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AMINO ACID ACCUMULATION AND INCORPORATION IN RAT INTESTINE *IN VITRO*

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SUMMARY

1. Rings of rat jejunum incubated *in vitro* accumulate a mixture of amino acids at a rate of about 3 μmoles/cm . hr. The rate of incorporation of the accumulated amino acid into the tissue protein corresponds to a rate of synthesis of 50 % of the protein of the whole wall in five days.

2. Replacement of the Na^+ in the NaCl of the incubation medium by choline or by Li^+ did not prevent amino acid accumulation by the tissue. However, replacement of the Na^+ by K^+ prevented the accumulation.

3. The accumulation of amino acids by rat jejunum *in vitro* proceeded at normal rates not only in the presence in the incubation medium of oxygen tensions below 10 Torr but also in the presence of 2,4-dinitrophenol. Reasons are given for supposing that the findings are compatible with the view that the energy upon which depend the processes of amino acid accumulation by the tissue could be derived from the movement of ions across cellular boundaries.

4. The amino acid incorporation into the tissue proteins was reduced to one tenth of the control rate in the presence of 2,4-dinitrophenol or by hypoxia so that the processes of incorporation depend upon energy derived from oxidative metabolism. In the presence of oligomycin the tissue respiration was depressed but the amino acid incorporation into the tissue protein was not inhibited. The view that the amino acid incorporation may be able to function with energy intermediates other than ATP is discussed.

INTRODUCTION

Most studies of amino acid uptake by the intestine have been made using single amino acids or simple mixtures (Wilson, 1962; Wiseman, 1964). Under physiological conditions, however, absorption of the products of protein digestion occurs from a mixture of all of the common amino acids and small peptides. There is also evidence that during absorption of glycine *in vitro* and of protein hydrolysate *in vivo*, there is incor-

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poration of the absorbed amino acids into the protein of rat intestine mucosal cells (Winnick, Friedberg & Greenberg, 1947; Dawson & Holdsworth, 1962). We have accordingly examined amino acid accumulation by rat intestinal mucosa from a mixture of eighteen amino acids. In addition we have measured the incorporation of the absorbed amino acids into the tissue protein and our findings with respect to the influence of the thyroid gland on the processes have been described (Bronk & Parsons, 1966). We have also attempted to discover, first, what effect, if any, changes in the ionic environment of the tissue had on the amino acid accumulation and the incorporation into protein, and secondly, the nature of the energy requirements for the two processes; these experiments are described here. A preliminary report of some of this work has already been published (Bronk & Parsons, 1965*b*).

METHODS

Rings of jejunum were cut from the small intestine of male albino rats of local stock (body weights range 260–300 g) as described earlier (Bronk & Parsons, 1965*a*). The rings were incubated in a medium containing (m-moles/l.): NaCl, 113; $NaHCO_3$, 25; KCl, 4·5; $MgSO_4$, 1·0; $CaCl_2$, 1·25; NaH_2PO_4, 1·8, and previously equilibrated with 95% air, 5% CO_2 (v/v), except for the incubations under hypoxic conditions (see below). To the incubation medium were added [^{14}C]amino acids together with either 1·0 or 0·2 mg/ml. of a mixture of eighteen unlabelled amino acids; the composition of the mixture is given in Table 1 of Bronk & Parsons (1966). For incubations in which a single labelled amino acid was used 1 mg/ml. of the fundamental mixture of the amino acids was added to the medium and the amount of the carrier (unlabelled) amino acid was reduced to give a total concentration of amino acid (carrier + label) which is indicated in the tables. For other incubations in which a labelled protein hydrolysate was used, 0·2 mg/ml. of the fundamental mixture was added as carrier. In either case 0·4 μc of the labelled material was added in a total incubation volume of 2·6 ml. In a few experiments no amino acid mixture was added so that a single amino acid only was present in the incubation medium at a total concentration of 0·06 or 0·12 mM. Except when noted, glucose was present in the incubation medium at a concentration of 500 mg/100 ml.

In cases where the NaCl in the incubation medium was replaced by either lithium, potassium or choline chloride, the same substitution was made for the solution used to rinse the intestinal segments from which the rings were cut.

The details of the methods used to measure the amino acid accumulation and incorporation into the tissue protein are those described by Bronk & Parsons (1966).

