revealed that the colon was infected with T. trichiura and that the lungs and liver were enlarged. The liver was tan in colour and, when cut, tiny scattered foci of greyish-tan discolouration about 1 mm in diameter were seen. Microscopically these proved to be fibrous granulomas enclosing numerous ova of C. hepatica in the periportal areas. Microscopic examination of the lungs did not reveal any parasites or granulomata. Additional diagnoses of interstitial pneumonitis and C. hepatica infection of the liver were then made. The authors believe that the C. hepatica infection was an incidental finding and that death was due to bronchopneumonia and gastroenteritis, although it would seem reasonable to assume that the debilitating effect of C. hepatica infection must have contributed to the patient's death.

Calle (1961) describes the eighth genuine case of human infection with C. hepatica. The patient was a twenty-month-old male infant who was admitted to hospital in North Carolina in the United States of America, with fever, lethargy and abdominal swelling. He was known to be a dirt eater. He was treated for sore throat and infected ears but there was no reduction in the fever. On the seventeenth hospital day a liver biopsy was done. The liver was seen to be purple-tinted, indurated and mottled, with numerous grey scars scattered throughout the organ. Microscopic examination of the scars revealed that they were granulomata situated in the portal areas of the liver and containing C. hepatica ova. These granulomata were surrounded by an

inflammatory response which had caused partial disruption of the liver architecture. Partially disintegrated worm fragments were seen but there was no calcification. The infection was treated with Delvex ® (dithiazanine iodide). The patient improved and the liver became smaller. Although liver biopsy was not performed again, the child appeared to be in good health ten months after the initial illness.

The ninth case report is also the second in South Africa. Kallichurum and Elsdon-Dew (1961) describe the case of a five-year-old girl who was admitted to hospital in Durban, Natal, with measles, bronchopneumonia and dysentry. Stool examination revealed T. trichiura, Trichomonas hominis and A. lumbricoides. There was apparently no clinical evidence of liver disease and no blood count was done. The patient died on the sixth day. At autopsy the liver was seen to be of normal size but studded throughout with gritty yellow-white specks 1 mm to 2 mm in diameter. Microscopically these appeared as foci of fibrosis and inflammation containing numerous ova of C. hepatica, but the surrounding liver architecture did not appear distorted.

Romero Garcia et al. (1962) provide the tenth report of human infestation with C. hepatica. The case is that of a twenty-two-month old female child from Guadalajara in Mexico. The family's living conditions were fairly primitive and there were dogs, cats and chickens close by which often strayed into the house. Rats and mice were also present. The child was known to be an earth-eater. For two months prior to hospital admission the child's symptoms had included fever, sweating, anorexia, vomiting, oedema, pale skin, palpitations, severe headaches, irritability, and loss of weight. Immediately prior to admission the mother had detected abdominal swelling and a mass in the abdomen. At a previous hospital, blood tests had been carried out and leukaemia diagnosed. Upon admission, for the second time to hospital, the general clinical picture was as already

described, and, additionally, the liver was found to be enlarged and painful to the touch. Blood tests and liver function tests revealed leucocytosis, eosinophilia and hypergammaglobulinaemia. Several intestinal parasites were found. These findings did not confirm the diagnosis of leukaemia. Hetrazen ® (diethylcarbamazine) was administered to deal with the parasitic infections and a steroid was also given in varying doses, from 2 mg to 15 mg per day. The steroid resulted in the fever disappearing and the hepatomegaly, eosinophilia and leucocytosis being reduced. If the steroid therapy was suspended the fever returned and the patient's general condition deteriorated. Twelve months after initiation of treatment, the steroid administration was stopped with no ill-effects. At this time a liver biopsy, by means of a laparotomy, was performed. The liver was seen to be enlarged, with many whitish-yellow nodules of 1 mm to 2 mm diameter scattered over the whole surface of the organ. Histology revealed numerous granulomata in the portal spaces. The granulomata contained eggs of C. hepatica. Fibrotic areas were present. There was no infiltration in the lungs. The patient was thus diagnosed as having an infection with C. hepatica. No further treatment for the infection was given.

Details of a case of human C. hepatica infection in Italy were published by Cislaghi and Radice (1970). A forty-month-old girl who had been ill intermittently for about two years was admitted to hospital, chronically ill. The major finding was liver enlargement and a liver biopsy was performed. The liver appeared cirrhotic with numerous grey scars scattered throughout the organ. Microscopically these areas were seen to be granulomata containing ova of C. hepatica, which were partially calcified. There was an inflammatory response but no adult worms were seen. The treatment is not detailed beyond the use of unspecified anthelmintic drugs, but the patient appears to have survived.

Silverman et al. (1973) give an account of the infestation of a seventeen-month-old infant girl from Bethal in the Transvaal. This constitutes the twelth case in the world literature and the third in South Africa. The child was admitted to hospital after suffering from cough and fever for a month. She was known to be a sand-eater. The liver and spleen were enlarged and percutaneous liver biopsies were taken. Histological examination showed numerous granulomata surrounded by inflammation and containing ova which were identified as C. hepatica. Apart from these features the liver parenchyma appeared normal. Treatment proceeded with oral iron for anaemia, and Hetrazan ® (diethylcarbamazine) against the parasitic infection. As the patient remained ill, Triostam ® (sodium antimonyl gluconate) was administered. This led to a general improvement in health. The patient was discharged. Four months later, a repeat biopsy revealed reduction of the fibrosis but ova were still present. One year after the initial admission the child was still well.

The final and most recent case to be reviewed is from Kaduna in Nigeria and is described by Attah et al. (1983). A twenty-seven-year-old woman complained of increased swelling in the right side of the abdomen for the previous two years. The liver was found to be markedly enlarged. Percutaneous liver biopsy revealed extensive fibrosis and inflammation surrounding ova of C. hepatica in the portal areas. There was some granulomatous reaction but it was not widespread. There was no calcification and no adult worms were seen. Treatment with potassium iodide as an outpatient was planned but the patient was lost to follow-up. Presumably she is still alive.

The true human cases of C. hepatica infection are summarised in Table 2.

TABLE 2 True Human Cases of C. hepatica Infection

CASE NO.	AUTHOR(S)	DATE OF CASE	LOCATION	PATIENT AGE	DIAGNOSIS	DEAD/ALIVE
1.	Dive et al. (1924)	1923	India	20yrs	At autopsy	Dead
2.	McQuown (1950)	1947	Louisiana, U.S.A.	17 mths	At autopsy	Dead
3.	Otto et al. (1954)	1951	Maryland, U.S.A.	7 yrs	At autopsy	Dead
4.	Turhan et al. (1954)	1953	Turkey	60 yrs	At autopsy	Dead
5.	Ewing and Tilden (1956)	1954	Hawaii, U.S.A.	15 mths	By liver biopsy	Alive
6.	Cochrane et al. (1957)	1956	Vanderbijlpark, R.S.A.	[illegible] mths	By liver biopsy	Alive
7.	Ward and Dent (1959)	1958	Louisiana, U.S.A.	2 yrs	At autopsy	Dead
8.	Calle (1961)	1959	North Carolina, U.S.A.	20 mths	By liver biopsy	Alive
9.	Kallichurum and Elsdon-Dew (1961)	1961	Durban, R.S.A.	5 yrs	By liver biopsy	Alive
10.	Romero Garcia et al. (1962)	1961	Guadalajara, Mexico	22 mths	By liver biopsy	Alive
11.	Cislaghi and Radice (1970)	1970	Milan, Italy	40 mths	By liver biopsy	Alive
12.	Silverman et al. (1973)	1972	Johannesburg, R.S.A.	17 mths	By liver biopsy	Alive
13.	Attah (1983)	1981	Kaduna, Nigeria	27 yrs	By liver biopsy	Alive

3. MATERIALS AND METHODS

3.1 Experimental Animals

All the animals used for experimental purposes were male Swiss-Webster White mice obtained from the Central Animal Unit at the University of the Witwatersrand. The mice usually weighed between 20 g and 35 g at the start of an experiment. The mice were housed in groups of five in solid-bottomed plastic cages with sawdust as bedding, and fed a diet of mouse cubes and water *ad libitum*. Every experimental mouse was given an individual number by means of ear punching, using the code shown in Figure 3.

3.2 Supply of C. hepatica Eggs

Initially eggs of *C. hepatica* were obtained by trapping black rats *Rattus rattus* in the vicinity of Johannesburg by means of large metal rat traps, killing them with ether and examining the liver of each animal for the characteristic fibrosis caused by *C. hepatica*. When an infected liver was found it was processed as in sections 3.3 and 3.4 and mice, as in section 3.1, were then infected with the parasite, as in section 3.6.1. Once the infection had been established in the laboratory it was maintained by routine passage in white mice.

3.3 Separation of Eggs for Embryonation

The infected liver was cut into small pieces and then pulverised using a pestle and mortar, in order to break open any fibrotic masses containing eggs. The resulting mixture was then flushed through a piece of fine gauze material with tap-water in order to remove as many large liver particles as possible. The filtrate was kept at 4°C and allowed to settle

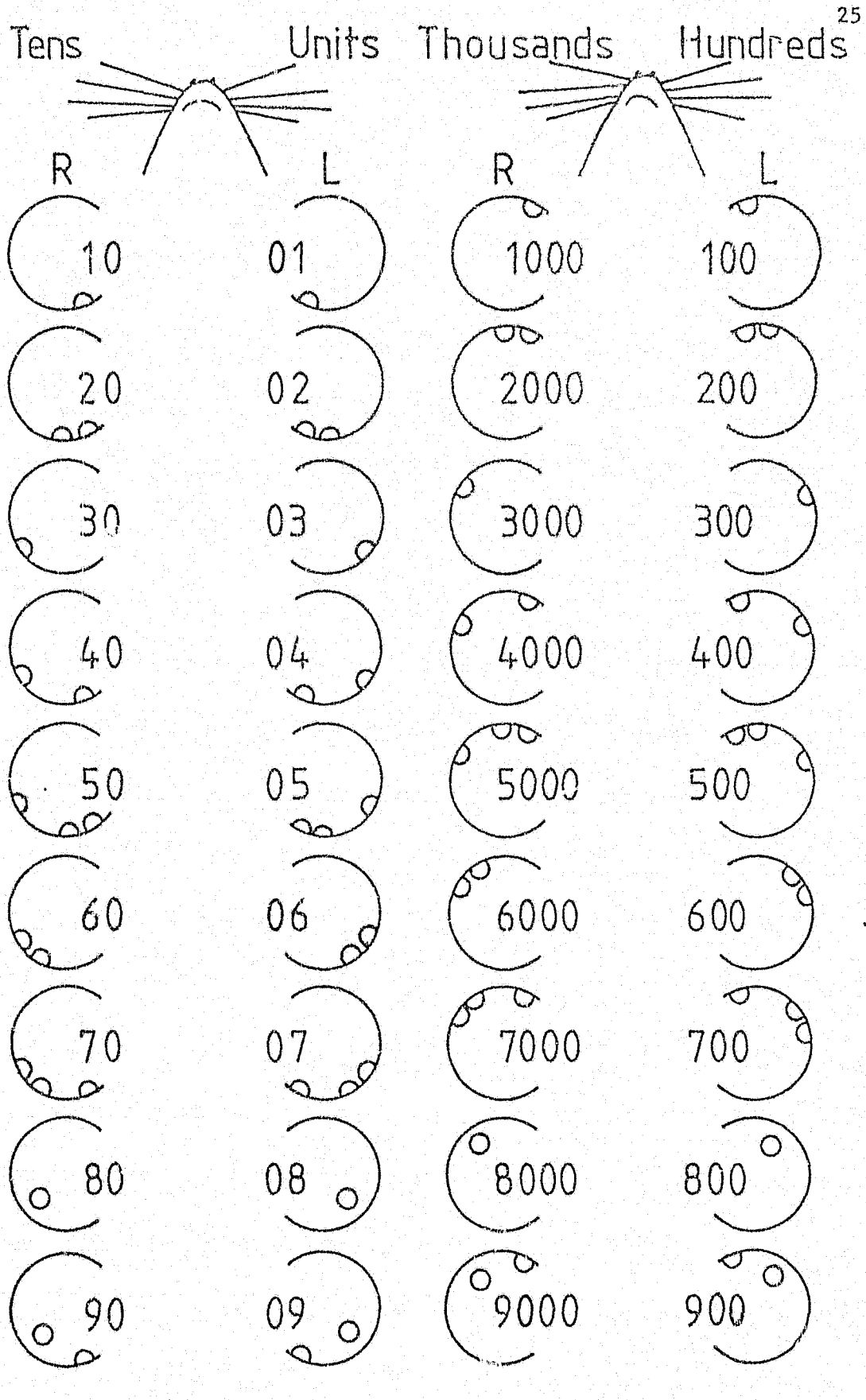

FIGURE 3 Mouse Ear Marking Code

(By courtesy of the Department of Toxicology and Reproductive Studies, Wyeth Laboratories Ltd., Maidenhead, Berks., U.K.)

for at least twelve hours. The supernatant was sucked off using a venturine pump, leaving the eggs, which sink in water, in the sediment at the bottom of the container. The container was then refilled with tap-water and replaced at 4°C. In order to wash the eggs as thoroughly as possible, the process of sedimentation and removal of the supernatant was repeated several times over a period of approximately seven days, until the supernatant was clear.

3.4 Embryonation

The clear supernatant of the washed eggs was discarded and the eggs, in a minimum of tissue debris, were placed in a petri dish containing a small amount of tap-water, which had been allowed to stand for at least two days. The petri dish containing the eggs was then placed for six to eight weeks in an incubator maintained at a temperature of between 27°C and 30°C (Shorb, 1931; Luttermoser, 1938a; Wright, 1961; Vollerthun, et al., 1974; Lämmler and Grüner, 1976; Zahner et al., 1976). During this time the eggs were aerated two to three times per week by means of a Pasteur pipette and dechlorinated water was added as required, so that the eggs did not dry out. After six to eight weeks the percentage embryonation was determined by microscopic examination of the eggs.

