These data clearly indicate that ethanol, both in vivo and in vitro, stimulates intestinal triglyceride synthesis from fatty acids and that this effect is associated with a marked increase in the activity of the microsomal enzyme system involved in fatty acid esterification. A similar but less striking influence of ethanol on fatty acid esterification in liver was previously reported from this laboratory (*14*). While the mechanism of this stimulation remains to be elucidated, it is important to note that these results were observed with ethanol concentrations comparable to those found in the intestinal lumen of social drinkers (*1*). The fact that pyrazole, an inhibitor of alcohol dehydrogenase, decreased the effect of ethanol on intestinal triglyceride synthesis, suggests that the mechanism of this stimulation is mediated, at least in part, by the metabolism of ethanol in the intestinal mucosa. Our results are consistent with the recent reports of Mistilis and Ockner (*9*, *10*), who observed that ethanol increases the triglyceride content of the intestinal mucosa and lymph. These workers suggested that lymph lipids may contribute to alcohol hyperlipemia and the alcohol-induced fatty liver.

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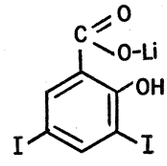
Glycoproteins: Isolation from Cell Membranes with Lithium Diiodosalicylate

Abstract. *A glycoprotein has been extracted in water-soluble form from human red cell membranes with lithium diiodosalicylate. After extraction of the membranes and phosphocellulose chromatography a homogeneous preparation is obtained which was 60 percent carbohydrate and 40 percent protein (by weight). The preparation contains AB and MN blood group antigens, receptors for influenza virus, and various phytohemagglutinins.*

Glycoproteins of cell membranes apparently have an important role in mediating interactions between cells (*1*). Although these complex molecules carry certain blood group antigens (*2*), transplantation antigens (*3*), and tumor-specific antigens (*4*), we nevertheless know little about their physical and chemical properties primarily because reliable methods for their isolation have not been available.

We now describe how lithium diiodosalicylate (LIS) can be used to



Lithium diiodosalicylate

extract glycoproteins in a biologically active and water-soluble form from red blood cell and other cell membranes. Studies in which LIS is used as a solubilizing agent were prompted by a report of Robinson and Jencks (*5*) which showed that LIS was an effective way to dissociate model peptide complexes.

Solutions of LIS (*6*) produce a striking effect on the gross structure of red cell ghosts, as shown by phase contrast microscopy. In 0.01 to 0.025*M* LIS the ghosts break up into small vesicles, and all membrane forms disappear when the concentration of LIS is raised to approximately 1.0*M*.

In addition to its ability to disrupt membranes, a property shared by many denaturing agents, LIS releases glycoproteins from the membrane fragments. Glycoproteins are extracted from membranes by LIS (0.1 to 0.3*M*), whereas much higher concentrations of guanidine or urea are ineffective (Fig. 1). The capacity of LIS to dissociate red cell membranes and release glycoproteins is greater than that of any salt so far tested. Probably LIS acts like a detergent, such as sodium dodecyl sulfate (SDS), but, unlike the latter, LIS can be removed after the extraction procedure is completed (*7*).

After glycoproteins are solubilized by LIS, several procedures can be used to purify the products. One convenient method for the isolation of the principal glycoprotein of human red blood cells is as follows:

Red cell membranes (*8*) were suspended in 0.3*M* LIS and 0.05*M* tris(hydroxmethyl)aminomethane (tris) hydrochloride, *p*H 7.5, at a concentration of approximately 25 mg of membrane protein per milliliter and stirred at room temperature for 15 minutes. Two volumes of distilled water were then added, and the turbid suspension was stirred for an additional 10 minutes at 4°C. After centrifugation at 45,000*g* for 90 minutes at 4°C, the supernatant, which contained most of the membrane proteins, was decanted and mixed with an equal volume of freshly prepared 50 percent

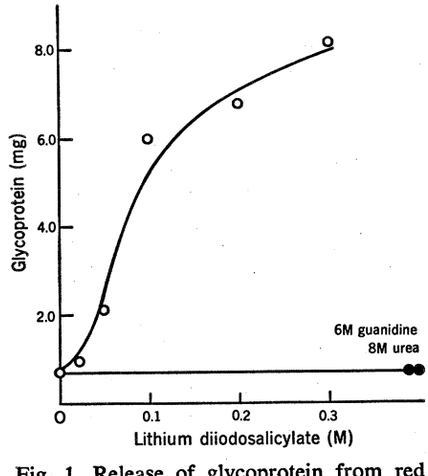


Fig. 1. Release of glycoprotein from red cell membranes. Portions of red blood cell ghost membranes were suspended in various concentrations of LIS–tris-hydrochloride buffer or in 8*M* urea or 6*M* guanidine-hydrochloride and mixed for 15 minutes at room temperature. An equal volume of cold phenol was added and the mixtures were centrifuged. Glycoprotein was isolated from the aqueous phases and quantified by dry weight measurements or by sialic acid analysis (*14*).

