Biodistribution of ^{64}Cu in inflamed rats following administration of two anti-inflammatory copper complexes

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Abstract

^{64}Cu was administered in two anti-inflammatory formulations to normal rats and to rats with 2 forms of local inflammation, namely (a) an acute paw oedema (elicited with carrageenan) or (b) a chronic granulomatous response to an implanted irritant (*Mycobacterium tuberculosis* in a polyurethane sponge). The copper formulations used were (i) a slow release one consisting of Cu(II) salicylate applied dermally with ethanol/DMSO and (ii) short acting hydrophilic complex (Cu(I)Cu(II)-penicillamine)$^{5-}$ given subcutaneously.

Three types of changes in copper biodistribution with these forms of inflammation were discerned based on determination of ^{64}Cu and copper content in the following organs: inflammatory locus (foot or sponge implant), kidney, liver, spleen, adrenals, brain, blood, thymus, heart, and skin (site of application). The most evident changes were in the kidneys, liver, spleen, adrenals, thymus and serum from animals with chronic granulomatous inflammation. In contrast, a short term acute inflammatory stress (carrageenan paw oedema) had little effect.

While copper D-penicillamine (applied subcutaneously) appeared to move as a bolus through the animals, the results with the percutaneous copper salicylate formulation are consistent with it providing a slow release source of copper(II). Exogenous ^{64}Cu from both formulations was sequestered at inflammatory sites (relative to serum). This may partly explain how applied copper complexes can be anti-inflammatory.

Introduction

The effects of copper in inflammation have recently been the subject of considerable attention. Attempts have been made to evaluate both the anti-inflammatory (AI) effects of applied copper salts and the role of endogenous copper complexes in chronic inflammatory disease. A large body of data obtained in man [1] and animals show that circulating levels of copper rise significantly during the course of many inflammatory processes (either acute or chronic). Copper may be involved in several of the biochemical pathways concerned with the inflammatory response [2], for example accelerating the conversion of arachidonic acid to prostaglandin [3, 4] or inhibiting kinin production [2]. Furthermore, copper-complexing agents can inhibit prostaglandin biosynthesis [2, 5].

Copper(II) complexes exhibit anti-inflammatory activity in animals when administered parenterally [6, 7]. The anti-inflammatory/anti-arthritic efficacy of percutaneous lipophilic copper(II) formulations has been described [8–10]. The biodistribution of ^{64}Cu, following its topical application in two lipophilic anti-inflammatory Cu(II) formulations, has been investigated in non-inflamed rats [11]. Here we report the distribution of ^{64}Cu in the tissues of inflamed and non-inflamed rats after administering it by two different routes, namely (i) the topical application of ^{64}Cu-salicylate in a non-aqueous solvent and (ii) subcutaneous dosing of water soluble ^{64}Cu-D-Penicillamine, Cu(I)$_8$Cu(II)$_6$ D-Pen$_{12}$ (the red-violet copper complex) [12–14]. Copper D-penicillamine was chosen as a well defined complex which is a non-irritant hydrophilic source of exchangeable copper. This copper complex has also been shown to have anti-inflammatory and antiulcer activity [15, 16].

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Materials and methods

Radiochemical

The ^{64}Cu labelled copper salicylate 100 mM (with respect to Cu(II) dissolved in ethanol/dimethyl sulphoxide/glycerol (3:1:1 v/v) (specific activity = 570 counts/µg copper) was prepared as described previously [11]. It was

administered percutaneously as a single dose of 6 ml/kg (equivalent to 0.6 mmole Cu(II)).

^{64}Copper-D-penicillamine complex was prepared in a 4:3 ratio [13] of D-penicillamine:Cu, by adding 1 volume of D-penicillamine in 0.15 M NaCl slowly to an equal volume of ^{64}Cu labelled CuCl$_2$ (50 mM) in 0.15 M NaCl and the pH was subsequently adjusted to 7.4. After neutralization the solution was a deep-red to purple colour and stable in air. The ^{64}Cu D-penicillamine solution (specific activity = 970 counts/μg Cu(II)) was injected subcutaneously at a dose of 2.5 ml/k (equivalent to 63 μmoles Cu(II)).

