AUSTRALIAN JOURNAL OF BIOLOGICAL SCIENCES

Editorial Report for 1979

Statistics

In 1979 the *Australian Journal of Biological Sciences* published 70 research papers in five issues and 662 pages. Though issues 4 and 5 were combined, the total number of pages published for the year was approximately the same as in previous years. In all, 24 of the 70 papers were listed in the endocrinology and reproductive biology section of the contents, 17 in the biochemistry section, 10 in the genetics section, 9 in the physiology section, 7 in the microbiology section and 3 in the cell physiology and ultrastructure section.

Submissions to the Journal

There were 90 submissions to the Journal in 1979, approximately the same number as in 1978. Of these 13 were rejected and 1 was withdrawn. There were 39 papers still under consideration at the end of the year. Approximately half of these were with authors for revision and the remainder were with referees. Each relevant submission was assessed initially by two referees. We would like to take this opportunity to thank those persons whose names are listed below for acting as referees and advisers during the year and also those referees who did not wish to have their names listed.

Invitations to submit papers to the Journal were inserted in newsletters of two learned societies during the year, namely the newly launched *Newsletter of the Australian Society for Reproductive Biology* and the *Newsletter of the Australian Society for Microbiology Inc.* At the same time it was pointed out that members of learned societies may receive copies of the Journal at one-third of the cover cost, providing lists are submitted through their secretaries. Thus, in 1980, though the cover price will be increased to $30, the cost to members will be only $10. This cost is usually tax-deductible.

Review Papers

Papers reviewing existing knowledge in a field and which indicate fruitful lines of research are now acceptable to the Journal. It is pleasing to report the publication of such a paper in the first issue for 1980. This paper was initially presented in the symposium on lactation which formed part of the proceedings of the annual conference of the *Australian Society for Reproductive Biology* which met in Perth in August 1979. It is also hoped to publish key-note addresses given at scientific conferences providing they meet Journal requirements.

Advisory Committee

Dr R. F. Seamark retired from the Advisory Committee at the end of the year and we would like to take this opportunity to thank him for his help during his term of office. His place on the Committee will be taken by Professor D. de Kretser, who is Professor of Anatomy at Monash University.

The other members of the Committee are as for 1978, namely Professor E. F. Annison, Department of Animal Husbandry, University of Sydney (Chairman), Professor K. C. Marshall, School of Microbiology, University of New South Wales, Dr I. J. O'Donnell, Division of Protein Chemistry, CSIRO, Dr W. J. Peacock, Division of Plant Industry, CSIRO, Dr Marilyn Renfree, Department of Environment and Life Sciences, Murdoch University, and Professor R. G. Wake, Department of Biochemistry, University of Sydney. Mr B. J. Walby, Editor-in-Chief, is *ex officio* a member of the Advisory Committee.

Notice to Authors

The 'Notice to Authors' was expanded during the year to include a paragraph on the Journal's requirements for the reporting of hormonal assays. The paragraph on Enzyme Nomenclature was also amended to state that the names of enzymes in submitted papers should conform to the Recommendations of the Nomenclature Committee of the IUB as published in 'Enzyme Nomenclature 1978' (Academic Press, Inc., New York, 1979).

The 'Notice to Authors' is included in every issue of the Journal and there is no doubt that the stricter the observance to the requirements listed therein the shorter will be the time between submission and publication which is now approximately 8 months. However, because of escalating typesetting costs, the Journal can no longer afford to set lengthy peptide or DNA sequences in type. In future these will be reproduced from the copy provided. Care should therefore be taken in the preparation of this original copy; its lay-out should have regard for the dimensions of the Journal page, and a carbon ribbon should be used for typescript.

Editorial Comment

It has been drawn to the Journal's attention that in the paper by T. L. J. Mann and O. Mayo entitled 'Response to partial selection on clean fleece weight in South Australian strong-wool Merino sheep. III. Genetic distance between flocks', which was published in Volume 31, Number 6, of the *Australian Journal of Biological Sciences*, statements made on p. 670, line 5 from bottom, and p. 676, line 9 from bottom, are open to misinterpretation and might possibly cast reflection on the scientific ability of the persons referred to in these statements. The Journal has been assured by the authors of the above paper that the 'misclassification' would probably have arisen from mistaken identification of flock status for the sheep bled rather than errors due to typing of the blood samples.

