Acta vet. scand. 1971, *12*, 335—343.

From the Departement of Special Pathology and Therapeutics, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

ALBUMIN METABOLISM IN CHRONIC FASCIOLA HEPATICA INFECTIONS OF CATTLE*

By

Peter Nansen

It has been repeatedly shown that infection with the trematode parasite Fasciola hepatica may lead to anaemia and changes in the serum protein pattern. During acute fascioliasis the changes are usually moderate, whereas during the chronic disease the serum protein profile may be markedly altered and there may be pronounced anaemia. One of the most conspicuous findings is hypoproteinaemia mainly due to lowered serum albumin levels. A bulk of evidence has indicated that in rabbits (*Jennings et al.* 1956, *Dargie et al.* 1968, *Holmes et al.* 1968 a, *Dargie* 1969) as well as in sheep (*Pearson* 1963, *Holmes et al.* 1968 a, b, *Sewell et al.* 1968) the main explanation of these findings is a loss of plasma and red cells into the lumen of the bile ducts. Immunoglobulin levels may be variously affected and usually there is hyperimmunoglobulinaemia. It has been shown in naturally infected sheep (*Nansen et al.* 1968) and heifers (*Nansen* 1970) that immunoglobulin synthesis is increased so that normal or even high levels can be maintained — despite accelerated breakdown rates.

During chronic bovine fascioliasis low serum albumin levels can be explained by a biliary loss of this protein. On the other hand, depressed serum levels could possibly also be secondary to decreased synthesis. Chronic fascioliasis in cattle is often accompanied by a marked cirrhosis of the liver. Since albumin is synthesized by the parenchymal cells of this organ the mainte-

* This work was supported by grants from Statens jordbrugs- og veterinærvidenskabelige Forskningsråd and Bernhard Bang's Mindefond.

nance of a normal synthetic rate could be critical. The present experiments were primarily designed to elucidate this point by studying the kinetics of radioisotopically labelled albumin.

MATERIAL

The study comprised 6 animals with chronic fascioliasis and 3 normal control animals. Ages of the infected animals ranged from 1 to 2½ years. Ages of controls ranged from 2 to 3½ years. One animal was a bull (J. no. 550/68), the others were heifers or cows. Breeds and body weights are listed in Table 1.

All infected animals except J. no. 569/68 came from 1 herd, where clinical fascioliasis had been a serious problem during several years. J. no. 569/68 came from a herd where subclinical fascioliasis was a well-known disease. The animals had been grazing on infected pastures during 1 season only. The present experiments were conducted in mid-winter. Thus, 3 or 4 months had passed since the last metacercariae might have been ingested. Consequently, the animals were examined during a typical, chronic phase of the disease. The animals were meagre, and a poor general condition had developed. J. nos. 432/66, 438/66 and 439/66 were even emaciated and disinclined to move. However, in all animals appetite was fairly good. Faeces were mostly normal and gastrointestinal helminths were considered of insignificant importance, except in J. no. 432/66. Necropsy of this heifer revealed a verminous abomasitis of a limited area.

Two heifers (J. nos. 438/66 and 439/66) were re-examined after approximately 2 months with radioiodinated bovine immunoglobulin-G. A detailed description of these experiments are given elsewhere (*Nansen* 1970).

METHODS

The albumin preparation used for labelling was a commercial product (Serum-Albumin vom Rind (trocken, "reinst")) obtained from Hoechst, Behringwerke AG. Labelling with carrier-free I^{131} was carried out according to the method described by *McFarlane* (1958). Radioactivity not bound to protein was removed on a resin column (Amberlite IRA 400). More than 99 % of the radioactivity in the final solution was precipitable with trichloroacetic acid. The mean ratio of iodine bound to protein was less than 1 atom per molecule. A small quantity of the labelled preparation was mixed with normal bovine serum and submitted to immuno-

