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GAMMA-GLUTAMYL TRANSPEPTIDASE ACTIVITY IN SHEEP SERUM: NORMAL VALUES AND AN EVALUATION OF ITS POTENTIAL FOR DETECTING LIVER INVOLVEMENT IN EXPERIMENTAL LUPINOSIS

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ABSTRACT

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A brief survey of the literature on gamma-glutamyl transpeptidase (γ–GT) activity is included in this study. The levels of activity in the serum of normal Merino sheep (13,6–32,4 mI.U./ml) were ascertained as a preliminary to following the activity through the entire course of experimentally induced ovine lupinosis, a hepatotoxicosis caused by *Phomopsis leptostromiformis* (Kühn) Bubák. The response of the serum level of γ–GT activity to the course of the disease was compared with that of glutamate oxaloacetic transaminase (GOT) and 2 liver function tests for the purpose of assessing its potential application in the study of this mycotoxicosis. Because the levels of activity of γ–GT were more valuable for the early diagnosis of low grade acute intoxication and the detection of chronic liver involvement while those of GOT gave better information on the development of severe acute hepato-cellular damage, these 2 enzymes, considered together, were found to give the best information on the course of the toxicosis. The changes in γ–GT activity during various stages of intoxication were also related to the histopathological lesions in the liver.

Résumé

L'ACTIVITÉ DE LA TRANSPEPTIDASE GAMMA-GLUTAMYLIQUE DANS LE SÉRUM DU MOUTON: TENEURS NORMALES ET UNE ESTIMATION DE SA VALEUR DÉTECTRICE DU RISQUE HÉPATIQUE EN LUPINOSE EXPÉRIMENTALE

La bibliographie au sujet de l'activité de la transpeptidase gamma-glutamylique (γ–GT) est brièvement passée en revue dans cette étude. Les niveaux de son activité dans le sérum de moutons de race Merinos (13,6–32,4 mI.U/ml) ont été déterminés avant de suivre son activité au cours d'une lupinose ovine expérimentale, se traduisant comme une hépatotoxicose due au Phomopsis leptostromiformis *(Kühn) Bubák. L'activité de la γ–GT au cours de la maladie a été comparée avec le taux de la transaminase-glutamate-oxal-acétique (GOT) ainsi qu'avec deux tests destinés à déterminer le fonctionnement hépatique, pour apprécier leur emploi dans l'étude de cette mycotoxicose. Parce que l'activité de la γ–GT facilitait un diagnostic précoce d'une intoxication aiguë mais légère et la mise en évidence d'une hépatose chronique, tandis que celle de la GOT permettait une meilleure connaissance d'une hépatose aiquë et grave, on estime que ces 2 enzymes considérées ensemble fournissent la meilleure appréciation du développement de la toxicose. Les variations dans l'activité de la γ–GT ont pu être mises en rapport avec les lésions histo-pathologiques du foie.*

LITERATURE SURVEY

The enzyme gamma-glutamyl transpeptidase (γ-GT) catalyses the transfer of γ-glutamyl groups from a γ-glutamyl peptide to other (acceptor) peptides or L-amino acids. Hanes, Hird & Isherwood (1950; 1952) characterized it and studied its properties. In 1965 the International Union of Biochemistry recommended the systematic name of γ-glutamyl transferase (E.C. 2.3.2.1.) for it but the use of the original trivial name has persisted.

Distribution in man and animals

The enzyme has a localized distribution in the body and in individual cells. Early work with tissue homogenates was carried out by a Polish group (Szewczuk & Orlowski, 1960; Szczeklik, Orlowski & Szewczuk, 1961; Orlowski, 1963) who found that the highest activity occurred in the kidneys and much less in the pancreas, liver, spleen and small intestine. If the human kidney can be regarded as having 100% activity, the other organs, respectively, would have 8,3; 3,9; 1,5 and 0,95%. Red blood cells were found to have no activity at all, nor did they find any in heart and skeletal muscle, lung and brain. A similar distribution also applied in the case of farm animals.

The precise localization within the cells was studied by histochemical methods by several workers. Albert, Orlowski & Szewczuk (1961) found very high activity in the brush border of cells lining the proximal convuluted tubules of human kidneys. In other animals there were variations in siting within the nephron, but all showed a high degree of renal γ-GT activity. In the liver the enzyme was found in the peripheral cells of the lobules, and, in the pancreas, in the outer secretory cells but not in the Islets of Langerhans. Ewen & Griffiths (1971) found that γ-GT occurred in the luminal borders of epithelial tissues such as the renal tubular cells, pancreatic ductules, jejunal epithelium and biliary tract epithelium. These results were broadly confirmed by Naftalin, Child & Morley (1969). Szewczuk (1966), Schmidt (1972), and Kuntz (1972), stated that the enzyme occurs in the tissues mainly in the form of a membrane-bound constituent of the microsomal fraction but that there is also a 'soluble' form in the cell cytoplasm.

Origin of plasma/serum γ-GT

In spite of the evidence of the comparatively high degree of γ-GT activity in the kidneys, there is virtually complete unanimity that the enzyme as found in the plasma is of hepatic and not renal origin, and that the elevation of its activity is due to liver and sometimes to pancreatic pathology (Orlowski, 1963). The γ-GT formed in the kidneys and small intestine is excreted in the urine and the faeces. Thiele (1973), in a study of human renal disease, concluded that there is no correlation between urine and plasma levels as had been stated by Orlowski (1963) since high plasma levels were not reflected in any changes in urinary γ-GT activity. In severe

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nephrotoxicity, such as is produced in dogs by the administration of ochratoxin, there was no rise in the serum levels (Szczech, Carlton & Tuite, 1973). Ford (1974) produced experimental kidney damage (with high blood urea) by giving mercuric chloride and there was similarly no change in the level of γ-GT activity in the serum.

Serum levels of γ-GT activity

The presence of γ-GT activity in normal human serum was reported by Orlowski & Szewczuk in 1961. Szczeklik *et al.* (1961) first reported the elevation of γ-GT activity in human serum as a result of hepatic disease. In 1963 Orlowski collated all the information obtained up to that time in a monograph. In the same year Orlowski and Meister reported that γ-glutamyl-ρ-nitroanalide was a good substrate as it was stable and simple to use. (It is still in fact the substrate of choice.) Before the use of this nitroanalide substrate the normal values given by various authors varied with the different methods employed. Szasz (1969) gave ranges of 4,5–24,8 mI.U./ml for males and 3,2–13,5 for females; Kuntz (1972), 6–28 for males and 4–18 for females. In 1972 Lum & Gambino in the United States, finding a male level of 13,6±6,9 (range 2–29) and a female level of 10,9±6,4 (range 2–26), did not consider the difference as statistically significant. The values found by Betro, Oon & Edwards (1973a) in Australia were fairly similar, viz. 13,5±6,3 for males and 9,2±4,2 for females. They considered 24 the male maximum and 18 the female.

There is no unanimity as to sex differences in normal serum activity in man. Of 17 available reports (including those before 1963) 10 contain the information that males have higher γ-GT activity than females, while 7 did not find any significant differences. In view of the sensitivity of γ-GT level to alcohol consumption (Rosalki & Rau, 1972, and Rollason, Pincherle & Robinson, 1972) one is tempted to think that the explanation for any sex differences there are may be attributed to this fact. Boone, Routh & Schrantz (1974) found the upper limit for males to be 38 mI.U./ml and for females 28 mI.U./ml.

