of reduced diphosphopyridine nucleotide cytochrome *c* reductase, diphosphopyridine nucleotide-linked glutamic and β-hydroxybutyric dehydrogenases and fumarase were also stabilized for varying periods by ageing homogenates at 0° with succinate.

7. When succinate-supplemented homogenates were centrifuged and the resultant particulate preparations resuspended in water, the stabilization effect was not apparent unless additional succinate was added to the resuspended preparations.

8. Heat denaturation of succinoxidase, reduced diphosphopyridine nucleotide oxidase and succinic dehydrogenase activities was overcome by heating homogenates in the presence of succinate.

9. Of a large number of compounds, many of which are closely related to succinic acid structurally, only *iso*citrate and citrate (as well as succinylcholine and succinimide) gave appreciable protection of the succinoxidase and reduced diphosphopyridine nucleotide oxidase systems, indicating that the structural requirements of the succinate-stabilization effect are very rigid.

10. It is suggested that the present findings are different from previous observations on the stabilization and protection of enzymic activities by various methods, and the results are discussed.

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REFERENCES

Albritton, E. C. (1952). In *Standard Values in Blood*, p. 91. Philadelphia and London: W. B. Saunders Co. Ltd.
Borei, H. (1950). *Biochem. J.* **47**, 227.
Eichel, H. J. (1954). *J. biol. Chem.* **206**, 159.
Eichel, H. J. (1955). *J. Protozool.* (suppl.), **2**, 13.
Eichel, H. J. (1956*a*). *J. biol. Chem.* **222**, 121.
Eichel, H. J. (1956*b*). *J. biol. Chem.* **222**, 137.
Eichel, H. J. (1956*c*). *Biochim. biophys. Acta*, **22**, 571.
Keilin, D. & Hartree, E. F. (1949). *Biochem. J.* **44**, 205.
Morton, R. K. (1955). In *Methods in Enzymology*, vol. 1, p. 25. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Nason, A. & Lehman, I. R. (1956). *J. biol. Chem.* **222**, 511.
Polis, B. D. & Shmukler, H. W. (1957). *J. biol. Chem.* **227**, 419.
Racker, E. (1950). *Biochim. biophys. Acta*, **4**, 211.
Sacktor, B. (1954). *J. gen. Physiol.* **37**, 343.
Scott, D. A. & Mendive, J. R. (1941). *J. biol. Chem.* **139**, 661.
Singer, T. P., Kearney, E. B. & Massey, V. (1956). In *Enzymes, Units of Biological Structure and Function*, p. 417. Ed. by Gaebler, O. H. New York: Academic Press Inc.
Watanabe, M. I. & Williams, C. M. (1953). *J. gen. Physiol.* **37**, 71.

The Absorption of Cystine and Cysteine from Rat Small Intestine

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Although there have been many reports in recent years of experiments designed to study the rates and mechanisms of the absorption of amino acids from the small intestine, cystine and cysteine do not appear to have been included in the compounds studied. This is perhaps due in part to the relative insolubility of cystine, and the fact that one of the more elegant techniques at present being used for such studies involves the use of isolated everted sacs (Wilson & Wiseman, 1954) of small intestine, which cannot be used for the study of the absorption of cysteine. This is because the oxidation of cysteine in oxygenated solution is rapid, and anaerobic conditions inhibit absorption processes involving 'active transfer' (Wilson & Wiseman, 1954) in this preparation.

In the work reported here cystine absorption has been studied with both the everted-sac technique and a lumen-perfusion technique *in situ*, whereas the absorption of cysteine has been studied with the latter only.

Hess (1949) has briefly reviewed earlier work in the absorption of cystine, which, however, had not been concerned with the elucidation of the nature of the absorptive process, but only with the overall rates of absorption after ingestion by animals of the DL- and L-isomers of the amino acid. In his own experiments, in which he fed rats by stomach tube with solutions of the sodium salts of cystine isomers and of L-cysteine, Hess (1949) found no significant differences between the rates of absorption of L-, D- or DL-cystine. *meso*Cystine was absorbed at a lower rate than the other isomers. L-Cysteine was absorbed more slowly than the L-, D- and DL-isomers of cystine. A preliminary report of the work described here was given by Neil (1957).

