

# THE SUITABILITY OF SOME COPPER PREPARATIONS FOR PARENTERAL COPPER THERAPY IN SHEEP

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## Summary

The storage of copper by adult Merino sheep of normal copper status has been measured in liver biopsy samples drawn at weekly intervals following subcutaneous and intramuscular injections of copper glycine cerate (90 mg Cu) and aqueous suspensions of copper calcium ethylenediaminetetra-acetic acid (EDTA) and copper methionine complexes (each 100 mg Cu) .

The glycine and EDTA preparations safely and effectively induced maximum copper storage within three weeks and one week respectively, with relatively mild local reactions to the subcutaneous injections and no visible or palpable reactions to the intramuscular injections.

The copper storage following copper methionine administration was highly variable, and a cold abscess developed at each injection site.

The EDTA suspension appeared suitable for mass inoculation.

## I. INTRODUCTION

In the search for a simple and long-lasting means of raising the copper status of deficient sheep and cattle, the effectiveness of parenteral injections of more than 80 copper compounds has been examined. The copper glycine complex has been considered the most satisfactory of these compounds, as it induces the safe and rapid absorption of copper with a minimal local reaction when injected in aqueous solution (Harvey and Sutherland 1953) or in a cerate consisting of beeswax-peanut oil (Cunningham 1957; Allcroft and Uvarov 1959; Dye and O'Harra 1959) or peanut oil-aluminium monostearate (Allcroft and Uvarov 1959).

The copper sodium complex with ethylenediaminetetra-acetic acid (EDTA) in aqueous solution produced rapid absorption of copper frequently accompanied by a lethal rise in blood copper concentration when injected in sheep (Harvey and Sutherland 1953) while, in cattle, the corresponding copper calcium complex incorporated in a mixture of peanut oil-aluminium monostearate induced painful, persistent local reactions with highly variable absorption of copper (Allcroft and Uvarov 1959).

More recently, experimental preparations of copper methionine ‡, a complex not previously reported upon, and Cu Ca EDTA in a more satisfactory suspension§ have been made available to us; this paper describes a comparison between these two preparations and a commercial cerate of Cu glycine¶.

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‡ An aqueous suspension containing 20 mg Cu/ml.

§ An aqueous suspension containing 50 mg Cu/ml.

¶ Squeeze tubes containing 45 mg Cu; Glaxo Laboratories (N.Z.) Ltd.

The identity of the organic moiety in these three complexes was established by paper electrophoresis and their copper content checked.

## II. METHODS

In blood, copper was estimated by the method of Tompsett (1934); and in all other tissues by the method of Clare, Cunningham, and Perrin (1945).

Liver samples were drawn by the aspiration biopsy procedure described by Dick (1944, 1952).

## III. EXPERIMENTAL

In pens, 16 adult Merino sheep of normal copper status and in prime condition were fed a mixture of wheaten and lucerne hay chaffs *ad libitum*. Their body weights were recorded at weekly intervals. With each copper preparation two sheep were injected subcutaneously over the ribs immediately behind the shoulder, and two deep into the brachiocephalus muscle of the neck. Four remained untreated. The rectal temperature of each animal was recorded at frequent intervals.

A measure of the rapidity of the absorption of copper from the injection site was attempted by estimating the concentration of copper in freshly drawn blood samples taken at half-hourly and then hourly intervals during the first day and less frequently thereafter. The degree to which the absorbed copper was stored in the liver was assessed in biopsy samples taken at weekly intervals, and the total amount stored then calculated from the concentration in the liver and the total weight of the organ (in nine out of ten sheep slaughtered at the end of the experiment, the relationship between the dry weight of the liver and the body weight was sufficiently close to 3 g/kg for this ratio to be used to estimate the dry weight of the liver of the remaining animals).

## IV. OBSERVATIONS

The body weights of the sheep remained relatively constant; no animal gained or lost more than 5 kg.

None of the injections produced a consistent nor marked increase in the blood copper concentration; all values fell within the normal range, the highest being approximately 50 per cent. greater than the pre-treatment level.

### (a) Local Reactions

Following the subcutaneous injection of Cu glycine, there was no early reaction, but a fibrotic area developed beneath the skin in each sheep which reached maximum dimensions (3 cm and 8 cm diam.) after 12 days. Subsequently, these diminished until they could no longer be felt after 37 and 50 days. By contrast, subcutaneous Cu Ca EDTA induced soft swellings (6 x 2 cm and 7 x 7 cm) within 3 days; these hardened, shrank considerably, and finally sloughed after 35 and 42 days, leaving small, clean scars. There was no visible or palpable reaction to the intramuscular injection of either Cu glycine or Cu Ca EDTA.

