The absorption, distribution and excretion of labelled copper in young pigs given different quantities, as sulphate or sulphide, orally or intravenously

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Barber, Braude, Mitchell & Cassidy (1955) showed that the addition of copper to a normal ration at a rate of 250 mg/kg resulted in an increased rate of gain in weight of growing pigs, and this observation has since been confirmed in many other experiments. Neither the mode of action of Cu nor the site at which it acts is known. Preliminary investigations at Shinfield (Anonymous, 1959) suggested that the improved rate of gain in weight produced by Cu given as sulphide was similar to that produced by the same amount of Cu given as sulphate, but subsequent work indicated that the sulphate was relatively much more effective (Barber, Braude, Mitchell & Porter, 1960). In none of the experiments, however, was the increased liver storage of Cu that results from high levels of Cu as sulphate in the diet observed in pigs given the same quantity of Cu as sulphide. The preliminary observations suggested that the action of Cu might be entirely in the gut, and one purpose of the work reported here was to investigate the relative absorptions of Cu from sulphate and sulphide.

Comar, Davis & Singer (1948), using ^{64}Cu as a tracer, reported studies of Cu distribution in cattle, but no similar studies have been done with pigs in spite of the interest in supplementing their rations with Cu. Mahoney, Bush, Gubler, Moretz, Cartwright & Wintrobe (1955) injected pigs with cupric acetate, but only to study Cu excretion.

In the experiments reported here, young pigs were given labelled Cu in the food as sulphate or sulphide at either high or low level or intravenous injections of labelled CuSO$_4$, in order to obtain information on the absorption, excretion and tissue distribution of Cu up to 24 h after administration. The content of labelled Cu in the blood was measured throughout the experiments and the distribution of Cu was also studied within the liver and in various blood fractions collected over 72 h.

EXPERIMENTAL

Four experiments were done to study Cu absorption, distribution and excretion in the pig, ^{64}Cu, a γ-ray-emitting isotope of Cu with a half-life of 12·8 h being used. In each experiment, two male litter-mate Large White pigs from the Institute's herd free from virus pneumonia were used. In a fifth experiment with three litter-mates the distribution of labelled Cu in the blood and blood fractions for a 72 h period was

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studied. The basal diet used was as described by Barber *et al.* (1955) and contained 6–7 mg Cu/kg air-dry diet as determined by the method of Andrus (1955). The diet was supplemented in some of the experiments with 250 mg Cu/kg air-dry diet by the addition of either cupric sulphide or sulphate. The labelled Cu was also given either as cupric sulphide or sulphate. At the lower level the total Cu added was 21–24 mg, making the total content of the diet about 40 mg Cu/kg air-dry diet, and at the higher level the total Cu added was 130–190 mg, making the total content of the diet 250–300 mg Cu/kg. The amounts given are shown in Table 1.

All pigs were penned and fed individually for 3 weeks before the 24 h experimental period. During the test period they were kept in individual metabolism crates. Before each experiment the pig was placed in the crate several times so that it could become accustomed to confinement, to handling and to being fed in the crate.

Measurement of radioactivity

Weighed samples of tissues and excreta were placed in flat-bottomed glass tubes (50×19 mm) and counted in a scintillation counter consisting of a well-type thallium-activated sodium-iodide crystal (external dimensions 50×30 mm; well dimensions $40 \cdot 4 \times 23 \cdot 8$ mm), an eleven-stage photomultiplier tube and conventional electronic equipment. It had previously been found that the counting rate observed was independent of sample size up to 5 ml and all samples counted therefore occupied a volume of 5 ml or less. Counting rates were corrected to zero time by reference to a published table (Wright, 1957) and for dead-time coincidence losses by reference to a curve previously constructed for the instrument.

The ^{64}Cu was made in the Harwell pile by irradiation of anhydrous $CuSO_4$ or anhydrous CuS. The Cu was irradiated to saturation and had a specific activity at zero time of 0·9 mc/mg. The $^{64}CuSO_4$ was first dissolved in water, the activity of a measured portion was counted, and from it the total activity in the administered material was calculated. As the ^{64}CuS was insoluble in water, a duplicate sample irradiated in the same can at the same time was used for reference. It was dissolved in warm conc. HNO_3 and a portion of suitable size was counted. The labelled Cu was administered about 8 h after removal from the pile.

Activities counted were in the range of 700–60 000 counts/min with a background of about 500 counts/min. The counting efficiency was approximately 5 % and all activities were calculated as counts/min at zero time.

