Veterinary Research Communications, 21 (1997) 169–185

CLINICAL AND PATHOLOGICAL STUDIES IN CATTLE WITH HEPATIC DISEASE

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ABSTRACT

West, H.J., 1997. Clinical and pathological studies in cattle with hepatic disease. *Veterinary Research Communications*, **21 (3)**, 169–185

In cattle with hepatic lipidosis, hepatic abscessation, leptospirosis, biliary calculi or fasciolosis, the progression of the disease was studied by serial measurements of serum total bile acid concentrations, plasma glutamate dehydrogenase, γ-glutamyltransferase, 5'-nucleotidase and leucine aminopeptidase activities *Terminalia avicennioides*
and by liver biopsy. Regardless of the cause of the hepatic disease, weight loss, anorexia, dullness and depression were consistent features. Signs of hepatic encephalopathy, such as blindness, head pressing, excitability, ataxia and weakness were less common and, together with pyrexia and jaundice, were grave prognostic signs. Plasma ammonia concentrations were significantly elevated compared to clinically normal cattle, but such changes were not always accompanied by a decline in plasma urea concentrations. In normal, healthy cattle, the plasma ammonia:urea concentration ratio is 9:1 and the plasma ammonia:glucose concentration is 11:1. In hepatic disease, a plasma ammonia:glucose ratio >40:1 or plasma ammonia:urea ratio >30:1, particularly with a rising total ketone body concentration and a declining glucose concentration, carried a guarded prognosis. The study suggested that other factors, such as hypokalaemia, alkalosis, short-chain volatile fatty acids, and false and true neurotransmitters, may be important in the pathogenesis of hepatic coma in cattle.

Keywords: ammonia, biopsy, blood, cattle, enzymes, hepatic disease, liver

Abbreviations: GD, glutamate dehydrogenase; γGT, γ-glutamyltransferase; H-E, haematoxylin and eosin; LAP, leucine aminopeptidase; 5'NT, 5'-nucleotidase; SBA, serum bile acid

INTRODUCTION

Cattle are prone to liver disease as the bovine liver is involved in many metabolic disorders as well as infectious and parasitic diseases because of its central role in metabolism.

Bovine acetonaemia is an extreme manifestation of a metabolic state which, in a milder form, is a common, subclinical occurrence in heavily producing post-parturient cows. It may be primary, secondary or subclinical, and is associated with hepatic lipidosis (West, 1990). Secondary ketosis accounts for a third of all cases and can lead to a chronic unresponsive ketosis in early lactation (Higgins and Anderson, 1983) that is difficult to reverse because of fat deposition in the liver, i.e. 'fat cow syndrome' or 'fatty liver disease'.

Cattle are also susceptible to hepatic abscessation, which may be solitary or multiple

as a result of rumenitis (Rubarth, 1960). Hepatic abscess formation is also associated with thrombosis of the caudal *vena cava* (Selman *et al.*, 1974). Solitary hepatic abscesses may occur as a result of traumatic reticulitis, and as multiple abscesses in calves following omphalophlebitis (Rubarth, 1960).

Leptospirosis in cattle, due to *Leptospira hardjo* (Little, 1981), causes interstitial nephritis, haemolytic anaemia, jaundice, septicaemia, abortion and liver damage. Fasciolosis is a problem in both calves and adult cows (Rowlands and Clampitt, 1979) in low-lying wet areas. Other causes of liver damage include ingestion of mycotoxins.

Ammonia is normally absorbed after its production from the lower intestinal tract and from the rumen in ruminants (Wolff *et al.*, 1972) and carried to the liver, where it is converted to urea (Sherlock, 1968). If the hepatic functional mass is reduced, ammonia may not all be converted to urea, and consequently the concentration of urea in the blood may be low and the concentration of ammonia in the blood will rise. This is usually a late occurrence in chronic or terminal liver disease (Sherlock, 1968).

Hepatic encephalopathy is a clinical syndrome characterized by an abnormal mental status that is associated with any severe hepatocellular insufficiency or major circulatory bypass of the liver. Hepatic coma appears to be multifactorial in origin in man (Sherlock, 1968) and large animals (Tennant *et al.*, 1973). High concentrations of ammonia in the blood have been associated with hepatic coma in cattle (Fowler, 1968; Finn and Tennant, 1974), often with a concurrent hypoglycaemia. It is associated with lesions in the central nervous system, such as astrocytosis. In the brain, ammonia is detoxified by astrocytes and eventually converted to glutamine, so glutamine concentrations may increase in the cerebrospinal fluid in hepatic encephalopathy (Fraser and Arieff, 1985).

The present study was undertaken to establish whether a relationship exists between plasma ammonia, urea, glucose and total ketone bodies in cattle with various liver lesions confirmed by enzymology and hepatic biopsy and also to assess 5′-nucleotidase and leucine aminopeptidase activity in plasma as a means of evaluating liver disease in cattle.

MATERIALS AND METHODS

Animals

The cattle, mainly Friesian or Friesian–Holstein, aged 3 weeks to 14 years, were referred to the University of Liverpool, Large Animal Hospital, because of suspected hepatic disease, which was considered a possible diagnosis on the basis of history and clinical examination. Histological examination of the liver was used as the ultimate criterion for group segregation.

The cattle were separated into groups. The 44 cattle in group 1 were suffering from hepatic lipidosis as part of acetonaemia or the 'fat cow syndrome'. Group 2 included adult cattle and calves with hepatic abscessation (18 cattle). Group 3 comprised 7 cattle with leptospirosis. The 2 cows in group 4 had biliary calculi. In group 5 there were 11

cattle with fasciolosis. Group 6 comprised 33 cattle with a history of weight loss, so that liver disease was suspected initially, and which were suffering from respiratory, cardiovascular, infectious or gastrointestinal conditions. Results were compared with those from non-pregnant and non-lactating cows ($n = 43$). The cattle came from different commercial herds and were hospitalized in individual loose boxes; they were fed hay and water *ad libitum* and concentrates were offered twice daily. They were weighed and their condition was scored on admission. Faeces were examined where appropriate for *Salmonella* spp., *Mycobacterium johnei*, *Campylobacter* spp., rotavirus, coronavirus, fluke eggs and malaena. Routine haematology and paracentesis were performed when necessary. In cases of suspected *Leptospirosis*, dark-ground illumination of urine and bacteriological sampling for spirochaetes was used.