MATERIALS AND METHODS

Animals. Laboratory stock male albino rats weighing 200–300 g. were used throughout.

Chemicals. L-Isomers of cystine, cysteine and methionine were laboratory-reagent grade (British Drug Houses Ltd., Poole, Dorset); D-isomers of cystine CFP (California Foundation Purity) and cysteine HCl CFP were obtained from the California Foundation for Biochemical Research, Los Angeles, Calif., U.S.A. Other compounds used were AnalaR, Polaritan (Hopkins and Williams Ltd.), or laboratory-reagent grade (British Drug Houses Ltd.).

Lumen-perfusion technique in situ. Rats were fasted for about 12–15 hr. and then anaesthetized with 50% (w/v) urethane solution by injection of 0·9 ml. intraperitoneally and 3 ml. subcutaneously/kg. body wt. If necessary, surgical anaesthesia was induced with ether just before dissection. Tracheal cannulation was always used. Dissection, intestinal cannulation and perfusion were by conventional techniques. The perfusion pressure measured at the proximal end of the perfused segment was kept at 9–11 cm. water throughout each experiment. The volume of perfusion fluid [Krebs & Henseleit (1932) bicarbonate saline solution omitting calcium and glucose ('bicarbonate saline')] was about 55 ml. Samples of 1 ml. were removed periodically for analysis and solution was added to the fluid reservoir to maintain constant volume. At the end of the perfusion period the segment of gut was removed, slit open longitudinally, and blotted gently but firmly between sheets of filter paper and weighed immediately. When cysteine was being studied the solution was equilibrated with N_2+CO_2 (95:5); in all other experiments O_2+CO_2 (95:5) was used.

Everted-sac technique (in vitro). This preparation was set up according to Wilson & Wiseman (1954). Slight modifications were introduced. Rats were anaesthetized and the 'eversion rod' was tied into the gut while the latter was still perfused with blood. The segment was then rapidly dissected free from mesentery and placed in a bath of bicarbonate saline at room temperature [gassed with O_2+CO_2 (95:5)], and eversion and isolation of the sacs were carried out in this bath. Incubation was at 37°. A fine sintered-glass gas bubbler was placed just beneath the sacs, which were gently bathed in the stream of fine bubbles of O_2+CO_2 (95:5). After incubation the sacs were removed from the bath, and gently blotted and the contents removed for analysis. The empty sacs were then treated as described above (at the end of the perfusion period).

Analytical methods

Water content, fat extraction and potassium analysis. The water contents of segments were obtained by drying the tissue at 105° to constant weight. Fat was extracted from the dried material by the method of Hastings & Eichelberger (1937). The fat-free dry residues were dissolved in nitric acid and the potassium concentrations in suitable dilutions determined by flame photometry. Both water and potassium contents were calculated on a fat-free-tissue basis. Where mucosal cells alone were required the sacs were slit open, gently pressed between filter paper, and dissected free from fat on a cold table and the mucosal cells carefully scraped from the muscle layers with a blunt spatula. Fat was not extracted from the dried residues of the cells before dissolving in nitric acid for potassium analyses.

Cystine and cysteine. These compounds were determined polarographically (Brdicka, 1933, 1938) with Tinsley polarographic equipment. The supporting buffer was made by mixing 1 vol. of aq. 0·125 M-NH_3 soln. with 2 vol. of 0·125 M-NH_4Cl soln., and then adding 2 ml. of saturated Na_2SO_3 soln./100 ml. of the mixture. To 3·9 ml. of this buffer were added $(1-x)$ ml. of water and x ml. ($\leqslant 1$ ml.) of solution containing 1–3 μg. of cystine (or cysteine). Immediately before the estimation 0·1 ml. of 0·1 M-$CoCl_2$ soln. was added.

