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DISTRIBUTION AND ACTIVITIES OF DICARBOXYLIC AMINO ACID TRANSAMINASES IN GASTROINTESTINAL MUCOSA OF RAT, MOUSE, HAMSTER, GUINEA PIG, CHICKEN AND PIGEON

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SUMMARY

The specific activities of glutamate–pyruvate transaminase (EC 2.6.1.2), glutamate–oxaloacetate transaminase (EC 2.6.1.1) and glutamate dehydrogenase (EC 1.4.1.3), per min per mg protein have been measured in tissue homogenates, particle-free supernatants and in total particulate fractions of the mucosa of the gastrointestinal tract and in liver of rat, mouse, hamster, guinea pig, chicken and pigeon. Compared with other procedures, the assay methods used yielded maximal values for the activities of the enzymes. In all tissues glutamate–oxaloacetate transaminase was similar to the activity in liver and there was little difference between the species. Glutamate–pyruvate transaminase was present in the small intestinal mucosa and liver of all the species studied, and in the mucosa of the large intestine of the rat and mouse. In rat and mouse, the specific activity of glutamate–pyruvate transaminase was several times greater than in the corresponding tissues of the other species. The specific activity of glutamate–pyruvate transaminase in the small intestinal mucosa of the rat was about twice that of the liver, while in the other species it was less than that of the liver. Glutamate–pyruvate transaminase and glutamate–oxaloacetate transaminases were present in both the particle-free supernatant and in the total particulate fractions of the mucosal and liver cells. Reasons are given for supposing that the soluble forms of the enzymes differ from those associated with the particles. Glutamate dehydrogenase was found in all tissues of the gastrointestinal tract, but with a specific activity only 10–20 % that of the liver.

INTRODUCTION

The small intestine of many animals transports monocarboxylic L-amino acids against a concentration gradient across the intestinal wall in vitro. However, when either glutamate or aspartate is present at the same initial concentration in the fluids bathing both sides of intestine in vitro, the dicarboxylic amino acids disappear from both fluids and are therefore not subjected to transepithelial transport. Because the glutamate and aspartate cannot be recovered as free amino acids in the intestinal wall after absorption in vitro, i.e. they are not accumulated in the tissue, it seems probable

that dicarboxylic amino acids are subjected to transamination in the intestinal mucosa during absorption [1, 2]. The occurrence of transamination during absorption by small intestine in vitro was first demonstrated by Matthews and Wiseman [3] who found that when either glutamate or aspartate was absorbed from mucosal fluid, alanine appeared in the serosal fluid. In experiments with other amino acids it was found, in contrast, that only the amino acid originally present in the mucosal fluid appeared in the serosal fluid. During the absorption of glutamate and aspartate in vivo from the small intestine of dog, cat, rabbit and rat, there is an increase in the alanine concentration in the portal venous blood [4–9].

When whole protein or either acid or enzymic hydrolysates of protein were fed to dogs [10–12], rats [13], pigs [14], sheep [15], or cayman [16] no appreciable increase in the glutamate concentration in the portal venous blood was observed. Since proteins contain up to 30% of the total amino acid residues as dicarboxylic amino acids, the small intestine appears to be exceedingly efficient in transaminating the large quantities of glutamate and aspartate presented to it in the diet. These findings underline the importance of transamination during the absorption of a protein meal.

Very little is known about the distribution of intestinal glutamate–pyruvate transaminase (EC 2.6.1.2) and glutamate–oxaloacetate transaminase (EC 2.6.1.1.) along the length of the intestinal tract, nor is there much information on the intracellular localization of these enzymes within the mucosal cells. There is also little information available on the relative activities of glutamate–pyruvate transaminase and glutamate–oxaloacetate transaminase in various species and their role in the absorption of glutamate and aspartate. The purpose of the work described here was therefore to carry out a survey of the distribution and specific activities of glutamate–pyruvate transaminase, glutamate–oxaloacetate transaminase and glutamate dehydrogenase (EC 1.4.1.3) in the gastrointestinal tract of various species. Our ultimate aim is to compare in a quantitative fashion the results obtained and reported here, with the transamination observed to take place during absorption when glutamate, aspartate, and peptides containing these amino acids, or an enzymic hydrolysate of casein is introduced into the intestinal lumen.

