GLUTAMATE TRANSAMINASE ACTIVITY IN SHEEP TISSUES, AND THE RESPONSE TO PROLONGED PROTEIN DEPLETION

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Summary

The glutamate transaminase activity of sheep tissues has been studied, along with the effect of such variables as age, sex, breed, and nutritional status of the animal. A wide range of normal activities has been found, with glutamic–oxaloacetic transaminase always more active than glutamic–pyruvic transaminase. Most activity centres in heart muscle, liver, kidney, and skeletal muscle; there appear to be sex and age differences, but little variation between breeds.

Prolonged protein depletion has been shown to result in significant alterations of tissue transaminase activity, not only in the liver but also in myocardium, skeletal muscle, spleen, and pancreas. In addition, a cofactor deficiency was caused by the experimental diet.

The implications of these findings have been discussed, in relation both to intermediary metabolism and to chemical pathology.

I. Introduction

The properties of the transaminases and their distribution in nature have been widely investigated and well reviewed (Braunstein 1947; Cohen 1951; Meister 1955), but relatively few studies have been directed to this aspect of tissue metabolism in sheep (Cornelius *et al.* 1959; Setchell 1961).

Undoubtedly, the transaminations involving glutamate play a key role in intermediate nitrogen metabolism (Braunstein 1959). Anabolically, glutamate and glutamine are recognized as the first organic products of nitrogen assimilation, and all the other amino acids may be synthesized from this stage by transaminations and secondary transformations of the primary amino acids. Catabolically, the glutamate transaminases occupy the principal paths of dissimilation of amino acid nitrogen to excretory end products. In addition, these enzymes are intimately linked with other important metabolic aspects of protein deficiency (gluconeogenesis, fat and carbohydrate metabolism) by their connections with the tricarboxylic acid cycle and other enzyme systems.

With these ramifications in mind, and with the knowledge that protein deprivation is a common occurrence amongst sheep in Australia, it was considered desirable to investigate the response of the tissue glutamate transaminases to prolonged protein depletion.

Further, the level of glutamic–oxaloacetic transaminase (GOT) and glutamic–pyruvic transaminase (GPT) in the blood has widespread use as a sensitive indicator of tissue necrosis (Wroblewski 1958). From this point of view, further information

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on the tissue transaminases would establish reference data for chemical pathology studies.

This paper, then, has the objects of determining the glutamate transaminase activity of sheep tissues, and ascertaining the changes in this activity consequent upon prolonged protein depletion.

II. Materials and Methods

The animals used in these experiments were, in the main, normal well-fed Merino ewes, 3–4 years of age. In addition, various other breeds and age groups and male animals have been investigated as indicated in the text.

Prolonged protein depletion was attained by restricting the experimental animals to a ration of 3 kg per week of chaffed wheat straw (digestible crude protein 0·5%) and water *ad libitum*, for a period of 9–10 weeks. This experimental diet was chosen to approximate that under drought conditions and represents an average *ad libitum* intake of this feed. The duration was shown by preliminary experiments to closely approach the maximum consistent with survival.

Organs and tissues required for the measurement of transaminase activity were obtained by excision from freshly slaughtered animals, weighed rapidly, and cooled in ice. Subsequent manipulation of the tissues was performed at 4°C. Representative samples of at least 10 g were selected from the same areas of tissue in each animal and after homogenizing in an all-glass apparatus were quantitatively diluted to an appropriate volume (25–50 ml) with 0·88M sucrose. This homogenate was centrifuged (200 *g*, 5 min) to remove collagen material present in some tissues. An aliquot of the supernatant was then assayed for GOT and GPT by the method of Reitman and Frankel (1957). The units (Reitman–Frankel units) were defined as indicated in the original procedure. Duplicate determinations were made, and sufficient control solutions were used to assure accuracy and reproducibility. In addition, internal standard controls were performed in parallel by means of the analysed control sera "Enzatrol"* and "Chemtrol"†.

In connection with this method of estimating tissue transaminases, the recent findings of Boyd (1961) and Borst and Peeters (1961) are noteworthy. Since the completion of the experimental work for this paper, they have reported that the mitochondria of rat liver and heart possess GOT in latent form, and that the mitochondrial enzyme has different substrate affinities from the GOT present in the cell sap fraction. Boyd (1961) further demonstrated that the Reitman–Frankel estimation was relatively insensitive to activated mitochondrial GOT. These observations probably indicate that the results obtained for these sheep tissues refer in large part to the soluble transaminases, with a minimum intervention from unmasked mitochondrial enzyme.