The contents of the sacs were found to contain protein, which interfered with this determination. To avoid this, 0·5 ml. of the solution from the sac was mixed with 0·5 ml. of 10% (w/w) $HClO_4$, and allowed to stand for a few minutes and then centrifuged. A suitable dilution of the supernatant was used for polarography. All determinations were carried out at least in duplicate.

As the polarographic method does not differentiate cystine and cysteine, it was necessary when studying the absorption of cysteine to use a method whereby the contents of cystine and cysteine could be obtained separately in the same solution. Cystine plus cysteine was determined polarographically, and then cystine alone after allowing the cysteine to react with iodoacetate. Cysteine was found by difference. A volume (0·5 ml.) of solution containing cysteine (0·04–0·4 mg.) was mixed with excess of 0·016 M-iodoacetate (0·1–0·8 ml.) and 2 drops of aq. 5 N-NH_3 soln. were added. The mixture was heated for 2 min. in a boiling-water bath, and cooled and diluted to 10 ml. This solution, or a suitable dilution, was used for polarographic analysis.

S-Carboxymethylcysteine was prepared by the method of Dickens (1933), and separated quantitatively from traces of cystine by electrophoresis. This compound, which is formed by the reaction between iodoacetate and cysteine, appears to be reduced at the dropping-mercury cathode. No sharp reduction potential was observed. However, the diffusion current in the region of the cystine catalytic-curve peak is very small under the conditions used and no correction was necessary. The peak of the cystine catalytic curve could almost invariably be accurately delineated, but the base line was not always so clear. In view of this difficulty the accuracy of the method was probably not greater than ±7% (outer limits).

Methionine. This was estimated by the method of McCarthy & Sullivan (1941).

Glucose. This was estimated by the method of Nelson (1944).

Histology. Samples of normal and perfused (or incubated) gut were fixed in formol-saline, and embedded and sections stained with haematoxylin and eosin.

RESULTS

Lumen-perfusion experiments in situ

Preparation control. (*a*) Potassium and water contents. In perfused segments of duodenum, jejunum and ileum the potassium content of the whole tissue remained practically normal. The water content of such segments was about 3% higher than that of control samples. Results of these analyses are shown in Table 1.

(*b*) Histology. Apart from slight oedema the mucosal epithelium was practically normal in sections from perfused segments.

(*c*) Glucose absorption. The well-established inhibition of active absorption of glucose by phlorrhizin in such preparations was used as a functional test. The inhibition was unequivocally demonstrated.

Absorption of cystine and cysteine. The absorption of cystine and cysteine was studied by following the decrease in amino acid concentration in the perfusion fluid. Solution containing cystine was prepared either by shaking with excess of solid cystine, followed by filtration, or by dissolving cystine in a small excess of sodium carbonate solution and adding this solution immediately to 'bicarbonate saline' through which O_2+CO_2 (95:5) was being passed. Allowance was made for the bicarbonate formed from the sodium carbonate when the solution was prepared. No racemization of L-cystine occurred during this procedure. One advantage of the preparation *in situ* over the everted-sac technique is that because the gut in the former is oxygenated by the blood flow it is possible to keep the perfusion fluid O_2-free. Cysteine was found to remain almost completely unoxidized in the perfusion *in situ* when N_2+CO_2 (95:5) was used for the gas lift and in the spaces above the fluid level in the reservoir.

The relations found between the rates of jejunal absorption of L-cystine and L-cysteine and the initial concentration of the amino acids in the perfusion fluid are shown in Figs. 1 and 2. In each case the relation was approximately linear, although there was some scatter of the results, especially at high initial concentrations. In the concentration ranges over which comparable results were obtained, the rate of absorption of L-cystine was found to be about 70% of that of L-cysteine. Substitution of N_2 for O_2 in the cystine-absorption experiments did not affect the rate of absorption of this amino acid. Estimations of both cystine and cysteine were performed on each sample taken during cysteine-absorption experiments.