METHODS

Materials

Sodium pyruvate, α-oxoglutarate, NADH, NAD^+, ADP, malate dehydrogenase, lactate dehydrogenase and glutamate dehydrogenase were obtained from Boehringer and Söhne GmbH., Mannheim, Germany. Bovine serum albumin from Armour Pharmaceutical Co. Ltd, Eastbourne, England.

Animals

Male albino rats (300–350 g), guinea pigs of both sexes (300–350 g), hamsters of both sexes (100–125 g), chickens (white Leghorn) of both sexes from 5 days to 3 weeks of age and adult mice and pigeons were used. Animals were of local laboratory stock maintained on a standard diet and unless specified, unfasted.

Preparation of homogenates

The abdomen of the decapitated animal is opened and the liver, proventriculus

(birds), stomach, duodenum, jejunum, ileum, caecum and colon removed. Hamster stomach is divided into two different and well defined parts. One part, proximal, is much paler than the other distal portion which is red and similar in appearance to the gastric mucosa of the other species. Both portions were examined separately. The luminal contents of the small intestine and colon were washed out with ice-cold phosphate buffer, concentration 10 mM, pH 7.4. The stomach, proventriculus and caecum were opened, and the contents washed away by agitating in a large volume of ice-cold phosphate buffer, concentration 10 mM, pH 7.4.

Intestinal segments were laid on absorbent paper, slit open longitudinally and the mucosa removed with a microscope slide. Gastric mucosa was plucked from the underlying muscle with forceps and the mucosa of the proventriculus was removed with a pair of scissors.

Whole liver and mucosal scrapings were homogenized in ice-cold phosphate buffer, concentration 10 mM, pH 7.4, for about 1 min at top speed with a motor-driven homogenizer (Silverson Machines Ltd, London). As judged by phase-contrast microscopy, this treatment was sufficient to break all cells. To certain homogenates from chicken, L-cysteine (final concentration 10 mM) was added to stabilize the soluble glutamate–pyruvate transaminase. Homogenates were filtered through nylon mesh and a portion of each was centrifuged at $100000 \times g$ for 30 min (Spinco model L, SW 39 rotor; Beckman Instruments Co.). To obtain maximal activities of glutamate dehydrogenase, homogenates were sonicated for 1 min at 100 W ('Ultrason' Sontegrator, M-147, M.S.E. Ltd), after which homogenates were centrifuged as described above. Homogenates, supernatants and resuspended sediments were used for enzyme analysis.

Glutamate–oxaloacetate transaminase activity

Measurement of glutamate–oxaloacetate transaminase activity was based on the method of Karmen [17] for serum. The enzyme mixture (total volume, 3.0 ml, pH 7.6) contained (final concentrations): sodium phosphate buffer (80 mM), NADH (160 μM), α-oxoglutarate (7 mM), L-aspartate (33 mM) and malate dehydrogenase (25 μg/3 ml). The reaction was started by adding L-aspartate (not α-oxoglutarate as stated by Bergmeyer and Bernt [17]). The mixture was maintained at 25 °C. Malate dehydrogenase was used as indicator enzyme to measure the oxaloacetate formed. The extent of oxidation of NADH to NAD^+ is proportional to the amount of oxaloacetate formed, and measured by the decrease in absorbance at 340 nm. Values were corrected for the oxidation of NADH due to the presence of glutamate dehydrogenase in the samples. Absorbance decreased linearly with time for at least 20 min after starting the reaction. For each sample two different amounts of enzyme were used, each in duplicate.

Glutamate–pyruvate transaminase activity

Measurement of glutamate–pyruvate transaminase activity was based on the 'optimum' method of Wroblewski and LaDue [18] for serum. The assay mixture (total volume, 3.0 ml, pH 7.6) contained (final concentrations): sodium phosphate buffer (80 mM), NADH (160 μM), lactate dehydrogenase (25 μg/3 ml), α-oxoglutarate (7 mM) and L-alanine (80 mM). The mixture was maintained at 25 °C. The reaction was started by adding L-alanine (not α-oxoglutarate as stated by Bergmeyer and

Bernt [18]). Lactate dehydrogenase was used as indicator enzyme to measure the pyruvate formed. The extent of oxidation of NADH to NAD^+ is proportional to the pyruvate formed, and measured as the decrease in absorbance at 340 nm. Values were corrected for the oxidation of NADH due to the presence of glutamate dehydrogenase in the sample. Absorbance decreased linearly with time for at least 20 min after starting the reaction. For each sample two different amounts of enzyme were used, each in duplicate.

