J. Physiol. (1958) 140, 148–155

THE ALANINE AND OXO ACID CONCENTRATIONS IN MESENTERIC BLOOD DURING THE ABSORPTION OF L-GLUTAMIC ACID BY THE SMALL INTESTINE OF THE DOG, CAT AND RABBIT *IN VIVO*

By K. D. NEAME and G. WISEMAN

From the Department of Physiology, University of Sheffield

(*Received* 12 *August* 1957)

It has been shown by Neame & Wiseman (1957) that the absorption of L-glutamic acid and aspartic acid by the dog's small intestine *in vivo* results in an increased alanine concentration in the blood passing through the intestine, and their findings suggest that when protein is being digested and glutamic acid liberated in relatively low concentration in the intestinal lumen almost all the glutamic acid is involved in the transamination reaction. These observations explain the finding of a lower level of glutamic acid than was expected in the portal blood after feeding casein to dogs (Dent & Schilling, 1949) and an examination of their chromatogram shows an unexpected increase in blood alanine.

The study of transamination by the small intestine *in vivo* has now been carried further and the experiments described below show that the phenomenon also occurs in the cat and rabbit. The mesenteric blood concentrations of pyruvate and α-oxoglutarate have also been investigated during the absorption of glutamic acid by the dog, cat and rabbit, but no significant relationship was found between any changes in their concentrations and the transamination that was occurring.

METHODS

Experimental procedure

Dogs weighing between 8 and 22 kg and cats and rabbits weighing between 1·6 and 3·0 kg were used, and were allowed only water during the 24 hr before the experiment. Pentobarbitone sodium B.P. was used as the anaesthetic, the dogs receiving about 50 mg/kg body weight (intravenously), the cats about 50 mg/kg body weight (intraperitoneally), and the rabbits about 60 mg/kg body weight (intravenously). Artificial respiration via an intratracheal cannula was maintained throughout all experiments by means of an 'Ideal' pump (C. F. Palmer, Ltd., London) and amounted to about 0·5 l./kg body weight/min in the case of dogs, about 0·6 l./kg body weight/min in the case of cats, and about 0·7 l./kg body weight/min for rabbits. The artificial respiration was begun as soon as possible after anaesthesia had been induced.

The abdomen was opened by a mid-line incision, and in readiness for the taking of blood from the mesenteric venous trunk the latter was located and carefully cleared distal to its junction with the gastrosplenic vein. The mesenteric artery was then cleared and a loose thread ligature

placed around it ready for ligation towards the end of the experiment (see below). The abdominal aorta was next cleared ready for the taking of an arterial blood sample. Then the small intestine was ligated in all three animals at about the middle of the duodenum, this being the approximate dividing line between blood draining into the gastrosplenic vein and blood draining into the superior mesenteric vein. The small intestine was further ligated just proximal to the ileocaecal junction in the case of the dog and cat, and in the case of the rabbit at a point level with the tip of the appendix, which lies in conjunction with the lower end of the ileum in this animal. A cannula was introduced into each end of the experimental portion of the small intestine (the part between the ligatures) and isotonic saline (at 37° C) was used to wash out the intestinal lumen. Any residual saline was then gently expelled from the intestinal lumen, a ligature was tied around each end of the experimental portion of intestine so as to exclude the cannulae, and the cannulae were then removed.

In the dog and cat the mesenteric venous blood collected comes from the small and large intestine, though mainly from the small intestine. In the rabbit, however, a large part of this mixed blood is derived from the large intestine, which, in this animal, has a relatively large bulk, and therefore the blood from the experimental portion of intestine may be considerably diluted by blood from the large intestine. In view of this, two series of experiments were carried out on the rabbit. In one series both the large and small intestine were left *in situ*, while in the other series that part of the intestine distal to the experimental portion of small intestine was removed immediately after opening the abdomen. In practice it was found necessary to leave *in situ* a short length of the colon, some of which has a mesentery in common with part of the small intestine.

