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A CLINICO-PATHOLOGICAL STUDY OF BILHARZIASIS IN SHEEP

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Abstract

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Six healthy sheep were artificially infested through skin exposure with graded doses of cercariae of *Schistosoma mattheei* Veglia & Le Roux, 1929.

By means of laboratory tests the development of lesions due to the disease could be monitored at weekly intervals, so as to shed light on its pathogenesis.

In spite of lowering of the albumin fraction the total serum protein tended to rise, mainly as the result of an increase of beta and gamma globulin concentrations.

Bromsulphalein retention was increased in the more acute cases due to portal venous tree obstruction but transaminase activity and bilirubin concentration rose only occasionally and transiently, indicating minimal and passing hepatocellular damage.

Anaemia developed, normocytic in character, indicating the effect of simple blood loss. Leucocyte counts were not significantly affected but occasional rises of eosinophile percentage indicated transient episodes of sensitivity.

Acid-base disturbances were absent and some loss of sodium and potassium through the bowel wall was demonstrated.

Renal and mineral metabolism were not affected.

Introduction

As has been pointed out by Coutinho (1968), schistosomiasis is one of the most important diseases in the world. It was first recorded in Egypt in pharaonic times and was transferred to China. From the Nile and Yangtze valleys it spread to the greater part of Africa and Asia, and through slave traffic to the Americas. In South Africa a number of schistosome species has long been recognized both in man and animals. In domestic ruminants the most important is *Schistosoma mattheei* Veglia & Le Roux, 1929, which is also pathogenic for man (McCully & Kruger, 1969). These authors made a detailed pathological study of 100 cases in sheep and 14 in cattle.

A paucity of clinico-pathological studies of the disease, particularly in sheep, exists. Only one such study has been carried out previously, by Lengy (1962) who produced an infestation with *S. bovis* (Sonsino, 1876) in a sheep by exposing one of its fore-limbs to 20,000 to 30,000 cercariae for 2 hours. He drew blood samples for examination weekly for some 16 weeks after exposure, and made the following estimations: haematological determinations, blood sugar, non-protein nitrogen, total serum proteins and albumin-globulin ratio, prothrombin time and icterus index. In addition the lamb was examined superficially for clinical signs, weighed periodically, and its rectal temperatures were recorded. Weekly faecal examinations were carried out to exclude the possibility that other helminth infestations were present.

There is a high incidence of the disease in some of the warmer regions of South Africa, notably Zululand and the Eastern and Northern Transvaal. The present study was therefore conducted under controlled conditions with the experimental animals free from other helminths, to study the effect of *S. mattheei* infestation alone both clinically and clinico-pathologically.

Materials and Methods

Nine healthy, parasite-free sheep, aged from 7 to 11 months, were used. Six of these (No. 1 to 6) were in the low dosage (chronic) experiment, and three (No. 7 to 9) in the higher dosage (acute) experiment. The first six were paired by breed and weight, one of each pair serving as an uninfested control throughout. Feed and water intake, temperature variations and other clinical details were recorded daily. The controls were given exactly the same amount of feed as was ingested by their paired experimental mates fed *ad lib.* the previous day. Details of the sheep used are given in Table 1.

After an initial period of over a week for the sheep to get accustomed to their individual pens, preliminary determinations were done of a selected battery of tests. These were continued weekly for some 26 weeks in the

Table 1 *Experimental sheep, with infestation, patency and survival data*

Sheep No.	Breed	Role	Initial weight (kg)	Period of Observation (days)	Lab. Determinations (No. of Test Series)		Effective Infestation (No. of (cercariae)	Onset of Patency (Days after Day "O")†	Survival Period (Days from Day "O" to Death)
					Prepatent	Patent			
1	Merino	Control	12.5	182	10	17	—	—	—
2	Merino	Infested	13	182	10	17	1,966	56	Did not die
3	Dorper*	Control	20.5	182	10	17	—	—	—
4	Dorper	Infested	21	182	10	17	3,976	56	227
5	Dorper	Control	27.7	182	10	17	—	—	—
6	Dorper	Infested	32	182	10	17	15,700	56	181
7	Dorper	Infested	19	74	8	5	30,900	50	67
8	Dorper	Infested	22.5	64	8	3	59,759	50	62
9	Dorper	Infested	21	64	8	3	89,931	50	58

*Dorper = Dorset Horn × Black Head Persian
†Day "O" = Date infestation commenced

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chronic experiment and for 10 to 12 weeks in the acute experiment.

The experimental sheep were infested roughly on the "doubling up" principle for total dose of cercariae, resulting in a series of effective infestations ranging from nearly 2,000 to nearly 90,000 cercariae in the six animals.