Biological

Male J.C. Lewis rats (average weight 250 $\pm$ 30 g; Gilles Plains Field Station, Adelaide, South Australia) were housed in metabolic cages. Where dermal application was the route of ^{64}Cu administration, animals were lightly anaesthetized and an area about 20 cm^2 shaved from the dorsum immediately below the neck. Carrageenan paw oedema was induced by injecting 0.1 ml of a 1% solution of sodium carrageenan (Marine Colloids) in 150 mM NaCl sub-plantar in both rear paws 1 hour before administering the ^{64}Cu. The control group for this experiment consisted of non-inflamed animals. In the first experiment, groups of both control and experimental animals ($n = 4$) were sacrificed at intervals of 2.5, 5, 24 and 48 hours, following drug administration. Organs were excised and specimens taken as described previously [11].

Animals for the sponge granuloma model of inflammation were prepared as follows [17]. Polyurethane foam, 7 mm in thickness, was cut to 15 × 15 mm blocks with an average weight of 40 mg. These sponges were treated with boiling ethanol, and were then impregnated with heat-killed human strain *Mycobact. tuberculosis* at a concentration of 0.5 mg/ml followed by air drying overnight at 37°C to constant weight. Before implantation the cubes were sterilized at 121°C for 15 minutes. One impregnated sponge was then implanted into each flank of the rat through a midline dorsal incision under ether anaesthesia by a sterile technique. Animals were treated with the copper drugs only once 5 days after the sponge implantation, then sacrificed 24 hours later and tissue samples taken. The control group for this experiment consisted of non-inflamed animals.

Analytical

Radioactivity was determined using an LKB Model 1260 Multigamma counter.

Tissue copper levels were measured by using a Varian Technicon atomic absorption spectrometer following digestion of the tissues with nitric acid [18] and expressed as μg of copper/gram of wet wt. tissue.

Percentage of relative specific activity of each tissue (% R.S.A.) was determined by:

$$\% \text{ R.S.A.} = \frac{[\text{counts/gram of tissue}]}{[\mu\text{g copper/gram of tissue}]}$$

$$\times \frac{100}{[\text{specific activity of}] \text{ the labelled complex}}$$

Results

Carrageenan paw oedema

Carageenan was chosen as an acute inflammagen to determine any changes in ^{64}Cu biodistribution due to a transient inflammatory stress.

With topically applied ^{64}Cu-salicylate. ^{64}Cu in sera (see Fig. 1a) increased steadily over the first 5 hours and was slightly elevated in the inflamed animals compared to the non-inflamed animals. After 24 hours, the level of ^{64}Cu in the serum of the inflamed animals was greater than in the non-inflamed animals. Radioactivity in the serum was elevated compared to whole blood in both cases.

Afer 48 hours, ^{64}Cu in the blood (Fig. 1a) was very low in the non-inflamed group and marginally lower in the inflamed animals. This pattern confirms the slow release and clearance of ^{64}Cu observed previously in non-inflamed animals [11].

Levels of ^{64}Cu in the kidney (Fig. 1b) were similar after 5 hours in both non-inflamed and inflamed animals, thereafter the rate of accumulation was greater in the non-inflamed than the inflamed animals. After 48 hours, there was no significant difference between the two groups.

Liver levels of ^{64}Cu were similar in both non-inflamed and inflamed animals over the first 2 days (see Fig. 1b). Hepatic ^{64}Cu increased steadily during the first 24 hours, then rose quite sharply to approximately twice this level by 48 hours. ^{64}Cu in the spleen increased slightly for the non-inflamed group after 2.5 hours and then declined (Fig. 1b). In the spleens of the inflamed animals ^{64}Cu rose to a peak at 5 hours, after which it fell to a level similar to that found in the non-inflamed animals and may be accounted for mainly by the ^{64}Cu in the serum.

