of protease inhibitors in sera and body tissues (7) make the presence of proteolytic substances in vaccine of doubtful clinical significance. Similarity of activator from tissue slices and activator produced by cell cultures has been noted(3). It has been proposed that fibrinolytic conditions *in vivo* are due to the activator from tissue slices(8). Most of the activator produced by cell cultures is released into the supernatant medium. It is possible that cells *in vivo* may release activator into extracellular fluid and be responsible for fibrinolytic conditions *in vivo*.

Summary. Assays of 25 metabolizing cell culture lines disclosed that 13 of these released an activator of plasminogen resembling tissue activator. Of 4 primary cell lines tested the 3 of kidney origin produced both activator and protease. Assay of a specific cell culture fluid, commercially prepared poliomyelitis vaccine, disclosed the presence of both activator and protease in many lots of vaccine.

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Enzymatic Determination of Glucose-6-phosphate in Tissue Extracts Containing Glutathione and Other Interfering Substances.* (25583)

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Enzymatic reduction of triphosphopyridine nucleotide (TPN) with glucose-6-phosphate dehydrogenase (G-6-PDH) can be used for quantitative determination of glucose-6-phosphate (G-6-P)(1). However, most preparations of G-6-PDH are contaminated with other enzymes which interfere with accurate determination of G-6-P, especially when carried out in crude tissue extracts. In the present report are described conditions under which G-6-P may be measured in tissue extracts by use of sufficiently purified G-6-PDH preparations and the selective inhibition of both hexokinase and glutathione reductase.

Methods. Three preparations of yeast G-6-PDH were gifts from C. F. Boehringer and Sons (Mannheim). A pure crystalline barium salt of G-6-P from Sigma Chemical Co. was converted to potassium salt before use. Oxidized glutathione (GSSG) was prepared from reagent grade glutathione (GSH) of Fisher Scientific Co. by the method of Rall and Lehninger(2). Formation of reduced TPN (TPNH) was followed at 340 mμ in Beckman DU Spectrophotometer, using 1 ml cell with light path of 1 cm. The reaction was carried out at room temperature in *tris*-(hydroxymethyl)aminomethane (Tris) buffer at pH 8.3 with sufficient G-6-PDH to oxidize 0.03 μmole of G-6-P in about 1 to 3 minutes in the presence of inhibitors to be described below. The blank solution contained Tris and the inhibitors but no enzyme. The reaction was initiated by addition of G-6-P or tissue extract to experimental and blank solutions, which brought the volumes to 1 ml. The initial baseline reading, corrected for dilution by substrate, was subtracted from the stable final reading, and the change in optical density was divided by 6.22 to obtain μmoles of TPNH

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TABLE I. Assay of G-6-P in Presence of GSSG.

GSSG	$HgCl_2$-GSH	Increase in optical density 2 min.	4 min.	6 min.	8 min.	10 min.	TPNH (μmole) 2 min.
—	—	.200	.200	.200	.200	.200	.032
+	—	.138	.104	.084	.072	.065	.022
+	+	.201	.200	.200	.200	.200	.032

Reaction mixture: 0.1 M Tris pH 8.3, G-6-PDH, TPN 0.15 μmole, G-6-P 0.032 μmole. GSSG 0.024 μmole and $HgCl_2$-GSH reagent 0.1 ml added as indicated. Means of duplicate assays.

produced(3). The completed blank mixtures had an optical density of about 0.040 when read against distilled water. No significant activity of 6-phosphogluconic dehydrogenase, phosphoglucoseisomerase or phosphoglucomutase was associated with the G-6-PDH preparations; glucose-1, 6-diphosphate and $MgCl_2$ were added when testing for phosphoglucomutase. A change in optical density of 0.001 or less in 10 minutes in presence of appropriate substrate was considered insignificant. $HgCl_2$-GSH reagent was prepared by mixing a freshly made solution of 3.2×10^{-3} M GSH with equal volume of 3×10^{-3} M $HgCl_2$; 0.1 ml of this mixture was used/reaction cell. Tissue extracts for G-6-P analysis were prepared by grinding frozen liver from non-fasted rat, or saline suspension of rat erythrocytes, or frozen frog leg muscles in cold 1 N $HClO_4$ with sand in a mortar. Precipitated proteins were centrifuged, and excess $HClO_4$ removed from supernatant fluid by adjusting the pH to 7.2 with KOH, chilling on ice, and centrifuging off the $KClO_4$. About 0.005 ml of a 0.02% aqueous solution of phenol red was added as indicator/ml of $HClO_4$ extract; in control studies, addition of phenol red to a neutralized extract did not alter results of the G-6-P determination.