Infestation was carried out over 5 to 7 days by placing their legs, previously scrubbed, into jars containing a known number of cercariae. The time allowed on each occasion was 30 min. After this the vessels were returned to the laboratory, iodine was added and the cercariae remaining in suspension were counted. The effective infestation was then calculated by difference. The effective total dosages are to be found in Table 1.

Day zero (Day "O") is the day on which artificial infestation was commenced and the word *patency* refers to the presence in the sheep of mature, egg-laying worms. Faecal examination was carried out frequently and the presence of viable schistosome eggs determined by hatching of miracidia, using the apparatus described by Kruger & Heitmann (1967). The word *prepatent* thus serves to indicate the period from Day "O" until the day miracidia could first be hatched, and *patent* the subsequent period as defined above.

It was soon found that there was some variation in the profiles of normal values between the individual experimental sheep. Similarly the controls tended to have their own sets of values. It was thus decided to let the infested sheep provide their own norms during the prepatent period and it was feasible to compare statistically the figures obtained before with those after the onset of patency.

The degree of significance of such differences was calculated by means of Snedecor's variance ratio "F" between the variance "between groups" (numerator) and the variance "within groups" (denominator). The method used is given by Hoffman (1963) and Lewis (1966).

For the laboratory work blood was collected with heparin as the anti-coagulant for all determinations except the haematology, for which EDTA (di-sodium ethylene diamine tetra-acetate) was used. The following estimations were done:–

Blood urea nitrogen: Titrimetric procedure of Hench & Aldrich (1926).
Blood sugar: Method of Folin & Wu (1920) as modified by Varley (1958).
Serum calcium: Method of Ferro & Ham (1957).
Serum inorganic phosphate: Method of Fiske & Subbarow (1925).
Total plasma protein: Biuret method of Weichselbaum (1946).
Albumin-globulin ratio: Method of Kingsley (1940).
Plasma pH: was determined with a Radiometer pH meter (Model 27) with a micro-electrode unit.
"Carbon dioxide content": By means of a Natelson microgasometer.
Plasma sodium and potassium: By flame photometry in association with a Zeiss PMQ II spectrophotometer.
Plasma chlorides: Method of Schales & Schales (1941).
Plasma alkaline phosphatase: Method of King & Armstrong (1934).
Serum glutamic oxalacetic transaminase: Method of King (1958).
Plasma bilirubin: Classical method of Malloy & Evelyn (1937) as modified (Varley, 1958).
Bromsulphalein retention: Colorimetric measurement of dye remaining in plasma ten minutes after intravenous injection of 5 mg per kg.
Haematology: Standard methods (Wintrobe, 1961) were used for red blood cell count, haemoglobin estimation and packed cell volume, as also for white blood cell count and differential white cell count. For determination of the erythrocyte sedimentation rate, however, Wintrobe tubes were set up at an angle of 50° from the horizontal, for one hour.
Electrophoresis: Filter paper procedure in an EEL apparatus (Evans Electroselenium Limited). Staining with bromophenol blue. Scanning with a Spinco Analytrol Model RB Densitometer.

Results

Blood urea nitrogen

Blood urea nitrogen levels tended generally to remain within normal limits until death was imminent. In the case of Sheep 7 the last determination was on the day of death, 17 days after miracidia were first hatched from the faeces. Sheep 8 died five days after the last available figure for blood urea nitrogen while Sheep 9 died one day later. Both Sheep 7 and 9, when near to death, showed distinct evidence of terminal nitrogen retention.

Blood sugar

Levels for this constituent remained within normal limits throughout.

Serum calcium, inorganic phosphate and magnesium

These determinations did not reveal deviations from the normal range at any stage.

Plasma proteins

Total plasma protein figures tended to rise from the onset of patency onwards.

In the chronic experiment Sheep 2, which had the smallest dose, showed a slight but statistically non-significant rise from a mean of 6.46 to 6.69 g/100 ml. In Sheep 4 the rise of means from 6.87 to 7.68 was highly significant and in Sheep 6 moderately so, from 6.56 to 7.31. A comparison of all prepatent with all patent figures showed a highly significant overall rise. The statistical data are given in Table 2 as are also those for the albumin-globulin ratios. In the latter the sheep with the lowest infestation (Sheep 2) went through a phase of very low values followed by recovery. Both the others showed a highly significant progressive drop after the beginning of patency. Comparing the prepatent findings of all three animals with all the patent figures

Table 2 *Statistical data of chronic group analysis of variance: "F" ratio*

Sheep	"F"	Degrees of Freedom	Probability	Significance
TOTAL PLASMA PROTEIN				
2	3.16	DF_1 1 DF_2 24	$P > 0.05$	None
4	21.5	DF_1 1 DF_2 24	$P < 0.001$	High
6	13.9	DF_1 1 DF_2 24	$0.001 < P < 0.01$	Moderate
All three	22.8	DF_1 1 DF_2 76	$P < 0.001$	High
ALBUMIN-GLOBULIN RATIO				
2	0.25	DF_1 1 DF_2 24	$P > 0.05$	None
4	22.5	DF_1 1 DF_2 24	$P < 0.001$	High
6	48.3	DF_1 1 DF_2 24	$P < 0.001$	High
All three	32.5	DF_1 1 DF_2 76	$P < 0.001$	High

the decrease in the albumin-globulin ratio was statistically highly significant.