A Beckmann model B spectrophotometer was used for all spectrophotometric measurements. Blood samples were collected by venipuncture, care being taken to avoid the use of haemolysed sera.

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TABLE 1

GLUTAMATE TRANSAMINASE ACTIVITIES IN MERINO SHEEP TISSUES

Activities are given in Reitman–Frankel (RF) units as mean values ± standard errors of the mean. Abbreviations in the tissue column refer to young male (YM), young female (YF), adult male (AM), adult female (AF), and tissues of protein-depleted adult females (D). Significant differences ($P<0\cdot05$) between the latter two tissues are indicated by asterisks. The young animals were approximately 6 months old, and adults were 3–4 years of age. No pyridoxal phosphate was added in these determinations

Tissue		No. in Group	Activity (RF units/mg wet wt.)		Specific Activity (RF units/mg protein)		Total Activity × 10^{-3} (RF units/total protein)	
			Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase	Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase	Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase
Heart	YM	3	62·6± 9·2	6·9 ±1·4	512 ± 52	56·8 ± 3·6	11,500±1,400	1,290±320
	YF	3	102·2±16·8	9·0 ±1·5	807 ±142	71·1 ±12·6	16,600±3,600	1,440±220
	AM	6	56·4± 7·9	5·7 ±0·7	480 ± 39	48·9 ± 6·7	11,200±4,100	1,640±630
	AF	16	75·0±16·8	10·8 ±2·9	597 ±143	88·6 ±25·3	13,600±3,400	2,010±980
	D	6	77·2±27·8	3·3 ±0·9*	560 ±256	29·8 ±18·6*	15,600±6,300	660±210*
Liver	YM	3	26·4± 4·8	1·3 ±0·2	179 ± 17	8·6 ± 1·6	14,200±3,300	686±130
	YF	3	42·3± 7·0	2·0 ±0·2	293 ± 39	13·8 ± 1·0	20,000±3,300	943± 81
	AM	6	28·7± 6·2	1·6 ±0·7	231 ± 31	8·7 ± 1·4	12,200±3,200	615±154
	AF	16	36·1±13·9	2·7 ±0·8	257 ±107	18·5 ± 5·1	18,300±8,500	1,280±410
	D	5	36·6±13·4	4·3 ±2·6	274 ± 99	31·2 ±10·7*	11,700±4,500	1,360±530
Skeletal muscle	YM	3	19·0± 1·9	3·6 ±0·4	149 ± 14	27·9 ± 3·3		
	YF	3	19·4± 9·3	5·0 ±1·0	140 ± 88	35·4 ± 8·0		
	AM	6	23·0± 1·1	3·8 ±0·6	179 ± 10	29·2 ± 4·5		
	AF	15	29·5± 6·1	2·7 ±0·9	217 ±145	19·3 ± 6·7		
	D	6	23·0± 7·1	3·6 ±1·0	323 ±138	32·7 ±10·1*		
Kidney	YM	3	15·8± 0·8	2·7 ±1·2	164 ± 10	31·4 ± 8·8	906± 130	156± 83
	YF	3	12·0± 2·2	4·3 ±0·6	111 ± 22	39·6 ± 6·3	480± 69	173± 28
	AM	6	11·9± 1·3	3·2 ±1·0	129 ± 11	30·1 ± 3·5	598± 158	135± 54
	AF	18	15·4± 1·3	2·6 ±0·9	153 ± 38	26·6 ± 9·4	675± 173	116± 48
	D	5	13·1± 5·0	3·7 ±1·3	106 ± 36	29·3 ±10·3	624± 176	181± 44

TABLE 1 (*Continued*)