List of Referees Consulted during the 1979 Calendar Year

A. Ashford
F. J. Ayala
F. J. Ballard
J. S. F. Barker
P. J. Barter
D. A. Barr
P. R. Baverstock
R. C. Bayly
F. J. Bergerson
B. M. Bindon
J. L. Black
R. J. Blagrove
J. Blair-West
J. W. Bowyer
A. D. Brown
A. H. D. Brown
N. W. Bruce
R. W. Burley
L. Cahill
J. Camakaris
P. R. Carnegie
I. J. Clarke
K. W. Cleland
R. I. Cox
C. C. J. Culvenor
I. A. Cumming
J. M. Cummins
J. C. Daniel
B. E. Davidson
E. S. Dennis
M. J. Dilworth
H. W. Doelle
E. R. Donovan
C. E. dos Remedios
M. R. Edwards
A. R. Egan
G. J. Faichney
R. J. Fairclough
I. R. Falconer
E. S. Finckh
J. K. Findlay
R. Frankham
I. R. Franklin
E. L. French
R. G. Garrett
A. H. Gibson
J. B. Gibson
F. C. Greenwood
G. W. Grigg
P. Hartmann
H. Hearnshaw
D. R. Hewish
A. J. Hilliker
A. A. Holland
B. W. Holloway
R. Hope
P. S. Hopkins
B. Howard
G. Howlett
D. Jackson
J. F. Jackson
I. G. Jarrett
P. D. Jeffrey
A. R. Johnson
S. J. Judd
G. J. Judson
I. R. Kennedy
R. W. Kerr
D. C. Klein
B. D. H. Latter
M. F. Lavin
V. W. K. Lee
G. C. Liggins
D. Lowther
G. H. McDowell
W. V. Macfarlane
H. A. McKenzie
J. A. McKenzie
A. G. MacKinlay
A. McLaren
R. H. Maddern
C. Matthews
G. M. E. Mayo
J. R. Mercer
N. W. Moore
J. F. Morrison
R. N. Murdoch
W. G. Murrell
P. Nagley
C. Nancarrow
D. J. D. Nicholas
J. V. Nolan
R. O'Brien
J. O'Shea
C. A. Parker
R. J. Pepperell
G. Phillipou
E. S. Pilkington
B. J. Potter
A. J. Pryor
J. M. Rendel
F. Robinson
G. E. Rogers
H. Rosenberg
T. L. Rothwell
I. J. Ryrie
J. M. Sabine
R. J. Scaramuzzi
R. K. Scopes
W. R. Scowcroft
G. J. Shanahan
K. D. Shortman
D. Shutt
G. S. Sidhu
M. W. Simpson-Morgan
T. C. Smeaton
R. M. Smith
V. A. Stanisich
T. Stelmasiak
B. A. Stone
L. L. Stubbs
K. W. Taylor
E. O. P. Thompson
J. A. Thomson
G. C. Wade
J. Wadsworth
D. M. Walker
G. J. Walker
A. L. C. Wallace
B. J. Wallace
C. Ward
K. A. Ward
A. B. Wardrop
J. R. E. Wells
J. G. White
R. Whittaker
M. J. Whitten
A. J. Williams
J. Willoughby
P. R. Wills
D. J. Winzor
E. F. Woods
J. Young

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Activity of Aspartate Carbamoyltransferase in Ovine Tissues

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Abstract

The activity of the enzyme aspartate carbamoyltransferase (ACT; EC 2.1.3.2) was studied in ovine tissues. Hepatic, ileal and duodenal tissues showed higher levels of activity (6–19 units) than skeletal muscle, cardiac muscle, lung, spleen, kidney and rumen wall (2–7 units). Low growth impetus skeletal muscles had higher activity (2·9 units) than high growth impetus muscles (1·8 units) taken from immature sheep but such differences were not present in mature sheep.

Refeeding of starved immature and mature sheep resulted in two- to threefold increases in activity of ACT in hepatic, ileal and duodenal tissues. These elevated levels of ACT activity were maintained for considerably longer (100 days in immature and 50 days in mature sheep) than the 30 days required for both groups of sheep to achieve liveweights similar to those of continuously grown animals.

The optimum pH for ACT activity from both liver and ileum was between pH 8·5 and 9·2. pH profiles showed that activity of ileal ACT was consistently lower than liver ACT in the pH range 6·0–8·5. Starch-gel electrophoresis indicated only one major band of enzyme activity in both liver and ileum. However, both enzymes migrated to different positions in the gel with ileal ACT being the faster migrating of the enzyme forms.