Table 2 shows the results of experiments in which the rates of absorption of the two amino acids were studied in various regions of the small intestine. The regions used were proximal duodenum, mid-jejunum and distal ileum. The rates of absorption of cystine clearly decreased in the order duodenum, jejunum, ileum, with a small overlap between the ranges observed for duodenum and jejunum. The rate of absorption of L-cysteine is also lower from the jejunum than from the duodenum.

Table 1. *Potassium and water contents of rat small intestine*

Normal and *in situ* lumen-perfused samples. Potassium contents are calculated on a fat-free dry-tissue basis, and water on fat-free fresh tissue. Standard deviations (S.D.) have not been calculated where less than five results were available.

Experiment	No. of experiments	Potassium, m-equiv./kg. Mean	Potassium, m-equiv./kg. S.D. or range	Water (%, w/w) Mean	Water (%, w/w) S.D. or range
		Controls			
Duodenum	5	435	21	78·8	0·97
Jejunum	17	420	22	78·7	0·97
Ileum	1	417	—	78·3	—
		Perfused			
Duodenum	16	457	24	81·7	0·93
Jejunum	20	448	23	81·5	1·36
Ileum	4	448	443–453	82·1	81·2–83·9

Table 2. *Absorption of L-cystine and L-cysteine from various regions of the rat small intestine* in situ

Region of gut	No. of experiments	Mean initial amino acid concentration (mM)	Mean absorption rate (μmoles/g. of fat-free dry tissue/hr.)	Range of rates
		L-Cystine		
Duodenum	5	0·875	75·0	57·1–92·5
Jejunum	8	0·821	47·5	36·7–58·8
Ileum	4	0·872	25·9	22·9–31·7
		L-Cysteine		
Duodenum	3	1·77	222	155–268
Jejunum	3	1·77	150	132–166

The presence of 0·3 mM-2:4-dinitrophenol (DNP) in the perfusion fluid had no influence on the absorption of D-glucose, L-cystine (Fig. 3) and L-methionine. The presence of DNP did not alter the potassium and water contents of the perfused segments. This is in contrast with the large loss of intracellular potassium and increase in water observed when everted sacs were incubated in bicarbonate saline containing DNP (0·3 mM) (Table 6).

The D-isomers of both cystine and cysteine were absorbed from rat duodenum at much lower rates than their L-isomers. Table 3 shows the results obtained. Comparison of the ranges of results obtained shows no overlap between the rates for L- and D-isomers in either case. These results provided

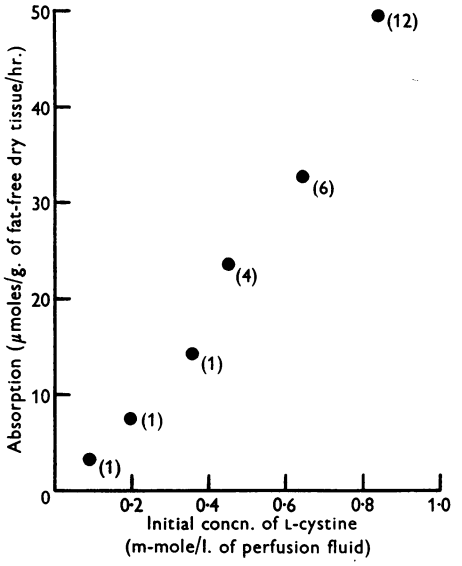

Fig. 1. Relation between the rate of absorption of L-cystine and the initial concentration in the perfusion fluid (*in situ* technique). Figures in parentheses are numbers of results, which were averaged to yield each point. Rates were calculated over 90 min. perfusion periods.

