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^{67}Cu for metabolic studies in milk cows

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Summary

Primarily because of its half-life, the longest-lived radionuclide of copper, ^{67}Cu ($t_{1/2}$ = 61.7 h), is often the isotope of choice for application in metabolic studies. However, large-scale production of ^{67}Cu is cumbersome. The ^{67}Cu used in this study was produced in a cyclotron by exposing a zinc target to a beam of accelerated protons. Radiochemical purification was achieved by solvent extraction, electroplating and ion-exchange, in that order. The resulting dilute acidic solution contained no measurable activity apart from ^{67}Cu and ^{64}Cu. After the injection in cows, the ^{64}Cu component of the preparation of radiocopper could be used during the first days of the experiment for external radioassay of the liver. The ^{67}Cu could be followed in metabolic products for over three weeks. It is concluded that the isotope ^{67}Cu in batches of about 100 μCi can be very well used for metabolic excretion studies even on large animals.

Introduction

The knowledge of copper metabolism in ruminants is far from complete. Many studies have focussed on the absorption phase (Underwood, 1971). The frequent occurrence of copper deficiency has been explained by the unavailability of copper compounds like CuS (Hartmans, 1974) that are formed in the forestomachs. For copper-molybdenum complexes it was postulated that these not only were formed in the rumen (Suttle, 1974), but might travel in the blood stream (Clawson et al., 1972). As for the further pathways in the metabolism nothing definitely is established. The liver uptake of radio-copper in milk cows has been measured under different feeding circumstances (Binnerts, 1964, 1965, 1966, 1967). Large urine volumes carrying much copper have been recorded in sheep (Marcilese et al., 1970), but are unknown in cows. Endogenous faecal losses have not been reported; they could be measured with radioactivity. Application of the isotope ^{64}Cu ($t_{1/2}$ = 12.8 h) will

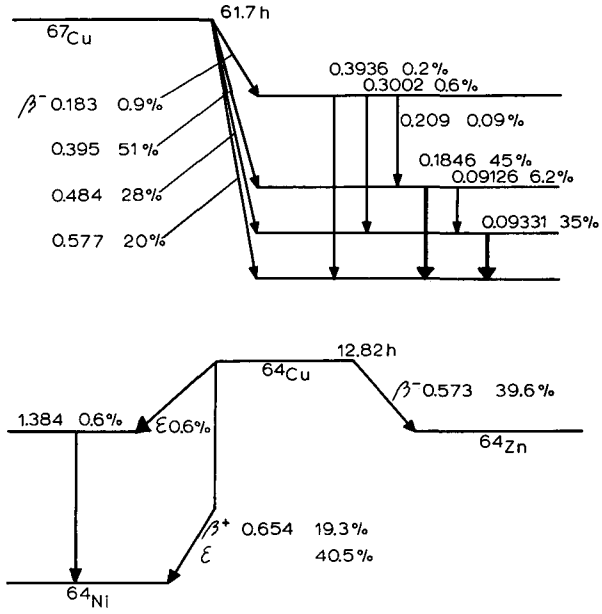

Fig. 1. Top: decay scheme of ^{64}Cu. Bottom: decay scheme of ^{67}Cu.

allow measurement for about 5 days (Binnerts, 1965), with growing errors towards the end of this period. Use of the longer-lived ^{67}Cu ($t_{1/2}$ = 61.7 h) allows these studies to cover much longer periods. This enhances the more uniform labelling of all metabolic products, in a better approach of steady state conditions. Apart from its longer half-life, also the radiation characteristics of ^{67}Cu make it the radionuclide of choice for efficient detection (Fig. 1).

Production and purification of ^{67}Cu; procedures

Various methods for production of ^{67}Cu are listed in Table 1. The use of enriched targets in two cases requires special measures to recover this highly expensive material. The photonuclear reaction employed at IKO with the 85 MeV linear electron accelerator refers to investigations of a preliminary though promising nature. Production of the ^{67}Cu used for the work described here was done by using the ^{68}Zn(p,2p)^{67}Cu reaction with protons of about 52 - 55 MeV. The threshold is 10.1 MeV; the cross section at 55 MeV has been reported to be about 5.3 mb (McGee et al., 1970). The metallic zinc targets of 2 mm thickness were clamped in between copper blocks cooled with water. This target arrangement could withstand beam currents of 5 - 10 μA. Irradiations generally lasted about two hours. At the end of bombardment the target was allowed to cool down during a period of about 2 days. After dissolution of the irradiated zinc in 12 *N* HCl in the presence of 5 - 10 mg Cu^{2+} and 0.1 mg Ga^{3+}, little NHO_3 is added to ensure proper oxidation

Table 1. Methods for ^{67}Cu production.