Chemical methods

Jugular venous blood samples were collected at the time of clinical diagnosis for measurement of serum bile acids (SBA) using the Enzabile (R) enzymatic method (Nycomed, Sheldon, Birmingham, UK), plasma glucose (by the guaiacum and glucose oxidase method), plasma urea (based on the cleavage of urease), plasma ammonia (by an enzymatic method on samples in EDTA), plasma total ketone bodies and plasma activities of glutamate dehydrogenase (GD, EC 1.4.1.3), γ-glutamyltransferase (γGT, EC 2.3.2.2), 5′-nucleotidase (5′NT, EC 3.1.3.5) and leucine aminopeptidase (LAP, EC 3.4.11.), all by standard methods (West, 1989, 1994, 1996).

Serial changes in these serum and plasma constituents were measured at regular 2- or 3-day intervals after appropriate treatment until clinical recovery or slaughter.

Statistical analysis

To be considered significant, each test value was compared with the mean $\pm$ 2SD of the mean. Serial clinical chemistry measurements were compared with those from normal, healthy, non-pregnant non-lactating cattle by unpaired *t*-tests. Intergroup comparisons for non-parametric data were made using the Mann–Whitney *U*-test (Armitage, 1971).

Liver biopsy

Liver biopsy samples were taken (Loosmore and Allcroft, 1951) through the 11th intercostal space at the time of blood sampling and fixed in 10% formol saline. The sections were stained with haematoxylin and eosin (H-E), periodic acid–Schiff (PAS) (with and without diastase treatment) and oil red O. The sections were examined under light microscopy and changes were recorded. Stereological analysis was used to determine the average percentage of fat in the liver parenchymal cells (Reid and Collins, 1980). Twenty fields were examined at $\times 1100$ in each biopsy from cows with hepatic lipidosis, using a 100-point eyepiece graticule.

RESULTS

Clinical and necropsy findings

The clinical findings are summarized in Table I. The classification into the different types of liver disease was made on the basis of the predominant pathological findings on liver biopsy and/or post-mortem examination (Table II). A total of 35 cattle in groups 1–5 died or were slaughtered.

Regardless of the cause of the hepatic disease, weight loss, anorexia, dullness and depression were consistent features (Table I). Cows with acetonaemia, but not the 'fat cow syndrome', had a high recovery rate, as did those with fasciolosis. Hepatic abscessation and leptospirosis with liver involvement carried a poor prognosis. The

TABLE I
Summary of clinical findings in cattle with liver disease at the time of diagnosis: percentage of cases showing each sign

	Acetonaemia ($n=44$)	Hepatic abscessation ($n=18$)	Leptospirosis ($n=7$)	Biliary calculi ($n=2$)	Fasciolosis ($n=11$)
Reduced appetite	100.0	94.0	100.0	100.0	100.0
Reduced milk yield	100.0	58.0 (rest dry)	100.0	100.0	38.0 (rest dry)
Weight loss	100.0	100.0	100.0	100.0	100.0
Dullness/depression	60.0	58.0	87.0	50.0	92.0
Nervousness	18.0	7.0	17.0	0	10.0
Tachycardia	12.0	22.0	75.0	0	65.0
Pyrexia	12.0	22.0	18.0	0	38.0
Decreased rumen movements	90.9	64.0	18.0	100.0	40.0
Diarrhoea	59.0	30.0	30.0	100.0	48.0
Ketones on breath/ milk/urine	100.0	33.0	42.0	100.0	48.0
Jaundiced mucous membranes	6.8	7.0	18.0	0	0
Good response to therapy	79.5	15.0	28.0	0	84.0
Headpressing	6.0	25.0	10.0	0	0
Salivation	13.6	5.0	0	0	0
Apparent blindness	0	15.0	0	0	0
Excitability	15.9	0	0	0	0
Ataxia	26.0	38.0	35.0	50.0	25.0
Weakness	23.6	60.0	30.0	0	0
Increased respiratory rate	0	18.0	0	0	0
Submandibular oedema	0	6.0	0	0	28.0
Distended jugular veins	0	6.0	0	0	0
Tenesmus	0	6.0	0	0	0

TABLE II
Summary of necropsy findings

Group	Diagnosis	Number necropsied out of total number in the group	Necropsy findings[a]
Group 1			
Hepatic lipidosis	'Fat cow syndrome'	9/11	Friable, enlarged, yellow fatty livers and adrenal gland. Fat deposition around the kidney and extensive fatty infiltration of the liver seen histogically. Fat vacuoles in epithelial cells of kidney tubules, especially corticomedullary junction (7) and medulla. Metritis (6), mastitis (4), endocarditis right atrioventricular valve (1), abomasal adhesions (1)
Group 2			
Hepatic abscessation	Caval thrombosis	3/3 adult cows	Caval thrombosis with multiple hepatic abscesses from which *Corynebacterium pyogenes* was cultured, chronic venous congestion of the liver and embolic spread to lungs, and multiple small abscesses
	Reticular foreign body and hepatic abscesses	3/3 adult cows	Metal up to 50 mm long in reticulum penetrating to the diaphragm with extensive adhesions and chronic peritonitis involving reticulum and left lobe of liver, vagus indigestion (2)
	Multiple hepatic abscesses	4/4 cows	Multiple hepatic abscesses due to *Fusiformis necrophorus* (1) and *C. pyogenes* (3)
	Multiple hepatic abscesses	6/6 calves	Omphalophlebitis, multiple liver abscesses, especially of left liver lobe, due to *F. necrophorus* (1) and *C. pyogenes* (5). Distended left hock (3), left stifle (2), left elbow (1), right hock (2) and left fetlock (1). Joints with erosion of articular cartilage (6) and ankylosis (2)
Group 3			
Leptospirosis		5/7 cows	Very enlarged yellow liver. Leptospira in kidney under dark ground illumination, interstitial nephritis, jaundiced carcase (1), dark red urine in bladder (1)

TABLE II (cont)

Group	Diagnosis	Number necropsied out of total number in the group	Necropsy findings[a]
Group 4			
Biliary calculi		2/2	Shrunken fibrosed liver, gall bladder virtually empty. Several calculi in the bile ducts throughout the liver, some with a hollow centre, of calcium carbonate and phosphate, stained with bile salts. Severe non-specific chronic enteritis of small intestines
Group 5			
Fasciolosis		3/11	Grossly thickened bile ducts in the liver, especially the ventral lobe, and a light burden of mature liver flukes. Some bile duct calcification, extensive fibrosis of hepatic parenchyma

[a]Numbers of animals shown in parenthesis when less than the number necropsied

post-mortem findings served to confirm the clinical diagnosis (Table II). *Status spongiosus* was observed in two cases of 'fat cow syndrome' and one of caval thrombosis.

