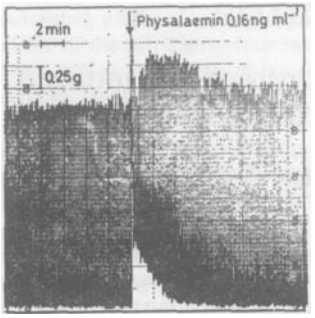

FIG. 2. The effect of physalaemin on guinea-pig ileum contractions to coaxial stimulation.

physalaemin. However, at these higher concentrations physalaemin caused marked contraction of the muscle by itself which masked the normal responses to electrical stimulation and drugs. At 1·25 ng ml^{-1} ($\simeq$ 0·9 10^{-9} M) of physalaemin, reversible increases were still observed for contractions to acetylcholine (14·6%) and nicotine (47·5%) but not to 5-HT.

Contractions to histamine were not potentiated by physalaemin at any of the concentrations tested (Fig. 1).

These observations show that physalaemin behaves like substance P and sensitizes ileal smooth muscle to cholinomimetics. The small increase in the size of the contractions produced by 5-HT was at variance with the depression of contractions of that agonist described by Beleslin & Varagić (1960) with crude substance P. Thus whether the mechanism of this sensitization observed with physalaemin is similar to that described for substance P remains to be determined.

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Evaluation of copper complexes as potential anti-arthritic drugs

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Recently copper aspirinate and copper-salicylate were compared with aspirin and several salicylates to evaluate the potential use of copper complexes as oral anti-inflammatory drugs (Rainsford & Whitehouse, (1976). Because it was felt that reported anti-inflammatory activity of a variety of Cu complexes (Sorenson, 1974) was at least partially due to irritation following subcutaneous administration, the anti-inflammatory and irritant activities of Cu aspirinate and Cu salicylate were determined after subcutaneous or oral administration. Results of those studies were interpreted as indicating that orally administered Cu salicylates may be no more effective than aspirin or salicylate and that irritation accounted for increased activity following subcutaneous dosing. However, I wish to caution against these interpretations in the light of the following considerations.

Anti-inflammatory studies. The use of the oral route and use, as a suspending agent, of acacia, which is acidic and also forms complexes with metals (The Merck Index), leaves open the possibility of partial or total Cu complex destruction before absorption and peripherial distribution. It was anticipated that gastric acid alone would cause at least partial destruction of these complexes so that the subcutaneous route was favoured (Sorenson, 1974). Acacia may have additionally prevented complex absorption by forming a quaternary complex or removing Cu from the complex, bringing about a release of the original complexing agent, i.e. aspirin or salicylic acid. If the existence of as much as possible of the free and intact complex in the dosage form is not assured it may not be possible to optimize the biological activity and adequately evaluate the hypothesis that Cu complexes are more active than the

parent complexing agent. To circumvent this potential interference I used a non-ionic surfactant, Tween 80 (Sorenson, 1974).

With the use of acacia and oral administration, Cu aspirinate still produced a greater reduction in both the urate and carrageenan foot oedemas than aspirin, the preparation of Cu salicylate, salicylic acid, Na salicylate and the preparation of Zn salicylate (see *oral* data in Table 1 of Rainsford & Whitehouse, 1976), but these anti-inflammatory activities might have been greater had acacia not been used. In a similar study Williams, Walz & Foye (1976) also found that Cu aspirinate and aspirin had about the same activity in the carrageenan model of inflammation but Cu aspirinate was about twice as active as aspirin in a model of adjuvant-induced inflammation when tragacanth, another acidic anionic suspending agent (The Merck Index), was used along with oral administration.

When these Cu complexes were given subcutaneously they were active at 1/5 to 1/6 of the oral doses, and Cu aspirinate, given at a smaller dose* than the others, was again the most active (see *subcutaneous* data Table 1 of Rainsford & Whitehouse, 1976). The use of acacia may also account for a need to use much larger amounts (42 mg kg^{-1}) of Cu aspirinate to observe activity compared to the original report of activity at 1 mg rat (8 mg kg^{-1}) using Tween 80 (Sorenson, 1974).