Since then there has been sustained clinical interest in the use of this procedure and a number of reports have appeared from countries all over the world. These have been concerned almost entirely with human medicine and the conclusions reached are briefly as follows: An elevation of γ-GT activity in the serum is the most sensitive and specific indicator of liver damage of all the tests used up to the present (Lukasik & Richterich, 1965; Nosslin, Aronsen & Hanson, 1966; Lukasik, Richterich & Colombo, 1968; Szasz, 1969; Szasz, Rosenthal & Fritsche, 1969; Swinnen, 1970; Kammeraat, 1970; Rosalki & Rau, 1972; Schmidt, 1972). Conditions involving an element of intra- or extrahepatic cholestasis give the highest values, while necrosis of liver cells, such as in intoxication with carbon tetrachloride or acute viral hepatitis, produces less elevation of γ-GT than of an enzyme such as glutamate pyruvic transaminase (Szczeklik *et al.*, 1961; Aronsen, Hanson & Nosslin, 1965; Nosslin *et al.*, 1966; Swinnen, 1970; Zein & Discombe, 1970; Lum & Gambino, 1972). γ-GT like alkaline phosphatase may be used for the detection of liver disease but as it is more specific, it is more useful for the differential diagnosis of clinical jaundice. Moreover it is unaffected by pregnancy, bone disease and immaturity of the patient (Lukasik & Richterich, 1965; Szasz *et al.*, 1969; Rosalki & Rau, 1972). γ-GT is constantly elevated in all chronic liver conditions and may persist for many weeks in cirrhosis (Orlowski, 1963; Rutenburg, Goldbarg & Pineda, 1963; Villa, Dioguardi, Agostini, Ideo & Stabalini, 1966; Cohen & McNamara, 1969; Ideo & Dioguardi, 1970). It has also been used for detecting metastatic invasion of the liver by malignant tumours (Szczeklik *et al.*, 1961; Rutenburg *et al.*, 1963; Lukasik & Richterich, 1965; Aronsen, Nosslin & Phil, 1967; Zein & Discombe, 1970; Schmidt, 1972). In general it has been found that, except in early acute hepatitis, γ-GT rises higher and stays elevated longer than other enzyme activities (Lukasik & Richterich, 1965; Villa *et al.*, 1966; Lukasik *et al.*, 1968). Acute and chronic pancreatic disease also affects serum levels, but they are as a rule fairly easily differentiated by clinical observation and by other tests (Rutenburg *et al.*, 1963; Orlowski, 1963; Lum & Gambino, 1972). Increased levels have been recorded after myocardial infarction (5–8 days later), myocardial ischaemia and congestive heart failure. Some authors, including Agostoni, Ideo & Stabalini (1965), Hedworth-Whitty, Whitefield & Richardson (1967) and Ewen & Griffiths (1971), believe that such increases may occur without liver involvement, but others (Naftalin *et al.*, 1969; Rosalki & Thompson, 1971; Jacobs, 1972; Coodley, 1972; Betro, Oon & Edwards, 1973b; Cook & Carter, 1973) are of the opinion that circulatory disturbances must involve the liver and that this organ cannot be ruled out as the source of increased enzyme activity. The latter view is probably correct as histochemical examination has shown that the myocard is virtually devoid of γ-GT activity. Ewen & Griffiths (1973) reported an elevation in neurological conditions, but Lum & Gambino (1972) are of the opinion that drugs used for therapy could have hepatotoxic properties or that drugs, such as diphenyl-hydantoin or phenobarbitone, could give rise to induction of microsomal enzymes. Pathological conditions of skeletal muscles also do not give rise to elevation of γ-GT (Rosalki & Thompson, 1971). The concept, therefore, that increased γ-GT activity is almost invariably the result of hepatic or pancreatic disease can thus be said to have universal acceptance.

The enzyme in serum has further advantages as a criterion for diagnosis. It is stable at room temperature for at least 2 days, at 4 °C for at least a week (Zein & Discombe, 1970) and at —20 °C it is stable virtually indefinitely according to many of the workers already mentioned who have studied its stability under various conditions. Joel & Curry (1974) found that even at a room temperature of 25 °C the enzyme was unimpaired for up to 72 h. Szasz (1969) kept serum samples with varying γ-GT activities at 20 °C for as long as a week without significant change. The absence of γ-GT from red cells is an additional advantage, as haemolysis often invalidates other enzyme activity determinations. After the introduction of γ-glutamyl-p-nitroanilide as the substrate (Orlowski & Meister, 1963), and of a kinetic photometric procedure (Szasz, 1969) the firm Boehringer Mannheim made available test kits, which basically embody the above improvements. The test is quickly carried out with reproducible results with the use of fairly simple laboratory equipment.

Very few studies of γ-GT activity in animals have been made. For cattle Findeisen (1972) gave 15,5±3 mI.U./ml and Unglaub (1974) 10,8±5,3 mI.U./ml. For horses, Unglaub, Afschar & Marx (1973) gave 13±7 mI.U./ml and for sheep, Ford (1974) gave

21,4±1,5 mI.U./ml for young Clun Forest wethers. He also presented figures (in units per g wet basis) for homogenized tissues from 4 sheep. These averaged *c.* 27 000 mI.U. for kidneys, 2 480 mI.U. for liver, 882 mI.U. for small intestine, 110 mI.U. for myocard, and 0 for skeletal muscles.

The present study was undertaken to establish the normal range of levels of γ-GT activity in the serum of clinically normal Merino sheep, and also to obtain an indication of the value of the test as a research tool in assessing the development and course of liver pathology in sheep poisoned with cultures of *Phomopsis leptostromiformis*.

Methods

To obtain normal values of γ-GT activity in the serum, 86 clinically normal Merino sheep of mixed sexes and ages were bled from the jugular vein.

Cultures of *P. leptostromiformis*, grown on maize seeds in the dark at 27 °C for 4 weeks (Van Rensburg, Marasas & Kellerman, 1975) were air-dried, milled and dosed as a watery suspension per stomach tube to 14 Merino sheep. Each of these sheep received a single lethal or sublethal dose as indicated in Table 1.

The sheep were fed on standard Onderstepoort rations and examined daily. Periodically the following standard chemical pathological determinations were done on the blood: serum urea nitrogen, serum glutamate oxaloacetic transaminase, bilirubin, bromosulphalein retention, γ-glutamyl transpeptidase, total plasma protein, glucose and serum calcium, sodium and potassium.

Some sheep were allowed to die naturally and the others were destroyed by intravenous injection of pentobarbitone sodium. At necropsy, specimens were taken from the livers, fixed in 10% buffered formalin, sectioned and stained with haematoxylin and eosin (HE), Van Gieson's stain (VG) and oil red O (ORO).

The activity of γ-GT was assayed by a slight modification of the kinetic method (Szasz, 1969) using γ-glutamyl-p-nitroanilide as substrate and spectrophotometrically measuring the rise of extinction during increasing concentration of *p*-nitroaniline as it is released from the substrate. The glutamyl residue was transferred to the acceptor glycyl glycine. The procedure was carried out at a temperature of 25 °C and a wavelength of 405 nm on a Beckman B spectrophotometer, using cuvettes with a 10 mm light path. Reagents were supplied by Boehringer Mannheim in γ-GT Monotest kits. The results were expressed as mI.U./ml.

