

Copper deficiency in rats fed upon raw meat

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Evidence given in earlier papers (Moore, Sharman, Constable, Symonds, Martin & Collinson, 1962; Moore, Impey, Martin & Symonds, 1963) has supported the view that a diet of raw beef produces both skeletal and haematological abnormalities in young piebald rats. The skeletal lesions appear to be due mainly, and probably entirely, to the low calcium content of the meat. Since Ca requirements are much higher in young than in mature rats, the effect of a meat diet on the bones can be demonstrated most readily in weanlings. Mature rats can subsist for long periods on diets of unsupplemented meat without developing noticeable skeletal abnormalities. The haematological lesions are those of a severe microcytic anaemia, as seen in dietary deficiency of iron. Associated abnormalities are loss of the normal brown Fe-containing pigment from the anterior surfaces of the incisor teeth and hypochromotrichia. The key element for preventing both the anaemia and the two associated abnormalities caused by a diet of raw meat was found not to be Fe, as first expected, but copper (Moore, 1962*a*). Indications have been reported that the severity of the lesions now associated with Cu deficiency may be reduced by an adequate, but not excessive, intake of Ca (Moore *et al.* 1962, 1963).

Adler (1958) observed anaemia in mice fed upon meat. Subsequently 'meat anaemia' has been studied extensively in the same laboratory by Ilan & Guggenheim (1960, 1962) and Ilan, Kende & Guggenheim (1960). Mice, but not rats, developed severe anaemia when fed upon raw skeletal muscle from animals of various species. Supplements of organs, particularly liver, were first tried for treating the anaemia and found to be effective. Eventually Cu was found to prevent or cure the anaemia (Ilan & Guggenheim 1961; Ilan, Guggenheim & Ickowicz, 1963; Guggenheim, Ilan, Fostick & Tal, 1963). Ca was also beneficial, but manganese was not. Both Cu and Mn improved the utilization of the small amount of Ca in meat for mineralization of the bone.

Moore (1962*b*) reported briefly the appearance of anaemia, dental depigmentation and hypochromotrichia in rats fed upon various forms of meat and also their prevention by Cu, but not by Mn or pantothenic acid. A preliminary account of breeding trials, which indicated the importance of Cu for lactation, was also given. Further experiments (Moore, 1963) showed that albino rats, as well as piebald, develop Cu deficiency when fed on raw meat. In piebalds cobalt was ineffective against the abnormalities, but they were usually prevented or ameliorated by 'domestic' cooking of the meat. Commercial processing appeared to be less effective. Evidence was

obtained that in meat-fed rats Cu causes the liver to be smaller in size than in un-supplemented rats, but to have a higher fat content.

This paper gives a detailed account of the effects of raw meat in producing anaemia, dental depigmentation and hypochromotrichia in rats. Evidence is included also of the beneficial effects of Cu and domestic cooking, and of the ineffectiveness of other nutrients and of two forms of commercial processing.

EXPERIMENTAL

Rats. Pure-bred, piebald rats were used for all experiments except one, in which some groups were albino. Except in one experiment the rats were all males; they were taken for experiment at a body-weight of about 70 g. At this stage of development they could survive for long periods without supplements of Ca, under conditions favourable for the appearance of the abnormalities due to Cu deficiency.

Plan of experiments. Expt 1 was carried out on female rats. Its aim was to prove that our routine diet of raw minced beef was capable, when supplemented with Ca, Cu and fat-soluble vitamins, of allowing prolonged and rapid growth. In this experiment, control rats received the stock diet used for many years in the breeding room at this laboratory. It consists of commercial rat cubes (wholemeal flour 46%, Sussex-ground oats 40%, white fish meal 8%, dried yeast 1%, dried skim milk 3%, with added vitamins A, D and E and trace elements). Milk was allowed *ad lib.* daily, and meat, liver, wheat (all cooked), carrots and cabbage (raw) were each given once weekly.

In each of four further experiments, groups were included that received raw minced beef, either without mineral supplements (negative controls) or with adequate supplements of Cu (positive controls). In the negative controls the abnormalities associated with Cu deficiency invariably developed. The points investigated in the various experiments were:

Expt 2. Effects of supplements of calcium pantothenate and of Mn; effects of substituting raw mutton or pork for raw beef.

