I am grateful to Dr. P. Goodwin and Dr. L. W. Smith for details of the case in which insulin was used in an attempt to procure an abortion. My thanks are also accorded to the 15 pathologists and 14 coroners from whose cases these details have been collected, and to Mr. E. R. Rutter, who has given invaluable assistance in the analyses.

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Preliminary Communications

Copper Deficiency in Rats Fed upon Meat

The severe skeletal lesions which occur in young rats fed upon meat, without supplements of calcium, have already been described (Scott and Scott, 1960; Moore and Sharman, 1960). Further experiments have indicated another consistent abnormality, marked hypochromotrichia, in those parts of the piebald rat in which the fur is normally pigmented. Thus the fur of the head, back, and shoulders became light grey instead of black. A third lesion was anaemia, which was observed in most of the animals.

Clear evidence, to be reported later, has now shown that the effect of a meat diet upon the bones depends upon the age of the rats when the meat diet is started. Weanlings invariably develop severe lesions, usually including fractures, within a few weeks. In contrast adult animals can subsist for long periods on the same diet without sustaining skeletal lesions of any great severity. Both young and adult rats, however, become grey when they are fed solely upon meat. In adults the greyness was prevented by the addition to the diet of 0.5% of calcium carbonate. In young rats, however, this supplement was only partially effective against hypochromotrichia, although skeletal lesions were completely prevented. Anaemia, in parallel with hypochromotrichia, was reduced in severity, but not completely prevented, in young rats which were given calcium supplements.

Crude ferrous carbonate, when added to the meat at the high level of 2.5%, completely prevented both hypochromotrichia and anaemia, irrespective of the calcium intake. In contrast this addition of iron to the diet intensified the skeletal lesions in young rats not given calcium. It appeared at one point of the investigation, therefore, that both the hypochromotrichia and anaemia might be due to deficiency of iron in the meat, or the poor absorption of its iron contents by the rat. In view of the beneficial action of calcium against these abnormalities it appeared that calcium can influence the metabolism of iron. For this latter conclusion there is indeed ample further evidence.

Previous work on achromotrichia or hypochromotrichia in rats, however, has never implicated iron deficiency among the possible causes. Deficiencies of pantothenic and *p*-aminobenzoic acids have been shown to cause greyness, but these substances could certainly not have been present as impurities of iron carbonate even in its crudest form. Copper, however, is a well-known contaminant of iron salts, and its deficiency has

already been found to cause hypochromotrichia. Thus Hundley (1950) has shown that black rats become grey when they are fed upon purified diets deficient in copper, and that the greyness can be prevented by the addition of as little as 2.5 µg. of copper per g. of diet.

The suspicion therefore arose that the prevention of achromotrichia by crude ferrous carbonate might be due to copper present as impurity. The main purpose of this article is to describe a key experiment in which rats were given meat without mineral supplements or with supplements of crude iron, or pure iron, or copper.

THE EXPERIMENT

The animals used were female piebalds, mean body weight 114 g. They were all given a basal diet of minced raw beefsteak (skeletal muscle with attached adipose tissues) found by analysis to contain about 17% of fat. Doses of vitamins A and D were allowed, but it was thought that the meat contained adequate amounts of other vitamins. Four groups, each of six rats, were made up as follows.

- Group 1: Minced steak only.
- Group 2: Minced steak, with the addition of 2.5% of crude ferrous carbonate.
- Group 3: Minced steak, with supplements of 2 mg. of iron per rat per day, as pure ferrous ammonium sulphate.
- Group 4: Minced steak, with the addition of 10 µg. of copper per g. of steak, as copper sulphate.

The skeletal development of the rats at the start of the experiment was sufficiently mature to exclude severe lesions, and all the animals survived in apparently good health for 83 days, when the experiment was ended. Differences in the pigmentation of the animals appeared after about 28 days, and became more obvious as time advanced. Depigmentation was first seen at the periphery of the black parts, with greying at the sides of the shoulders and at the posterior end of the back. In many animals the hair on the crown of the head remained black long after other areas had become grey, with a sharp line of demarcation. Eventually most of the rats not given effective supplements became completely grey in those areas which are normally black.

Clear-cut differences were found between the incidences of hypochromotrichia in the different groups. All the rats which were undosed or were dosed with pure ferrous ammonium sulphate showed depigmentation. In contrast all those rats given a high intake of crude iron carbonate or small doses of copper remained normally pigmented. Fig. 1 shows the head of a typical rat of group 1 given no supplement. The marked depigmentation may be compared with the normal pigmentation of a rat dosed with copper (Fig. 2).