3.5 Egg Counting - for Embryonated Eggs

A modified McMaster counting technique was used (Ministry of Agriculture, Fisheries and Food, 1971; Vollerthun et al., 1974; Lämmler and Grüner, 1976; Dunn, 1978). A known volume of eggs in solution was added to zinc chloride solution with a specific gravity of approximately 1.6 gcm^{-3}. A solution of this nature was obtained by dissolving 1 300 g of zinc chloride in one litre of distilled water. The minimum ratio of egg suspension to zinc chloride solution had to be 1:14 (eg. 3 ml of egg suspension and 42 ml of zinc chloride

solution), otherwise it appeared that not all the eggs would float. If the concentration of eggs was very high it was better to increase the ratio to 1:28, or even more, in order to facilitate counting. The zinc chloride/egg suspension mixture was thoroughly mixed and a sample rapidly transferred to the counting chamber of a McMaster slide using a Pasteur pipette. The eggs were allowed to rise to underneath the upper surface of the slide and were then counted microscopically. Usually three McMaster chambers were counted and an average egg count per chamber used in the calculations. The McMaster slide is shown in Figure 4. As the counting chamber is 1 cm square and 0.15 cm deep, the formula for the number of eggs per ml of egg suspension is given by:

$$\frac{x\ \frac{(y+z)}{y}\ .\ 100}{15}$$

where x = average egg count
y = size of egg suspension sample in ml
z = amount of zinc chloride suspension added in ml

Knowing the percentage embryonation of the eggs, as determined in section 3.4, the number of embryonated eggs per ml could be calculated. Thereafter, a solution containing 150 eggs in 0.2 ml could be prepared for administration to the test animals.

3.6 Experimental Design

C. hepatica eggs were administered to the experimental mice on day one of the experiment (section 3.6.1) and their body

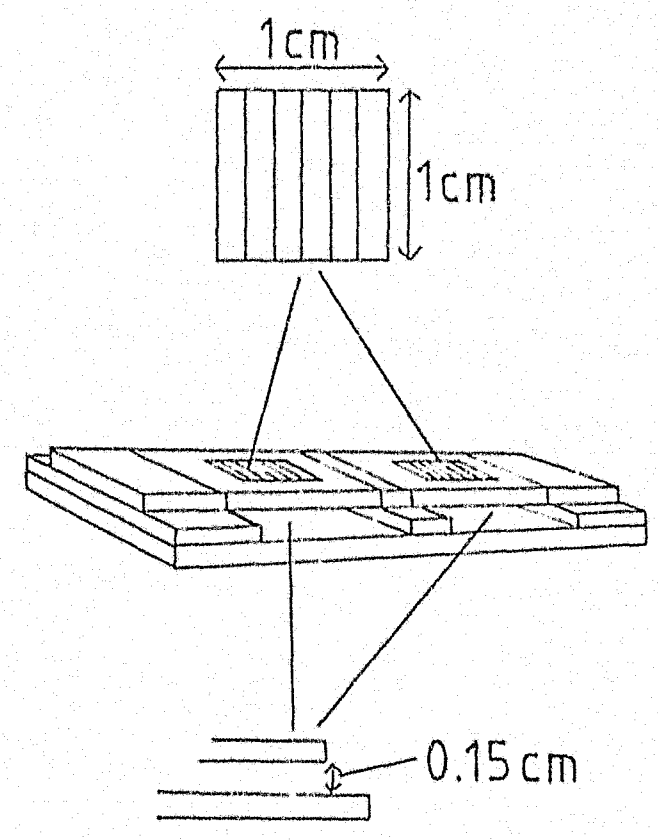

FIGURE 4 McMaster Slide

weights recorded. On days fourteen to eighteen post inoculation the drugs were administered to the mice according to their daily body weights (section 3.6.2). On day twenty-nine post inoculation each animal was weighed, anaesthetised with ether, and then its jugular vein was severed for exsanguination so that the liver did not act as a reservoir for blood and make the liver weights inconsistent. The body cavity was opened up, any gross abnormalities noted, and the liver removed and weighed. The livers from each group of mice were photographed. Each liver was wrapped individually in a plastic bag and labelled with the experiment code, mouse number, cage number, drug and dose-level. The mouse livers were frozen at -70°C for a minimum of seventy-two hours in order to render the C. hepatica eggs uninfective (Wright, 1961). The livers were then processed by means of a modified liver digestion technique (section 3.6.3). The number of eggs per gram of liver was calculated and the results evaluated (sections 3.6.4 and 3.6.5).

Pilot experiments were first performed with each drug. This involved using very high dose-levels of the test drug to determine whether or not the drug had any potential use against C. hepatica. Three dose-levels were used in each case, doses at each level being administered to five mice from days fourteen to eighteen of the infection. A control group of five infected but untreated mice was used. For drugs which appeared to be having an advantageous effect on the infection, further experiments were performed using much lower dose-levels.

3.6.1 Infection of Test Animals

The test animals were inoculated per os with approximately 150 embryonated C. hepatica eggs, by means of a blunted metal cannula attached to a 1 ml disposable plastic syringe. The dosing syringe is illustrated in

Figure 5 and the dosing procedure in Figures 6a and 6b. Since the eggs sink rapidly in water, care had to be taken to shake the bottle containing the eggs before filling the syringe, in order to administer the required number of eggs as accurately as possible.

3.6.2 Drug Administration

A list of drugs used, the manufacturers and other pertinent information is given in Appendix A. Each drug came in one of the following forms:

(i) Powder soluble in water
(ii) Powder insoluble in water
(iii) Suspension miscible with water
(iv) Suspension not miscible with water

A powder which was insoluble in water was suspended in a solution of gum tragacanth by means of a homogeniser. A suspension not miscible with water was supplied with a suitable fluid for dilution, which was also used as a placebo for the control animals where applicable. The drugs were supplied in known concentrations. Consequently, the required dose-levels could be prepared so that each drug was administered in an amount of 1 ml fluid per 100 g body weight. Thus, if the dose-level was 500 mg/kg, then each 1 ml of the solution to be administered contained 50 mg of the drug. The drugs were prepared daily and given to the test animals as in section 3.6.1. Drugs were administered in ascending order of concentration and a new syringe was used for each drug, the dosing needle having been thoroughly washed. Control groups were dosed with distilled water, gum tragacanth or placebo, according to which had been used for drug administration in the particular experiment concerned.

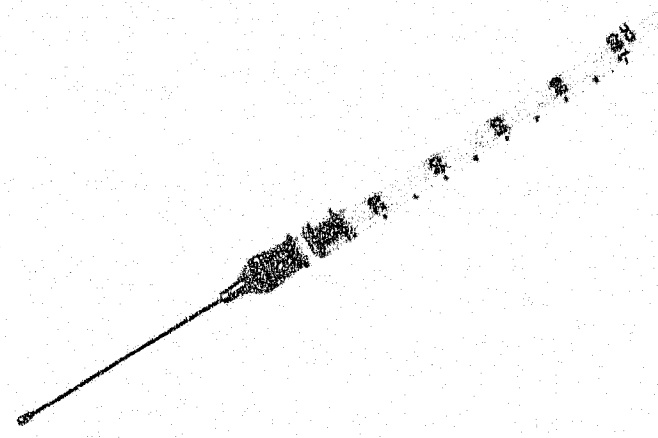

FIGURE 5 Dosing Syringe

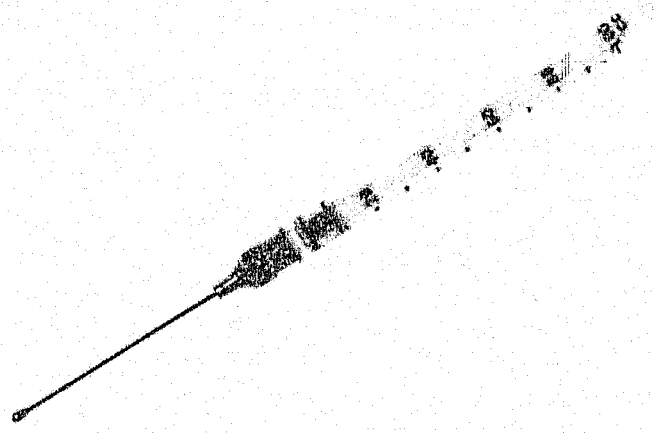

FIGURE 5 Dosing Syringe

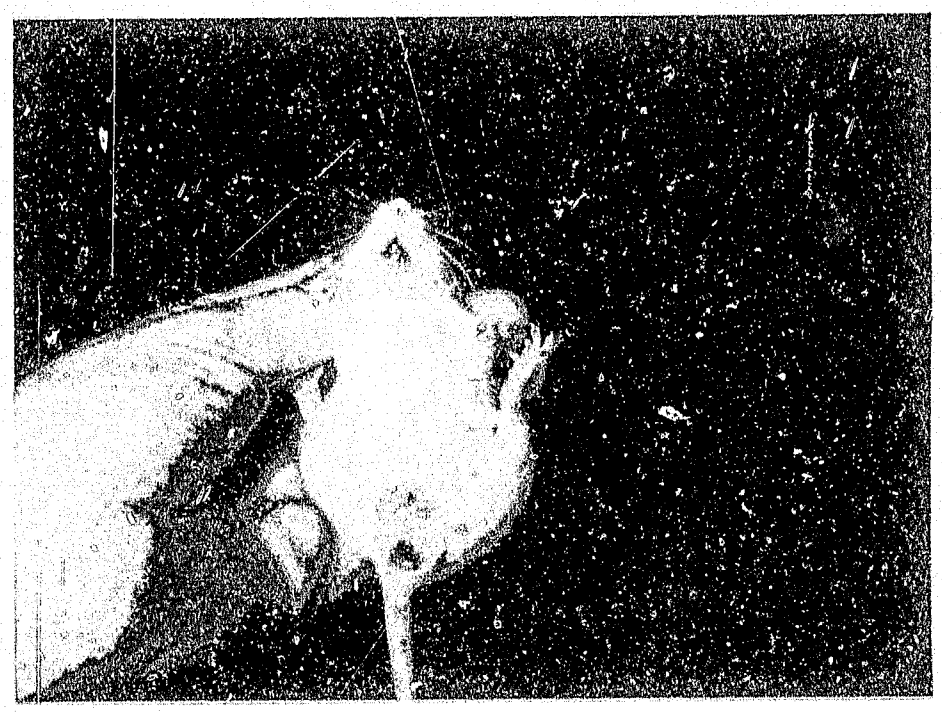

FIGURE 6a Dosing Procedure - I

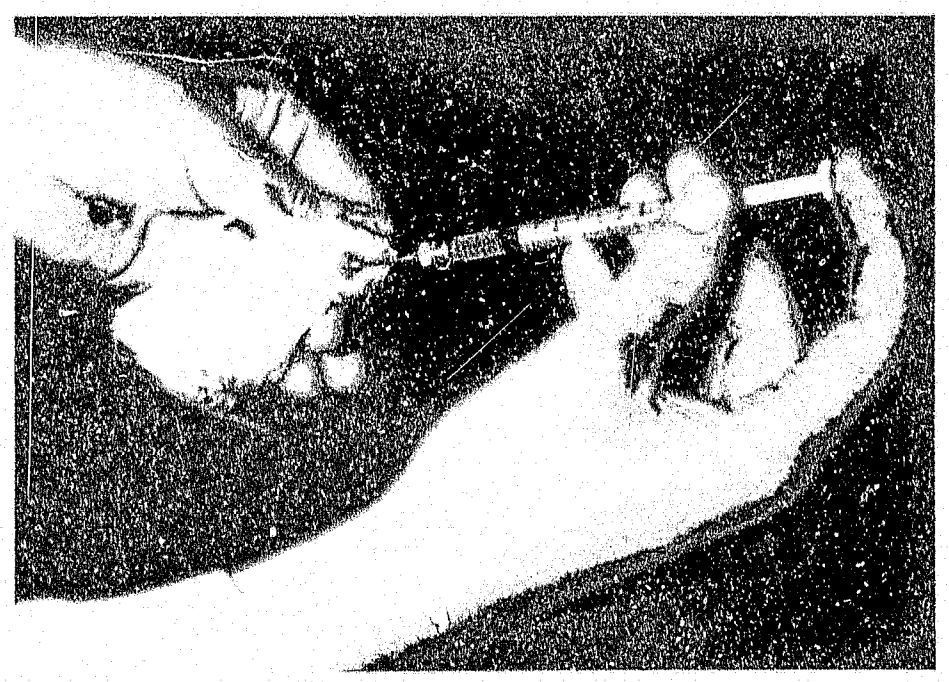

FIGURE 6b Dosing Procedure - II

3.6.3 Modified Liver Digestion Technique

The technique described by Matsusaki (1951) (cited by Vollerthun et al., 1974; Lämmler and Grüner, 1976; Zahner et al., 1976) was modified and used as follows:

A solution containing 0.7 g pepsin, 6 ml normal (molar) hydrochloric acid and 94 ml distilled water was placed in a 250 ml round-bottomed flask. The liver was cut into small pieces and added to the solution, the flask being clearly labelled. The flask was held in a water-bath maintained at 37.5°C and stirred by means of a vibrating stirrer obtained from Chemapec (Pty) Ltd., P.O. Box 9051, Johannesburg, South Africa. The apparatus is shown in Figure 7. The flask was stirred for a minimum of three hours, or until all the fibrotic masses had been broken up. The resulting solution was made up to 200 ml with distilled water and placed in a sealed flask at 4°C until required for examination.

3.6.4 Egg Counting - for Liver Examination

The egg suspension was thoroughly mixed and a 3 ml sample removed with a graduated pipette. 42 ml of 1.6 gcm^{-3} zinc chloride solution was added to this sample. This solution was thoroughly mixed and a sample taken quickly with a Pasteur pipette and run into a McMaster counting chamber. Three samples were examined microscopically and the eggs counted as described in section 3.5.

