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OBSERVATIONS ON THE MECHANISM OF ABSORPTION OF COPPER BY THE SMALL INTESTINE

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Copper is an essential element in animal and probably in human nutrition. In animals, copper deficiency leads to anaemia, bone disorders, abnormalities of pigmentation and hair growth, and demyelination (Adelstein & Vallee, 1961). In man, though hypocupraemia occurs in a variety of conditions, and copper absorption is defective in small intestinal disease (Sternlieb & Janowitz, 1964), there are no recognized signs of copper deficiency. One pathological condition occurring in man is associated with an excess of copper in the body—hepatolenticular degeneration or Wilson's disease. In this, it is probable that copper absorption is excessive, though whether this is a primary feature of the disease, or merely a secondary disorder, is unknown (Zimdahl, Hyman & Cook, 1953; Matthews, 1954; Bearn & Kunkel, 1955; Bush, Mahoney, Markowitz, Gubler, Cartwright & Wintrobe, 1955; Thompson & Cumings, 1964). Investigation of disordered copper absorption is hampered by the fact that very little is known about the normal mechanism of absorption of this metal. Scheinberg & Morell (1957) based a hypothesis to explain over-absorption of copper in Wilson's disease on the assumption that copper was absorbed by simple diffusion—though this hypothesis was subsequently retracted for other reasons (Scheinberg, 1961). However, Gitlin, Hughes & Janeway (1960) concluded from experiments on mice that the absorption of copper included a rate-limiting component, and could not be the result of simple diffusion alone. If the normal mechanism of copper absorption were better understood, it should be easier to form hypotheses of how this mechanism might be deranged in human disease, and to test them by clinical investigations. This paper describes the first stage of an investigation of the physiology of copper absorption, in which hamster small intestine *in vitro* is used, and provides evidence that it involves a special mechanism dependent on metabolic energy.

METHODS

All experiments were carried out with female golden hamsters (*Mesocricetus auratus*), weighing 70–110 g. The animals were not starved beforehand. They were fed on Oxoid Diet 41B, supplemented with cabbage. Analysis of the diet indicated that the average daily copper intake was between 20 and 40 μg.

Sac techniques. Two techniques were used (1) simple everted sacs (Wilson & Wiseman, 1954), filled with 0·3–0·5 ml. of medium and incubated with gentle shaking in conical flasks containing 3–5 ml. of medium, and (2) everted sacs cannulated at one end (Crane & Wilson, 1958) as modified by Crampton & Matthews (1964), filled with 0·3–0·5 ml. of medium and suspended in tubes containing 15 ml. of medium. The technique of Crane & Wilson has the advantages that samples can be removed from the interior of the sac during the course of the experiment, and since there is no shaking, less damage is caused to the intestinal mucosa during prolonged incubation. Fluid inside everted sacs is termed 'serosal fluid', and that on the outside 'mucosal fluid'. Incubations were at 37° C for periods of 1–4 hr, and were carried out with oxygen as the gas phase unless otherwise stated. Sacs (2–3 cm in length) were prepared from different regions of the small intestine, the total length of which is approximately 40 cm. Starting at the pylorus, the first 10 cm was termed upper segment, (*U*), the next 15 cm, mid-segment, (*M*), the next 10 cm, lower segment, (*L*), and the final 5 cm, terminal segment, (*T*). If the intestine was appreciably longer or shorter than 40 cm, the segments were located in similar proportions.

At the end of the experiments, mucosal fluid from simple sacs was centrifuged and the supernatant analysed. The final volume of serosal fluid was measured by draining and weighing and, after centrifugation, the supernatant analysed. With Crane & Wilson sacs, samples of mucosal and serosal fluids were taken at intervals by means of a fine polythene cannula, and at the end of the experiments the sacs were emptied completely by the same means. The volumes of the samples were determined by weighing. Samples of mucosal fluid were centrifuged before analysis. After the sacs had been emptied, they were gently blotted and the final wet weight noted; the dry weight was found by drying for 2 hr at 110° C. The medium used for mucosal and serosal fluids contained glucose (200 mg/100 ml.), NaCl (145 mM), KCl (5 mM), $MgCl_2$ (2 mM) and tris (2-amino-2-hydroxymethylpropane-1:3 diol) buffer (10 mM) at pH 7·2. When copper (as $CuCl_2$ or $^{64}CuCl_2$) was added, it was added to the mucosal fluid only, unless otherwise stated. When inhibitors were used, they were added to both mucosal and serosal fluids.

Ultrafiltration of serosal and mucosal fluids was carried out by placing a sample (0·5–1 ml.) in a small bag of dialysis tubing, and centrifuging for 1 hr at about 2500 *g*/g in tubes containing a sintered glass plate to support the bag.