electrophoresis using rabbit anti bovine serum. By autoradiography it was found that the preparation exclusively contained labelled albumin. The general experimental procedure described in detail by *Nielsen* (1966) or *Nansen* (1970) was followed: The labelled preparation (dose: approx. 200 μCi) was injected intravenously through a polythene catheter. After 15 min. a plasma sample was drawn for estimation of plasma volume. Plasma samples were taken daily during the first week, then on alternate days during the next 2 weeks. Serum samples for the quantitation of albumin and total protein were drawn biweekly. Lugol's iodine solution was given routinely to prevent thyroid uptake of radioiodine. Counting of radioactivity was performed in a thallium-activated NaI scintillation well-counter at the end of each experiment. Calculation of metabolic data was performed according to the method of *Nosslin* (1966), which is based upon the mathematical description of the plasma radioactivity disappearance curve. Intravascular degradation was assumed, and a metabolic steady state condition was likely to exist in all animals. Steady state criteria were based upon biweekly serum albumin determinations, weight control and daily clinical observation of the animals. The renal function was considered to be normal in all experimental animals.

Serum albumin was determined by immunoquantitation, using the method described by *Mancini et al.* (1965). The preparation of monospecific antiserum and the technical procedure is described elsewhere (*Nansen*). Total serum protein was determined by a micro-Kjeldahl method. Various haematological and biochemical analyses were performed by conventional laboratory procedures.

Two infected heifers (J. nos. 432/66 and 569/68) were killed immediately after the experimental period. Two others (J. nos. 438/66 and 439/66) were killed approximately 3 months after the albumin experiment when they had been re-examined in immunoglobulin-G experiments (*Nansen*). J. nos. 550/68 and 551/68 were killed after 3 and 5 months respectively. The livers were submitted to a macroscopical and microscopical examination*. Bile ducts were cut open, and adult flukes were removed and counted.

* The author is grateful to Tage Møller, D.V.M. (Dept. General Pathology and Pathological Anatomy of the Royal Veterinary and Agricultural University, Copenhagen), who carried out histology.

RESULTS

The degree of anaemia and hypoalbuminaemia and the size of fluke-burdens suggested that the infected animals could be arranged into 2 groups. Thus, it appears from Tables 1 and 2 that there was moderate anaemia and hypoalbuminaemia in 3 low-level infected animals (J. nos. 550/68, 551/68 and 569/68). In 3 heavily infected animals (J. nos. 432/66, 438/66 and 439/66) haemoglobin and albumin levels were considerably lowered. Liver function tests in the infected animals did not deviate much from those of the controls (Table 1). S-GOT levels in the infected animals were only slightly elevated compared with the controls, bilirubin levels were normal in all animals and only 2 animals (J. nos. 432/66 and 438/66) exhibited decreased elimination rate of bromsulphalein.

Table 1. Breed, body weight, haemoglobin, liver function tests and fluke-burden in 6 animals with chronic fascioliasis and 3 normal control animals.

	Animal J. no.	Breed	Weight (kg)	Haemoglobin (g/100 ml)	S-GOT transaminase (S.-F. units)	Total bilirubin (mg/100 ml)	BSP clearance ($T^1/_2$, min.)	Number of adult flukes
Infected animals	550/68	Jersey	240	8.0	120	0.5	4.0	120
	551/68	Cross-breed	360	7.6	120	0.4	3.1	150
	569/68	RDM	199	9.8	97	0.3	2.3	95
	432/66	SDM	329	7.1	128	0.3	6.7	470
	438/66	RDM	245	6.8	102	0.4	7.6	630
	439/66	Cross-breed	185	5.9	106	0.3	3.9	620
Controls	442/66	Cross-breed	338	11.2	50	0.4	2.7	0
	565/68	Jersey	323	10.4	83	0.5	2.4	0
	566/68	Jersey	361	10.3	69	0.4	3.7	0

It appears from Table 2 that the infected animals, despite lowered serum albumin, had normal total serum protein levels. An explanation as to this finding may be looked for in the fact that all diseased animals (except J. no. 550/68) had positive formolgel reactions, indicating hyperimmunoglobulinaemia.

