Reports

Selective Removal of Nonprotein Sulfhydryl Compounds from Biological Systems

Many important biological systems depend on sulfhydryl groups for their function. Maintenance of a balanced oxidation-reduction system is of great importance, and a reservoir of readily oxidizable thiols such as glutathione is frequently necessary to protect the sulfhydryl groups on various enzymes and other proteins. In the course of experimentation, however, it is often desirable to deplete this reservoir. The sulfhydryl groups of the reservoir may be inactivated by the use of iodosobenzoate, chloromercuribenzoate, or N-ethyl maleimide, but these agents have an affinity for the sulfhydryl of enzymes as well as for the sulfhydryl of glutathione. Hence, the selective inactivation of nonprotein sulfhydryl groups is often impossible. This problem was encountered during our studies on the relationship of glutathione to adrenal cortical function, and a technique was devised which may find wide application (*1*). Its basis is to render chloromercuribenzoate insoluble, so that it may be enclosed in a dialysis casing and used to trap the sulfhydryl compounds of low molecular weight which dialyze out of a solution or suspension of proteins.

It was found that sodium *p*-chloromercuribenzoate could be tightly bound by its carboxyl group to Dowex 2-X resin, 25–50 mesh, leaving the mercury end free. The resin was first converted to the chloride form, then placed into a small flask surmounted by a filter funnel. Into another flask was weighed a quantity of sodium *p*-chloromercuribenzoate (*p*CMB) crystals in 25 percent excess of the milliequivalents represented by the weight of resin. Water was added to the highly insoluble *p*CMB crystals, and the flask was shaken for a few minutes. After the undissolved crystals had been allowed to settle, the supernatant was poured through the funnel onto the resin, which was stirred continuously with a magnetic stirrer. After a minute or two, the aqueous supernatant was poured back into the flask containing the *p*CMB crystals. It is of greatest importance to avoid any contact between the resin and crystals. This procedure was repeated until the resin supernatant showed the presence of excess *p*CMB. This was determined by adding 0.5 ml of supernatant to 0.5 ml of a standard solution of glutathione (9.3 mg/100 ml) and measuring the resulting concentration of glutathione by amperometric titration (*2*). The process was continued until there was no further uptake of *p*CMB. Ninety to 92 percent of the theoretical amount of *p*CMB was eventually bound to the resin.

Stability studies with the *p*CMB-resin showed no change in sulfhydryl-combining power after the solution was shaken with 1.0*N* HCl or 1.0*N* NaOH. Storage in distilled water at 4°C for periods up to 60 days, storage for several days at room temperature, or drying out of the resin had no effect on its activity as long as it was kept in the dark. The presence of tetrasodium ethylenediamine tetraacetate in the test solution did not affect sulfhydryl-binding capacity.

A small dialysis bag containing 8 g of *p*CMB-resin was placed in a vial with 45 ml of a sulfosalicylic acid extract of rat liver, and the vial was sealed and shaken mechanically at about 30 oscillations per minute. Hourly amperometric titration of an aliquot of filtrate showed that 40 percent of the liver sulfhydryl was removed in 4 hours and that 93 percent was removed in 6 hours. In another experiment a homogenate of rat liver was prepared in saline. A portion of this homogenate was boiled briefly and filtered through paper. The dialysis bag was filled with 500 mg of *p*CMB-resin, and the residual air was displaced with boiled filtrate. The bag was then placed in a small screw-cap vial containing 1.0 ml of liver homogenate to which had been added 114,000 count/min of S^{35}-radioglutathione. A series of such vials was placed in a tubular incubator rotating on its long axis at 13 rev/min. At intervals, small aliquots of homogenate were removed and treated with 10-percent sulfosalicylic acid; the radioactivity of the extract was measured by liquid scintillation counting (*3*).

In several trials, an average of 26.5 percent of the radioactivity was removed in 30 minutes, 37 percent in 60 minutes, and 46.5 percent in 90 minutes. Longer dialysis times were not investigated. From these data it is evident that dialyzable sulfhydryl compounds can be selectively removed from tissue homogenates or other protein-containing media.

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References and Notes

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2. J. W. Goldzieher, W. B. Rawls, M. A. Goldzieher, *J. Biol. Chem.* 203, 519 (1953).
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Elution of Chromium-51 from Labeled Hemoglobins of Human Adult and Cord Blood

The erythrocyte of the newborn infant apparently has the same life-span as, or possibly one slightly shorter than, that of the adult, as determined by the method of differential agglutination (*1, 2*), but measurements with Cr^{51}-labeled red cells seem to indicate a much shorter life-span (*3*). It has been pointed out by Mollison (*2*) that this apparent discrepancy may

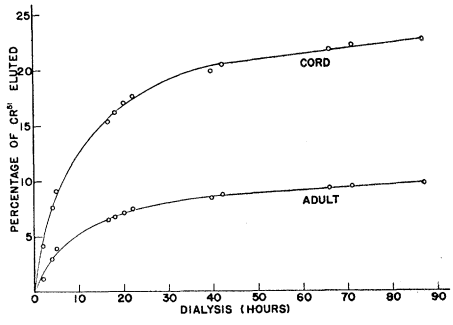

Fig. 1. Relative rates of elution of Cr^{51} from human adult and cord blood hemoglobin solutions upon dialysis against 0.9 percent NaCl. Initial Cr^{51} activity of the samples: $15.9 \times 10^6 \pm 0.2 \times 10^6$ counts per minute per gram of hemoglobin.

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