Estimation of total copper and ^{64}Cu. Chemical analysis of copper was carried out by a modification of the method of Rice (1960). Samples of mucosal and serosal fluids were treated as for plasma. Tissue and food samples were ashed at 530° C for 36 hr, and to the residue 0·5 N-HCl (0·5 ml.) was added, followed by gentle heating to dryness. Further HCl (0·5 ml.) was added, and heated just to fuming. After cooling 0·5 N-HCl (0·5 ml.) containing 0·13 mg oxalyldihydrazide was added, with approximately 50 mg of citric acid crystals. Concentrated NH_4OH (0·25 ml.) and 50 % vol./vol. acetaldehyde (0·25 ml.) were added. After 30 min, ion exchange water (1 ml.) was added, and the optical density of the solution read at 542 mμ in 1 cm cuvettes in a Unicam SP 600 spectrophotometer. Appropriate reagent and EDTA blanks were run.

$^{64}CuCl_2$ was obtained from the Radiochemical Centre, Amersham. Owing to its low specific activity, no carrier was added. Thus the specific activity varied from experiment to experiment, and for this reason results were expressed as μg of ^{64}Cu, and not as counts/sec. The activity of the samples was determined in an Ecko well-type crystal scintillation counter, and appropriate corrections were made for background and decay.

RESULTS

Initial copper content of the intestinal wall

The copper content of the intestinal wall in different regions of the small intestine is shown in Table 1. In three experiments in which the mucosa of a length of intestine from the lower segment was scraped off and analysed separately from the underlying muscle layer, no significant difference was found between the copper content (per g dry wt.) of the mucosal and muscle layers.

TABLE 1. The copper content of the small intestinal wall in different regions. The values are expressed as the mean ± S.E. The number of observations is given in brackets

Segment of intestine	Approx. distance from pylorus (cm)	Copper content of intestinal wall (μg/g dry wt.)
Upper	0–10	33·4 ± 6·0 (5)
Mid	10–25	22·5 ± 4·7 (5)
Lower	25–35	24·4 ± 4·7 (5)
Terminal	35–40	29·1 ± 3·3 (5)

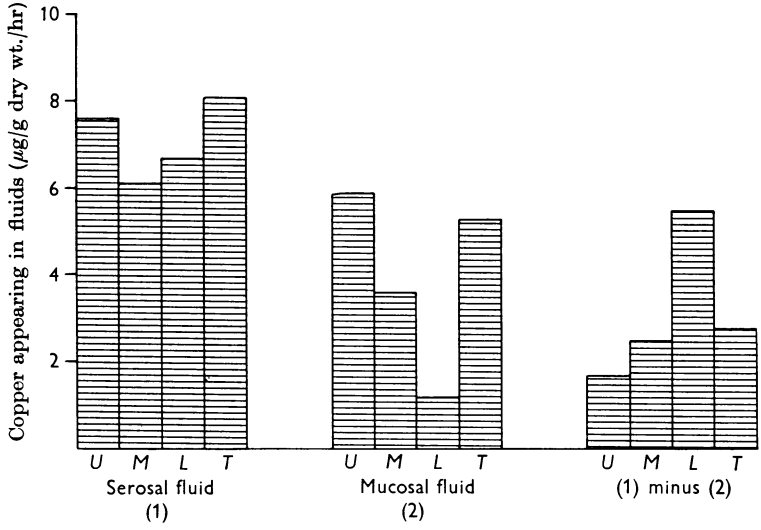

Fig. 1. The amounts of copper appearing in serosal and mucosal fluids in different regions of the small intestine, using simple sacs incubated under O_2 for 1 hr. *U*, Upper segment; *M*, mid segment; *L*, lower segment; *T*, terminal segment. The results are the means of 6 experiments, each using four sacs from one animal.

Transport of copper in different regions of the small intestine

The movement of copper initially present in the gut wall into the mucosal and serosal fluids, in different regions of the small intestine, was investigated by incubating simple everted sacs for 1 hr in medium containing no

added copper. The amounts of copper passing into the serosal fluid did not differ greatly in different regions, but the amount passing into the mucosal fluid was much less in the lower segment than in the others, so that most of the copper leaving the gut wall in this region passed in the serosal direction (Fig. 1).

