THE UPTAKE OF AMINO ACIDS BY ISOLATED SEGMENTS OF RAT INTESTINE

I. A SURVEY OF FACTORS AFFECTING THE MEASUREMENT OF UPTAKE

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SUMMARY

Techniques have been developed for the determination of the uptake of a large number of amino acids on a single preparation of segments from isolated small intestine of rats. The uptake can be followed by the fall in amino acid concentration of the external medium provided that a correction is made for water exchange between the tissue and the medium. As measured in this way the uptake may include both accumulation within, and metabolic transformation by the tissue. From recovery studies, metabolic transformation is likely to be of importance only in the uptake of L-ornithine, L-arginine, L-aspartic acid, L-glutamic acid and L-glutamine. The amino acids that are completely recoverable from the tissue after uptake show similar time-progresses of uptake with a cessation of net uptake suggestive of a steady state. The uptakes of all the amino acids, with the possible exceptions of L-arginine and L-lysine, are inhibited by 2,4-dinitrophenol. It is concluded that comparisons of rates of uptake may be made by determination of the uptake at 4 min.

INTRODUCTION

Isolated preparations of tissue from small intestine will transfer certain L-amino acids against a concentration gradient[1–6]. Similar preparations will also take up L-histidine and concentrate it within the tissue[7]. These results would indicate a transport mechanism requiring energy. Impairment of the concentrating process when the supply of metabolic energy is impaired, as in anoxia[6] and in the presence of cyanide or 2,4-dinitrophenol[4,7], is supporting evidence of an active process for transport of amino acids in intestinal tissue. In investigating the rate of such a process a number of factors may affect the result obtained. Since the range of conditions for the physiological activity of the tissue is limited, these factors cannot be eliminated, and so must be assessed rather than controlled. The purpose of the present paper is to survey the uptake of amino acids to seek information on some factors of possible effect on the determination of uptake.

EXPERIMENTAL METHODS

Preparations of tissue

Segments of intestine were prepared from starved, young female rats (120–170 g) as described by AGAR *et al.*[7]. In some experiments on water exchange the procedure of these authors was followed in detail, using rats starved 18–24 h and washing the segments by at least 5 min preincubation. Otherwise the rats were starved 40–48 h, and the tissue was washed twice in 50 ml of Krebs bicarbonate–Ringer buffer[8] (pH 7.4) containing 0.5 % glucose, gassed with CO_2–O_2 (5:95). When the tissue was taken from more than one animal, it was all mixed in the first washing which was continued for 5 min after the addition of the last lot of tissue; it was then transferred to the second vessel for a further washing of 10 min. This gave a total preincubation time of 15–21 min, the latter time being that for the tissue from the first rat. On removal from the washing vessel the segments were drained and turned over once on hardened filter paper before transfer to the incubation medium.

Conditions of incubation

The tissue was incubated in a medium of Krebs bicarbonate–Ringer buffer (pH 7.4) containing 0.5 % glucose and amino acids as required, shaken in 25-ml conical flasks, with a gas phase of CO_2–O_2 (5:95). Samples were taken before and at intervals, usually 4, 10 and 50 min, after the addition of tissue. The dry weight was determined after drying the tissue overnight at 110°. The uptake of amino acid in μmole/g dry weight was calculated from the decrease in concentration of the external solution, making allowance for the change in volume of medium resulting from the exchange of water between the tissue and the medium under the conditions of the experiment.

In experiments with amino acids at 10 m*M*, 0.15–0.20 g (dry wt.) of tissue was incubated in 4 or 5 ml of medium. For concentrations above 10 m*M*, the volume was 3 ml. At 1 m*M* concentration, 0.1 g (dry wt.) was incubated in 5 ml. A correction was made for the amino acid released from control tissue incubated in amino acid-free buffer. The magnitude of this correction varied with the tissue and the amino acid, ranging from 1 μmole/g dry weight (*e.g.* valine, methionine) up to 5 μmoles/g dry weight (alanine). The release of amino acids from the tissue was not greatly affected by the presence of other non-metabolised amino acids in the medium.

Investigation of water exchange

The exchange of water was followed by weighing samples of tissue after they had been carefully freed of surplus water by being turned over on hardened filter paper. The difference between the weights before (preincubation fresh weight) and after (post-incubation wet weight) the final incubation period was taken as the exchange of water by the tissue. Replications were made with each batch of tissue, the number of replications depending on the type of experiment.