Dullness and depression were frequently observed, whereas other signs of hepatic encephalopathy, such as blindness, head pressing, excitability, ataxia, and weakness were less common (Table I). These signs had to be differentiated from nervous ketosis, listeriosis and bovine spongiform encephalopathy. Signs of hepatic encephalopathy were most frequently observed in 'fat cow syndrome' and caval thrombosis, but dullness and depression were features of at least 60% of cases, even in those which recovered.

Histopathology

In cows with hepatic lipidosis, H-E sections showed necrosis of single cells and large droplet vacuolation, mainly in the centrilobular and mid-zonal areas. In oil red O-stained sections, small fat droplets could be seen in the periportal cells. In severe cases, the entire lobule was affected. Mitotic figures were more obvious in less severely affected livers. Within the hepatocytes, the fat was usually present as a single large droplet displacing the nucleus to the periphery. In more severely affected livers, fatty cysts were occasionally observed near the central vein, surrounded by lymphocytes and

histiocytes; usually there was only one per lobule. With PAS-stained sections there was a lack of glycogen staining throughout the lobule, which was more severe in the centrilobular and mid-zonal areas. There was no glycogen staining in the most severely affected livers. Stereological analysis of liver samples showed that the percentage of fat in the liver parenchyma varied from 20% to 80% (mean 55.6 μm^3 fat/100 μm^3 liver cell $\pm$2.1 SEM).

Hepatic biopsy was not attempted in calves with suspected hepatic abscesses, in order to avoid puncturing the abscesses. In cows, either samples were obtained with normal liver structure and glycogen staining or, in some, mononuclear cellular infiltration was seen with necrosis of surrounding cells or venous congestion. In 5 of the 7 cows with leptospirosis, centrilobular swollen vacuolated cells were seen. These cows did not recover (Table II). The less severe cases had hepatocellular necrosis and loss of glycogen staining.

In cows with biliary calculi or fasciolosis, some of the liver samples yielded normal liver and glycogen staining. Others showed fibrosis and reduced glycogen staining. In the cows with biliary calculi, hepatic fibrosis and non-specific enteritis were present and in-contact cows had a peripheral eosinophilia of ~25%. The liver biopsies from cows with lesions not affecting the liver (group 6) were normal.

Clinical chemistry

The concentration of total SBA (Figure 1) was elevated in both diffuse and localized liver lesions in cattle and was persistently raised during the recovery phase. Overall values much greater than 100 µmol/L carried a guarded prognosis. The concentration of GD was elevated in the acute phase of liver disease and persistently raised in chronic lesions and had a high hepatic specificity. The activity of γGT was raised, especially in discrete liver lesions (hepatic abscesses), and persistent. In hepatic abscessation the GD and γGT activities were higher in young calves, probably because the lesions were acute, whereas in adults the lesions were found at post-mortem examination to be of a more chronic nature. The lack of response by these enzymes in cases of biliary calculi reflected the chronicity of the lesion (Figures 2 and 3). Comparing γGT, 5′NT and LAP activities overall, γGT and 5′NT had similar specificity, but γGT was more elevated than 5′NT and more persistent during the course of disease in a variety of liver lesions, while the concentration of LAP was not markedly elevated.

In cows with hepatic lipidosis at diagnosis, the plasma glucose concentrations were $<$2.25 µmol/L and total ketone bodies were $>$3000 µmol/L. Glucose concentrations fell significantly in the acute phase of hepatic lipidosis, abscessation and leptospirosis (Figure 4). They returned to normal on recovery only in hepatic lipidosis, while total ketone bodies decreased in parallel to the increase in blood glucose concentrations. Glucose concentrations in blood were most elevated in diffuse hepatic lesions. Total ketone bodies were high in the acute phase of liver disease, returning to normal as the appetite was regained, but there were wide individual fluctuations. In unresponsive cases, the total ketone bodies remained high and fluctuating, often with a terminal hyperglycaemia and a raised concentration of urea in the plasma.