Method	Nuclear reaction	Energy	Target	Yield	Reference
Cyclotron	^{64}Ni (α, p) ^{67}Cu	E_α = 40 MeV	enr. 96% ^{64}Ni (nat.:1.08 %)	2.8 μCi/μAh	Sternlieb et al. (1961)
Reactor	^{67}Zn (n, p) ^{67}Cu	fast n	enr. 90 % ^{67}Zn (nat.: 4.11 %)	12 mCi/5d × 0.1 g	O'Brien (1969)
e$^-$-accelerator	^{68}Zn (γ, p) ^{67}Cu	$E_\gamma \lesssim$ 35 MeV	natural Zn (18.6 % ^{68} Zn)	40 μCi/100 μA × 1 g	Marceau et al. (1970)
		$E_\gamma \lesssim$ 85 MeV	natural Zn (18.6 % ^{68}Zn)	100 μCi/100 μAh × 1 g	unpublished work (IKO)
Cyclotron	^{68}Zn (p, 2p) ^{67}Cu	$E_p \approx$ 55 MeV	natural Zn (18.6 % ^{68}Zn)	30 μCi/μAh	this work

states. Radioactive gallium (^{66}Ga, ^{67}Ga) was removed from the strongly hydrochloric-acid solution by extraction with di-isopropylether (Nachtrieb & Tryxell, 1949). The aqueous phase containing the radio-copper was evaporated to near dryness whereafter the residual chlorides were converted into nitrates by heating with concentrated nitric acid. The nitrates were taken up in about 150 ml H_2O + 6 ml concentrated H_2SO_4. The copper was electroplated within one hour from this solution on a cylindrical (Ø = 5 cm) platina foil (5 cm high) as the anode and a platina wire as the cathode. Stirring during electroplating reduced the amounts of coprecipitated zinc and cobalt activities. To remove residual radiochemical impurities the copper was dissolved in nitric acid, converted to the chloride and taken up in 9 *N* HCl containing 1 mg Co^{2+} as a hold-back carrier. The final purification was achieved by ion-exchange chromatography on Dowex I-X8 (1 cm Ø × 40 cm) conditioned with 6 *N* HCl. Cobalt activities and other residual contaminations were removed by elution with 6 *N* HCl. The radiocopper fraction was recovered by elution with a few millilitres of 1 *N* HCl (Kraus & Nelson, 1956). Thick target yields for ^{67}Cu amounted to 30 μCi/μAh taken at the end of bombardment. The corresponding figure for ^{64}Cu produced simultaneously primarily by the reaction ^{67}Zn(p,α)^{64}Cu is in the order of 3.5 mCi/μAh. Overall radiochemical yields were nearly 100 %. Preparations made according to the procedure ascribed above did not contain any measurable radiochemical impurities as proven by gamma-ray spectroscopy with Ge(Li) solid state detectors, at different times during decay of such sources of ^{67}Cu (Fig. 2).