In all except control experiments a neutralized 2% L-glutamic acid solution (6–10 ml./kg body weight) at 37° C was introduced into the upper end of the lumen of the experimental portion of intestine and gently massaged down the intestine so as to distribute it evenly. The abdominal wall was then closed after a thermometer had been inserted, and the animal left undisturbed for 30 min. At the end of this period the abdomen was reopened, the closed tip of a pair of Doyen's scissors passed under the portion of mesenteric vein previously cleared, and the vein raised on the blunt edge of the scissors so as to obstruct any back-flow of blood along the mesenteric vein during collection of the venous sample. The blood was drawn from the mesenteric vein into a syringe moistened with heparin solution. When sufficient had been obtained (10–20 ml.) the vein was clamped and the ligature round the mesenteric artery tied to prevent loss of blood into the intestinal vessels (although this was not necessary in the case of the dog). About 1 ml. of the blood taken (to be used for oxo acid estimation) was transferred rapidly into each of three 15 ml. weighed centrifuge tubes, each containing 3 ml. of distilled water and 4 ml. of 10% trichloroacetic acid. The exact volume of blood added to each tube was determined by weighing. The remainder of the venous sample (to be used for glutamic acid and alanine estimations) was not deproteinized until after an arterial blood sample had been taken from the abdominal aorta, and after the experimental portion of intestine had been removed and drained of its contents. The time interval between the taking of the mesenteric venous sample and the arterial sample was usually 1·5–2 min and never more than 2·5 min. In view of the instability of pyruvate in freshly drawn blood (Bueding & Wortis, 1940; Long, 1944) the exact time interval between the venepuncture and the deproteinization of the venous blood was noted; the time between the taking and the deproteinization of the arterial blood was made to correspond with this interval in order to compensate for any losses in the pyruvate between the drawing of the blood and its deproteinization. This time interval was usually between 1 and 1·5 min and never more than 2 min.

In control experiments the same procedure was carried out, except that the small intestine was left empty after the initial washing out with saline.

Chemical methods

Amino acid solutions. The L-glutamic acid was a commercial sample (L. Light and Co., Ltd.) used without further purification, and gave the theoretical yield of gas on decarboxylation. It was

dissolved in distilled water with the aid of NaOH and the final solution was adjusted to about pH 6 (chlorophenol red as indicator).

Treatment of blood samples. The initial treatment of the blood samples for the estimation of oxo acids has been described above. After weighing the tubes to which the blood had been added, the deproteinized blood in each tube was thoroughly mixed, centrifuged at 2500 r.p.m. for 10 min and the supernatant filtered. Blood for glutamic acid and alanine estimation was well stirred and deproteinized as described by Neame & Wiseman (1957).

Recovery of residual amino acids from the intestinal lumen. At the end of each experiment the experimental portion of intestine was drained and washed out with isotonic saline. A sample was deproteinized and its glutamic acid content estimated as described by Neame & Wiseman (1957).

Estimation of amino acids. L-Glutamic acid was estimated by the manometric method of Gale (1945) and alanine by the colorimetric method of Alexander & Seligman (1945) as modified by Christensen, Riggs & Ray (1952).