Serum glutamate oxaloacetic (aspartic) transaminase (GOT) activity was measured by the method of King (1958) and the results given in King units. Bromosulphalein (BSP) retention was measured colorimetrically as a percentage remaining in the plasma 10 min after an intravenous injection of 5 mg/kg (Cornelius, Holm & Jasper, 1958).

The concentration of conjugated and unconjugated bilirubin (TBr) was estimated by a modification of the classical method (Malloy & Evelyn, 1937) and expressed in mg/100 ml.

Results

Normal values for γ-GT in sheep serum

When a distribution curve was drawn of the 86 values of γ-GT activity in sheep serum, the values were grouped symmetrically around the mean in a gaussian or 'normal' distribution. In such a distribution 95% of the readings could be expected to fall in the range of the mean ± 2 standard deviations (SD). Since the mean was 23±4,7, this range is 13,6–32,4 mI.U./ml.

The values were also analysed in another way, i.e. by constructing a percentage ogive (or cumulative relative frequency polygon). The 50 percentile reading gave a mean of 22,5 and the 5–95 percentile a range of 14–31,5 mI.U./ml. These results are in close agreement with the more usually employed method above.

γ-GT activity in the serum of sheep suffering from experimentally induced lupinosis

Experiment 1

The chemical pathological changes in the blood of 3 sublethally intoxicated sheep were studied for 72 days (Table 1: Sheep 1–3).

TABLE 1 Dosage rates and survival times of sheep in Experiments 1–3

Sheep No.	Dose* (g/kg)	Survival period (days)	Manner of death E=Euthanised D=Died
1.....	2	72	
2.....	2	72	
3.....	4	72	
4.....	3	5	D
5.....	3	5	D
6.....	3	5	D
7.....	8	4	D
8.....	8	3	D
9.....	3	10	D
10.....	3	1	E
11.....	3	2	E
12.....	3	5	E
13.....	3	5	E
14.....	3	16	E

* Dose of *P. leptostromiformis* culture material

The clinical signs, chemical pathological changes, and pathological findings were typical of lupinosis as described by Gardiner (1967) and Van Warmelo, Marasas, Adelaar, Kellerman, Van Rensburg & Minne (1970). In the present study, the most conspicuous chemical pathological changes were: increased activities of serum γ-GT and GOT, retention of BSP and elevation of the TBr levels in the blood (Fig. 1, 2 & 3). GOT activity started to rise within a day or two of administering the toxic culture and it remained elevated for *c.* 35–45 days. Maximal activity, i.e. an increase equivalent to *c.* 100%, was recorded about 7 days after the animals were dosed. The activity of γ-GT started to rise almost immediately after the sheep were dosed and it remained elevated for 60, or more, days. Maximal activity was recorded *c.* 18 days after the culture material was administered. This peak represented an increase of 300–800% over the initial levels of activity.

Two peaks of activity were recorded in Sheep 1 and Sheep 3. The first, a small one occurring 2–3 days after dosing, was followed by a 2nd higher peak that reached maximal elevation *c.* 18 days after the animals were dosed. BSP was retained above normal limits, and the level of TBr in the blood was elevated between approximately the 2nd and 20th days. These two curves came to a peak when the GOT activity was about maximal and γ-GT was on the downward slope of the first peak.

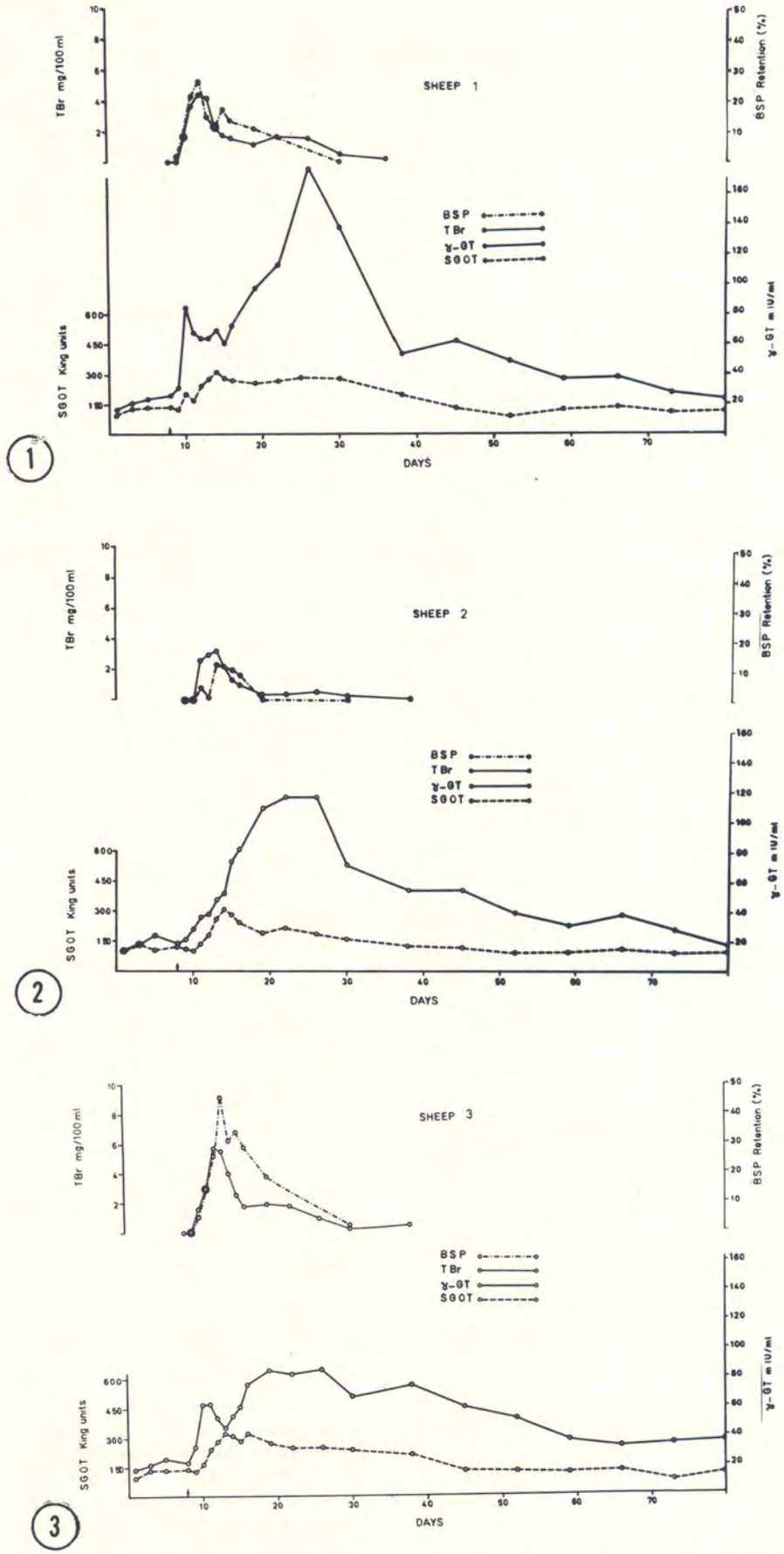

FIG. 1–3 Chemical pathological changes in the blood of sheep exposed to a single sublethal dose* of *Phomopsis leptostromiformis* culture material

* ↑ time of dosing

The activity of γ-GT in the blood increased sooner, reached proportionally higher levels and remained elevated longer, than that of GOT. From the limited data of this experiment it would therefore appear that γ-GT is a more sensitive test for liver damage of this type. It is also interesting to note that γ-GT activity was still markedly elevated when the liver function tests and GOT activity had returned to normal. As this property could be useful in the diagnosis of chronic liver disorders it was further investigated in Experiment 3.