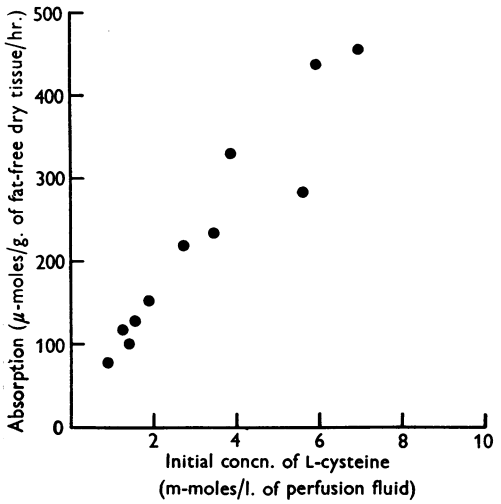

Fig. 2. Relation between the rate of absorption of L-cysteine and the initial concentration in the perfusion fluid. Points represent individual experiments. Rates were calculated over 90 min. perfusion periods.

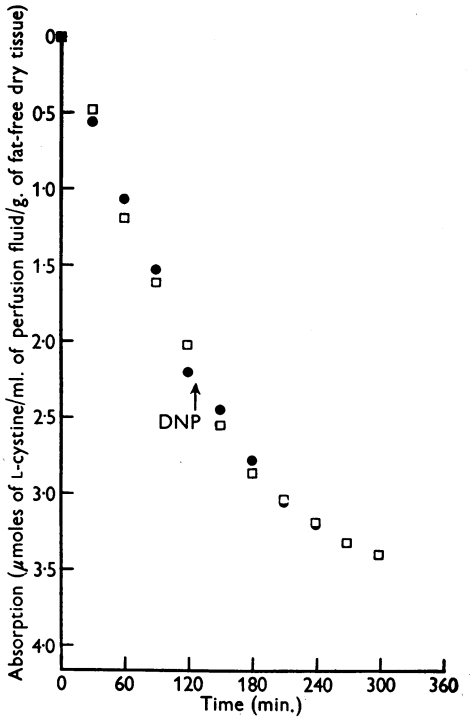

Fig. 3. Absorption of cystine from rat jejunum (*in situ* technique). □, L-Cystine alone; ●, control period followed by injection of DNP (to make 0·3 mM). Starting concentrations, volumes of perfusion fluid and weights of the fat-free dry segments for the two experiments were within 5% of each other.

Table 3. *Rates of absorption of* D- *and* L-*isomers of cystine and cysteine from rat duodenum* in situ

Number of experiments is given in parenthesis. Values given are absorption rates calculated from 90 min. perfusion periods. Starting concentrations of amino acids in perfusion fluids were similar for the experiments with D- and L-isomers in each pair.

	Mean absorption rates (μmoles/g. of fat-free dry tissue/hr.)	
	L-Isomers	D-Isomers
Cystine	80 (5)	27 (4)
Cysteine	222 (3)	57 (3)

preliminary evidence that an active process was involved in the absorption mechanism for the L-isomers in each case.

Everted-sac experiments in vitro

Preparation control. (*a*) Potassium and water contents. At the end of incubation for 1 hr. at 37° in bicarbonate saline, the intestinal sacs were found to have lost about 10% of their normal potassium content. The water content rose from about 78·5% to about 84% (Table 4). Before using the preparation further, it was necessary to find out whether these changes represented a continuous deterioration of the tissue or whether a new steady state was set up in which the potassium and water contents remained constant, but at levels different from those of the normal tissue.

Five sacs were therefore prepared from a single length of jejunum and the whole tissue was analysed after various times of incubation. Fig. 4 shows the results of such an experiment.

Loss of potassium and accumulation of water evidently commenced immediately after dissection of the segment. Small changes were found to have occurred by the time eversion was complete, a matter of about 2–4 min. after the start of dissection. The main changes occurred between eversion and the end of the first 10 min. of incubation. Thereafter both the potassium and the water contents of the whole tissue remained relatively constant.