Tissue		No. in Group	Activity (RF units/mg wet wt.)		Specific Activity (RF units/mg protein)		Total Activity × 10^{-3} (RF units/total protein)	
			Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase	Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase	Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase
Spleen	YM	3	6·1± 0·5	0·40±0·01	51·4± 12·3	3·4 ± 1·5	373± 56	24·3± 3·5
	YF	3	5·1± 0·6	0·74±0·15	42·0± 9·7	7·1 ± 2·4	234± 29	33·5± 1·3
	AM	6	3·7± 0·3	0·62±0·46	37·2± 7·7	5·3 ± 1·9	238± 40	39·5± 7·1
	AF	18	5·6± 1·3	0·72±0·25	51·4± 12·7	5·8 ± 2·0	385± 143	39·1± 16·0
	D	2	8·7± 1·7	1·00±0·31	78·8± 16·0	8·9 ± 2·5	755± 280	97·2± 35·1*
Pancreas	YM	3	5·6± 0·5	0·81±0·14	61·7± 16·4	8·8 ± 2·0	177± 26	25·2± 2·1
	YF	3	3·8± 0·8	0·40±0·06	55·0± 14·2	5·9 ± 1·7	442± 190	9·0± 3·8
	AM	6	2·9± 0·2	0·36±0·06	31·1± 8·1	3·9 ± 1·1	662± 105	8·2± 1·4
	AF	18	4·6± 1·1	0·83±0·24	49·0± 12·0	8·94± 2·7	137± 36	24·8± 6·5
	D	1	7·4*	1·4*	105·0*	20·4*	220*	42·0*
Lung	YM	3	3·6± 0·1	0·53±0·07	28·2± 7·0	5·2 ± 1·4	1290± 270	195 ± 30
	YF	3	4·7± 1·0	0·60±0·07	37·5± 9·2	5·8 ± 1·4	1880± 500	272 ± 68
	AM	6	2·7± 0·3	0·41±0·04	22·0± 1·1	3·8 ± 0·9	940± 140	145 ± 29
	AF	16	4·6± 1·8	1·03±0·22	39·6± 10·6	7·9 ± 2·0	1710± 890	270 ±178
	D	6	3·2± 1·0	0·93±0·37	31·2± 8·2	6·3 ± 1·5	1910± 570	488 ±205
Brain	YF	6	14·0± 2·0	0·49±0·06	204 ± 61	5·5 ± 2·6	1300± 280	42·5± 7·7
	AF	18	20·3± 5·9	0·72±0·29	267 ± 83	8·9 ± 4·6	2330± 320	86·3± 23·5
	D	1	21·0	1·29	294	17·9	2260	108

Reagents used were A.R. grade wherever possible. Pyridoxal phosphate was prepared from pyridoxal hydrochloride by the method of Heyl *et al.* (1951), and the resultant concentration determined by the method of Wada and Snell (1961). Whereas the addition of this pyridoxal phosphate to the homogenates of normal tissues resulted in no alteration of transaminase activity, its addition to the homogenates of some protein-depleted tissues caused an increase of activity. In these cases therefore, an excess of cofactor was used (Meister, Sober, and Peterson 1954) to elucidate the maximum reactivation.

TABLE 2

SERUM TRANSAMINASE LEVELS

Activities are given in Reitman–Frankel (RF) units per millilitre of serum and are expressed as mean values ± standard error of the mean

Serum from:	Glutamic–Oxaloacetic Transaminase (RF units/ml)	No. of Observations	Glutamic–Pyruvic Transaminase (RF units/ml)	No. of Observations
Merino rams	99±20	12	14·6±3·9	12
Merino ewes	97±45	18	12·7±7·3	18
Merino lambs (male)	142±35	6	19·7±4·0	6
Merino lambs (female)	111±22	6	16·0±3·6	6
Corriedale rams	101	1	15·0	1
Corriedale ewes	120±26	8	16·0±2·5	8
Dorset Horn rams	139±20	3	12·0±1·0	4
Border Leicester ewe	120	1	18·0	1
Border Leicester lambs (female)	130±11	4	12·0±2·0	4
Southdown lambs (female)	129± 2	2	15·0±2·6	2

For the protein nitrogen determinations, weighed samples (5 g) of the tissues were extracted, first with 10 volumes of ethanol–ether (3 : 1 v/v) and then with 25 volumes of 5% trichloroacetic acid at 90°C for 15 min. This was followed by microKjeldahl digestion of the residue and subsequent nesslerization (Hawk, Oser, and Summerson 1954).

III. RESULTS

The data, presented in Table 1, demonstrate the levels of transaminase activity in the various tissues investigated, and give a basis for assessing normal ranges and for comparing the effects of age and sex. Besides listing activities in terms of wet weight, they have also been expressed on the basis of units per milligram of protein (specific activity), and units per organ (total activity). The purpose of these different methods of expression was to clarify the relative status of enzyme protein and total protein.

Comparing the effects of age and sex on activities, it can be seen that while there is considerable overlapping of the standard errors, differences due to age and sex are indicated. Individual cases are best considered by reference to Table 1, but the general tendency is for female tissues to have higher activities than male tissues, with this sex difference greater in adults than in the younger animals.