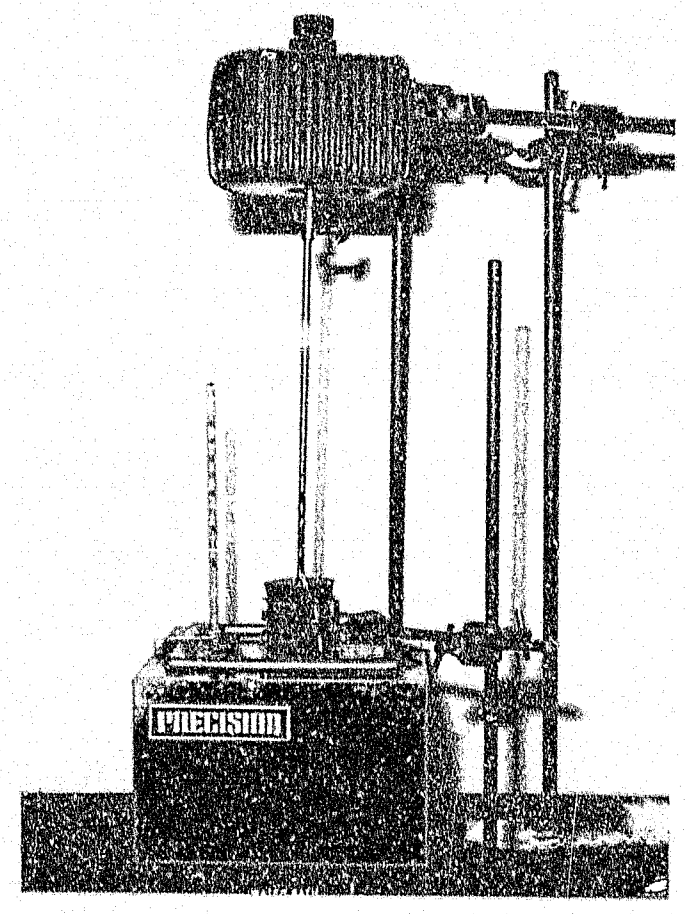

FIGURE 7 Apparatus for Liver Digestion

3.6.5 Evaluation of Results and Statistics

The formula for the weight of liver examined per McMaster chamber (Wm) is given by:

$$Wm = \frac{W \cdot 3 \cdot 0.15}{200 \cdot 45}$$

$$Wm = W \cdot 5 \cdot 10^{-5}$$

Where W = weight of the mouse liver in grams.

The number of eggs per gram of liver (EL) is given by:

$$EL = \frac{\bar{x}}{Wm}$$

Where $\bar{x}$ = average egg count per McMaster chamber.

The percentage reduction in the number of eggs present in the liver is given by:

$$\% \text{ Reduction} = 100 - \frac{\text{(Average EL for given drug/dose-level)}}{\text{(Average EL for Control group)}} \cdot 100$$

The liver weight per 10 g body weight was calculated for each mouse, and the Dunnett's test, for comparison of treatment means with the control, was performed to compare liver weights between dosed and control groups (Dunnett, 1964).

A linear regression analysis was utilized to investigate the relationship between the number of eggs per gram of liver and the liver weight.

3.7 Scanning Electron Microscopy (SEM)

For drugs which proved to be effective, as determined by the egg counts from the low-dose experiments, groups of five mice were infected with C. hepatica, as in section 3.6.1, and treated with drugs, as in section 3.6.2. The dose-levels used were the lowest effective dose-levels for the appropriate drugs, as determined by the low-dose experiments. The mice were killed on day nineteen post inoculation by means of ether. A control group of five mice was also infected with C. hepatica and dosed with distilled water.

Livers were removed and some of the worms extracted by microdissection. Worms were immediately fixed in glutaraldehyde at room temperature (to prevent excessive shrinkage) for a minimum of one hour. The worms were then washed in cacodylate buffer and post-fixed in osmium tetroxide at 4°C for at least one hour, or until the worms had turned black. The worms were then washed in buffer and dehydrated at 4°C through an ascending alcohol series. The worms were critical point dried, mounted and gold-coated before examination under the scanning electron microscope (after Wright, 1978).

4. RESULTS

4.1 Effects of Drugs

A summary of the high-dose pilot experiment results is given in Table 3 and the control data in Table 4. Full experimental data from which these tables are derived are given in Appendix B. In order for the drug to be deemed effective, it was required to cause more than 99 per cent reduction (against the control) in the number of eggs present in the livers of the experimental mice. The column scoring the liver appearance is, admittedly, a subjective measurement, but as can be seen from the photographs which follow, the liver appearance (including apparent fibrosis, colour and texture) is a reasonable indication of the degree of infestation with C. hepatica. In the pilot experiments, four drugs showed the required degree of efficacy, and these were then subjected to low-dose trials. They were albendozole (Valbazen ®), febantel (Rintal ®), mebendazole (Vermox ®) and oxfendazole (Systamex ®). Also in the case of these drugs, the Dunnett's test to determine any significant difference between the control and treated liver weights is recorded as being highly significant at one per cent, i.e. there is a very significant difference between the control and treated liver weights, which has not arisen by chance.

The results of the low-dose experiments are given in Table 5 and the control data in Table 6. Full experimental data from which these tables are derived is given in Appendix C. The lowest dose-level in these experiments was chosen so that the total dose administered fell within, or was very near to, the dose-levels recommended by the manufacturers.

TABLE 3 Results of Pilot Experiments

DRUG NAME	DOSE mg/kg X5	NUMBER OF MICE TO SURVIVE	LIVER APPEARANCE[1]	EGGS/ g LIVER		DUNNETT'S TEST	CONTROL GROUP
				$\bar{x} \times 10^4$	% REDUCTION		
Albendazole (Valbazen ®)	125	5	++	0.1	99.8	0.01	B
	250	5	++	0.2	99.7	0.01	B
	500	5	++	0.3	99.6	0.01	B
Amoscanate	125	5	0	163.1	20.2	N.S.	E1
	250	5	0	150.7	26.3	N.S.	E1
	500	2	0	255.8	0	N.S.	E1
Febantal (Rintal ®)	62.5	5	++	0.1	99.9	0.01	C
	125	5	++	0.4	99.8	0.01	C
	250	5	++	0.2	99.9	0.01	C
Mebendazole (Vermox ®)	6.25	5	++	0.1	99.9	0.01	A1
	12.5	5	++	0.1	99.9	0.01	A1
	25	5	++	0.1	99.9	0.01	A1
Niclosamide (Lintex ®)	125	4	0	153.6	9.5	N.S.	C
	250	4	0	135.8	20.0	N.S.	C
	500	5	0	67.5	60.2	N.S.	C
Oxamniquine (Vansil ®)	125	5	0	90.3	0	N.S.	E2
	250	5	0	74.5	1.3	0.01	E2
	500	2	0	73.2	3.1	N.S.	E2
Oxfendazole (Systamex ®)	56.25	5	+	0	100	0.01	D
	112.5	5	+	0	100	0.01	D
	225	5	+	0	100	0.01	D
Oxyclozanide (ICI Liver Fluke Remedy ®)	85.0	5	0	26.6	65.8	N.S.	B
	170	5	0	40.7	47.6	N.S.	B
	340	4	0	5.3	93.1	N.S.	B
Piperazine adipate (Ascaradina ®)	125	5	0	123.7	0	N.S.	B
	250	5	0	124.3	0	N.S.	B
	500	5	0	175.6	0	0.05	B
Piperazine citrate (Rid ®)	125	5	0	196.0	28.4	N.S.	A
	250	5	0	110.3	59.7	N.S.	A
	500	5	0	248.6	9.1	N.S.	A
Piperazine dihydrochloride (Wazine ®)	125	2	0	100.0	0	N.S.	D
	250	4	0	133.9	0	N.S.	D
	500	4	0	157.1	0	N.S.	D
Praziquantel (Droncit ®)	50.0	5	0	72.9	73.4	N.S.	A
	100	5	0	229.7	16.1	N.S.	A
	200	0	-	-	-	-	A
Pyrantel (Combantrin ®)	125	5	0	66.7	75.6	N.S.	A
	250	5	0	86.5	68.4	N.S.	A
	500	5	0	117.0	57.3	N.S.	A
Rafoxanide (Ranide ®)	62.5	5	+	66.4	60.9	0.01	C
	125	3	+	70.1	58.7	0.01	C
	250	0	-	-	-	-	C

(Continued)

TABLE 3 (Continued) .....

[1] Key To Liver Appearance Symbols

0 : No apparent difference in appearance from the control livers.

+ : Some reduction in fibrosis apparent.

++ : Considerable reduction in fibrosis.

TABLE 4 Results for Pilot Experiment Control Groups

CONTROL GROUP	NUMBER OF MICE TO SURVIVE	$\bar{x}$ EGGS/g LIVER x 10^4
A1	5	120.7
A	5	273.7
B	5	77.6
C	5	169.7
D	5	87.9
E1	5	204.3
E2	5	75.5

TABLE 5

Results of Low-Dose Experiments

DRUG NAME	TOTAL RECOMMENDED DOSE mg/kg	EXPERIMENTAL DOSE-LEVEL		NUMBER OF MICE TO SURVIVE	LIVER APPEAR-ANCE[1]	EGGS/g LIVER		DUNNETT'S TEST	CONTROL GROUP
		DOSE mg/kg	TOTAL DOSE mg/kg			$\overline{X} \times 10^4$	% REDUCTION		
Albendazole (Valbazen ®)	3.8 - 7.5	1.88	9.4	5	0	26.9	20.5	N.S.	F
		3.75	18.75	5	0	20.0	41.1	N.S.	F
		7.5	37.5	5	0	14.2	78.7	N.S.	F
		15.0	75.0	5	0	6.1	91.8	N.S.	F
		30.0	150	5	+	0.1	99.8	0.01	H
		60.0	300	5	++	0	100	0.01	H
		120	600	5	++	0	100	0.01	H
Febantel (Rintal ®)	5 - 20	1.25	6.25	5	0	40.6	0	N.S.	F
		2.5	12.5	5	0	47.7	0	N.S.	F
		5.0	25.0	5	0	34.3	0	N.S.	F
		10.0	50.0	5	+	4.1	87.9	0.05	F
		15.0	75.0	5	+	5.6	90.2	0.01	H
		30.0	150	5	++	0.1	99.9	0.01	H
		60.0	300	5	++	0	100	0.01	H
Mebendazole (Vermox ®)	8.6 - 17.1	3.13	15.63	5	+	0.2	99.7	0.01	J
		6.25	31.25	5	++	0	100	0.01	J
		12.5	62.5	5	++		100	0.01	J
Oxfendazole (Systamex ®)	5	1.25	6.25	5	+	45.1	0	N.S.	F
		2.5	12.5	5	+	41.3	0	N.S.	F
		5.0	25.0	5	+	28.6	15.7	N.S.	F
		10.0	50.0	5	+	11.2	67.0	N.S.	F
		12.5	62.5	5	+	0.1	99.8	0.01	H
		25.0	125	5	+	0.1	99.9	0.01	H
		50.0	250	5	++	0	100	0.01	H

(Continued) . . . . . .

TABLE 5 (Continued) . . . . . .

[1] Key To Liver Appearance Symbols

0 : No apparent difference in appearance from the control livers.

\+ : Some reduction in fibrosis apparent.

++ : Considerable reduction in fibrosis.

TABLE 6 Results for Low-Dose Experiment Control Groups

CONTROL GROUP	NUMBER OF MICE TO SURVIVE	$\bar{x}$ EGGS/g LIVER x 10^4
F	5	33.9
H	5	56.8
J	5	66.9

For each drug a trend may be seen, which will be described below. In order for the experimental photographs to be assessed objectively, reference should be made to Figures 8 and 9 which are photographs of livers of uninoculated and inoculated control mice, respectively. Bearing in mind that the scales are different, the uninoculated mouse livers (Figure 7) are dark blood red in colour and are generally smaller than the inoculated controls (Figure 8). The inoculated control mouse livers are paler, because of the fibrotic masses of eggs located within the liver parenchyma. These livers are heavier than normal and firm to the touch, not soft and easily damaged as is a normal liver.

4.1.1 Albendazole (Valbazen ®); Figures 10 - 19

The lowest dose of this drug administered to mice was 1.88 mg/kg (total dose 9.40 mg/kg). At this level the livers closely resembled the inoculated control livers in colour and texture; the Dunnett's test was not significant and the egg count was reduced by only 20.5 per cent. If Figures 10 - 14 (dose-levels from 1.88 mg/kg to 30.0 mg/kg) are examined, a progression indicating an improvement in the condition of the liver may be seen. The fibrosis is gradually reduced until at 30.0 mg/kg there are only a few white fibrotic areas visible, the livers being predominantly a healthy colour and more natural in texture. At 30.0 mg/kg the egg burden is reduced by 99.8 per cent and the Dunnett's test shows a highly significant difference in weight between treated and control livers. Figures 15 - 19 (dose-levels from 60.0 mg/kg to 500 mg/kg) show livers which are almost completely healthy, having only a few small pockets of fibrosis.

4.1.2 Febantel (Rintal ®) ; Figures 20 - 29

For this compound the lowest dose administered was 1.25 mg/kg (total dose 6.25 mg/kg). At this level the livers were identical to those of the inoculated controls in colour, texture and apparent abundant fibrosis. The Dunnett's test was not signifant and there was no reduction in egg count. If one examines Figures 20 - 25 (dose-levels from 1.25 mg/kg to 30.0 mg/kg) a similar picture of improvement in the liver condition to that for albendazole, may be seen. There are no visible differences between Figures 20, 21 and 22 (1.25 mg/kg, 2.50 mg/kg and 5.00 mg/kg respectively), and in fact these livers show no numerical reduction in the number of eggs present. However, at 10.0 mg/kg (Figure 23), the fibrosis can be seen to be slightly reduced; and at 15.0 mg/kg and 30.0 mg/kg (Figures 24 and 25) the liver is seen to be normal in colour (and normal as regards texture), with only a few pale fibrotic areas visible. It is at 30.0 mg/kg that the egg count is reduced by more than 99 per cent. The Dunnett's test also reflects these changes, from being 5 per cent significant at 10.0 mg/kg to highly significant (one per cent) at 15.0 mg/kg and 30.0 mg/kg. Figures 26 - 29 (dose-levels from 60.0 mg/kg to 250.0 mg/kg) show livers which are almost normal. Fibrosis is almost entirely absent at the high dose-levels.

4.1.3 Mebendazole (Vermox ®) ; Figures 30 - 33

Mebendazole (Vermox ®) is the most effective anthelmintic against G. hepatica investigated to date. This drug is highly effective against this parasite at as little as 3.13 mg/kg (total dose 15.63 mg/kg). Figure 30 (3.13 mg/kg) shows normal-coloured livers with a few pale fibrotic patches. This picture continues in Figures 31 - 33 (dose-levels 6.25 mg/kg to 25.0 mg/kg).

The Dunnett's test also shows the difference in the liver weights (as compared to the controls) to be highly significant at all the dose-levels used.

4.1.4 Oxfendazole (Systamex ®) ; Figures 34 - 43

The lowest dose of this drug to be administered was 1.25 mg/kg (total dose 6.25 mg/kg). Figures 34 - 37 (dose-levels from 1.25 mg/kg to 10.0 mg/kg) reveal livers which still have large amounts of fibrosis present, although in some livers it is reduced, giving rise to a pale, mottled appearance. At dose-levels of 5.0 mg/kg and 10.0 mg/kg there is some reduction in the egg counts, although the Dunnett's test remains non-significant. Between 10.0 mg/kg and 12.5 mg/kg (Figures 37 and 38) there is a dramatic change. At 12.5 mg/kg the livers have a more normal blood-red colour and fibrosis is greatly reduced. The number of eggs present is reduced by 99.8 per cent at 12.5 mg/kg; and the Dunnett's test shows a highly significant difference between the control and experimental liver weights. The liver appearance continues to improve into the higher dose-levels (Figures 39 - 43; dose-levels from 25.0 mg/kg to 225 mg/kg), revealing livers of normal colour and size, and having only a few pale fibrotic patches.