^{64}Cu in the adrenals, thymus, brain and heart (see Fig. 1c) all showed a marked peak at 2.5 hours The accumulation of ^{64}Cu in the heart and brain was very similar for both inflamed and non-inflamed groups of animals. After 24 hours, there was no significant difference in ^{64}Cu levels in these two organs taken from either the inflamed and non-inflamed groups. A high level of ^{64}Cu was sequestered by the adrenal glands: the level in the inflamed group was marginally higher than the control at 2.5 hours. Although some of the adrenal ^{64}Cu content may be attributed to its blood content, this alone does not account for the levels observed.

^{64}Cu content of the thymus was greater in the non-inflamed animals than in the inflamed animals after 2.5 hours; however, after 5 hours, it was very

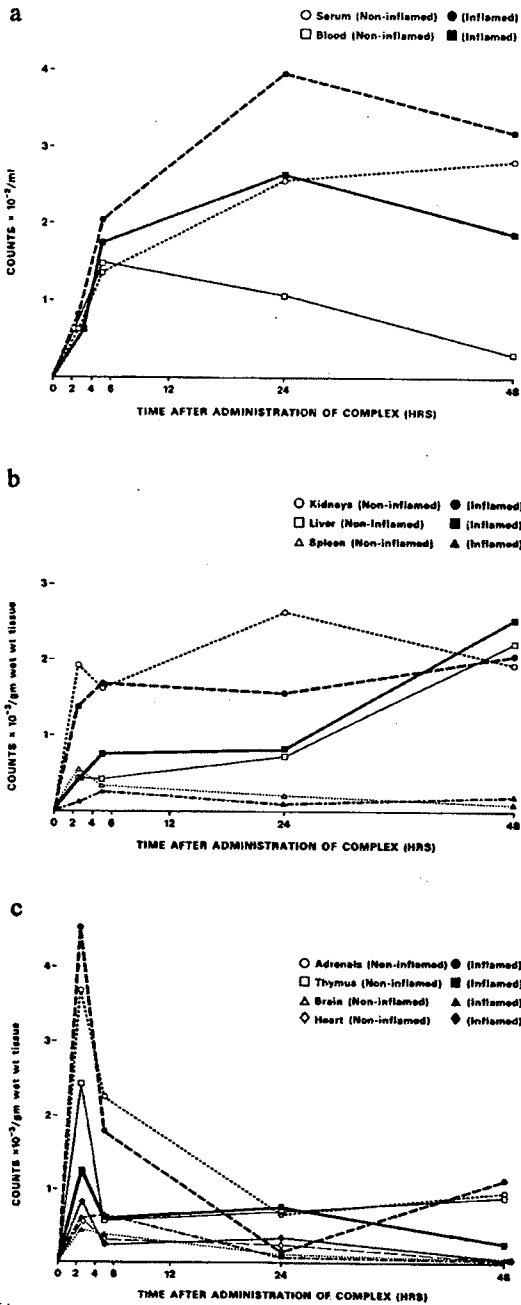**Figure 1**
Distribution of ^{64}Cu applied as topical ^{64}Cu-salicylate in ethanol/DMSO onto carrageenan-inflamed and non-inflamed rats in (a) serum and whole blood, (b) kidneys, liver and spleen, (c) adrenals, thymus, brain and heart.
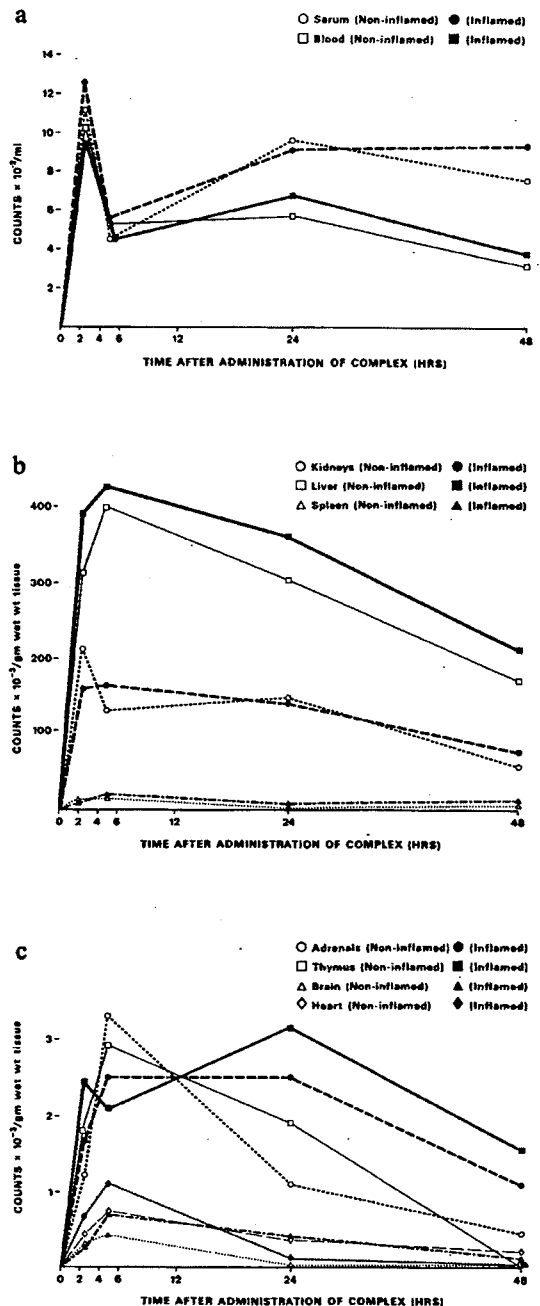
Figure 2
Distribution of ^{64}Cu injected subcutaneously as ^{64}Cu-penicillamine in carrageenan-inflamed and non-inflamed rats (a) serum and whole blood, (b) kidneys, liver and spleen, (c) adrenals, thymus, brain and heart.