Estimation of oxo acids. Pyruvic acid and α-oxoglutaric acid were estimated by the method of Goodwin & Williams (1952) with certain modifications. 6 ml. of each blood filtrate was pipetted into a 50 ml. separating funnel, which was then brought to 25° C in a water-bath (10 min being needed for this purpose). 1 ml. of 0·1% (w/v) 2:4-dinitrophenylhydrazine in 2 N-HCl was added and the separating funnel allowed to stand at room temperature for 25 min. The 2:4-dinitrophenylhydrazones of the oxo acids were then extracted with 4 ml. of diethyl ether by shaking for 1 min. The two layers were allowed to separate and the aqueous (lower) layer discarded. In some cases the ethereal layer was not clear but, after draining off most of the aqueous layer, clearing could be induced by giving two or three firm shakes to the separating funnel. The ethereal layer was extracted with 4 ml. of 15% (w/v) Na_2CO_3 by shaking for 1 min and the Na_2CO_3 layer transferred to a test-tube by means of a Pasteur pipette. 2 ml. of this was treated with 1 ml. of 3 N-NaOH and the optical density was estimated in a Hilger Uvispek spectrophotometer at 414 mμ (isosbestic point) and at 443 mμ (maximal absorption of pyruvic acid 2:4-dinitrophenylhydrazone). Standard solutions of pyruvate and α-oxoglutarate were taken through this entire procedure and all oxo acid estimations carried out on the same day as the experiment. The values for total oxo acid concentrations obtained from replicate samples were usually within 1%.

Paper chromatography of oxo acids. The remainder of each Na_2CO_3 extract was acidified by the dropwise addition of conc. HCl until effervescence no longer occurred and the yellow colour of the solution had become pale. The 2:4-dinitrophenylhydrazones were then extracted with 4 ml. of diethyl ether and the ethereal layer transferred to a shallow tube, evaporated to dryness *in vacuo* at room temperature, and stored at −20° C until required for chromatography. (Evaporation to dryness is effected more rapidly if the last stages of the acidification are carried out carefully with dilute HCl.) The 2:4-dinitrophenylhydrazones were dissolved in about 0·5 ml. of diethyl ether, and 0·1 ml. applied to Whatman No. 1 filter paper and dried in a stream of warm air. Ascending chromatograms were run for 36 hr using butanol saturated with N-NH_4OH as solvent (Cavallini, Frontali & Toschi, 1949).

Oxo acid standards. Commercial samples (L. Light and Co. Ltd.) of sodium pyruvate and α-oxoglutaric acid were used as standards without further purification. The purity of the sodium pyruvate was found to be 81% by the manometric method described by Umbreit, Burris & Stauffer (1949). The purity of the α-oxoglutaric acid was assessed by the manometric method of Krebs (1950), and found to be 97%.

Identification of parts drained by the vein used for venepuncture

The animals used for this purpose were killed by an overdose of pentobarbitone sodium B.P. administered as described previously. The abdomen was opened, the mesenteric vein identified and a loose ligature tied around it. The vascular system of the intestine was washed out with isotonic saline introduced into the aorta or mesenteric artery (using a pressure of about 140 mm Hg), depending upon the size of the animal. A cannula connected to a pressure bottle containing blue Neoprene Latex (B. B. Chemical Co., Ltd., Leicester) from which all air bubbles were care-

fully excluded, was then inserted into the portal vein as near the liver as possible. A pressure of about 20 mm Hg was then exerted on the Neoprene Latex to induce passage into the intestinal vessels. As soon as the Neoprene Latex had reached the antimesenteric side of the intestine the pressure was released, a ligature tied around the portal vein distal to the tip of the cannula, and the cannula removed. The stomach, intestine and spleen were then removed *in toto* and placed in a 5% solution of formaldehyde acidified with HCl (the pH being about 2). After 2–4 hr the specimen can be dissected and the vessels exposed and examined. The site used for venepuncture for the blood samples during an absorption experiment is easily identified.

RESULTS

The results show that during the absorption of glutamic acid by the small intestine of the dog, cat and rabbit there is an increase in the concentration of alanine in the mesenteric blood as it passes through the small intestine. The phenomenon can be easily demonstrated in the dog and cat even when the large intestine is left *in situ* (as is shown by the results in Tables 1 and 2). In the case of the rabbit, however, when the entire intestine is left *in situ* the difference between the alanine concentration in the arterial and the mesenteric venous blood during the absorption of glutamic acid shows either no change (Table 3; Expts. 1–3 and 6) or a definite rise (Table 3; Expts. 4, 5 and 7). However, when the large intestine of the rabbit is removed before the experimental period has begun the mesenteric venous blood shows a much greater

TABLE 1. Alanine, pyruvate and α-oxoglutarate concentrations in arterial (Art.) and mesenteric venous (Mes.) blood taken 30 min after the introduction of 2% L-glutamic acid solution into the lumen of the small intestine of the dog. (Small and large intestine *in situ*. In the control experiments the small intestine was left empty.)