4.1.5 Other Drugs Tested ; Figures 44 - 53

Figures 44 - 53 show the livers of mice from the highest dose groups of all the remaining drugs tested. None of these drugs caused any significant reduction in egg numbers. The photographs show livers which are similar in appearance (and texture) to the inoculated control mouse livers shown in Figure 9.

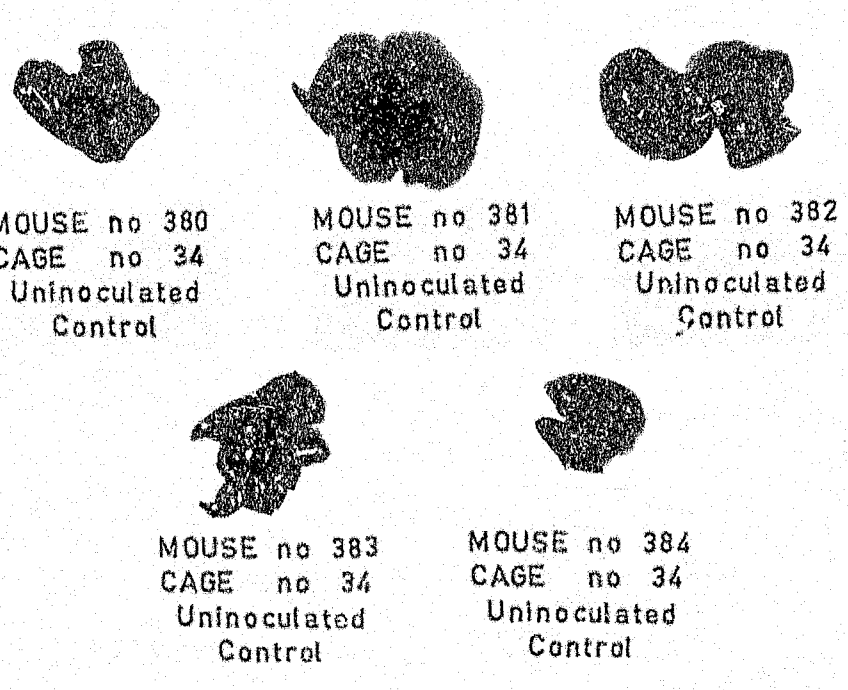

FIGURE 8 Livers from Uninoculated Control Mice
(Note dark blood red colour)
Scale Bar indicates 25 mm

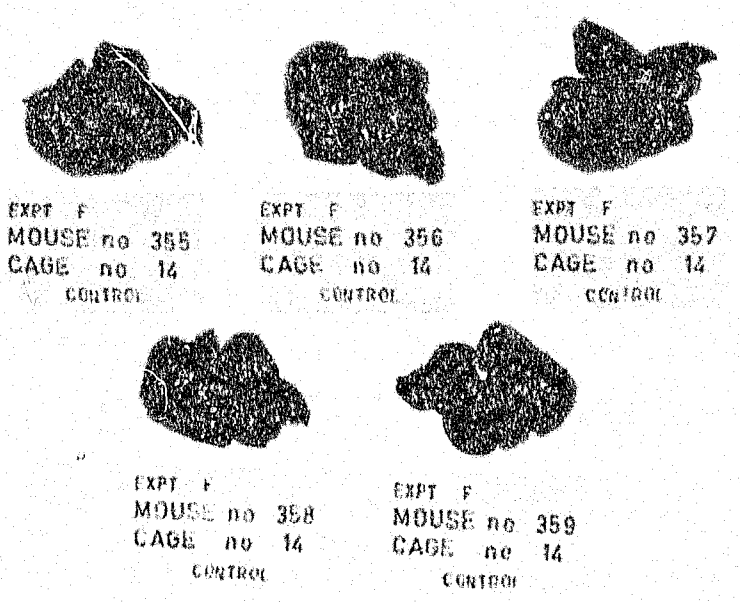

FIGURE 9 Livers from Control Mice Infected with C. hepatica
(Note paler colour due to fibrotic egg masses within liver parenchyma, and increased size)
Scale bar indicates 25 mm

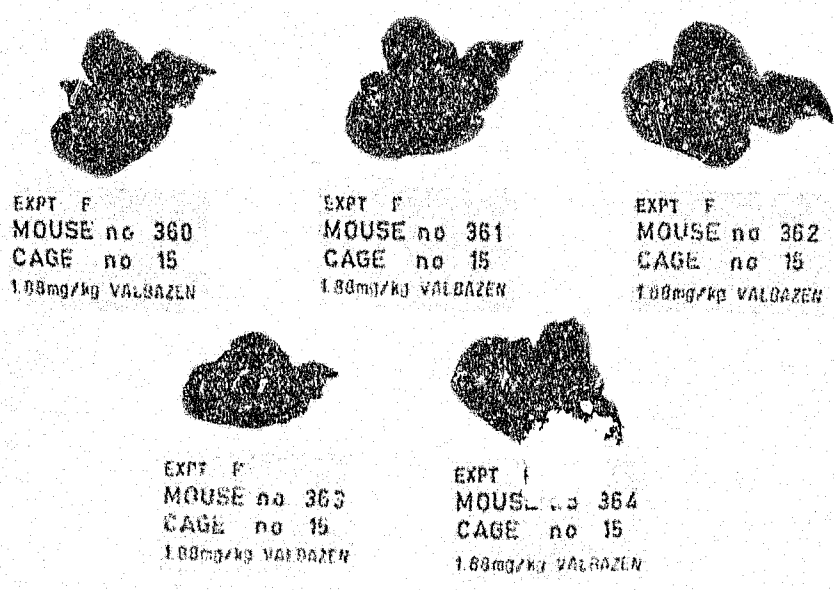

FIGURE 10 Livers from Mice Dosed with 1.88 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm ⊢——⊣

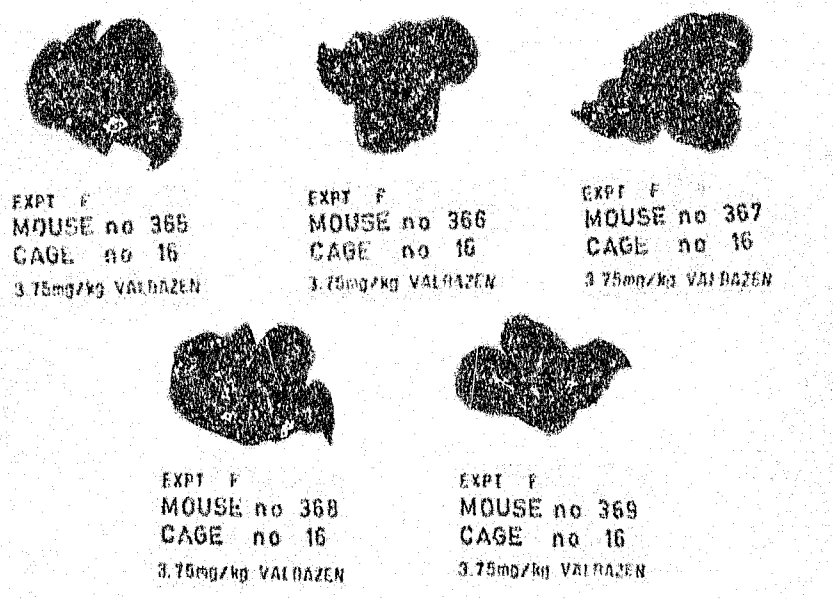

FIGURE 11 Livers from Mice Dosed with 3.75 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm ⊢——⊣

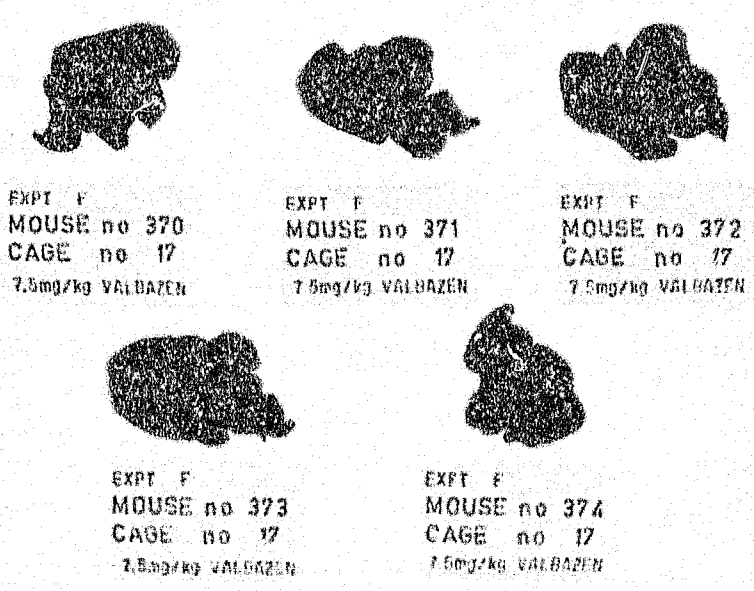

FIGURE 12 Livers from Mice Dosed with 7.50 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

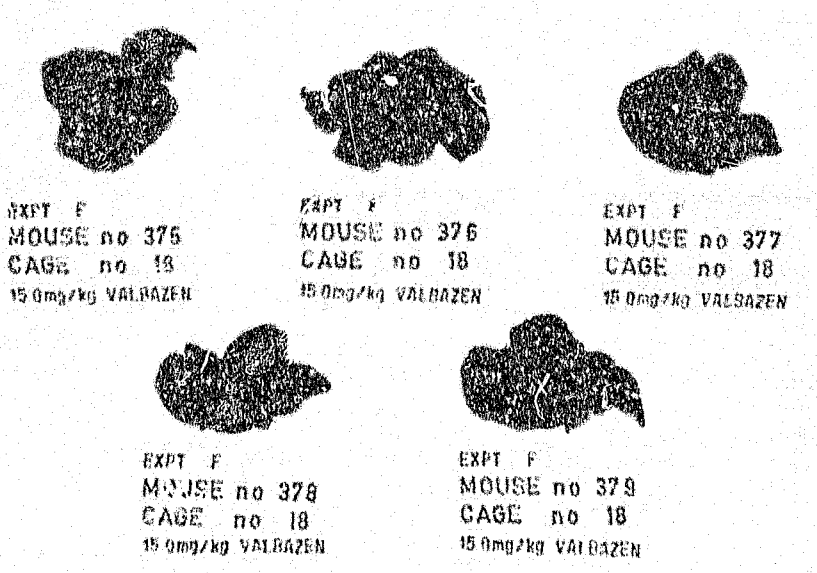

FIGURE 13 Livers from Mice Dosed with 15.0 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

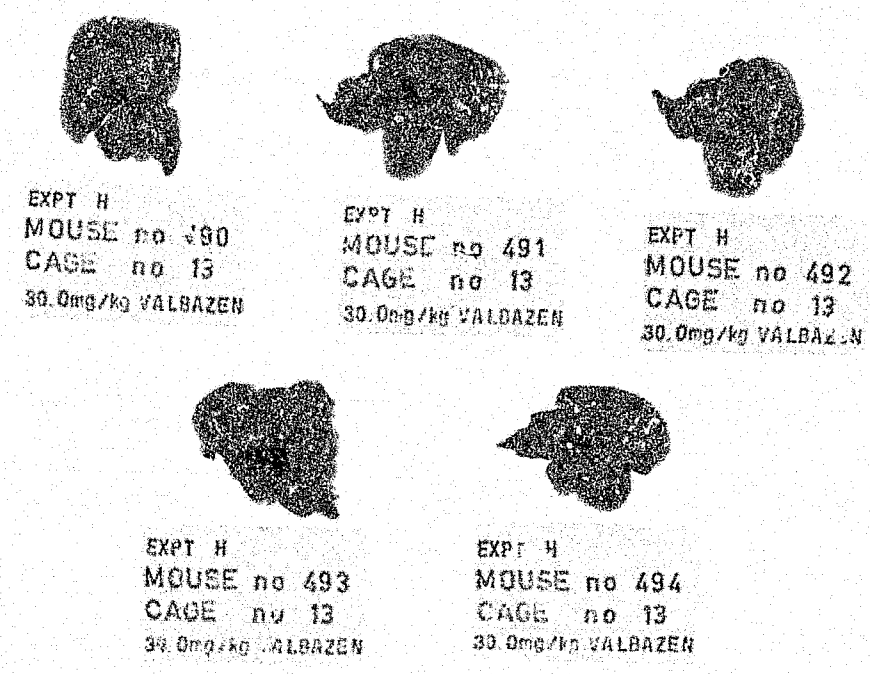

FIGURE 14 Livers from Mice Dosed with 30.0 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

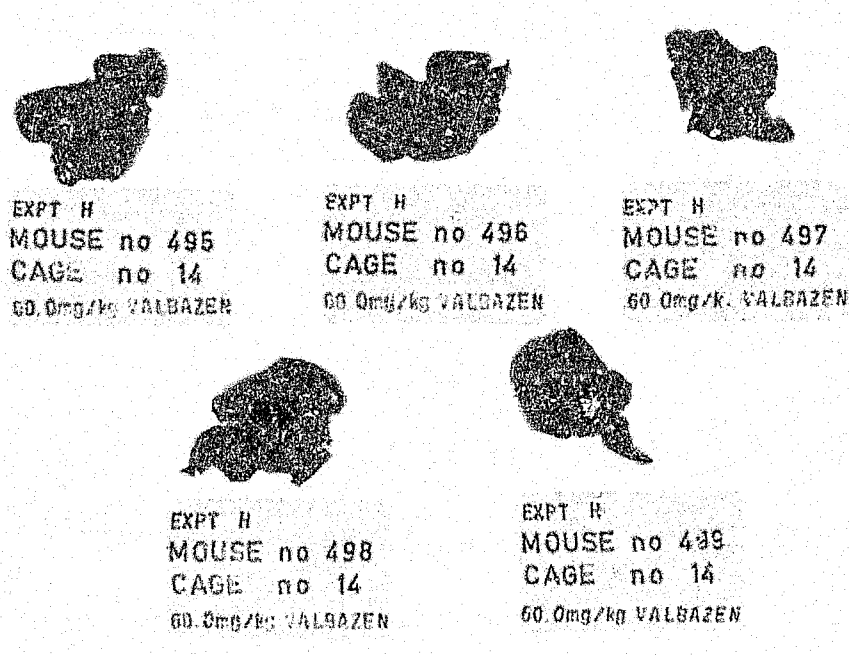

FIGURE 15 Livers from Mice Dosed with 60.0 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