It is important to correct for the presence of ammonia in tissue homogenates and in the commercial enzyme preparation used. This necessity follows from the fact that ammonia increases the oxidation of NADH through its participation in the glutamate dehydrogenase reaction:

$$NH_3 + \alpha\text{-oxoglutarate} + NADH + H^+ \rightleftarrows \text{glutamate} + NAD^+ + H_2O$$

The enzyme glutamate dehydrogenase is present in all the tissues examined (see below).

Glutamate dehydrogenase activity

Measurement of glutamate dehydrogenase activity was based on the method of Schmidt [19] for serum. The assay mixture (total volume, 3.0 ml, pH 7.6) contained (final concentrations): sodium phosphate buffer (80 mM), NADH (160 μM), α-oxoglutarate (7 mM), NH_4Cl (110 mM), EDTA (300 μM) and ADP (1.6 mM). The assay mixture was maintained at 25 °C. The oxidation of NADH to NAD^+ is directly proportional to the reduction of the substrate and was followed by the decrease in absorbance at 340 nm. Values were corrected for the oxidation of NADH in the absence of substrate. Absorbance decreased linearly with time for at least 20 min after starting the reaction. For each sample two different amounts of enzyme were used, each in duplicate.

Protein

Protein was determined by the method of Lowry et al. [20] using bovine serum albumin as a standard.

Expression of results

Results are expressed in terms of specific activity and as per cent of total activity in soluble and bound form. Units of specific activity are nmoles /mg protein per min. To obtain estimates of the soluble and bound fractions, the activity observed in soluble and particulate fractions of 1-ml aliquots of whole homogenate were compared with that of the whole homogenate.

RESULTS

Glutamate–oxaloacetate transaminase

Table I shows the specific activity of glutamate–oxaloacetate transaminase present in homogenates of mucosae from the gastrointestinal tract and for comparison that of homogenates of liver of different species. Glutamate–oxaloacetate transaminase was present in all the tissues studied of six species, namely rat, mouse, guinea pig, hamster, chicken and pigeon. The liver and gastric mucosa (or mucosa of avian

TABLE I

GLUTAMATE-OXALOACETATE TRANSAMINASE

Specific activity of glutamate–oxaloacetate transaminase in whole mucosal homogenates of tissues of gastrointestinal tract and in liver of fed rats, mice, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S.E. (No. of observations), —, tissue not present in these species. Units of specific activity are nmoles of substrate transaminated/mg protein per min.

Tissue	Specific activity					
	Rat	Mouse	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	—	481 ± 7 (3)	622 ± 35 (3)
Stomach	395 ± 18 (4)	365 (2)	282 ± 18 (4)	115 ± 7 (4)* 297 ± 10 (4)**	—	—
Duodenum	244 ± 14 (4)	314 (2)	132 ± 10 (3)	114 ± 4 (4)	131 ± 3 (3)	220 ± 4 (3)
Jejunum	176 ± 7 (4)	276 (2)	109 ± 6 (5)	108 ± 1 (4)	113 ± 4 (3)	144 ± 9 (3)
Ileum	134 ± 6 (5)	203 (2)	111 ± 4 (4)	97 ± 3 (4)	113 ± 13 (3)	128 ± 1 (3)
Caecum	141 ± 16 (4)	185 (2)	152 ± 7 (4)	81 ± 6 (4)	135 ± 9 (3)	—
Proximal colon	134 ± 5 (5)	181 (2)	141 ± 7 (4)	96 (2)	158 (2)	—
Distal colon	202 ± 22 (3)	—	152 ± 2 (3)	72 (2)	—	—
Liver	468 ± 42 (4)	540 (2)	227 ± 13 (4)	512 ± 26 (4)	301 ± 14 (3)	415 ± 44 (3)

* Non-glandular portion.

** Glandular (acid-secreting) portion.

proventriculus) possessed the highest activities while the intestinal mucosal tissues from duodenum to distal colon contained lower, but still substantial, amounts of glutamate–oxaloacetate transaminase. A notable feature was the similarity in content of glutamate–oxaloacetate transaminase of the small and the large intestinal mucosa. Data show no substantial differences between tissues of omnivores (rat, mouse, hamster) and herbivores (guinea pig), or between mammalian and avian species.