Expt 3. Effects of substituting cooked beef, corned beef or canned pork for raw beef; substitution of albino for piebald rats.

Expt 4. Effects of supplements of cobalt, of reducing the Cu supplement or of cooking the beef.

Expt 5. Effects of reducing the Cu supplement or of cooking the beef by autoclaving.

Raw minced steak. Prime chine, or shoulder steak, from British-reared steers, was supplied by a local butcher. He was instructed to include such portions of attached adipose tissues as are usually sold with the steak, but to exclude all bone. The steak was minced first by the butcher and reminced to a fine state on arrival at the laboratory. It was then kneaded by hand into a uniform paste and stored in glass vessels in a refrigerator until required for feeding the rats. Fresh bulk supplies of meat were prepared twice weekly.

The various supplements, other than the fat-soluble vitamins, were mixed into the meat during the kneading stage. Portions of meat, in freshly washed food-pots, were given daily to the rats. Daily inspection of the food left over by the rats indicated that

the material was eaten uniformly, without rejection of fat particles. The excess of food from the previous day was discarded.

Determination of fats in the steak, on samples taken at different times, varied only slightly from a mean of 17%. Moisture was 62% and protein (by difference, neglecting non-protein extractives and ash) 21%. About 65% of the calories supplied by the steak, therefore, came from fat. The steak contained 12.2 mg Ca, 4.9 mg Fe and 160 μ g Cu per 100 g wet weight.

Cooked steak. In Expt 3 minced steak was cooked in an enamel saucepan. The meat was stirred in the saucepan with half its own weight of tap water. The saucepan was then covered with a porcelain plate, and the meat was gently boiled with occasional stirring for 90 min. It was calculated that the tap water contributed about 2.5 mg Ca/100 g original meat.

In Expt 4 the saucepan was replaced by a closed glass casserole and the tap water by distilled water. The meat was again cooked for 90 min, but in an oven. In Expt 5 the meat was placed in the glass casserole, without addition of water, and was autoclaved for 20 min at a pressure of 10 lb/in².

All the cooking processes caused the meat to shrink and fat to be extruded. After cooking, the meat and surrounding fat and fluid were placed in a refrigerator, which caused the fat to solidify and the fluid to form a jelly. The mixture was then stirred up well before it was given to the rats.

The first two cooking processes, in which water was added to the meat and partly removed by evaporation, resulted in a net addition of 34% of water. The autoclaving caused an increase of 3%.

Other kinds of meat. Raw mutton and pork, both from the shoulder, were obtained from the butcher in large pieces and were finely minced and kneaded in the laboratory. The mutton contained 21.3 g fat, 60.0 g moisture, 18.7 g protein and 128 μ g Cu/100 g. The pork contained 10.3 g fat, 70.4 g moisture, 19.3 g protein and 101 μ g Cu per 100 g. According to food tables (McCance & Widdowson, 1960), mutton and pork resemble beef in containing only small amounts of Ca. Their Fe contents appear to be about half that of beef.

Canned corned beef contained 14.3 g fat, 51.7 g moisture, 34.0 g protein and 137 μ g Cu/100 g. According to its commercial label, it came from Argentina and was made from cooked beef, salt, sugar, sodium nitrate and sodium nitrite. Canned chopped pork contained 22 g fat, 50 g moisture, 28 g protein and 101 μ g Cu/100 g. It came from Denmark and was made from chopped pork, potato starch, salt, sugar and sodium nitrate. Both these products were minced finely before they were given to the rats. Ca and Fe were not determined.

Supplements. Cu was mostly added to the meat given to the positive control groups as sulphate at the level of 10 μ g Cu/g, but certain groups in Expts 4 and 5 received smaller Cu supplements in the same form. Mn was tested at the level of 30 μ g, and Co at 10 μ g/g meat, both as sulphate. Pantothenic acid was given as its Ca salt, at the level of 20 μ g of the salt/g. All the rats were given adequate weekly doses of vitamins A, D, E and K separately from the rest of their diet.