Experiment 2

The chemical pathological changes in the blood of 6 fatally poisoned sheep were studied (Table 1: Sheep 4–9). All the sheep succumbed after developing typical signs of lupinosis. Five animals (Sheep 4–8) died within 6 days of being dosed, i.e. during the initial or smaller peak of γ-GT activity (Fig. 4–8). In most of these cases, while the γ-GT activity was diminishing after the first peak, GOT activity was at a plateau or still ascending, and BSP and TBr levels were near to maximal elevation. The most long-lived case, Sheep 9, died after 10 days, i.e. at a stage roughly corresponding to the end of the first peak of γ-GT activity. GOT activity seemed to have fallen off and so also the level of TBr in the blood, but BSP values were nearly maximally elevated and γ-GT activity was on the decline after the first peak. This diminution of γ-GT activity before death could easily be misinterpreted as recovery.

Experiment 3

Five sublethally intoxicated sheep (Sheep 10–14) were destroyed as described below (Table 1).

Sheep 10 was destroyed as soon as the first meaningful increase of γ-GT activity in the blood was recorded, i.e. while the other tests (GOT, BSP and TBr) were still normal (Fig. 10). No clinical signs of intoxication were apparent, and at autopsy the only macroscopically observable lesion was a mild hepatosis manifested as a slight swelling of individual lobules. Histopathological examination of HE stained liver sections revealed a decrease in basophilic, and an increase in eosinophilic staining properties of the hepatocytes. The eosinophilia occurred diffusely but was particularly prominent in the centrilobular regions. Most hepatocytes had undergone severe hydropic degeneration (Fig. 15), and, in a small proportion of them, the periphery of the cytoplasm was occupied by granules resembling hyaline droplets. Furthermore, the hepatocytes were swollen, a mild disseminate neutrophile infiltration was present, the portal tracts were oedematous, and the lymph vessels were well filled. The sections stained negatively for lipids with ORO stain and there was a normal reaction with VG stain for collagen.

The first meaningful elevation of γ-GT activity was in this case associated with eosinophilia and hydropic degeneration of the hepatocytes. It is interesting to note that such slight hepatic damage could be detected and that this occurred before notable changes in the other tests were observed. From this limited experiment it would appear, therefore, as if γ-GT activity could be a very sensitive indicator of mild subclinical hepatic disorder in sheep.

Sheep 11 was autopsied at a slightly later stage of intoxication, i.e. when the activity of γ-GT was estimated to be near the apex of the first peak and as the other tests were just beginning to register liver disorder (Fig. 11). The sheep was destroyed when the first clinical signs of acute intoxication, viz. inappetence and reduction in ruminal movements appeared. Although the level of TBr in the blood was 1 mg/100 ml, icterus was not clinically apparent. The liver was pale, individual lobules were swollen, lobulation was distinct and the consistency of the liver parenchyma was reduced. Mild stasis of the caecum was present. With few exceptions the histopathological lesions in the liver of Sheep 11 closely resembled those of Sheep 10. The chief difference was that the hyaline droplets in the hepatocytes of Sheep 11 were more abundant, more prominent, and more clearly defined (Fig. 16). Less conspicuous differences were the appearance of a few small randomly distributed areas of necrosis, evidence of autophagocytosis in some hepatocytes, mild infiltration of portal tracts with round cells, and slight proliferation of bile ducts.

At the height of the first peak of γ-GT activity the most important hepatic lesions were moderate hyaline droplet degeneration and early focal necrosis of hepatocytes.

Sheep 12 was destroyed when it was estimated that γ-GT was diminishing after the first peak and the activity of GOT was near maximal level. The animal displayed typical signs of lupinosis, viz. apathy, weakness, anorexia, constipation, ruminal atony and icterus. At autopsy the principal lesions were icterus, hepatosis, and stasis of the large intestine. The liver was discoloured a yellow-brown, and was swollen and friable. Histopathological examination revealed severe diffuse fatty degeneration of the hepatocytes and fairly conspicuous more or less periportally arranged areas of midzonal necrosis (Fig. 17). In the necrotic areas the fatty changes were less pronounced, the cytoplasm of the hepatocytes stained eosinophilically, hyaline droplets were present, yellow pigment granules were seen in some cells, and a small number of nuclei were karyorrhectic. One or more cells in each of these necrotic areas contained mitotic figures. Large oval vesicular nuclei surrounded by a basophilic matrix (evidence of early fibroblastic proliferation) were present in several of the necrotic areas (Fig. 18). All the hepatocytes except those in the necrotic areas stained positively for fat with ORO stain, and there was a mild positive reaction to VG stain in those necrotic areas where the oval vesicular nuclei were present.

GOT activity reached a height when the hepatocytes had undergone severe fatty changes and some necrosis had taken place. At this stage γ-GT activity was already declining and the first signs of organization were seen.

Sheep 13 was autopsied at the estimated end of the 1st and the beginning of the 2nd peaks, i.e. in the saddle between the two peaks. (Fig. 13). The sheep was destroyed *in extremis* after developing typical signs of acute lupinosis. Grossly, the pathological changes resembled those of Sheep 12, the most conspicuous histopathological liver lesion being severe diffuse fatty degeneration of the hepatocytes. This change was particularly obvious in the centrilobular regions and, to a lesser extent, in the periportal areas. Some hepatocytes throughout the lobules also contained small numbers of hyaline droplets and pigment granules. Small focal areas of coagulative necrosis consisting of one or a few hypatocytes were scattered randomly in the lobules.