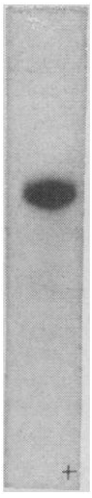


Fig. 2. Electrophoretic pattern of purified glycoprotein. Samples were treated with 1 percent sodium dodecyl sulfate and 0.01*M* phosphate buffer and subjected to electrophoresis on either 5 or 10 percent acrylamide gels in 0.1 percent SDS and 0.1*M* phosphate buffer, *p*H 7.0 (*15*). Duplicate gels stained with Coomassie blue for protein or periodic acid–Schiff reagent for carbohydrate show a single major component.

phenol in water. The mixture was stirred at 4°C for 15 minutes and centrifuged at 4000*g* for 1 hour at 4°C in a swinging bucket rotor. The centrifuged material separated into two phases; the upper (aqueous) phase contained most of the soluble glycoprotein. This was removed and dialyzed against several changes of distilled water at 4°C over a period of 24 to 36 hours. The dialyzed material, which sometimes was slightly turbid, was freeze-dried. The dry material was suspended in cold 100 percent ethanol and mixed for 1 to 2 hours in the cold and centrifuged to collect the precipitate. The ethanol washing procedure was repeated three times. The washed sediment was suspended in distilled water and dialyzed against water in the cold overnight. The material was then centrifuged at 10,000*g* for 30 minutes at 4°C; the clear supernatant contained the soluble glycoprotein.

The glycoprotein solution obtained by this procedure may contain a small amount of contaminating protein that is not removed during the phenol partitioning step. These contaminants can be removed by acidifying the solution with citric acid to *p*H 3.5 and then passing it through a phosphocellulose column equilibrated with 0.02*M* sodium citrate, *p*H 3.6. The glycoproteins do not bind to the cellulose under these conditions and emerge as a single peak.

Approximately 35 to 50 mg of glycoprotein can be extracted from 450 ml of human blood. This amounts to 3 to 4 percent of the original dry membranes and represents at least 70 to 80 percent of the total glycoprotein of the red cell membrane.

The glycoprotein prepared by this procedure appears to be a single molecular species, as judged by acrylamide gel electrophoresis (Fig. 2) in two different systems and by analysis of tryptic and cyanogen bromide peptides and COOH-terminal analysis (*9*). The monomeric unit has an apparent molecular weight of 55,000 (*10*), and the purified glycoprotein has A, B, and MN blood group activities and also carries the receptors for influenza viruses, phytohemagglutinin, and the wheat germ agglutinin (*11*). These activities were measured by hemagglutination-inhibition assays and by precipitin reactions with purified lectins (*12*).

These activities can also be demonstrated after the purified glycoprotein is extracted with a mixture of chloroform and methanol (2 : 1, at room temperature). This treatment removes contaminating lipids and eliminates the possibility that the blood group activities are due to glycolipids. The glycoprotein is still completely soluble in water after this treatment.

The above-described procedure can be used for the isolation of glycoproteins from other membranes. It has been used to extract proteins from lymphoid cells, platelets, liver cell membranes, and various human tumors, and there is evidence that it is effective for isolating tumor-specific antigens from human colonic tumors (*13*).

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7. LIS has a molar extinction coefficient of approximately 4×10^3 at 323 nm, and the presence of small amounts can be detected spectrophotometrically. The glycoprotein prepared according to this procedure has no detectable bound LIS.
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Diethyl Pyrocarbonate: Formation of Urethan in Treated Beverages

Abstract. *Isotope dilution analyses with tritium-labeled diethyl pyrocarbonate show that the carcinogen urethan is formed in orange juice, white wine, and beer. Commercial use of the antimicrobial food additive diethyl pyrocarbonate can result in urethan concentrations of 0.1 to 0.2 milligram per liter in orange juice and of the order of 1 milligram per liter in white wine and beer.*

Diethyl pyrocarbonate (DEP),

$$H_5C_2\text{–O–CO–O–CO–O–}C_2H_5$$

also known as Baycovin, is a widely used antimicrobial food additive for beverages such as fruit juices, wine, and beer (*1, 2*). Diethyl pyrocarbonate hydrolyzes rapidly in water to form ethanol and carbon dioxide, but in the presence of suitable compounds it also reacts to some extent with these compounds forming carbethoxy derivatives and ethyl esters (*1, 3*). The hydrolyses and other reactions are completed within less than 24 hours after the addition of DEP to water, aqueous solutions, or beverages (*1, 2, 4*). A number of investigations on the formation of carb-