PROCEDURE

Expt 1 lasted for 14 weeks, the others for about 7 weeks. Before being killed, the rats were anaesthetized with ether and then bled from the inferior vena cava. Red-cell count, haemoglobin and packed cell volume were measured by routine methods.

The upper incisor teeth were extracted, and the degree of pigmentation of their anterior surfaces was assessed by comparison with five pairs of 'standard teeth'. These had been selected as showing even graduations between completely depigmented teeth (white) and normal teeth (dark brown). As before (Moore *et al.* 1962), a score of 4 was given for each normal tooth and 0 for each completely white tooth, making a maximum of 8 and a minimum of 0 for each pair. Dental depigmentation is well known not to be a specific effect of Cu deficiency: it can be caused by several dietary defects, notably by lack of vitamin A or vitamin E (Moore, 1943).

The degree of hypochromotrichia was assessed in our earlier experiments by inspecting the hair on the head, shoulders and back, which was recorded as being normal or slightly grey, grey or very grey. Later we selected, in each series of experiments, the rats with the darkest hair and the rats with the most grey hair. Three other rats to show the smoothest possible gradient between the two extremes were then chosen. Pl. 1 shows a set of rats' carcasses selected for this purpose. The scoring, to be consistent with that for dental pigmentation, was 4 for normal hair and 0 for maximum greyness. Attempts to keep a set of carcasses as permanent standards were defeated by changes in the colour of the hair during storage.

It was not a major aim in these experiments to study further the Ca deficiency induced by meat diets. At autopsy, however, the usual gross signs of Ca deficiency were noticed in many animals. In certain groups in Expt 5, superficial observations were supplemented by determinations of the ash contents of the bones.

RESULTS

Expt 1. Fig. 1 shows the curve for the mean weight increases of six young female rats, fed upon raw beef-steak supplemented with Ca (0.5% CaCO₃) and Cu (10 µg/g). The lower curve relates to a control group of five similar rats given the stock diet. The weight increases were significantly greater ($P < 0.01$) in the rats fed upon meat. At least part of the higher body-weight in the meat-fed animals was due to greater fat storage. Although body lengths did not differ significantly between groups, the mean girth, measured round the abdomen, was 19.3 cm in the meat-fed rats against 17.0 cm in those given stock diet ($P < 0.01$). The mean weight of the combined perinephric and periuterine adipose tissues averaged 36.2 g in the meat-fed rats against 26.4 g in those given the stock diet ($P < 0.01$). Throughout this experiment the rats in both groups remained in good health, as evidenced by normal activity and sleek coats.

Expts 2-5. Our results, summarized in Tables 1-4, indicated that anaemia, dental depigmentation and hypochromotrichia always developed in rats fed on raw meat and were always prevented by adequate supplements of Cu. 'Domestic' cooking also prevented these abnormalities almost completely.

Efficacy of Cu. In Expts 2, 3 and 4 the mean percentages of haemoglobin in the blood of piebald rats fed on raw beef without Cu supplements were 9.35, 5.31 and 8.27. In the animals given Cu, 10 $\mu\text{g/g}$ meat, the corresponding percentages were 16.52, 14.43 and 15.18. In the albino rats in Expt 3 haemoglobin percentages averaged 7.65 in the unsupplemented group and 14.18 in the rats given Cu. For red-cell counts the difference between the rats with and without Cu was less striking, but the differences for packed cell volume were similar to those for haemoglobin.