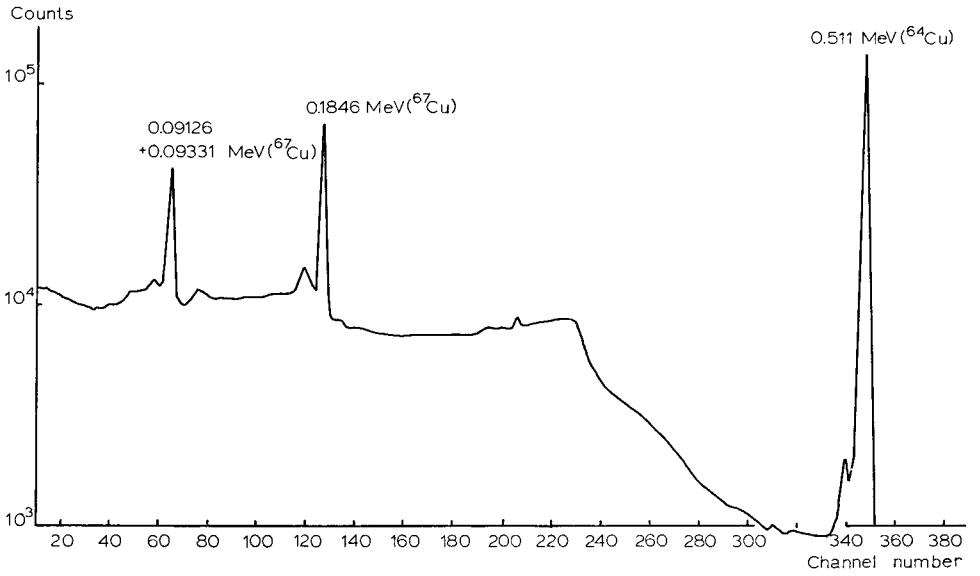

Fig. 2. Gamma ray spectrum of ^{67}Cu: source measured on a Ge(Li) detector about two days after the end of cyclotron exposure.

Preparations of ^{67}Cu were dispatched as hydrochloric acid solution. For injection they were brought at pH 4.5 with dilute NaOH and then converted to the glycine complex.

Typical ^{67}Cu preparations contained approximately 100 μCi of activity in a 10 ml volume. The glycine complex at pH 4.5 with the low total copper concentration present, would hardly show a noticeable blue colour. The specific activity was in the order of 10 μCi per mg of copper, a figure that seemed acceptable for large animals (with some effort it could be made up to 100 times higher if desired). After the intramuscular injection of a total of approx. 8 ml in 1 to 3 animals, appropriate standard solutions were prepared from the remaining fluid, usually containing 0.5 to 1.0 % of the administered dose. Milk, urine and faeces were collected twice daily, and representative subsamples were prepared. Samples and standards were counted alternatively in each 3.00-litre volumes in large Marinelli beakers placed on a NaI(Tl) detector inside a thick-walled lead pot. In the gamma-counter two counting channels were available, which were used for ^{64}Cu and ^{67}Cu, respectively.

Table 2. Excretion in faeces, milk and urine, as measured simultaneously in the ^{64}Cu and the ^{67}Cu channels.

Date[1]	Sample	^{67}Cu channel (% dose)	^{64}Cu channel (% dose)
5 July M	faeces	0.037	0.037
	milk	0.348	0.343
	urine	0.241	0.242
5 July A	faeces	0.364	0.364
	milk	0.130	0.124
	urine	0.040	0.039
6 July M	faeces	0.276	0.270
	milk	0.096	0.090
	urine	0.037	0.035
6 July A	faeces	0.189	0.178
	milk	0.049	0.046
	urine	0.029	0.026
7 July M	faeces	0.331	0.308
	milk	0.061	0.052
	urine	0.038	0.038
7 July A	faeces	0.124	0.128
	milk	0.042	0.034
	urine	0.021	0.018
8 July M	faeces	0.388	0.404[2]
	milk	0.049	0.035[2]
	urine	0.029	0.045[2]

[1] M: morning 07h00; A: afternoon 17h00 sampling. The injection had been performed at 4 July 18h00.

[2] Low count rates at this time lead to uncertain values. At three days after the injection the ^{64}Cu counts are usually stopped.

Experimental results; Discussion

Samples of excretion products

Already at the day of injection, by means of the NaI(T1) detector, the two low-energy gamma peaks of ^{67}Cu, 93.3 and 184.6 keV, were well separated from the 511 keV annihilation radiation of ^{64}Cu. As can be seen from the results in Table 2 the occurrence of some ^{64}Cu during the first days of the experiments could be used to show that results in analysis of faeces samples, urine and milk were identical relative to the standard solutions, irrespective of measuring in the ^{64}Cu or in the ^{67}Cu channels. These results also show that the low-energy radiation suffers no noticeable self-absorption differences in the large urine and faeces samples relative to the radiation from standard solutions.

It should be noted that especially towards the end of the experiments due attention must be given to the natural background level in the various substrates. So, for instance, urine can contain fairly high concentrations of potassium, resulting in Compton scatter in the range of the measuring channels originating from the 1.4 MeV gamma radiation of the isotope ^{40}K. During an experiment with standardized feeding the contributions from natural and fallout isotopes will be practically constant as long as the animals are healthy, so that a single sample for the 'blank' value, taken before the start of the experiment with radio-copper, usually will suffice to make the necessary correction. Samples with suspected different composition may be stored in a freezer for establishing the background count rates after sufficient decay of the radiocopper.