	Alanine (μmole/100 ml.)		Pyruvate (μmole/100 ml.)		α-Oxoglutarate (μmole/100 ml.)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt.)	% of amount introduced
1	133	151	43	41	0	0	4649	580	87
2	56	79	22	22	4	5	11430	820	65
3	79	113	23	23	2	1	9250	840	69
Control 1	75	66	41	37	1	1	—	—	—
Control 2	42	46	9	9	0	0	—	—	—

TABLE 2. Alanine, pyruvate and α-oxoglutarate concentrations in arterial (Art.) and mesenteric venous (Mes.) blood taken 30 min after the introduction of 2% L-glutamic acid solution into the lumen of the small intestine of the cat. (Small and large intestine *in situ*. In the control experiments the small intestine was left empty.)

	Alanine (μmole/100 ml.)		Pyruvate (μmole/100 ml.)		α-Oxoglutarate (μmole/100 ml.)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt.)	% of amount introduced
1	78	124	19	26	5	5	1403	830	94
2	108	169	21	24	2	2	2320	800	86
3	25	51	10	11	1	1	1706	1070	95
Control 1	47	40	14	12	1	1	—	—	—
Control 2	64	63	15	16	1	2	—	—	—

alanine concentration than the arterial blood during the absorption of glutamic acid (Table 4). The reason for this variability in the experimental findings in the case of the rabbit when the entire intestine is left *in situ* is that the contribution of venous drainage from the large intestine to the common mesenteric vein is so much greater in the rabbit than in the dog or cat. Determination of the parts of the intestine drained by the vein from which venous blood samples were collected showed that in the dog and cat the vein used for venepuncture drained mainly the small intestine, and only a small amount of blood came from the large intestine. In the rabbit, however, where the large intestine comprises much of the bulk of the total intestine, the venous drainage from the small intestine appeared to be only slightly greater than the venous drainage from the large intestine. In the experiments carried out on the rabbit after the removal of the large intestine, blood from the small intestine was no longer diluted by blood from the large intestine. A rise in alanine concentration in the blood passing through the intestine could then be clearly demonstrated. The results with the rabbit show the importance of determining the area drained by the common mesenteric vein in this type of experiment.

TABLE 3. Alanine, pyruvate and α-oxoglutarate concentrations in arterial (Art.) and mesenteric venous (Mes.) blood taken 30 min after the introduction of 2% L-glutamic acid solution into the lumen of the small intestine of the rabbit. (Small and large intestine *in situ*. In the control experiment the small intestine was left empty)

	Alanine (μmole/100 ml.)		Pyruvate (μmole/100 ml.)		α-Oxoglutarate (μmole/100 ml.)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt.)	% of amount introduced
1	126	124	66	34	3	5	1090	450	41
2	119	119	53	46	2	3	998	590	38
3	92	99	37	33	2	2	1450	630	52
4	130	147	50	40	7	6	1440	480	43
5	135	163	65	48	2	3	1600	670	52
6	154	159	62	49	3	5	1260	440	37
7	131	181	68	55	2	2	1690	840	62
Control	99	90	53	37	2	1	—	—	—

TABLE 4. Alanine, pyruvate and α-oxoglutarate concentrations in arterial (Art.) and mesenteric venous (Mes.) blood taken 30 min after the introduction of 2% L-glutamic acid solution into the lumen of the small intestine of the rabbit. (Only small intestine *in situ*. In the control experiments the small intestine was left empty)