Preparation of labelled copper sulphide

It was found that about 20 % of the ^{64}Cu in the ^{64}CuS used in Expt 2 was soluble in distilled water or in 0·1 N-HCl. In subsequent experiments this soluble ^{64}Cu was converted into the sulphide before use. The 30 mg of irradiated CuS were transferred from the Polythene ampoule to a tapered centrifuge tube, 5 ml 0·1 N-HCl were added, and H_2S was bubbled into the mixture. A black precipitate formed immediately. Treatment with H_2S was continued for 10 min, the mixture was centrifuged and maintained at a temperature of 70° for 10 min and H_2S was then passed into the clear

Table 1. *Treatment of pigs*

Expt no.	Pig no.	Live weight (kg)	Method of administration of ^{64}Cu	Form of administered Cu*	Pre-experimental diet	Copper administered at the beginning of the experimental period				Total Cu content of diet (mg/kg)†
						Present in basal meal (mg)	Added Cu (mg)	Labelled Cu (mg)	Total (mg)	
1	1063	30·4	By mouth	CuSO$_4$	Basal	4	0	20	24·0	38
	1065	29·1	By mouth	CuSO$_4$	Basal	4	157	20	181	284
2	1061	28·6	By mouth	CuS‡	Basal	4	0	20	24·0	38
	1064	28·6	By mouth	CuS‡	Basal	4	150	33	187	294
2a	1201	18·4	By mouth	CuS	Basal	2·7	0	18·4	21·1	46
	1197	21·6	By mouth	CuS	Basal	3·0	108	19	130	260
3	1126	26·6	By mouth	CuS	High-CuS	3·5	125	18·7	147	249
	1127	32·0	By mouth	CuSO$_4$	High-CuSO$_4$	4	144	20	168	264
4	1141	27·7	By injection	CuSO$_4$	Basal	(3·5)	0	0·8	0·8	(6)
	1143	28·6	By injection	CuSO$_4$	High-CuSO$_4$	(3·5)	(150)	1·2	1·2	(260)
5	1296	35·4	By mouth	CuSO$_4$	Basal	5	149	20	174	255
	1298	30·7	By mouth	CuS	Basal	4	119	18·6	141	252
	1295	32·5	By injection	CuSO$_4$	Basal	(4·5)	0	1·2	1·2	(7)

* Labelled CuSO$_4$ and CuS were anhydrous, whereas the remainder of the added Cu was in the form of CuSO$_4$.5H$_2$O or CuS.H$_2$O.

† Dietary Cu (values in parentheses) was not considered in the calculation of the distribution of labelled Cu in pigs injected with labelled Cu.

‡ Labelled CuS used in this experiment was found to be impure and 20% of the Cu was soluble in water (see p. 60).

supernatant layer. No further precipitate was formed. The tube was centrifuged again, and the supernatant solution was removed. The residue was stirred with 5 ml o·1 N-HCl previously saturated with H_2S and centrifuged, and the supernatant solution was removed. The residue was re-suspended in 5 ml o·1 N-HCl, saturated with H_2S, centrifuged and finally re-suspended in 5 ml boiled distilled water. All losses of ^{64}Cu into the supernatant solutions were measured to permit calculation of the total dose given to each pig.

Methods of administering labelled copper

The methods of administering Cu and the form in which it was administered, together with figures for the total levels of Cu given by mouth or injected, are summarized in Table 1.

The ^{64}Cu as sulphate or sulphide was given at approximately 2 p.m. mixed with half the daily ration based on the Shinfield restricted scale (Braude & Mitchell, 1951). A marker of ferric oxide was included in this meal. The second half of the daily ration was given at about 7 a.m. next morning, but contained no ^{64}Cu other than the small amounts not eaten at the previous meal.

In Expt 4 all the [64]$CuSO_4$ was dissolved in distilled water (0·08 mg Cu/ml water), and 1 ml of the solution was injected into an ear vein. The pigs were fed immediately before this injection, and the same time schedule as in the feeding experiments was then followed. In Expt 5, methods of administration of Cu both orally and intravenously were as described for the other experiments, but the experiment was continued for 72 h.

Methods of taking samples

In all experiments blood samples were taken from the anterior vena cava by the method of Carle & Dewhirst (1942) at 0·5, 1, 2, 3, 4, 7, 10, 16, 21 and 24 h after administration of labelled Cu. In Expt 4 blood samples were also taken 4 min after the injection. In Expt 5 blood samples were taken at intervals up to 72 h after the administration of labelled Cu.

Urine and faeces were collected each time they were voided, and a sample of each excretion was counted separately.

The pigs were slaughtered at 24–24·5 h after the beginning of the experiment by shooting with a humane killer and were immediately bled. Samples of various tissues were taken for counting: eight from the liver at the centre and at the periphery of each of the four lobes; one each at the anterior pole of the cortex of the right kidney, at the intermediate lobe of the lung, at the left ventricular wall of the heart, at the dorsal pole of the spleen, from the cerebrum, the right psoas muscle, the back fat and skin from the mid-region of back, the left first rib, the pancreas, the bile and bladder contents. The bladder contents were included in the total urinary excretion. Before samples were taken the back fat and skin were frozen at $-25°$ to facilitate separation. Samples of the gut wall were also taken: one from the fundic region of the stomach, three at a distance of approximately 4 m from each end and in the centre of the small intestine, one from the caecum, and two at approximately 30 cm from each end of the colon.