In the acute group the same type of change was found. The mean total plasma proteins in Sheep 7 rose from 6.7 to 8.1, in Sheep 8 from 6.8 to 7.7. and in Sheep 9 from 6.9 to 7.9 g/100 ml. Examined statistically, the differences in individual sheep were not very significant, probably as a result of the small number of determinations between the onset of patency and death. Comparing all values of the three sheep before patency with those afterwards, the rise was found to be highly significant. This is evident from Table 3.

TABLE 3 *Statistical data of acute group analysis of variance: "F" ratio*

Sheep	"F"	Degrees of Freedom	Probability	Significance
TOTAL PLASMA PROTEIN				
7	9.3	DF_1 1 DF_2 10	$0.01 < P < 0.05$	Poor
8	4.6	DF_1 1 DF_2 8	$P > 0.05$	None
9	7.03	DF_1 1 DF_2 7	$0.01 < P < 0.05$	Poor
All three	21.2	DF_1 1 DF_2 29	$P < 0.001$	High
ALBUMIN-GLOBULIN RATIO				
7	47.5	DF_1 1 DF_2 10	$P < 0.001$	High
8	40	DF_1 1 DF_2 8	$P < 0.001$	High
9	5.3	DF_1 1 DF_2 7	$P > 0.05$	None
All three	57.2	DF_1 1 DF_2 29	$P < 0.001$	High

There was, however, a great drop in the albumin concentration to as low as 0.85 g/100 ml before death, while the globulins rose to levels high enough to raise total protein levels. Analysis of variance between albumin-glubulin ratios before and after patency showed a high degree of significance for the decrease during the latter period. This applied to Sheep 7 and 8 individually and to the overall values. Sheep 9 died too soon after patency to allow of a satisfactory statistical comparison.

The relevant data are also presented in Table 3.

Filter paper electrophoresis of serum

The changes in mean levels of the different electrophoretic fractions, together with the statistical significance of the changes, are summarized in Tables 4 and 5 for the chronic and acute groups respectively. These means of values before patency as well as those after patency are given for the individual sheep and for each group of three. In each case the statistical significance is indicated.

The direction of change can, in each case, be derived from the change of the means.

Plasma pH and "carbon dioxide content"

These basic indices of acid-base balance remained within normal limits during the entire period of the experiments, the only exception being in Sheep 7 where the "CO_2-content" dropped to 17 mM/l on the day of death. In the case of Sheep 8 it was normal the day before death.

Plasma electrolytes

Both sodium and potassium concentrations in the chronic group remained within normal limits while those in the acute group tended to fall during the patency stage. The mean sodium figures for Sheep 7, 8 and 9 were respectively 152.9 and 145, 154.5 and 146,

TABLE 4 *Electrophoretic fractions in chronic group before and after onset of patency: Means and statistical significance of changes*

Sheep No.	Alb.-Glob. Ratio	Albumin	Alpha-1	Alpha-2	Beta	Gamma
2	1.23–0.91	3.54–3.19	0.21–0.27	0.67–0.84	0.37–0.50	1.63–1.91
	$P < 0.05$	None	None	$P < 0.01$	None	None
4	1.43–0.95	4.01–3.71	0.26–0.39	0.69–0.82	0.40–0.54	1.5–2.2
	$P < 0.01$	None	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.01$
6	1.75–1.04	4.16–3.62	0.21–0.26	0.66–0.82	0.37–0.48	1.14–1.97
	$P < 0.001$	$P < 0.01$	None	$P < 0.05$	None	$P < 0.01$
All three	1.44–0.97	3.88–3.51	0.23–0.30	0.67–0.83	0.38–0.50	1.46–2.02
	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$

TABLE 5 *Electrophoretic fractions in acute group before and after onset of patency: Means and statistical significance of changes*