TABLE II

INSOLUBLE GLUTAMATE-OXALOACETATE TRANSAMINASE

Specific activity of glutamate–oxaloacetate transaminase in particulate material from mucosal homogenates of tissues of gastrointestinal tract and in liver of fed rats, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S E. (No. of observations); —, tissue not present in these species. Units of specific activity are nmoles of substrate transaminated/mg protein per min.

Tissue	Specific activity				
	Rat	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	147	191 ± 14 (3)
Stomach	110 ± 23 (3)	83 ± 6 (3)	83 (2)* 31 (1)**	—	—
Duodenum	127 ± 23 (3)	38 ± 5 (4)	43 (2)	48	108 ± 4 (3)
Jejunum	101 ± 24 (3)	40 ± 7 (3)	78 (2)	50	80 ± 6 (3)
Ileum	81 ± 13 (3)	34 ± 1 (3)	36 (2)	28	72 (2)
Caecum	96 (2)	101 ± 7 (3)	52 (1)	91	—
Proximal colon	66 (2)	41 ± 7 (3)	56 (1)	38	—
Distal colon	69 (2)	40 (2)	63 (1)	—	—
Liver	264 (2)	74 (2)	233 (2)	—	258 ± 25 (3)

* Non-glandular portion.

** Glandular (acid-secreting) portion.

TABLE III

SOLUBLE GLUTAMATE–OXALOACETATE TRANSAMINASE

Specific activity of glutamate–oxaloacetate transaminase in high-speed supernatants of homogenates of mucosal tissues of gastrointestinal tract and in liver of fed rats, mice, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S.E. (No. of observations), —, tissue not present in these species. Units of specific activity are nmoles of substrate transaminated/mg protein per min.

Tissue	Specific activity					
	Rat	Mouse	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	—	799 ± 12 (4)	1074 ± 58 (3)
Stomach	565 ± 40 (4)	499 (2)	425 ± 22 (4)	158 ± 2 (3)* 470 ± 46 (3)**	—	—
Duodenum	312 ± 10 (4)	441 (2)	219 ± 14 (5)	171 ± 7 (3)	218 ± 13 (3)	328 ± 17 (3)
Jejunum	252 ± 18 (4)	329 (2)	201 ± 8 (5)	142 ± 3 (3)	178 ± 10 (3)	222 ± 23 (3)
Ileum	181 ± 11 (4)	283 (2)	213 ± 2 (4)	142 ± 8 (3)	183 ± 12 (3)	249 (2)
Caecum	171 ± 9 (3)	298 (2)	222 ± 6 (4)	118 ± 6 (3)	195 ± 6 (3)	—
Proximal colon	221 ± 13 (4)	267 (2)	250 ± 7 (3)	137 (1)	235 ± 6 (3)	—
Distal colon	296 (2)	—	270 ± 1 (3)	124 (1)	—	—
Liver	735 ± 58 (3)	680 (2)	357 ± 40 (3)	776 ± 8 (3)	521 (2)	—

* Non-glandular portion.

** Glandular (acid-secreting) portion.

Tables II and III show, respectively, the specific activities of the total particulate and soluble matter from the tissues studied. The results again showed a remarkable uniformity throughout the tissues studied in all six species. The specific activities of the soluble fractions were higher than those of the whole homogenates, while the specific activities of the total particulate fractions were lower than those of the homogenates. Between 10 and 25% of the glutamate–oxaloacetate transaminase activity was found in particulate matter (Table IV).

TABLE IV

GLUTAMATE–OXALOACETATE TRANSAMINASE

Percentage of particle-bound glutamate–oxaloacetate transaminase in mucosal homogenates of tissues of gastrointestinal tract and in liver of fed rats, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S E. (No. of observations); —, tissue not present in these species.

Tissue	Particle bound (%)				
	Rat	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	17 (2)	14 ± 1 (3)
Stomach	10 ± 2 (3)	11 ± 1 (3)	22 ± 3 (3)* 6 (2)**	—	—
Duodenum	13 ± 3 (3)	13 ± 1 (3)	12 (2)	20 (2)	19 ± 1 (3)
Jejunum	20 ± 5 (3)	15 ± 2 (2)	21 ± 2 (3)	25 (2)	19 ± 2 (3)
Ileum	18 ± 1 (3)	13 ± 1 (3)	15 ± 1 (3)	19 (2)	18 ± 5 (3)
Caecum	22 (2)	26 ± 3 (3)	23 ± 3 (3)	25 (1)	—
Proximal colon	10 (2)	12 ± 1 (3)	20 ± 3 (3)	10 (1)	—
Distal colon	13 (2)	10 (2)		—	—
Liver	22 (2)	12 (2)	15 ± 2 (3)	22 (1)	22 ± 1 (3)

* Non-glandular portion.