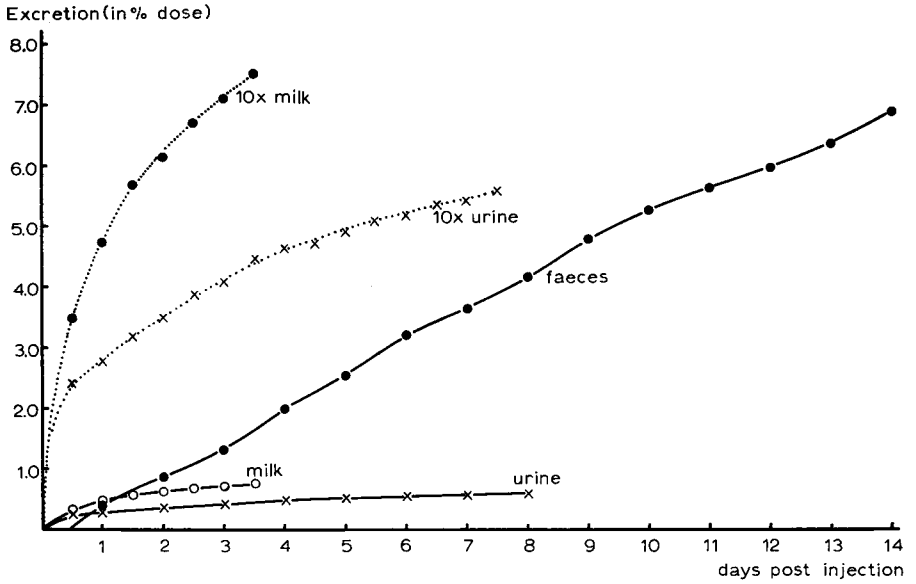

Fig. 3. Accumulated excretions of radio-copper (cow W 12).

After the period of a couple of days for simultaneous measurement of ^{64}Cu and ^{67}Cu, the further measurements were performed solely on the ^{67}Cu radiation. An illustration of the proportions of faecal, urine and milk excretions is given in Fig. 3. For milk after the first day following the injection and for urine the excretions were very small indeed. The faecal losses were larger, but in this experiment the daily quantities remained well below 1 % of the administered dose. The rather high copper status in most cows, resulting from feeding copper-containing concentrates, prevented higher labelling of the endogenous fecal copper excreted into the faeces.

External liver measurement

In these large animals the low-energy ^{67}Cu radiation does not penetrate sufficiently to allow in vivo liver measurement. During the first three days after injection, however, the ^{64}Cu component may serve very well for this purpose, and liver uptake measurements can be performed as described before (Binnerts, 1964, 1965, 1966, 1967). Also the amounts of radioactivity remaining at the injection site and the quantities in the cannulae and syringes will be easily measured with the ^{64}Cu radiation. The contribution of the ^{67}Cu from the latter undeep sources as well as from the usual small plastic bottles with standard solutions will, of course, be no problem when gamma-ray energy discrimination is used. In experiments with the usual 1 mm Pb plate in front of the scintillation crystal (Binnerts, 1964, 1965, 1966, 1967), some contribution of the ^{67}Cu remained, but it could easily be corrected for by extrapolation of the activity-time curve. In Fig. 4 an example of such a correction is given and in Fig. 5 the effect of correction on the liver uptake measurements is indicated.

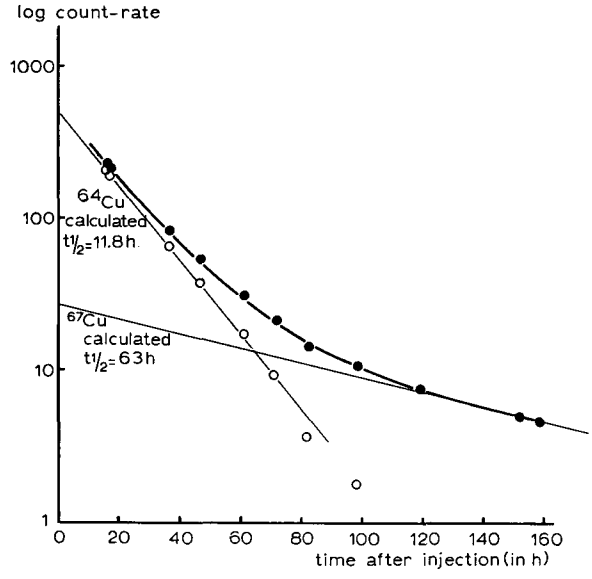

Fig. 4. Decay of a copper standard solution in a 100 ml plastic bottle placed in front of a scintillator crystal and separated from the crystal by 1 mm of lead.

