# Copper metabolism in milch cows. II. Some precautions to be taken when working with radio-copper

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## 1. Introduction

The isotope $^{64}Cu$ has a half-life of 12.8 hrs (WAY *et al.*, 1955), emitting $\beta^+$, $\beta^-$ and a few gamma rays of 1.34 MeV, which are considerably reinforced by the secondary 0.5 MeV gamma radiation from the $\beta^+$-annihilation. The emission of gamma rays permits external measurement *in vivo* over body compartments, especially the liver, which is the main storage organ for copper. The short half-life excludes long-term experiments, but even 8—10 half-lives are sufficient to reveal many interesting aspects of copper metabolism. On the other hand, the short half-life is an advantage in so far as contamination risks are limited. Thus it is even thought possible to make use of the widespread occurrence of copper deficiency in cattle and to perform experiments with $^{64}Cu$ on ordinary farms.

It was found, however, that due consideration should be given to the source of radio-copper utilised. In the literature COMAR and co-workers (FRIERSON *et al.*, 1952) report the presence of long-lived contaminants in pile-irradiated copper. They present evidence for the existence in the samples of small amounts of $^{65}Zn$, $^{59}Fe$ and probably $^{110}Ag$, among other unknown constituents. In their experiments the impurities were found to be concentrated in (rat) urine, and they were said to become appreciable after ca. 10 half-lives of radio-copper had elapsed. For this reason LASSITER *et al.* (1960), who prepared their copper samples in the same way, limited their observations to 96 hrs in cattle studies.

## 2. Experimental

In starting the series of experiments to be described here, I used commercial $^{64}Cu$-preparations. Two of these samples, ordered at an interval of about four months, were studied in some detail.

### 2.1. Commercial $^{64}Cu$-preparations with medium specific activity

These preparations, listed as 0.25 mC/mg Cu, in amounts of a few mC per animal, were considered sufficiently low in non-radioactive copper to leave the internal body compartments of the cow relatively undisturbed. The daily intake of a cow is in the region of 150 mg of Cu. For special purposes, another more potent preparation can be supplied commercially, but this is about three times more expensive and only provides a few times less copper. This preparation is not considered here.

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The decay of a sample of the medium specific-activity preparation is recorded in FIG. 1. The other sample gave almost identical results. Also included in the figure are the graphs for a special preparation of copper(I)iodide from this sample and the resulting filtrate. Although no attempt was made to work up these samples quantitatively, they can be easily compared with same original copper activity of the steep decay line. The $\beta$—-radiation was measured with extremely thin samples, using a constant geometry. The purification resulting in a product at least three times as pure as the original one, was a subsidiary result of preparing the relatively insoluble Cu(I)iodide, which, when administered orally, is assumed to be more readibly available to cows than the soluble Cu(II)salts.

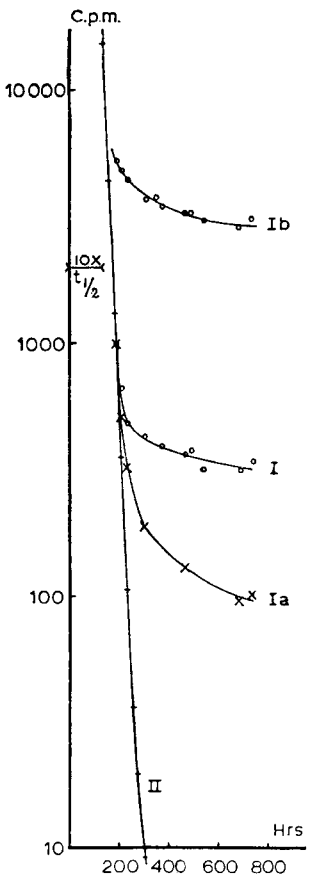



FIG. 1
Decay of a commercial radio-copper preparation (I) and of a briefly irradiated sample (II). Also included in the graph are the decay of a copper-iodide preparation (Ia) and the resulting filtrate (Ib)

In order to prepare Cu(I)iodide, the Cu(II)chloride supplied after addition of 100 mg $AgNO_3$-carrier was treated with an excess of hydrochloric acid. The filtrate was poured through a sintered glass filter and the treatment repeated. The resulting silver precipitate was washed and found to contain a small amount of definitely long-lived radioactivity. The combined filtrates were neutralized to pH3 with ammonia (indicator thymol blue), the calculated quantity of KI-thiosulphate was added and a green precipitate of copper iodide was formed by dropwise addition of further ammonia. A careful control with some drops of excess potassium iodide-thio was exercised to