** Glandular (acid-secreting) portion.

Glutamate–pyruvate transaminase

Table V shows the specific activities of glutamate–pyruvate transaminase measured in homogenates of mucosae of the gastrointestinal tract and in homogenates of liver from rat, mouse, guinea pig, hamster, pigeon and chicken.

The distribution of glutamate–pyruvate transaminase is very different from that of glutamate–oxaloacetate transaminase (Table I). In all six species the liver and small intestinal mucosa contained the highest specific activities with little or none

TABLE V

GLUTAMATE–PYRUVATE TRANSAMINASE

Specific activity of glutamate–pyruvate transaminase in whole homogenates of mucosal tissues of gastrointestinal tract and in liver of fed rats, mice, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S.E. (No. of observations), —, tissue not present in these species. Units of specific activity are nmoles of substrate transaminated/mg protein per min.

Tissue	Specific activity					
	Rat	Mouse	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	—	0	0
Stomach	130 ± 10 (5)	81 (2)	30 ± 3 (4)	12 ± 1 (3)*	—	—
				24 ± 2 (3)**		
Duodenum	271 ± 9 (4)	87 (2)	26 ± 2 (4)	56 ± 8 (3)	47 ± 6 (7)	28 ± 5 (3)
Jejunum	281 ± 18 (4)	148 (2)	37 ± 4 (4)	42 ± 1 (3)	13 ± 1 (7)	15 ± 1 (3)
Ileum	199 ± 7 (5)	120 (2)	48 ± 2 (4)	25 ± 3 (3)	6 ± 1 (7)	21 ± 2 (3)
Caecum	34 ± 3 (5)	22 (2)	0	7 ± 1 (3)	0	—
Proximal colon	42 ± 2 (5)	19 (2)	0	0	0	—
Distal colon	29 (1)	—	0	0	—	—
Liver	132 ± 10 (3)	201 (2)	35 ± 1 (3)	156 ± 10 (3)	16 ± 3 (8)	51 ± 6 (3)

* Non-glandular portion.

** Glandular (acid-secreting) portion.

TABLE VI

SOLUBLE GLUTAMATE–PYRUVATE TRANSAMINASE

Specific activity of glutamate–pyruvate transaminase in high-speed supernatants of homogenates of mucosal tissues of gastrointestinal tract and in liver of fed rats, mice, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S.E. (No. of observations); —, tissue not present in these species. Units of specific activity are nmoles of substrate transaminated/mg protein per min.

Tissue	Specific activity					
	Rat	Mouse	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	—	0	0
Stomach	189 ± 7 (4)	123 (2)	52 ± 6 (4)	14 ± 0 (4)*	—	—
				45 ± 3 (4)**		
Duodenum	415 ± 2 (4)	130 (2)	50 ± 3 (4)	89 ± 11 (4)	18 ± 6 (6)	40 ± 6 (3)
Jejunum	453 ± 37 (4)	264 (2)	73 ± 6 (4)	71 ± 10 (3)	6 ± 1 (8)	19 ± 2 (3)
Ileum	292 ± 20 (4)	202 (2)	96 ± 2 (4)	40 ± 7 (3)	3 ± 0 (5)	27 ± 2 (3)
Caecum	62 ± 5 (3)	40 (2)	0	12 ± 1 (3)	0	—
Proximal colon	58 ± 4 (3)	41 (2)	0	19 (1)	0	—
Distal colon	—	—	0	—	—	—
Liver	184 (2)	278 (2)	46 (2)	237 ± 14 (4)	81 ± 10 (4)	66 ± 8 (3)

* Non-glandular portion.

** Glandular (acid-secreting) portion.

present in the large intestinal mucosa. The mammalian stomach also contained glutamate–pyruvate transaminase with appreciable activity. The specific activities of glutamate–pyruvate transaminase in the gastrointestinal mucosae from rat were nearly double those in the corresponding tissues from mouse, but were 5–7-fold greater than those in hamster and guinea pig, and 10–20 fold greater than the activities in chick and pigeon.

