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.

PAIGE K. BESCH
JOSEPH W. GOLDZIEHER
SHIRLEY MCCORMACK
Department of Endocrinology, Southwest Foundation for Research and Education, San Antonio, Texas

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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

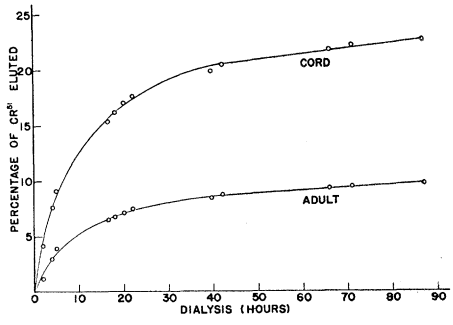

Fig. 1. Relative rates of elution of Cr^{51} from human adult and cord blood hemoglobin solutions upon dialysis against 0.9 percent NaCl. Initial Cr^{51} activity of the samples: $15.9 \times 10^6 \pm 0.2 \times 10^6$ counts per minute per gram of hemoglobin.

All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in Science **125**, 16 (4 Jan. 1957).

be due to a difference in the rates of elution of Cr^{51} from the cells.

Since the labeling of erythrocytes with Cr^{51} involves attachment of the metal to hemoglobin (*4*), and since the hemoglobins of normal adult red cells (mainly hemoglobin A, *5*) differ markedly from the predominating hemoglobin (hemoglobin F) in the red cells of the newborn (*6*), it was decided to determine whether a difference exists in the ability of the respective hemoglobin to retain the Cr^{51} label.

Erythrocytes, obtained from the blood of normal adults and from the umbilical cord of normal full-term infants, were washed with saline to remove plasma proteins, then resuspended in suitable volumes of saline to bring the hemoglobin concentrations to the same level (about 12 g percent). Equal volumes (5 to 15 ml) of the two types of washed cell suspensions were incubated with equal amounts of radioactive Cr^{51} (280 to 980 μc as $Na_2Cr^{51}O_4$) for about 2 hours at 37°C. The labeled cells were repeatedly centrifuged and washed, with one or two prolonged periods (3 to 24 hours) of contact between cells and saline, till the supernatants exhibited a very low degree of radioactivity, as measured in a well-type scintillation counter. After removal, by this means, of essentially all unbound Cr^{51} from the cells, the latter were hemolyzed by the addition of 1 vol of water and 0.4 vol of toluene. After centrifugation to remove stroma, the concentration and radioactivity of the clear hemoglobin solutions were determined. Suitable aliquots containing approximately equal amounts of the Cr^{51} label were subsequently dialyzed over periods of time ranging from 65 to 213 hours against 12 or more changes of saline, each of 100 ml. The radioactivity of the dispersion medium was finally measured, and appropriate correction was made for decay of the isotope.

Under strictly comparable experimental conditions, an unequivocal difference was evident in the rate at which Cr^{51} was eluted from the two types of hemoglobin solutions. In a typical experiment (Fig. 1), the percentage of the isotope eluted from cord blood hemoglobins after 87 hours of dialysis was 2.3 times that eluted from adult hemoglobin, whereas the amount of Cr^{51} bound per unit concentration of hemoglobin was approximately the same. In these experiments, the ratio of percentage of Cr^{51} eluted from cord hemoglobins to percentage of Cr^{51} eluted from adult hemoglobins ranged from 2.0 to 2.9. Variations in the absolute percentages of chromium eluted (ranging from 17 to 27 percent of the total for cord and from 5.9 to 13.5 percent for adult) did not affect the net results. Most of the elution, moreover, occurred during the first 24 to 37 hours of dialysis, confirming the work of Gray and Sterling (*4*).

Whether or not the hemoglobins show the same differentiation in the intact red cell is still uncertain, but the results reported here lend indirect support to Mollison's suggestion that much of the difference in survival rates of red cells of the adult and of the newborn, as determined with Cr^{51}, is only apparent. It is probable that molecular differences in these hemoglobins are responsible. This aspect and its implications for thalassemia (Hb F) and other hemoglobin diseases are receiving further attention (*7*).

H. J. SUDERMAN
F. D. WHITE
L. G. ISRAELS

Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Canada

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Reversal of Virus-Caused Stunting in Plants by Gibberellic Acid

Reports on the use of gibberellic acid in overcoming genetic (*1*) and physiologic (*2*) dwarfism suggested testing whether it could induce growth of plants stunted by certain virus diseases. Three leafhopper-borne viruses that cause severe stunting in susceptible plants were chosen for the experiment. Corn stunt virus of the Mesa Central strain (*3*) was inoculated into seedlings of hybrid sweet corn, *Zea mays,* of the variety Country Gentleman, by means of viruliferous *Dalbulus maidis.* The Eastern strain of aster yellows virus was introduced into seedlings of China asters, *Callistephus chinensis,* by the leafhopper *Macrosteles fascifrons.* Wound tumor virus was transferred to seedlings of crimson clover, *Trifolium incarnatum,* by *Agalliopsis novella.* Six weeks after inoculation, severely stunted plants were grouped according to size and sprayed with a freshly prepared water solution of gibberellic acid at 100 ppm (*4*). A control group of similar sized plants was sprayed with distilled water. Both groups were maintained on the same greenhouse bench.

Gibberellic acid was reapplied twice, at weekly intervals. The striking effect of the treatment is shown in Figs. 1–3. The photographs were taken 1 week after the last application. The treated plants had resumed growth, while diseased controls had remained stunted. The internodes of yellowed asters and stunted corn elongated to twice the original length, while the clover petioles increased 3 times in length and assumed an erect position during the 4-week period. In corn, the reversal of stunting became visible 48 hours after the first application, while in asters and clover it was noticed only after 5 days.

It is apparent that, under the conditions of the test, gibberellic acid influenced significantly the growth of virus-stunted plants. Although stunting could be overcome to a considerable degree by three applications of gibberellic acid, diseased plants retained other signs of virus infection. Leafhopper vectors were

Fig. 1. China asters infected with aster yellows: (left) untreated control; (right) treated with gibberellic acid.

Fig. 2. Hybrid sweet corn infected with corn stunt: (left) untreated control; (right) treated.

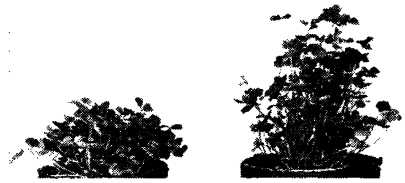

Fig. 3. Crimson clover with wound tumor: (left) untreated control; (right) treated. [Photos for Figs. 1–3 by J. A. Carlile]