Table 1
Biodistribution of ^{64}Cu in feet, faeces and skin in carrageenan inflamed and normal rats.

Tissue	Group	2.5 h	5 h	24 h	48 h
Cu(II)-salicylate					
Feet	Norm.	3.79	5.74	2.34	7.36
Feet	Infl.	3.30	2.97	1.42	6.10
Faeces	Norm.	N/D[b]	N/D	102	397
Faeces	Infl.	N/D	N/D	83.0	358
Skin	Norm.	N/D	N/D	69.6	75.7
Skin	Infl.	N/D	N/D	60.5	64.8
Cu(I)Cu(II)-D-penicillamine					
Feet	Norm.	0.85	0.36	0.23	0
Feet	Infl.	1.59	0.31	0.31	0
Faeces	Norm.	N/D	N/D	5.19	56.1
Faeces	Infl.	N/D	N/D	3.12	86.9

[a] Counts/g wet tissue × 10^3 corrected for radiodecay of ^{64}Cu.
[b] N.D.: not determined.

similar and quite low in both groups. This was probably due to equilibration with the serum ^{64}Cu. The ^{64}Cu in the thymus of the non-inflamed animals continued at this level for the following 43 hours, whereas the ^{64}Cu in the inflamed animals dropped from 24 to 48 hours, which may be due to involution of the thymus under the inflammatory stress. ^{64}Cu levels in the paws were not significantly different between non-inflamed and inflamed groups (Table 1). This may be a result of contamination resulting from topical application of the ^{64}Cu-salicylate. To avoid this problem, an implanted sponge granuloma model was also employed.