Table VI shows the specific activities in the soluble fraction from the tissues studied. Again the results show the great interspecies variation. In each mammalian species the specific activity of the soluble fraction was up to 2-fold higher than that of the homogenate (Table V), while the activity in the total particulate fraction when compared with homogenates was very small. In mammals at least 90% of the total activity of unit volume of homogenate appeared to be associated with the soluble fraction (unpublished experiments). In homogenates of avian tissues, on the other hand, a more substantial proportion of the total glutamate–pyruvate transaminase activity present in unit volume of homogenate was particle bound (Tables VII and VIII). Thus in homogenates of chick tissues 70–80% of the homogenate activity was associated with the particulate fraction, and in pigeon tissues homogenates 30–40% was particle bound (Tables VII and VIII).

In tissue homogenates, the activity of mitochondrial glutamate–pyruvate

TABLE VII

GLUTAMATE–PYRUVATE TRANSAMINASE

Specific activity of glutamate–pyruvate transaminase in homogenates, high-speed supernatants and particulate fractions of mucosal tissues of gastrointestinal tract and in liver of fed chickens and percentage of total activity of soluble and particulate glutamate–pyruvate transaminase. Values are of means ± S.E. (No. of experiments). Units of specific activity are nmoles of substrate transaminated /mg protein per min.

Tissue	Specific activity			Total activity (%)	
	Homogenate	Soluble	Particulate	Soluble	Particulate
Duodenum	47.3 ± 5.9 (7)	18.4 ± 5.9 (6)	69.4 ± 11.0 (6)	22 ± 6 (6)	78 ± 6 (6)
Jejunum	13.3 ± 1.3 (7)	6.2 ± 1.0 (8)	19.3 ± 2.0 (7)	28 ± 4 (8)	72 ± 4 (8)
Ileum	5.5 ± 0.7 (7)	2.5 ± 0.3 (5)	11.4 ± 1 4 (4)	21 ± 2 (4)	79 ± 2 (4)
Liver	15.6 ± 2.7 (8)	8.1 ± 1.0 (4)	22.4 ± 1.9 (4)	29 ± 4 (5)	71 ± 4 (5)

TABLE VIII

GLUTAMATE–PYRUVATE TRANSAMINASE

Specific activity of glutamate–pyruvate transaminase in homogenates, high-speed supernatants and particle fractions of mucosal tissues of gastrointestinal tract and in liver of fed pigeons and percentage of total activity of soluble and particulate glutamate–pyruvate transaminase. Values are means + S.E. of three experiments. Units of specific activity are nmoles of substrate transaminated/mg protein per min.

Tissue	Specific activity			Total activity (%)	
	Homogenate	Soluble	Particulate	Soluble	Particulate
Duodenum	28.3 ± 4.8	39.9 ± 6.2	15.3 ± 1.2	71 ± 5	29 ± 5
Jejunum	15.4 ± 0.6	18.5 ± 1.9	21 7 ± 2 7	66 ± 6	34 ± 6
Ileum	21.4 ± 1.5	26.9 ± 2.3	19.4 ± 1.6	59 ± 3	41 ± 3
Liver	51.1 ± 5.6	65.7 ± 8.3	44.8 ± 8.8	64 ± 4	36 ± 5

transaminase is evidently completely exposed, since sonication of the particulate fraction did not further increase the enzyme activity. Also, extraction of the mitochondrial glutamate–pyruvate transaminase proved to be very difficult. For these reasons the data presented are felt to provide good estimates of the specific activities of soluble and mitochondrial enzymes of mammalian tissues. However, in preliminary experiments, it was found that the soluble enzyme from chick tissues was very labile, virtually no activity remaining after storage for 24 h at 4 °C. This lability raised the possibility that the proportion of particulate glutamate–pyruvate transaminase activity in chick intestinal mucosal homogenates might have been exaggerated. Addition of L-cysteine (final concentration 10 mM) to the homogenizing medium appeared, in fact, effectively to stabilize the enzyme; glutamate–pyruvate transaminase activity was measured within 10 min after homogenization and remained constant for at least 24 h. This lability in the absence of L-cysteine was not due to the presence in chick small intestine of an inhibitor to glutamate–pyruvate transaminase because when an homogenate from small intestine of chick was added to an homogenate from rat jejunum the activity observed was the sum of the activities measured in each homogenate separately. In the mixture of the two homogenates the decrease in glutamate–pyruvate transaminase activity with time was similar to that observed in the untreated homogenate from chick jejunum.