Experiments were also carried out with simple everted sacs to determine the rate of transport of ^{64}Cu across the intestinal wall, and uptake of ^{64}Cu by the wall, in different regions. In the first series of experiments, ^{64}Cu was

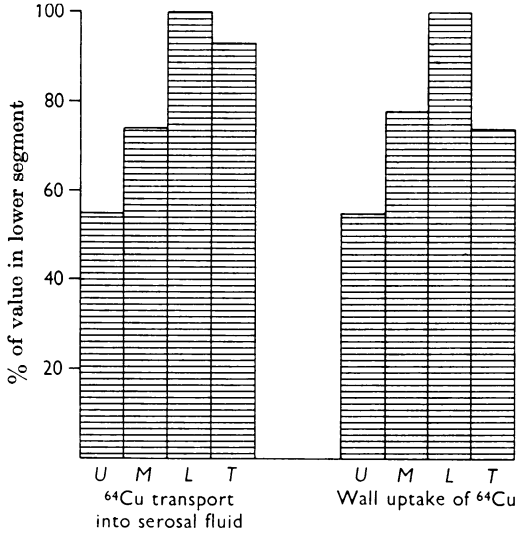

Fig. 2. Transport of ^{64}Cu from mucosal fluid into serosal fluid, and wall uptake of ^{64}Cu from mucosal fluid in different regions of the small intestine. The experiments were carried out with simple sacs incubated under O_2 for 3 hr; ^{64}Cu was initially present on the mucosal side only, in concentrations ranging from 0·5 to 3·0 μg/ml. The results were obtained from ten experiments, each using four sacs from one animal. They were calculated as μg ^{64}Cu/g dry wt./hr and are expressed as a percentage of those obtained in the lower segment.

initially present in the mucosal fluid only, and the amount transported to the serosal fluid was measured. These experiments were carried out at several different concentrations of ^{64}Cu. As the patterns of wall uptake and transport into the serosal fluid in different regions were qualitatively similar at all concentrations, though the absolute amounts involved varied, the results have been combined, and rates of uptake and transport are expressed as a percentage of those obtained in the lower segment, in which wall uptake of ^{64}Cu and transport from the mucosal to the serosal side were greatest (Fig. 2). While the amounts of unlabelled copper appearing in the mucosal fluid varied in different segments, as described above, they were

small in relation to the amounts of added ^{64}Cu (< 2%), so that alterations in specific activity in the mucosal fluid could not account for the results. In the second series of experiments, ^{64}Cu was initially present in the serosal fluid only, and transport into the mucosal fluid was measured. Figure 3 compares the transport rates of ^{64}Cu from the mucosal to the serosal side, and from the serosal to the mucosal side, at an initial concentration of 0·5 μg/ml., and shows the corresponding wall uptakes of ^{64}Cu. Transport from the mucosal to the serosal side was greater than transport in the opposite direction in all segments, and this difference was particularly

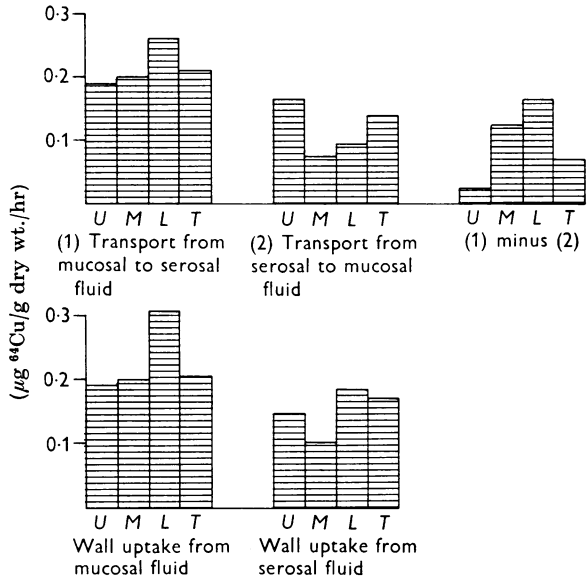

Fig. 3. Transport of ^{64}Cu from mucosal to serosal fluid and from serosal fluid to mucosal fluid, and corresponding wall uptake of ^{64}Cu, in different regions of the small intestine. The experiments were carried out with simple sacs incubated under O_2 for 3 hr; ^{64}Cu was initially present either on the mucosal side only (mean of four experiments each using four sacs from one animal), or on the serosal side only (mean of three experiments each using four sacs from one animal), at a concentration of 0·5 μg/ml.

marked in the lower segment. In four experiments at an initial ^{64}Cu concentration of 2 μg/ml. (not illustrated), a rather similar pattern was found, but in these, transport from the serosal to the mucosal side in the upper segment (mean 1·5 μg/g dry wt./hr) exceeded that from the mucosal to the serosal side (mean 0·54 μg/g dry wt./hr). As movement of unlabelled copper from the intestinal wall in the serosal direction, and transport of ^{64}Cu across the intestinal wall in this direction both preponderated in the lower segment, subsequent investigations were confined to this region.