Gastric mucosal and parenteral irritancy studies. Rainsford & Whitehouse (1976) also evaluated gastric irritation of solutions which originally contained neutral copper complexes after acidification (final concentration was 20 mM HCl) to prevent gastric emptying. Based upon this procedure it is assumed that other solutions studied were also acidic. Since HCl converts these complexes to the free complexing agent and the metal chloride salt, no gastric irritation observed in these experiments can be exclusively attributed to a metal complex. It is well known that salicylic acid, acetylsalicylic acid and metal chloride salts cause gastric irritation but this has no bearing on the question of gastric irritation by the original metal complex. It would seem that this experimental design is best not used to evaluate the compounds of interest. In an experiment where suspensions were not first made acidic, aspirin produced a 50% or greater incidence of gastrointestinal erosions at doses of 100 to 300 mg kg^{-1} while Cu aspirinate caused no erosions in doses up to and including 1380 mg kg^{-1} (Williams & others, 1976).

Since increased potency following subcutaneous administration was attributed by Rainsford & Whitehouse (1976) in part to irritation, they attempted to assess the parenteral irritancy of copper complexes. Although it is unclear whether solutions tested contained 20 mM Cu (II), as stated, or 20 mM HCl, a final pH of 6 was given for them. The significance of these results are also questioned on grounds that irritation obtained on injection of acidic solutions of what were originally 'neutral Cu solutions' does not relate to the question of irritancy of the neutral Cu compounds in the original solutions.

Rainsford & Whitehouse also reported that several other Cu preparations including $CuCl_2$ (a potent gastric irritant), which were irritant parenterally but not when given orally, had anti-oedemic activity when given subcutaneously but were virtually inactive when given orally. These observations were interpreted to support the suggestion of Bonta (1969) that the anti-oedemic activity of Cu-containing compounds was due to irritation at the injection site. However, Bonta (1969) had no argument to rule out the possibility that although the Cu-containing substances caused irritation at the site of oral or subcutaneous absorption, after being absorbed they could act by virtue of their own physiological anti-inflammatory effect. This is consistent with Bonta's (1976) more recent statement concerning his certainty that Cu plays an important role as an anti-arthritic agent.

Additional data have been provided which do not support the suggestion of a toxic-irritant-induced mechanism of action to account for the anti-inflammatory activities of Cu complexes (Sorenson, 1976). Copper acetate, which caused marked irritation even on subcutaneous injection of the lowest active dose, was only active in the carrageenan foot oedema model of inflammation while a number of Cu complexes were also active in the cotton wad granuloma and adjuvant arthritis models of inflammation after subcutaneous administration of non-irritating doses. Adrenalectomy (cotton wad granuloma model) did not prevent anti-inflammatory activity. Intravenous administration did not eliminate the anti-inflammatory activity. Irritant-induced behavioural effects including central nervous system stimulation or depression was not observed after subcutaneous injection of large doses of Cu complexes.

Anti-ulcer studies. Since potent anti-ulcer activity has been observed for suspensions of copper complexes which were not first made acidic (Sorenson, 1976), it is suggested that the anti-ulcer activities observed by Rainsford & Whitehouse (1976) might have been greater had they not added hydrochloric acid which may have caused some chemical decomposition of their complexes.

In support of the clinical use of Cu complexes as anti-arthritic drugs it is known that serum concentrations of a Cu-containing oxidase, caeruloplasmin, as well as albumin and amino acid Cu complexes, increase as a primary response to irritaiton, inflammation or infection

* 100 μmol of Cu $(aspirin)_2$ was given subcutaneously by Rainsford & Whitehouse (1976). This was calculated to be 42 mg kg^{-1} of copper aspirinate. Since copper aspirinate is actually Cu_2 $(aspirinate)_4$ (Manojlovic-Muir, 1967; Williams & others 1976) and has a molecular weight of 844 daltons and not 422 daltons, which would be the molecular weight of Cu $(aspirinate)_2$, only 50 μmol of copper aspirinate was actually used.

(Wintrobe, Cartwright & Gubler, 1953; Underwood, 1971). It is now suggested that these play a role in mediating normal physiological anti-inflammatory responses which bring about tissue repair and remission. In support of this suggestion many Cu complexes have been shown to be active anti-inflammatory agents in animal models of inflammation. It has also been suggested that clinically used anti-inflammatory agents bring about remission as a result of Cu complex formation *in vivo* (Sorenson, 1976).

This is supported by the clinical effectiveness of a preparation of Cu-salicylate (Permalon, Albert Chemical Co., Wiesbaden) which was used for 21 years, from 1950 to 1971, in therapy of patients with a variety of rheumatic and degenerative diseases (Hangarter & Lubke; 1952; Hangarter 1966, 1974), Treatment was accomplished by intravenous administration of Permalon without pathological changes, abnormal reactions or gastrointestinal disturbances. These results seem revelant to the potential 'copper' toxicity associated with the clinical use of copper aspirinate or any other copper complex.

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