Results. When determination of G-6-P was carried out in presence of added GSSG, there was a rapid initial reduction of TPN accompanied by slower reoxidation of TPNH formed (Table I), which indicated contamination with glutathione reductase. A mixture of GSH and $HgCl_2$ could inhibit glutathione reductase rather selectively. Concentrations of $HgCl_2$ and GSH as low as 10^{-6} M inhibited completely the glutathione reductase activity associated with G-6-PDH, but these compounds at concentration of 1.5×10^{-4} M did not appreciably inhibit G-6-PDH, and produced only about 40% inhibition of hexokinase. $HgCl_2$ alone did not produce the same type of differential inhibition, and an excess of ethylenediaminetetraacetic acid (EDTA) did not reverse or prevent inhibition of glutathione reductase. The $HgCl_2$-GSH reagent, when mixed with Tris buffer at pH 8.3, retained its ability to inhibit glutathione reductase after standing 5 hours at room temperature; longer intervals were not tested.

When extracts of rat liver and erythrocytes were analyzed for G-6-P, there was interference by the relatively high concentration of GSSG in these tissues (Table II). The $HgCl_2$-GSH reagent effectively prevented reoxidation of TPNH, as indicated by the stable and higher optical density values obtained. An extract of frog leg muscle showed no tendency to reoxidize TPNH, and no significant difference in G-6-P content was found when

TABLE II. Determination of G-6-P in Tissue Extracts.

Tissue	$HgCl_2$-GSH	Increase in optical density 1 min.	2 min.	3 min.	4 min.	5 min.	10 min.
Liver	—	.030	.014	.006	.003	.0	.0
"	+	.045	.045	.046	.045	.045	.045
Erythrocytes	—	.005	.001	.0	.0	.0	
"	+	.011	.012	.012	.011	.011	
Muscle	—					.095 ±.0009	.095 ±.0008
"	+					.096 ±.0006	.096 ±.0006

Reaction mixture: Tris, TPN, and G-6-PDH as in Table I, plus 0.1 ml 0.1 M EDTA adjusted to pH 8.3 with KOH. Extracts correspond to 14.2 mg liver, 29.3 mg muscle or 0.013 ml packed RBC. Means of duplicate assays for liver and RBC; quadruplicate determinations for muscle, ± stand. error of mean.

muscle extracts were assayed with or without $HgCl_2$-GSH reagent (Table II).

Two of the G-6-PDH preparations had significant hexokinase activity, which poses a problem, since glucose and ATP are present in most tissue extracts. However, when $MgCl_2$ was omitted and EDTA added, hexokinase was completely inhibited without appreciable loss of G-6-PDH activity(4).

To test recovery of G-6-P, a measured amount was added to a $HClO_4$ extract of muscle prior to neutralization; a 70% increase in G-6-P content was observed in quadruplicate determinations, and this agreed within 1% with the theoretical value.

Discussion. Steiner and Williams(5) recently showed that GSSG in liver extracts interferes with enzymatic assay of G-6-P. They were able to remove the GSSG with Dowex 50. G-6-P was separated from free glucose in their extracts by precipitation with barium and alcohol. The present procedure makes these additional steps in determination of G-6-P unnecessary.

The important but separate problem of obtaining tissue extracts without stimulating glycogenolysis has not been considered in this presentation.

Summary. A method is described for measurement of glucose-6-phosphate in tissue extracts by use of glucose-6-phosphate dehydrogenase. Interference resulting from contamination of this enzyme with glutathione reductase was abolished by addition of a mixture of $HgCl_2$ and glutathione. Hexokinase, another contaminating enzyme which interfered with the determination, was inhibited by removing Mg ion. Phosphoglucomutase and phosphoglucoseisomerase were not present in significant amounts.

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Regulation of Cholesterol Mobilization in Mice by Bile Acids.* (25584)

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In a study of effects of several bile acids on cholesterol levels and synthesis rates in mice (1), 2 of these substances—cholic and hyodeoxycholic acids—had definite but opposing effects. Cholic acid caused substantial increase in liver cholesterol levels, which triggered 50% decrease in rate of acetate-1-C^{14} incorporation into liver total cholesterol. On the other hand, hyodeoxycholic acid decreased liver cholesterol, and greatly increased synthesis rate. Since previous experiments(2,3) had shown that cholic acid greatly retards mobilization of accumulated mouse liver cholesterol, it seemed of interest to test the effect of hyodeoxycholic acid on the same parameter to see if it would effectively increase the rate of cholesterol mobilization.

Procedures. Two hundred twenty Webster strain female, albino mice, weighing 18-20 g, were placed on a synthetic basal cholesterol free diet (CFD)(4) for 2 weeks, and then divided into 2 groups. Fifty mice received unsupplemented basal diet (CFD), while remaining 170 mice were placed on diet of CFD plus 0.5% cholic acid and 1% cholesterol. At end of 3-week cholesterol build-up, 10 normal mice and 10 mice from the build-up group were sacrificed for liver and carcass choles-

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