Recovery of amino acid taken up

Tissue (1.0–1.2 g wet weight) prepared normally was incubated in 5 ml of the appropriate medium of buffer + 10 m*M* amino acid, or buffer alone for the control. After 50 min, 3 ml of the incubating medium was withdrawn and made up to 8 ml

for estimation. The remaining medium was withdrawn, combined with three subsequent ethanolic extracts of the tissue and made up to 8 ml. A further four washings were made with 2 ml volumes of 70% ethanol, then combined and made up to 8 ml. 5-ml samples of the two initial media (with and without amino acid) were each made up to 8 ml. Any turbidity which appeared in the extracts on standing was removed by centrifugation.

The amount of amino acid disappearing from the external medium was determined from the concentration of the medium before and after incubation. On the samples obtained by pooling equal volumes of the appropriate solutions, the initial and final contents of amino acid in the whole system were estimated in triplicate. For the initial content, the sample was derived from the unincubated amino acid-containing medium, plus the medium and extracts from the control incubation. For the final content, the sample was derived from the unincubated amino acid-free medium, plus the medium and extracts from the incubation with amino acid. The difference between initial and final amounts of the amino acid has been taken as the loss due to the metabolism of the amino acid by the tissue. Amino acids formed as products of metabolism were detected by comparing the chromatograms. Where the formation of an amino acid appeared likely from this comparison, the initial and final amounts of it were estimated quantitatively.

Tissue extracted as above adsorbs lysine strongly and does not release it to further washing with 70% ethanol. Extraction with 1% Na_2CO_3 (w/v) in 70% ethanol yielded higher recoveries of lysine, ornithine and arginine.

Estimation of amino acids

Except for proline, and in some cases for histidine, the concentration of amino acids was measured by a paper chromatographic method[9]. 0.1–0.2 μmole of amino acid was applied to the paper and the chromatograms developed with a descending solvent, using *n*-butanol–acetic acid–water (4:1:1, v/v) for all amino acids except alanine, for which *n*-propanol–water (4:1, v/v) was used. Replicates were found to be within a range of $\pm 2\%$ of the mean. For histidine, the results were similar to those from the method of MACPHERSON[10]. Proline was estimated by the method of CHINARD[11]. Although the colour development was markedly stimulated by the presence of other amino acids, the effect did not seriously interfere with the determination of the uptake of proline.

RESULTS AND DISCUSSION

Water exchange

Preliminary experiments indicated that uptake of water occurs during the period of preincubation and that it is substantially complete within 15 min. After preincubation while the tissue is out of the aqueous medium for distribution, a release of water on to the surface of the tissue occurs. This water is conveyed with the tissue to the incubation medium, when a renewed uptake of water begins. The dilution of the incubation medium at a given time will depend on the counter-balancing effects of the initial addition of water and its removal by the renewed uptake. For a net release of W ml/g dry weight of tissue the correction to the uptake determined from a final amino acid concentration of C_2 m*M* would be $-WC_2$ μmole/g dry

weight. To allow this correction the exchanges of water between tissue and medium were studied using the same procedures and times as in determining the uptake of amino acids.

Results are presented in Tables I and II. The release after 4 min incubation is significantly less (at the 1% level) for tissue preincubated 5 min than for tissue preincubated 10 min, while there is not a significant difference between the effects of 10 and 15 min preincubations (Table I). With tissue preincubated 15 min and

TABLE I

WATER EXCHANGE BY SEGMENTS OF RAT INTESTINE

Segments of small intestine, from rats starved 24 h were preincubated for the given times, then three samples from each rat were transferred for incubation for the given times. Water exchange determined as described in text.

Period of preincubation (min)	*Period of final incubation (min)*	*Number of samples*	*Average change of wt. (g/g preincubation fresh wt.)*	*Average dry wt. (% preincubation fresh wt.)*
5	4	36	—0.040	—
5	10	30	—0.026	—
5	15	30	—0.021	—
5	25	30	+0.006	—
5	50	26	+0.031*	16.9**
10	4	24	—0.075	—
15	4	39	—0.074	16.8***
15§	4§	33§	—0.087§	16.0§

* Values for one rat giving negative water exchange deleted, since not homogenous with the remainder.