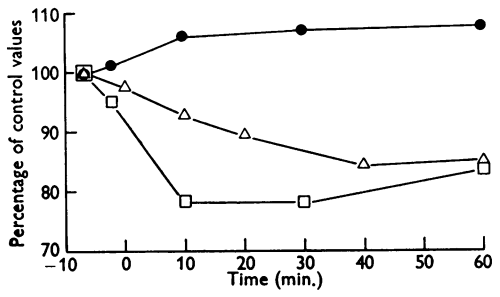

Fig. 4. Variation of potassium and water contents of everted-sac tissue with time. Incubation was in bicarbonate saline; 37°; O_2+CO_2 (95:5); 1 hr. Negative values on the abscissa represent times between cutting off intestinal blood supply (at about −7 min.) and start of incubation (at zero time). □, Potassium and ●, water contents of whole tissue; △, potassium contents of mucosal cells. All ordinate values are expressed as percentages of the appropriate normal control values.

Further experiments were carried out to determine whether these changes involved the mucosal cells. The rate of loss of intracellular potassium from these cells was found to be more gradual than that from the whole tissue, a new steady state being established between 20 and 40 min. of incubation. The potassium content of the mucosal cells at the end of incubation for 1 hr. was about 85% of the control value (Fig. 4).

(*b*) Histology. Comparisons of the normal and incubated tissues showed that there was a certain amount of oedema of the epithelium in the latter. This was more pronounced in the cells nearer the tips of the villi. The mucosal cells originally covering the tips of some of the villi appeared to have been sloughed off. The cells within the crypts were practically normal. It is possible that cells were stripped from the villi tips during the eversion procedure.

(*c*) Transfer of L-methionine. The transfer of L-methionine from the mucosal side to the serosal side of both hamster and rat small intestine has been shown to involve an active transport mechanism (Wilson & Wiseman, 1954; Wiseman, 1955). The absorption of this substance was considered particularly suitable as a control functional test in the present case. Concentration gradients of a similar magnitude to those found by Wiseman (1955) were observed.

Conclusions from the preparation controls. (*a*) and (*b*) above indicate that the physiological state of the rat small intestine was not as well preserved in the everted-sac technique as in the lumen-perfusion technique *in situ*. However, the establishment of a new steady state observed in (*a*) above, and the creation of concentration gradients in the experiments with L-methionine appeared to indicate that the tissue was still functional. It was decided therefore to use the preparation for further studies on the nature of the transport of cystine across the rat-intestinal wall, although bearing in mind the limitations of the technique.

Table 4. *Control experiments on everted sacs of rat jejunum*

Calculation of potassium and water contents are as for Table 1. Sacs were incubated in bicarbonate saline; 37°; O_2+CO_2 (95:5); 1 hr. Number of experiments is given in parentheses.

	Potassium (m-equiv./kg.)		Water (%, w/w)	
	Mean	S.D.	Mean	S.D.
Normal tissue	420	22 (17)	78·7	0·97 (17)
Sacs incubated in bicarbonate saline alone	393	14 (6)	83·6	0 (3)
Mean values for all incubated jejunal sacs	382	23 (23)	84·3	0·58 (19)

Cystine transfer. Everted sacs of rat small intestine were prepared and incubated in bicarbonate saline containing cystine. The initial cystine serosal/mucosal concentration ratio was 1·0. After 1 hr. the ratio was redetermined. Table 5 illustrates the results obtained with the L- and D-isomers of cystine. It is evident that L-cystine was transported from the mucosal side to the serosal side of the sacs against a concentration gradient. No such transport of D-cystine occurred. The reasons for the decrease in the serosal/mucosal concentration ratio in the latter case was almost certainly due to the transport of water from the mucosal to the serosal side (Wilson & Wiseman, 1954).