Subcutaneous Cu methionine produced a flat diffuse swelling in one sheep within 3 days; this gradually subsided and, after 12 days, a consolidated subcutaneous mass could be felt in both sheep injected by this route. Abscess formation was unmistakable after about 3 weeks and 6 weeks. These sheep were

sacrificed at 43 and 51 days, and an abscess excised from the under surface of the skin in one, and from the fat immediately beneath the thin layer of cutaneous muscle in the other. Each abscess consisted of well-formed fibrous capsule enclosing about 50 ml of grey-green sterile pus which included deep blue foci of clearly recognizable Cu methionine.

No reaction could be detected for about 5 weeks following intramuscular Cu methionine, but then swellings developed at the injection sites. When these two sheep were killed after 43 and 49 days, cold abscesses were dissected from the deep muscle layers; the pus was sterile, but only that from sheep No. 352 contained visible pockets of the copper complex.

None of the reactions described appeared to be painful. The rectal temperatures all fell within the range 101-103 ° F and did not vary by more than 1° F for any one sheep.

(b) Liver Copper

In Fig. 1, the increase in liver copper concentration has been plotted for each sheep. In the four untreated sheet, this concentration increased very slightly during

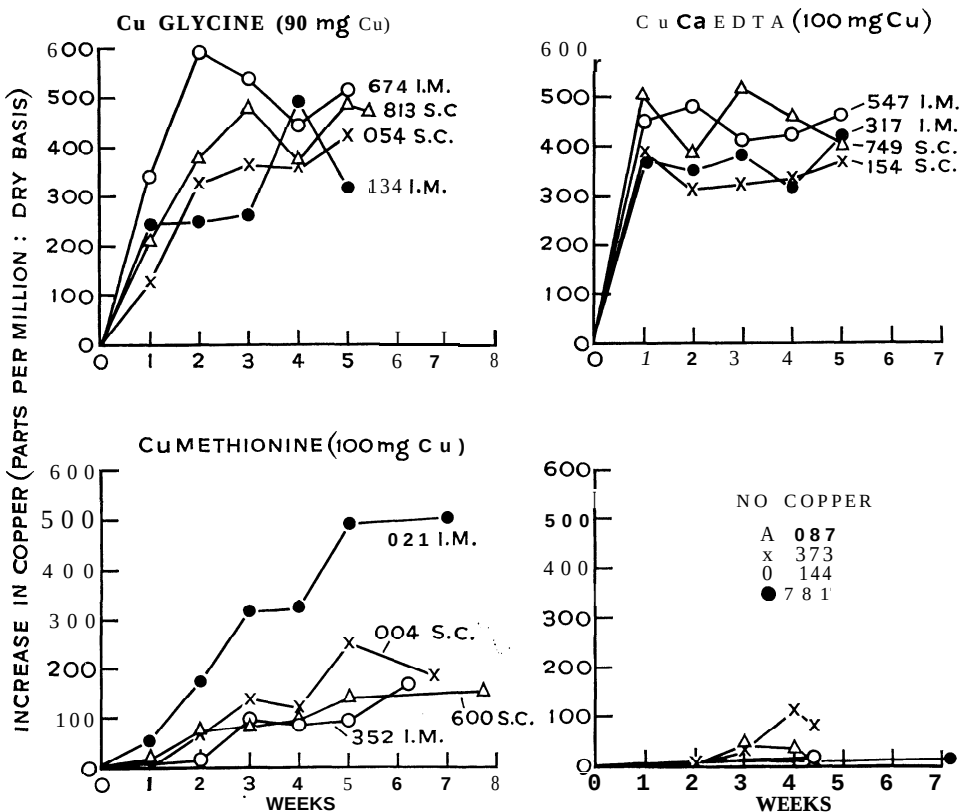


Fig. 1—Increase in liver copper concentration following injections of copper complexes in sheep.

i.m. = intramuscular; s.c. = subcutaneous.

the experimental period, so presumably the diet itself induced a similar small increase in the injected animals.

Following Cu glycine injections by either route, the copper concentration in the liver increased steadily for about 3 weeks until a high proportion of the dose was stored, while Cu Ca EDTA injections by both routes induced maximum storage within a week of similar proportion of the copper administered. Appreciable storage resulted from one subcutaneous and one intramuscular injection of Cu methionine, but in the other two sheep the amount of copper stored in the liver was either meagre or negligible.

For each sheep the calculated amount of copper and the percentage of the injected dose stored in the liver are shown in Table 1, together with the supporting data.