Relevant metabolic data are listed in Table 2. The vascular albumin pools (g/kg) in the heavily infected group were in the low range of the controls. The fractional catabolic rates of the heavily infected group exceeded those of the other groups. Al-

Table 2. Protein levels and metabolic data of I^{131}-albumin in 6 animals with chronic fascioliasis and 3 normal control animals.

	Animal J. no.	Total serum protein (g/100 ml)	Serum albumin (g/100 ml)	Plasma volume (ml/kg)	Vascular albumin pool (g/kg)	Fractional catabolic rate (%/day)	Albumin synthesis or degradation (g/kg/day	Trans-capillary escape rate (%/day)	Ev:Iv ratio	Total albumin pool (g/kg)
Infected animals	550/68	7.07	2.25	46.7	1.06	10.1	0.11	91	1.08	2.20
	551/68	7.54	1.98	66.4	1.31	7.6	0.10	81	0.82	2.38
	569/68	6.02	2.23	52.8	1.16	7.0	0.08	55	0.96	2.27
	Mean	6.88	2.15	55.3	1.18	8.2	0.10	76	0.95	2.28
	432/66	6.96	1.57	48.0	0.75	12.6	0.09	60	0.97	1.49
	438/66	7.30	1.54	54.0	0.87	11.0	0.10	49	0.68	1.45
	439/66	7.37	1.63	49.1	0.79	10.6	0.08	53	1.08	1.64
	Mean	7.21	1.58	50.4	0.80	11.4	0.09	54	0.91	1.53
Controls	442/66	6.85	2.56	31.5	0.79	10.2	0.08	58	1.10	1.67
	565/68	7.53	2.40	42.6	1.02	9.4	0.10	63	1.22	2.26
	566/68	7.30	3.13	67.0	1.99	7.7	0.15	99	1.21	4.40
	Mean	7.23	2.70	47.0	1.27	9.1	0.11	73	1.18	2.78

bumin degradation or synthesis (expressed as g/kg/day) of the 3 groups were comparable and in the order of 0.1 g/kg/day. Extravascular:intravascular ratios were low in both infected groups compared with the controls, which means that the proportion of albumin in extravascular pools was low in the fluke-infected animals.

The pathological lesions of the infected livers were typical for the manifest chronic disease and essentially similar to detailed descriptions given by *Nieberle & Cohrs* (1961), *Supperer* (1964), *Keck & Supperer* (1967), *Dow et al.* (1967) and *Rahko* (1969). The left lobe was atrophied to various degrees. In J. nos. 432/66, 438/66 and 439/66 this lobe was extremely fibrotic, containing insignificant amounts of parenchyma. The other parts of the livers, especially the right lobe, were enlarged. On 2 animals necropsy was performed immediately after the experimental period and weight of livers was determined: J. no. 432/66 = 10.1 kg, J. no. 569/68 = 4.4 kg. Expressed as a percentage of body weight these figures were 3.1 and 2.2 respectively. Even in view of decreased body weights these figures were considered high (weight of normal liver is appr. 1—1½ % of body weight).