Transport of copper in the lower small intestine using Crane & Wilson sacs

The movement of copper from the intestinal wall of the lower segment into the mucosal and serosal fluids was further investigated by incubating Crane & Wilson sacs for 1 hr with no added copper. The mean amount of copper passing into the serosal fluid was substantial (12·6 μg/g dry wt. intestine, S.E. 1·3, $n = 26$) and equal to about half the original content of the intestinal wall (Table 1). There was a significant though rather poor correlation between the copper passing into the serosal fluid and the original content of the wall as indicated by analysis of short lengths of gut from sites adjacent to those from which each sac was taken ($r = 0{\cdot}48$, $P < 0{\cdot}02 > 0{\cdot}01$, $n = 25$). The amounts passing into the mucosal fluid were too small for accurate estimation (mean *ca.* 1·4 μg/g dry wt. intestine, $n = 26$). In four experiments, the serosal fluid was sampled at 1½ hr and again at 3 hr, but after 1½ hr there was no further increase in its copper concentration. To determine the effect of addition of copper to the mucosal fluid, paired sacs were made from the lower segment of the small intestine in the same animal, and incubated for 1 hr under similar conditions, except that in one, no copper was added, and in the other, copper was added to the mucosal side (Figs. 4 and 5). At an initial copper concentration of 1 μg/ml. there was no significant effect on the amount of copper passing into the serosal fluid, in spite of a large increase in the copper content of the intestinal wall. At an initial concentration of 3 μg/ml. the copper passing into the serosal fluid was reduced (Table 2). In one experiment at an initial copper concentration of 1 μg/ml. the fluids were ultrafiltered at the end of 1 hr; only 37% of the copper in the mucosal fluid, and only 11% of that in the serosal fluid, was ultrafilterable.

Experiments were also carried out with ^{64}Cu to determine its rate of transport into the serosal fluid and uptake by the intestinal wall, at different initial concentrations in the mucosal fluid. These were run for 3 hr. The course of a representative experiment is shown in Fig. 6. The rate of uptake of ^{64}Cu by the wall, as indicated by the fall in concentration in the mucosal fluid, was most rapid during the first half of the experimental period, while the concentration of ^{64}Cu in the serosal fluid increased in an approximately linear way. In general, the lower the initial concentration of ^{64}Cu in the mucosal fluid, the higher was the final serosal concentration in relation to this, but in no case did the final concentration in the serosal fluid exceed that in the mucosal fluid. The results (Figs. 7, 8) suggested that transport of ^{64}Cu into the serosal fluid was subject to a limiting rate, which was approached at an initial mucosal concentration of 1 μg/ml., whereas the wall uptake of ^{64}Cu was still increasing at 3 μg/ml., the

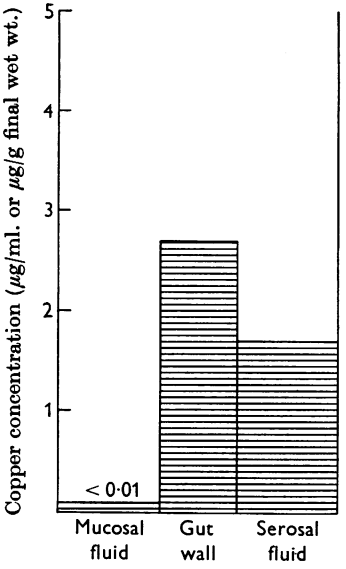

Fig. 4

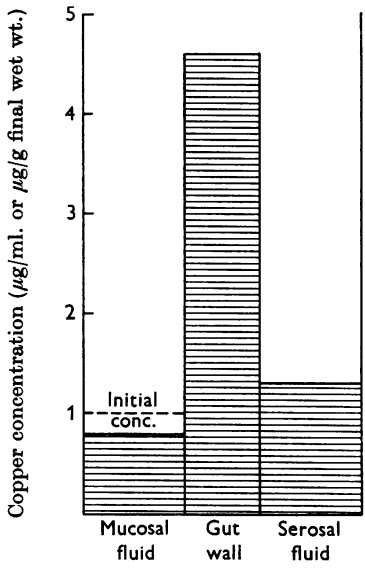

Fig. 5

Fig. 4. Final copper concentrations in mucosal fluid, serosal fluid and intestinal wall, using a Crane & Wilson sac incubated under O_2 for 1 hr with no added copper in the medium.

Fig. 5. Final copper concentration in mucosal fluid, serosal fluid and intestinal wall, using a Crane & Wilson sac incubated under O_2 for 1 hr with copper added to the mucosal fluid at an initial concentration of 1 μg/ml. The sacs used in the experiments illustrated in Figs. 4 and 5 were taken from adjacent sites in the lower small intestine of the same animal.

TABLE 2. The effect of addition of copper to the mucosal fluid on appearance of copper in the serosal fluid and on final wall copper in Crane & Wilson sacs. In Expts. 1–6, the concentration of copper added to the mucosal fluid was 1 μg/ml.; in Expts. 7–9, it was 3 μg/ml.