The oxygen consumption of the intestinal tissue was measured polarographically as described by Bronk & Parsons (1965*a*). When the tissue was incubated under hypoxic conditions, the incubation medium was first equilibrated with a gas mixture containing 95% (v/v) N_2, 5% (v/v) CO_2 and an atmosphere of the same gas mixture was maintained in the supernatant gas phase over the medium during incubation. The oxygen tension in the medium was followed polarographically during the incubation and the mean values observed are given in the tables.

Chemical materials. The radioactive compounds used were those specified previously (Bronk & Parsons, 1966). The oligomycin used was kindly provided by Dr M. Johnson, University of Wisconsin and tetra-iodothyroacetic acid was obtained from the Aldrich Chemical Co. Inc., Milwaukee, Wisconsin.

Expression of results. The values given for amino acid accumulation represent the total amount of amino acid found in the tissue at the end of the incubation and therefore include both free amino acids and the amino acids which have been incorporated into protein. The tissue/medium values (T/M) give the ratio of the concentration of free amino acid in the tissue water to that in the medium at the end of the incubation period. The incorporation of amino acid into protein is expressed both in terms of the number of moles incorporated or as the fraction (percentage) of the total absorbed amino acid which was found incorporated into the protein at the end of the incubation period.

RESULTS

The influence of ionic environment on amino acid accumulation and incorporation

In Tables 1–3 are given values obtained for amino acid accumulation by rings of rat jejunum from a mixture of eighteen amino acids over 4 and 8 min when various cations were substituted for the Na^+ of the NaCl in the incubation medium. The data show that the replacement of Na^+ by K^+ in the incubation medium abolished the accumulation of the labelled

TABLE 1. The influence of cations in incubation medium containing mixture of amino acids on accumulation of leucine by rings of rat intestine *in vitro*. Initial leucine concn. 0·06 mM

Predominant cation in incubation medium	Leucine transported n-moles/mg protein		T/M	
	4 min	8 min	4 min	8 min
Na^+	0·474 ± 0·040 (7)	0·798 ± 0·092 (8)	1·43 ± 0·10 (7)	2·13 ± 0·27 (8)
K^+	0·208 ± 0·036 (5)	0·281 ± 0·035 (5)	0·60 ± 0·11 (5)	0·71 ± 0·11 (5)
Li^+	0·394 ± 0·059 (6)	0·539 ± 0·051 (6)	1·20 ± 0·21 (6)	1·57 ± 0·16 (6)
Choline	1·07 ± 0·11 (6)	1·45 ± 0·10 (6)	3·38 ± 0·37 (6)	4·00 ± 0·31 (6)

TABLE 2. The influence of cations in incubation medium containing mixture of amino acids on accumulation of lysine by rings of rat intestine *in vitro*. Initial lysine concn., 0·06 mM

Predominant cation in incubation medium	Lysine transported n-moles/mg protein		T/M	
	4 min	8 min	4 min	8 min
Na^+	0·356 ± 0·034 (8)	0·598 ± 0·072 (8)	1·33 ± 0·14 (8)	2·20 ± 0·19 (8)
K^+	0·117 ± 0·019 (8)	0·193 ± 0·020 (8)	0·44 ± 0·09 (8)	0·68 ± 0·07 (8)
Choline	0·384 ± 0·020 (5)	0·937 ± 0·133 (5)	1·22 ± 0·09 (5)	2·86 ± 0·42 (5)

TABLE 3. Influence of cations in incubation medium containing mixture of amino acids on accumulation of amino acids in protein hydrolysate by rings of rat intestine *in vitro*

Predominant cation in incubation medium	Total amino acid transported n-moles/mg protein		T/M	
	4 min	8 min	4 min	8 min
Na^+	11·2 ± 1·21 (10)	20·4 ± 1·40 (10)	1·48 ± 0·18 (10)	2·57 ± 0·25 (10)
K^+	4·1 ± 0·84 (6)	8·1 ± 1·29 (6)	0·41 ± 0·07 (6)	0·86 ± 0·14 (6)
Choline	17·0 ± 2·6 (5)	24·0 ± 3·9 (6)	1·89 ± 0·29 (5)	2·73 ± 0·46 (6)

amino acids by the intestinal wall when either labelled lysine or leucine was followed or when the whole labelled hydrolysate was used. This is shown by the fact that the T/M ratios failed to exceed unity in the K^+ medium. With all experiments of both 4 and 8 min duration, the rate of transport was cut to about one-third of the control values by the substitution of K^+ for the Na^+.