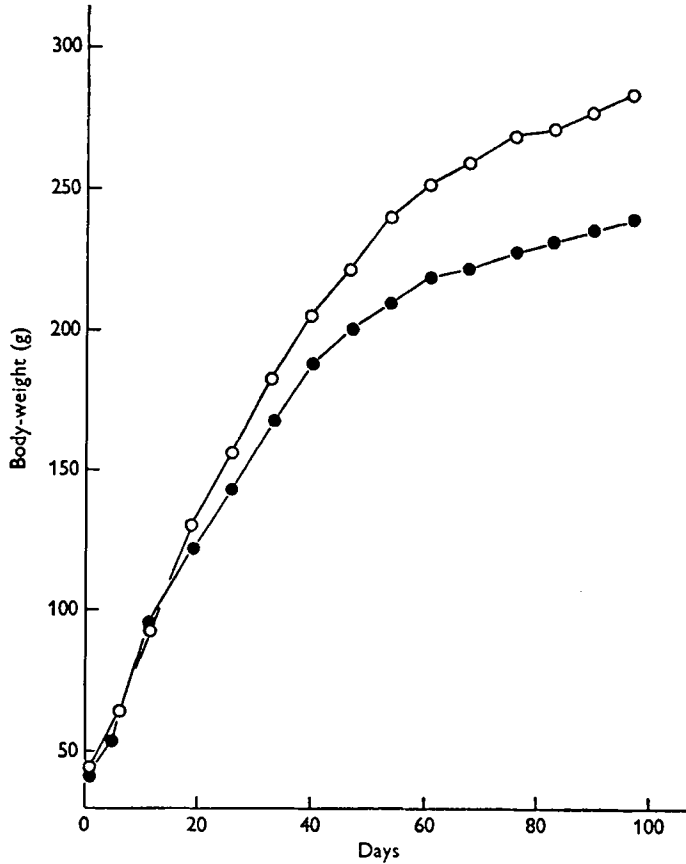


Fig. 1. Weight increases of young female piebald rats fed either upon stock diet, or upon raw beef-steak adequately supplemented with copper, calcium and fat-soluble vitamins. ●—●, mean body-weights for five rats fed on stock diet; ○—○, mean body-weights for six rats fed on steak.

In the same experiments the mean scores for dental pigmentation in piebald rats, without mineral supplements, were 1.2, 1.0, 3.0, compared with 8.0, 7.5 and 8.0 when Cu was given. In the albinos the mean scores were 1.3 without Cu and 8.0 with Cu.

Hypochromotrichia was invariably prevented by Cu at the 10 μg level. With a diet of raw meat without Cu, the first signs of greyness were usually seen within 14 days.

Minimal dose of Cu. In groups of rats given 1 (Expt 4), 2.5 or 5.0 (Expt 5) μg Cu/g meat, haemoglobin percentages averaged 11.41, 13.77 and 13.86, compared with 8.27,

Table 1. *Expt 2. Mean findings for body-weight, hair colour, dental pigmentation and blood examinations in groups, each of five male piebald rats, fed on raw beef (with or without supplements of copper, calcium pantothenate or manganese) or on raw mutton or pork*

Group no.	Diet	Supplement ($\mu\text{g/g}$ diet)	Days on diet	Body-weight (g)		Hair on head*	Dental pigmentation (max. 8)	Red blood cell count	Hb (g/100 ml)	Packed cell volume
				Initial	Final					
1†	Raw beef	0	63	71	168	VG	1.2	7.99	9.35	37.5
2‡	Raw beef	Cu, 10	63	72	192	N	8.0	8.14	16.52	53.1
3	Raw beef	Ca pant. 20	57	68	154	VG	0.8	5.62	6.66	22.3
4§	Raw beef	Mn, 30	56	69	176	G or VG	3.8	6.55	8.35	28.2
5	Raw mutton	0	53	71	165	SG-VG	1.3	6.35	7.39	27.5
6	Raw pork	0	52	70	153	SG-VG	1.3	6.66	6.05	22.8
	Pooled standard error of mean							± 0.47	± 0.43	± 1.5

* VG, very grey; G, grey; SG, slightly grey; N, normal.

† Blood was examined 7 days after the final weighing. During this interval two rats died. Results for blood relate to three rats only.

‡ Blood from all five rats was examined 7 days after the final weighing.

§ Two rats died. Means for final weights and blood values relate to three remaining rats only.