On the basis of activity per wet weight, the transaminase concentration is greatest in heart muscle, liver, skeletal muscle, and kidney. Both GOT and GPT follow a similar relationship, with the exception that the ratio GPT/GOT is higher than average in the case of the lung, and lower than average with the brain.

TABLE 3

ACTIVATION OF TRANSAMINASES IN DEPLETED TISSUES BY PYRIDOXAL PHOSPHATE

Conditions of enzyme assay: α-oxoglutarate 2 mM; aspartic acid 200 mM or alanine 200 mM; sodium–potassium phosphate buffer, pH 7·4, 100 mM; pyridoxal phosphate 0·02 mM; 0·2 ml of 0·88M sucrose homogenate; total volume 1·2 ml; temperature 37°C; incubation period 60 min for GOT, 30 min for GPT. Results are given as the mean percentage increase of activity in the homogenates of depleted tissues upon incubation with excess pyridoxal phosphate

Tissue	Mean Percentage Increase of Activity	
	Glutamic–Oxaloacetic Transaminase	Glutamic–Pyruvic Transaminase
Skeletal muscle	101	0
Kidney	70	0
Heart	69	0
Brain	32	0
Liver	29	0
Spleen	23	0
Pancreas	0	0
Lung	0	0

Expressing the enzyme concentrations as specific activity and total activity somewhat alters the relative position. Most noteworthy are the high specific activity of brain GOT, the relatively low specific activity of liver GPT, and the high total activity of the lungs.

A statistical evaluation was made in order to test the significance of the changes in activity observed in the protein depletion of adult female Merino sheep. It was found that the differences were significant in several instances, which are indicated in the table by asterisks. The response to depletion was an increase of activity, in general, but heart muscle, on the contrary, showed a significant decrease in GPT levels.

Besides illustrating the range of normal values and the effects of age and sex on serum transaminases, Table 2 also shows the values obtained in different breeds.

The effect of this variable was studied, firstly, because marked species differences have been reported in the literature (Wroblewski 1958) and, secondly, because the values reported by Setchell (1961) for Corriedale wethers were considerably lower than the values for Merinos. Little variation among breeds occurred in our experiment.

In comparing the activities of tissues before and after protein depletion, the influence of the addition of cofactor to the homogenate was studied. None of the GPT activities were altered by this procedure, but some of the GOT activities in depleted tissues were considerably increased (Table 3). The degree of this pyridoxal reactivation would presumably differ with individual animals and their nutritional history, but the values recorded here refer to the standard conditions of the experiment.

IV. Discussion

From an examination of Table 1, it is apparent that there is a wide range of normal transaminase activity in all the tissues investigated. The tissues showing the greatest activity (on units per wet weight basis) were the heart, liver, kidneys, and the skeletal muscle. This was consistent with reported results on other domestic animals (Cornelius *et al.* 1959), and nominates these tissues as the most likely origin of pathological elevation in the serum glutamate transaminases. Further, in agreement with Cornelius *et al.* (1959), the ratio of GPT/GOT in the sheep liver is considerably less than in the liver of man and certain other animals. This may explain why hepatic necrosis in sheep is not associated with an increase of serum GPT (Setchell 1961).

Using the rat as an experimental animal, previous workers (Beaton, Curry, and Veen 1957; Rosen, Roberts, and Nichol 1959; Soberon and Sanchez 1961) have studied the alterations of liver transaminases during conditions conducive to protein catabolism and gluconeogenesis. Although most of these investigations were concerned with the early stages of these processes, large alterations of activity were found. In this study the maximum period of protein depletion was employed for comparison with the normal activities of the sheep tissues, and with the likelihood of even greater alterations in these terminal stages, results in this paper have been expressed in three ways: activity per wet weight, activity per unit weight of protein, and activity per organ. The rationale was that considerable changes in activity under these conditions were most likely to be caused by a synthesis or destruction of protein, and that these methods of expressing the results would give a clearer indication of the relative status of the apoenzyme. An increase of total activity has been assumed to imply net synthesis, and a decrease of total activity, destruction of the enzyme involved. An increase in specific activity could signify sparing of the enzyme relative to overall protein catabolism, or a relative increase in protein synthesis. A decrease in specific activity would signify the converse of these situations.