** Based on values for 6 rats, 17 samples.

*** Based on values for 6 rats, 18 samples.

§ Samples from rats starved 48 h.

incubated 4 min, the release is significantly less (at the 5% level) for tissue from rats starved 24 h than that for tissue from starved 48 h (Table I). There was no significant difference beween the variance of samples taken from a single rat and of samples taken from the pooled tissue of 4 rats. In the experiments with tissue, pooled for 15–21 min preincubation, from four rats starved 48 h, the results summarized in Table II show no significant differences at the 5% level in the effect of the pairs of incubation treatments on the net release of water. The significantly greater release for no incubation than for 4 min incubation supports the observation that water is released during the handling of the tissue and is taken up on incubation.

For corrections in this paper only the values for tissue, from rats starved 48 h, pooled for 15–21 min preincubation have been required. Since the effects of the various incubation treatments in Table II are not significantly different from the corresponding ones with L-histidine alone, all corrections have been made on the basis of the average values for the net release after incubation in the presence of 10 m*M* L-histidine. Equating grams of weight lost with millilitres of water released, these values are 0.76 ml/g dry weight for no incubation, 0.64 ml/g dry weight for 4 min incubation, 0.46 ml/g dry weight for 10 min incubation and 0.59 ml/g dry weight for 50 min incubation. For uptake at 4 min, determined at 10 m*M*, this would correspond to a correction of —6.4 μmoles/g dry weight, with an extreme range of

TABLE II

WATER EXCHANGES BY SEGMENTS OF RAT INTESTINE INCUBATED IN THE PRESENCE OF L-AMINO ACIDS

Segments of small intestine, from 4 rats starved 48 h, pooled and preincubated 21–15 min and then incubated for the given time with L-amino acids at 10 m*M* and 2,4-dinitrophenol as noted. Water exchange determined as described in text. Treatments bracketed together were performed on samples from the same preparation of tissue. The statistical significance of the differences between these treatments are given (5% = significant at 5% level; N.S. = not significant at 5% level).

Additions to buffer plus glucose	*Period of final incubation (min)*	*Number of samples*	*Average change of wt. (g/g dry wt.)*		*Average dry wt. (% preincubation fresh wt.)*
Histidine	4	12	0.65		15.2
Isoleucine	4	8	0.68		16.0
Histidine	4	4	0.69	N.S.	15.6
Isoleucine	4	4	0.61		15.5
Histidine	4	6	0.58	N.S.	16.0
Histidine + isoleucine	4	6	0.61		16.1
Histidine	4	4	0.68	N.S.	15.4
Histidine + DNP	4	4	0.63		15.0
Histidine	10	4	0.46	N.S.*	16.1
Histidine + DNP	10	4	0.64		15.1
Histidine	50	2	0.59	N.S.	14.8
Histidine + DNP	50	2	0.69		14.2
Histidine**	4	4	0.62	5%	16.4
Histidine**	Nil	4	0.76		16.7
Histidine***	50	4	0.32	N.S.	15.6
Histidine + isoleucine***	50	4	0.43		15.7

* Large difference required for significance due to one anomalous figure which cannot be rejected.

** 4 samples from the pooled tissue of 2 rats.

*** Samples of segments preincubated 10–5 min, from rats starved 24 h.

error of ± 2.5 μmoles/g dry weight as shown from the extreme ranges of a total of 52 determinations including those in the presence of L-isoleucine and 2,4-dinitrophenol.

The net decrease in weight, for tissue from rats starved 48 h, over 50 min incubation following 15–21 min preincubation might suggest that the water released during the distribution procedure has not been replaced. However, it seems likely that this loss of weight may represent a loss of tissue, since the dry weight as a percentage of the preincubation fresh weight has dropped considerably (Table II). Such loss of tissue is observed to occur after long periods of incubation and may be interpreted as a shedding of the mucosa. The correction has been applied since the material lost may also have a diluting effect. For the greater uptake at 50 min than at earlier times, the relative magnitude of the correction is small.