Table 2. *Expt 3. Mean findings for body-weight, hair colour, dental pigmentation and blood examinations in groups, each of six male rats, fed on raw beef (with or without copper), cooked beef, canned corned beef or canned chopped pork*

Group no.	Diet	Supplement ($\mu\text{g/g}$ diet)	Days on diet	Body-weight (g)		Hair on head*	Dental pigmentation (max. 8)	Red blood cell count	Hb (g/100 ml)	Packed cell volume
				Initial	Final					
7†	Raw beef	0	51	73	168	VG	1.0	5.43	5.31	26.5
8	Raw beef	Cu, 10	51	73	216	N	7.5	8.43	14.43	48.7
9	Cooked beef	0	50	70	212	SG-N	5.8	8.41	13.82	49.9
10	Corned beef	0	49	71	113	G	1.7	6.80	8.14	32.2
11	Canned pork	0	50	70	142	G	0.2	5.34	5.62	23.7
	Pooled standard error of mean							± 0.21	± 0.32	± 1.1
12‡	Raw beef	0	42	68	181	—	1.3	6.31	7.65	29.8
13	Raw beef	Cu, 10	42	68	206	—	8.0	7.57	14.18	45.0
	Pooled standard error of mean							± 0.42	± 0.41	± 1.6

* VG, very grey; G, grey; SG, slightly grey; N, normal.

† One rat died. Final weightings and results for blood are for five rats only.

‡ Results for blood relate to five rats only.

Table 3. *Expt 4. Mean findings for body-weight, hair colour, dental pigmentation and blood examinations for groups, each of six male piebald rats, fed on raw beef (with or without supplements of copper or cobalt) or cooked beef*

Group no.	Diet	Supplement ($\mu\text{g/g}$ diet)	Days on diet	Body-weight (g)		Hair on head*	Dental pigmentation (max. 8)	Red blood cell count	Hb (g/100 ml)	Packed cell volume
				Initial	Final					
14	Raw beef	0	49	71	169	VG-G	3.0	6.45	8.27	29.6
15	Raw beef	Cu, 10	48	71	192	N	8.0	7.12	15.18	46.1
16	Raw beef	Cu, 1	47	72	183	VG-G	5.8	7.28	11.41	37.9
17	Raw beef	Co, 10	48	72	151	VG	1.8	4.39	5.76	20.2
18	Cooked beef	0	49	70	177	SG-N	7.0	7.28	14.53	43.9
		Pooled standard error of mean						± 0.18	± 0.33	± 0.97

* VG, very grey; G, grey; SG, slightly grey; N, normal.

Table 4. *Expt 5. Mean findings for body-weight, hair colour, dental pigmentation and blood examinations in groups, each of six male piebald rats, fed on raw beef (with or without supplements of copper) or cooked beef*

Group no.	Diet	Supplement ($\mu\text{g/g}$ diet)	Days on diet	Body-weight (g)		Pigmentation		Red blood cell count	Hb (g/100 ml)	Packed cell volume
				Initial	Final	Hair on head	Incisor teeth (max. 8)			
19	Raw beef	0	46	72	160	0.3	0.3	5.11	7.22	21.8
20*	Raw beef	Cu, 5	47	71	196	3.2	7.3	6.81	13.86	44.7
21	Raw beef	Cu, 2.5	46	72	190	2.0	6.5	7.76	13.77	42.9
22*	Cooked beef	0	47	72	202	3.5	7.6	6.60	13.82	43.9
		Pooled standard error of mean						± 0.21	± 0.34	± 0.83

* One rat died. All results relate to remaining five rats only.

7.22 and 7.22 in the corresponding groups given no Cu. The influence of Cu on the blood picture was therefore apparent at all these levels. Evidence that the 1.0 and 2.5 μg levels were not quite adequate, however, could be seen in the obvious greyness of several of the rats given these low doses. As a further indication that the 1.0 μg level was inadequate, decidedly low haemoglobin percentages of 9.95, 9.70 and 10.20 were observed in three of the six rats dosed. The lowest individual haemoglobin percentage in a total of twenty-three rats given Cu at the 10 μg level was 13.35. Even the 5 μg level was not quite adequate on the evidence of slight hypochromotrichia in three out of five rats. To illustrate the extreme severity of the anaemia developed in some of the undosed animals given raw beef, values may be quoted for one of the rats in Expt 4. The red blood cell count was 3.73 million, haemoglobin 4.25 g/100 ml and packed cell volume 16.9%. Several other rats, undosed or dosed with inactive substances, had equally severe anaemia.