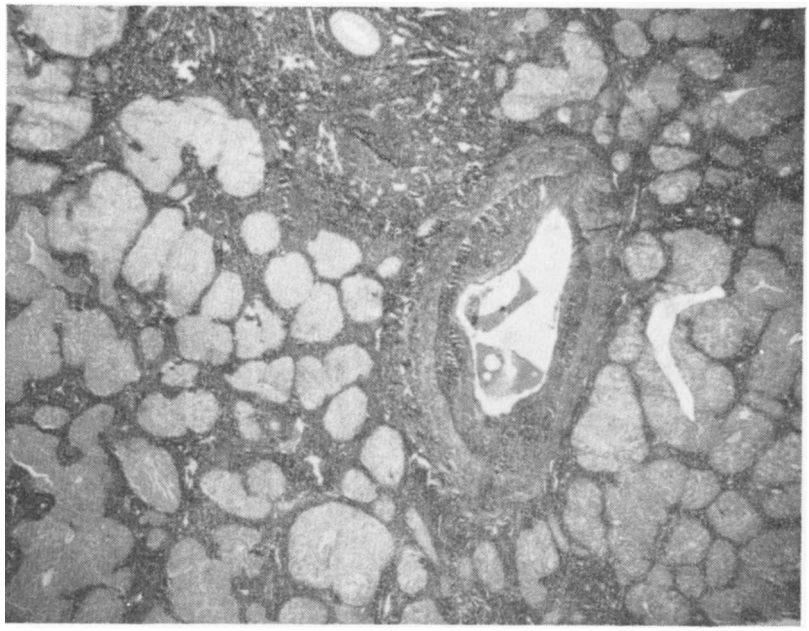

Figure 1. Hepatic cirrhosis in bovine fascioliasis (J. no. 569/68). Pronounced perilobular fibrosis. Enlarged bile duct with epithelial hyperplasia (Jh. v. G., Magn. 10 ×).

Throughout the livers there was a marked fibrosis, producing mainly a perilobular type of cirrhosis (see Fig. 1). Bile duct changes comprised i.a. duct wall fibrosis, pronounced epithelial hyperplasia and calcium depositions. These changes are described in detail elsewhere (*Nansen* 1970).

DISCUSSION

The present experiments have thrown light upon some traits of albumin metabolism in chronically fluke-infected cattle. Probably, the most interesting finding is that albumin synthesis is *not* severely disturbed despite pronounced fibrosis of the liver. This is in keeping with liver function tests, which revealed normal or only slightly reduced hepatic function. The most reasonable explanation as to these findings is that the liver size has increased so that a normal parenchymal cell mass could be maintained despite an over-all fibrosis. Obviously, the right liver lobe is particularly responsible for this enlargement. Whether the synthesis of albumin might be only slightly decreased is difficult to evaluate. Since the synthetic rate is expressed in relative terms,

i.e. g/kg/day this parameter is highly influenced by the nutritional stage of the individual animal. The chronically infected animals studied here were meagre, and thus the synthesis is overestimated, when comparison is made with the well-nourished control animals. Preferably, the synthetic rate and other relative values should be referred to a more fixed parameter such as surface area, although, here also undecipherable factors may be involved, i.e. differences in body-build will make comparison between individual animals or breeds somewhat difficult. From the present experiments it seems justified to conclude that albumin synthesis in the fluke-infected animals proceeded at a normal or slightly decreased rate.

In rabbits (*Dargie et al.* 1968, *Dargie* 1969) and in sheep (*Nansen et al.* 1968) it has been shown that the chronic F. hepatica infection may be associated with increased synthesis of albumin. This contrasts clearly with the observation made here. The difference can be attributed to the varying host reaction to the parasite. In sheep the liver reaction to migrating flukes is essentially regenerative with parenchymal hyperplasia and comparatively slight fibrosis (*Dow et al.* 1968). In cattle, on the other hand, fibrosis is marked and regeneration is less pronounced (*Dow et al.* 1967). These histopathological changes continue in the chronic phase of the disease.

Accelerated albumin turnover was found in animals with high fluke-burdens. This is consonant with similar experiments on the metabolism of immunoglobulin-G (*Nansen* 1970). Two animals of the present study (J. nos. 438/66 and 439/66) were re-examined in these experiments and exhibited a marked hypercatabolism of bovine IgG slow, presumably due to a biliary loss. It should be emphasized that immunoglobulin synthesis, unlike albumin synthesis, was increased so that normal or high serum levels were found despite accelerated catabolism.

REFERENCES

Dargie, J. D.: Pathophysiology of Fasciola hepatica infections in rabbits. Thesis, Glasgow 1969.

Dargie, J. D., P. H. Holmes, J. M. MacLean & W. Mulligan: Pathophysiology of fascioliasis in the rabbit. Studies on albumin turnover. J. comp. Path. 1968, 78, 101—105.

Dow, C., J. G. Ross & J. R. Todd: The pathology of experimental fascioliasis in calves. J. comp. Path. 1967, 77, 377—385.

Dow, C., J. G. Ross & J. R. Todd: The histopathology of Fasciola hepatica infections in sheep. Parasitology 1968, *58*, 129—135.