Sheep No.	Alb.-Glob. Ratio	Albumin	Alpha-1	Alpha-2	Beta	Gamma
7	1.12–0.8	3.56–3.1	0.31–0.35	0.87–1.3	0.51–1.0	1.4–1.2
	$0.001 < P < 0.01$	None	None	None	$0.001 < P < 0.01$	None
8	1.07–0.55	3.5–2.9	0.34–0.5	0.9–1.5	0.44–1.25	1.64–1.6
	$0.01 < P < 0.05$	None	$0.001 < P < 0.01$	$P < 0.001$	$P < 0.001$	None
9	0.93–0.6	3.29–3.1	0.29–0.3	1.0–1.4	0.51–0.9	1.82–2.2
	None	None	None	$0.001 < P < 0.01$	None	None
All three	1.03–0.66	3.44–3.02	0.31–0.40	0.93–1.40	0.49–1.08	1.63–1.56
	$0.001 < P < 0.01$	$0.01 < P < 0.05$	None	$0.001 < P < 0.01$	$P < 0.001$	None

and 153.6 and 147.8 mEq/l. For potassium the respective mean levels were 5.03 and 4.25, 5.4 and 4.3, 5.33 and 4.35 mEq/l.

The significance of these drops was poor to moderate when viewed individually but when all the prepatent levels together were compared with all the patent levels the fall in concentration was highly significant. The analysis of variance for the two cations is presented in Table 6.

TABLE 6 *Statistical data analysis of variance: "F" ratio*

Sheep	"F"	Degrees of Freedom	Probability	Significance
PLASMA SODIUM				
7	8.28	DF_1 1 DF_2 9	$0.01 < P < 0.05$	Poor
8	22.6	DF_1 1 DF_2 7	$0.001 < P < 0.01$	Moderate
9	18.5	DF_1 1 DF_2 7	$0.001 < P < 0.01$	Moderate
All three	38.9	DF_1 1 DF_2 27	$P < 0.001$	High
PLASMA POTASSIUM				
7	9.12	DF_1 1 DF_2 9	$0.01 < P < 0.05$	Poor
8	7.14	DF_1 1 DF_2 7	$0.01 < P < 0.05$	Poor
9	4.2	DF_1 1 DF_2 7	$P > 0.05$	None
All three	19.23	DF_1 1 DF_2 27	$P < 0.001$	High

Chloride figures remained within the normal range.

Alkaline phosphatase

This determination did not prove to be of value because of the great lability of ovine alkaline phosphatase activity (Cornelius, 1963).

Serum glutamic oxalacetic transaminase

The activity of this enzyme usually remained within normal limits in all the sheep, but showed occasional isolated peaks above 140 King units.

Plasma bilirubin

Only fractional and irregular rises of conjugated and unconjugated bilirubin above zero were noted and did not present any particular pattern.

Bromsulphalein retention

At no stage was there any increase in bromsulphalein retention in the chronic group but in the acute one there was an immediate reaction to the presence of mature worms. In these three sheep (7, 8 and 9) all determinations done after miracidia could be demonstrated, showed progressively elevated values at a high level of significance (see Table 7). The comparative means for the three sheep were respectively 3.25 and 14, 3.4 and 17.8, and 3.4 and 12.5 per cent retention after 10 min.

TABLE 7 *Statistical data analysis of variance: "F" ratio*

Sheep	"F"	Degrees of Freedom	Probability	Significance
BROMSULPHALEIN RETENTION				
7	8.27	DF_1 1 DF_2 11	$0.01 < P < 0.05$	Moderate
8	39.1	DF_1 1 DF_2 9	$P < 0.001$	High
9	64.7	DF_1 1 DF_2 9	$P < 0.001$	High
All three	48.8	DF_1 1 DF_2 33	$P = 0.001$	High

Haematology

In the chronic group the erythrocyte sedimentation rate remained unaffected throughout in Sheep 2 and 4 but was increased in Sheep 6 (with the heaviest infestation) at a high level of significance ($P < 0.001$), the means rising from 3.6 to 7.6 mm/h.

This trend was even more striking in the acute group with progressively heavier infestation. While all prepatent readings were below 5 mm, all patent ones were in excess of this figure and reached a maximum of 29, 36 and 37 mm respectively before death in Sheep 7, 8 and 9. Statistical evaluation is given in Table 8.

TABLE 8 *Statistical data analysis of variance: "F" ratio*

Sheep	"F"	Degrees of Freedom	Probability	Significance
ERYTHROCYTE SEDIMENTATION RATE				
7	14.14	DF_1 1 DF_2 11	$0.001 < P < 0.01$	Moderate
8	36.2	DF_1 1 DF_2 9	$P < 0.001$	High
9	61.4	DF_1 1 DF_2 9	$P < 0.001$	High
All three	78.0	DF_1 1 DF_2 33	$P < 0.001$	High

The packed cell volume, red blood cell count and haemoglobin values were unaffected in Sheep 2 and 4, and in fact showed a very gradual improvement over the period of observation as they did in their paired, uninfested, controls. Sheep 6, however, showed a highly significant decline in all three indices. This development of anaemia was even more marked in all three sheep in the acute group during the patent period. Statistically, as will be seen from Table 9, significance appeared to be erratic in individual cases, no doubt as a result of the usual technical errors in red blood cell counts and haemoglobin estimations. Overall, however, the decrease in all three sets of figures was highly significant.