Introduction

Studies of metabolic adaptations to protein malnutrition indicate that both body and organ growth, and increase in cell number, are depressed, whereas differentiated functions, such as protein synthesis, are less affected (Widdowson and McCance 1963; Patt *et al.* 1964; Winick and Noble 1966; Dallman and Manies 1973). In the rat, protein deficiency has contrasting effects on DNA and RNA synthesis in proliferating liver cells. Whereas DNA synthesis is depressed, RNA production is maintained and responds rapidly to an inducing stimulus (Shaw and Fillios 1968; Siimes and Dallman 1974). The activity of aspartate carbamoyltransferase (ACT; EC 2.1.3.2), which catalyses the first step in the biosynthesis of pyrimidines required for RNA synthesis, was found to be reduced in the liver of rats starved for 2 days but almost doubled in rat liver after partial hepatectomy (Durkin and Nishikawara 1971).

Rate of protein synthesis is positively related to RNA concentration (Garlick *et al.* 1976). Protein turnover is much slower in the tissues of large animals (e.g. man, Picou and Taylor-Roberts 1969; sheep, Buttrey *et al.* 1975; and pigs, Garlick *et al.* 1976) than in small animals such as rats (Millward 1970). Furthermore, rates of protein synthesis differ from as much as 20% protein renewal per day in some visceral organs to 4% per day in the skeletal muscle of the same animal (Garlick *et al.* 1976).

Despite this association between ACT activity, RNA synthesis and protein turnover, little attention has been given to the activity of ACT in tissues other than rat liver. This paper reports a study of ACT in tissues from sheep whose diets were designed to promote continuous, negative and compensatory growth.

Materials and Methods

Animals

A group of 51 Dorset Horn × Merino wether lambs, approximately 7 months of age and 20 kg liveweight, were divided at random into two groups, namely continuous growth (group A) and compensatory growth (group B). Rations for these groups were planned to permit continuous growth in group A but the food intake of lambs in group B was progressively restricted to ensure that they lost liveweight. These lambs (group B) were subsequently refed by offering them the same amount of food as those in group A. When the sheep in each group had reached approximately 43 kg liveweight, 10 from each group were subjected to immediate dietary restriction to reduce their liveweight (group C). These animals were refed by offering them the same food intake as those continuously grown (group A). There were seven slaughterings of sheep from groups A and B and five of sheep from group C. Group A animals were slaughtered on days 0, 56, 96, 166, 222, 278 and 386; group B animals were subjected to progressive feed restriction for a total of 56 days (final dry matter intake was 159 g/day) and slaughtered on days 56 (starvation), 60, 96, 166, 222, 278 and 386. On day 186 the food supply to group C animals was abruptly restricted (185 g/day) and animals were slaughtered on days 222 (starvation), 229, 234, 278 and 386. Further details of the experimental design and procedures together with the growth patterns and body composition of the sheep have been presented by Thornton *et al.* (1979).

Tissue Sampling

Immediately after slaughter the skin was removed and the abdominal and thoracic cavities opened to permit samples of 10–20 g of the following tissue to be taken:

(i) *Skeletal muscle*—samples from the centre regions of the M. semimembranosus, M. semitendinosus, M. flexor carpi radialis and M. flexor carpi ulnaris were excised with scissors. The first two of these muscles have been classified as high growth impetus and the second two as low growth impetus by Lohse *et al.* (1973);
(ii) *Liver*—part of the caudal lobe was taken;
(iii) *Cardiac muscle*—from the apex of the heart;
(iv) *Alimentary tract*—the digesta was washed from the lumen of the tract by gentle hosing. Samples of the duodenum, ileum and rumen wall were taken, freed of adipose and connective tissue, and blotted dry;
(v) *Others*—samples of the spleen, kidneys and lungs were also taken. All of these samples were immediately frozen in liquid nitrogen and stored at −20°C for subsequent enzyme assay.

Enzyme Preparation

Homogenates were prepared from the thawed tissues in an Ultra-Turrax homogenizer, with 3 ml per gram of tissue of 0·25 M sucrose, 0·05 M Tris–HCl (pH 7·5), 0·025 M KCl and 0·005 M $MgCl_2$. The homogenates were centrifuged at 75 000 *g* for 60 min at 4°C to yield the soluble extract without any appreciable loss in enzyme activity. This cytosol extract was used as the enzyme source in comparative tissue assays (fraction 1).