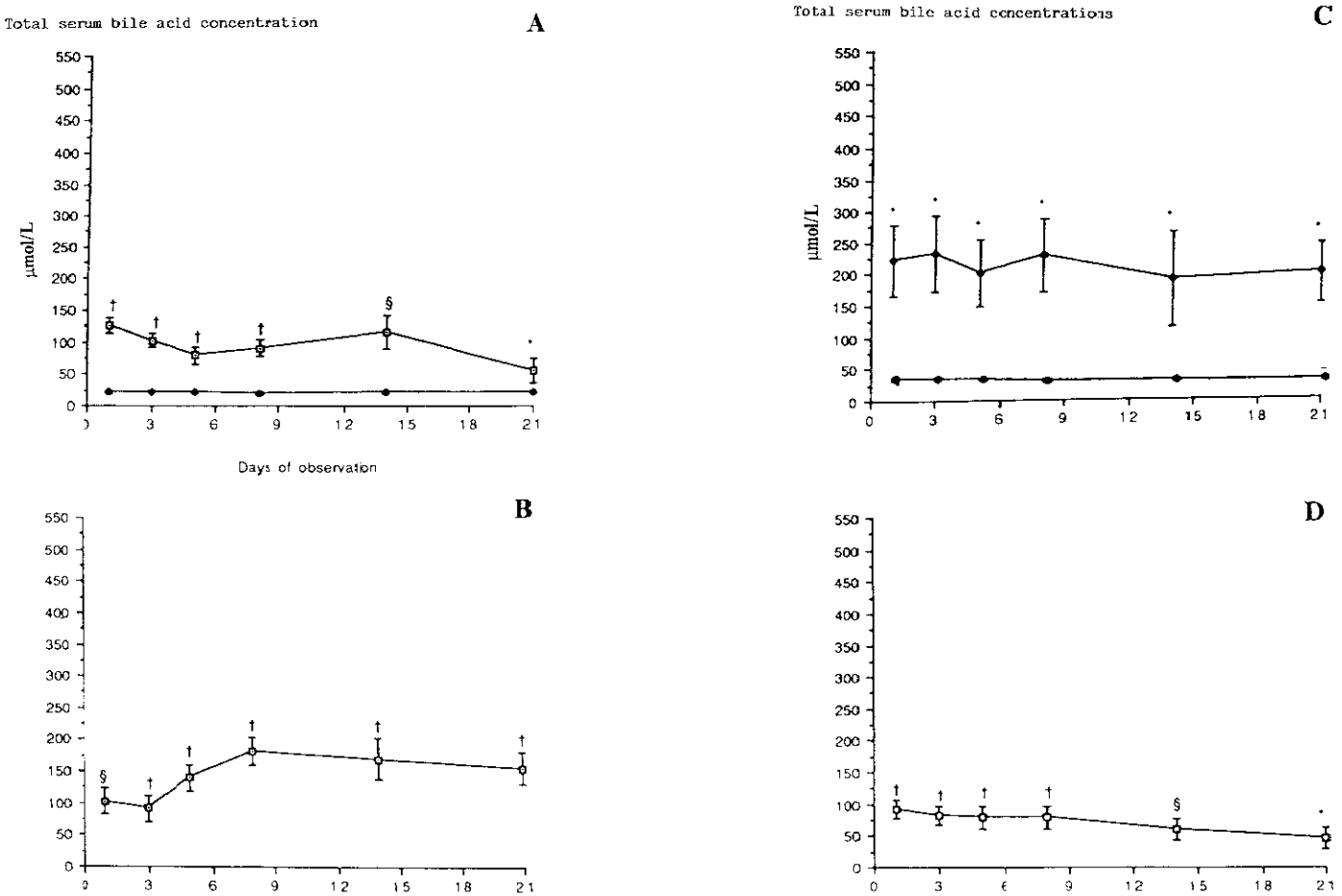

Figure 1. Total serum bile acid concentrations in cattle with different liver diseases during the period of observation. Results are mean ± SEM. (A) Cows with hepatic lipidosis ($n = 44$) are compared to control, non-pregnant, non-lactating cows ($n = 43$). (B) Hepatic abscessation ($n = 18$). (C) Cattle with leptospirosis ($n = 7$) compared with cattle without liver disease ($n = 33$). (D) Cattle with fasciolosis ($n = 11$) *Significant at $p < 0.05$; §significant at $p < 0.01$; ‡significant at $p < 0.001$