TABLE 9 *Statistical data analysis of variance: "F" ratio*

Sheep	"F"	Degrees of Freedom	Probability	Significance
HAEMATOCRIT				
7	16.01	DF_1 1 DF_2 11	$0.001 < P < 0.01$	Moderate
8	33	DF_1 1 DF_2 9	$P < 0.001$	High
9	45.3	DF_1 1 DF_2 9	$P < 0.001$	High
All three	85.3	DF_1 1 DF_2 33	$P < 0.001$	High
RED BLOOD CELL COUNT				
7	19.2	DF_1 1 DF_2 11	$0.01 < P < 0.001$	Moderate
8	3.85	DF_1 1 DF_2 9	$P > 0.05$	None
9	22.7	DF_1 1 DF_2 9	Very nearly $P = 0.001$	High
All three	32.6	DF_1 1 DF_2 33	$P < 0.001$	High
HAEMOGLOBIN				
7	8.47	DF_1 1 DF_2 11	$0.01 < P < 0.05$	Poor
8	29.2	DF_1 1 DF_2 9	$P < 0.001$	High
9	13.03	DF_1 1 DF_2 9	$0.001 < P < 0.01$	Moderate
All three	41.7	DF_1 1 DF_2 33	$P < 0.001$	High

From haematocrit values of generally around 29 to 34 the percentage went down to 22, 22 and 25 for the three sheep.

In calculations of mean corpuscular volume it was found that there was no significant change in this index at any stage of developing anaemia.

White blood cell counts and differential counts similarly revealed no particular tendencies. Eosinophile leucocytes did not follow any consistent pattern; there were flare-ups at single determinations or for periods of up to 4 weeks, followed by a return to normal figures.

Clinical observations

In the lightly infested animals (Sheep 2, 4 and 6) of the chronic experiment no deviant clinical signs were observed at any time. However, in the acute group (Sheep 7, 8 and 9), the faecal pellets at about 50 days after Day "O" became confluent, fluid and slimy. Isolated blood flecks appeared in the stools and after a few days very fluid faeces impregnated with blood were much in evidence.

A few days before death, which took place 67, 62 and 58 days after initial infestation in Sheep 7, 8 and 9 respectively, the animals became recumbent. Breathing became increasingly laboured, and moist râles were striking on auscultation of the thorax. This was accompanied by rapid beating of the heart. The very fluid diarrhoea was frankly haemorrhagic.

Rectal temperatures fluctuated more or less within the normal range, with no effect due to the disease discernible.

Water intake increased steadily with increase of body size and the advancing summer months. In the chronic experiment it was noted, when comparing infested sheep with their paired mates, that Control Sheep 1 consistently did not finish the amount of feed that Sheep 2 had had the previous day. In the final weeks there was a considerable difference. Their weight gains also reflected the same pattern and lightly infested Sheep 2 at the end of the experiment weighed some 7 kg more than its control.

Between Sheep 3 and 4 there was a similar finding. At the end the infested sheep weighed 2.5 kg more than its control. Sheep 5 and 6, however, ran a more or less parallel course as far as weekly food intake and weight gain were concerned.

In the acute experiment all three sheep showed good increases in food intake and gains in weight until the onset of patency, when both dropped sharply and progressively till the time of death.

Discussion

The most significant pathological changes following infestation with *S. mattheei* in sheep and cattle have been described by McCully & Kruger (1969) as resulting from the presence of ova and dead schistosomes in the branches of the intrahepatic portal vein. The host reaction is of a granulomatous nature following initial thrombosis. From this there is a localized lymphoid proliferation which destroys the wall of the vein, and which remains after the parasite has been removed by the reactive process. Some necrosis of adjacent liver cord cells is also present.

They have further described the following macroscopical findings at necropsy: depletion and serious atrophy of fat, signs of anaemia, ascites, hydrothorax, hydropericardium, the presence of nodules and thrombi in the liver, and small red foci oozing fresh blood in the mucous membrane of the abomasum, small intestine, caecum and rectum.

The point has been made by several authors reporting on infestations with *S. mansoni* (Sambon, 1907) in man (Aufses, Schaffner, Rosenthal & Herman, 1959; Coutinho & Loureiro, 1960; Mousa, Atta, El Rooby, El Garem, Saif & El Abdin, 1966; Coutinho, 1968) that the disease is essentially mesenchymal in nature and that the liver parenchyma is spared till the terminal stages. This fact determines some of its clinical, pathological and biochemical features. The observation is substantially supported in the case of sheep with *S. mattheei* in the present study, as will be shown later.