To the liver and ileum fractions, solid ammonium sulfate was added to 30% saturation and the suspension stirred at 4°C for 20 min. The suspension was centrifuged at 20 000 *g* for 20 min and the precipitate discarded. The supernatant was then brought to 60% saturation with ammonium sulfate following the same procedure, and the resulting precipitate dissolved in 0·01 M imidazole buffer pH 7·2 containing 1 mM EDTA (fraction 2). This fraction retained approximately 85% of the total original activity while enzyme activity/mg protein was increased fivefold in comparison to fraction 1 as a result of this treatment. Fraction 2 was used in the pH and electrophoresis studies.

Enzyme Assay

The assay for ACT was a modification of the method described by Bresnick and Mosse (1966). Each tissue fraction (0·2 ml) was preincubated for 5 min at 37°C in 2·0 ml 0·4 M Tris–HCl buffer (pH 9·2) and 0·3 ml 50 mM L-aspartic acid.

The reaction was initiated by addition of 0·2 ml of freshly prepared 0·7 mM dilithium carbamyl phosphate (Boehringer), and incubated for 20 min. One unit of ACT activity is defined as the amount that produces 1 nmole carbamyl aspartate per 30 min per milligram protein at 37°C under these conditions. The reaction was stopped by the addition of 0·3 ml 4 M perchloric acid and the reaction tubes were placed in an ice bath for 5 min to coagulate the denatured proteins, which were then removed by centrifugation at 5000 *g* for 20 min. The carbamyl aspartate formed was colorimetrically determined in a portion of the deproteinized material by the method II procedure of Prescott and Jones (1969).

Suitable enzyme blanks, obtained by adding 0·3 ml 4 M perchloric acid before the addition of enzyme, were run for each tissue sample and the results were corrected for absorbance due to endogenous carbamyl compounds.

The protein concentration of the enzyme extracts was estimated by the method of Gornall *et al.* (1949), with bovine serum albumin (Sigma Chemical Co.) as the reference standard.

Starch-gel Electrophoresis

This was performed according to the technique described by Scopes (1968), using a discontinuous Tris–borate–EDTA system at pH 8·6. Gels were routinely run at 6 mA, 180 V for 5 h at 0°C. At the completion of the run, each starch block was cut into 1-cm sections, eluted with 0·01 M imidazole buffer (pH 7·2) containing 1 mM EDTA, and the extracts tested for enzyme activity.

Results

ACT Activity in Tissues

There were differences in ACT activity between the muscles of the five immature sheep of mean liveweight 23·1 kg which were slaughtered at the commencement of the experiment (day 0), as indicated in the following tabulation (mean values ± s.e. expressed as nmol carbamyl aspartate produced in 30 min per milligram protein at 37°C):

High impetus growth muscle		Low impetus growth muscle	
M. semimembranosus	1·87±0·22	M. flexor carpi radialis	2·95±0·32
M. semitendinosus	1·79±0·19	M. flexor carpi ulnaris	2·81±0·32

Low growth impetus muscles showed significantly higher activity ($P < 0·001$) than high growth impetus muscles. There were also significant differences ($P < 0·001$) between sheep. However, differences between the muscles within each of these classifications were not significant. Relatively low levels (2–6 units) of activity in skeletal muscle were maintained throughout the experiment and over the entire experiment there were no significant differences between muscles, muscle classifications (low *v.* high impetus) or between treatments (groups A, B and C; see Fig. 1 and Table 1). Progressive starvation resulted in lower levels of ACT activity in skeletal muscles of immature sheep than in those of continuously grown controls. Refeeding of these starved sheep resulted in immediate increases in the level of ACT in skeletal muscle and these elevated levels were generally but not consistently maintained (see Fig. 1*b*). Abrupt starvation followed by refeeding of mature sheep did not invoke the same responses in level of ACT activity in skeletal muscle as was observed in immature sheep. Values for starved mature sheep were similar to those of controls and refeeding was without significant effect on the ACT activity of skeletal muscle.