Other differences between the groups are summarized in the Table. It will be seen that mean blood haemoglobin was higher in the two groups which received either copper sulphate or impure ferrous carbonate than in the group which received the pure ferrous ammonium sulphate salt or no supplement. Moreover, weight increases with supplements of copper sulphate were greater than when no supplement or the pure ferrous salt was given. Statistical tests between group 1 (no supplement) and group 4 (CuSO_4) indicated that the difference was significant ($0.02 < P < 0.05$). On the other hand, weight gains were much lower in the rats given 2.5% of ferrous carbonate than in the group with no

Mean Values for Gain in Body Weight, R.B.C. Counts, and Haemoglobin in Groups of Six Female Rats Fed Upon Minced Beefsteak, With or Without Various Supplements

Group No.	Mineral Supplement	Hypochromotrichia	R.B.C. Count (mill.)	Blood Hb mg./100 ml.	Gain in Body Weight (g.)
1	None	+	8.3	13.4	86
2	Crude FeCO_3	—	6.2	14.4	36
3	Pure $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$	+	7.8	13.3	82
4	CuSO_4	—	7.6	15.8	129

supplement, or indeed in any of the other groups. This action of an excess of ferrous carbonate in the absence of calcium supplements in retarding weight increases was in agreement with the results of earlier experiments.

COMMENT

Further experiments have confirmed the potency of pure copper sulphate in preventing hypochromotrichia and promoting growth in rats fed upon meat. It is clear, therefore, that a diet of beefsteak induces in rats a state of copper deficiency. Moreover, this deficiency is plainly evident even when the accompanying calcium deficiency has not reached a stage productive of severe skeletal lesions. Obviously the next question to consider is how the copper deficiency is induced. The beef was purchased twice weekly from a local butcher, and doubtless came from many different healthy animals. It seems most unlikely, therefore, that the deficiency in the rat was related to a similar dietary deficiency in the cattle from which the meat was obtained. Chemical estimations of copper, kindly carried out by Dr. J. M. Walshe, gave a copper content of $1.6 \mu\text{g./g.}$ wet weight of the mince, a value consistent with previous findings for such material. On the assumption that each rat ate 25 g. of mince daily the intake of copper was about $40 \mu\text{g.}$ daily. Early work by Schultze, Elvehjem, and Hart (1934) indicated that $10 \mu\text{g.}$ of copper daily was sufficient to cure anaemia in copper-deficient rats.

It appears, therefore, that the signs of copper deficiency in rats fed upon steak are not due to an absolute lack of copper in the diet but must be due to some defect in the absorption or utilization of copper. Schultze *et al.* (1934) found that copper was ineffective when given to rats in the form of its haematoporphyrin derivative. No information seems to be available about whether any of the copper present in beef muscle is so combined. Another possibility is that the high phosphate content of meat may depress the absorption of copper, and indeed of other minerals. The action of calcium carbonate in preventing hypochromotrichia in adult rats is in favour of this view.

The level of dosing of copper sulphate to the rats of group 4 gave a total intake of $250 \mu\text{g.}$ per rat daily, which was obviously sufficient to overcome any difficulties in absorption. The impure iron carbonate given to the rats of group 2 contained, according to Dr. Walshe, $324 \mu\text{g./g.}$, which gave a total daily intake of $202 \mu\text{g.}$ This was not far removed from the dose given to group 4, and was again sufficient to overcome difficulties in absorption. The calcium carbonate found in previous work to prevent hypochromotrichia in adult rats contained only $12.2 \mu\text{g./g.}$, and when included as 0.5% of the diet contributed a daily dose of only about $1.5 \mu\text{g.}$ of copper. The calcium carbonate therefore did not exert its action against hypochromotrichia by virtue of its copper content.

In the classical experiments of Hart *et al.* (1928), in which the haematopoietic potency of copper in rats

was first demonstrated, whole milk was used as the basal diet. The present work is somewhat alarming in showing that another important staple food, beefsteak, is also deficient in this trace element. Obviously, white sugar and fats must also be deficient. There may be grounds for uncertainty, therefore, whether human diets always contain adequate amounts of copper, derived from the remaining foods. A point for emphasis is that rats deficient in copper, apart from their grey hair, remained lively and apparently in good general health. The small defects in the rates of weight increase, and in haemoglobin levels, could only be established between groups, and would not have been significant in individual rats. A relative deficiency of copper may therefore adversely affect health in minor ways without necessarily becoming lethal. Premature achromotrichia is certainly

FIG. 1.—Rat fed for seven weeks on a diet of minced beefsteak without mineral supplements. Note depigmentation of the fur on head, back, and shoulders.

FIG. 2.—Rat fed for seven weeks on a diet of minced beefsteak supplemented with $10 \mu\text{g.}$ of copper, as copper sulphate, per g. of meat. Pigmentation normal.

not uncommon in human populations. Further research seems desirable on the copper contents of human dietaries, and on the intricate interrelationships between the absorption and metabolism of copper, iron, and calcium.