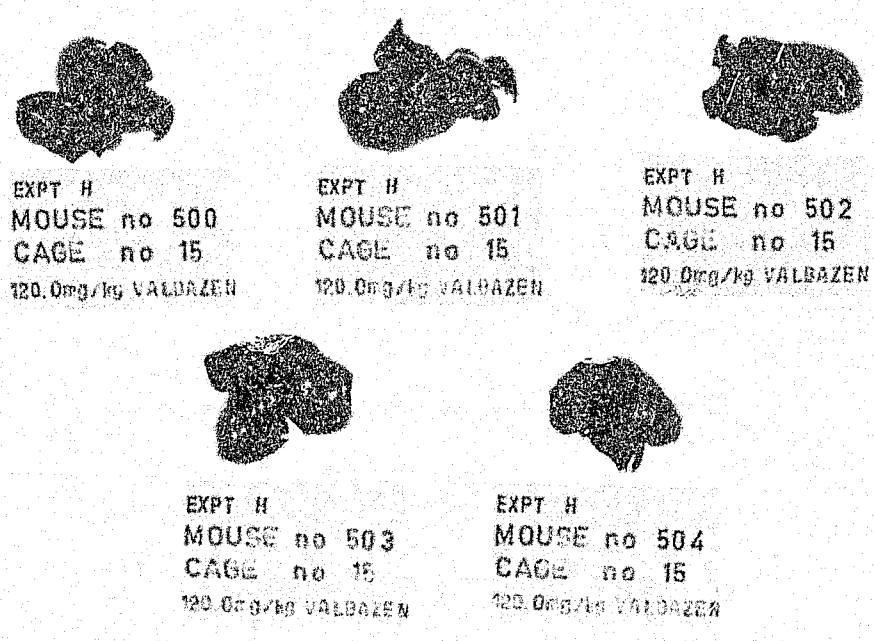

FIGURE 16 Livers from Mice Dosed with 120 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

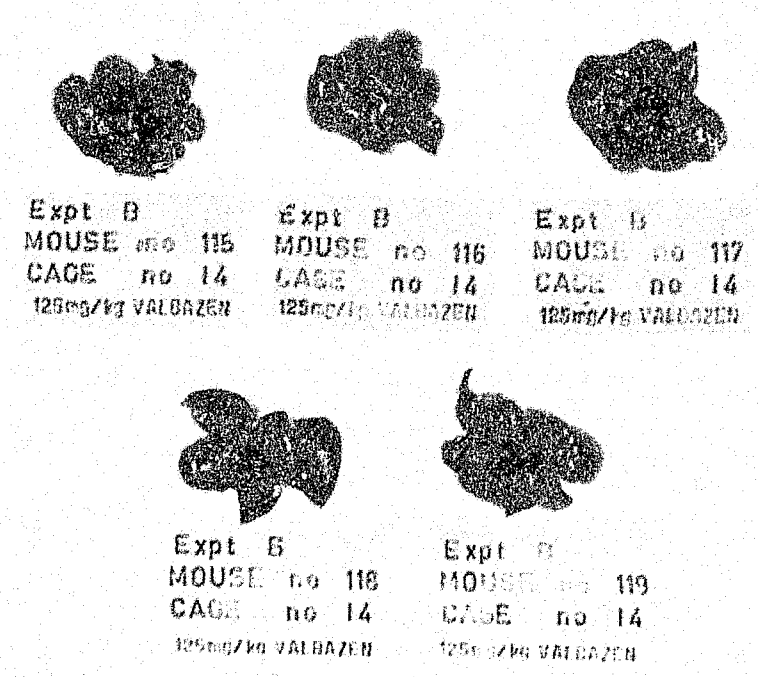

FIGURE 17 Livers from Mice Dosed with 125 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

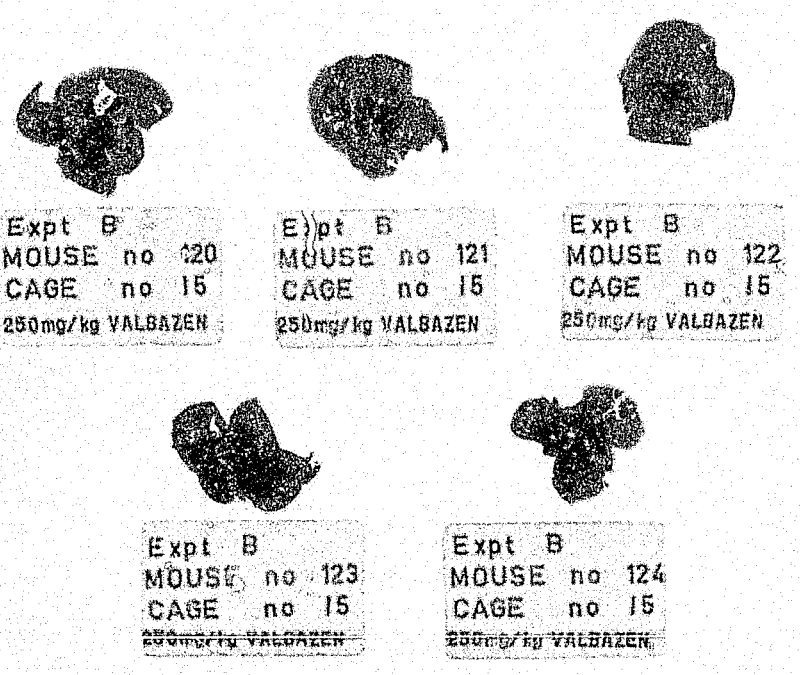

FIGURE 18 Livers from Mice Dosed with 250 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

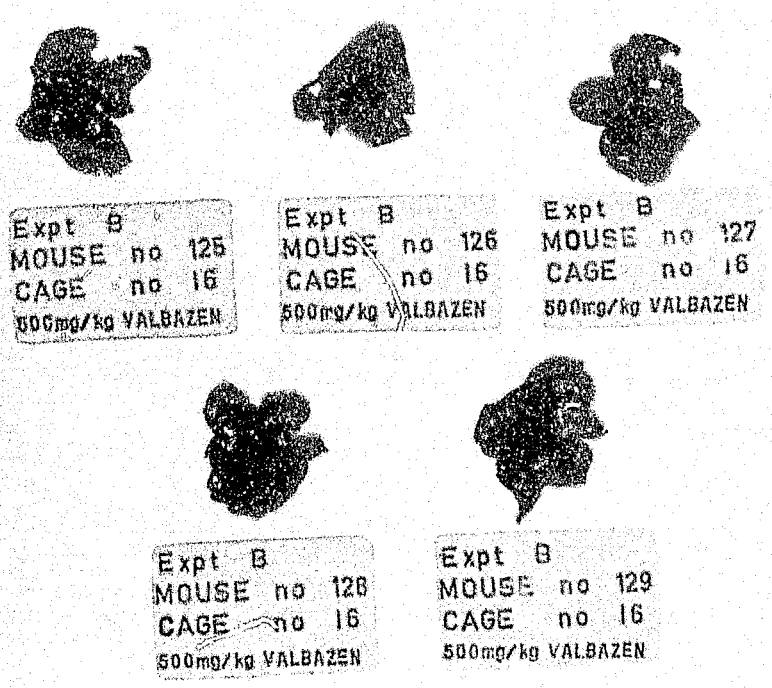

FIGURE 19 Livers from Mice Dosed with 500 mg/kg Albendazole (Valbazen ®)

Scale bar indicates 25 mm

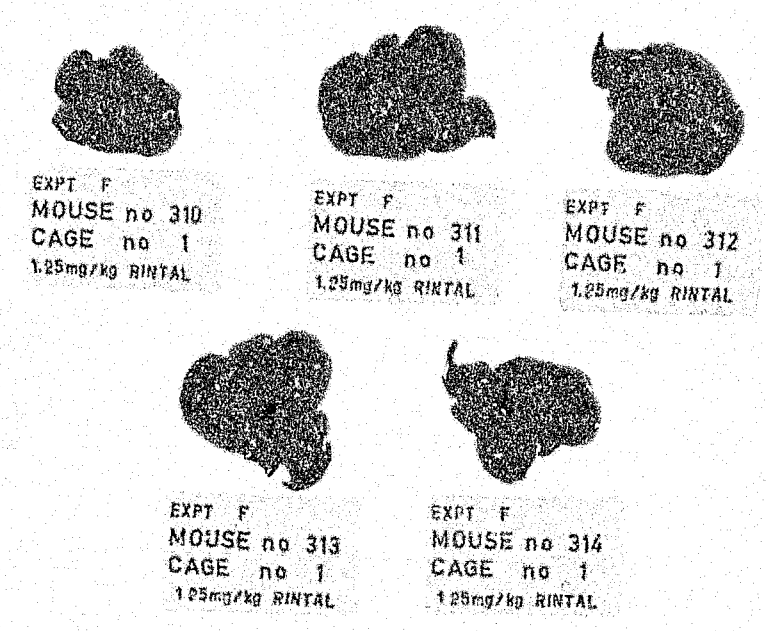

FIGURE 20 Livers from Mice Dosed with 1.25 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

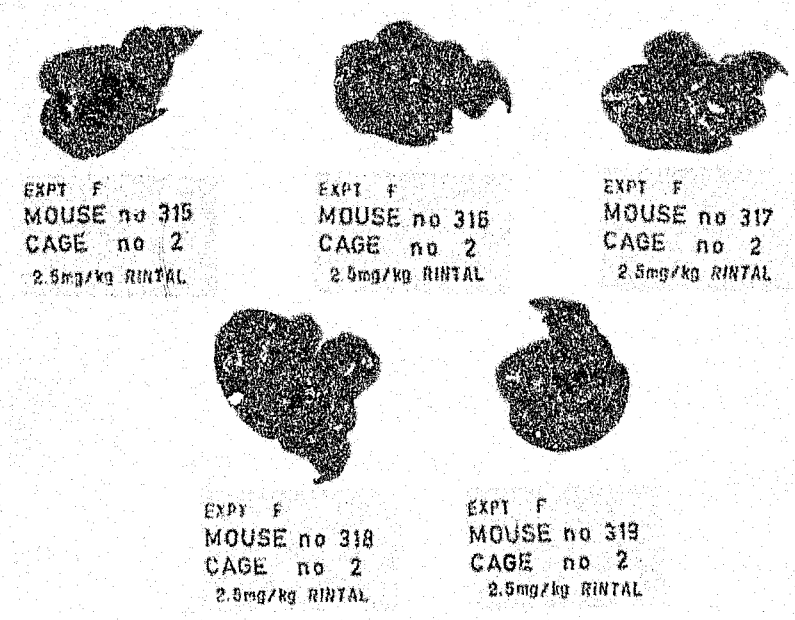

FIGURE 21 Livers from Mice Dosed with 2.50 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

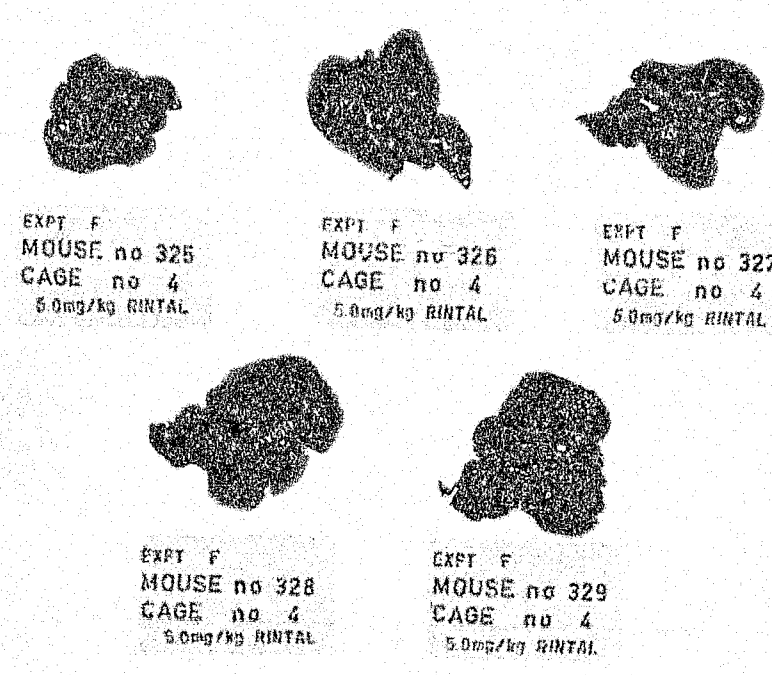

FIGURE 22 Livers from Mice Dosed with 5.00 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

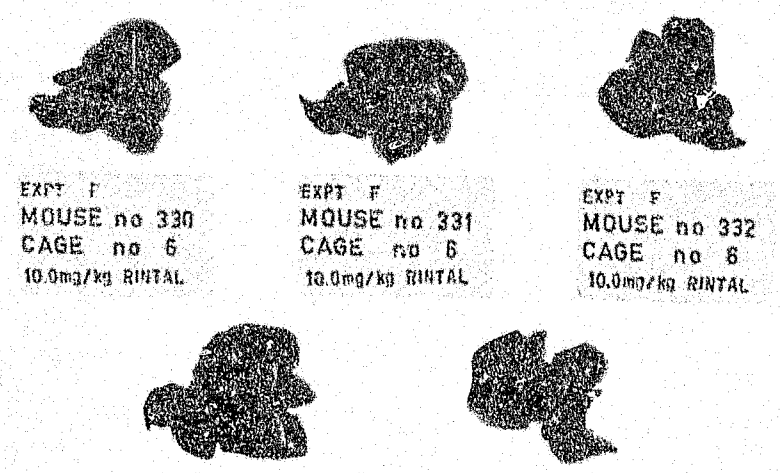

FIGURE 23 Livers from Mice Dosed with 10.0 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