In contrast to these findings, when the Na^+ was replaced either by Li^+ or by choline, significant accumulation of the amino acids was obtained after 8 min. Values were found for the transport in the presence of Li^+

TABLE 4. The influence of ionic environment on leucine incorporation into protein of rat jejunum during incubation *in vitro*. Initial leucine concn., 0·06 mM

Predominant cation in incubation medium	Leucine incorporated n-moles/mg protein		Incorporation (%)	
	4 min	8 min	4 min	8 min
Na^+	0·034 ± 0·005 (8)	0·140 ± 0·017 (8)	7·9 ± 1·5 (7)	19·1 ± 3·3 (8)
K^+	0·027 ± 0·004 (5)	0·062 ± 0·006 (5)	14·2 ± 2·9 (5)	22·6 ± 1·8 (5)
Li^+	0·029 ± 0·003 (6)	0·056 ± 0·004 (6)	8·7 ± 1·9 (6)	10·7 ± 1·1 (6)
Choline	0·057 ± 0·006 (6)	0·193 ± 0·020 (6)	5·6 ± 0·8 (6)	13·5 ± 1·4 (6)

TABLE 5. The influence of ionic environment on lysine incorporation into protein of rat jejunum during incubation *in vitro*. Initial lysine concn., 0·06 mM

Predominant cation in incubation medium	Lysine incorporated n-moles/mg protein		Incorporation (%)	
	4 min	8 min	4 min	8 min
Na^+	0·029 ± 0·007 (8)	0·094 ± 0·030 (8)	8·3 ± 1·5 (8)	13·9 ± 2·9 (8)
K^+	0·012 ± 0·003 (8)	0·027 ± 0·004 (8)	12·4 ± 3·0 (8)	15·7 ± 1·9 (8)
Choline	0·016 ± 0·003 (5)	0·072 ± 0·017 (5)	4·4 ± 0·9 (5)	7·8 ± 1·2 (5)

TABLE 6. The influence of ionic environment on amino acids of protein hydrolysate: incorporation into protein of rat jejunum during incubation *in vitro*

Predominant cation in incubation medium	Amino acids of protein hydrolysate incorporated n-moles/mg protein		Incorporation (%)	
	4 min	8 min	4 min	8 min
Na^+	0·50 ± 0·05 (10)	2·31 ± 0·34 (10)	4·8 ± 0·5 (10)	11·2 ± 1·3 (10)
K^+	0·31 ± 0·02 (6)	0·64 ± 0·03 (6)	8·8 ± 1·4 (6)	9·0 ± 1·5 (6)
Choline	0·40 ± 0·03 (5)	0·90 ± 0·13 (6)	2·6 ± 0·34 (5)	4·6 ± 1·1 (6)

which were less than those obtained with Na^+, while in the presence of choline values for the transport were regularly found which were higher than with Na^+. The extent to which the rate of amino acid transport into the tissue was increased by the substitution of choline for Na^+ depended upon which labelled compound was studied and on the length of incubation.

The effects of these same changes in the ionic composition of the incubation medium on the amino acid incorporation into the tissue proteins are shown in Tables 4–6. The rate of incorporation in general followed the

changes produced in the rate of amino acid transport, as if the rate of transport tended to be rate limiting to the processes of incorporation. Thus, the decrease in transport caused by replacing the Na^+ in the medium by K^+ resulted in approximately the same decrease in incorporation into protein so that the percentage incorporation was not altered significantly. However, when Li^+ was substituted for Na^+ there was a significant depression of the percentage of the amino acid which was incorporated into the protein. The substitution of choline also inhibited the incorporation of the amino acids into protein in the case of the labelled hydrolysate after 8 min of incubation.

TABLE 7. Influence of sugars on accumulation and incorporation of leucine from mixture of amino acids by rings of jejunum incubated *in vitro* for 8 min. Initial leucine concentration 0·06 mM. Sugar concentration 500 mg/100 ml.