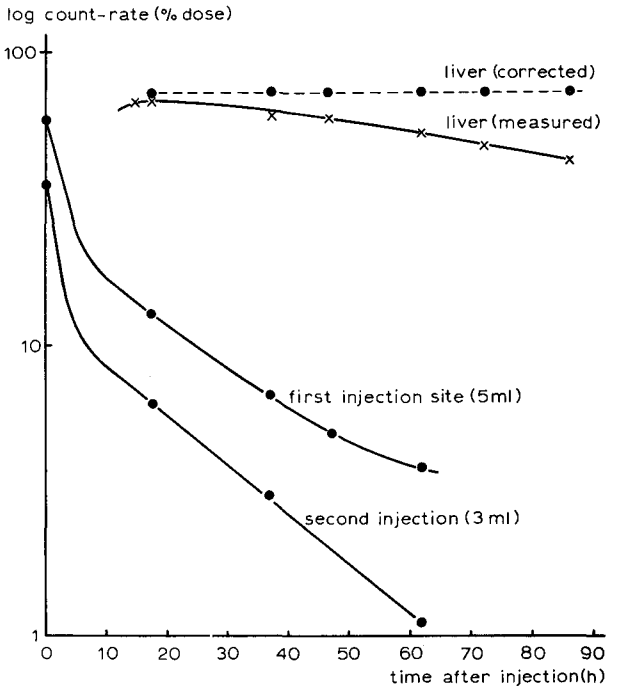

Fig. 5. Movement of radio-copper from the injection sites and from the liver as measured in vivo by scintillation counting. The liver measurements need correction for the ^{67}Cu contribution from the standards.

The resulting nearly horizontal line for the ensuing liver discharge is usual in cows with a large store of non-radioactive liver copper.

Samples from the intestinal tract

Samples of the gastrointestinal fluids could be counted in the same way as are the milk and urine samples. Sampling sites were the proximal and distal duodenum in one cow and the proximal duodenum and the ileum in two other cows. Spot samples of approximately 250 ml each were collected with hourly intervals during the day, and the composite sample of 3 litres was counted. Such experiments could be performed on four successive days in the second week of the trials. The difference between the count rates from both sampling sites would indicate the contribution of the bile in copper excretion. Before such comparisons could be made, however, the count rates had to be corrected for the relatively large concentration effects, resulting from the considerable water movement along the intestinal tract. For the correction non-absorbable markers were used: chromium oxide paper and complexes of yttrium, as described elsewhere (Binnerts & Boer, 1975). The results of some experiments are given in Table 3. It can be seen in Table 3 that even before

Table 3. Radio-copper contents in intestinal samples of cow Witschoft 12.

Injection date	Sampling date	Proximal duodenum[1]	Distal duodenum[1]	Fecal content[2]	
27 Sept.	2 Oct.	4.10	5.93		
	3 Oct.	4.09	6.90		
	4 Oct.	—	1.86	6.59	—
4 July	8 July	2.70	6.72	6.63	5.42
					5.94
	10 July	3.36	3.46	6.77	4.69
	11 July	4.14	7.07	4.11	5.21
					4.86
	12 July	5.20	5.71	5.35	5.44
					5.24
	15 July	2.90	2.48	3.36	6.77
					5.78
	16 July	—	—	4.58	3.58
					3.11

[1] Results expressed in promille of the dose per 10.0 g marker (the daily administered quantity was 10.0 g of Cr_2O_3).

[2] First faeces column: as measured, and totalled per day, second column as expressed per Cr_2O_3 content, separate for morning and evening sample.

the site of bile secretion there is a considerable endogenous excretion of radio-copper. The origin of this material is unknown; it is improbable that backflow of bile would account for the radioactivity in the proximal part of the duodenum, because in all samples the amount of bile was very low. For quantitative calculations the expression in terms of percentage of the injected dose should be changed into terms of mg copper. This transformation needs the knowledge of specific activities which are fairly difficult to obtain for copper in the intestinal tract (Binnerts, 1974). Further work is devoted to this aspect of the endogenous copper, as measured with the isotope ^{67}Cu.

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