Copper and vitamin A concentrations in the blood of normal and Cu-poisoned sheep

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1. In experimental sheep the culmination of chronic copper poisoning, as manifested by the onset of haemolysis and greatly increased blood Cu levels, was accompanied by much-reduced plasma concentrations of retinol, which was not due merely to the dilution of the plasma by the products of broken corpuscles. A further instance can therefore be added to the series of inverse relationships already known to apply between Cu and retinol.

2. In specimens of blood taken from normal sheep in the field, however, a direct correlation between Cu and retinol was found, indicating that the inverse relationship is not universally applicable in all physiological and nutritional conditions.

3. The liver of a single lamb that died from swayback, associated with Cu deficiency during pregnancy, contained only traces of Cu, but had a retinol content at the top of the normal range. The livers of two lambs that had been dosed with Cu, but had failed to develop haemolytic crises, contained high concentrations of Cu, with retinol within the normal range.

Moore (1969, 1970) has pointed out that retinol and copper behave similarly in being stored preferentially in the liver, and being carried in blood plasma in combination with similar, but different α_2-globulins (Glover & Walker, 1964; Kanai, Raz & Goodman, 1968). Otherwise the interrelationships of these two nutrients are often inverse. Physiological or pathological factors that tend to increase the concentration of one of them often decrease the concentration of the other. Thus in human maternal blood in the later stages of pregnancy the level of Cu is greatly increased (Krebs, 1928) but retinol decreases (Bodansky, Lewis & Lillienfeld, 1943; Lund & Kimble, 1943). In the foetal liver of experimental animals the concentration of retinol, under normal nutritional conditions, is usually a small fraction of that pertaining in the maternal liver (Dann, 1932), but for Cu this relationship is reversed in most mammals (Cunningham, 1931; Brückmann & Zondek, 1940). In the blood of healthy men the mean for vitamin A is usually some 20% higher than for women in the same population (Kimble, 1938–9; Leitner, Moore & Sharman, 1960), but for Cu the mean is 10% higher for women than for men (Cartwright, 1950). In both sexes fever is usually associated with a greatly decreased blood concentration of retinol (Lindqvist, 1938) but Cu is increased

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(Wintrobe, Cartwright & Gubler, 1953). In nephrosis, one of the few human diseases associated with raised blood concentrations of retinol (Kagan, Thomas, Jordan & Abt, 1950), Cu is decreased (Krebs, 1928; Cartwright, Gubler & Wintrobe, 1954).

All the above findings, although consistent in their total effect, were obtained in separate investigations on either retinol or Cu. Studies by Owen (1965) and Owen, Proudfoot, Robertson, Barlow, Butler & Smith (1965) on relationships between retinol and Cu in the blood and liver of kids, therefore, gain importance from being carried out simultaneously on the same animals. In blood specimens taken from kids aged 5–23 d a statistically significant inverse relationship between retinol and Cu was found.

In an intensive study of the Cu status of the sheep in Northern Ireland, investigations have been included of the pathological and biochemical changes underlying chronic Cu poisoning (Todd, Gracey & Thompson, 1962; Todd & Thompson, 1963). When sheep ingest massive amounts of Cu over long periods their first reaction is to store the excess in the liver. This process may continue for weeks or months, but eventually a sudden crisis develops in which Cu is transferred in large amounts from the liver into the blood-stream. This release may occur spontaneously, or may be precipitated by various forms of stress, such as cold, fright or undue physical activity. The flooding of Cu into the blood-stream is accompanied, or closely followed, by severe haemolysis, icterus, anaemia and various biochemical abnormalities.

Cu poisoning in sheep, therefore, presented interesting possibilities for the further study of Cu–retinol interrelationships. In addition, specimens were collected from normal sheep and from one sheep with swayback caused by Cu deficiency.