	No added Cu (Control)		Cu added	
Expt. no.	Cu appearing in serosal fluid (μg/g dry wt./hr)	Final wall Cu (μg/g dry wt.)	Cu appearing in serosal fluid (μg/g dry wt./hr)	Final wall Cu (μg/g dry wt.)
1	13·3	15·5	8·3	85·5
2	15·0	12·9	12·4	48·0
3	3·9	10·6	4·1	101·0
4	5·1	9·1	7·7	32·5
5	21·9	18·5	18·0	36·5
6	6·4	11·4	7·5	39·5
Mean ± S.E.	10·9 ± 2·9	13·0 ± 1·4	9·7 ± 2·0	57·2 ± 10·7
7	14·3	16·9	8·3	99
8	18·9	21·7	7·7	197
9	18·6	14·0	6·9	153
Mean	17·3	17·5	7·6	150

highest concentration used. To see whether ^{64}Cu could be concentrated in the serosal fluid against a gradient, experiments were made starting with equal concentrations of ^{64}Cu in mucosal and serosal fluids and were run for 4 hr. In these, the concentration of ^{64}Cu fell in both fluids throughout the experimental period.

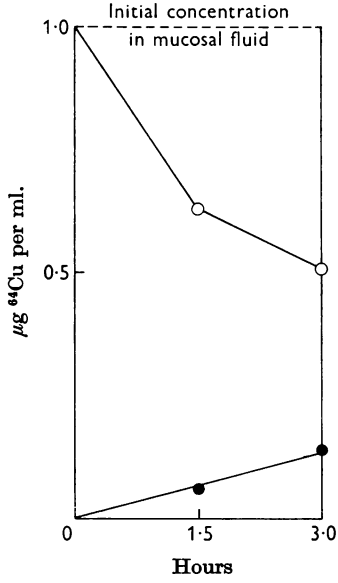

Fig. 6. The time course of an experiment using a Crane & Wilson sac incubated under O_2 for 3 hr., with ^{64}Cu added to the mucosal fluid at an initial concentration of 1 μg/ml. ○——○, ^{64}Cu concentration in mucosal fluid; ●——●, ^{64}Cu concentration in serosal fluid.

In one experiment, at an initial concentration of 3 μg/ml. in the mucosal fluid, ^{64}Cu was measured separately in the mucosal and muscle layers; the concentration was much higher in the mucosa (270 μg/g dry wt.) than in the muscle (100 μg/g dry wt.).

Comparison of the results obtained with ^{64}Cu and those obtained by estimation of total copper showed that the amounts of copper transported across the intestinal wall from mucosal to serosal fluid as indicated by ^{64}Cu, were small in relation to the amounts of unlabelled copper passing from the intestinal wall into the serosal fluid. A direct comparison was made in two experiments run for 1 hr at an initial ^{64}Cu concentration of 1 μg/ml. in the mucosal fluid. The mean transport of ^{64}Cu to the serosal fluid was 2·7 μg/g dry wt., whereas the mean total copper appearing in this fluid was 28·7 μg/g dry wt. These findings were consistent with the failure to show a significant increase in total copper in the serosal fluid when copper was added to the mucosal side.

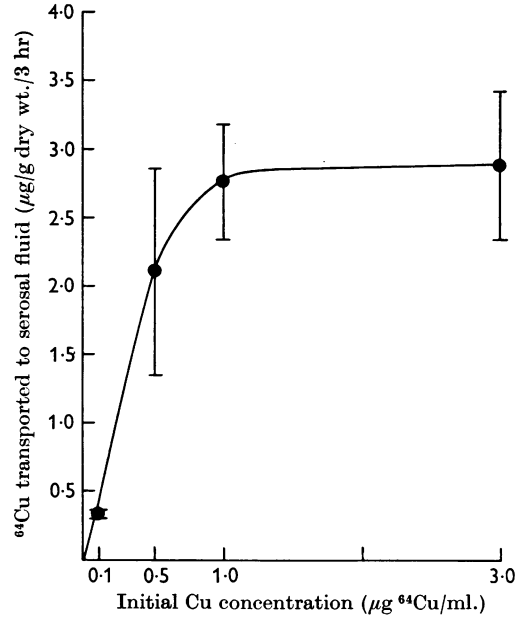

Fig. 7. The relation between the initial concentration of ^{64}Cu in the mucosal fluid and the rate of transport of ^{64}Cu into the serosal fluid, using Crane & Wilson sacs incubated under O_2 for 3 hr. Each point represents the mean of 5–8 experiments. The vertical lines represent the S.E.