Influence of Cu on body-weight increase. In all our experiments adequate doses of Cu augmented the mean body-weight increase (Table 5). The rates of growth with Cu, however, were still much below those to be expected if supplements of both Cu and Ca had been given.

Table 5. *Mean increases in body-weight, during the whole experimental period, in groups of rats fed on raw beef with or without supplements of copper*

Expt no.	Cu supplement ($\mu\text{g/g}$ meat)	Mean increase in body-weight (g)	Cu supplement ($\mu\text{g/g}$ meat)	Mean increase in body-weight (g)
1	0	97	10	120
2	0	95	10	143
2 (albinos)	0	113	10	138
3	0	98	10	121
4	0	88	5	125

Mutton and pork. Both these meats, eaten raw, resembled beef in producing anaemia, dental depigmentation and hypochromotrichia. The preventive action of Cu was not tested.

Inefficacy of calcium pantothenate, Mn and Co. The abnormalities attributable to Cu deficiency were not prevented by supplements of 20 μg calcium pantothenate/g (Table 1), which was tried because pantothenic acid deficiency can give rise to hypochromotrichia (György & Poling, 1940; Unna & Sampson, 1940). Mn, 30 $\mu\text{g/g}$, was ineffective, except possibly for slight protection against dental depigmentation. Co, 10 $\mu\text{g/g}$, was also ineffective. Since the abnormalities in the group given Co appeared to be somewhat more severe than in the negative controls, the potency of Co as an antagonist to Cu requires further investigation.

Effect of cooking. Three tests with stewed (Expts 3 and 4) or autoclaved (Expt 5) meat all indicated that the ability of the meat to produce Cu deficiency had been lost. Thus haemoglobin percentages were 13.82, 14.53 and 13.82 as compared with 5.31, 8.27 and 7.22 in the corresponding negative control groups. No signs of Cu deficiency were observed, other than slight greyness or dental depigmentation in some animals.

Commercial processing. Results for commercially cooked meats were different. In the groups given corned beef and chopped ham the mean percentages of haemoglobin were 8.14 and 5.62 respectively, compared with 14.43 in the positive group. In both the groups given processed meats the hair was grey and the teeth almost completely depigmented. Growth increases were much lower than in the rats given raw beef without Cu. Preliminary experiments have indicated that the low weight increases in rats fed upon corned beef, unlike those in rats fed upon raw beef, cannot be fully corrected by combined supplementation with Ca and Cu.

Calcification. The ash contents of the undried femurs were measured in the rats of groups 19 (raw beef only), 20 (raw beef + 5 μg Cu/g) and 22 (autoclaved beef) (Table 4). The mean percentages were 13.1 ± 0.87 , 13.9 ± 0.73 and 13.6 ± 0.47 . These values may be compared with the mean value of 42% previously found in rats given adequate supplements of Ca continuously (Moore *et al.* 1963). Neither supplements of Cu nor cooking, therefore, had any significant influence in raising the low ash content of the bones. There were, indeed, indications that the supplements of Cu sometimes increased the incidence of rib fracture, presumably a reflection of the slightly increased growth rate.

Size, and fat content, of livers. In all these experiments the weight of the liver was recorded, and in many instances its fat content was measured. In sixteen of the negative control rats given raw beef, the liver averaged 6.6% of the body-weight, with a mean fat content of 4.9%. For sixteen rats that received raw beef with 10 μg Cu/g, the corresponding percentages were 4.8 and 7.5. Thus the effect of Cu deficiency was to increase the size of the liver, in relation to body-weight, and to decrease its fat percentage.

DISCUSSION

Our results indicate that the abnormalities regularly developed by young rats fed upon raw meat (beef, mutton or pork) include anaemia, dental depigmentation and hypochromotrichia. On the evidence of experiments with beef only, all these abnormalities may be prevented either (1) by the addition of adequate supplements of Cu to the meat, or (2) by cooking the meat.