A comparison of the results of enzyme activity in normal and depleted sheep tissues does not show as great a change as exhibited in the rat under less severe conditions, but in spite of this and the wide variation of normal values, significant differences were demonstrated in many cases. In protein-depleted sheep, both the

liver and skeletal muscle demonstrated a sparing of GPT protein relative to the total protein content of these tissues, and net synthesis was indicated for both GOT and GPT apoenzymes in the pancreas. By contrast, preferential destruction of GPT was indicated in the myocardium.

Under the experimental conditions of dietary restriction, the glutamate transaminases could be expected to play an important role in satisfying energy requirements by the provision of tricarboxylic acid cycle substrates from the amino acid pool (Rosen, Roberts, and Nichol 1959). Whilst appreciating the pitfalls of applying *in vitro* measurements to *in vivo* interpretations (Webb 1961), it seems reasonable to assume that this importance is reflected in the way the relative status of GPT protein, in particular, is improved in many of the tissues under these conditions. This observation is in agreement with the results in monogastric experimental animals (Awapara 1953; Rosen, Roberts, and Nichol 1959).

Rosen, Roberts, and Nichol (1959) have suggested that GPT represents a rate-limiting step in the conversion of protein to carbohydrate. Sheep, however, are largely dependent on fatty acid metabolism for the maintenance of tricarboxylic acid cycle activity (Gallagher and Buttery 1959). This bias away from carbohydrate energy sources might explain why GPT is low in activity in the ruminant liver, and also why increases in GPT in sheep tissues, even under these conditions of protracted protein deprivation, were not of the same order of magnitude as those in the rat at a much earlier stage of depletion (Rosen, Roberts, and Nichol 1959).

As mentioned in Section III, the heart shows a different response from the other tissues, and a preferential destruction of GPT apoenzyme occurs in this tissue under conditions of protein depletion. Assuming, again, that GPT represents a rate-limiting step in the conversion of protein to carbohydrate (Rosen, Roberts, and Nichol 1959), this would appear to imply that there is a decreasing call of pyruvate as an energy source, and a possible explanation lies in the utilization of alternative sources of energy in preference. It is known that the heart, in non-ruminant animals, obtains its supply of energy largely from non-carbohydrate sources, particularly acetoacetate (White *et al.* 1959). Under the conditions of this experiment, the supply of ketone bodies is increased, and there may be preferential use of these as an energy source, reducing the requirement for GPT and resulting in a reduction of GPT activity.

Protein depletion produced fewer significant differences in GOT than in GPT activities (Table 1). However, comparison here is complicated by the demonstration that some of the tissues from depleted animals were deficient in cofactor (Table 3). In those depleted tissues where the apoenzyme was reactivated by incubation with pyridoxal phosphate, the maximum activity obtained merely brought the results within the normal range. Thus, though no quantitative comparison is possible, it seems on the results available that GOT is less sensitive than GPT to this stimulus of protein deprivation. This would be in accord with findings in the rat (Beaton, Curry, and Veen 1957).

The pyridoxal phosphate deficiency observed in depleted tissues is of interest because of the peculiar position of ruminants with regard to the requirements for vitamin B_6. The adult ruminant does not require B vitamins (including pyridoxine)

in its diet, these being synthesized by the rumen microorganisms in excess of the normal requirements (McElroy and Goss 1939, 1940*a*, 1940*b*, 1940*c*, 1941). Normally, therefore, one would expect no deficiency of this type. The diet of the experimental animals, however, besides being of low protein content, also was low in the amount of utilizable carbohydrate, and this latter deficiency would tend to reduce the microbial synthesis of pyridoxine (Porter 1961). Since there is little storage for vitamin B_6, and considerable binding at non-functional sites (Meister, Sober, and Petersen 1954), the deficiency would soon become apparent.

There are two points of interest in the localization of this deficiency (Table 3). Firstly, the effect was greatest in skeletal muscle, heart muscle, and the kidney, which are amongst the most actively transaminating tissues. Secondly, the deficiency showed at this stage in some of the GOT, but none of the GPT, activities. This would follow from the higher concentrations of GOT, and also from the fact that GOT has a much higher content of pyridoxal phosphate than GPT (Green, Leloir, and Nocito 1945).

While the glutamate transaminations are undoubtedly the most consequential of the metabolic reactions which are dependent on pyridoxal phosphate, this compound is also a cofactor for a wide range of important reactions (Braunstein 1959). Therefore, the occurrence of pyridoxal phosphate deficiencies in protein-depleted sheep might implicate other important processes such as the biosynthesis of sulphur amino acids and the tetrapyrrolic biocatalysts, and fat metabolism.

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