in a subsequent and extensive study of rabbits concluded that the deposits, from 3 days to 18 months after administration of the gold-containing drug, have a constant composition with Au:S:P ratios of 1:1:3 (Ghadially et al., 1978). A recent extended X-ray absorption fine structure (EXAFS) study of isolated auosomes from the rat kidney after both chronic and acute administration of gold has determined that gold is bound to sulphur in the ratio of 1:1.8 (Elder et al., 1983).

We now report on the morphology and analysis of gold deposits in human synovial membranes removed from patients at the time of joint replacement. The patients had received therapy with Myocrisin (gold thiomalate) for rheumatoid arthritis for varying periods up to 18 months before the operation. Tissues for analysis were immediately fixed in 2% (v/v) glutaraldehyde buffered with 0.1M cacodylate at pH 7.3, dehydrated through graded alcohols and embedded in Epon. Thin sections of unstained material or of material stained with uranyl acetate and lead citrate were examined in a Philips 400 electron microscope at 80 kV. Analysis of specimens tilted at 30° was with a Microtrace Si(Li) energy-dispersive detector and spectra were processed using Link systems 860 Computer and ‘Quantem/FLS’ program. Spectra of gold metal, Myocrisin (Au:S = 1) and Auranofin (Au:S:P = 1:1:1) were recorded and used as standards.

These studies show that the gold deposits are not homogeneous in either morphology or composition. In the synovial membrane the gold is present in synovial-intimal cells and in macrophages. It is found in both electron-lucent and electron-dense lysosomes. Gold is present in both fine particles (c. 3 nm) on filamentous structures within the lysosomes or as larger particles (c. 30 nm) which are often grouped in clusters. The region of the spectrum which has the PKA, PKM and S np for the tissue deposits and for the standards is shown in Fig. 1. There was no indication that the gold in synovial-membrane cells was associated with phosphorus. The amount of sulphur associated with the gold was variable and in some deposits none was detected. Somewhat more sulphur appeared to be associated with the smaller particles than the larger. All deposits were amorphous to electron diffraction.

Gold deposits within the lysosomes of synovial-membrane cells do not, therefore, appear to possess a constant Au:S:P ratio like the deposits described in rabbits. This is of some interest as it has been suggested that gold-containing anti-rheumatic drugs may function by inactivating lysosomal hydrolases (Menninger & Burkhardt, 1983). Formation of gold-thiol complexes would seem a likely way in which gold could modify the structure of a protein and may explain the formation of the antibodies to gold-thiol compounds found in some human patients (Bretza et al., 1983). The results which we present in this communication show that the gold which accumulates within lysosomes is particulate and not always complexed to the most likely ligands phosphorus and sulphur, but is probably also present as metallic gold. It would seem likely that deposition in this form in lysosomes reflects the fate of inactivated drug.

We thank Mr. P. J. Stiles for supplying us with specimens from human joints; Miss D. Chescoe and Mrs. J. Mullervy for assistance in electron microscopy. Financial support was provided by the Arthritis and Rheumatism Council.

Abbreviations used: T3, tri-iodothyronine; T4, thyroxine; PAS, periodate-orthamine/Schiff; TSH, thyroid stimulating hormone (thyrotropin).
dence of toxic damage but did show the classical symptoms of hyperactivity caused by, for example, elevated levels of TSH, antibodies to the TSH receptor or low dietary levels of iodine (Anderson, 1980). Accordingly we speculated that the reduction in serum T4 coupled with no change or even an increase in serum T3 was due to interference in the iodination of tyrosine residues in the thyroglobulin molecules. Measurement of the iodine concentration in the lysosomes, which are responsible for degradation of iodinated thyroglobulin molecules taken up from the colloid and the release of free thyroid hormone, showed, however, a slight increase in iodine concentration. Hence there is no evidence for interference of hypolipidaemic drugs in the formation of thyroid hormones and the alteration in serum levels which we observe would seem to be associated with changes in the peripheral metabolism and removal of circulating thyroid hormones.

The authors thank Mrs. J. Mullevy and Miss J. Howarth for their excellent preparation of specimens for electron and light microscopy respectively. Our thanks also to Professor P. Grasso, Professor J. W. Bridges and Professor John Faccini for useful discussion of the results.


Studies on the mechanism of action of a drug-carrier-antibody conjugate

MARTIN C. GARNETT, ELISABETH JACOBS, M. J. EMBLETON and ROBERT W. BALDWIN
Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD, U.K.

Chemotherapy has played a useful role in the treatment of neoplasms for a large number of years. However, the efficacy of the cytotoxic agents is limited by their lack of specificity between normal and neoplastic tissues resulting in debilitating and life-threatening side effects. If these drugs could be specifically directed to the correct cells, e.g. by using antibody/antigen or other specific interactions (Ghose & Blair, 1978), there are prospects for improved therapeutic efficiency. This could either be manifest as improved cancer management or reduced side effects.

We recently reported the synthesis and properties of a conjugate (Garnett et al., 1983) by using an anti-osteogenic sarcoma monoclonal antibody, 791T/36 (Embleton et al.,

![Fig. 1. Inhibition of toxicity of MTX-HSA-791T/36 conjugate by lysosomotropic and methotrexate transport inhibitors](image-url)

(a) Effect of 10mM-ammonium chloride (——) on the incorporation of selenomethionine by 791T cells treated with either methotrexate (●) or conjugated methotrexate (▲). (b) Effect of folic (——) or folinic (——) acids on the incorporation of selenomethionine by 788T cells treated with either 100ng of methotrexate/ml (●) or conjugated methotrexate (▲). Neither folic or folinic acid affected selenomethionine uptake by untreated cells.

Vol. 12