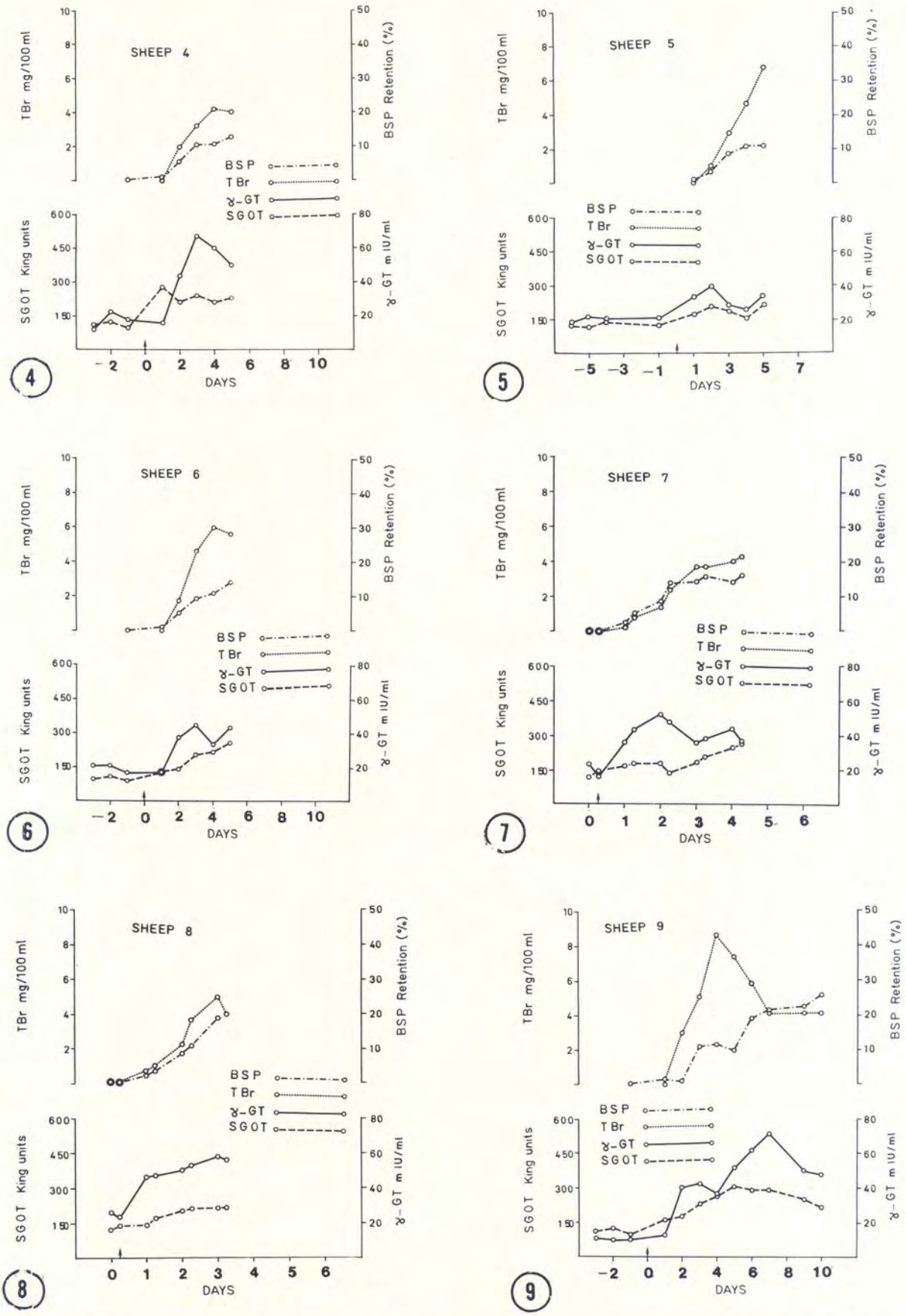

FIG. 4–9 Chemical pathological changes in the blood of sheep poisoned with a lethal dose* of *Phomopsis leptostromiformis* culture material

* ↑ time of dosing

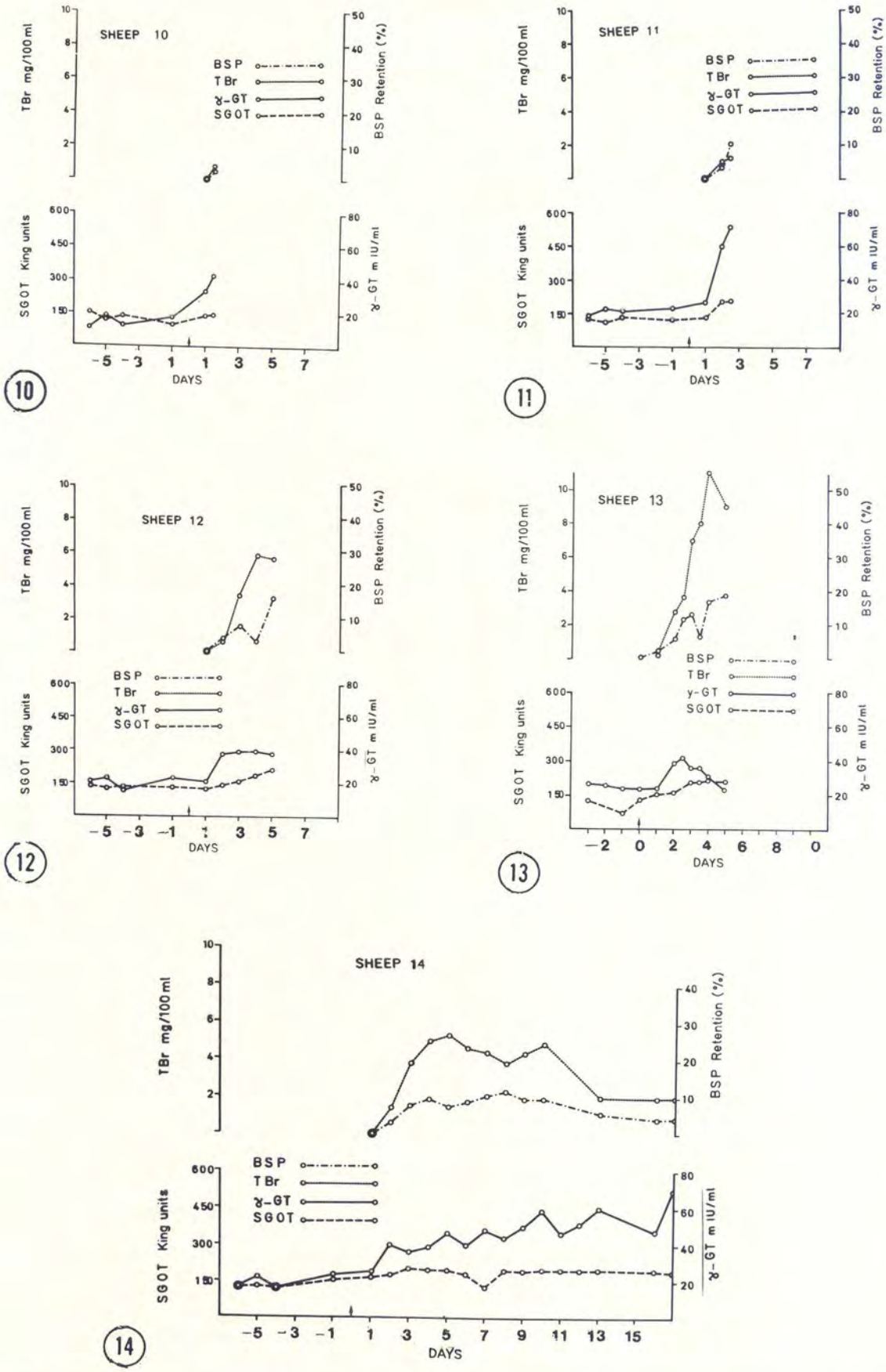

FIG. 10–14 Chemical pathological changes in the blood of sheep that were destroyed at predetermined times after receiving a single dose* of *Phomopsis leptostromiformis* culture material

* ↑ time of dosing

These areas contained karyorrhectic nuclei and were lightly infiltrated with neutrophiles. Cells with vesicular oval nuclei and little cytoplasm were seen in a few of the necrotic areas, but, although these cells were tentatively identified as fibroblasts, no colagen was demonstrable with VG stain. Mild bile duct proliferation, slight bile stasis and a light round cell infiltration completed the histopathological picture.