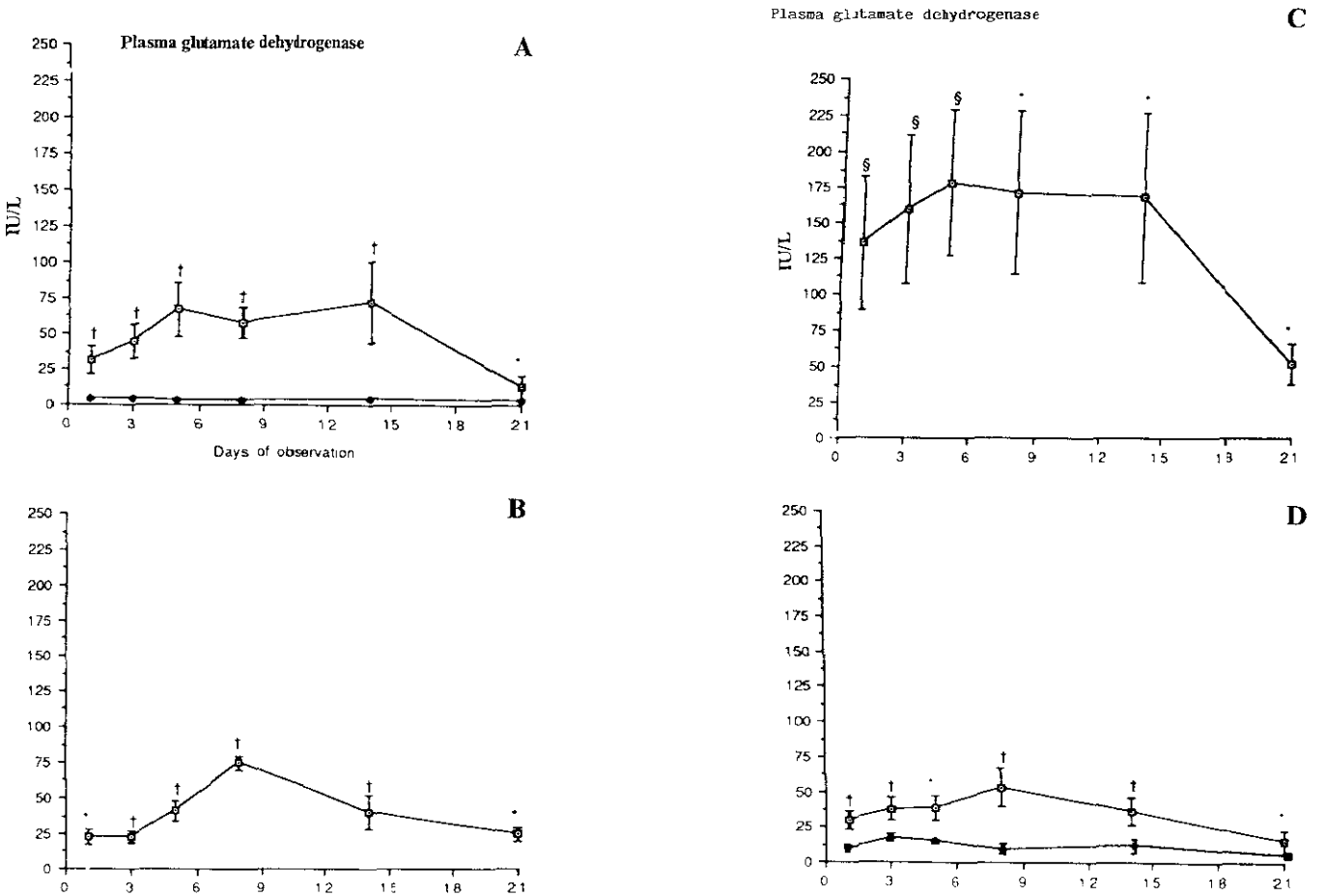

Figure 2. Plasma glutamate dehydrogenase activities in cattle with different liver diseases during the period of observation. Results are mean ± SEM. (A) Cows with hepatic lipidosis ($n = 44$) are compared with non-pregnant, non-lactating cows ($n = 43$). (B) Cows with hepatic abscessation ($n = 18$). (C) Cows with leptospirosis ($n = 7$). (D) Cows with fasciolosis ($n = 11$) compared with cows without liver disease ($n = 33$) *Significant at $p < 0.05$; §significant at $p < 0.01$; ‡significant at $p < 0.001$

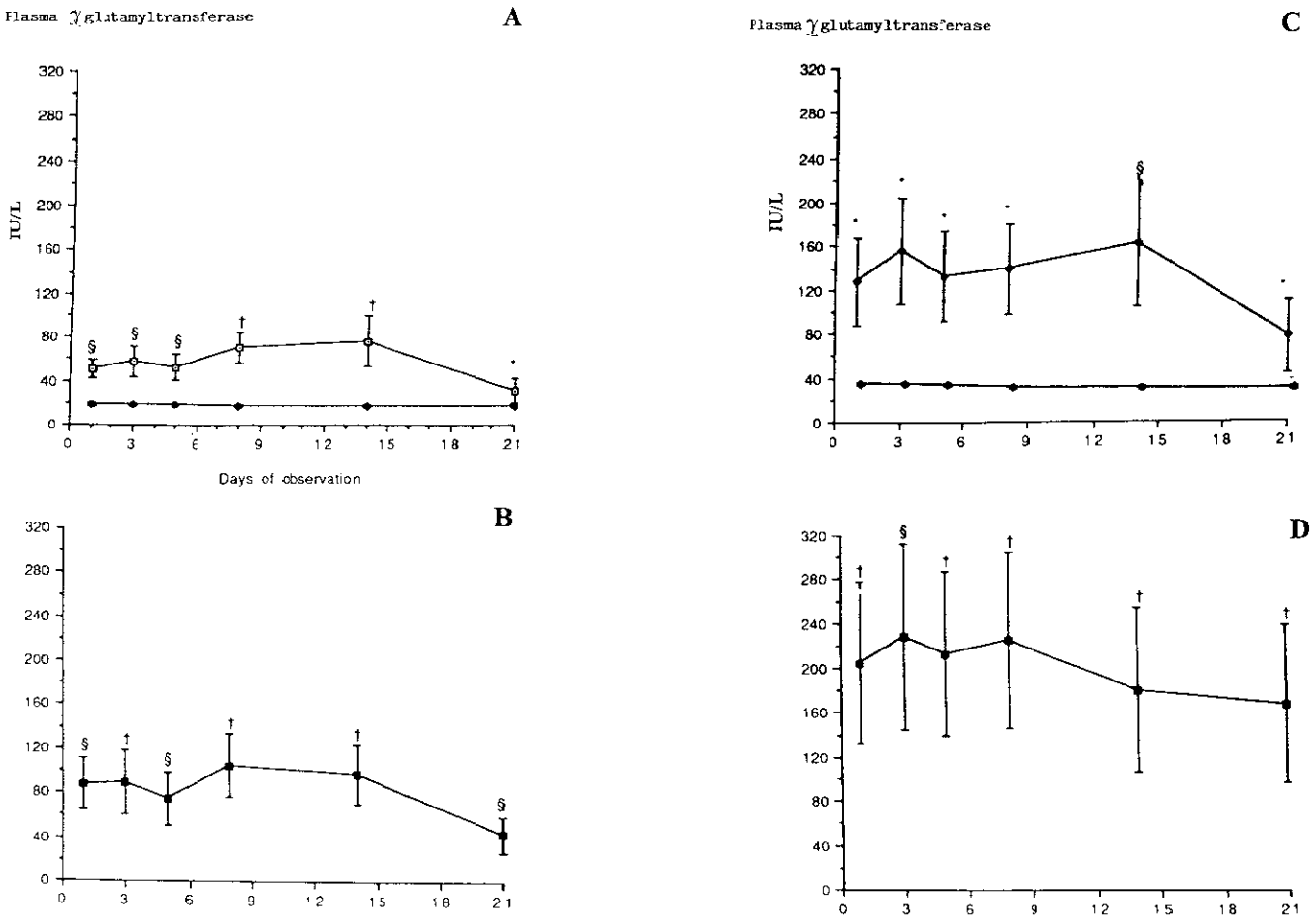

Figure 3. Plasma γ-glutamyltransferase activities in cattle with different liver diseases during the period of observation. Results are mean ± SEM. (A) Cows with hepatic lipidosis ($n = 44$) compared with non-pregnant, non-lactating cows ($n = 43$). (B) Cows with hepatic abscessation ($n = 18$). (C) Cows with leptospirosis ($n = 7$) compared with cattle without liver disease ($n = 33$). (D) Cows with fasciolosis ($n = 11$); *Significant at $p < 0.05$; §significant at $p < 0.01$; ‡significant at $p < 0.001$