With parenteral ^{64}Cu-D-penicillamine. A similar trend in levels of ^{64}Cu was observed for blood and serum in both inflamed and non-inflamed groups over the first 5 hours (see Fig. 2a). Both exhibited a marked rise during the first 2.5 hours, falling to half that level after 5 hours. The ratio of ^{64}Cu/ml in whole blood to ^{64}Cu/ml serum of both inflamed and non-inflamed groups was almost 1:1, both at 2.5 and 5 hours. Since serum ^{64}Cu was not greater than ^{64}Cu in the whole blood, this would imply some form of ^{64}Cu retention by the erythrocytes. However, by 24 and 48 hours, the ^{64}Cu in whole blood would appear to be predominantly in the serum. Although after 24 hours there was more ^{64}Cu in the blood of the inflamed animals compared to the non-inflamed group, the levels of ^{64}Cu in serum of both non-inflamed and inflamed groups were very similar. The magnitude of the ^{64}Cu level observed at 2.5

hours may be attributed to a 'surge' effect, where a bolus of ^{64}Cu-D-penicillamine travels rapidly through the cardiovascular system. This may be correlated with urinary elimination of the intact purple complex which is apparent within 20 minutes of administration. Approximately 50% of the injected dose is cleared via the kidneys within 6 hours.

Levels of ^{64}Cu in the kidneys of both non-inflamed and inflamed animals were very similar (see Fig. 2b). The rates of accumulation were similar to that of the liver, although observed levels of ^{64}Cu were considerably lower. There was a rapid rise of ^{64}Cu in the liver over the initial 5 hours, which gradually dropped to approximately half that value after 48 hours. This decline in hepatic ^{64}Cu correlated with a drop in serum ^{64}Cu. There was no significant difference in hepatic ^{64}Cu levels in either the inflamed or non-inflamed rats. Since the observed organ level of radioactivity cannot be accounted for by its blood volume alone (particularly at 24 hours), we may assume a selective uptake/absorption.

The low level of radioactivity in the spleen over the period of the study may be substantially accounted for by blood levels of ^{64}Cu. Similarly, radioactivity levels in the heart and brain may be largely attributed to their blood content. In addition, the rate of accumulation/excretion of the ^{64}Cu was the same for both organs. The initial rapid increase in adrenals and thymus in the non-inflamed rats fell to lower levels after 48 hours, whereas in the inflamed animals, the ^{64}Cu remained at a higher level for a longer duration.

Initially, ^{64}Cu levels rose rapidly in the paws (Table 1). Although there was a difference between non-inflamed and inflamed animals after 2.5 hours, some of the activity may well be due to the accompanying oedema. From 5 hours onward, there was no difference between the two groups as might be expected from a short term inflammation.

A comparison of ^{64}Cu and copper levels in the carrageenan and sponge granuloma models of inflammation after 24 hours

Figures 1 and 2 show that a 'steady state' appears to have been reached in most organ systems by 24 hours. This follows an initial purge effect that was quite pronounced in the case of the copper D-penicillamine. Selection of a 24 hour sampling time enabled a valid comparison of ^{64}Cu distribution in the various organs and thus between the two models of inflammation. The

Table 2

Tissue copper concentration and relative specific activity of ^{64}Cu in the carrageenan and sponge granuloma models of inflammation, 24 hours after dosing with [^{64}Cu]-salicylate.

Tissue	Control[a]		Carrageenan		Sponge	
	μg/g + S.E.	% R.S.A.[*]	μg/g + S.E.	% R.S.A.	μg/g + S.E.	% R.S.A.
Kidney	13.8 ± 1.5	39	12.5 ± 0.8	24	8.7 ± 0.4	51
Liver	5.7 ± 0.5	31	5.9 ± 0.4	33	5.8 ± 0.5	61
Spleen	9.4 ± 3.2	07	8.6 ± 4.3	04	5.3 ± 2.9	13
Adrenal	48.0 ± 9.5	23	38.6 ± 12.4	01	5.9 ± 1.7	37
Brain	6.7 ± 1.2	02	7.7 ± 1.2	01	2.4 ± 0.3	22
Blood	1.0 ± 0.4	11	1.9 ± 0.4	23	2.6 ± 0.2	14
Thymus	22.9 ± 5.2	07	48.8 ± 17.5	05	16.6 ± 4.2	27
Heart	7.4 ± 0.3	03	7.7 ± 0.8	02	4.1 ± 0.2	09
Serum	1.1 ± 0.1	41	1.3 ± 0.1	53	1.9 ± 0.1	19
Capsule[b]	–	–	–	–	2.0 ± 0.3	100
Sponge	–	–	–	–	3.9 ± 0.3	46
Skin[c]	1.2 ± 0.4	–	112 ± 17.0	100	200 ± 22.2	90