Glutamate dehydrogenase

Table IX shows the specific activity of glutamate dehydrogenase in sonicated mucosal homogenates of some tissues of the gastrointestinal tract and also in sonicated homogenates of liver from rat, guinea pig, hamster, chick and pigeon. Activities were measured according to the method of Schmidt [19], except that ADP (final concentration 1.6 mM) was added to the assay mixture to obtain maximal activities [21, 22]. In all five species examined, the liver possessed the highest specific activity,

TABLE IX

GLUTAMATE DEHYDROGENASE

Specific activity of glutamate dehydrogenase in mucosal tissues of gastrointestinal tract and in liver of fed rats, guinea pigs, hamsters, chickens and pigeons. Values are of means ± S.E. (No. of observations), —, tissue not present in these species. Homogenates were sonicated for 1 min. Units of specific activity are nmoles of NADH oxidized/mg protein per min

Tissue	Specific activity				
	Rat	Guinea pig	Hamster	Chicken	Pigeon
Proventriculus	—	—	—	61 ± 4 (7)	93 (1)
Stomach	66 ± 3 (4)	77 ± 3 (4)	61 ± 3 (3)*	—	—
			65 ± 3 (3)**		
Duodenum	122 ± 11 (4)	85 ± 10 (3)	139 ± 7 (4)	74 ± 4 (5)	57 (2)
Jejunum	106 ± 4 (4)	99 ± 3 (3)	91 ± 5 (4)	63 ± 5 (4)	63 (2)
Ileum	183 ± 13 (4)	89 ± 2 (4)	103 ± 0 (3)	87 ± 3 (3)	75 (2)
Caecum	111 ± 11 (4)	54 ± 1 (4)	130 ± 10 (3)	57 ± 4 (5)	—
Proximal colon	124 ± 11 (4)	50 ± 5 (3)	126 ± 12 (3)	100 ± 6 (3)	—
Distal colon	122 (1)	68 ± 5 (3)	81 ± 8 (3)	—	—
Liver	725 ± 36 (4)	867 ± 55 (4)	720 ± 55 (4)	818 ± 38 (4)	594 (2)

* Non-glandular portion

** Glandular (acid-secreting) portion.

while the mucosal tissues from stomach to distal colon each contained much lower levels of glutamate dehydrogenase activity (about 10% of the liver specific activity). The gastric, small and large intestinal mucosae had similar activities, and there was little interspecies variation.

DISCUSSION

Distribution of glutamate–oxaloacetate transaminase and glutamate–pyruvate transaminase in the gastrointestinal tract

In all tissues of the gastrointestinal tract studied, glutamate–oxaloacetate transaminase was found to be present with relatively high specific activities and distributed in a similar fashion in all tissues from all the species studied.

Glutamate–pyruvate transaminase was also found in the mucosa of the small intestine of all the species examined, in the stomach of mammals and in the large intestinal mucosa of the rat and the mouse. There is considerable species variation in the specific activities of the various tissues studied. Thus, the glutamate–pyruvate transaminase activities of the small intestine of rat and mouse were much higher than those in the other four species. Furthermore, an unexpected finding was that the glutamate–pyruvate transaminase activity of rat small intestinal mucosa was more than twice that in rat liver tissue.

An important finding was that the distribution of glutamate–pyruvate transaminase was rather selective, and quite different from the distribution of glutamate–oxaloacetate transaminase. Most of the glutamate–pyruvate transaminase activity was confined to the small intestine, with little or none in the large intestinal mucosa; in contrast, glutamate–oxaloacetate transaminase was more uniformly distributed in the tissues of the gastrointestinal tract. Also, glutamate– oxaloacetate transaminase did not show the large interspecies variation found for glutamate–pyruvate transaminase.