SUMMARY

Young rats fed on a diet of raw minced beefsteak developed signs of copper deficiency as evidenced by hypochromotrichia, slight anaemia, and a slight reduction in the rate of weight increase.

The deficiency could be prevented by the addition to the meat of copper sulphate or of impure ferrous carbonate containing copper.

Chemical analyses showed that the meat contained the expected amounts of copper. A defect in its absorption, probably due to the high phosphate content of the meat, therefore seemed probable.

In adult rats hypochromotrichia could be prevented, and in young rats partially prevented, by the addition of calcium carbonate to the diet. The calcium carbonate contained only traces of copper, and presumably acted by counteracting the high intake of phosphate.

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Medical Memoranda

Fatal Marrow Aplasia During Chlorpropamide Therapy

Although it is difficult to obtain any accurate indications of the extent to which chlorpropamide is at present prescribed, the risk of serious marrow damage arising from its administration is evidently small. Nevertheless, the possibility should be borne in mind during the initial stages of treatment.

CASE REPORT

A woman aged 63 at death had been under the care of Dr. C. S. D. Don at Manchester Royal Infirmary for diabetes mellitus since October, 1958. As simple dietary restriction proved unsuccessful, tolbutamide (0.5 g. t.d.s.) was given. The initial response was satisfactory, but from January, 1960, appreciable hyperglycaemia developed. Because of this, chlorpropamide (375 mg. daily) was substituted for tolbutamide at the beginning of June, 1961.

On July 21 a rash appeared on the chest and neck, followed four days later by a sore throat, which was unrelieved by penicillin therapy. The patient was referred to Monsall Hospital by her family doctor on July 27 as a possible case of diphtheria. On examination she was somewhat dehydrated and dyspnoeic, and was unable to speak clearly. Her neck was erythematous, swollen, and

tender, while her tonsils were enlarged and covered by thick membranous slough. Rales were audible over both lung bases, and the percussion note was impaired on the left side. Glycosuria was absent, and the blood sugar was 110 mg./100 ml. The total leucocyte count was 250/c.mm., of which only 5% were neutrophil polymorphs. Initial treatment with chemotherapy and intravenous Hartmann's solution was followed on July 28 by transfusion of 1 pint (570 ml.) of fresh blood, but she collapsed and died the same day. A blood count shortly before death gave the following results: Hb. 13.9 g./100 ml. (94%); total leucocytes 200/c.mm. (neutrophil polymorphs 1%, lymphocytes 97%, monocytes 2%); platelets 64,000/c.mm.

At necropsy there was extensive pharyngeal ulceration with oedema of surrounding structures, including the larynx, and tracheobronchitis with pulmonary congestion and oedema. The heart was flabby and dilated, and there was atheroma of the aorta and coronary arteries. Two small chronic peptic ulcers were present in the pyloric antrum. The liver was moderately fatty. The only other abnormalities were congestive splenomegaly and mild nephrosclerosis. Marrow films were unfortunately not obtained until 72 hours after death and were of poor quality. However, it was evident that cellularity was reduced and that the majority of nucleated cells were lymphocytes, plasma cells, and reticulum cells. Only small numbers of erythroblasts and myeloid cells were present, most of them fairly mature, and megakaryocytes were difficult to find.

These appearances, in conjunction with the platelet count during life, suggest that there was a general disturbance of haemopoietic activity rather than a selective suppression of granulocyte production, although the latter was the important clinical factor.

COMMENT

The initial oral hypoglycaemic agent, carbutamide, was soon discarded because of the frequency of toxic marrow damage. It was replaced by tolbutamide, which has proved to be remarkably free from side-effects, and so far as is known only one case of fatal aplastic anaemia has been reported (Jost, 1959). However, tolbutamide has only a mild and short-acting hypoglycaemic action, and its effect often diminishes with time. In the last three years chlorpropamide has been widely used, since it has a greater and more prolonged hypoglycaemic effect, and often proves successful in cases no longer responsive to tolbutamide. Like the latter, it is a sulphonylurea. A variety of toxic effects, including mental and visual disturbances, dyspnoea, skin rashes, and jaundice, have been recorded, but the incidence of these may have been related to the rather large doses given in early trials. The only previous report of fatal marrow damage appears to be that of Karlin (1960) in a man of 59 who developed agranulocytosis after three weeks of therapy (500 mg. daily). Karlin, however, draws attention to several reports of non-fatal haematological reactions, and to these may be added that of Grace (1959), who recorded thrombocytopenic purpura within five days of beginning therapy (500 mg. t.d.s.). It has been pointed out that the frequency of these reactions increases with the dose administered, and Bloom (1961) thinks that the dose should never exceed 500 mg. daily. In the case reported the amount administered was well within this limit.

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