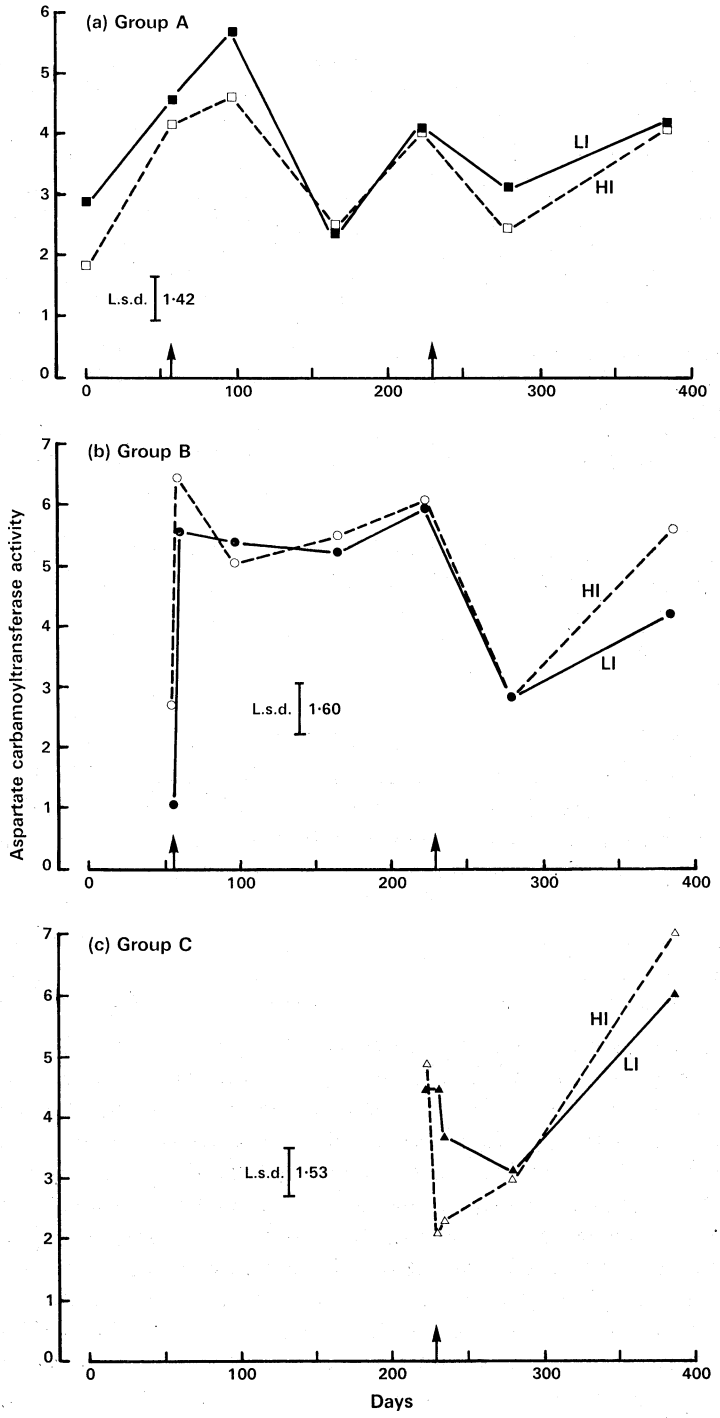


Fig. 1. Aspartate carbamoyltransferase activity (expressed as nmol carbamyl aspartate produced in 30 min per milligram protein) of the fraction 1 preparation of high growth impetus (HI) and low growth impetus (LI) muscles of animal groups A, B and C throughout the experiment. Vertical arrows indicate when refeeding commenced. L.s.d., least significant difference at $P < 0{\cdot}05$.

Mean values for ACT activity tended to initially decline and then rise in skeletal muscle of refed mature sheep. ACT activity was consistently lower ($P < 0{\cdot}001$) in skeletal muscle than in cardiac muscle.

Table 1. Aspartate carbamoyltransferase activity in organ and muscle tissue from sheep

Values are means ± s.e. Values for group A (continuously grown) are the mean of 21 sheep, those for group B (immature, starved–refed) are the mean of 20 sheep and those for group C (mature, starved–refed) are the mean of 10 sheep. The liveweights of group A sheep ranged from 23·1 kg (day 0) to 59·8 kg (day 386); of group B from 16·9 kg (day 56—starved) to 57·7 kg (day 386); of group C from 30·8 kg (day 222—starved) to 58·7 kg (day 386). See Table 4, Thornton *et al.* (1979) for complete growth and body composition data. Results are expressed as nmol carbamyl aspartate produced in 30 min per milligram protein at 37°C. Differences between groups for any tissue were not significant

Tissue	Group A	Group B	Group C
Spleen	6·06±1·40	6·27±2·17	5·64±1·82
Kidney	4·36±1·81	4·76±2·08	3·56±1·18
Rumen wall	4·37±1·07	4·26±1·36	3·34±0·75
Cardiac muscle	7·17±2·15	6·77±3·55	5·90±1·31
Lung	2·90±0·90	1·63±0·55	2·44±0·46
M. semimembranosus	4·14±1·86	5·19±1·70	3·62±1·68
M. semitendinosus	3·06±0·86	3·51±1·23	4·60±2·07
M. flexor carpi radialis	3·80±0·96	4·49±1·41	3·98±1·32
M. flexor carpi ulnaris	4·06±1·04	3·91±1·16	4·94±1·52