Samples of the gut contents were taken from the composite contents of the stomach

and of the caecum and from sites in the small intestine and colon from which the tissue samples were taken. In Expts 2*a*, 3 and 4, samples were also taken from the composite contents of two approximately equal sections of the colon, as the first two experiments had shown a wide variation in the concentration of labelled Cu at these two sites of sampling. As the contents of this section of the gut represented a large proportion of the total Cu in pigs given labelled Cu, the additional refinement in sampling seemed desirable. Results with the two methods of sampling were, however, similar.

The weights of blood, muscle, fat, bone, skin, brain and pancreas were calculated on the basis of McMeekan's (1940) data for 16-week-old male pigs averaging 31 kg in weight. Other organs, including stomach and caecum, parts of the small intestine and colon and the entire gut contents were weighed separately.

Ultrafiltration of gut contents

Samples of gut contents were centrifuged to give supernatant fractions which were removed and filtered under vacuum through Visking Cellophane tubing. The activity of measured portions of the ultrafiltrates obtained in this way was counted to give an indication of the levels of soluble Cu in the gut contents.

Fractionation of whole blood

To study the distribution of labelled Cu, whole blood, to which both heparin and oxalate had been added, was centrifuged, and the plasma was removed. The cells were washed once with 5 ml of 0·9 % saline solution, and washings and cells were counted separately. In the complete fractionation of the plasma, in Expts 2*a* and 5, 1 mg Cu as cupric acetate in 0·1 ml solution was added to 1 ml plasma, and the protein was precipitated by addition of 4 ml absolute ethanol. The supernatant layer and the protein obtained by centrifuging were counted. These steps were repeated without added Cu; as similar results were obtained, no additional Cu was used in Expt 5, in which albumin and globulin fractions of the blood protein were separated. The methods of Pillemer & Hutchinson (1945), who used methanol to precipitate the globulin, and of Bush, Mahoney, Markowitz, Gubler, Cartwright & Wintrobe (1955), who used ammonium sulphate for that purpose, were applied. The method of Pillemer & Hutchinson (1945) was slightly modified in that the plasma was centrifuged at 20° and not at 0°. The globulin precipitate and albumin-containing supernatant liquid were assayed for ^{64}Cu activity. Electrophoresis of a selection of the globulin and albumin samples obtained by either the ammonium-sulphate or the methanol method of fractionation indicated that the separation of these two components was not sharp, a small fraction of one sometimes contaminating the other. The two methods of fractionation also tended to give different results, particularly for blood taken 48 h after dosing. A portion of the plasma was subjected to ultrafiltration through a Cellophane membrane to give an indication of the level of unbound Cu in the plasma. The activities of the samples of blood plasma taken 1 h after the pigs had received copper sulphide were too low for them to be measured in the protein fractions. The activity decayed to such an extent 72 h after dosing that fractionation of the blood was not attempted.

RESULTS AND DISCUSSION

The tissue distribution of the labelled Cu in the pigs slaughtered 24 h after dosing in Expts 1–4 is given in Table 2. The figures for the dose given represent the total Cu in the 2 p.m. meal of the pigs given ^{64}Cu by mouth, but they represent only the injected Cu in Expt 4. As there are no published data on Cu distribution in the pig, results are given for individual pigs to allow more detailed consideration of the Cu levels.

Absorption and excretion

In Expts 1 and 2 pigs nos. 1063 and 1065 received by mouth labelled Cu in the form of cupric sulphate and pigs nos. 1061 and 1064 received the impure cupric sulphide of which a considerable proportion of the Cu was soluble (see p. 60). Presumably because of this soluble Cu the results for the two pairs were closely similar. As shown in Table 2, pigs nos. 1063 and 1061, which received the labelled Cu at the lower level (24 mg), absorbed 8·7 and 9·7% of it; pigs nos. 1065 and 1064, which received the labelled Cu at the higher level (about 185 mg), absorbed only 2·9 and 4·6% of it, which represented, however, a total weight about three times as great as that absorbed by pigs nos. 1063 and 1061. Pig no. 1127 in Expt 3, whose diet had contained supplementary cupric sulphate for 3 weeks before the experiment, absorbed 3·8% of a dose of labelled Cu given at the high level (168 mg) in the form of sulphate. The degree of absorption was similar to that by pigs nos. 1065 and 1064, whose previous diet had contained no supplementary Cu.

In Expts 2a and 3, pigs nos. 1201, 1197 and 1126 received their labelled Cu as cupric sulphide free from soluble Cu. Pig no. 1201 which received it at the lower level (20 mg) absorbed 1·9% of it, and pig no. 1197, which received it at the higher level (125 mg), absorbed 2·1%. Pig no. 1126, whose diet had previously contained supplementary cupric sulphide, absorbed only 1·1% of a dose of labelled Cu given at the higher level (145 mg as CuS). These figures represent absorption rates of 20–70% of those shown by pigs given the labelled Cu as sulphate.