Evidence for nitrogen retention in the blood was lacking in this series. Terminal rises of blood urea nitrogen in the acute cases were no doubt due to dehydration resulting from intestinal loss of fluid and blood and consequently reduced renal blood flow. This observation was supported by a simultaneous rise in the concentration of total plasma protein just before death. Lengy (1962) found normal to low values in *S. bovis* infestation of one sheep and Aufses *et al.* (1959) found both urine analysis and blood urea nitrogen to be normal in human *S. mansoni* infestation.

Blood glucose remained normal in this series, as Aufses *et al.* (1959) also found in man. Lengy (1962), however, in his single sheep found it to be higher in the later stage of the disease.

Plasma determinations of calcium, inorganic phosphate and magnesium revealed no changes, presumably because homeostatic mechanisms could cope with the degree of malabsorption which may have been present after the onset of diarrhoea in the acute cases.

The plasma proteins have received rather more attention than other aspects in the sparse literature of laboratory tests in schistosomiasis. From papers by Aufses *et al.* (1959), Coutinho & Loureiro (1960), Loureiro, Lima & Coutinho (1964) and Lees (1968) on *S. mansoni* infestation in man and that of Lengy (1962) mentioned above, it is always found that albumin concentration becomes depleted and that there is a rise of globulins, mostly gamma globulin. This was also found in the present study to a significant extent in all the infested animals except Sheep 2, which had the lowest degree of infestation. Total plasma levels were found to be generally within normal limits and quite often above normal. In this study there was mostly a greater gain in the total globulin moiety than loss of albumin. Both the alpha fractions contributed to this gain to a small extent while the beta globulins had an overall tendency to increase. This amounted to very roughly 30 per cent in the chronic and 100 per cent in the acute group. The rise of gamma globulins was, however, more striking in the chronic group than in the acute since, presumably, there was more time for the development of antibodies in the former.

Leland (1961) reviewed an extensive series of parasitic infestations in different animal species. Marked changes in the serum proteins were recorded. Total serum proteins were in some instances decreased while, quite frequently, they were normal or increased depending on the rise in total globulins. Changes in globulin fractions were manifested singly or in various combinations and in either direction, depending on the host and parasite concerned. The precise mechanisms responsible for these changes he described as "largely conjectural". Gamma globulin was generally increased while albumin was regularly depleted.

In *S. mansoni* infestation, specifically, the earliest reports on serum protein fractionation, in hamsters, mice and albino rats were given by Evans, Stirewalt &

MacKenzie (1955) and Evans & Stirewalt (1958). These authors found a steady rise in total protein to be due to gamma and beta-2 increases. Alpha-2 also steadily increased as the result of infestation and this was considered by Leland (1961) to be in keeping with the general concept that alpha globulin increases when any considerable inflammation or tissue destruction is present, irrespective of cause. Sadun & Walton (1958) concluded from fractionation of sera from humans with proven *S. japonicum* (Katsurada, 1904) infestation that there were "significant increases in total protein and in the relative proportions of alpha-2, beta and gamma globulin fractions, with a corresponding decrease in the relative proportions of albumin".

In the present study the albumin-globulin ratio was estimated by two methods: chemical (salt) fractionation and filter paper electrophoresis. In spite of some discrepancies the results were substantially in agreement and it was clear that there was a progressive fall in the ratio as a result of the infestation.

Acid-base balance was at no time disturbed since plasma pH and "CO_2 content" as well as chlorides remained within normal limits throughout. The tendency for the cations, sodium and potassium, to fall in the acute group was regarded as reflecting a loss of these electrolytes through the bowel walls. In the chronic cases which did not manifest diarrhoea, the figures remained within normal limits.

For the assessment of hepatocellular involvement serum glutamic oxalacetic transaminase activity and bilirubin levels were determined with substantially negative results. The oxalacetic transaminase fell generally within normal limits (up to about 140 King units) (Malherbe, 1960), with occasional transient rises to abnormal levels. As sampling was generally done once a week only detection of such possibly fleeting episodes of some hepatocellular necrosis could depend on an accident of timing. The finding by McCully & Kruger, (1969) of some necrosis in liver cells adjacent to granulomatous reactions to ova or to adult schistosomes could explain occasional rises. Occasional and small rises of conjugated or unconjugated bilirubin above zero were regarded in the same light.

Bromsulphalein retention remained unaffected in the chronic group but in the acute one there was an immediate and progressive increase after the onset of patency. This was clearly caused by the extensive incidence of thrombi and granulomas involving the vascular tree of the intrahepatic portal venous system, as described in detail by McCully & Kruger (1969).