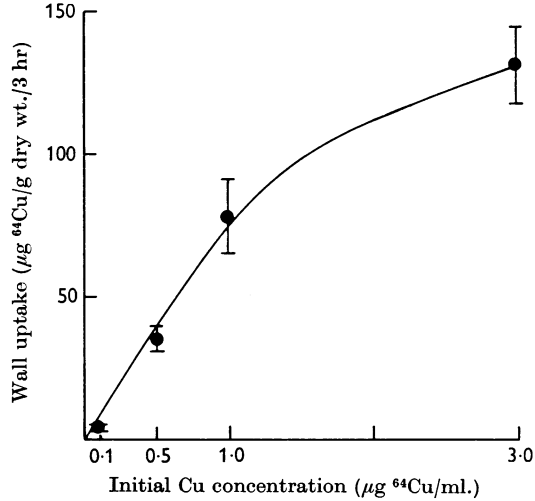

Fig. 8. The relation between the initial concentration of ^{64}Cu in the mucosal fluid, and uptake of ^{64}Cu by the intestinal wall, using Crane & Wilson sacs incubated under O_2 for 3 hr. Each point represents the mean of 5–8 experiments. The vertical lines represent the S.E.

The effects of anoxia and metabolic inhibitors

The effects of anoxia and 2,4-dinitrophenol (DNP) on the total copper passing into the serosal fluid, and on the final copper content of the sac wall, are shown in Table 3. The amounts of copper passing into the serosal fluid were only slightly reduced in the absence of added copper, but in the presence of copper in the mucosal fluid inhibition became more marked.

TABLE 3. The effects of anoxia and DNP on appearance of copper in the serosal fluid and on final wall copper in Crane & Wilson sacs

(The sacs were incubated for 1 hr; in each experiment the control and inhibited sacs were taken from adjacent sites in the lower small intestine of the same animal.)

			Control sacs		Inhibited sacs	
Inhibitor	Cu concentration in mucosal fluid (μg/ml.)	No. of expts.	Cu appearing in serosal fluid (μg/g dry wt./hr)	Final wall Cu (μg/g dry wt.)	Cu appearing in serosal fluid (μg/g dry wt./hr)	Final wall Cu (μg/g dry wt.)
N_2	Nil	9	5·4* ± 0·8	11·5 ± 1·2	4·9 ± 1·7	12·4 ± 0·8
N_2	0·25	2	15·3 —	25·8 —	8·8 —	28·2 —
N_2	0·5	2	14·9 —	33·3 —	7·3 —	27·5 —
N_2	1·0	1	10·0 —	40·4 —	4·5 —	68·7 —
DNP 10^{-4}M	Nil	5	12·4 ± 1·5	16·1 ± 2·4	8·3 ± 2·3	18·9 ± 3·6
DNP 10^{-4}M	0·25	2	21·7 —	20·1 —	9·7 —	28·4 —
DNP 10^{-4}M	1·0	2	33·7 —	66·0 —	13·0 —	73·0 —

* Mean ± S.E.

TABLE 4. The effect of anoxia and metabolic inhibitors on transport of ^{64}Cu into the serosal fluid and on wall uptake of ^{64}Cu by Crane & Wilson sacs. (Experimental procedure as in Table 3)

			Control sacs		Inhibited sacs	
Inhibitor	^{64}Cu concentration in mucosal fluid (μg/ml.)	No. of expts.	^{64}Cu transported to serosal fluid (μg/g dry wt./hr)	^{64}Cu uptake by wall (μg/g dry wt.)	^{64}Cu transported to serosal fluid (μg/g dry wt./hr)	^{64}Cu uptake by wall (μg/g dry wt.)
N_2	0·25	3	0·38	10·0	0·03	10·0
N_2	1·0	3	1·8	67·3	0·07	96·0
DNP 10^{-4}M	0·25	3	0·26	7·7	0·02	9·4
DNP 10^{-4}M	1·0	2	0·89	72·0	0·15	57·4
NaIAA 10^{-3}M	0·25	2	0·30	10·5	0·03	8·9
NaIAA 10^{-3}M	1·0	2	1·8	43·0	0·12	37·7
NaF 10^{-2}M	1·0	3	0·82	83·0	0·13	33·0
KCN 10^{-3}M	0·25	2	0·18	12·5	0·48	3·7
KCN 10^{-3}M	1·0	1	0·63	103	4·4	47·0

The effects on the final wall content of copper were small, though there was a tendency for this to increase, consistent with the reduction in copper passing into the serosal fluid.

The effects of anoxia and metabolic inhibitors on transport of ^{64}Cu from mucosal to serosal fluids, and on wall uptake of ^{64}Cu, are shown in Table 4.

Anoxia, DNP, NaF and iodoacetate (NaIAA) all caused a great reduction in transport into the serosal fluid. Neither anoxia, DNP nor NaIAA caused a significant alteration in wall uptake, though NaF caused a large reduction. KCN also caused a reduction in wall uptake, accompanied by an *increase* in the ^{64}Cu appearing in the serosal fluid.