Although Sheep 13 was more acutely intoxicated than Sheep 12, the main histopathological features of the two cases were similar. In both the liver damage had apparently reached maximal proportions and the very first signs of recovery were visible.

Sheep 14 was autopsied at the estimated height of the second peak of γ-GT activity. At this stage TBr (2 mg/100 ml) was mildly elevated, some BSP (10%) was retained and GOT (187 KU) activity was slightly above the normal limits (Fig. 14). Apart from mild icterus and emaciation, there was little evidence of intoxication; the sheep was eating well and had gained 1 kg live mass in a week. At the post mortem examination the most conspicuous macroscopic lesions, viz. atrophy and mild fibrosis of the liver, mild icterus and brown pigmentation of the kidneys, were confirmed by the histopathological examination. The liver was conspicuously fibrotic, especially in the portal areas, and, in some of these areas, the connective tissue was being actively displaced by regenerating hepatocytes. A small number of these new cells showed evidence of megalocytosis, sometimes with intranuclear pseudo inclusions, and a few were polynucleates (Fig. 19 and 20). Focal areas of fatty degeneration were evident, especially around the central veins. These areas sometimes stretched into the midzonal region, giving the effect of a paracentral distribution. The lobules were mildly but diffusely infiltrated with neutrophiles and, around the portal tracts, a mild round cell reaction was observed.

The point of maximal γ-GT activity was reached when fibroplasia was marked and regeneration was taking place. At this stage the results of the other tests were returning to normal.

Discussion

For practical purposes, the normal values for γ-GT activity in a group of 86 South African Merino sheep of mixed ages and sexes, were found to fall in a range of 14–32 mI.U./ml with a mean of about 23. This was comparable with a smaller and more homogeneous group of British (Clun Forest) sheep studied by Ford (1974), who found a mean value of 21,4 and SD of 1,5 mI.U./ml. With 2 SDs about the mean, the range would be about 18,4–24,4. These enzyme levels in the serum of man and of farm animals other than sheep are given in the literature survey and show some variations, though not large ones, when compared with those of sheep.

A pattern in the changes of γ-GT activity was discernible in the serum of sheep throughout the course of lupinosis induced in them experimentally by lethal or sublethal doses of *P. leptostromiformis* cultures. These changes consisted of an early rise to a primary peak followed by partial remission, and then a steady rise to a secondary peak of greater magnitude than the first. After that there was a long slow decline of this enzyme's activity over a number of weeks. It remained above the normal range long after the other parameters and the clinical condition of the sheep had returned to normal. The double peak was not invariably present but it was surmised that this could be the result of a failure to estimate it at the right time for detection, so flattening that part of the graph. GOT activity usually rose about 1 day after that of γ-GT and continued to rise steadily over several days to reach its highest point at approximately 1 week. This point coincided roughly with the 'saddle' between the two γ-GT peaks. After that, it declined to normal much more rapidly than did γ-GT. The liver function tests in this series, represented by BSP retention and TBr concentration, rose more or less concomitantly with the rise in GOT but subsided more rapidly. In the lethally intoxicated animals there appeared to be a tendency for them to die in the 'saddle' period between the 2 γ-GT peaks and when the other parameters indicated maximum liver dysfunction.

At present we can only speculate on the significance of the rise and fall of γ-GT activity found in this investigation. The initial, very prompt rise to the first peak could be due to microsomal induction at a time of minimal morphological changes in the hepatocytes. This mechanism has been mentioned in connection with the therapeutic use of anticonvulsive drugs like phenobarbitone and diphenylhydantoin (Lum & Gambino, 1972) and after consumption of alcohol (Rosalki & Rau, 1972 and Rollason *et al.*, 1972). This aspect should be investigated further.

The decline of activity from the first peak to the 'saddle' during the time of greatest damage to liver cells and the low point of liver function could be ascribed to exhaustion of γ-GT production. Rutenburg *et al.* (1963) found that cirrhotic patients in hepatic coma generally had lower γ-GT elevations than those not in coma. The lower γ-GT elevation in severe viral hepatitis or advanced cirrhosis were thought to result from decreased hepatic synthesis of the enzyme due to extensive liver cell necrosis. In a later study of this aspect in chronic liver disease, Villa *et al.* (1966) were of the opinion that the fall of serum γ-GT was related "to the exhaustion of the intracellular viable structures involving the synthesis of proteins". These authors also found lower γ-GT activity in advanced atrophic cirrhosis than in mild cases with enlarged livers.

The subsequent rise to the secondary peak could result from the fibroblastic activity and perhaps an element of transient obstruction both of which have been well documented as reasons for elevated serum activity of γ-GT. Alternatively, the increase in γ-GT activity could be related to hepatocellular regeneration.

The following pattern emerged after correlating the γ-GT findings at different stages of the intoxication with histopathological picture: The first discernible rise which related to eosinophilia and some hydropic degeneration of the hepatocytes, occurred before meaningful changes in the other laboratory tests were registered. At the first peak of activity, hyaline droplet degeneration and early necrosis were the most prominent hepatocellular lesions. The decline of activity into the 'saddle' coincided with fatty degeneration and more severe necrosis. The progressive increase of these lesions was accompanied by a steady rise in GOT activity and deteriorating liver function as evidenced by rising BSP retention and TBr concentration.