Holmes, P. H., J. D. Dargie, J. M. MacLean & Mulligan: The anaemia in fascioliasis: Studies with ^{51}Cr-labelled red cells. J. comp. Path. 1968 a, *78*, 415—420.

Holmes, P. H., J. D. Dargie, J. M. MacLean & W. Mulligan: Albumin and globulin turnover in chronic ovine fascioliasis. Vet. Rec. 1968 b, *83*, 227—228.

Jennings, F. W., W. Mulligan & G. M. Urquhart: Radioisotope studies on the anemia produced by infection with Fasciola hepatica. Exp. Parasit. 1956, *5*, 458—468.

Keck, G. & R. Supperer: The changes occurring in the bileducts in chronic bovine fascioliasis. Vet. Med. Rev. 1967, No. 2/3, 322—329.

Mancini, G., A. O. Carbonara & J. F. Heremans: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965, *2*, 235—254.

McFarlane, A. S.: Efficient trace-labelling of proteins with iodine. Nature (Lond.) 1958, *182*, 53.

Nansen, P.: Metabolism of bovine immunoglobulin-G. A clinical and pathophysiological study. Thesis, Copenhagen 1970.

Nansen, P., L. Eriksen, M. G. Simesen & K. Nielsen: Chronic fascioliasis in sheep. II. Metabolism of ^{131}I-labelled albumin and ^{125}I-labelled immunoglobulin-G. Nord. Vet.-Med. 1968, *20*, 651—656.

Nieberle, K. & P. Cohrs: Lehrbuch der speziellen pathologischen Anatomie der Haustiere. (Textbook of special pathological anatomy of domestic animals). G. Fischer Verlag, 4th Ed., Jena 1961.

Nielsen, K.: Gastrointestinal protein loss in cattle. A clinical and pathophysiological study. Thesis. Carl Fr. Mortensen, Copenhagen 1966.

Nosslin, B.: In Applications of Tracer Theory of Protein Turnover Studies. J. nucl. Biol. Med. 1966, *10*, 3—30.

Pearson, I. G.: Use of the chromium radioisotope ^{51}Cr to estimate blood loss through ingestion by Fasciola hepatica. Exp. Parasit. 1963, *13*, 186—193.

Rahko, T.: The pathology of natural Fasciola hepatica infection in cattle. Path. Vet. 1969, *6*, 244—256.

Sewell, M. M. H., J. A. Hammond & D. C. Dinning: Studies on the aetiology of anaemia in chronic fascioliasis in sheep. Brit. vet. J. 1968, *124*, 160—170.

Supperer, R.: Zum Wirt-Parasit-Verhältnis des grossen Leberegels, Fasciola hepatica L. (The host-parasite relationship of Fasciola hepatica). Dtsch. tierärztl. Wschr. 1964, *71*, 668—670.

SUMMARY

The metabolism of I^{131}-albumin was examined in chronically fluke-infected animals in which anaemia and hypoalbuminaemia were prominent features. Animals with high fluke-burdens exhibited increased

turnover rates of albumin, presumably due to a loss of blood into the bile ducts. Despite pronounced hepatic fibrosis the animals had a normal or only slightly decreased synthetic rate of albumin. This was in keeping with results from liver-function tests and is presumably explained by enlargement of the liver, resulting in maintenance of a near-normal parenchymal cell mass.

SAMMENDRAG

Albuminomsætning ved kronisk infektion med Fasciola hepatica hos kvæg.

Omsætningen af J^{131}-albumin er undersøgt hos dyr med kronisk distomatose. Disse dyr havde udtalt anæmi og hypoalbuminæmi. Kraftigt inficerede dyr havde forøgede relative omsætningshastigheder af albumin, hvilket formentlig skyldtes et blodtab til galdegangene. Til trods for en udtalt hepatisk fibrose havde dyrene en normal eller kun let nedsat albumin syntese. Dette var i overensstemmelse med leverfunktionsprøver og kan antagelig forklares ved en forstørrelse af leveren, resulterende i en normal eller kun let formindsket masse af parenchymceller.

(Received June 11, 1970).