Low levels of activity (2–7 units) were found in lung, spleen, kidney and rumen wall (see Table 1) and imposed treatments were without significant effect on activity in any of the tissues (see Table 1). However, hepatic, ileal and duodenal tissues showed high levels of ACT activity (6–19 units; see Fig. 2). There were no significant differences in activity between these three tissues from continuously grown animals (group A; 'controls') and values ranged from 7 to 11 units. Starvation of immature sheep did not depress activity below these 'control' levels but starved mature sheep showed 20·5, 25·0 and 26·7% less ACT activity, in liver, ileum and duodenum respectively, than continuously grown animals killed at the same period of the experiment. Refeeding resulted in sharp increases in activity (two- to threefold) in all of these tissues in both groups of sheep (B and C; see Fig. 2). These elevated levels of ACT activity were maintained, for 130 days in immature sheep and for 80 days in mature sheep, during refeeding even though compensatory growth permitted each group to achieve the liveweight of continuously grown animals in 30 days (Thornton *et al.* 1979).

pH Optima

The pH profiles showed marked differences in the range pH 6·0–8·5, with ileum ACT having a lower range of activity than the enzyme from liver at each pH value. However, the pH optima for both enzymes was between pH 8·5 and 9·2 (Fig. 3).

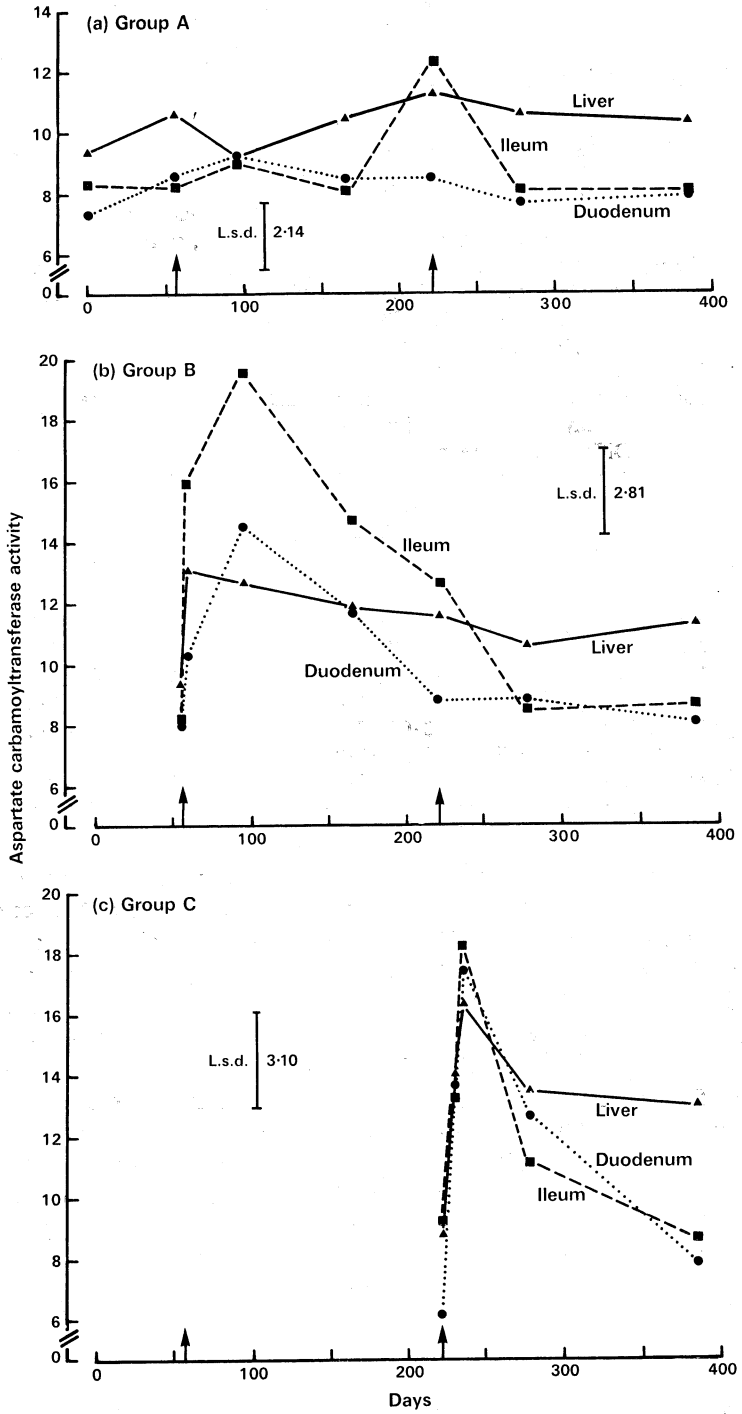
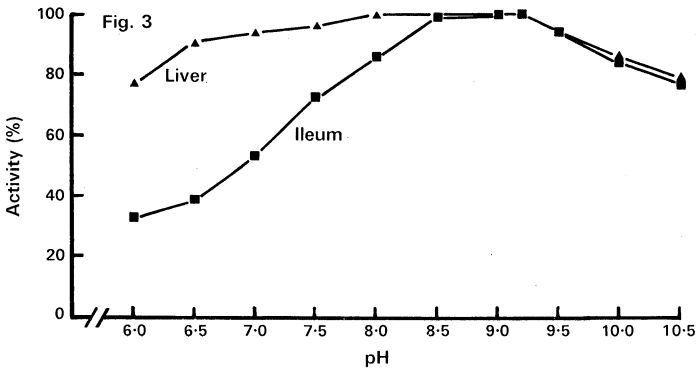