The percentage distribution of the labelled Cu in the digestive tract, which may have been Cu in process of absorption, excretion, re-absorption or tissue storage, is shown in Table 3. The major sites of Cu transfer across the gut wall appeared to be the small intestine and the colon; in the former, the highest concentration occurred in the end section. In pigs given labelled cupric sulphate by mouth, more was found in the small intestine than in the colon, whereas in pigs given labelled cupric sulphide by mouth, the reverse was true. The stomach and caecum walls contained a mean of 5·7 and 9·6% of the total Cu found in the gut tissues of the eight pigs given Cu by mouth.

The appearance of labelled Cu in the faeces occurred less than 2 h after dosing. This Cu was probably from excretion into the lower gut; when the labelled Cu was injected (Expt 4) it appeared in the faeces 30–100 min after the dosing. Large amounts of labelled Cu appeared in the faeces 17–20 h after labelled cupric sulphate had been given by mouth and at approximately the same time as the ferric-oxide marker given in the feed. In the pigs given labelled Cu as sulphide, large quantities appeared in the faeces 6 h 42 min, 7 h 18 min and 8 h 24 min after the dosing, whereas the ferric-oxide

Table 2. *Distribution of labelled copper 24 h after administration of ^{64}Cu*

	1	1	2	2	2a	2a	3	3	4	4
Expt no.	1063	1065	1061	1064	1201	1197	1126	1127	1141	1143
Pig no.										
Pre-experimental period (3 weeks)										
Diet	Basal	Basal	Basal	Basal	Basal	Basal	High-CuS	High-CuSO₄	Basal	High-CuSO₄
Mean daily gain (kg)	0·48	0·45	0·40	0·43	0·43	0·43	0·37	0·57	0·43	0·45
Food eaten per kg gain (kg)	2·49	2·57	2·84	2·68	1·92	2·10	2·83	2·08	2·57	2·47
Experimental period										
Initial weight of pig (kg)	30·4	29·1	28·6	28·6	18·4	21·6	26·7	32·0	27·7	28·6
Diet	Basal	High-CuSO₄	Basal	High-CuS	Basal	High-CuS	High-CuS	High-CuSO₄	Basal	High-CuSO₄
Method of administration of ^{64}Cu	By mouth	By mouth	By mouth	By mouth	By mouth	By mouth	By mouth	By mouth	By injection	By injection
Form of Cu administered	CuSO₄	CuSO₄	CuS*	CuS*	CuS	CuS	CuS	CuSO₄	CuSO₄	CuSO₄
Cu given at beginning of experimental period (mg)†	24·0	181	24·0	187	20·3	125	145	168	0·8	12
Cu absorbed: mg	2·081	5·289	2·339	8·532	0·378	2·615	1·530	6·355	—	—
as percentage of dose	8·7	2·9	9·7	4·6	1·9	2·1	1·1	3·8	—	—
Cu recovered after 24 h (mg):										
Blood	0·223	0·560	0·206	0·828	0·045	0·323	0·118	0·231	0·058	0·045
Tissues										
Liver	0·746	1·941	1·069	3·006	0·087	0·749	0·470	2·776	0·350	0·617
Kidney	0·117	0·273	0·162	0·581	0·014	†	0·036	0·180	0·029	0·030
Lung	0·029	0·044	0·023	0·054	0·003	0·014	0·008	0·019	0·008	0·003
Heart	0·006	0·016	0·005	0·022	0·001	0·009	0·001	0·008	0·002	0·002
Spleen	0·002	0·004	0·001	0·005	§	0·003	0·001	0·002	§	§
Pancreas	0·007	0·019	0·005	0·027	0·002	0·016	0·002	0·037	0·001	0·001
Bile	0·001	0·020	0·002	0·007	§	0·005	§	0·010	0·001	0·001
Brain	0·001	0·004	0·001	0·004	§	0·001	0·001	0·002	§	§
Muscle	0·220	0·577	0·133	0·558	0·033	0·321	0·093	0·785	0·037	0·033
Fat	0·156	0·342	0·091	0·428	0·023	0·189	0·073	0·152	0·034	0·025
Skeleton	‡	‡	0·104	0·489	0·021	0·165	0·068	0·166	0·037	0·021
Skin	0·142	0·370	0·116	0·521	0·021	0·185	0·079	0·187	0·045	0·027
Stomach	0·025	0·063	0·025	0·078	0·006	0·048	0·026	0·114	0·005	0·003
Small intestine	0·191	0·438	0·129	0·549	0·039	0·268	0·122	1·680	0·025	0·020
Caecum	0·036	0·115	0·088	0·172	0·008	0·045	0·033	0·131	0·004	0·004
Colon	0·165	0·369	0·158	1·146	0·073	0·259	0·387	0·408	0·014	0·009
Total	1·844	4·595	2·112	7·647	0·331	2·277	1·402	6·057	0·592	0·796
Urine (24 h period)	0·014	0·134	0·011	0·057	0·002	0·015	0·010	0·067	0·012	0·004
Intestinal contents										
Stomach	0·602	3·482	0·921	2·435	1·564	7·818	4·777	3·923	0·002	0·003
Small intestine	0·434	3·148	0·981	2·255	0·804	2·395	1·242	3·784	0·016	0·074
Caecum	1·133	7·855	2·239	6·227	0·914	12·442	4·196	10·750	0·011	0·022
Colon	12·537	92·637	9·901	98·730	4·781	36·950	116·594	107·001	0·101	0·217
Faeces (24 h period)	1·564	32·009	2·240	12·022	5·421	28·248	28·653	31·027	0·031	0·049
Total	16·270	139·131	16·282	121·669	13·484	87·853	155·462	156·485	0·161	0·365
Uneaten food	—	—	—	—	0·770	5·003	1·817	—	—	—
Total labelled Cu recovered:										
mg	18·351	144·420	18·611	130·201	14·632	95·471	158·809	162·840	0·823	1·210
as percentage of dose	76·5	79·8	77·5	69·6	72·1	76·4	109·5	96·9	102·9	100·8