Sugar in incubation medium	No. of observations	Leucine n-moles/mg protein		T/M	Incorporated (%)
		Transported	Incorporated		
Glucose	8	0·798 ± 0·092	0·140 ± 0·017	2·13 ± 0·27	19·1 ± 3·3
None	8	0·774 ± 0·139	0·165 ± 0·026	2·00 ± 0·38	23·4 ± 2·8
Galactose	9	0·741 ± 0·105	0·138 ± 0·014	1·96 ± 0·32	20·8 ± 2·4

TABLE 8. Influence of concentration, sugars and amino acid mixture on accumulation and incorporation of leucine from mixture of amino acids by rings of rat jejunum incubated *in vitro* for 8 min. Except where indicated incubation medium contained mixture of amino acids

Leucine concentration (mM)	Sugar in incubation medium	No. of observations	Leucine n-moles/mg protein		T/M	Incorporated %
			Transported	Incorporated		
0·12	Glucose	9	1·16 ± 0·23	0·171 ± 0·017	1·67 ± 0·40	17·7 ± 2·1
0·24	Glucose	9	2·13 ± 0·26	0·280 ± 0·045	1·52 ± 0·19	13·4 ± 1·8
0·12	None	6	1·96 ± 0·24	0·196 ± 0·032	2·92 ± 0·36	9·8 ± 1·1
0·12	Glucose: amino acid mixture omitted	6	9·16 ± 0·94	0·399 ± 0·056	14·74 ± 1·55	4·6 ± 0·8

Effects of sugars. The data presented in Table 7 show that omission of glucose from the incubation medium did not cause any significant change in the accumulation of leucine by the intestinal tissue from a concentration of 0·06 mM. However, from a concentration in the incubation medium of 0·12 mM, the rate of transport of leucine was significantly increased after 8 min by the absence of glucose (Table 8). The substitution of galactose for glucose produced no significant change in the accumulation of amino acids by the intestinal walls.

The omission of glucose from the medium produced no striking effects on amino acid incorporation into the protein although at concentration of 0·12 mM the percentage incorporation of the leucine was significantly depressed. The data show that the lower percentage incorporation of the

absorbed amino acids into tissue protein was due to the increased transport under these conditions. The substitution of galactose for the glucose in the incubation medium was likewise without effect on incorporation into the protein.

Effects of amino acid concentration. A fourfold increase in the amino acid concentration in the medium resulted in approximately a threefold increase in the accumulation of leucine (Tables 7 and 8). Even at the highest concentration the value of T/M was significantly greater than unity ($P < 0{\cdot}05$). From these observations it is clear that the processes underlying the accumulation of leucine were not saturated at the concentrations of the amino acid which were used.

A fourfold increase in the leucine concentration produced a threefold increase in the rate of incorporation of leucine into the protein. At the higher concentrations of leucine in the incubation medium there was therefore a decrease in the percentage of the absorbed amino acid which was incorporated into protein.

TABLE 9. Effect of presence of amino acid mixture on accumulation and incorporation of lysine by rings of rat jejunum incubated *in vitro* for 8 min. Initial lysine concentration 0·06 mM

Complete amino acid mixture in medium	No. of observations	Lysine n-moles/mg protein		T/M	Incorporated (%)
		Transported	Incorporated		
Present	8	0·598 ± 0·072	0·094 ± 0·030	2·20 ± 0·19	13·9 ± 2·8
Absent	4	1·99 ± 0·08	0·190 ± 0·022	7·88 ± 0·30	9·5 ± 1·0

Effect of the presence of amino acid mixture. The accumulation of both leucine and lysine in the absence of the amino acid mixture was also examined (Tables 8 and 9). Under these conditions the rate of accumulation of the individual amino acids was increased; the accumulation of leucine was 4–8 times, and the accumulation of lysine was 3 times greater than that found in the presence of the mixture. After 8 min leucine was accumulated to give a T/M value of 14·7 while for lysine it was 7·9. The inclusion of the complete amino acid mixture in the incubation medium therefore inhibited leucine accumulation by 75–88% and the accumulation of lysine by 70%. When a mixture of amino acids is absorbed the differences between rates of accumulation of individual amino acids therefore appear to be reduced. In the absence of the amino acid mixture, there was an increase in the rate of amino acid incorporation into protein. However, the rate of incorporation of leucine and lysine was only doubled when the mixture of amino acids was omitted (Tables 8 and 9) and the percentage incorporation of the amino acids into the tissue protein for both amino acids was significantly reduced ($P < 0{\cdot}05$). These obser-

vations suggest that changes in amino acid incorporation into protein in the intestinal wall will reflect the changes in the amino acid transport into the tissue only in cases when a mixture of amino acids is being absorbed.