## V. DISCUSSION

Copper glycine induced satisfactory absorption and storage from both the intramuscular and subcutaneous injections, and the slight, local subcutaneous reaction was less than in the case of the other two copper complexes.

The EDTA complex by both routes gave rise to satisfactory copper storage most rapidly, and without either the excessive hypercupremia and attendant risk reported (for Cu Na EDTA) by Harvey and Sutherland (1953) in sheep or the erratic copper absorption and severe local reactions reported by Allcroft and Uvarov (1959) in cattle. The subcutaneous inoculations did produce local necrosis, but the small areas of skin that separated from these sites were found to contain only about 1 mg Cu. The suspension employed appeared to be suitable for mass inoculation by means of an automatic hypodermic syringe connected to a portable reservoir.

Intramuscular injections of Cu glycine sometimes produce persistent deep-seated lesions (Allcroft and Uvarov 1959), but no similar lesions could be detected at autopsy after 15 weeks in our sheep which had received either Cu glycine or Cu Ca EDTA.

The Cu methionine preparation proved unsuitable not only for subcutaneous injection but also when delivered deep into the muscle of the neck. The cold abscess formed in each instance trapped a considerable proportion of the injected dose, viz. sheep No. 600, 70 mg Cu; No. 004, 60 mg; No. 352, 45 mg; and No. 021, 8mg. Abscesses formed also in four sheep subsequently injected either with 5 c.c. or with 2 c.c. of the Cu methionine preparation subcutaneously in the brisket region or intramuscularly in the rump.

## VI. ACKNOWLEDGEMENTS

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TABLE 1

COPPER STORAGE IN THE LIVERS OF SHEEP INJECTED WITH COPPER COMPLEXES

| Sheep No.                                    | Final Body Weight (kg) | Injection Route | Cu Concentration in Liver (p.p.m. dry basis) |         |             | Dry Weight of Liver (g) | Cu Storage in Liver       |                       |
|----------------------------------------------|------------------------|-----------------|----------------------------------------------|---------|-------------|-------------------------|---------------------------|-----------------------|
|                                              |                        |                 | Pre-treatment                                | Maximum | Post mortem |                         | Approximate Increase (mg) | Approximate % of dose |
| GLYCINE (90 mg Cu)                           |                        |                 |                                              |         |             |                         |                           |                       |
| 813                                          | 42                     | Sub-cutaneous   | 137                                          | 620     | 612         | 126*                    | 61                        | 70                    |
| 054                                          | 61                     | (s.c.)          | 262                                          | 685     |             | 180                     | 76                        | 80                    |
| 674                                          | 43                     | Intra-muscular  | 125                                          | 720     |             | 130                     | 77                        | 80                    |
| 134                                          | 61                     | (i.m.) i.m.     | 322                                          | 815     |             | 180                     | 89                        | 100                   |
| ETHYLENEDIAMINETETRA-ACETIC ACID (100 mg Cu) |                        |                 |                                              |         |             |                         |                           |                       |
| 749                                          | 57                     | s.c.            | 111                                          | 630     |             | 170                     | 88                        | 90                    |
| 154                                          | 65                     | s.c.            | 200                                          | 590     |             | 200                     | 78                        | 80                    |
| 547                                          | 51                     | i.m.            | 446                                          | 930     |             | 150                     | 73                        | 70                    |
| 317                                          | 69                     | i.m.            | 288                                          | 705     |             | 210                     | 88                        | 90                    |
| METHIONINE ( 100 mg Cu)                      |                        |                 |                                              |         |             |                         |                           |                       |
| 600                                          | 50                     | s.c.            | 72                                           | 214     | 224         | 132*                    | 19                        | 20                    |
| 004                                          | 63                     | s.c.            | 520                                          | 770     | 705         | 185*                    | 46                        | 50                    |
| 352                                          | 45                     | i.m.            | 315                                          | 412     | 480         | 123*                    | 12                        | 10                    |
| 021                                          | 61                     | i.m.            | 385                                          | 877     | 890         | 118*                    | 58                        | 60                    |
| NO TREATMENT                                 |                        |                 |                                              |         |             |                         |                           |                       |
| 087                                          | 53                     | —               | 76                                           | 120     | 81          | 182*                    | 8                         |                       |
| 373                                          | 58                     | —               | 164                                          | 275     |             | 170                     | 19                        |                       |
| 144                                          | 50                     | —               | 233                                          | 246     |             | 150                     | 2                         |                       |
| 781                                          | 47                     | —               | 51                                           | 65      |             | 140                     | 2                         |                       |

\* Determined after slaughter; the remaining weights calculated as 3 g/kg body weight.

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