* Impure CuS containing 20 % Cu soluble in water and 27 % Cu soluble in 0·1 N-HCl (see p. 60).   † Corrected for uneaten food.   ‡ Not determined.   § Less than 0·0005 mg.

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marker appeared in the usual time of 16–20 h. This increased rate of movement of the labelled Cu when supplied as sulphide cannot be explained at present.

Labelled Cu usually appeared in the first sample of urine excreted after dosing, the earliest appearance being after 10 min.

Ultrafiltration of samples of gut contents from pigs receiving Cu orally either as sulphate or sulphide indicated the presence of some free Cu^{2+}, but the concentration in the gut contents of pigs given cupric sulphate was approximately six times as high as for those receiving cupric sulphide.

Table 3. *Mean percentage distribution of labelled copper in tissues of the digestive tract 24 h after administration of* ^{64}Cu

	Stomach	Intestine*	Caecum	Colon†
Three pigs given $CuSO_4$ by mouth	5·7	54·0	8·7	31·6
Three pigs given CuS by mouth	5·9	31·7	6·6	55·8
Three pigs given CuS‡ by mouth	5·2	30·2	15·4	49·2
Two pigs given $CuSO_4$ by injection	9·8	54·0	9·5	26·7

* Mean value for three locations.
† Mean value for two locations.
‡ Contained some soluble copper (see Table 1).

In Expt 4 (pigs nos. 1141 and 1143, Table 2), 83 and 70 % of the injected Cu were still present in the blood, tissues and urine at the end of 24 h. Of the amounts excreted in the urine, faeces and gut contents, that in the urine represented only 1 and 7 % of the total in each pig. It would appear from Table 3 that the site of Cu transfer through the gut walls was again predominantly in the small intestine and colon, as these tissues contained 54 and 27 % of the labelled Cu in the gut tissues of the injected pigs. Dukes (1947) reported that the mean daily bile secretion of several species was 15–30 ml/kg body-weight. Calculated from the upper limit, bile secretion for 24 h would have been 830 and 860 ml for pigs nos. 1141 and 1143 and could have accounted for 40 and 34 % of the total labelled Cu in the gut. It is unlikely that the concentration of labelled Cu in the bile was as high throughout the entire 24 h period as it was at slaughter, and these figures are therefore undoubtedly high. Mahoney *et al.* (1955), from experiments in which the bile duct was blocked, concluded that, although excretion of Cu could be mainly through the intestinal walls and the kidney, the bile could also represent a major route of excretion.

Blood levels

The amounts of labelled Cu circulating in the blood 24 h after administration are shown in Table 2. It is seen that the values for pigs that had been receiving supplementary Cu for 3 weeks before the experiments tended to be lower, indicating that dilution had probably occurred. The curves in Figs. 1, 2 and 3 represent the levels of labelled Cu in the total circulating blood up to the time of slaughter 24 h after dosing. It will be seen that the levels in all pigs given the dose orally continued to rise for 16–24 h. The blood levels of labelled Cu in the pigs given Cu as sulphate at the lower level were approximately five times as high as in pigs given the same dose of Cu as sulphide. In the pigs given Cu as sulphate at the higher level, the blood levels of
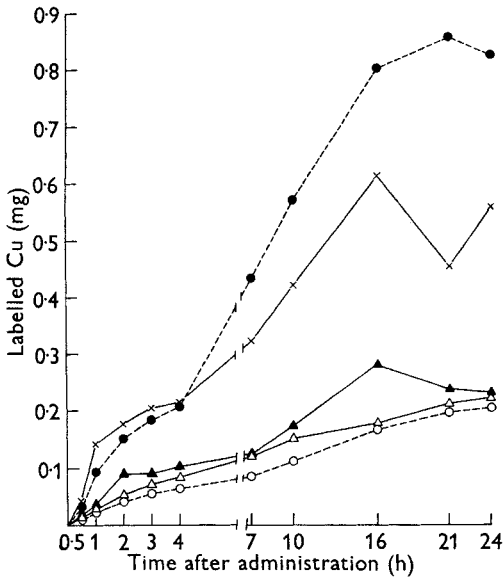
Table 4. *Distribution of labelled copper in fractionated whole blood*