Effects of unfavourable metabolic conditions on amino acid accumulation and incorporation

In order to investigate the nature of the processes which provide the energy for the amino acid accumulation and incorporation, three sets of experiments were undertaken. In the first set the effects were examined of certain metabolic inhibitory substances on the accumulation and incorporation by the jejunum of the amino acids present in the labelled hydrolysate added to the fundamental mixture. The findings of these experiments are given in Tables 10 and 11. In the second set of experiments also

TABLE 10. The influence of inhibitors on amino acid accumulation by rat jejunum during incubation for 4 or 8 min *in vitro*. Labelled hydrolysate present

Additions	Total amino acid transported n-moles/mg protein		*T/M*	
	4 min	8 min	4 min	8 min
None	11·2 ± 1·2 (10)	20·4 ± 1·4 (10)	1·48 ± 0·18 (10)	2·57 ± 0·25 (10)
5×10^{-4} M 2,4-dinitrophenol	13·0 ± 1·4 (9)	22·2 ± 2·9 (9)	1·43 ± 0·15 (9)	2·50 ± 0·30 (9)
10 μg/ml. oligomycin	19·4 ± 2·3 (9)	32·7 ± 2·7 (9)	2·29 ± 0·33 (9)	3·72 ± 0·31 (9)
5×10^{-5} M TETRAC	—	31·9 ± 2·9 (6)	—	3·51 ± 0·30 (6)
5×10^{-5} M phlorrhizin	—	39·6 ± 4·4 (5)	—	4·39 ± 0·58 (5)

TABLE 11. The influence of inhibitors on amino acid incorporation into protein of rat jejunum during incubation for 4 or 8 min *in vitro* in the presence of a mixture of amino acids. Labelled hydrolysate present

Additions	Total amino acids incorporated n-moles/mg protein		Incorporation (%)	
	4 min	8 min	4 min	8 min
None	0·50 ± 0·05 (10)	2·31 ± 0·34 (10)	4·8 ± 0·5 (10)	11·2 ± 1·3 (10)
5×10^{-4} M 2, 4-dinitrophenol	0·16 ± 0·04 (9)	0·26 ± 0·04 (9)	1·4 ± 0·42 (9)	1·4 ± 0·3 (9)
10 μg/ml. oligomycin	1·01 ± 0·19 (9)	2·32 ± 0·45 (9)	5·0 ± 0·6 (9)	6·9 ± 1·0 (9)
5×10^{-5} M TETRAC	—	1·45 ± 0·22 (6)	—	4·5 ± 0·35 (6)
5×10^{-5} M phlorrhizin	—	2·27 ± 0·21 (5)	—	5·9 ± 0·52 (5)

using the hydrolysate, the effects of hypoxia and of low temperature of incubation (28° C) were followed. The results of these experiments are given in Tables 12 and 13. In the third set of experiments the effects of dinitrophenol and oligomycin as chemical metabolic inhibitors and of hypoxia were observed on the accumulation and incorporation of [^{14}C]-leucine in the presence of the amino acid mixture. The findings of these experiments are shown in Table 14.

The effects on the amino acid transport processes are clearly distinguished from those on the incorporation of the amino acids into the protein. None of the inhibitors examined completely prevented the amino acid accumulation, for in all cases values of T/M were obtained which were significantly greater than unity. In fact, three of the substances tested,

TABLE 12. The influence of hypoxia and low temperature on amino acid accumulation by rat jejunum during incubation for 4 or 8 min *in vitro*. Labelled hydrolysate present

Additions	Total amino acid transported n-moles/mg protein		T/M	
	4 min	8 min	4 min	8 min
Hypoxic incubations				
Average P_{O_2}: 0–4 min 40 mm Hg; 0–8 min 54 mm Hg	13·0 ± 1·7 (6)	23·9 ± 2·4 (6)	1·46 ± 0·20 (6)	2·66 ± 0·29 (6)
Average P_{O_2}: 0–8 min 7·5 mm Hg	—	19·9 ± 1·9 (6)	—	2·26 ± 0·23 (6)
Incubation at 28° C	7·2 ± 0·5 (6)	12·6 ± 1·0 (6)	0·80 ± 0·06 (6)	1·36 ± 0·11 (6)