Variability of rates of uptake

Fig. 1 shows an example of the variations in the rate of uptake of L-alanine by a single preparation of tissue. Because of the lesser variation in the uptake determined at 4 min, as compared with those determined at later times, it was decided to base estimates of rate of uptake on that at 4 min. A further advantage of measuring uptake at 4 min, as compared with later times, is that the net rate of entry will be closer to the initial rate of entry into the tissue. For the uptake at 4 min of L-alanine at 10 mM, two single preparations of tissue showed ranges of 38–44 μmoles/g dry

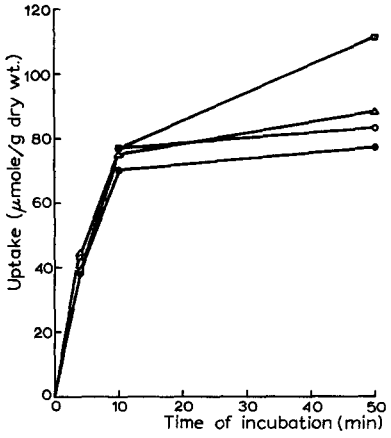

Fig. 1. Replicates of the uptake of L-alanine. Segments of small intestine incubated with L-alanine at 10 mM.

weight for four replicates, and 43–50 μmoles/g dry weight for six replicates, as compared with a range of 35–56 μmoles/g dry weight for sixteen values in different preparations of tissue. In experiments at 1 mM, the uptake was determined at 4 min only. In one preparation of tissue five replicates of the uptake of L-alanine gave the range 9.7–11.5 μmoles/g dry weight, while in another preparation of tissue four replicates of the uptake of L-isoleucine gave the range 11.0–15.5 μmoles/g dry weight.

Since the variation between preparations is greater than the variation within a single one, it was thought best to make single determinations for each amino acid on several preparations of tissue with determinations for uptake of as many amino acids as possible on each. Up to thirteen amino acids have been compared on the one preparation of tissue.

Recovery of the amino acid taken up

To distinguish whether the observed uptake might arise by metabolic transformation of the amino acid rather than by its accumulation within the tissue, studies were made of the extent to which the amino acids were recoverable from the tissue after the uptake period. To allow the maximum effect of metabolism they were made after 50 min incubation.

Inspection of the results in Table III shows that there is considerable metabolism of L-ornithine, L-arginine, L-aspartic acid, L-glutamic acid and L-glutamine, while the remaining amino acids tested are fully recoverable within the limits of experi-

TABLE III

THE RECOVERY OF L-AMINO ACID DISAPPEARING FROM THE MEDIUM

A known wet weight (1.0–1.2 g) of tissue incubated for 50 min with L-amino acid at 10 m*M*, or without amino acid for control tissue. Recovery of amino acid disappearing from the medium determined as described in text.

Amino acid	*% recovery of amino acid disappearing from the medium*
Lysine	76
Lysine	95*
Ornithine	40
Ornithine	56*
Arginine	9
Arginine	36*
Aspartic acid	5
Glutamic acid	9
Asparagine	119
Glutamine	19
Glycine	102
Serine	84
Proline	112
Proline	109
Histidine	93
Alanine	93
Phenylalanine	97
Valine	104
Isoleucine	103
Methionine	111
Leucine	106

* Tissue washed with 1 % w/v Na_2CO_3 in 70 % ethanol.

TABLE IV

THE PERCENTAGE OF L-AMINO ACID DISAPPEARING FROM THE MEDIUM RECOVERED AS α-AMINO-NITROGEN IN VARIOUS AMINO ACIDS

Experimental details as described in text.

Amino acid incubated with tissue	*Recovery as % of the disappearance of incubated amino acid from the medium*					
	Arginine	*Ornithine*	*Aspartic acid*	*Glutamic acid*	*Glutamine*	*Alanine*
Arginine	9, 36	56, 26				
Ornithine	Nil	40				29
Aspartic acid			5	Nil		58
Glutamic acid			Nil	9		57
Glutamine				7	19	47

mental error. From Table IV it will be seen that the alanine appears as the main product containing α-amino nitrogen. From the limitations of the chromatographic separation used, it is not excluded that glycine is metabolised to serine, but conversion of L-serine to glycine is excluded.

In so far as comparisons are possible, the results agree with those of WISEMAN *et al.*[12,13]. The complete recovery of L-asparagine and the substantial disappearance of L-glutamine are in contrast to findings for guinea pig intestine *in vitro*[14]. The absence of asparaginase activity in extracts of rat intestinal tissue has been noted[15].

The finding that lysine is strongly adsorbed on to ethanol-extracted tissue and

that washing the tissue with sodium carbonate in aqueous ethanol increases the recovery of the positively charged amino acids suggests that an adsorption to anionic groups of the tissue may contribute to the value determined for the uptake of these amino acids.