[a] The control group consisted of shaved, non-inflamed animals, dosed with ^{64}Cu-salicylate applied twice for a total of 6 ml/kg.
[b] The capsule consists of inflammatory tissue surrounding the sponge implant.
[c] At site of ^{64}Cu application = Cu-salicylate in ethanol–DMSO–Glycerol.
[*] A measure of exchangeability of endogenous copper with applied ^{64}Cu (see experimental).

Table 3

Tissue copper concentration and relative specific activity of ^{64}Cu in the carrageenan and sponge granuloma models of inflammation, 24 hours after dosing with [^{64}Cu]-D-penicillamine.

Tissue	Control[a]		Carrageenan		Sponge	
	μg/g + S.E.	% R.S.A.[*]	μg/g + S.E.	% R.S.A.	μg/g + S.E.	% R.S.A.
Kidney	17.5 ± 0.7	86	19.1 ± 1.6	76	13.4 ± 0.9	100
Liver	24.3 ± 1.2	100	28.2 ± 1.2	100	23.1 ± 0.4	100
Spleen	4.1 ± 1.2	01	2.9 ± 0.3	19	2.5 ± 0.1	80
Adrenal	49.8 ± 19.9	02	34.5 ± 7.2	07	7.1 ± 1.2	21
Brain	3.3 ± 0.4	01	3.9 ± 0.4	12	2.5 ± 0.3	06
Blood	1.75 ± 0.3	31	2.3 ± 0.4	31	2.5 ± 0.4	51
Thymus	11.9 ± 4.3	16	28.6 ± 8.7	11	6.2 ± 1.1	25
Heart	5.9 ± 0.4	07	5.7 ± 0.8	03	5.9 ± 0.3	50
Serum	1.1 ± 0.1	90	1.6 ± 0.2	60	1.8 ± 0.1	100
Capsule	–	–	–	–	1.6 ± 0.2	100
Sponge	–	–	–	–	2.5 ± 0.3	47

[a] The control group consisted of normal, non-inflamed animals, dosed once, subcutaneously with ^{64}Cu-D-penicillamine at 12.5 mg/kg.
[*] A measure of exchangeability of endogenous copper with applied ^{64}Cu.

carrageenan inflammation is short term (4–6 hours) and mostly oedemic. The sponge granuloma model [17] was chosen as an alternative inflammatory stress because of its substantially longer duration (6 days) and proliferative character. Tables 2 and 3 provide comparative data on both total and radioactive copper per gram of wet weight tissue or body fluid in both these inflammatory models.

With ^{64}Cu-salicylate. (i) In carrageenan inflamed animals: while no change in copper concentration was observed in kidney tissue, there was a slight decrease in the % relative specific activity of ^{64}Cu (% R.S.A.) (i.e. % exchangeable copper) compared to non-inflamed animals (Table 2). This may be due to reduction of exchangeable copper by an increase in non-exchangeable binding sites. Copper concentrations and % R.S.A. in liver, spleen, adrenals, thymus, brain and heart were the same in both non-inflamed and inflamed

groups. Serum copper concentrations were similar in both groups, with a marginal increase in the high % R.S.A. in the inflamed group.

(ii) In the sponge inflamed animals: when the levels of copper in the kidneys of animals with granulomatous inflammation were compared to those in the kidneys of either the carrageenan-inflamed or non-inflamed groups an increase in % R.S.A. but a decrease in the copper content was found. This increased specific (radio)activity would suggest that copper was being exchanged, then mobilized (or excreted) from the kidney. This was in contrast to the carrageenan-treated group, where a reduced % R.S.A. was seen. Liver concentrations of copper were very similar between the two inflamed groups and normal animals. The high % R.S.A., however, would indicate a greater turnover of ^{64}Cu in this organ. In all other tissues, with the exception of serum, the % R.S.A. rose in the granuloma group compared to the carrageenan and non-inflamed groups. However, the actual copper content decreased or remained constant, indicating a higher turnover than in the non-inflamed group. Conversely, compared to either the carrageenan or the non-inflamed groups, the % R.S.A. in the serum of the sponge granuloma group was less, while the copper concentration actually rose, indicating a loss of exchangeable copper from the serum.