TABLE 13. The influence of hypoxia and low temperature on amino acid incorporation into protein of rat jejunum during incubation for 4 or 8 min *in vitro* in the presence of a mixture of amino acids. Labelled hydrolysate present

Additions	Total amino acid incorporated n-moles/mg protein		Incorporation (%)	
	4 min	8 min	4 min	8 min
Hypoxic incubations				
Average P_{O_2}: 0–4 min 40 mm Hg; 0—8 min 54 mm Hg	0·34 ± 0·03 (6)	0·99 ± 0·05 (6)	3·0 ± 0·6 (6)	4·4 ± 0·5 (6)
Average P_{O_2}: 0–8 min 7·5 mm Hg	—	0·13 ± 0·03 (6)	—	0·7 ± 0·2 (6)
Incubation at 28° C	0·31 ± 0·07 (6)	0·93 ± 0·14 (6)	4·5 ± 1·0 (6)	7·4 ± 0·9 (6)

TABLE 14. The influence of inhibitors and hypoxic conditions on [^{14}C]leucine accumulation in presence of amino acid mixture and incorporation during 8 min incubations

Additions	Amino acid accumulation		Amino acid incorporation	
	Leucine transported n-moles/mg protein	T/M	Leucine incorporated n-moles/mg protein	Incorporation (%)
None	0·798 ± 0·092 (8)	2·13 ± 0·27 (8)	0·140 ± 0·017 (8)	19·1 ± 3·3 (8)
5×10^{-4} M 2,4-dinitrophenol	0·493 ± 0·042 (6)	1·59 ± 0·14 (6)	0·010 ± 0·002 (6)	2·1 ± 0·4 (6)
10 μg/ml. oligomycin	0·918 ± 0·155 (6)	2·62 ± 0·47 (6)	0·119 ± 0·013 (6)	13·6 ± 1·3 (6)
Hypoxic incubations average P_{O_2} 6·5 mm Hg	0·452 ± 0·015 (6)	1·41 ± 0·05 (6)	0·017 ± 0·002 (6)	3·7 ± 0·6 (6)

oligomycin, tetra-iodothyroacetic acid (TETRAC), and phlorrhizin, caused a significant increase in the transport rate when the movements of the amino acids in the labelled hydrolysate were examined. When the tissue was incubated at 28° C the amino acid accumulation was reduced to a rate of about 60% of that found at 38° C. In addition it was found that

amino acid accumulation continued over a period of 8 min under conditions of virtual anoxia, namely from an incubation medium in which the average P_{O_2} was less than 10 Torr.

In contrast to these effects on the accumulation of the amino acids by the tissue, it was found that the addition of 2,4-dinitrophenol (DNP) to the incubation medium, or incubation in the presence of low oxygen tensions almost completely abolished the incorporation of the assimilated amino acids into the tissue protein. Incubation in the presence of TETRAC caused some reduction in the incorporation into protein, but both oligomycin and phlorrhizin were without effect on the incorporation. The relative effects of inhibitory conditions on the transport and incorporation of the amino acids in the hydrolysate are summarized in Table 15.

TABLE 15. The relative effects of inhibitors and hypoxia on amino acid transport and protein synthesis by rings of rat jejunum during incubation *in vitro* for 8 min in the presence of amino acid mixture. Labelled hydrolysate added

Additions	% of control at 8 min	
	Amino acid transport	Amino acid incorporation
None	100	100
5×10^{-4} M DNP	109	11
10 μg/ml. oligomycin	160	100
5×10^{-5} M TETRAC	156	63
5×10^{-5} M phlorrhizin	194	98
Average P_{O_2} 54 mm Hg	117	43
Average P_{O_2} 7·5 mm Hg	98	6

When the accumulation and incorporation of leucine were followed, essentially the same effects were observed. In the presence of DNP and under hypoxic conditions, amino acids were accumulated to give values of T/M which were significantly greater than unity. In this case there appeared to be some reduction in the absolute rate of amino acid transport into the tissues. On the other hand the amino acid incorporation into protein was reduced to a value which was 10% of the control level. Oligomycin did not significantly alter either the rate of amino acid transport or the rate of incorporation of amino acid into protein.