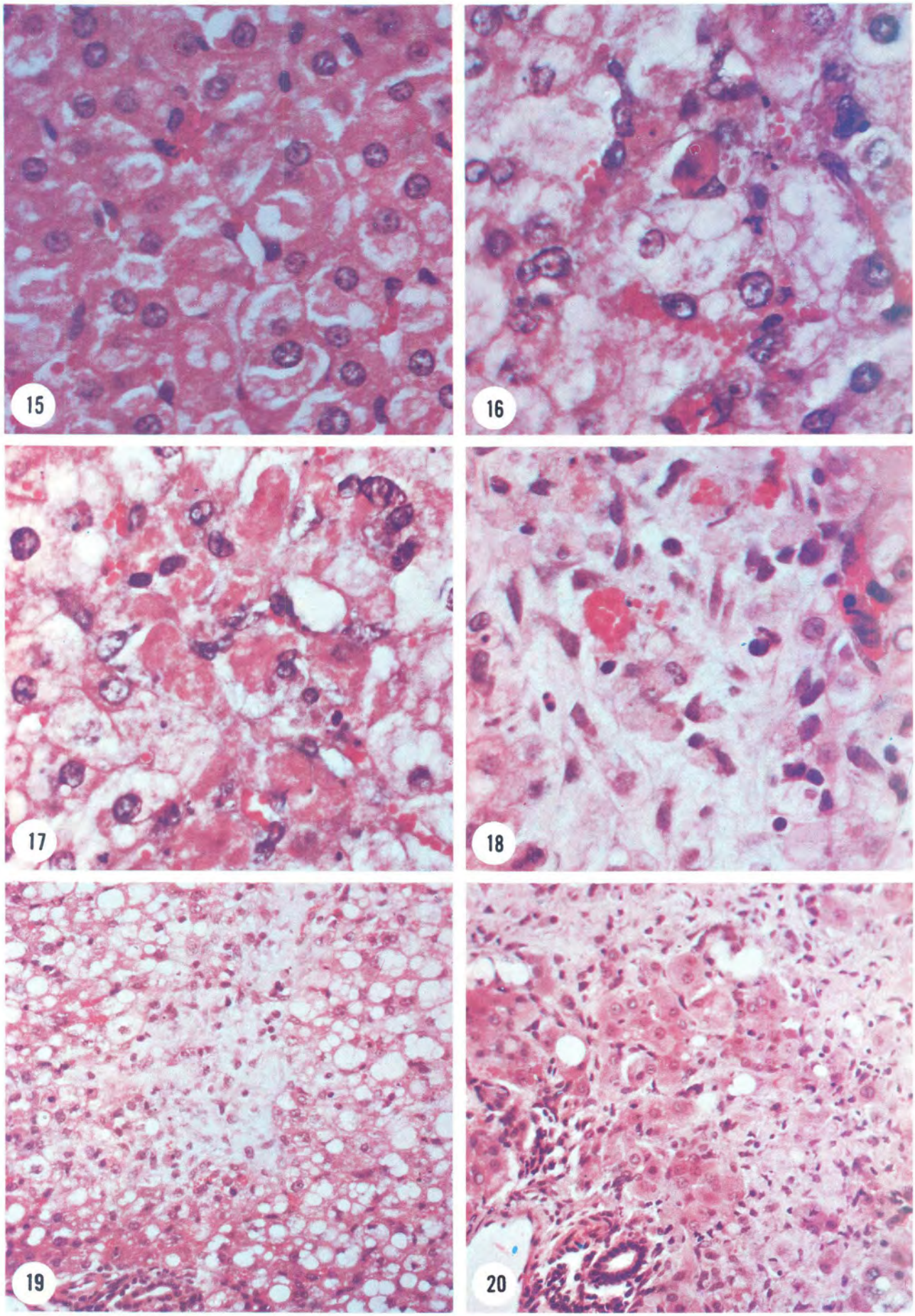

FIG. 15 Swollen hepatocytes showing diffuse eosinophilia and hydropic degeneration (HE×600)
FIG. 16 Hepatocytes showing hyaline droplet degeneration, fatty changes, hydropic degeneration and necrosis of individual cells (HE×600)
FIG. 17 Focal necrosis of hepatocytes surrounded by cells showing fatty changes (HE×600)
FIG. 18 Focal area of necrosis bordered by proliferating fibroblasts (HE×600)
FIG. 19 Area of fibrosis bordered by hepatocytes showing severe fatty changes (HE×200)
FIG. 20 Fibrous tissue being replaced by regenerating hepatocytes (HE×200)

A comparison of GOT and γ-GT activities in lupinosis appears to lead to a conclusion that these enzyme tests should be used in conjunction with each other since GOT would give the best information on the course of development of severe acute hepatocellular damage, while γ-GT would more promptly and sensitively detect the lower grades of early acute intoxication. For the diagnosis of chronic lupinosis, especially when sheep are in the recovery stage, γ-GT activity is the test of choice.

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References

AGOSTONI, A., IDEO, G. & STABILINI, R., 1965. Serum γ-glutamyl transpeptidase activity in myocardial infarction. *British Heart Journal*, 27, 688–690.

ALBERT, Z., ORLOWSKI, MARIAN & SZEWCZUK, A., 1961. Histochemical determination of gamma-glutamyltranspeptidase. *Nature, London*, 191, 767–768.

ARONSEN, K. F., HANSON, A. & NOSSLIN, B., 1965. The value of γ-glutamyl transpeptidase in differentiating virus hepatitis from obstructive jaundice. *Acta Chirurgica Scandinavica*, 130, 92–99.

ARONSEN, K. F., NOSSLIN, B. & PIHL, B., 1967. The value of glutamyl transpeptidase in detection of liver metastases in patients with abdominal cancer. *Scandinavian Journal of Clinical & Laboratory Investigation*, 19, Suppl. 100, 107.

BETRO, M. G., OON, R. C. S. & EDWARDS, J. B., 1973a. Gamma-glutamyl transpeptidase in disease of the liver and bone. *American Journal of Clinical Pathology*, 60, 673–678.

BETRO, M. G., OON, R. C. S. & EDWARDS, J. B., 1973b. Gamma-glutamyl transpeptidase and other liver function tests in myocardial infarction and heart failure. *American Journal of Clinical Pathology*, 60, 679–683.

BOONE, D. J., ROUTH, J. I. & SCHRANTZ, R., 1974. γ-glutamyl transpeptidase and 5′-nucleotidase. Comparison as diagnostics for hepatic disease. *American Journal of Clinical Pathology*, 61, 321–327.

COHEN, M. I. & McNAMARA, HELEN, 1969. The diagnostic value of gamma-glutamyl transpeptidase in children and adults with liver disease. *Journal of Pediatrics*, 75, 838–842.

COODLEY, E. L., 1972. Evaluation of γ-glutamyl transpeptidase in myocardial infarction. *Journal of the American Medical Association*, 20, 217–219.

COOK, VIRGINIA P. & CARTER, N. K., 1973. Nonutility of measurement of serum gamma-glutamyl transpeptidase activities in diagnosis of myocardial disease. *Clinical Chemistry*, 19, 774–776.

CORNELIUS, G. E., HOLM, L. W. & JASPER, D. E., 1958. Bromsulphalein clearance in normal sheep and in pregnancy toxemia. *Cornell Veterinarian*, 48, 305–312.

EWEN, LILIAN & GRIFFITHS, J., 1971. Patterns of enzyme activity following myocardial infarction and ischemia. *American Journal of Clinical Pathology*, 56, 614–622.

EWEN, LILIAN & GRIFFITHS, J., 1973. γ-glutamyl transpeptidase: elevated activities in certain neurologic diseases. *American Journal of Clinical Pathology*, 59, 2–9.

FINDEISEN, RENATE, 1972. Untersuchungen über die Aktivität der γ-Glutamyl-Transpeptidase im Serum gesunder und kranker Rinder. Inaugural-Dissertation: Doctor Medicinae Veterinariae Hochschule Hannover.

FORD, E. J. H., 1974. Activity of gamma-glutamyl transpeptidase and other enzymes in the serum of sheep with liver or kidney damage. *Journal of Comparative Pathology*, 84, 231–243.

GARDINER, M. R., 1967. Lupinosis. *Advances in Veterinary Science*, 11, 85–138.

HANES, C. S., HIRD, F. J. R. & ISHERWOOD, F. A., 1950. Synthesis of peptides in enzymic reactions involving glutathione. *Nature, London*, 166, 288–292.

HANES, C. S., HIRD, F. J. R. & ISHERWOOD, F. A., 1952. Enzymic transpeptidation reactions involving γ-glutamyl peptides and α-amino-acyl peptides. *Biochemical Journal*, 51, 25–35.

HEDWORTH-WHITTY, R. B., WHITFIELD, J. B. & RICHARDSON, R. W., 1967. Serum γ-glutamyl transpeptidase activity in myocardial ischaemia. *British Heart Journal*, 29, 432–438.

IDEO, G. & DIOGUARDI, N., 1970. Gamma-glutamyl transpeptidase as a diagnostic aid. *Lancet*, 2 (1970), 1036–1037.

INTERNATIONAL UNION OF BIOCHEMISTRY, 1965. Enzyme nomenclature. Amsterdam, New York and Barking: Elsevier Publication Co.