The sponges had been implanted in the animals 5 days before administering the ^{64}Cu-salicylate. The copper concentrations in the sponges were greater than that of the surrounding connective tissue capsule or the serum. However, the % R.S.A. was markedly greater for the capsules than for the sponges.

With ^{64}Cu-D-penicillamine. (i) In the carrageenan inflamed animals: there was no apparent difference in copper concentrations or % R.S.A. in kidney, liver, adrenals, thymus and heart tissue between either the non-inflamed or inflamed groups (Table 3). Although there was no difference in copper content of brain or spleen tissue, the two organs contained more ^{64}Cu in the inflamed group than in the non-inflamed group. In the inflamed group, serum showed an increase in copper content with an overall decrease in the % R.S.A. compared to the non-inflamed group.

(ii) In sponge inflamed animals: while the renal copper concentration decreased in the sponge group compared to both non-inflamed and carrageenan groups, the % R.S.A. rose (Table 3). A similar trend was also seen for adrenals and thymus tissue. Spleen copper concentrations were not significantly different in the three groups; however, the % R.S.A. rapidly rose to a level well above that of the carrageenan group. Copper levels in liver tissue were the same in both inflamed groups and the controls, with 100% R.S.A. indicating a possible saturation of liver binding sites. It would appear that the brain is not a target organ for this copper formulation, as both the copper concentration and the % R.S.A. remained very low for all groups of animals.

Serum copper levels were lowest in the non-inflamed animals, raised in the carrageenan group and highest in the sponge granuloma group. The percentage exchangeable copper was very high in the serum of both non-inflamed and granuloma groups but much lower in the carrageenan group. The copper concentrations in the sponge implants were greater than those of the surrounding tissue capsule. These results are almost identical with those obtained using copper salicylate (see Table 2).

Discussion

The results of the carrageenan study show that copper was absorbed from both routes of administration and then distributed systemically. The ^{64}Cu in the D-penicillamine complex rapidly circulated through the animal. However, after 24 hours, the tissue ^{64}Cu levels attained stable levels. In contrast ^{64}Cu-salicylate was slowly released from its site of administration but again the tissue ^{64}Cu levels were stable after 24 hours.

Carrageenan inflammation caused little change in the biodistribution of ^{64}Cu in animals treated with either topical ^{64}Cu-salicylate or subcutaneous ^{64}Cu-D-penicillamine, except for minor changes in blood thymus and adrenals. The ^{64}Cu level in the adrenals following ^{64}Cu-salicylate treatment may be attributed in part to the ^{64}Cu serum levels attained; however, this does not explain the total ^{64}Cu levels found in this tissue and may then imply a selective absorption of copper by the adrenals, not necessarily related to anti-inflammatory activity. The anti-inflammatory efficacy of copper salicylate has been shown to be independent of adrenal function [9]. Alternatively, the observed level of ^{64}Cu may be explained by the adrenals possessing a high concentration of exchangeable copper, or a higher affinity for copper. One difficulty in computing changes in radioactivity of the adrenals per standard mass was the variable weights of these organs, ranging

from 20 to 80 mg (2 adrenals). When the radio-activity is converted to counts per gram of wet tissue, small variations in weight can then make a substantial difference to the final value (c.p.m./g). A small difference in the blood/serum levels of ^{64}Cu between carrageenan inflamed and non-inflamed animals was observed after administering ^{64}Cu-salicylate in a slow release formulation. However, this was not the case with the parenteral ^{64}Cu-D-penicillamine. Probably this is because most of the copper in the copper-D-penicillamine complex is systemically translocated while remaining within this complex, while the Cu(II) derived from the copper-salicylate is mainly transported in the circulation as complexes with albumin, peptide or aminoacids.