Interaction of Cu and Fe. Explanations of these clear-cut findings can still only be hypothetical. Anaemia and dental depigmentation, we may notice, are bound up with Fe metabolism, since both haemoglobin and the brown dental pigment contain this element. There is as yet no evidence, however, to suggest that Fe is concerned in preventing hypochromotrichia, whereas the role of Cu in the formation of melanin pigments is well known (Underwood, 1956).

Readily absorbable Cu is presumably required both for those roles in which it aids the utilization of Fe and for those in which it has direct action on the tissues. At least part of the Cu held in animal tissues is known to be combined with protein (Frieden, 1962), and for this reason it may be poorly absorbed during digestion. It seems probable, therefore, that the same beneficial effect may be produced either by supplying inorganic Cu or by releasing as a result of cooking the Cu naturally present in the meat from its binding to protein.

This theory may be reminiscent of the long-standing conception that Fe occurs in food partly in ionizable, readily available form, and partly in combined form, not readily available. Cooking may increase the proportion of available Fe (Shackleton & McCance, 1936). How far the availability of Fe from foods depends on their containing readily available Cu, rather than ionizable Fe, is now open to question. Early work by Schultze, Elvehjem & Hart (1934) showed that Cu in the form of its haemoporphyrin is poorly absorbed by rats. A matter requiring further investigation is the failure of the commercially cooked meats, corned beef and canned pork, to prevent the signs of Cu deficiency.

Cu and fat metabolism. One of the effects of cooking on meat is to liberate fat from the tissues. It is possible, therefore, that the ease of digestion of the fat, by the rat, may be changed. How far these changes in the state of the dietary fat can affect the Cu-deficiency syndrome cannot be assessed. It is clear, however, from our results on weight and fat content of the liver that Cu can affect fat metabolism, either directly or indirectly.

Cu requirements. Schultze *et al.* (1934) found that 5 μg Cu daily was enough to promote haemoglobin formation in anaemic rats, but considered that an intake of 10 μg was desirable. Recently Warren (1962) has given 50 μg as the daily requirement for the prevention of Cu deficiency in rats fed upon milk. A rat eating 30 g raw steak daily, without Cu supplements, must ingest about 48 μg Cu. This intake, if efficiently absorbed, would obviously be adequate. Our experiments also indicated that an extra intake of inorganic Cu at the rate of 1 $\mu\text{g}/\text{g}$ meat, totalling 30 μg daily, was sufficient to prevent all but minor signs of deficiency in most of the rats.

It appears, therefore, that the Cu of raw meat is present in a form unavailable to the rat and that its liberation could explain the beneficial effects of cooking the meat. Apparently the protein in the meat did not chelate, or otherwise immobilize, additional Cu added to the meat after the death of the bovine. From our earlier experiments (Moore *et al.* 1963) we may also deduce that a diet of raw meat does not interfere with the absorption of Ca, added as carbonate.

General. The production of hypochromotrichia in rats fed on raw but not on cooked beef recalls reports of the production of pale-coloured hair ('cotton fur') and anaemia in mink given diets containing raw fish (Helgebostad & Martinsons, 1958; Stout, Oldfield & Adair, 1960*a, b*). In parallel with our results, abnormalities were prevented by cooking the fish. Cu, however, appeared to be ineffective against 'cotton fur'.

The ability of Cu to improve the growth rates of rats, observed consistently in our experiments, brings to mind observations that addition of Cu has been found beneficial for the fattening of pigs (Braude, Townsend, Harrington & Rowell, 1962). The pigs received 250 μg Cu/g dry diet, which consisted mainly of cereal products. There is no evidence that the diet without the mineral mixture was deficient in Cu.