Water transport

Table 5 shows that as the initial concentration of ^{64}Cu in the mucosal fluid was increased, water transport into the serosal fluid progressively diminished. Water transport was also markedly reduced or abolished when sacs were incubated with nitrogen as the gas phase, or in the presence of metabolic inhibitors.

TABLE 5. Water transport into serosal fluid at increasing concentrations of copper in the mucosal fluid. (Crane & Wilson sacs incubated under O_2 for 3 hr. The number of observations is given in brackets)

Initial ^{64}Cu concentration in mucosal fluid (μg/ml.)	Water transport into serosal fluid (μg/g dry wt./hr)
0·1	3070* ± 107 (5)
0·5	2190 ± 540 (8)
1·0	1620 ± 530 (5)
3·0	980 ± 310 (5)

* Mean ± S.E.

Wet and dry weights of sac walls

The relation of final wet weight to dry weight varied with the experimental conditions. When strips of intestinal wall from the lower segment were rinsed in saline, lightly blotted, and wet and dry weights determined, mean dry weight was 20·1% of wet weight (S.E. 0·25, $n = 18$). In the walls of Crane & Wilson sacs from the same region, incubated under O_2 for 1 hr, and treated similarly, dry weight was only 14·2% of final wet weight (S.E. 0·4, $n = 29$), showing that considerable swelling took place during incubation. In the presence of DNP, 10^{-4}M, swelling tended to be greater (dry wt. 12·4% of wet wt., S.E. 0·8, $n = 4$). The walls of simple sacs from the lower segment, incubated under O_2 for 1 hr, swelled more than those of Crane & Wilson sacs (dry wt. 12·7% of wet wt., S.E. 0·4, $n = 6$).

DISCUSSION

It is clear that the results are not compatible with the hypothesis that copper is transported across the intestinal wall by 'simple diffusion'. This is shown by the differences in copper transport in different regions of the intestine, the lack of a linear relation between initial concentration of

copper and rate of transport across the intestinal wall, and the effects of anoxia and metabolic inhibitors.

The experiments on transport of ^{64}Cu across the intestinal wall in different regions showed that in the lower segment transport from the mucosal to the serosal side was greatest, whereas transport in the opposite direction was least. Transport of copper from the intestinal wall into the serosal fluid also predominated in this region. These results strongly suggest that, in the hamster, the site of maximal absorptive ability for copper is in the lower small intestine.

The experiments with Crane & Wilson sacs from the lower segment confirmed that the passage of copper out of the intestinal wall was mainly unidirectional, showing that it was unlikely to be the result of simple diffusion. This conclusion was supported by the fact that addition of copper to the mucosal side at 1 μg/ml. had no significant effect on the total copper passing into the serosal fluid, in spite of a large increase in copper concentration in the intestinal wall. For this reason, transport of copper across the gut wall from mucosal to serosal fluids could not be studied by using unlabelled copper alone. The use of ^{64}Cu showed that small amounts of copper did cross the intestinal wall during the experimental period. This transport was not proportional to the copper concentration on the mucosal side, or to the concentration of copper taken up by the intestinal wall, and approached a limiting rate when the initial copper concentration on the mucosal side was raised to 1 μg/ml. In contrast, wall uptake of ^{64}Cu was still increasing when the concentration was raised to 3 μg/ml. This showed that passage of ^{64}Cu across the intestinal wall could not be the result of uptake by the wall from the mucosal fluid, followed by simple diffusion into the serosal fluid. It suggests that uptake of ^{64}Cu by the intestinal wall, and transport of ^{64}Cu from the wall into the serosal fluid involve separate processes, and that transport into the serosal fluid is by a mechanism of limited capacity that can be saturated at relatively low concentrations. There was no evidence that copper could be transported across the intestinal wall against a gradient. Though the situation illustrated in Fig. 5 superficially resembles the end-result when a substrate has been transported from mucosal to serosal fluids against a concentration gradient, the high concentration in the serosal fluid was almost entirely due to transport of copper originally present in the intestinal wall, and did not represent transport *across* the wall during the experimental period. Moreover, nearly all the copper found in the serosal fluid was non-diffusible.

The effects of anoxia and metabolic inhibitors on wall uptake of ^{64}Cu were not the same as their effects on transport of ^{64}Cu into the serosal fluid, which emphasizes the differences between these processes. Neither

anoxia, DNP, nor iodoacetate had any appreciable effect on wall uptake of copper from the mucosal side, but they produced marked inhibition of transport of ^{64}Cu into the serosal fluid. The reduction of wall uptake of ^{64}Cu by NaF was probably at least partly a mechanical effect, since this inhibitor caused appreciable mucosal desquamation. The anomalous effects of KCN are possibly to be explained by the fact that cyanide forms a very stable complex with copper, and this may displace equilibria involving copper ions. The results suggest that uptake of copper by the intestinal wall may be mainly due to binding of the metal at the cell surface or within the cells, while the process of transport to the serosal fluid requires metabolic energy.