Copper contained in blood fractions (mμg/ml whole blood)

Expt no.	Pig no.	Treatment	Sampling time (h)	Whole blood	Cells	Ultra-filtrate	Plasma	Protein $+Cu^{2+}$*	Protein-free serum $+Cu^{2+}$*	Plasma protein	Protein-free serum	Ammonium sulphate Albumin	Ammonium sulphate Globulin	Methanol Albumin	Methanol Globulin	Mean albumin-bound Cu (%) (X)	Mean globulin-bound Cu (%) (Y)	$\frac{X}{Y}$†
2a	1201	Low-CuS diet	2	1·6	0·4	—	1·4	1·2	‡	1·2	‡	—	—	—	—	—	—	—
	1197	High-CuS diet	3	22·0	4·8	—	17·2	16·4	2·6	17·2	‡	—	—	—	—	—	—	—
	1201	Low-CuS diet	21	33·7	0·4	—	30·5	22·8	1·6	—	—	—	—	—	—	—	—	—
	1197	High-CuS diet	21	275	5·9	—	192	170	9·2	—	—	—	—	—	—	—	—	—
5	1296	High-CuSO₄ diet	1	26·6	0·8	7·2	29·0	—	—	—	—	11·9	3·0	11·7	6·2	45	17	3·0
	1295	CuSO₄ injected	1	70·2	4·1	12·8	70·7	—	—	—	—	35·9	7·7	32·0	11·4	49	14	3·3
	1296	High-CuSO₄ diet	18	120	6·6	4·3	117	—	—	—	—	58·1	37·6	45·4	45·9	43	35	1·3
	1298	High-CuS diet	18	177	4·8	10·0	161	—	—	—	—	79·4	48·7	71·3	74·0	43	37	1·3
	1295	CuSO₄ injected	18	38·8	1·8	3·5	39·0	—	—	—	—	15·1	15·3	16·0	13·9	41	38	1·1
	1296	High-CuSO₄ diet	30	92·5	2·8	5·0	101	—	—	—	—	54·0	25·3	40·0	43·6	51	37	1·5
	1298	High-CuS diet	30	170	1·9	4·6	—	—	—	—	—	54·7	73·2	97·1	38·8	45	33	1·6
	1295	CuSO₄ injected	30	40·7	0·4	0·5	34·9	—	—	—	—	11·5	14·5	16·7	9·7	35	30	1·3
	1296	High-CuSO₄ diet	48	81·6	—	—	—	—	—	—	—	15·0	65·6	50·9	17·3	39	51	0·8
	1298	High-CuS diet	48	170	—	—	—	—	—	—	—	61·9	93·4	—	—	36	55	0·7
	1295	CuSO₄ injected	48	39·6	—	—	—	—	—	—	—	9·5	20·6	19·0	10·4	36	39	0·9

* See p. 63.
† The ratio of labelled Cu in the albumin fraction to that in the globulin fraction calculated from the mean values obtained by the two methods of fractionation.
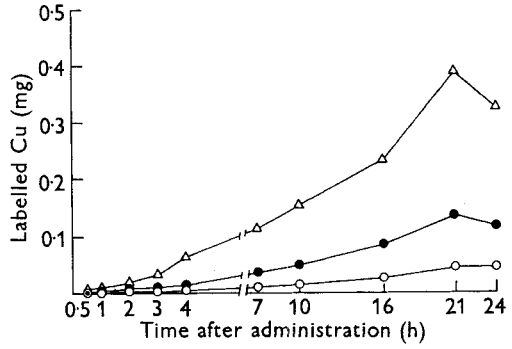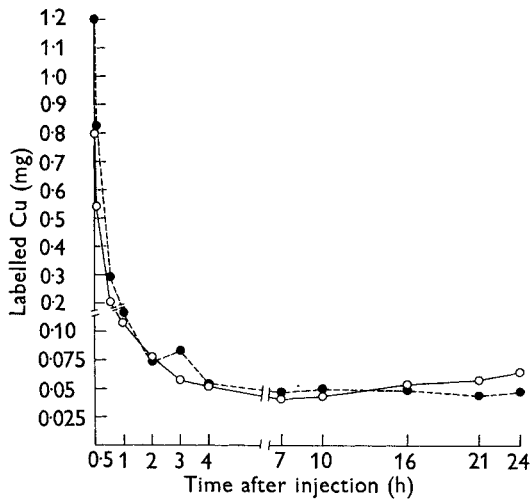‡ No measurable radioactivity.

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Amounts of labelled copper in the blood of pigs after oral administration of copper sulphate or impure copper sulphide. △—△, copper sulphate, low dosage; ▲—▲, copper sulphate, high dosage after 3 weeks preliminary high-level treatment; ×—×, copper sulphate, high dosage; ○- - - -○, copper sulphide, low dosage; ●- - - -●, copper sulphide, high dosage.