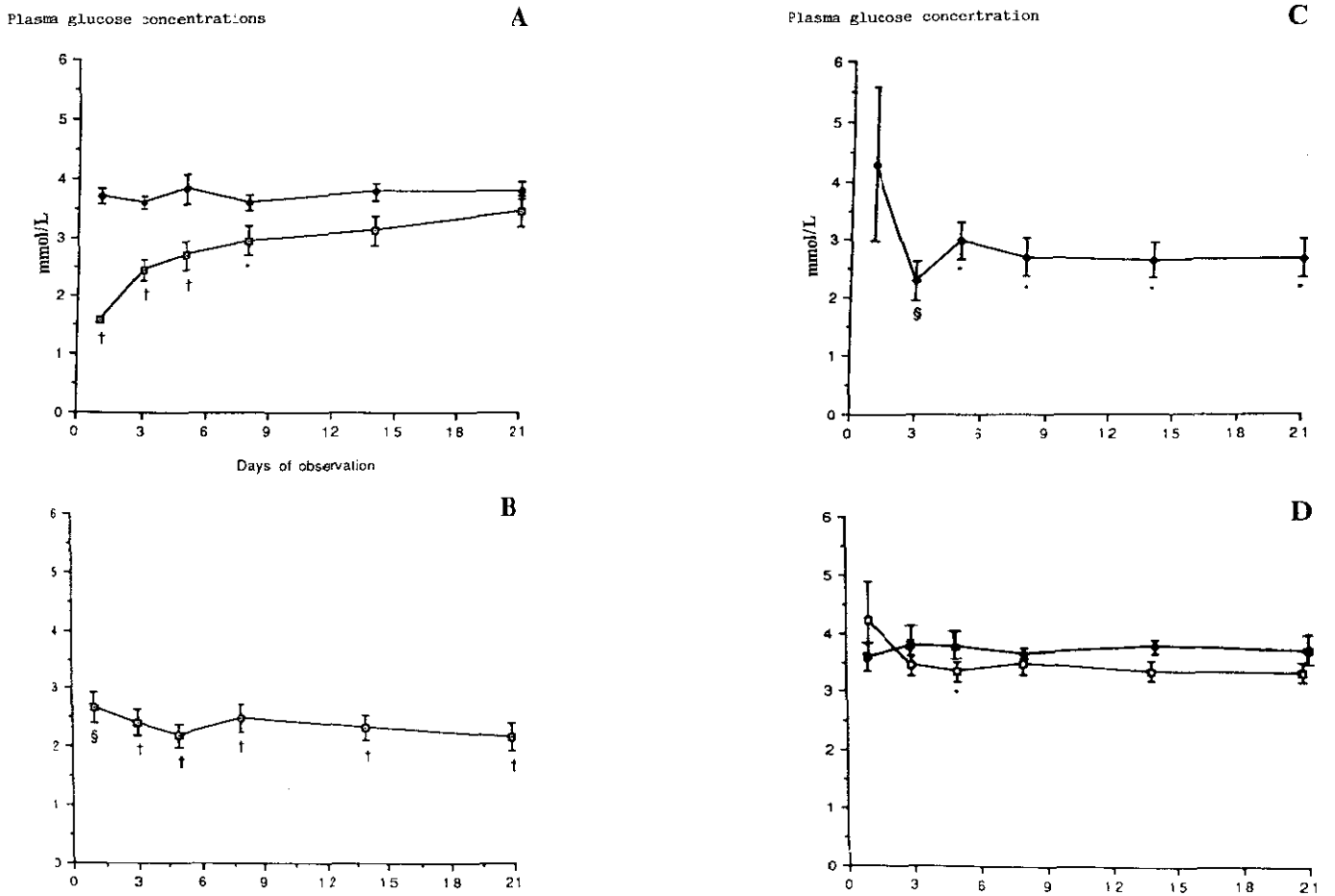

Figure 4. Plasma glucose concentrations in cattle with different liver diseases during the period of observation. Results are mean ± SEM. (A) Cows with hepatic lipidosis ($n = 44$) compared with non-pregnant, non-lactating cows ($n = 43$). (B) Cows with hepatic abscessation ($n = 18$). (C) Cows with leptospirosis ($n = 7$). (D) Cows with fasciolosis ($n = 11$) compared with cattle without liver disease ($n = 33$) *Significant at $p < 0.05$; §significant at $p < 0.01$; ‡significant at $p < 0.001$