Fig. 2. Aspartate carbamoyltransferase activity (expressed as nmol carbamyl aspartate produced in 30 min per milligram protein) of the fraction 1 preparation of liver, ileum and duodenum for animal groups A, B and C. L.s.d., least significant difference at $P < 0{\cdot}05$. Vertical arrows indicate when refeeding commenced.

Starch-gel Electrophoresis

Electrophoresis of both liver and ileum ACT, with subsequent assay for enzyme activity, showed that both enzymes migrated towards the cathode at pH 8·6 (Fig. 4). No enzyme migrated towards the anode at this pH. Only one slow-migrating major band of enzyme activity existed for both liver and ileum. Regardless of the strength of electric current used to electrophoretically separate the enzymes from these sources both enzymes consistently appeared to migrate to different positions in the gel, with ileum ACT being the faster-migrating of the two enzyme forms.



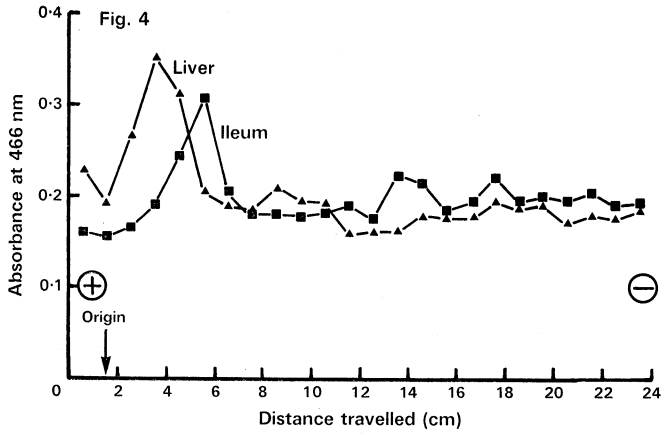


Fig. 3. Effect of pH on aspartate carbamoyltransferase activity (50–55 units per milligram protein) of the fraction 2 preparation of liver and ileum. The reaction mixture (2·7 ml) contained 0·3 M buffer (phosphate, pH 6–8; Tris–HCl, pH 8–9·2 and Tris-Glycine pH 9·5–10·5). Experimental details are given in the text.

Fig. 4. Starch-gel electrophoresis pattern, at pH 8·6, of aspartate carbamoyltransferase from liver and ileum. 0·1–0·2 mg enzyme (50–55 units per milligram protein; fraction 2 preparation) were used for each assay. Experimental details are given in the text.

Discussion

ACT activity was present in all the sheep tissues assayed, with the highest levels in ileum, duodenum and liver. These tissues exhibit a more rapid turnover of RNA

and protein than skeletal muscle (Garlick *et al.* 1976) which in this study showed a low level of ACT activity. This low level of ACT activity together with the inconsistent response of skeletal muscle ACT to successive periods of starvation–weight loss/refeeding–compensatory growth indicates that ACT activity estimates on skeletal muscle are of little value as an indication of protein metabolism in that tissue. These results are in general agreement with the relative ACT activities of various rat tissues (Lowenstein and Cohen 1956) but activity values for sheep tissues are up to six times lower than corresponding values for rat tissues reported by Bresnick and Mosse (1966). This finding is consistent with the lower rate of protein turnover in the tissues of larger animals than in small animals (Buttrey *et al.* 1975; Garlick *et al.* 1976).