These results show that the sponge granuloma model of inflammation, can substantially change the distribution of endogenous copper. It is therefore possible to propose models of dynamic change in copper biodistribution as a result of inflammatory stress, rather than simply considering total copper or labelled levels alone. Three types of change in copper status could be distinguished.

Type I. No change in copper concentration but an increase in the % R.S.A. This would suggest that the bio-exchangeability of the tissue-associated copper increases as a result of inflammation. Another possible inference is that the tissue-association copper is mobilized as a result of the inflammation and replaced by exogenous copper from the drugs. An example of this is provided by the liver tissue from copper salicylate-treated animals with the sponge granulomae.

Type II. A decrease in actual concentration of copper with a concomitant increase in the % R.S.A. This could occur when the rate of mobilization from the tissue exceeds the rate of repletion by exogenous copper following inflammatory stress. An example of this mode of copper redistribution is provided by the adrenals from copper-treated animals with sponge granulomae.

Type III. No change in copper concentration or the % R.S.A. This may mean that either the organ is not a target tissue for exogenous copper or that the endogenous copper is not readily exchanged during the inflammatory stress. Alternatively, the organ has been saturated with labelled copper such as the liver tissue in all three experimental groups treated with ^{64}Cu-D-penicillamine.

The longer-term more chronic, inflammation associated with the sponge granuloma shows more Type I and Type II changes in organs such as kidney, liver, spleen, adrenals, thymus and serum. In contrast, the short-term acute inflammatory stress of the carrageenan paw oedema, after 24 hours, gave mostly Type III changes. Thus significant differences in biodistribution patterns may only be triggered by long standing inflammatory stress such as accompanies the implantation of irritant impregnated sponges (to elicit a granuloma).

Both ^{64}Cu salicylate and ^{64}Cu-D-penicillamine produced similar changes in biodistribution patterns of ^{64}Cu in kidney, spleen, adrenals and thymus tissue. Exceptions to this pattern of duplication were seen in other tissues, however, notably the liver, brain, serum and heart. The high liver concentration of copper and high % R.S.A. in the serum may reflect saturation of their copper-binding sites by ^{64}Cu-D-penicillamine, following the subcutaneous injection of this highly-charged water-soluble copper(I)–copper(II) complex which then moves as a single bolus through the animal. Conversely, ^{64}Cu-salicylate being a lipophilic compound administered percutaneously, provides a slow release formulation consistent with the lower % R.S.A. in most tissues.

With both ^{64}Cu-salicylate and ^{64}Cu-D-penicillamine, the concentration of copper in the connective tissue capsule surrounding the sponge was similar to that of the serum, reflecting a possible equilibrium. However, the very high % R.S.A. of the capsule (100%) for both ^{64}Cu-salicylate and ^{64}Cu-D-penicillamine indicated that all of the copper in this tissue was probably due to copper from the drugs alone. These results suggest that copper moved to inflammatory sites and furthermore that the copper *in situ* was readily exchangeable. This was particularly apparent in the ^{64}Cu-salicylate treated animals where there was a comparatively low level of ^{64}Cu in the serum, probably indicating targeting of this drug to inflammatory sites.

The copper concentrations in the sponges from animals treated with both ^{64}Cu-salicylate and ^{64}Cu-D-penicillamine were higher than in the corresponding samples of blood or granulomae capsules. With the relatively high % R.S.A. (47%) found in these sponges these results would indicate that exogenous ^{64}Cu from both copper formulations was sequestered at inflammatory sites. This may partly explain how applied copper complexes elicit their anti-inflammatory effect when admin-

istered parenterally [6, 7] or percutaneously [8–10].

Related studies on the biodistribution of ^{64}Cu in rats with a systemic inflammatory disease (adjuvant induced polyarthritis) will be the subject of another report.

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