The effect of DNP on the preparation was also studied, since the inhibition of absorption processes by this substance has been cited as evidence in favour of the indirect involvement of 'a phosphorylation process' in active transport mechanisms (Fridhandler & Quastel, 1955). Jejunal sacs were incubated in bicarbonate saline alone and in bicarbonate saline containing 0·3 mM-DNP. At the end of 1 hr. the sacs were briefly examined macroscopically and the potassium and water contents determined. Similar experiments were carried out in which, at the end of the incubation period, the mucosal cells were gently scraped off the muscle layers and analysed separately. Table 6 shows the results obtained. It is clear that DNP caused a severe loss of intracellular potassium and an increased accumulation of water in the gut. The total potassium content of the mucosal cells alone was reduced to about 50 % of the normal level.

Macroscopic examination of the tissue after exposure to solution containing DNP revealed a curious 'granular' appearance of the mucosa. The tissue had the appearance of having grossly deteriorated, and the potassium and water analyses confirmed this conclusion. In view of the gross generalized damage to the tissue caused by the presence of DNP in the solution, further experiments planned to study the effects of DNP on cystine transport with this preparation were abandoned.

Table 5. *Demonstration of active transport of* L-*cystine by rat small intestine*

Sacs were incubated in bicarbonate saline; 37°; $O_2 + CO_2$ (95:5); 1 hr.

Region of small intestine	No. of sacs	Initial concn. (mM)	Mean concn. gradient after 1 hr.
	L-Cystine		
Duodenum	2	0·62	2·38
Jejunum	3	0·85	2·90
Ileum	2	0·62	1·57
	D-Cystine		
Jejunum	3	0·77	0·84
Jejunum	3	0·80	0·93

DISCUSSION

The two rat-intestine preparations used in these studies differed considerably from one another both in the condition of the tissue during the experiments and in the factors which affected the rates of transfer of metabolites across the gut wall.

The intestinal tissue in the lumen-perfusion technique appeared to remain practically normal, as judged by the potassium and water contents and histological examination, during the period of the experiments. The everted-sac technique, however, caused marked changes in the tissue, by the same criteria. Even so, it was clear that the sac tissue was not deteriorating at an appreciable rate after the establishment of a new steady state. It was concluded therefore that both preparations could be used for absorption studies, although recognizing clearly that the states of the tissues differed from each other, and from normal tissue, at least in the respects revealed by the control tests.

The absorption routes of metabolites were different in the two preparations. In the technique *in situ* the route approximated to normal, but in the everted-sac technique the amino acids had to traverse the whole intestinal wall. However, the conditions were more reproducible in the technique *in vitro* than in the technique *in situ*, because the latter was subject to such factors as variation in the blood flow perfusing the gut capillaries, amounts of anaesthetic required, degree of shock (Guthrie & Quastel, 1956) and degree of distension of the segment during perfusion.

Table 6. *Effect of* 2:4-*dinitrophenol on incubated sacs of rat jejunum*

Mucosal cells were removed after incubation of the whole preparation. Sacs were incubated in bicarbonate saline; 37°; $O_2 + CO_2$ (95:5); 1 hr. Calculations of potassium and water contents for *A* were as for Table 1. Mucosal cells were not fat-extracted.

Experiment	No. of sacs	Potassium (m-equiv./kg.)	Water (%, w/w)
A. Whole preparation			
Bicarbonate saline alone	3	395	83·6
Bicarbonate saline + DNP (0·3 mM)	3	215	85·2
B. Mucosal cells			
Bicarbonate saline alone	3	436	—
Bicarbonate saline + DNP (0·3 mM)	2	224	—

These points are emphasized because it is important to compare the results of absorption studies in relation to the techniques used. This is illustrated below, where the disparity between the present results and those of Hess (1949) is considered.