The one exception appears to be lung tissue which in sheep showed a low level of enzyme activity more typical of that found in skeletal muscle than in visceral organs. Rat lung tissue showed levels of ACT activity some four times that of leg muscle and half that of liver (Lowenstein and Cohen 1956). In addition, a major difference was noted between the purification procedure of sheep liver and rat liver enzymes. Whereas rat liver ACT was precipitated by a 0–30% ammonium sulfate fractionation (Bresnick and Mosse 1966), the sheep liver and ileum enzymes were fractionated by 30–60% ammonium sulfate in this study. The majority of enzyme activity in all sheep tissues was recovered in the soluble fraction (75 000 *g* supernatant), which is in accord with the subcellular distribution of the enzyme in rat liver (Oliver *et al.* 1969) and mouse spleen (Inagaki and Tatibana 1970).

The ACT activities in tissues from continuously grown animals were consistently within the same range and exhibited no trends throughout the duration of the experiment despite a threefold increase in liveweight (Thornton *et al.* 1979). Thus valid comparisons can be made between levels of ACT activity in the tissues of animals in different nutritional states and at various stages of the experiment. Changes in the level of enzyme activity in response to nutritional changes were greatest in liver, ileum and duodenum. No significant changes in activity in these tissues from immature sheep were demonstrated as a result of 56 days of progressive starvation. This finding is in agreement with the results of Munro (1969) and others (Shaw and Fillios 1968) who have shown that short-term starvation depresses total hepatic RNA content, but not the rate of synthesis, which may even increase. Thus the rate of *de novo* pyrimidine synthesis and ACT activity would not be expected to alter markedly as a result of this progressive starvation treatment. The abrupt and prolonged (36 days) restriction of food intake of mature sheep resulted in slightly reduced (20–27%) levels of ACT in liver, ileum and duodenum. Durkin and Nishikawara, (1971) also found a 24% reduction in the level of ACT in rat liver following a short-term (2-day) starvation period.

When immature sheep were refed, the level of ACT in the liver, ileum and duodenum increased rapidly, and was 20–97% higher than in the corresponding control tissues. Durkin and Nishikawara (1971) reported that in rat liver growth was stimulated by high dietary protein without a corresponding change in ACT activity and presumably, therefore, in pyrimidine biosynthesis, while in regenerating hepatic tissue the ACT activity per milligram tissue increased markedly. Hence, compensatory growth in these refed sheep appears to be more akin to the rapid growth following partial hepatectomy than to growth stimulated by change from a low to a high protein diet in which the level of ornithine transcarbamylase and not ACT is reported to increase markedly.

The frequency of slaughterings of both immature and mature sheep undergoing compensatory growth was not sufficient to define the precise rates of ACT induction associated with refeeding. However, there appeared to be little evidence of increased inducibility of enzyme response during repeated starve–refeed episodes. Increased inducibility has been noted for other enzymes, including glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and malate dehydrogenase (EC 1.1.1.37) and this phenomenon has been referred to as 'metabolic memory' (Szepesi 1973) or 'overshoot' (Wurdeman *et al.* 1978).

In metabolic regulation, enzyme activity may be altered in response to nutritional factors by changes in the catalytic activity/unit weight of enzyme protein without change in the amount of enzyme protein, or by increase in the intracellular level of the enzyme, as is commonly observed in rapidly growing tissues (Kim and Cohen 1965; Gorlich and Heise 1967). The former phenomenon may be observed with multimeric enzymes which catalyse rate-limiting reactions in metabolic pathways. Mammalian and avian ACT from various tissues have been found in association with two macromolecular components of different molecular weights (Oliver *et al.* 1969; Inagaki and Tatibana 1970; Koskimies *et al.* 1971). In the present experiment different results for pH–activity and electrophoretic profiles indicate that tissue-specific ACT forms appear to be associated with sheep liver and ileum. Thus the apparent multimeric nature of the enzyme may be an essential factor in its regulatory function in different tissues of the sheep.

Acknowledgments

The authors gratefully acknowledge the technical assistance of T. W. Larsen and A. L. Neish.

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