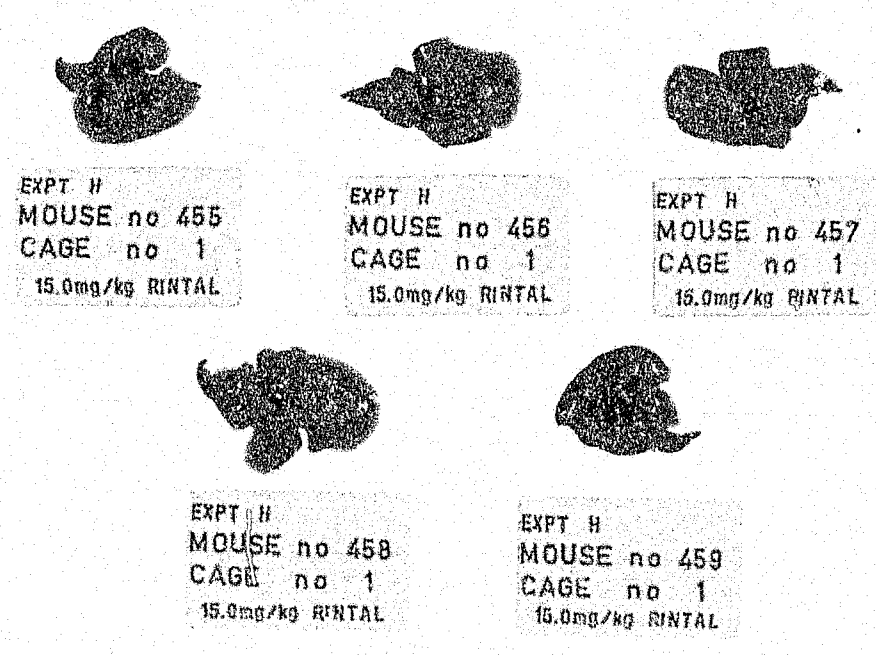

FIGURE 24 Livers from Mice Dosed with 15.0 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

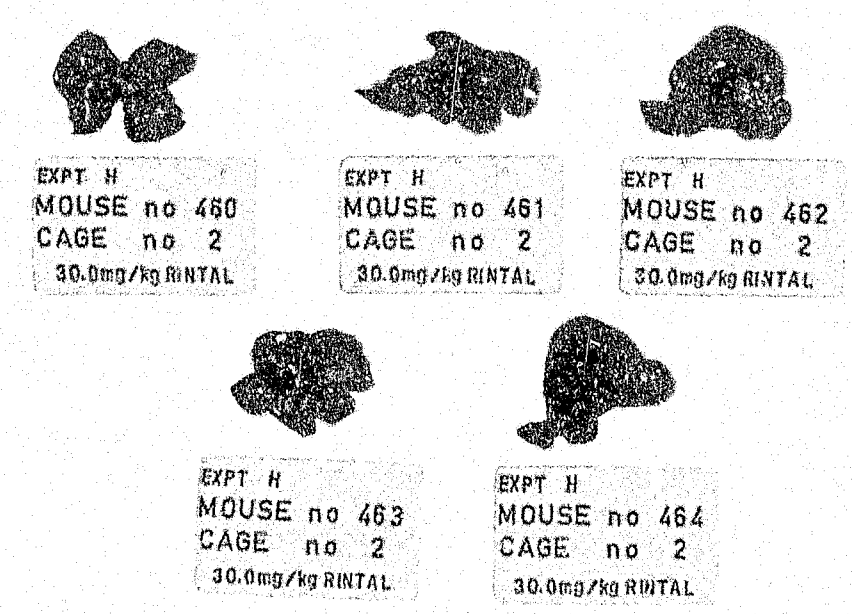

FIGURE 25 Livers from Mice Dosed with 30.0 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

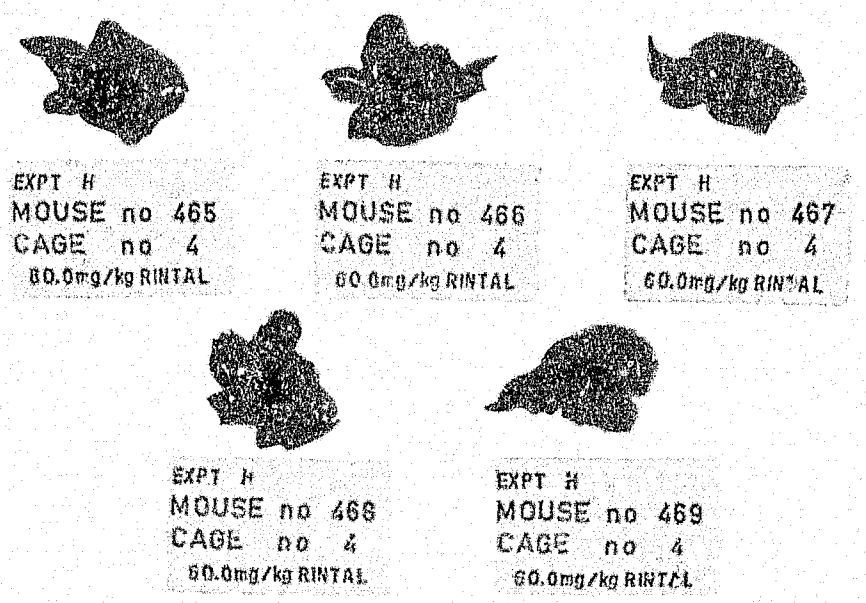

FIGURE 26 Livers from Mice Dosed with 60.0 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

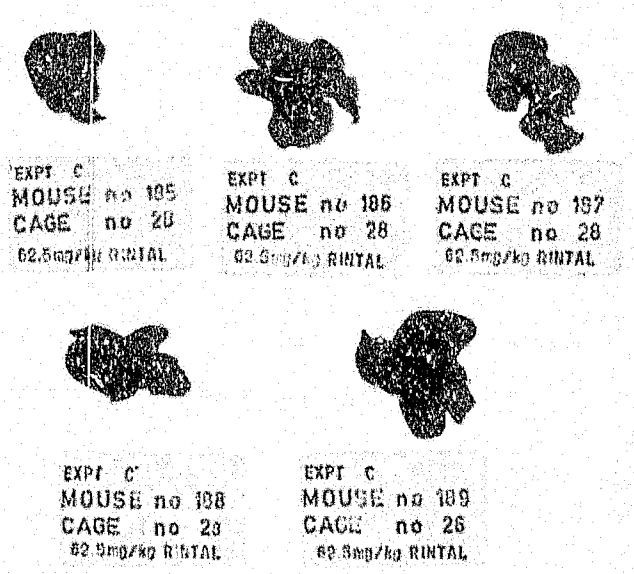

FIGURE 27 Livers from Mice Dosed with 62.5 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

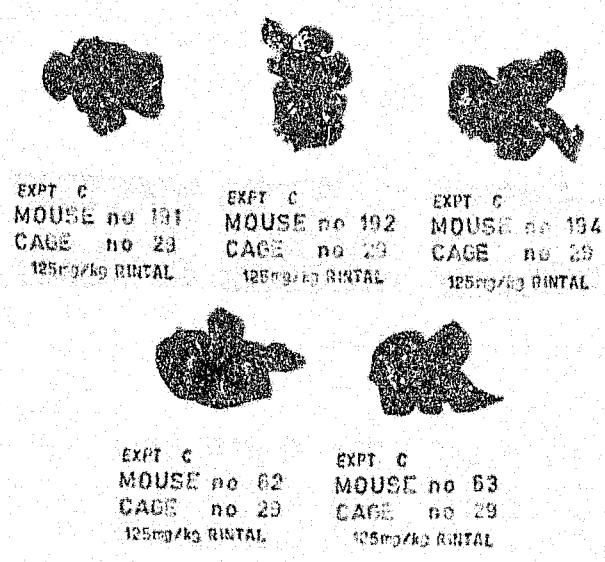

FIGURE 28 Livers from Mice Dosed with 125 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

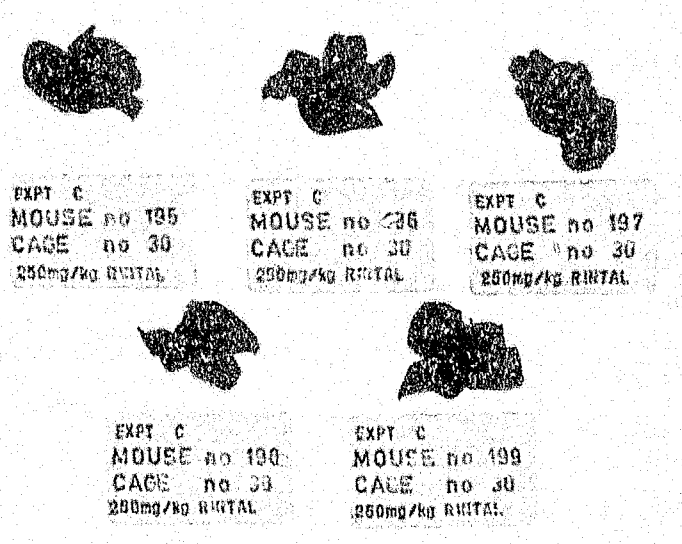

FIGURE 29 Livers from Mice Dosed with 250 mg/kg Febantel (Rintal ®)

Scale bar indicates 25 mm

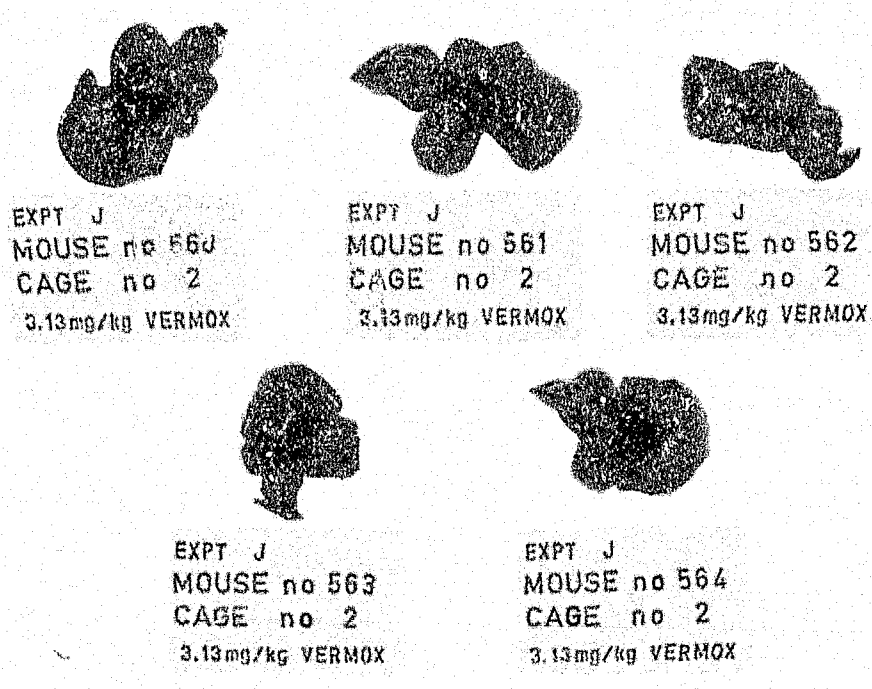

FIGURE 30 Livers from Mice Dosed with 3.13 mg/kg Mebendazole (Vermox ®)

Scale bar indicates 25 mm

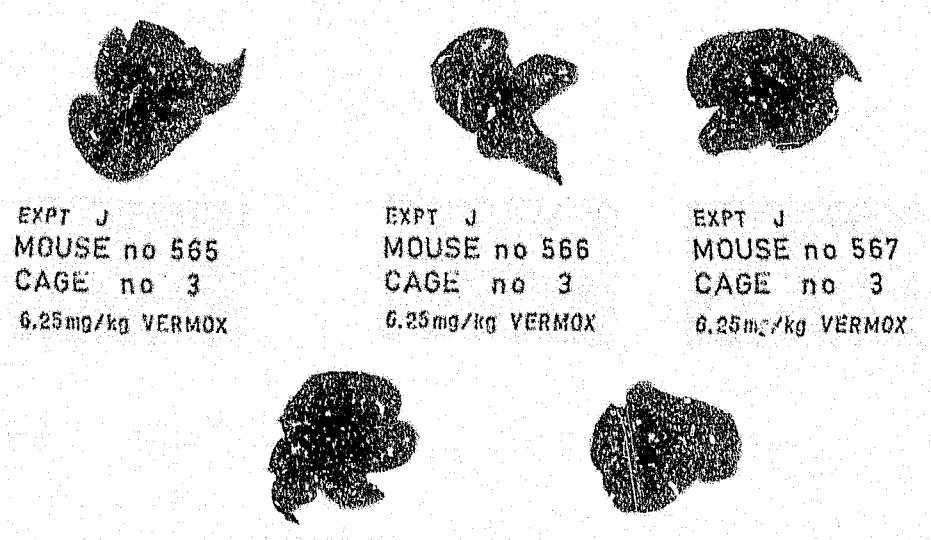

FIGURE 31 Livers from Mice Dosed with 6.25 mg/kg Mebendazole (Vermox ®)

Scale bar indicates 25 mm

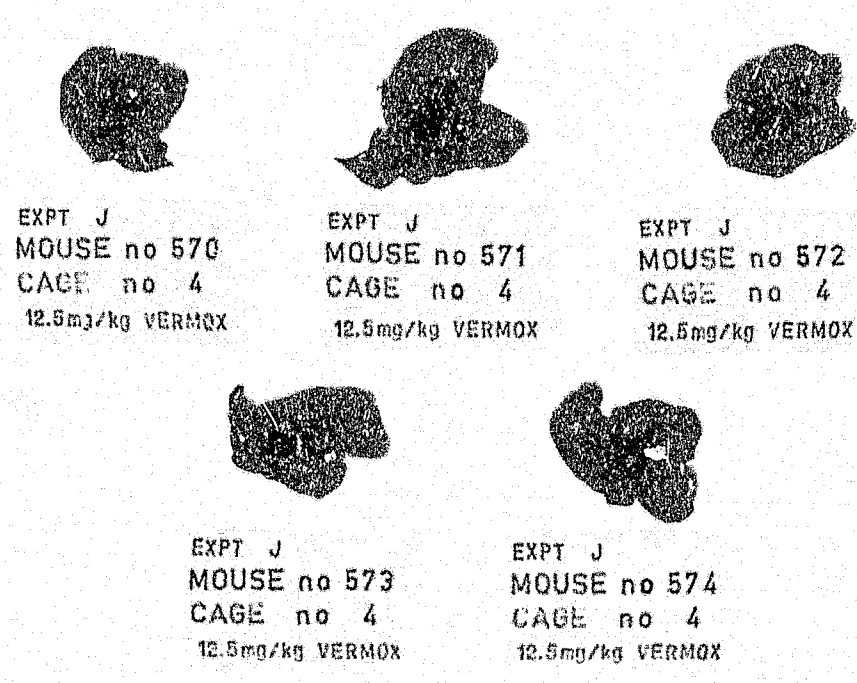

FIGURE 32 Livers from Mice Dosed with 12.5 mg/kg Mebendazole (Vermox ®)