JACOBS, W. L. W., 1972. γ-glutamyl transpeptidase in disease of the liver, cardiovascular system and diabetes mellitus. *Clinica Chimica Acta*, 38, 419–434.

JOËL, R. & CURRY, SUSAN, N., 1974. Stability of gamma-glutamyl transferase in serum. *Clinical Chemistry*, 20, 914–915.

KAMMERAAT, C., 1970. Serum gamma-glutamyl transpeptidase: een gevoelige indikator van hepatobiliaire aandoeningen. *Nederlands Tijdschrift voor Geneeskunde*, 114, 1814–1816.

KING, E. J., 1958. Routine methods for the estimation of transaminase. *Journal of Medical Laboratory Technology*, 15, 17–22.

KUNTZ, E., 1972. Aktuelle laborchemische Leberfunktionsdiagnostik. Teil II. *Münchener Medizinische Wochenschrift*, 114, 830–835.

LUKASIK, S. & RICHTERICH, R, 1965. Comparison of the diagnostic value of serum alkaline phosphatase and γ-glutamyl transpeptidase in biliary obstruction. *Archivum Immunologiae et Therapiae Experimentalis*, 13, 580–585.

LUKASIK, S., RICHTERICH, R. & COLOMBO, J. P., 1968. Der diagnostische Wert der alkalischen Phosphatase der Leucinaminopeptidase und der γ-Glutamyl-Transpeptidase bei Erkrankungen der Gallenwege. *Schweizerische Medizinische Wochenschrift*, 98, 81–83.

LUM, G. & GAMBINO, S. R., 1972. Serum gamma-glutamyl transpeptidase activity as an indicator of disease of the liver, pancreas or bone. *Clinical Chemistry*, 18, 358–362.

MALLOY, H. T. & EVELYN, K. A., 1937. The determination of bilirubin with the photo electric colorimeter. *Journal of Biological Chemistry*, 119, 481–490.

NAFTALIN, L., CHILD, VALMA J. & MORLEY, J., 1969. Observations on the site of origin of serum γ-glutamyl transpeptidase. *Clinica Chimica Acta*, 26, 297–300.

NOSSLIN, B., ARONSEN, K. F. & HANSON, A., 1966. The value of glutamyl transpeptidase in liver diagnosis. *Scandinavian Journal of Clinical & Laboratory Investigation*, 18, Suppl. 92, 178–180.

ORLOWSKI, MARIAN, 1963. The role of γ-glutamyl transpeptidase in the internal diseases clinic. *Archivum Immunologiae et Therapiae Experimentalis*, 11, 1–61.

ORLOWSKI, MARIAN & MEISTER, A., 1963. γ-glutamyl-p-nitroanilide or new convenient substrate for determination and study of L- and D-glutamyl transpeptidase activities. *Biochimica et Biophysica Acta*, 73, 679–681.

ORLOWSKI, MARIAN & SZEWCZUK, A., 1961. A note on the occurrence of γ-glutamyl transpeptidase in the serum. *Clinica Chimica Acta*, 6, 430–432.

ROLLASON, J. G., PINCHERLE, G. & ROBINSON, D., 1972. Serum gamma-glutamyl transpeptidase in relation to alcohol consumption. *Clinica Chimica Acta*, 39, 75–80.

ROSALKI, S. B. & RAU, D., 1972. Serum γ-glutamyl transpeptidase activity in alcoholism. *Clinica Chimica Acta*, 39, 41–47.

ROSALKI, S. B. & THOMSON, W. H. S., 1971. Serum gamma-glutamyl transpeptidase in muscle disease. *Clinica Chimica Acta*, 33, 264.

RUTENBURG, A. M., GOLDBARG, J. A. & PINEDA, E. P., 1963. Serum γ-glutamyl transpeptidase activity in hepatobiliary or pancreatic disease. *Gastroenterology*, 45, 43–48.

SCHMIDT, ELLEN, 1972. γ-glutamyl transpeptidase. *Deutsche Medizinische Wochenschrift*, 97, 584.

SWINNEN, J., 1970. New colorimetric micromethod for the estimation of γ-glutamyl transpeptidase activity in biological samples. *Zeitschrift fur Klinische Chemie und Klinische Biochemie*, 8, 557–563.

SZASZ, G., 1969. A kinetic photometric method for serum γ-glutamyl transpeptidase. *Clinical Chemistry*, 45, 124–136.

SZASZ, G., ROSENTHAL, P. & FRITSCHE, W., 1969. Die γ-Glutamyl-Transpeptidase Aktivität im Serum bei hepatobiliären Erkrankungen. *Deutsche Medizinische Wochenschrift*, 94, 1911–1917.

SZCZECH, G. M., CARLTON, W. W. & TUITE, J., 1973. Ochratoxicosis in Beagle dogs 1. Clinical and clinicopathological findings. *Veterinary Pathology*, 10, 135–154.

SZCZEKLIK, E., ORLOWSKI, MARIAN & SZEWCZUK, A., 1961. Serum γ-glutamyl transpeptidase activity in liver disease. *Gastroenterology*, 41, 353–369.

SZEWCZUK, A., 1966. A soluble form of γ-glutamyl transpeptidase in man. *Clinica Chimica Acta*, 14, 608–614.

SZEWCZUK, A. & ORLOWSKI, MARIAN, 1960. The use of α-(N-γ-DL-glutamyl)-aminonitriles for the colorimetric determination of a specific peptidase in blood serum. *Clinica Chimica Acta*, 5, 680–685.

THIELE, K. G., 1973. γ-Glutamyltranspeptidase-Aktivität im Urin bei Gesunden und Nierenkranken. *Klinische Wochenschrift*, 51, 339–345.

UNGLAUB, W., 1974. Vorläufige Mitteilung zu klin. chem. Blutserumwerten (γ-GT, anorganisches Phosphat, GOT und Gesamtbilirubin) von trächtig gewordenen und von güst gebliebenen Stuten. *Tierärtzliche Umschau*, 29, 192–194.

UNGLAUB, W., AFSCHAR, A. & MARX, D., 1973. Die Aktivität der γ-GT (gamma-Glutamyltranspeptidase) in Serum des Rindes. *Deutsche Tierärtzliche Wochenschrift*, 80, 131–134.

VAN RENSBURG, I. B. J., MARASAS, W. F. O. & KELLERMAN, T. S., 1975. Experimental *Phomopsis leptostromiformis* mycotoxicosis in pigs. *Journal of the South African Veterinary Association*, 46, 197–204.

VAN WARMELO, K. T., MARASAS, W. F. O., ADELAAR, T. F., KELLERMAN, T. S., VAN RENSBURG, I. B. J. & MINNE, J. A., 1970. Experimental evidence that lupinosis of sheep is a mycotoxicosis caused by the fungus *Phomopsis leptostromiformis* (Kühn) Bubák. *Journal of the South African Veterinary Medical Association*, 41, 235–247.

VILLA, L., DIOGUARDI, N., AGOSTONI, A., IDEO, G. & STABILINI, R., 1966. Prognostic value of serum γ-glutamyl transpeptidase activity in liver disease. *Enzymologia Biologia et Clinica*, 7, 109–114.

ZEIN, M. & DISCOMBE, G., 1970. Serum gamma-glutamyl transpeptidase as a diagnostic aid. *Lancet*, 2 (1970), 748–750.

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