EXPERIMENTAL AND RESULTS

Materials and methods

Blood was obtained by venepuncture from sheep either at pasture in rural parts of Ulster or kept experimentally at Stormont. Cu was estimated in the whole blood by the colorimetric method of Brown & Hemingway (1962) and haemoglobin by the cyanmethaemoglobin method. Packed cell volume (PCV) was measured by micro-haematocrit, and aspartate amino transferase (GOT), methaemoglobin (MHb) and urea as in previous work (Todd & Thompson, 1963). For retinol estimations plasma was separated from the blood and sent by post to Cambridge, where the antimony trichloride method was applied according to the technique of Kimble (1938–9) as modified by Yudkin (1941). Since sheep plasma is virtually devoid of carotenoids, however, a correction for their contribution to the antimony trichloride reaction was unnecessary. For the extraction of retinol from liver a modification of the method of Davies (1933) was used.

Sheep at pasture

Specimens of blood were collected from twenty-three ewes at pasture in the region of Enniskillen and Claudy; the mean blood concentrations (μg/100 ml) of Cu and retinol were:

Region	No. of ewes	Cu	Retinol
Claudy	15	72·4	38
Enniskillen	8	90·4	44

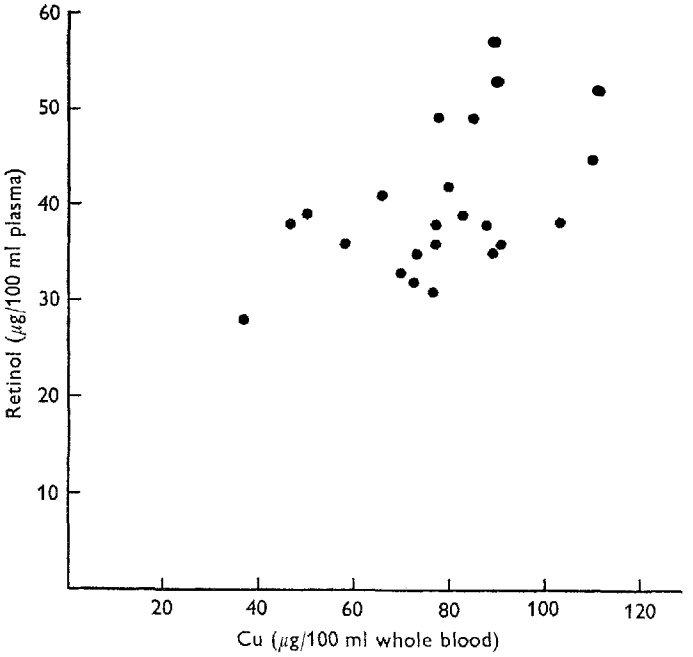

Fig. 1. Direct correlation, in sheep at pasture, between concentrations of retinol in blood plasma and copper in whole blood.

The combined results for ewes from both regions showed (Fig. 1) a direct relationship between the concentrations of Cu and retinol in the blood ($r = 0{\cdot}511$, $P < 0{\cdot}02$).

Chronic Cu poisoning

Difficulty in the experimental production and observation of the haemolytic crisis resulting from Cu poisoning in sheep can arise not only from the long latent period before its development but also from the speed of the pathological process once it has started. Sheep may sicken and die before arrangements for the collection of blood specimens can be made. Other sheep may develop symptoms of illness other than the typical picture of Cu poisoning. Out of several groups of young sheep kept under experimental conditions at Stormont only three developed toxic crises.

Lamb A. Although this animal was kept under experimental conditions the cause of its poisoning by Cu was accidental. Inadvertently a group of lambs was fed on a diet containing calf pellets, with 37 μg Cu/g, instead of sheep nuts. The total diet, which also included cereals, contained 17·5 μg Cu/g, which induced poisoning in this one animal. It was treated by the removal of the calf nuts from its diet and by dosing with 50 mg sodium molybdate and 1 g sodium sulphate daily.

Serial results for blood Cu and retinol are shown in Fig. 2. At the commencement of the observations blood Cu was already about five times the normal average, whereas retinol was reduced to about one-quarter of the normal average. Treatment with $Na_2MoO_4 + Na_2SO_4$ was followed by repeated reductions in blood Cu until, after 6 d, a steady value of about twice the normal average was reached. As Cu fell the plasma retinol rose, eventually reaching a level within the normal range.