Time-progress of uptake

In preliminary experiments time-progress curves were determined for the uptake of L-histidine at a number of concentrations. In all these curves the rate of uptake decreased in a similar manner with time, so that there was little increase in the total uptake after 20–30 min. For the other amino acids determinations at a single concentration (10 m*M*) were made to compare the time-progress of uptake with that of L-histidine in the same preparation of tissue. The results of two such experiments are presented in Figs. 2 and 3. These figures show two types of curve; those shaped

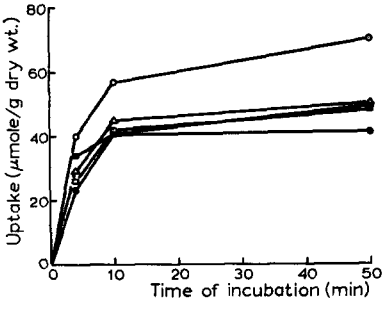

Fig. 2. The time-progress of uptake of various L-amino acids. Segments of small intestine incubated with L-amino acid at 10 m*M*. O—O, L-histidine; ■—■, L-phenylalanine; △—△, L-methionine; □—□, L-leucine; ●—●, L-isoleucine.

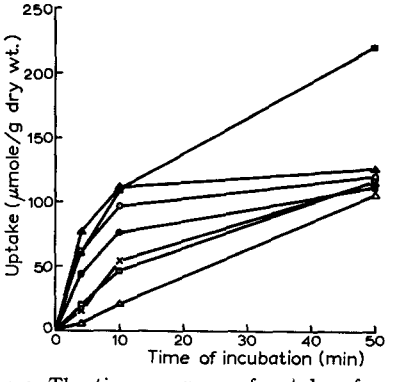

Fig. 3. The time-progress of uptake of various L-amino acids. Segments of small intestine incubated with L-amino acid at 10 m*M*. ▲—▲, L-asparagine; O—O, L-histidine; ■—■, L-glutamine; ●—●, L-proline; □—□, L-glutamic acid; ×—×, L-arginine; △—△, L-aspartic acid.

similarly to the curves for L-histidine uptake and those showing a more continuous uptake with time. The amino acids, L-glutamine, L-arginine, L-glutamic acid and L-aspartic acid, showing the second type of curve are among those which are metabolised by the tissue. A summary of the results of these and a number of other similar experiments is presented in Table V as the averages of the ratios of uptake at 10 min to uptake at 4 min and of the ratios of the uptake at 50 min to uptake at 4 min. These other experiments confirmed that the amino acids which are not completely recoverable show a continued uptake, whereas those which are largely recoverable show a tendency to reach a point where net uptake ceases. An exception in the latter class, however, is L-lysine which shows a more continuous uptake than L-histidine. For those other amino acids which are not metabolised, the similarity in the shape of the curves is indicated by the similarity of the two sets of ratios.

The existence of a steady state, *i.e.* influx = efflux, after net uptake has ceased is suggested by results[7] on the release of accumulated L-histidine. In terms of a hypothesis of a constant rate of influx and a rate of efflux increasing with the amount of amino acid taken up, the similarity of the shapes of the curves would indicate

TABLE V

AVERAGE RATIOS OF THE UPTAKE AT 10 min TO UPTAKE AT 4 min AND OF UPTAKE AT 50 min TO UPTAKE AT 4 min FOR L-AMINO ACIDS AT 10 m*M*

Segments of small intestine incubated with L-amino acid at 10 m*M*. Sampled for determination of uptake at 4, 10 and 50 min. Ratios calculated for individual experiments. Individual ratios averaged and presented as the mean ± S.E.M., for given number of experiments.