The usually normal levels of transaminase activity with occasional peaking would suggest either slight necrotic involvement of the liver or short duration of the process, while the prolonged retention of bromsulphalein in the plasma clearly resulted from mechanical interference to the flow of blood through the intrahepatic portal veins.

There has been a number of reports on the portal hypertension of schistosomiasis due to obstruction of the intrahepatic veins and venules, including those of Aufses *et al.* (1959), Cheever & Warren (1964), Cheever (1965) and Coutinho (1968), all with *S. mansoni* involvement. It was concluded by Coutinho from haemodynamic data obtained from human patients that hepatosplenic *S. mansoni* infestation represented "the prototype of an intrahepatic presinusoidal block with a parasinusoidal component in advanced cases".

The presence of ascitic fluid in the peritoneal cavity, the extensive incidence of hydrothorax, hydropericardium and serous atrophy of subcutaneous and depot fat described by McCully & Kruger (1969) could be ascribed to the hypoalbuminaemia, with portal hypertension playing its usual role in the abdominal cavity.

The haematological examination showed an absence of anaemia in Sheep 2 and 4 (with the lowest degree of infestation) but increasingly severe anaemia of normocytic type occurred in Sheep 6 and the three acute animals in accordance with the degree of infestation. This was associated with marked and increasing acceleration of erythrocyte sedimentation. Normocytic anaemia is a usual result of blood loss for any reason so that this finding is consonant with the blood loss from the intestinal tract as described by McCully & Kruger (1969) and as seen clinically in the present study.

The total leucocyte count was not affected significantly in any direction. Eosinophiles similarly did not follow any consistent pattern since flare-ups were recorded at single determinations or for up to 4 weeks running, returning to normal figures in between and afterwards. The question of sensitization clearly merits a separate investigation. McCully & Kruger (1969) in the histopathological part of their study, which included these sheep, found varying evidence of sensitivity, ranging from a type of delayed hypersensitivity to a superimposed more marked sensitivity characterized by concentrations of eosinophiles around miracidia-containing ova or shells of ova present in the centre of mature granulomas. This was accompanied by necrosis of adjacent liver cord cells and necrosis of masses of eosinophiles. Weekly sampling for differential counts was probably too random to give any clear picture of eosinophile activity.

Summary

The effect of artificial infestation of healthy sheep with known numbers of cercariae of *S. mattheei* was studied by means of weekly collection of suitable samples from the sheep and of laboratory performance of a battery of tests designed to elucidate the pathogenesis of the disease.

The results of these tests before the onset of patency were compared with those after miracidia could be hatched from faeces and subjected to analysis of variance.

The following determinations gave results within the normal range throughout and were thus unaffected by the infestation: blood urea nitrogen, blood sugar, plasma calcium and inorganic phosphate, plasma pH, "carbon dioxide content" and chlorides, alkaline phosphatase, total white cell count and mean corpuscular volume.

Bromsulphalein retention was increased in the acute cases with the highest degrees of infestation but not in the more chronic ones.

Total plasma protein was increased in all cases except in the lowest infestation and this was accompanied by decreased albumin-globulin ratios. The increases in globulins were chiefly in the beta and gamma fractions while hypoalbuminaemia was a regular feature.

Sodium and potassium in plasma were reduced in the acute cases (highest infestations) due to enteric loss in diarrhoea.

Transaminase activity and bilirubin estimations showed occasional and transient increases but the general impression was given of minimal hepatocellular involvement.

Haematological examination revealed degrees of normocytic anaemia in keeping with the degree of infestation.

The total leucocyte count revealed no particular trends and eosinophilia was no more than an occasional and transient phenomenon.

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REFERENCES

AUFSES, A. H., SCHAFFNER, F., ROSENTHAL, W. S. & HERMAN, B. E., 1959. Portal venous pressure in "pipestem" fibrosis of the liver due to schistosomiasis. *Am. J. Med.* 27, 807–810.

CHEEVER, A. W., 1965. A comparative study of *Schistosoma mansoni* infections in mice, gerbils, multimammate rats and hamsters. I. The relation of portal hypertension to size of hepatic granulomas. *Am. J. trop. Med. Hyg.* 14, 211–226.

CHEEVER, A. W. & WARREN, K. S., 1964. Hepatic blood flow in mice with acute hepato-splenic schistosomiasis mansoni. *Trans. roy. Soc. trop. Med. Hyg.* 58, 406–412.

CORNELIUS, C. E., 1963. Liver function. *In:* Clinical biochemistry of domestic animals. Ed. Cornelius C. E. & Kaneko, J. J. New York & London: Academic Press.