Several features of the present work suggest the possibility that there are two separate modes of transport of copper from the mucosal to the serosal side of the intestine. (1) The transport of ^{64}Cu from the mucosal to the serosal side continued for at least 3 hr, whereas the transport of unlabelled copper from the intestinal wall into the serosal fluid did not continue for longer than $1\frac{1}{2}$ hr. (2) The transport of ^{64}Cu from the mucosal to the serosal side, though subject to a limiting rate, increased as the concentration of ^{64}Cu in the mucosal fluid was increased, whereas the transport of unlabelled copper from the wall into the serosal fluid was not significantly altered by a low concentration of copper in the mucosal fluid (1 μg/ml.) and was reduced by a higher one (3 μg/ml.). (3) The transport of ^{64}Cu from the mucosal to the serosal side was very strongly inhibited by anoxia and DNP, whereas the transport of unlabelled copper from the wall into the serosal fluid was only moderately reduced. The findings might be due to the existence of a rapid transport mechanism of relatively small capacity (indicated by the transport of ^{64}Cu), and a second mechanism with a 'lag period', in which copper is taken up from the mucosal side but only released to the serosal side after an interval of some hours—so that all that is observed of the process during the experimental period is the phase of release of copper previously taken up by the intestinal wall. Such a hypothesis requires further evidence to confirm it, but is felt to be consistent with the present observations.

Several additional points remain to be mentioned. The cause of the decrease in water transport as the mucosal concentration of copper was increased is uncertain—but it may have been a secondary effect connected with inhibition of transport of Cl^-, since copper reduces the transport of this ion across frog skin (Ussing & Zerahn, 1951). Since water transport into the serosal fluid is reduced or abolished in the presence of anoxia or metabolic inhibitors, it might be argued that reduction of copper transport into the serosal fluid is secondary to this. One possibility is the accumulation of copper in the submucosal and muscle layers, and

subsequent failure to reach the serosal fluid owing to absence of water flow. However, when no copper was added to the preparation, the movement of copper from the gut wall was not appreciably affected by anoxia (Table 3), although water transport was abolished. This suggests that such an explanation is inadequate.

Comparison of the final wet weights with the dry weights of sac walls showed that the degree of swelling varied according to the experimental conditions. Consequently, final wet weight, though used by some authors, is regarded as an unsatisfactory basis for expressing transport rates when these are to be compared under different conditions. Initial wet weight is an inconvenient measurement for technical reasons, so all results have been expressed on a dry weight basis except where it was intended to illustrate relative concentrations in the intestinal wall and the surrounding fluids at the end of an experiment, e.g. Figs. 4 and 5.

It now appears that several metals, including calcium and iron (Schacter, Dowdle & Schenker, 1960; Dowdle, Schacter & Schenker, 1960) are absorbed from the small intestine by active mechanisms which require metabolic energy and are capable of transport against an electrochemical gradient. The present results show that a special mechanism is also concerned in the absorption of copper, as suggested by the results of Gitlin *et al.* (1960). Though active transport in the sense of movement against a gradient was not demonstrable under our conditions, the mechanism appears to be dependent on metabolic energy. Our results suggest that in the hamster the site of maximum absorptive ability for copper is in the lower small intestine, whereas Sachs, Levine, Hill & Hughes (1943) stated that copper absorption in dogs took place only from the upper small intestine, and the rapidity of copper absorption in man also suggests absorption from the upper part of the small intestine. This might be due to species differences such as those observed in the site of absorption of calcium (Schacter *et al.* 1960), but it should also be borne in mind that the site of maximum absorptive ability for a substance is not *necessarily* the main site of absorption in the intact animal (Crampton & Matthews, 1964).

SUMMARY

1. The mechanism of copper absorption was studied using sacs of hamster small intestine *in vitro*.

2. It was found that transport of ^{64}Cu from the mucosal to the serosal fluids, and passage of copper originally present in the intestine wall to the serosal fluid, predominated in the lower small intestine, suggesting that the site of maximum absorptive ability for copper is in this region.

3. The relation between the concentration of ^{64}Cu in the mucosal fluid,

and the rate of transport of ^{64}Cu to the serosal fluid, showed that the rate tends to a maximum at higher concentrations. Wall uptake of ^{64}Cu continued at a concentration at which transport across the intestinal wall had reached a maximal rate.

4. Anoxia and 2,4-dinitrophenol inhibited transport of copper into the serosal fluid, but had no effect on wall uptake of copper from the mucosal side.

5. It was concluded that copper absorption cannot be the result of simple diffusion. Uptake of copper from the mucosal side is probably the result of binding to sites on the cell surfaces or within the cells, but transport to the serosal side must involve a special mechanism or mechanisms dependent on metabolic energy.

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