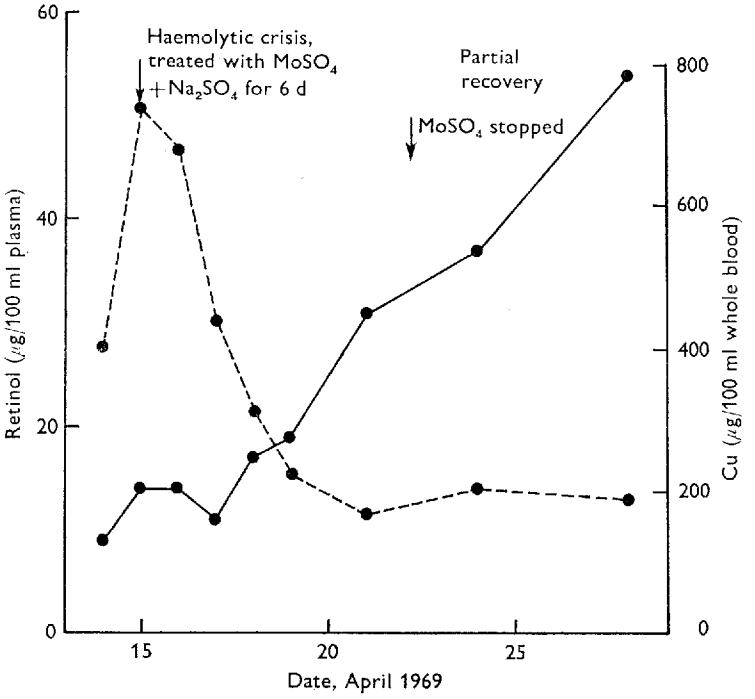

Fig. 2. Concentrations of retinol and copper in the blood of lamb A, accidentally poisoned with copper. ●—●, retinol; ●--●, Cu.

Table 1. *Lamb A: blood analyses in accidental copper poisoning*

Date, April 1969	Treatment	GOT (i.u./l)	PCV (%)	Hb (g/100 ml)	MHb (g/100 ml)	Urea (mg/100 ml)
14	None	2840	48	—	—	56
15	Mo+SO_4	1640	42	17·4	0·91	63
16		1500	35	15·5	1·89	71
17		1160	28	13·4	1·89	185
18		820	25	13·8	0·76	324
19		720	21	8·9	0·68	373
21		680	19	8·6	0·54	469
24	None	420	17	7·7	0	678
28		1040	27	10·1	0	754
29		990	22	9·7	0·31	650

GOT, aspartate aminotransferase; PCV, packed cell volume; Hb, haemoglobin; MHb, methaemoglobin.

Other biochemical findings for this animal are given in Table 1. At the start of the crisis, and when the greatly increased blood Cu and decreased retinol were first observed, the haematocrit (PCV) was still 50%. Nevertheless the dark-brown colour of the blood clearly indicated that it was abnormal, presumably in containing MHb and bile pigments (Todd & Thompson, 1963). Coincidentally with the effect of $Na_2MoO_4 + Na_2SO_4$ in restoring the Cu:retinol ratio towards normality the PCV steadily declined, eventually to only 20% with the plasma then yellow in colour. During the treatment with $Na_2MoO_4 + Na_2SO_4$ a rapid rise in blood urea had occurred, which persisted even after this treatment was stopped. Eventually the animal became