Amino acid	*Average ratio Uptake at 10 min / Uptake at 4 min*	*Average ratio Uptake at 50 min / Uptake at 4 min*
Lysine	2.6 ± 0.50 (2)	3.2 ± 0.80 (2)
Ornithine	2.1 ± 0.44 (3)	2.9 ± 0.38 (3)
Arginine	2.3 ± 1.00 (2)	6.5 ± 0.05 (2)
Aspartic acid	2.6 ± 0.67 (3)	9.4 ± 2.99 (3)
Glutamic acid	2.3 ± 0.05 (2)	4.7 ± 0.90 (2)
Asparagine	1.5 (1)	1.7 (1)
Glutamine	2.0 ± 0.20 (2)	3.4 ± 0.20 (2)
Glycine	1.6 ± 0.10 (4)	1.7 ± 0.17 (4)
Serine	1.5 ± 0.10 (2)	1.9 ± 0.16 (2)
Proline	1.6 ± 0.10 (2)	2.4 ± 0.07 (2)
Histidine	1.7 ± 0.07 (9)	1.8 ± 0.08 (9)
Alanine	1.6 ± 0.05 (13)	1.8 ± 0.09 (13)
Phenylalanine	1.4 ± 0.10 (2)	1.5 ± 0.07 (2)
Valine	1.6 ± 0.00 (2)	1.7 ± 0.10 (2)
Isoleucine	1.8 ± 0.06 (3)	1.9 ± 0.17 (3)
Methionine	1.5 ± 0.00 (3)	1.9 ± 0.21 (3)
Leucine	1.5 ± 0.10 (2)	1.8 ± 0.10 (2)

TABLE VI

THE EFFECT OF 2,4-DINITROPHENOL ON THE UPTAKE OF L-AMINO ACIDS

Segments of small intestine incubated with L-amino acid (10 m*M*). Test experiments carried out with $2 \cdot 10^{-4}$ *M* 2,4-dinitrophenol, controls no 2,4-dinitrophenol. Test tissue preincubated for 10 min with $2 \cdot 10^{-4}$ *M* 2,4-dinitrophenol, control tissue preincubated for 10 min without 2,4-dinitrophenol. Sampled for determination of uptake at 4, 10 and 50 min. Uptake in presence of 2,4-dinitrophenol expressed as percentage of uptake in control.

Amino acid	*Uptake in the presence of $2 \cdot 10^{-4}$ M DNP (% uptake in control)*		
	4 min incubation	*10 min incubation*	*50 min incubation*
Lysine	86, 110	97, 84	94, 108
Ornithine	19, 80	51, 82	53, 77
Arginine	132	140	53
Aspartic acid	54	95	52
Glutamic acid	62	64	57
Asparagine	24	28	10
Glutamine	48	52	72
Glycine	42	38	26
Serine	25	52	48
Proline	60	42	37
Histidine	59, 74, 83	50, 69, 62	10, 71, 36
Alanine	58, 40	53, 40	26, 29
Phenylalanine	21	35	31
Valine	41	37	50
Isoleucine	33, 60	50, 64	46, 46
Methionine	27	49	50
Leucine	46	41	60

similar relationships between rate of efflux and amount of amino acid taken up for each amino acid. Such might be expected to be the case if the major part of efflux were in a water stream discharging the amino acid in solution at a rate proportional to the amount present internally. The similarity would seem unlikely to be the result of efflux by diffusion, or by reversal of the uptake process, since the rate of these processes might be expected to be more greatly affected by the differences in the properties of the different amino acids.

While the tendency to attain a steady state as a result of increased efflux seems the most probable interpretation of the curves observed, it is also possible that the rate of influx decreases. This might occur through damage to the tissue on prolonged incubation. An inhibition of uptake due to amino acid previously taken up seems unlikely[16]. The similar shape of the curves, indicated by similarity of the various ratios between the uptakes at different times, implies that the uptake is at the same stage for the different amino acids, so that determinations of uptake made for the same time of incubation should be comparable as equivalent measures of the rate of uptake.

The effect of 2,4-dinitrophenol on the uptake of amino acids

As shown in Table VI, 2,4-dinitrophenol inhibits the uptake of all of the L-amino acids tested, except lysine and arginine. The inhibition may be taken to indicate that the total process of net uptake requires energy from respiration. However, the requirement might be quite indirect; for example, to maintain the integrity of the tissue as a whole. The absence of inhibition might indicate the absence of a requirement for energy from respiration, but with the known high glycolytic activity of intestinal tissue[17,18] a dependence of uptake on the presence of high energy phosphate compounds cannot be eliminated using 2,4-dinitrophenol alone. With these reservations, it might be inferred that, for L-lysine and perhaps L-arginine, the process of uptake does not have a requirement for useful energy from respiration. This requirement is indicated for the other amino acids tested, but for those which are not fully recoverable from the tissue no conclusion can be drawn as to whether the effect is on accumulation within the tissue or on metabolism. These results extend and confirm previous findings[4,7,14].

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