Fig. 2. Amounts of labelled copper in the blood of pigs after oral administration of copper sulphide. ○—○, low dosage; ●—●, high dosage after 3 weeks preliminary high-level treatment; △—△, high dosage.

Fig. 3. Amounts of labelled copper in the blood of pigs after injection of copper sulphate. ○—○, pigs on low-copper-sulphate diet; ●—●, pigs on high-copper-sulphate diet.

labelled Cu were only twice as high as in those given the same dose of Cu as sulphide (see also Table 2).

In the two pigs injected with labelled Cu, 65–68% of the dose was found in the blood after 4 min. This is a higher percentage than that observed by Comar *et al.* (1948) in the bovine animal, in which only 36% of the dose was present in the blood after 5 min. In the pigs, approximately 25% of the initial dose of labelled Cu was still present in the blood 30 min after the injections. The concentration fell for 4 h and then
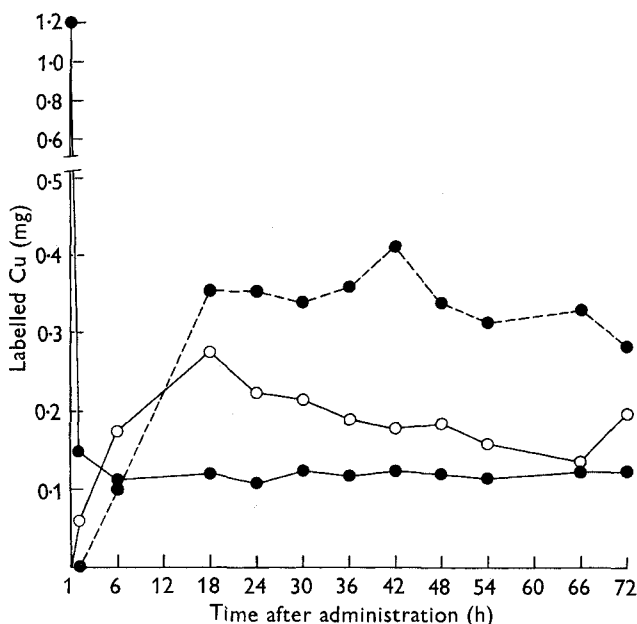
Fig. 4. Amounts of labelled copper in the blood of pigs for a 72 h period after administration of copper sulphate orally or by injection or of copper sulphide orally. ○—○, copper sulphate orally; ●- - - -●, copper sulphide orally; ●—●, copper sulphate by injection.

remained nearly constant for a further 20 h. Most of the injected Cu was presumably removed by the liver, as the livers of the two pigs in Expt 4 still contained 44 and 51% of the dose 24 h after administration. Only 22 and 31% of the Cu was excreted into the gut or the urine during the 24 h period. As excretion was continuous into the urine and presumably into the digestive tract, the liver stores of Cu apparently maintained the blood Cu at a relatively constant level from 4 to 24 h or even to 72 h after the dose, as shown in Fig. 4.

In Expt 5 the maximum concentration of labelled Cu in the blood of pig no. 1296, which had received it at the higher dose (174 mg) in the form of cupric sulphate, was not as high as it had been in the blood of pigs similarly treated in previous experiments. It is possible that this was the result of ordinary biological variation.

As shown in Table 4, most of the labelled Cu in the blood was in the plasma proteins, only a small amount being carried in the cells. The fraction present in the free state was also small, except in the period immediately after dosing. The albumin

fraction of the blood bound 35–50% of the total labelled Cu circulating during the period up to 48 h after the dose.

One h after administration of Cu the albumin fraction contained about three times as much labelled Cu as the globulin fraction, but after 18, 30 and 48 h the albumin fraction contained only 0·7–1·6 times as much labelled Cu as the globulin fraction. This change in relative levels of labelled Cu in the two fractions resulted largely from an increase in the percentage of labelled Cu associated with the globulin fraction, for the percentage of labelled Cu associated with the albumin remained relatively constant. It is possible that a Cu-containing globulin, probably ceruloplasmin, as suggested by Bush, Mahoney, Gubler, Cartwright & Wintrobe (1956), is formed by the liver or by some other organ and that this protein causes the increase in the globulin-bound Cu.

In previous studies there has been little agreement on the combination of Cu with the serum proteins. Moustgaard & Højgaard Olsen (1951) found that 3 h after administration of ^{64}Cu to pigs approximately 10% of the radioactivity was in the globulin fraction and 90% in the albumin fraction. Bush et al. (1956) reported that in man the Cu in transport was associated with albumin, the remainder being firmly bound to α_2-globulin. The fraction associated with albumin could pass readily and rapidly into the red blood cells.