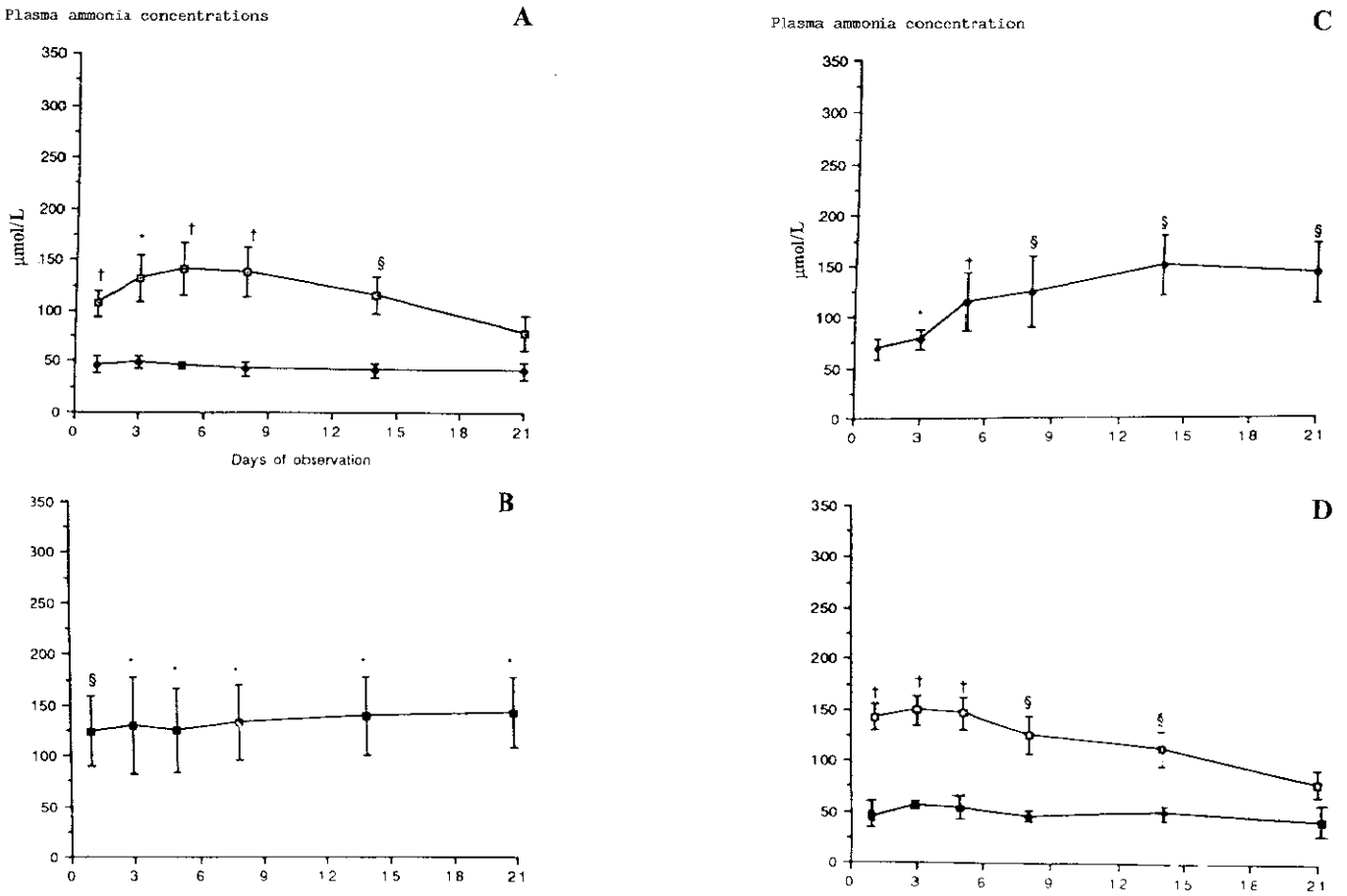

Figure 5. Plasma ammonia concentrations in cattle with different liver diseases during the period of observation. Results are mean ± SEM. (A) Cows with hepatic lipidosis ($n = 44$) compared with non-pregnant, non-lactating cows ($n = 43$). (B) Cows with hepatic abscessation ($n = 18$). (C) Cows with leptospirosis ($n = 7$). (D) Cows with fasciclosis ($n = 11$) compared with cattle without liver disease ($n = 33$) *Significant at $p < 0.05$; §significant at $p < 0.01$; ‡significant at $p < 0.001$

Analysis of the concentration of urea in the plasma was most useful in the early phase of acute hepatic lipidosis, returning to normal on recovery. In all types of lesions in the liver, there were wide individual variations. Plasma ammonia concentrations were elevated during the progression of all types of hepatic lesions (Figure 5) and fell on recovery in hepatic lipidosis and fasciolosis, but were raised in end-stage hepatic disease.

In normal, healthy cattle, the plasma ammonia:urea ratio was 9:1 and plasma ammonia:glucose ratio was 11:1. In hepatic disease, a plasma ammonia:glucose ratio >40:1 or plasma ammonia:urea >30:1, particularly with a rising total ketone body concentration, carried a guarded prognosis.

DISCUSSION

Clinical and necropsy findings

In each case, the diagnosis was made on clinical grounds and laboratory investigations including liver biopsy and/or necropsy were used to confirm the diagnosis and to aid prognosis. Clinical signs of liver disease were generally non-specific, i.e. depression, dullness, anorexia, weight loss and reduced milk yield (Table I). Liver damage was irreversible at the point at which anorexia became complete, which supports the observations of Spence (1978) in the 'fat cow syndrome'. In the present study, jaundice and pyrexia were grave prognostic signs. Further study is needed to elucidate the biochemical mechanisms responsible for the condition becoming irreversible. Treatments were deliberately standardized to facilitate comparison in the different types of disease conditions. It was not, therefore, possible to evaluate different treatments, except to say that the treatment was considered to be appropriate in each case.

The clinical findings, including the presence of intercurrent illness and response to therapy, agreed with those published for primary acetonaemia, secondary acetonaemia due to a displaced abomasum (Wallace, 1975) and 'fat cow syndrome' (Higgins and Anderson, 1983).

The clinical and post-mortem findings in cows with hepatic abscessation secondary to caval thrombosis (Table II) concurred with those of Selman and colleagues (1974). They were often associated with previous rumenitis caused by *Actinomyces (Corynebacterium) pyogenes*, *Streptococci* (Rubarth, 1960) and *Fusiformis necrophorus* (Jensen *et al.*, 1954). The non-specific signs, such as pyrexia, anorexia, depression, decreased milk production, weakness and abdominal pain resulting from the toxaemia seen in the early stages of acute multiple hepatic abscessation in cows, have been described previously (Jensen *et al.*, 1954; Rubarth, 1960). The anorexia (8 animals), emaciation and diarrhoea (5 cows) seen has also been reported previously (Rubarth, 1960). Solitary or multiple abscesses may not be associated with clinical signs (Rubarth, 1960) and, coupled with the duration of experimentally induced liver abscesses (Jensen *et al.*, 1954), may explain the recovery of 2 cows following antibiotic therapy. The clinical and necropsy findings in cows with traumatic reticulitis and calves with omphalophlebitis are typical of hepatic involvement (Rubarth, 1960).

The course of the disease and the good response to dihydrostreptomycin in two cows in group 3 was similar to that described in *Leptospira hardjo* infection by Little (1981). The clinical and biopsy features in the 5 animals that died were suggestive of *L. hardjo* infection, which causes an interstitial nephritis (Sullivan, 1974) and hepatic necrosis (Table II).

The presence of multiple calculi in the biliary ducts throughout the liver, but not in the gall bladder, in the animals with cholelithiasis (group 4) was unusual. In view of the herd's history of fasciolosis, the eosinophilia in in-contact cows, the hepatic fibrosis and non-specific enteritis, it seems likely that these calculi represent an end stage of *Fasciola hepatica* infection (West and Hogg, 1988). The clinical and post-mortem changes in the overt cases of fasciolosis agreed with the observations of Simesen and Nansen (1974).

Liver biopsy

The observed fatty infiltration of the liver with glycogen deletion was expected in both primary acetonaemia and 'fat cow syndrome' (Reid and Collins, 1980), in which up to 70% of the total hepatocyte volume may be fat. The fact that fatty infiltration of the liver was so extensive within such a short time of calving is further evidence that the process commences well before calving (West, 1989, 1990). The degree of fatty infiltration of the liver provided a valuable guide to prognosis.

