The Binding of Aurothiomalate to Serum Proteins in vitro

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Gold compounds, especially sodium aurothiomalate ('Myocrisin'), are used extensively in the treatment of rheumatoid arthritis, and idiosyncratic side effects, notably bone marrow depression sometimes occur. Although the changes in the concentration in the plasma during therapy have been studied extensively, little information is available on the qualitative and quantitative aspects of its binding to serum macromolecules. A large proportion of the information that is available on the interaction of gold and serum proteins is contradictory, probably owing to the variety of forms in which the gold has been
administered. For example previous studies have claimed that serum gold is bound mainly to fibrinogen, α'-globulin (Lawrence, 1961), β-lipoprotein and 7S-γ globulin (Coke, 1963).

Studying the distribution in rabbit sera after injection of sodium aurothiomalate in vivo, McQueen & Dykes (1969) showed that the gold was mainly bound to albumin. Mascarenhas et al. (1972) found that when human serum was incubated with gold chloride in vitro, 95% of the gold was bound to albumin.

The present study investigated the ability of human, rat and foetal calf serum to bind gold, when incubated with varying concentrations of aurothiomalate in vitro.

Samples (4.5 ml) of foetal calf serum (Flow Laboratories Ltd., Irvine, Ayrshire, U.K.), normal human serum or rat serum (Sprague-Dawley, male) were incubated at room temperature for 1 h or longer with 0.5 ml of an aqueous solution of sodium aurothiomalate (May and Baker Ltd., Dagenham, Essex, U.K.) containing a small proportion of drug labelled with $^{195}$Au (The Radiochemical Centre, Amersham, Bucks., U.K.). Samples

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Fig. 2. Effect of concentration of aurothiomalate on the percentage of free gold

Details are as in Fig. 1.
(4.0 ml) of the mixtures were applied to a column (2.5 or 2.6 cm × 82–98 cm) of Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) and eluted with 0.01 M-Tris-0.002 M-citrate buffer, pH 8.6, containing 0.55 M-NaCl and 0.005 % (w/v) NaNO₃.

The eluate was monitored for absorbance at 280 nm and assayed for γ-emission from ¹⁹⁵Au. On the basis of the absorbance profile the radioactivity was quantified with respect to being bound to albumin, bound to non-albumin protein or 'essentially free'. The latter peak corresponds to Ψ, (total column volume) on Sephadex G-200 and experiments with Sephadex G-25 indicate that, if it is bound at all, the molecular weight of the complex would be less than 1000.

The effect of varying the concentration of the drug on the proportion of drug bound to albumin and 'free drug' after 24 h preincubation can be seen in Figs. 1 and 2 respectively. At low concentrations most of the drug was bound to albumin for all the sera, a finding that was confirmed by polyacrylamide-gel electrophoresis. The proportion of drug that was 'free' was a function of the dose and duration of preincubation. However, the binding capacity of the sera showed marked species differences. For example rat sera showed the greatest ability to bind the drug, whereas foetal-calf serum showed the least tendency (Fig. 2). It should also be noted that the binding capacity varied between different human volunteers.

For the various human sera, the different extents of binding can be mainly attributed to different concentrations of albumin (Fig. 1). On the other hand, the gold-binding capacity/unit of albumin of rat and foetal-calf sera is somewhat greater than that for human sera (Fig. 1). A further species difference exists in the fact that the degree of non-albumin binding in human and foetal calf sera is greater than in rat sera. Although it has been generally considered that the interaction of aurothiomalate and serum proteins involves thiol groups (Gerber, 1964), in the present studies the binding of aurothiomalate (20 μM) to human sera was not altered by cysteine (100 μM), 2-mercaptoethanol (100 μM) or p-hydroxymercuribenzoate (1.2 mM).

Previous workers have been singularly unsuccessful in relating serum concentrations of gold to toxicity or therapeutic value of aurothiomalate. It is possible that the relative acute binding capacity of the serum, i.e. the amount of 'free' drug at any given dose, may be a better indication of the possible benefit or complications of chrysotherapy. This hypothesis has yet to be verified.


The Effect of Cephaloridine on Enzyme Excretion into the Urine

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The value of serum enzyme measurements in medical diagnosis is now well established (Wilkinson, 1962) and in many cases the tissue or tissues injured can be detected from serum enzyme and isoenzyme determinations. In some kidney diseases, the activity of enzymes excreted into the urine is increased although enzyme measurements in the urine appear to be of more limited diagnostic value than those in the serum (Raab, 1972). Enzymes are also present in the urine of animals and their excretion may be increased when the kidneys are damaged by toxic materials (Raab, 1968; Robinson et al., 1967; Ellis et al., 1973). Previous work in our laboratory has also shown that quite marked elevations of the activities of some enzymes in the urine occur when known and powerful nephrotoxins are given to rats (Wright & Plummer, 1974). In the present communication we report the effect on enzyme excretion into the urine of administering the antibiotic