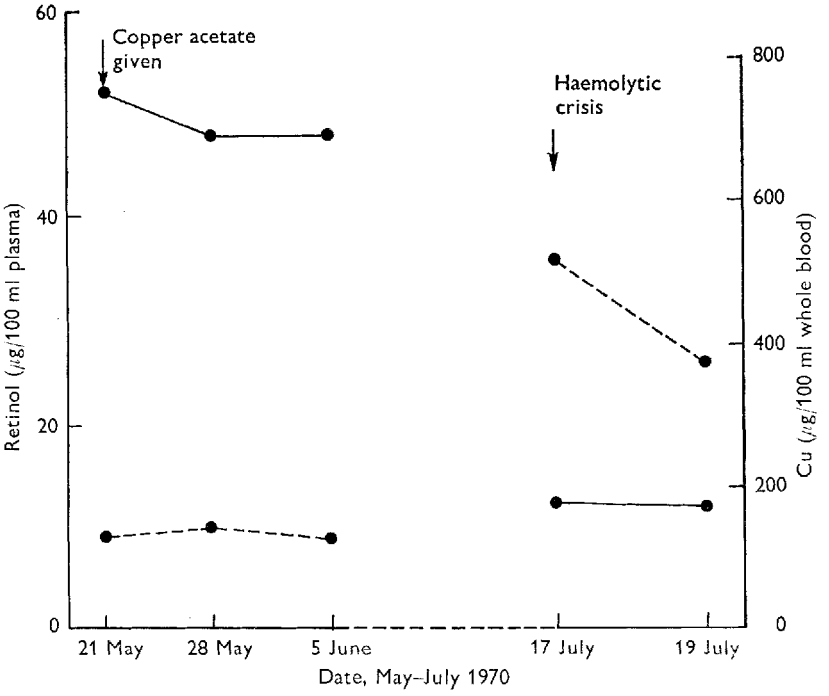

Fig. 3. Concentrations of retinol and copper in the blood of lamb B, experimentally poisoned with copper. ●—●, retinol; ●--●, Cu.

moribund, presumably from uraemia, and was slaughtered. The liver was found to contain 316 μg Cu/g, and only 12 μg retinol/g. The low retinol reserve was probably due to the low content of the experimental diet (only about 0·06 μg/g) combined with the immaturity of the animal (age at death, 145 d).

The haematological and other biochemical changes in this animal showed some unusual features for Cu poisoning. Usually in the haemolytic crisis the PCV falls to 10–15% within 2–3 d. Possibly the treatment with $Na_2MoO_4 + Na_2SO_4$ delayed, but did not finally prevent, the fall in PCV. High blood urea concentrations occur regularly in the terminal stages of Cu poisoning (Todd & Thompson, 1963).

Lamb B. This animal was bought in the Mourne region in October 1969 when about 3 months old. It was kept at Stormont and given diets containing subtoxic amounts of Cu until 21 May 1969. Daily doses of 1 g copper acetate were then administered until a haemolytic crisis began about 28 d later. Dosing with Cu was then stopped, but not in time for the animal's survival. Findings for Cu and retinol over the relevant period are shown in Fig. 3. Again, a greatly increased concentration of blood Cu was accompanied by greatly reduced retinol. Death ensued before a normal Cu–retinol relationship could be restored. Specimens of liver and kidney contained 149 and 76 μg Cu/g respectively. As in lamb A, the fall in plasma retinol could not be explained on the basis of changes in PCV.

Lamb C. This animal had the same history as lamb B. Its haemolytic crisis occurred 22 d after toxic dosing with Cu had started and from this point dosing was stopped.

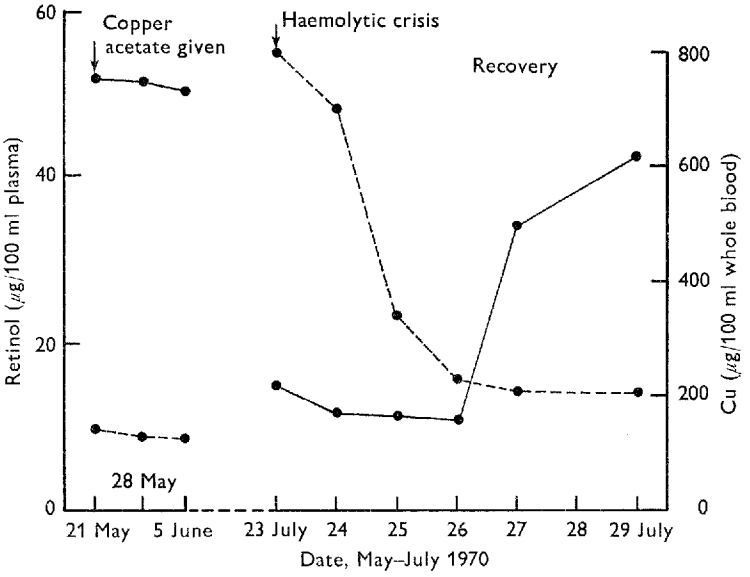

Fig. 4. Concentrations of retinol and copper in the blood of lamb C, experimentally poisoned with copper. ●—●, retinol; ●--●, Cu.