The subcellular distribution of glutamate–oxaloacetate transaminase and glutamate–pyruvate transaminase in the gastrointestinal tract

Glutamate–oxaloacetate transaminase. The tissue and subcellular distribution of the two glutamate–oxaloacetate transaminase isoenzymes was similar in all tissues; of the total glutamate–oxaloacetate transaminase activity present in homogenates of each tissue examined 10–25% was associated with the particulate matter. Subcellular fractionation of a mucosal homogenate of rat jejunum indicated that most of this particulate activity was localized in the mitochondrial fraction (unpublished work). Other investigators have reported that in most tissues of rat, mouse and man (including the small intestine of the rat) the larger portion of the total glutamate–oxaloacetate transaminase activity was associated with the mitochondrial fraction [23–25]. The lower values for particulate glutamate–oxaloacetate transaminase observed here in tissues of the gastrointestinal tract and in liver was probably due to the partial solubilization of the mitochondrial enzyme before fractionation.

Glutamate–pyruvate transaminase. In the mucosae of the gastrointestinal tract and the liver of mammals the particulate enzyme contributed only a very small proportion (5–10%) to the total glutamate–pyruvate transaminase activity present in unit volume of homogenate. In the rat, this particulate activity was confined to the

mitochondrial fraction of the intestinal mucosal cell (unpublished work). These findings are in agreement with the results obtained by Swick et al. [26] for rat liver. The much higher values reported for mitochondrial glutamate–pyruvate transaminase by Kafer and Pollak [27], and by Katunuma et al. [28], can partly be explained by the fact that these workers did not correct their values for the relatively high rate of oxidation of NADH by glutamate dehydrogenase in the presence of ammonia.

In contrast to mammals, in avian small intestinal mucosa and liver a much more substantial proportion of the total glutamate–pyruvate transaminase activity of the homogenates was particle bound (Tables VII and VIII). No further fractionation was performed so that it is not known to which particular structure this activity was confined. Generally, very little is known about the occurrence and subcellular distribution of aminotransferases in avian tissues.

We conclude that there is thus evidence for the presence of at least two different varieties of glutamate–oxaloacetate transaminase and glutamate–pyruvate transaminase in mucosae of the gastrointestinal tract, one in the cytosol and one in the particulate fractions of the cell.

Possible functions for glutamate–pyruvate transaminase and glutamate–oxaloacetate transaminase in the gastrointestinal tract

The distribution of glutamate–pyruvate transaminase suggests that the significance of this enzyme in the small intestine may be related to the transamination of glutamate during absorption. The rise in the glutamate–pyruvate transaminase level of rat small intestine which starts shortly after birth supports this view [29]. Likewise, a possible function of glutamate–oxaloacetate transaminase in the small intestine may be related to the absorption of aspartate. Glutamate and aspartate do not occur in the portal blood in appreciable amounts in contrast to their high concentrations in dietary proteins: it is therefore thought that amino-*N* of the dicarboxylic amino acid is transported from the intestine to the liver in the form of alanine. However, unlike glutamate–pyruvate transaminase, the distribution of glutamate–oxaloacetate transaminase (with the highest specific activity in the stomach and no difference between small and large intestine) does not correlate with what is known of the location of amino acid absorption. Whatever its role in amino acid absorption, glutamate–oxaloacetate transaminase must have other functions in the gastrointestinal tract.

Distribution and role of glutamate dehydrogenase in the gastrointestinal tract

In spite of the large differences in the nature of the diet of the species examined (omnivore, herbivore, avian) there is remarkably little variation in the distribution and activity of glutamate dehydrogenase in the gastrointestinal tract. The specific activities found here for glutamate dehydrogenase in rat small intestine and liver are similar to those found by other workers.

In liver and kidney, the tissues with the highest activities, the reaction catalysed by glutamate dehydrogenase is of central importance. Glutamate formation from α-oxoglutarate and ammonia permits the synthesis of non-essential amino acids, in particular aspartate, by transamination, while the reverse sequence of reactions results in the oxidative deamination of the amino acids with the formation of α-oxoglutarate and ammonia. In the presence of glucose, rat small intestine does not measurably degrade glutamate, as measured by the ammonia liberated during absorption (un-

published experiments). Nor does rat intestine appear to be of importance for the synthesis of glutamate. Thus the addition in vitro of NH_4^+ and either α-oxoglutarate or pyruvate to the luminal fluid results in only a minor increase in the alanine appearing on the serosal side (unpublished experiments). Glutamate dehydrogenase in the gastrointestinal tract has no apparent role in relation to the breakdown and synthesis of amino acids of dietary origin.

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