Finally, we must emphasize that our experiments were carried out under conditions known to favour the appearance of the abnormalities under investigation. Thus our rats were young and were not given supplements of Ca. As already mentioned (Moore *et al.* 1963), adult rats develop hypochromotrichia, but not anaemia or severe

dental depigmentation, when given raw beef without mineral supplements. Ca without Cu is effective against hypochromotrichia in adults (Moore *et al.* 1963). In young rats Ca, in adequate but not excessive doses, reduces the severity of anaemia and dental depigmentation, but does not prevent hypochromotrichia. The mechanism by which Ca affects the metabolism of Cu, which in turn affects the metabolism of Fe, remains unexplained (Moore *et al.* 1962). The high proportion of the calorie intake derived from fat, and the ability of the rat to thrive under these conditions if given adequate mineral supplements, have already been mentioned (Moore *et al.* 1963).

SUMMARY

1. Young male piebald rats weighing 70 g were fed on a basal diet of raw minced beef, without mineral supplements. At this stage of development, the degree of calcium deficiency attained over a period of 7 weeks was seldom fatal, and fairly rapid preliminary growth occurred.

2. All the negative control rats developed anaemia, dental depigmentation and hypochromotrichia.

3. Supplements of copper, as sulphate, at 10 $\mu\text{g/g}$ beef, invariably and completely prevented the anaemia and associated abnormalities. Weight gains were always increased by Cu. Doses of Cu down to 1.0 μg were at least partly effective.

4. Manganese, cobalt and calcium pantothenate (30, 10 and 20 $\mu\text{g/g}$) were ineffective.

5. Cooking the beef, which when unsupplemented contained 1.6 μg Cu/g, virtually prevented the anaemia and associated lesions.

6. Abnormalities similar to those seen in rats fed on raw beef were also observed in rats fed on raw mutton or pork or on commercially canned beef or pork.

7. The livers of rats fed on raw beef, without Cu, were larger, as a percentage of the body-weight, than those of rats given Cu. The percentage of fat in the livers was usually somewhat higher when Cu was given than when it was withheld.

8. Albino rats given raw beef also developed anaemia and dental depigmentation, which were prevented by Cu.

9. Young female piebald rats, fed on raw steak supplemented with both Cu and Ca, remained in good health. Their weight increases were significantly greater than those of control rats fed on a stock diet.

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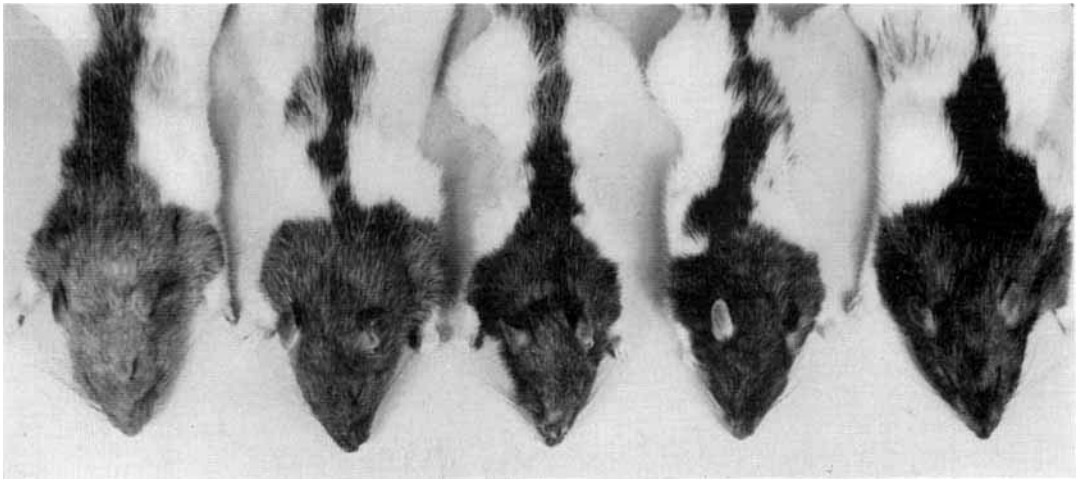
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EXPLANATION OF PLATE

Carcasses of rats graded for protection against hypochromotrichia. The rat on the extreme right, score 4, had been fed on raw beef, supplemented with 10 μg copper/g. The rat on the extreme left, score 0, had been fed on raw beef, without copper supplementation. The remaining rats were chosen to show intermediate grades of protection.



0

1

2

3

4