COUTINHO, A., 1968. Hemodynamic studies of portal hypertension in schistosomiasis. *Am. J. Med.* 44, 547–556.

COUTINHO, A. & LOUREIRO, P., 1960. Aspectos bioquimicos da insufficiencia hepática na esquistossomose mansonica hépato-esplenica. *O Hospital* 58, 885–902.

EVANS, A. S. & STIREWALT, M. A., 1958. Serologic reactions in *Schistosoma mansoni* infections. IV. Comparative ionographic study of sera of hamsters, mice and albino rats. *Expl Parasit.* 7, 165–177.

EVANS, A. S., STIREWALT, M. A. & MACKENZIE, M., 1955. Serologic reactions in *Schistosoma mansoni* infections. II. Cercarial behaviour in electrophoretically separated fractions of sera of infected and uninfected mice. *Expl Parasit.* 4, 419–426.

FERRO, P. V. & HAM, ANNA B., 1957. A simple spectrophotometric method for the determination of calcium. *Am. J. clin. Path.* 28, 208–217.

FISKE, C. H. & SUBBAROW, Y., 1925. The colorimetric determination of phosphorus. *J. biol. Chem.* 66, 375–400.

HENCH, P. S. & ALDRICH, M., 1926. Urea retention. A simple method for its estimation by the mercury combining power of blood. *Archs intern. Med.* 38, 474–488.

HOFFMANN, R. G., 1963. Statistics for medical students. Springfield, Ill.: Chas C. Thomas.

KING, E. J., 1958. Routine methods for the estimation of transaminase. *J. med. Lab. Technol.* 15, 17–22.

KING, E. J. & ARMSTRONG, A. R., 1934. A convenient method of determining serum and bile phosphatase activity. *Can. med. Ass. J.* 31, 376–381.

KINGSLEY, G. R., 1940. A rapid method for the separation of serum albumin and globulin. *J. biol. Chem.* 133, 731–735.

KRUGER, S. P. & HEITMANN, L. P., 1967. Studies on bilharzia. 1. The development of an apparatus to hatch miracidia. *Jl S. Afr. vet. med. Ass.* 38, 191–196.

LEES, R. E. M., 1968. Symptoms and clinical and laboratory findings in 123 cases of schistosomiasis mansoni in St. Lucia. *J. trop. Med. Hyg.* 71, 40–43.

LELAND, S. E., 1961. Blood and plasma volume, total serum protein, and electrophoretic studies in helminthic diseases. *Ann. N.Y. Acad. Sci.* 94 : 1, 163–182.

LENGY, J., 1962. Some observations on the biochemistry and haematology of *Paramphistomum microbothrium* and *Schistosoma bovis* infection in lambs. *Refuah vet.*, 19, 115–111.

LEWIS, A. E., 1966. Biostatistics. New York: Reinhold Publishing Corporation.

LOUREIRO, P., LIMA, C. A. & COUTINHO, A., 1964. Biochemical aspects of schistosomiasis mansoni. *Proc. VII. internat. Congr. trop. Med. Malar.* 2, 72–73.

MCCULLY, R. M. & KRUGER, S. P., 1969. Observations on bilharziasis of domestic ruminants in South Africa. *Onderstepoort J. vet. Res.* 36, 1, 129–161.

MALHERBE, W. D., 1960. The value of the determination of transaminase activity in plasma as a screening test for liver disease in animals. *Jl S. Afr. vet. med. Ass.*, 31, 159–171.

MALLOY, H. T. & EVELYN, K. A., 1937. The determination of bilirubin with the photoelectric colorimeter. *J. biol. Chem.* 119, 481–490.

MOUSA, A. H., ATTA, A. A., EL ROOBY, A., EL GAREM, A. A., SAIF, M & EL ABDIN, A. Z., 1966. Hepatic blood flow in hepatosplenic bilharziasis. *J. trop. Med. Hyg.* 69, 45–50.

SADUN, E. H. & WALTON, B. C., 1958. Studies on the host parasite relationships to *Schistosoma japonicum*. II. Quantitative changes in the concentration of serum proteins in humans and rabbits. *Am. J. trop. Med. Hyg.* 7, 500–504.

SCHALES, O. & SCHALES, SELMA S., 1941. A simple and accurate method for the determination of chloride in biological fluids. *J. biol. Chem.* 140, 879–884.

VARLEY, H., 1958. Practical clinical biochemistry. 2nd Edit. London: Wm. Heinemann Medical Books Ltd.

WEICHSELBAUM, T. E., 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am. J. clin. Path.* 16, T.S. 40–49.

WINTROBE, M. M., 1961. Clinical Hematology. 5th Edit. London: Henry Kimpton.

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