Scale bar indicates 25 mm

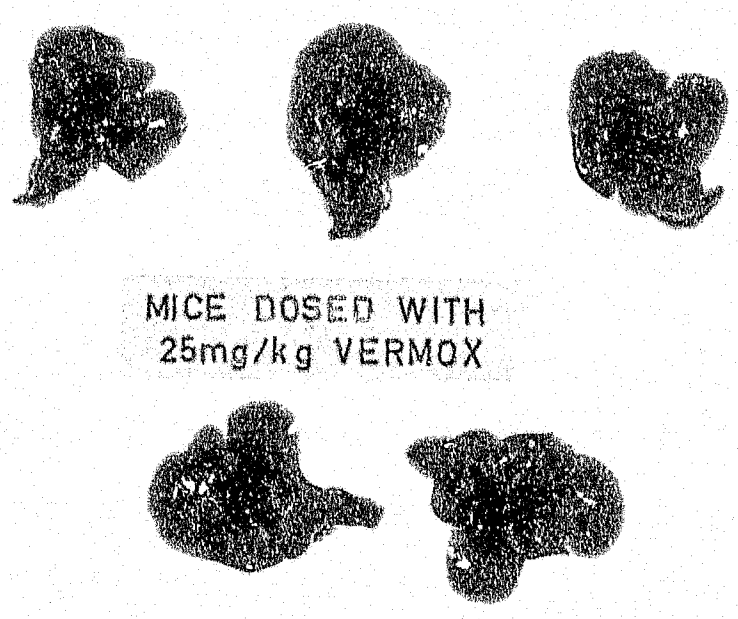

FIGURE 33 Livers from Mice Dosed with 25.0 mg/kg Mebendazole (Vermox ®)

Scale bar indicates 25 mm

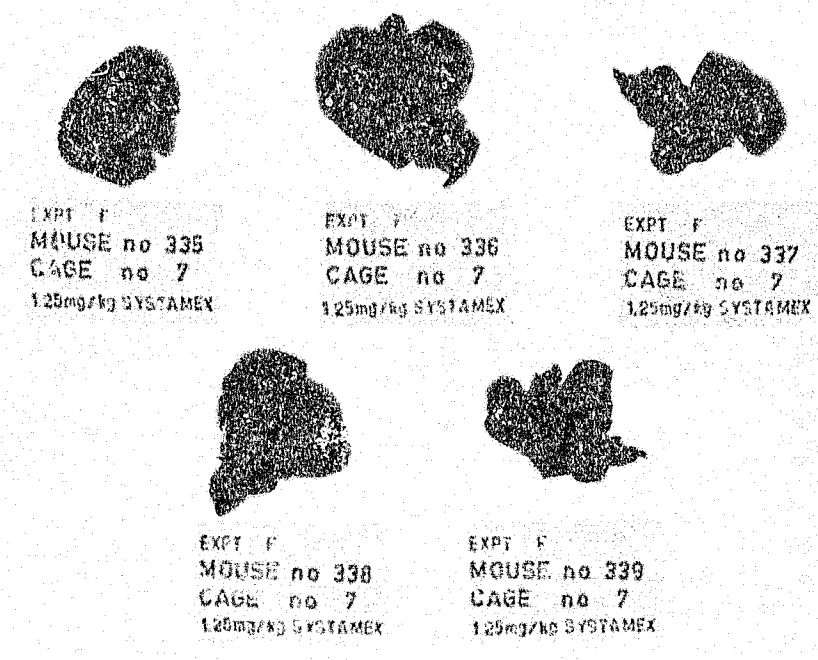

FIGURE 34 Livers from Mice Dosed with 1.25 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

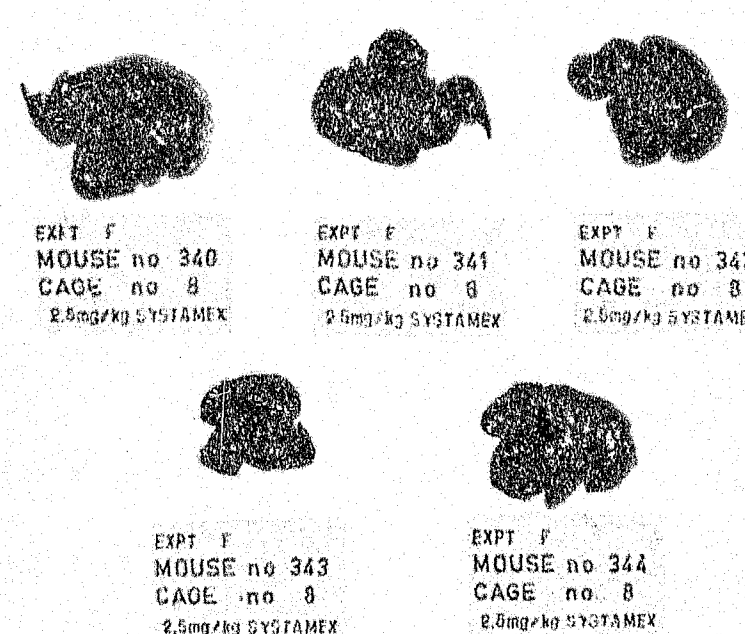

FIGURE 35 Livers from Mice Dosed with 2.50 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

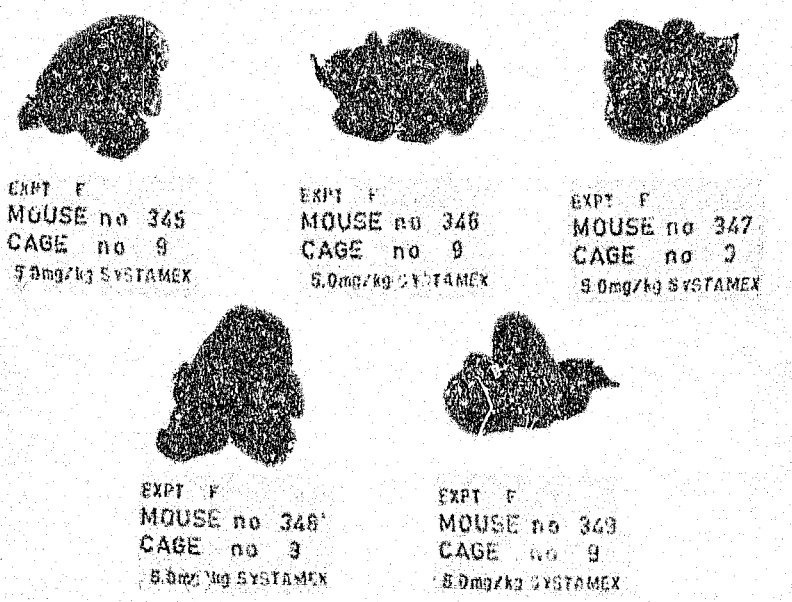

FIGURE 36 Livers from Mice Dosed with 5.00 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

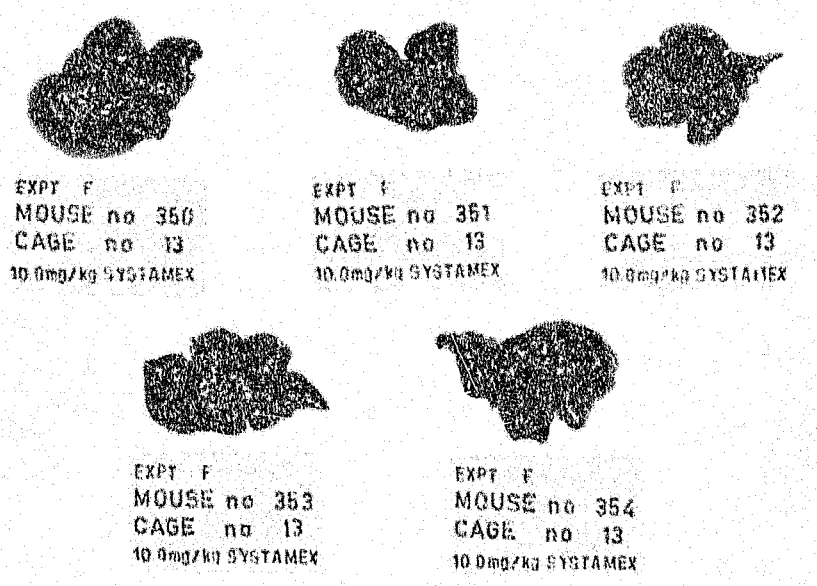

FIGURE 37 Livers from Mice Dosed with 10.0 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

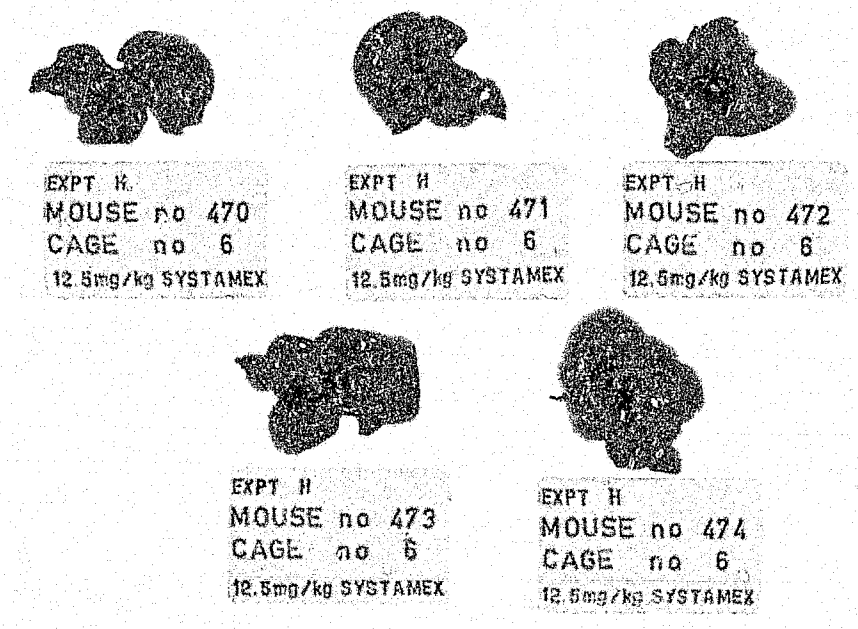

FIGURE 38 Livers from Mice Dosed with 12.5 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

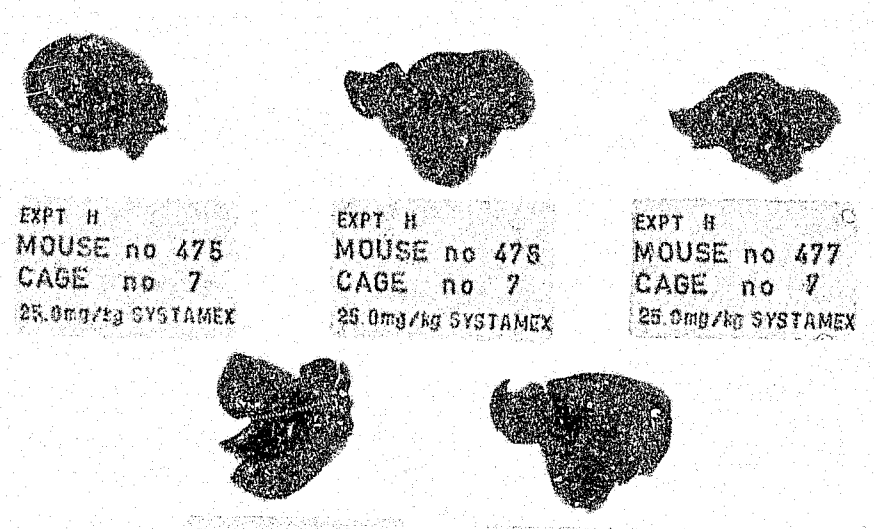

FIGURE 39 Livers from Mice Dosed with 25.0 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

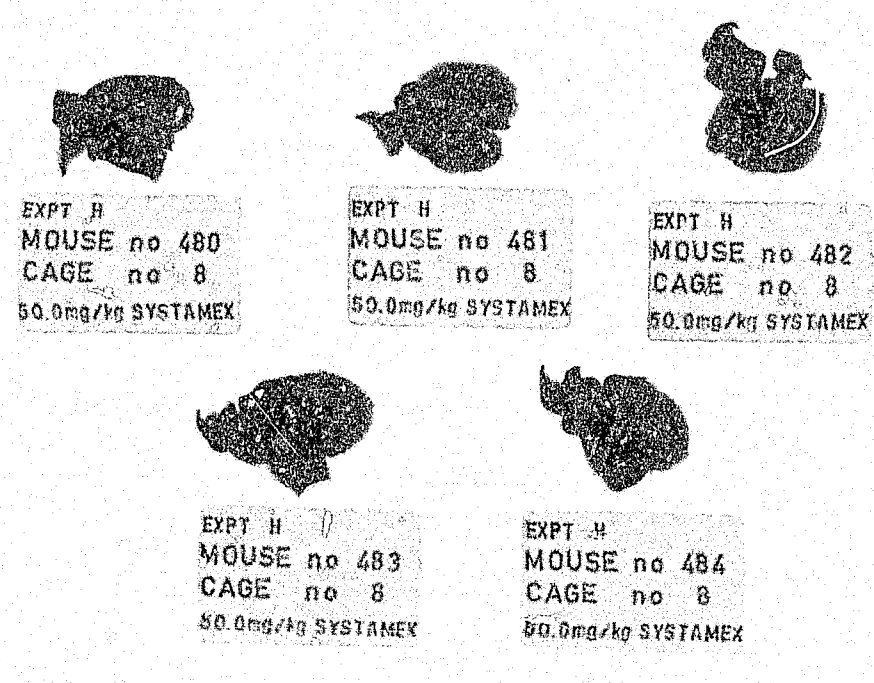

FIGURE 40 Livers from Mice Dosed with 50.0 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