The approximately linear relations between rates of absorption and concentration of both L-cystine and L-cysteine, which were observed with the technique *in situ*, do not exclude 'active-absorption' mechanisms. If, as suggested by Wiseman (1955) for the hamster small intestine, there exists a common mechanism for absorption of α-monoaminomonocarboxylic acids it seems likely that the capacity of this system would be large. If L-cystine is absorbed by this same mechanism the concentration required to 'saturate' it might well be far beyond the highest concentration limit imposed by the solubility of the amino acid (about mM). In this case there would be no practical range over which the rate of absorption would become independent of the concentration. The upper limit of concentration used in the experiments on L-cysteine was about 850 mg./l. (7 mM). This was also apparently too low to 'saturate' the active mechanism if, as is suggested by the later experiments, L-cysteine is actively absorbed.

Fisher & Parsons (1949), using an isolated loop of rat intestine *in vitro*, showed that the rate of absorption of glucose decreased with increasing distance from the pyloric sphincter. The present experiments indicate that a similar phenomenon occurs with L-cystine and L-cysteine. However, this may in part be due to variation in blood flow, and therefore in amino acid clearance, in different regions of the intestine in the technique *in situ*. It is conceivable also that the rates of absorption, especially of amphoteric molecules, would vary appreciably with changes in pH of the fluid bathing the lumen of the intestine. *In vivo* there is a considerable pH gradient along the small intestine during digestion and absorption, whereas in the experiments reported here absorption rates were determined in various regions only at pH 7·4.

The results obtained with D- and L-isomers of both amino acids with the technique *in situ* strongly suggest active absorption mechanisms. The results of experiments with D- and L-cystine in the everted-sac technique support this conclusion.

The reasons for the disparity between the findings reported here and those of Hess (1949) are not clear. In the feeding experiments *in vivo* of Hess (1949) the sodium salts of the amino acids, which are alkaline in aqueous solution, were used and it is difficult to know what effect such solutions would have on the mucosa of the stomach and small intestine before the pH had been reduced by the secretions of the alimentary canal. Further, there is the possibility that the amounts of cystine isomers (about 100–300 mg.) fed to the rats as sodium salts (the volumes of the solutions were not given) would lead to precipitation of free cystine in the gut if the pH was reduced below about 8. Such an occurrence would make the interpretation of the results very difficult.

SUMMARY

1. The absorption of isomers of cystine and cysteine from the small intestine of the rat has been studied with a lumen-perfusion (*in situ*) and an everted-sac technique.

2. Tissue potassium and water contents, histological examination and application of functional tests have been used to assess the condition of the intestinal tissue in the preparations.

3. The rates of absorption of both L-cystine and L-cysteine were found to increase approximately linearly with increasing concentration. The rates of absorption from duodenum, jejunum and ileum were found to decrease in that order.

4. The absorption of L-cystine but not D-cystine has been shown to be an active process. Evidence is presented which shows that a similar process is probably also involved in the absorption of L-cysteine.

5. Previous work which demonstrated no difference between the rates of absorption of the D- and L-isomers of cystine is discussed in the light of the experiments reported here.

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REFERENCES

Brdicka, R. (1933). *Coll. Trav. chim. Tchécosl.* **5**, 238.
Brdicka, R. (1938). *J. Chim. phys.* **35**, 89.
Dickens, R. (1933). *Biochem. J.* **27**, 1141.
Fisher, R. B. & Parsons, D. S. (1949). *J. Physiol.* **110**, 281.
Fridhandler, L. & Quastel, J. H. (1955). *Arch. Biochem. Biophys.* **56**, 424.
Guthrie, J. E. & Quastel, J. H. (1956). *Arch. Biochem. Biophys.* **62**, 485.
Hastings, A. B. & Eichelberger, L. (1937). *J. biol. Chem.* **117**, 73.
Hess, W. C. (1949). *J. biol. Chem.* **181**, 23.
Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
McCarthy, T. E. & Sullivan, M. X. (1941). *J. biol. Chem.* **141**, 871.
Neil, M. W. (1957). *Biochem. J.* **65**, 41P.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Wilson, T. H. & Wiseman, G. (1954). *J. Physiol.* **123**, 116.
Wiseman, G. (1955). *J. Physiol.* **127**, 414.