	Alanine (μmole/100 ml.)		Pyruvate (μmole/100 ml.)		α-Oxoglutarate (μmole/100 ml.)		Glutamic acid absorbed		
Expt. no.	Art.	Mes.	Art.	Mes.	Art.	Mes.	(μmole)	(μmole/kg body wt.)	% of amount introduced
1	104	153	36	30	10	11	935	580	42
2	217	286	87	57	9	6	2350	940	67
3	208	297	45	25	9	10	1910	710	60
4	153	230	67	41	7	13	1460	660	50
Control 1	127	124	76	53	4	3	—	—	—
Control 2	125	114	40	32	4	4	—	—	—

In control experiments with the small intestine empty there was no appreciable difference in the alanine concentration between the arterial blood and mesenteric venous blood.

In some of the experiments on dogs, cats and rabbits in which the whole intestine was left *in situ*, glutamic acid was determined in both arterial and venous blood at the end of the experimental period. This was not possible in experiments on rabbits with the large intestine removed, owing to insufficient blood. In the dog the mesenteric venous concentration of glutamic acid was from 25 to 83 μmole/100 ml. more than that in the arterial blood. With the cat the mesenteric venous concentration was from 57 to 144 μmole/100 ml. higher than the arterial level. In both these animals the arterial concentration of glutamic acid at the end of the experiments was about 140 μmole/100 ml., compared with a value of about 90 μmole/100 ml. in the control experiments. In the rabbit with the whole intestine *in situ* there was no significant difference between the arterial and mesenteric venous concentrations of glutamic acid at the end of the experimental period. These results show that, with the amounts of glutamic acid introduced into the lumen of the intestine in these experiments, some glutamic acid does appear in the blood passing through the intestine of the dog and cat.

As the glutamic acid left in the experimental portion of intestine at the end of the experimental period was determined, the amount which had disappeared from the lumen during this period could be calculated. In the case of the dog it was found that an average of 74% of the glutamic acid introduced had disappeared by the end of 30 min; in the cat an average of 92% disappeared, and in the rabbit an average of 49% disappeared.

The concentrations of pyruvate and α-oxoglutarate in the mesenteric venous blood from the experimental portion of intestine were not appreciably different from their concentrations in the arterial blood, except in the case of the rabbit, where the arterial pyruvate concentration was higher than the mesenteric venous concentration under both experimental and control conditions.

The oxo acid 2:4-dinitrophenylhydrazones derived from the blood samples collected at the end of the experimental periods were examined by one-dimensional paper chromatography. It was found that only pyruvate and α-oxoglutarate were present in arterial and venous blood collected both from control loops of intestine and from intestine absorbing glutamic acid.

DISCUSSION

The results show that when glutamic acid is absorbed from the small intestine of the dog, cat and rabbit, a rise in the concentration of alanine occurs in the blood passing through the intestine. This is clearly demonstrable in the case of the cat and the dog in spite of dilution of the blood from the small intestine

with blood from the large intestine. In the rabbit, however, the difference between the concentration of alanine in the mesenteric venous blood and in the arterial blood varies considerably from one experiment to another when the whole intestine is left *in situ*. This is probably due to a variable dilution of the blood from the small intestine by the comparatively large volume of blood flowing from the large intestine. During glutamic acid absorption in the rabbit with the large intestine removed the mesenteric venous concentration of alanine was always considerably higher than the arterial concentration and the rise in alanine content of the blood was greater than in the experiments with the dog and cat.

The concentrations of pyruvate and α-oxoglutarate in the blood passing through the intestine are unchanged by the transamination that occurs after introducing glutamic acid into the intestinal lumen. A consideration of the transamination reaction suggests that the rise in alanine concentration in the venous blood would be accompanied by an increase in α-oxoglutarate concentration and a concomitant fall in the pyruvate concentration, but this was not supported by the experimental results. A fall in venous pyruvate concentration occurred in the rabbit only and was unrelated to the rise in alanine concentration, since it occurred even in control experiments when no glutamic acid was introduced into the intestinal lumen.

