(4.0ml) of the mixtures were applied to a column (2.5 or 2.6cm x 82–98cm) 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) NaN₃.

The eluate was monitored for absorbance at 280nm and assayed for γ-emission from ⁹⁹ᵐAu. 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 Vₑ (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 24h 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 μM).

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...
Fig. 1. *Excretion of enzymes into rat urine after injection of cephaloridine (2g/kg)*

(- - - -) represents the upper limit of normal enzyme activity. *(a)* Alkaline phosphatase activity; *(b)* lactate dehydrogenase activity; *(c)* acid phosphatase activity; *(d)* glutamate dehydrogenase activity.

*Materials and methods*

Cephaloridine (400mg) was given as a single subcutaneous injection to eight male Wistar rats of 200g weight. Urine samples free of faecal contamination were then collected from these rats housed in perspex restraint cages for 12h, and the urine was prepared for enzyme assay (Leathwood & Plummer, 1969). Serum was collected after puncturing the jugular vein of rats anaesthetized with ether. The animals were then killed while still under the anaesthetic.

The activities of the enzymes, lactate dehydrogenase, glutamate dehydrogenase, acid phosphatase and alkaline phosphatase in urine and serum were then determined under...
Table 1. *Serum enzyme activities 42h after injection of cephaloridine*

Details are given in the text. The values are means of eight determinations ± s.d.

<table>
<thead>
<tr>
<th>Serum enzyme activity (μmol/min per ml)</th>
<th>No. of rats</th>
<th>Lactate dehydrogenase</th>
<th>Acid phosphatase</th>
<th>Alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>894 ± 218</td>
<td>15.8 ± 6.4</td>
<td>173 ± 39</td>
</tr>
<tr>
<td>Injected rats</td>
<td>6</td>
<td>811 ± 328</td>
<td>16.5 ± 8.5</td>
<td>72 ± 24*</td>
</tr>
</tbody>
</table>

* Highly significant decrease in activity, *P* < 0.001.

Conditions found to be optimal for rat urine (Leathwood & Plummer, 1969). The output of enzymes in the urine was expressed as nmol/min excreted per h and the average excretion recorded graphically. The upper limit of normal was taken to be the average excretion rate of 100 normal rats plus two standard deviations (Wright & Plummer, 1974). Experimental values greater than these were considered abnormal and indicative of kidney damage.

Isoenzymes of acid and alkaline phosphatase were separated on polyacrylamide-gel electrophoresis and detected as previously described (Wright et al., 1972a,b).

**Results and discussion**

Urine was collected from one group of rats at 0–12 h, 24–36 h and 48–60 h after injection and from another group at 12–24 h, 36–48 h and 60–72 h postinjection. The results of the enzyme measurements on these samples are combined in Fig. 1. The excretion of lactate dehydrogenase was abnormal in all urines except the first with a peak output at 24–36 h. Alkaline phosphatase excretion was normal except at 36–48 h after administration of the drug. Glutamate dehydrogenase excretion was also marginally raised immediately after injection but had returned to normal by 60 h. Acid phosphatase remained below the upper limit of normal throughout the experiment and actually appeared to be depressed in the 24 h immediately after injection. The sequential release of lactate dehydrogenase before alkaline phosphatase suggests a permeability change which precedes a structural change in the membrane at which time the alkaline phosphatase is also released. The increased glutamate dehydrogenase in the urine marks the involvement of mitochondria and Silverblatt et al. (1970) have noted mitochondrial swelling in rabbit kidney cells after cephaloridine injection, together with changes in the structure of the cell membrane. The normal or low acid phosphatase activity suggests that lysosomal injury is not the primary cause of the kidney lesion and that kidney lysosomes remain intact up to at least 3 days postinjection.

The enzymes in the urine after injection of cephaloridine do not appear to arise from the blood since none of the serum enzyme activities were raised (Table 1). Serum lactate dehydrogenase and acid phosphatase were normal and the alkaline phosphatase was significantly depressed after cephaloridine administration. The reason for the decreased enzyme activity in the serum is not an increased filtration through the glomerulus since the electrophoretic patterns of alkaline phosphatase in the 36–48 h urine samples was the same as that from normal rat urine and quite unlike the serum isoenzymes (Wright et al., 1972a).

In the case of cephaloridine, measurements of enzymes in the urine provide a useful index of kidney damage and are more valuable in this respect than those in the serum.

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Adult Brain Nerve-Ending Content and Acetylcholinesterase Activity in Rats Growth Retarded for Different Periods in Early Life

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An important question in developmental paediatrics concerns the neurological and intellectual outcome of children who were growth retarded in utero and consequently 'small-for-dates' at birth. A rat model for the small-for-dates baby has been proposed (Adlard et al., 1973) in which increasingly severe nutritional growth retardation is applied from conception to 5 days of postnatal age when the rat brain has reached a stage of growth and development comparable with the human brain at term (Dobbing & Sands, 1973).

Functional abnormalities in the growth-retarded brain might be reflected in altered synaptic interconnectivity (Cragg, 1972). A possible approach to this problem may be the quantification of brain nerve-ending particles (synaptosomes). Since nerve-ending diameter is not altered by growth retardation (Cragg, 1972; Gambetti et al., 1972) brain synaptosomal protein content may reflect total numbers of synaptic connections.

Undernutrition during the major period of rat brain growth results, after rehabilitation, in a whole brain acetylcholinesterase activity (per unit weight) higher than in controls (Adlard & Dobbing, 1971b; Im et al., 1971). It is of interest to determine whether this phenomenon occurs (a) after growth retardation to 5 days only and (b) in regions of widely differing acetylcholinesterase activity (striatum and cerebellum).

Rats were of a black and white hooded strain. Undernourished maternal rats were fed an approx. 50% ad libitum diet from conception to 25 days post partum as previously described (Adlard et al., 1973). At birth litters were decreased to 8 animals (5 males, 3 females). When 5 days of age whole litters were fostered between mothers in one of three ways: (i) between control, well-nourished mothers (control offspring); (ii) from a control to an underfed mother (infant and weanling restriction: IWR offspring); and (iii) from an underfed to a control mother (foetal and neonatal restriction: FNR offspring). Rats were weaned when 25 days old. Females only were used in the present study. After weaning, control and FNR young were fed ad libitum, but IWR young received a restricted quantity of food (3.5–4.5 g/day) until 42 days, after which they were also fed ad libitum.

At 12 weeks of age rats were killed and the brains dissected into cerebellum, striatum and residual brain (brainstem, midbrain and cerebral hemispheres excluding the striatum). Residual brain was homogenized in 9 vol. of 0.32M-sucrose–1 mM-EDTA (pH 7.4) and used for preparation of a synaptosomal fraction (Gray & Whittaker, 1962). Synaptosomal protein was determined (Lowry et al., 1951) with bovine serum albumin as