Chronic venous congestion is often observed in caval thrombosis (Selman *et al.*, 1974), as it was in the present study, and as are cellular infiltration of the portal tracts and hepatic necrosis in hepatic abscessation. Some cattle with hepatic abscessation appeared to have a normal liver on biopsy because of chance sampling between the lesions. A small liver biopsy specimen may not detect calcification, but some degree of fibrosis and loss of glycogen was seen in fasciolosis (group 5) and in cases with biliary calculi (group 4). In group 6, the liver biopsies were normal, that is there were no false positive results. Overall, hepatic biopsy was most helpful in diffuse lesions.

Clinical chemistry

Measurement of the SBA concentrations improved the diagnostic efficacy of routine hepatic tests in the detection of hepatobiliary disease. The range of SBA values for the various disease groups was wide, with considerable overlap between the groups. Concurrent evaluation of combinations of test results improved the overall diagnostic performance of estimations of SBAs. The individual interpretation of bile acid values was useful in detecting impaired hepatic function, particularly in diffuse lesions (e.g. lipidosis, leptospirosis) but they were less valuable in the differential diagnosis of hepatobiliary disease. Serial measurements of SBA concentrations provide a good guide to the prognosis of different liver lesions in cattle and their high stability on storage at –20°C is an analytical advantage.

The SBA concentrations were expected to be low owing to the effective hepatic

clearance of bile acids. The lack of a diurnal effect is an advantage and was expected as the cows were fed *ad libitum* (West, 1991), although controversy exists as to whether there is diurnal variation in the concentrations of total SBAs in cattle (Abdelkader and Ropstad, 1989).

The assessment of SBAs, when used in conjunction with other tests of hepatic disease, was useful in establishing a definitive diagnosis owing to certain patterns that develop in specific disorders. An increase in total SBA concentration is likely to be due to hepatic necrosis and cholestasis (West, 1991). The concentrations remained high in terminal hepatic disease and were often high in the recovery phase, when most plasma enzymes had returned to normal. SBA values over 45 μmol/L warrant morphological diagnosis of liver disease by biopsy (Figure 1).

The study indicated that GD was persistent in chronic liver injury and that γGT, 5′NT and LAP may be elevated in intra- and extrahepatic cholestasis in cattle. Certainly, experimental evidence exists that γGT is released in biliary tract damage (Simesen and Nansen, 1974; Craig *et al.*, 1978). 5′NT leaks into the plasma in cholestasis in ruminants (Rowlands and Clampitt, 1979) and LAP is high in the plasma in cholestasis in man (Rutenberg *et al.*, 1958). The pattern of enzyme release in cattle may be altered in chronic advanced liver lesions. In this study, γGT and 5′NT had similar specificity but γGT was more persistent in chronic liver damage. LAP was insufficiently sensitive to be of value, which may be because the enzyme is released into tissues and only slowly leaks into plasma, or because low activities exist in the liver. Measurement of 5′NT conferred little advantage over that of γGT, particularly considering the widespread distribution of 5′NT (Ford and Adam, 1981).

The clinical chemistry results obtained in the present study depended on the chronicity of the lesion and a disadvantage inherent in the protocol was that the cattle were presented at different stages of clinical illness, so the results can only show trends. For example, the activities of GD and γGT are sensitive indicators of acute liver cell damage in experimental fasciolosis during the migration phase but often fall after the flukes enter the bile ducts (Rowlands and Clampitt, 1979) at 8–10 weeks post-infection. In some cases, particularly of hepatic abscessation, variation between individuals was high, probably because lesions were at different stages of development.

The changes in clinical chemistry were greater in calves compared to cows with hepatic abscessation (group 2), probably because the abscesses were at a more acute stage in calves, as determined from the history and post-mortem examination.

Glucose, total ketone bodies and urea are an indication of the liver's synthetic function (Wolff *et al.*, 1972).

High total ketone body concentrations were a grave prognostic sign, confirming the observations of Spence (1978). High concentrations of ammonia in the plasma were related to hypoglycaemia, which was expected (Finn and Tennant, 1974).

There was a positive correlation between the early signs of hepatic encephalopathy, hyperammonaemia and liver failure. Overall, plasma ammonia concentration was a sensitive, specific indicator of hepatic disease in cattle, although the concomitant low plasma urea concentration anticipated (Sherlock, 1968; Wolff *et al.*, 1972) because of the liver's reduced synthetic ability was often not apparent. This supports the view that other factors may be important in the pathogenesis of hepatic coma; γ-aminobutyric

acid or false neurotransmitters (James *et al.*, 1979; Jones *et al.*, 1984; Fraser and Arieff, 1985) have been postulated in man. Hypoglycaemia was a reliable indicator of the degree of hepatic damage in cattle. High total ketone body concentrations were a grave prognostic sign in cattle, but their role in the pathogenesis of hepatic coma has yet to be established (Takahashi *et al.*, 1966). The course of disease in animals with signs of hepatic coma was relatively short and may explain the absence of the anticipated lesions in the central nervous system (Markson and Terlecki, 1968; Finn and Tennant, 1974).

Status spongiosus of the brain stem has been described in pyrrolizidine alkaloid poisoning of cattle (Markson and Terlecki, 1968; Finn and Tennant, 1974) and sheep (Hooper, 1972), in copper toxicity in sheep (Howell *et al.*, 1974) and in hepatic coma in man (Mossakowski, 1965). Experimental studies on infusion of intravenous ammonium acetate in sheep confirmed a relationship between hyperammonaemia and cerebrospinal degeneration and vacuolation (Hooper, 1972). *Status spongiosus* was not frequently observed in this study, as it is a terminal change and the course of the disease in animals with signs of hepatic coma was relatively short.

ACKNOWLEDGEMENTS

The author thanks Mr G. Hynes for technical assistance with some of the clinical chemistry measurements, the veterinary surgeons in practice who referred the cattle for this study, the Department of Veterinary Pathology for cutting the histological sections, and Miss J.A. Appleton for typing the manuscript.

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(Accepted: 9 July 1996