Respiration of rat jejunum in the presence of 2,4-dinitrophenol and of oligomycin

In Table 16 are shown the rates of respiration of rings of rat jejunum observed under the conditions in which the effects of inhibitors were examined. The data show that the respiration was unaffected by the presence of DNP, but that it was depressed in the presence of oligomycin. The significant inhibition ($P < 0{\cdot}05$) of the tissue respiration by the

oligomycin is taken to be evidence that the oligomycin penetrated into the tissue sufficiently to influence the respiratory processes.

TABLE 16. The influence of 2,4-dinitrophenol and oligomycin on the respiration of rings of rat jejunum incubated in the presence of glucose and 0·2 mg/ml. of the amino acid mixture

Addition	Respiration (μl./mg dry wt./hr)
None	7·56 ± 0·44 (11)
5×10^{-4} M 2,4-dinitrophenol	6·61 ± 0·44 (9)
10 μg/ml. oligomycin	6·28 ± 0·33 (9)

DISCUSSION

Rates of amino acid transport and of protein synthesis. The highest rates of amino acid transport into the tissues and of amino acid incorporation into the protein which were observed in this study were obtained with [^{14}C]protein hydrolysate. The maximum rate of transport was observed at the relatively low amino nitrogen concentration in the incubation medium of 1·7 mM and had a value of about 0·17 μmoles of amino acid/mg protein.hr. Taking 17·4 mg/cm for the wt./unit length of the intestine (Bronk & Parsons, 1965*a*) this is equivalent to an absorption of 2·9 μmoles amino acid/cm.hr in the jejunum. If this rate obtained over the whole length of intestine, it would, at the concentration used, provide for the absorption of over 1 g amino acid/day for the whole animal, which is clearly in the physiological range.

The maximum rate of amino acid incorporation which we observed was 0·65 μmoles/mg protein.day although we made no systematic attempt to estimate the limiting rate of incorporation. This rate would provide for synthesis of 50 % of the cell protein of the whole wall in approximately five days. On the further assumption that the mucosal tissue constitutes approximately one half of the intestinal wall (Bronk & Parsons, 1965*a*) and that only the mucosal tissue incorporated the amino acids into protein during the experiments, this figure represents a rate adequate for synthesis of 50 % of the mucosal cellular protein in about 60 hr. This rate is similar to the figure obtained for mammalian cells in tissue culture (Harris & Watts, 1958; Eagle, Piez, Fleischman & Oyama, 1959).

Effects of sugars. With low concentrations of leucine, the omission of glucose from the incubation medium was without significant effect on the accumulation of the amino acid. The fact that the rate of transport of the leucine was significantly increased by the absence of glucose only at the higher concentration of amino acid suggests that there might be some competition between the amino acids and the sugar for the processes underlying the entry of the substances into the cell, as has been postulated by Newey & Smyth (1964). However at the concentrations used, we were

unable to discover any effect on the amino acid accumulation by the presence of galactose in the incubation mixture.

Influence of ions on amino acid accumulation. According to Crane (1965) the transport of amino acids into the intestinal mucosal cells requires the presence of Na ions in the mucosal fluid. In our experiments in which the Na^+ of the NaCl of the incubation medium was replaced by either K^+, Li^+ or choline, the Na^+ concentration in the incubation medium was of the order of 25 mM. In the presence of this concentration of Na ions, amino acid accumulation was observed in the tissue when the predominant cation in the incubation fluid was either lithium or choline; no accumulation was observed when the predominant cation was K^+. Since the mucosal tissue normally contains a high concentration of K^+ (Brown & Parsons, 1962) it seems significant that the only condition in which amino acid accumulation failed to occur was that in which the gradient of K ions was reduced across the limiting membrane of the cells.

Possible energy sources for amino acid accumulation by rat jejunal wall. Our data reveal that amino acid accumulation by rat intestine *in vitro* will occur over a period of 8 min in the presence of 2,4-dinitrophenol or under anoxic conditions. Since rat intestinal mucosa is a tissue which exhibits a high rate of aerobic glycolysis (Dickens & Weil-Malherbe, 1941; Wilson, 1962) one possible conclusion with respect to the energy necessary for the processes of amino acid accumulation is that this may be derived from ATP provided by the glycolytic pathway. Certainly oxidative phosphorylation does not appear to be an obligitory source of the energy for the amino acid transport by rat jejunum *in vitro*. On the other hand, the observation that the accumulation of leucine from a concentration of 0·12 mM in the presence of the amino acid mixture is increased over an 8 min period in the absence of glucose indicates that the accumulation does not depend upon the presence of glucose as an exogenous substrate.