From these results it would appear that the normal metabolism of the intestinal cells overshadows or compensates for any changes in the oxo acid concentrations of the blood brought about as a result of the transamination of the absorbed glutamic acid. The alanine added to the blood during its passage through the glutamic acid-absorbing intestine is usually considerably more than the amount of pyruvate found in the arterial blood supplying the intestine and must come from the general metabolism of the intestinal cells. If all the α-oxoglutarate produced as a result of the transamination appeared in the mesenteric venous blood its concentration would be increased about tenfold. The site of the transamination in the intestine is as yet undetermined, but the most likely position would be in the mucosal epithelial lining.

It is stimulating to find that a phenomenon first discovered from *in vitro* experiments on the rat intestine (Matthews & Wiseman, 1953) is now shown to occur *in vivo* in the dog (Neame & Wiseman, 1957), cat and rabbit.

SUMMARY

1. The absorption of L-glutamic acid by the small intestine of the dog, cat and rabbit has been studied *in vivo*.

2. The absorption of glutamic acid is accompanied by a rise in the concentration of alanine in the blood passing through the intestine in all three animals. In the rabbit this can only be demonstrated consistently after removal of the large intestine.

3. The concentrations of pyruvate and α-oxoglutarate in the blood passing through the intestine in the dog and cat remain unaffected by the absorption of glutamic acid and the transamination involved.

The authors wish to thank Miss P. A. Maurer for her valuable assistance during the experiments. Part of the expenses was defrayed by a grant to the authors from the Beaverbrook Research Fund (K. D. N.) and from the Medical Research Fund of the University of Sheffield (G. W.). Our thanks are due to L. Light and Co., Ltd, for generous gifts of amino acids.

REFERENCES

ALEXANDER, B. & SELIGMAN, A. M. (1945). A colorimetric method for the microdetermination of α-alanine in blood. *J. biol. Chem.* **159**, 9–19.

BUEDING, E. & WORTIS, H. (1940). The stabilization and determination of pyruvic acid in the blood. *J. biol. Chem.* **133**, 585–591.

CAVALLINI, D., FRONTALI, N. & TOSCHI, G. (1949). Determination of keto-acids by partition chromatography on filter paper. *Nature, Lond.*, **163**, 568–569.

CHRISTENSEN, H. N., RIGGS, T. R. & RAY, N. E. (1952). The concentrative uptake of amino-acids by erythrocytes *in vitro*. *J. biol. Chem.* **194**, 41–51.

DENT, C. E. & SCHILLING, J. A. (1949). Studies on the absorption of proteins: The amino-acid pattern in the portal blood. *Biochem. J.* **44**, 318–333.

GALE, E. F. (1945). Studies on bacterial amino-acid decarboxylases; the use of specific decarboxylase preparations in the estimation of amino-acids and in protein analysis. *Biochem. J.* **39**, 46–52.

GOODWIN, T. W. & WILLIAMS, G. R. (1952). Studies in vitamin A: The effect of vitamin A deficiency on the pyruvate and α-keto-glutarate levels of rat blood. *Biochem. J.* **51**, 708–714.

KREBS, H. A. (1950). Manometric determination of L-aspartic acid and L-asparagine. *Biochem. J.* **47**, 605–614.

LONG, C. (1944). The stabilization and estimation of pyruvic acid in blood samples. *Biochem. J.* **38**, 447–452.

MATTHEWS, D. M. & WISEMAN, G. (1953). Transamination by the small intestine of the rat. *J. Physiol.* **120**, 55*P*.

NEAME, K. D. & WISEMAN, G. (1957). The transamination of glutamic and aspartic acids during absorption by the small intestine of the dog *in vivo*. *J. Physiol.* **135**, 442–450.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1949). *Manometric Techniques and Tissue Metabolism.* Minneapolis: Burgess Publishing Co.