Tissue distribution

As shown in Table 2, the absorbed Cu was widely distributed in the tissues of all animals, regardless of the total amount absorbed. The pattern of distribution of Cu in the tissues was similar for all pigs. There is no indication of its function in the various tissues. Intravenously injected Cu was also widely distributed and soon after injection reached an approximately constant level in the blood. These observations agree with those of Comar et al. (1948) on cattle.

The concentrations of labelled Cu, from which the total amounts in the tissues were calculated, were highest in liver and kidney but relatively low in all other organs. The total liver content was lower in the pigs given cupric sulphide than in those given cupric sulphate. This is in general agreement with the results of feeding experiments reported by Barber et al. (1960), when it was observed that the addition of cupric sulphate to the diet at a concentration of 250 mg Cu/kg (giving a dose to the pig of 250–750 mg Cu/day according to age) increased the levels of Cu in the liver, whereas the same dose of Cu as sulphide did not.

The muscle accounted for more Cu than the fat, skeleton or skin, each of which had a similar concentration of labelled Cu. The tissues of the digestive tract contained 20–40% of the total labelled Cu present in the tissues and urine (Table 2).

Cu distribution in liver lobes

Table 5 gives the values, expressed as a percentage of the mean value for the whole liver, for labelled Cu in eight areas representing two sections of the four lobes of the pig's liver. The levels of labelled Cu differed greatly, not only between lobes, but also between the peripheral and central portions of each lobe, which agrees with the results of chemical analyses previously carried out at this Institute (unpublished) and with

those of Cassidy & Eva (1958). Standard deviations were reasonably small, indicating considerable uniformity in distribution from liver to liver.

The growth response obtained by the addition of Cu to the diets of pigs, which has been firmly established by many experiments and has now been shown to be greater with Cu given as the sulphate than as the sulphide, could be due either to a systemic effect resulting from the absorbed Cu, to the effect of soluble Cu acting within the digestive tract or to both. The results of these experiments do not indicate in which of these ways the action takes place.

Table 5. *Distribution of labelled copper in the liver lobes of ten pigs expressed as a percentage of the mean count in the whole liver*

Lobe	Section	Mean value with standard deviation
Right lateral	Central	120·1 ± 8·7
	Periphery	97·0 ± 8·2
Right median	Central	112·5 ± 4·7
	Periphery	93·5 ± 5·3
Left median	Central	106·9 ± 7·4
	Periphery	86·3 ± 7·6
Left lateral	Central	102·4 ± 6·3
	Periphery	81·9 ± 4·1

SUMMARY

1. Four pairs of male Large White pigs, 12–13 weeks old, averaging 27 kg each in weight, were given labelled copper by mouth at two levels (about 21–24 mg or 130–190 mg), either as sulphate or as sulphide, and a fifth pair was intravenously injected with labelled Cu as sulphate. The pigs were kept individually in metabolism crates for 24 h after the dose, and were then slaughtered. In another experiment with three pigs the distribution of labelled Cu in the blood and blood fractions for a 72 h period after the administration of ^{64}Cu by mouth or injection was studied.

2. The percentage of labelled Cu absorbed averaged 5·1 in three pigs given cupric sulphate and 1·7 in three pigs given pure cupric sulphide. Absorption rates, expressed as percentages of the total doses, decreased in pigs given sulphate, but not in pigs given sulphide, as levels of labelled Cu given by mouth increased. A diet supplemented with 250 mg Cu/kg as sulphate given for 3 weeks before administration of the labelled Cu had little effect on absorption rates. When Cu as sulphide was given in the same way, however, the absorption of labelled Cu appeared to be reduced.

3. Whether the pigs received the Cu by mouth, or by vein, Cu transfer across the gut wall apparently occurred mainly in the small intestine and the colon, more in the intestine when the Cu was given as sulphate and more in the colon when it was given as sulphide. The stomach and caecum walls also contained appreciable quantities of labelled Cu.

4. The faeces were the major route of Cu excretion, only a small fraction of the total occurring in the urine. It was calculated that the bile could account for up to 40 % of the excretion.

5. Labelled Cu appeared in the blood at a comparatively high level within 30 min after oral administration of sulphate and at a lower level between 30 min and 1 h after sulphide. The blood levels continued to rise for 16–24 h after oral administration of Cu, either as sulphate or sulphide, and then remained relatively stable for a further 48 h. Injected ^{64}Cu was removed rapidly from the blood during the first 4 h, and the level then remained stable up to 72 h after injection. The liver was the major site of storage, containing 44–51 % of the total dose of labelled Cu 24 h after intravenous injection.

6. The labelled Cu was transported mostly by the plasma proteins, the blood-cells transporting less. Over 48 h, the labelled Cu in the albumin fraction remained a relatively constant proportion of the total in the plasma, whereas the proportion of labelled Cu in the globulin fraction increased.

7. There was wide variation in the levels of labelled Cu between different lobes and between different parts of the same lobe, but approximately the same distribution was found in all livers.

8. The response in rate of weight gain of pigs given high levels of Cu appears to be related to the amount of soluble copper in the gut, but these experiments do not indicate whether the site of action is systemic, enteric or both.

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