An alternative source of energy for the accumulation of the amino acids is that which could be derived from the movements of ions down electrochemical gradients existing across cellular boundaries (Riggs, Walker & Christensen, 1958; Christensen, 1962; Vidaver, 1964; Eddy & Mulcahy, 1965). Under this scheme the maintenance of the necessary ionic gradients would depend upon some form of ionic pumping, the energy for which would be derived from some energetic intermediate of either oxidative or anaerobic metabolism. A feature of this sort of hypothesis is the possibility of the maintenance of the amino acid accumulation for so long as the 'down hill' movements of ions continued. The accumulation might thus continue in the absence of metabolism, until the ionic battery was, as it were, discharged.

In the case of the small intestine of the rat there is evidence that the

tissue K^+ can be maintained for appreciable periods of time during incubation *in vitro* under hypoxic conditions. Thus Brown & Parsons (1962) showed that the K^+ of the jejunal mucosa is maintained when the tissue is incubated under hypoxic conditions *in vitro* for 20 min in the presence of glucose. In other experiments similar to those of Brown & Parsons it has been found that after 10 min incubation under hypoxic conditions *in vitro* and in the absence of exogenous substrate, the K^+/unit dry wt. of the intestinal mucosa of rat jejunum has fallen to $82 \pm 2(6)\%$ of the initial value, while in the presence of 28 mM glucose the value found for the tissue potassium after 10 min survival *in vitro* also under hypoxic conditions was $93 \pm 4\ (6)\%$ of the initial value (D. S. Parsons, unpublished observations). We conclude that our findings are consistent with the view that the energy for the processes of amino acid accumulation by rat jejunum *in vitro* could be derived from the movement of ions such as K^+ across cellular boundaries. On the other hand our findings do not exclude the possibility that the uptake of amino acids by rings of rat jejunum is also associated with the movement of ions such as Na^+ or Li^+ down electrochemical gradients.

Possible energy sources for incorporation of amino acids into protein of rat jejunal wall. Our finding that, under hypoxic conditions and also in the presence of DNP the incorporation of the amino acids into the tissue protein (as perchloric acid precipitable material) virtually stops, indicates that this incorporation depends upon energy derived from oxidative metabolism and cannot function at an appreciable rate with ATP derived from glycolysis as the energy source.

We have also found that oligomycin does not depress the rate of incorporation of amino acids into the tissue protein. Similar observations have been made on the processes of amino acid incorporation into the protein of isolated mitochondria (Bronk, 1963; Truman & Löw, 1963; Kroon, 1964).

Current views of the mode of action of oligomycin are that it prevents the generation of ATP by oxidative phosphorylation (Lardy, Johnson & McMurray, 1958). On the basis that oligomycin penetrated to the mitochondria because in its presence the respiratory rate was depressed, one can say that the incorporation of amino acids into tissue protein can proceed with energy sources derived from intermediates of oxidative phosphorylation other than ATP. If this view is correct and ATP is not an obligatory energy source for the incorporation of absorbed amino acids, this may simply indicate that there are two alternative processes through which energy can be used to activate amino acids. Thus when ATP formation is stopped the rate of the alternative process could increase to prevent any reduction in the over-all rate of incorporation. However, if we

suggest this hypothesis we are required to explain how it is that the ATP available from glycolysis under conditions of hypoxia was incapable of operating an ATP-consuming activation process for the incorporation of the amino acids. Hendler (1965) has proposed that some protein synthesis in intact cells can occur by a process distinct from that observed in isolated ribosome preparations, and need not require free ATP as an energy source. If ATP can be utilized as an energy source for the amino acid incorporation processes, but, as suggested by Hendler (1965) the protein synthesis occurs in the membranes, it is possible that the ATP derived from glycolysis might not reach the site of protein synthesis in the membranes. In this case the lack of inhibition of the incorporation observed in the presence of oligomycin still requires that the amino acid incorporation processes are able to function using energy sources which are derived from oxidative phosphorylation, but are other than ATP.

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