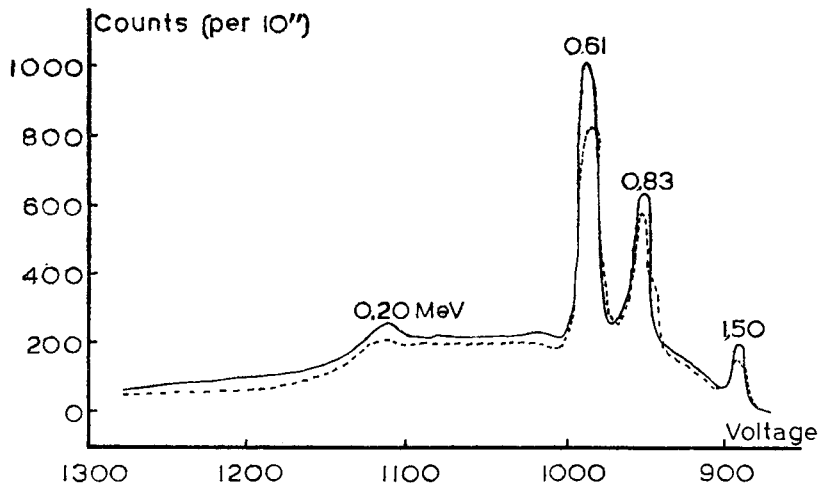
prove the completeness of the reaction. The fluid was decanted and filtered through soft filter paper, the precipitate being washed with water, pressed out between glass sticks and immediately tamped into gelatin capsules for oral administration (the capsules were double with a layer of Kleenex tissue paper between them to prevent the risk of solubilisation of the gelatine outer capsule). Previous experiments had shown that this ensures rapid separation with a minimum risk of radiation exposure, resulting in a finely dispersed, readily filtrable precipitate. The recovery, checked by gamma radiation, was approximately 92 %, most of the additional radioactivity remaining as copper in the filtrate and washings.

Inspection of FIG. 1 shows that a distinct deviation from the straight logarithmic decay occurs at later than 12 half-lives of $^{64}Cu$, calculated from the time of administration (an additional two half-lives may have elapsed in shipment *etc.*, so that ca. 14 half-lives will have elapsed from the end of irradiation), but that the number of residual counts is quite considerable even with the small angle efficiency of the normal $\beta$-counter.

It is not expected that a single experiment will cause any difficulties as regards contamination hazards, but if the experiment with a number of cows is to be repeated at the same experimental farm, the residual radioactivity may build up to an objectional level. Because of the unknown decay scheme of the impurities, only rough estimates can be made of the residual contamination. A residual activity of several tenths of a microcurie may be expected from every 10 mC of $^{64}Cu$. This will be deposited mainly on the land in the form of manure. The amount can be considerably reduced by purification procedures. According to the results obtained by FRIERSON *et al.* (1952), electrodeposition of the radio-copper will virtually remove all radio contaminants. Nevertheless, the impurities were subjected to research.

The *$\beta$-measurements* covered 75 days. By that time an apparently constant decay was observed with a half-life of 87.1 days. FRIERSON *et al.* (1952) report the presence of

FIG. 2. Gamma spectrogram of the long-lived contaminants in the filtrate of the copper-iodide precipitation. Some radio-silver from the preceding chloride precipitation is also included



shorter-lived isotopes $^{69}$Zn and $^{76}$As as well as long-lived $^{65}$Zn. Similar combinations of isotopes could easily give apparent half-lives of intermediate value.
*Chemical separation* according to the $H_2S$-scheme produced no additional evidence. Many separations could not be duplicated even after addition of 100-mg amounts of carriers, so it is thought probable that lesser known and rare elements are involved.
A *gamma spectrogram* was made mainly from the filtrate after precipitation of copper. This is shown in FIG. 2. The recording was taken three months after the separation and repeated nine months afterwards. As the figure shows, the decay rates are low; the relatively most rapid decay occurred at the 0.20 MeV peak, with $t_{½}$ somewhere near 300 days, the 0.61 MeV having at $t_{½}$ of approximately two years, the 0.83 MeV of four years and the 1.5 MeV of five years. The low radiation intensity at 0.5 MeV indicates the absence of appreciable quantities of $\beta$+-emitters. No definite conclusion can be drawn at present on the nature of the contaminants, but the measurements will be repeated after a sufficient lapse of time.

### 2.2. Preparation of radio-copper by short-time irradiation in a high neutron flux

Samples of 50 mg of very pure copper wire (Analytical Reagent Grade, electrolytically prepared) melted in quartz were exposed for 20 minutes to a neutron flux of $> 10^{13}$ cm$^{-2}$ sec$^{-1}$. A calculated amount of 8—10 mC of radioactivity would result. After cooling and several hours of transport, during which the short-lived products ($^{66}$Cu; $t_{½}$ 5.1 min.) decomposed, the copper was dissolved in 0.5 ml of concentrated nitric acid, brought to volume and neutralised dropwise with dilute NaOH to a pH of approximately 4.0—4.5.
In some preparations the neutralisation was only carried out to pH3 and a charge of 100 mg of Na-acetate added, while stirring until the materials were completely dissolved.
As FIG. 1 shows, the advantage of the shortly-irradiated samples is the almost complete absence of any long-lived contaminant. Even after allowing a difference of a factor 4 for a few half-lives during additional transport, the commercial preparation contains at least 30 times more impurities. This could be explained by contaminants in the irradiated copper, formation of a higher proportion of long-lived nuclides, and contamination during preparation and handling. A higher proportion of long-lived nuclides will be formed in a lower-flux reactor during the long exposure required to obtain the saturation level of radio-copper. During saturation of the copper the production of long-lived nuclides continues linearly with time. Moreover, the long contact will permit more diffusion of fission products into the sample.
It is clear that even in repeat experiments with the relatively large amount of radio-copper needed for large animals, no contamination need to be feared from the shortly-irradiated $^{64}$Cu. An additional advantage is the possibility of extending the measurements over longer periods of time. The $^{64}$Cu from short-time irradiations is therefore recommended for all metabolic studies.

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