The method of Davies *et al.* (1969) was used to measure the stability and proteolytic activity of liver lysosomes from rats treated with suramin or aurothiomalate 24 hr. before receiving an intravenous injection of denatured ^{125}I -labelled albumin. Control rats received protein only. A liver fraction containing lysosomes was prepared, resuspended and incubated as described by Davies *et al.* (1969). By sampling the incubation mixture over a 2 hr. period, two parameters were measured, the percentage of the total radioactivity becoming trichloroacetic acid-soluble and the percentage of the total radioactivity remaining trichloroacetic acid-insoluble but becoming non-sedimentable. These values provide information on the rate of intra-lysosomal proteolysis and the extent of lysosome breakage or leakage.

Suramin and aurothiomalate both decreased the proteolytic activity of the lysosome suspensions. Suramin given in doses of 50, 100 and 250 mg./kg. body wt. decreased the production of acid-soluble radioactivity to 90, 49 and 17% of the control value. Aurothiomalate in doses of 25, 50 and 100 mg./kg. body wt. gave values 37, 32 and 31% of controls. Suramin had no effect on lysosome stability, but aurothiomalate at the two higher doses gave some increase in the albumin released from lysosomes during the incubation.

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Observations on [^{35}S]Sulphated Insulins

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Treatment of insulin with concentrated sulphuric acid at low temperatures results in the formation of sulphated insulin preparations in which the aliphatic and aromatic hydroxyl groups have been converted into acid sulphate esters (Reitz, Ferrel, Fraenkel-Conrat & Olcott, 1946). Such preparations show unimpaired hormonal activity *in vivo* as measured by blood sugar reactions (Glendening, Greenberg & Fraenkel-Conrat, 1947). Moloney, Aprile & Wilson (1964) prepared sulphated insulin

under these conditions and showed it to be less antigenic and less neutralizable by antibodies than unmodified insulin. This material was successfully used in the treatment of patients with insulin-resistant diabetes mellitus (Little & Arnott, 1966; Menczel, Levy & Bentwich, 1966).

In view of the relatively non-specific nature of the sulphation reaction it appeared of interest to examine the homogeneity of sulphated insulin by electrophoresis. Crystalline bovine insulin (a gift from Boots Pure Drug Co. Ltd., Nottingham) was sulphated by reaction with $\text{H}_2^{35}\text{SO}_4$ according to the method of Reitz *et al.* (1946), and the product was subjected to paper electrophoresis on Whatman 3MM paper in the presence of 33% (v/v) acetic acid, pH 1.7. Nine well-defined and approximately equidistant bands were observed on staining, eight of which were radioactive. The nine bands covered a range from +6 units (native bovine insulin) to -2 units (sulphated insulin containing eight sulphate groups/molecule). Sulphation of insulin by pyridinium sulphonic acid gave 13 such bands (Sluytermann & Kwestroo-van den Bosch, 1960).

Preparations of sulphated insulin molecules carrying the same number of sulphate groups were isolated by elution and freeze-drying after preparative paper electrophoresis. Testing of the biological activity of these preparations by the mouse-convulsion procedure showed that the introduction of one sulphate group/molecule gave no significant lowering in potency; introduction of further groups resulted in a lowering of up to 70% in potency. The immunological reactivity of the sulphated preparations with bovine insulin antiserum from guinea pigs was tested by the double-antibody method in competition with [^{125}I]iodinated insulin. Sulphation of insulin was found to decrease the reactivity of insulin with antibodies, the extent of the decrease depending on the number of sulphate groups introduced.

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