Fig. 4 shows that blood Cu rose during the crisis, but retinol was much reduced. After dosing with Cu had stopped, blood Cu fell rapidly and retinol rose. Again, changes in PCV could not explain the fall in retinol. Although the after-effects of haemolysis persisted for several weeks the animal eventually recovered.

Cu and retinol in sheep's liver

The finding of a high concentration of Cu, but a low concentration of retinol, in the liver of an animal accidentally poisoned with Cu (lamb A) has already been mentioned. Liver was also collected from two lambs which were dosed for long periods with copper acetate but which became ill without developing the lesions typical of Cu poisoning. For Cu, values of 453 and 1168 μg/g were found, and for retinol 125 and 121 μg/g.

Estimations were also made on liver from a lamb from the Claudy region which had suffered from swayback, at first diagnosed clinically and confirmed histologically. Cu concentration was only 9 μg/g, but retinol concentration was high at 280 μg/g. All these values for retinol fall within the normal range for sheep (Moore & Payne, 1942).

DISCUSSION

Our results on chronic Cu poisoning add another example to the list of physiological and pathological conditions in which Cu and retinol in the blood are inversely related. Our findings for normal sheep, however, show that this inverse relationship is not universal, and that a direct relationship sometimes applies. In the liver no relationship between the two nutrients could be seen; retinol reserves within the normal range were present in specimens that contained virtually no Cu, or much more than the normal amount.

A complete explanation of these divergent findings cannot yet be offered. We already know, however, that the concentration of retinol in the blood is largely dependent on the synthesis, presumably in the liver, of its carrier 'retinol binding protein' (RBP). Similarly, Cu is carried in the blood plasma in combination with protein, although here the situation is complicated by the presence of the metal, unlike the vitamin, in substantial amounts in both the corpuscles and plasma. According to evidence already reviewed, the Cu and retinol complexes must be responsive to various stimuli, including hormones. Damage to the liver can be expected to interfere with the syntheses of the complexes.

Normal nutritional and physiological conditions, in which neither the storage capacity of the liver for Cu or retinol nor its synthesizing power for their carrier proteins are exceeded, may sometimes favour a direct relationship between the two nutrients. Diets well supplied with Cu may also be well supplied with retinol, or its provitamins. In sheep eating the same grass the concentrations of both nutrients in the blood may depend directly on factors such as the individual's appetite and digestive efficiency.

Pressure on the mechanisms for the synthesis of carrier proteins, however, may affect differently the amounts of each nutrient carried in the blood, with the possibility of competition for the synthesis of the respective carrier proteins. In Cu poisoning we may reasonably infer that the known injury to the liver reduces its ability to mobilize retinol into the blood-stream. The ability to mobilize Cu, however, is not lost, which suggests that excessive demands for the carrier of Cu are met at the expense of failure in the production of RBP. Gross liver damage and excessive demands for the storage and transport of Cu are probably two aspects of the same pathological picture.

The finding of Owen *et al.* (1965) of an inverse relationship between Cu and retinol concentrations in the blood of kids was made in animals intentionally deprived of Cu. These authors suggest that an abnormal accumulation of retinol occurred through its inefficient absorption by the Cu-deficient liver and drew attention to other claims that Cu may facilitate the storage of retinol (Šimek, Mandel, Trávníček & Syřínek, 1961; Anthony & Nix, 1965; Idris & Haag, 1965). In the liver of our one Cu-deficient sheep, however, a high-retinol reserve was found.

Finally, research in two bordering fields may possibly be relevant. Pollard & Bieri (1958) reported that the haemolysed blood of young rats and rabbits was destructive to retinol, although blood from older animals was less destructive. The exact nature of this mechanism has not yet been elucidated, but obviously in Cu poisoning the possible destruction of retinol, as opposed to a failure in mobilization, must not be overlooked. The Cu content of the corpuscles may well influence the destructive effects of their haemolysed products on the plasma retinol. Balakhowskii & Drozdova (1957), quoted by Wolf & Johnson (1960), suggested that the keratinization in vitamin A deficiency is due to a disturbance in oxidation processes, and that carotenoid compounds act by controlling the catalytic action of Cu in the conversion of cysteine into cystine.

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