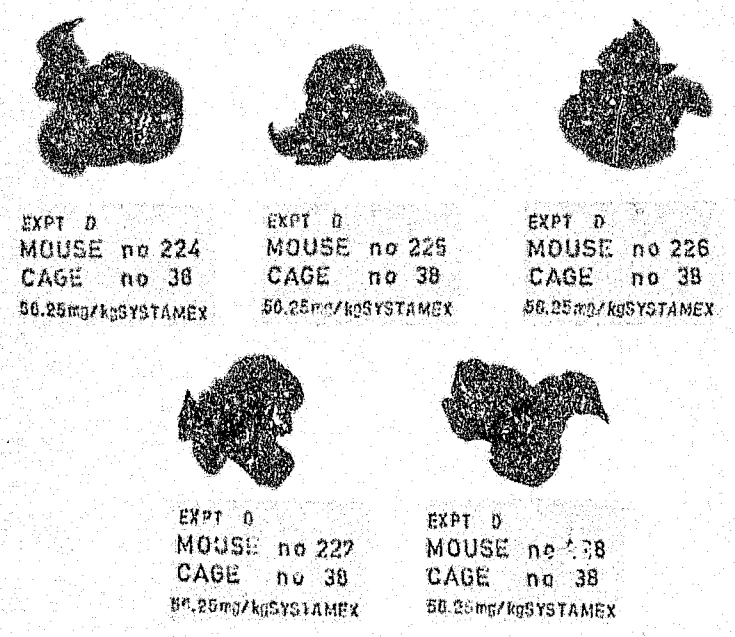

FIGURE 41 Livers from Mice Dosed with 56.25 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

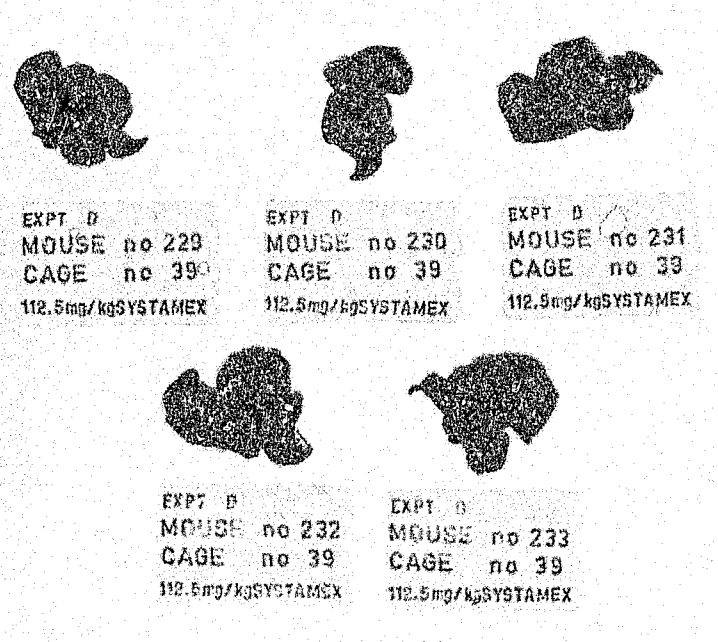

FIGURE 42 Livers from Mice Dosed with 112.5 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

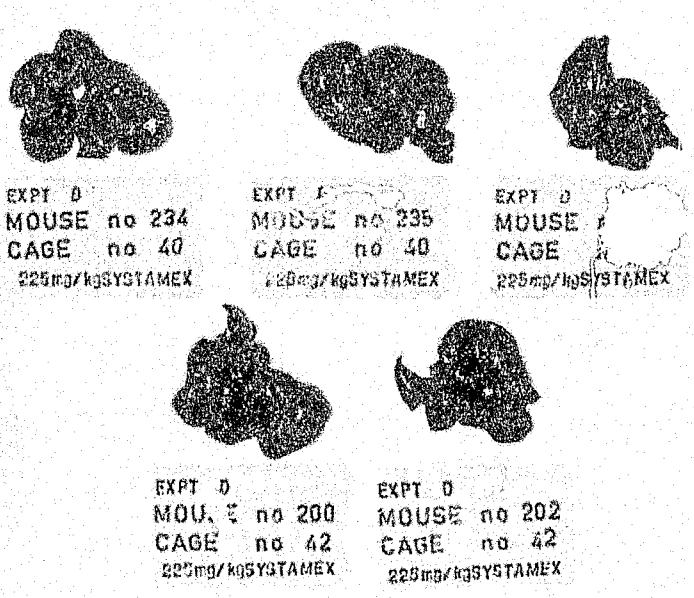

FIGURE 43 Livers from Mice Dosed with 225 mg/kg Oxfendazole (Systamex ®)

Scale bar indicates 25 mm

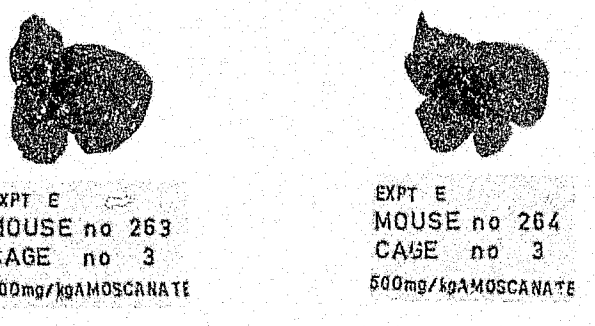

FIGURE 44 Livers from Mic ·d with 500 mg/kg Amoscanate

Scale bar indicates 25 mm

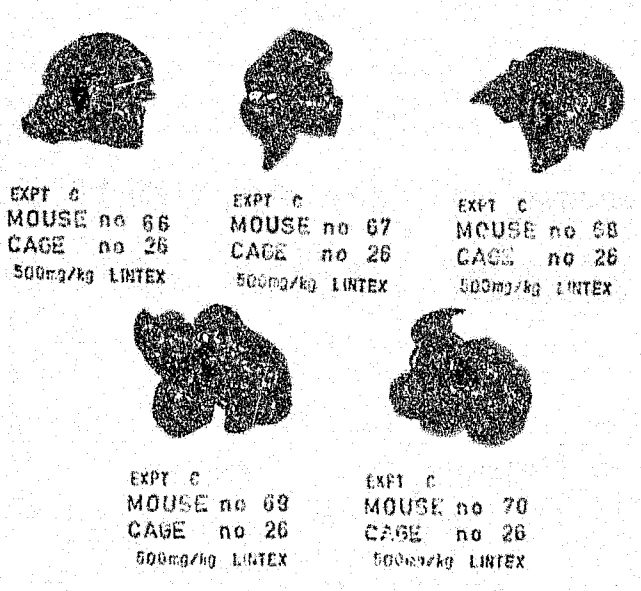

FIGURE 45 Livers from Mice Dosed with 500 mg/kg Niclosamide (Lintex ®)

Scale bar indicates 25 mm

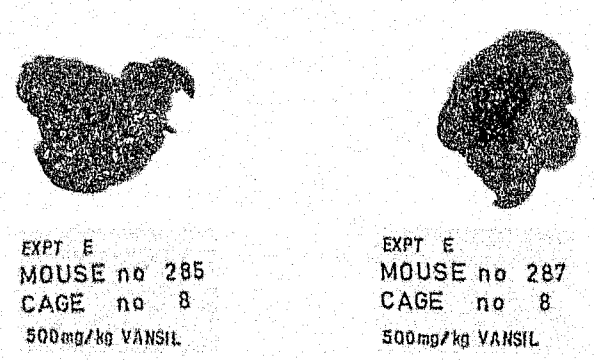

FIGURE 46 Livers from Mice Dosed with 500 mg/kg Oxamniquine (Vansil ®)

Scale bar indicates 25 mm

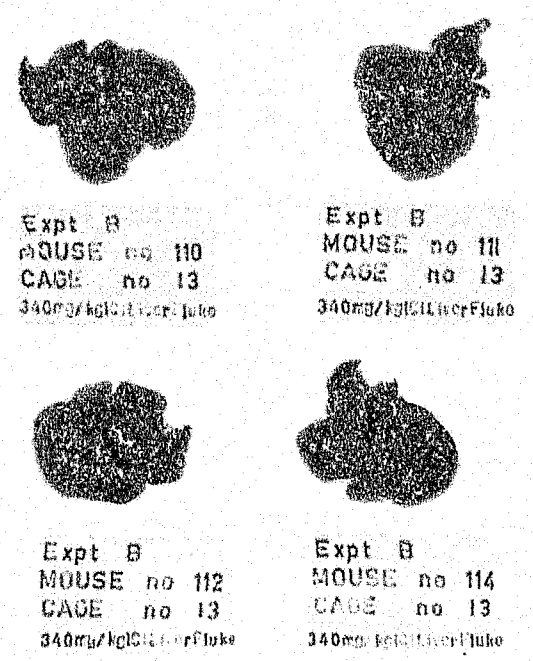

FIGURE 47 Livers from Mice Dosed with 340 mg/kg Oxyclozanide (ICI Liver Fluke Remedy ®)

Scale bar indicates 25 mm

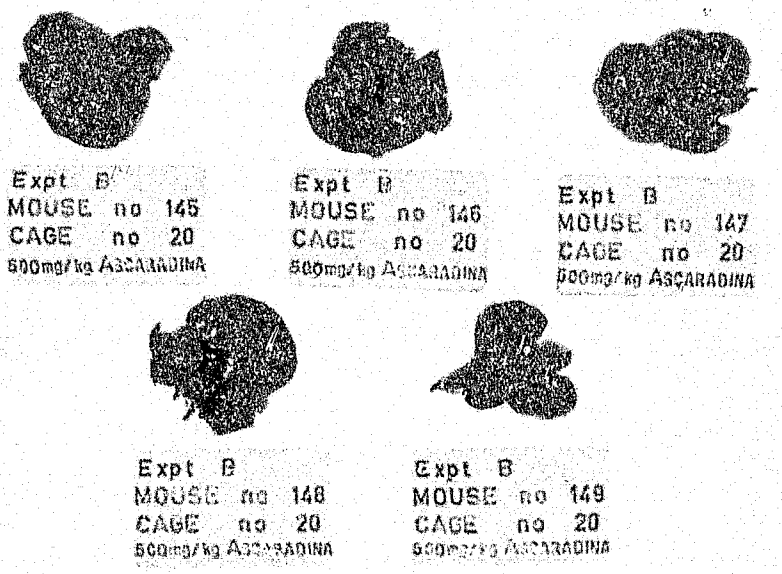

FIGURE 48 Livers from Mice Dosed with 500 mg/kg Piperazine Adepate (Ascaradina ®)

Scale bar indicates 25 mm

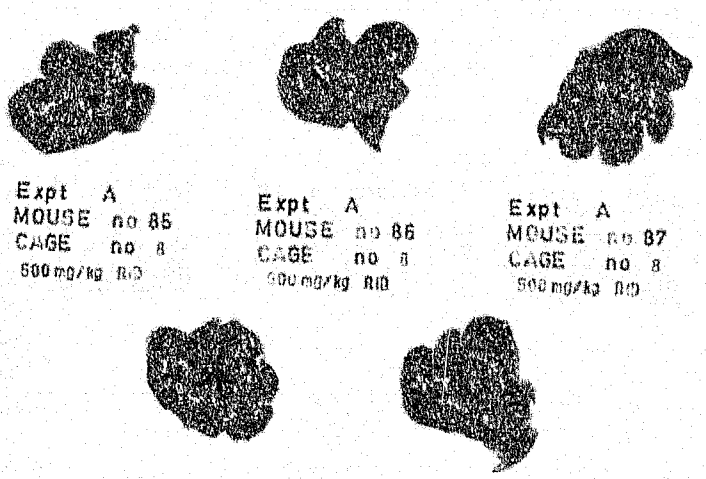

FIGURE 49 Livers from Mice Dosed with 500 mg/kg Piperazine Citrate (Rid ®)

Scale bar indicates 25 mm

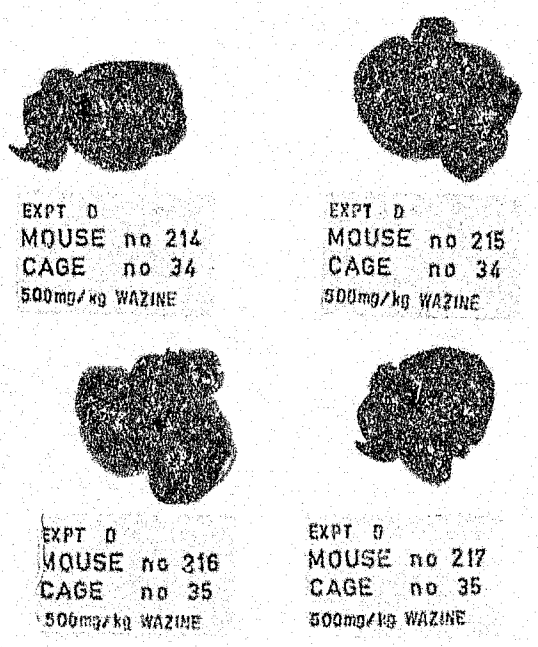

FIGURE 50 Livers from Mice Dosed with 500 mg/kg Piperazine Dihydrochloride (Wazine ®)

Scale bar indicates 25 mm

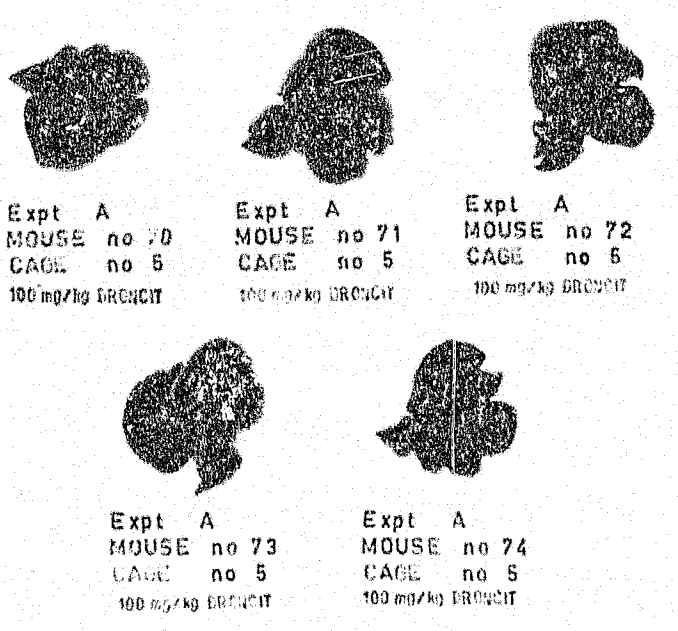

FIGURE 51 Livers from Mice Dosed with 100 mg/kg Praziquantel (Droncit ®)

Scale bar indicates 25 mm