change in acid-base state, as such does not alter the blood NPSH level as shown by the rat experiments reported here.

Summary and conclusion. Since(1) acetone and glutathione in aqueous solution or added to whole blood did not form any compound not readily reversible under physiologic conditions; (2) the development of ketosis following starvation in human subjects did not result in a lowered blood non-protein sulfhydryl; and, (3) induced metabolic acidosis or alkalosis in rats led to similar results, the decreased blood glutathione in diabetic ketosis must be explained on grounds other than the combination of thiols with ketone bodies, or some simple relation to acid-base balance.

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Effect of Galactose on Urinary Electrolyte Excretion in Man.* (28887)

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Acute ingestion of ethanol is followed by an increase in urinary excretion of magnesium and calcium and a decrease in urinary excretion of potassium(1-3). The mechanism (or mechanisms) responsible for these alterations in urinary electrolyte excretion is undetermined.

Rats ingesting a 60% galactose diet excrete more urinary calcium and magnesium than do pair-fed animals ingesting a 60% glucose diet(4). Galactose ingestion in man was found to have an effect on urinary excretions of magnesium, calcium and potassium similar to that of alcohol ingestion. The implications of these findings in suggesting a common mechanism for the observed changes in urinary cation excretion are discussed.

Methods. Normal male subjects between the ages of 22 and 42 were fasted overnight and then given orally 20 cc of water per kg body weight. Throughout each test, the volume of water excreted was replaced by an equal volume of intravenous and oral fluids. Following a priming infusion, a sustaining solution containing inulin and PAH in 5% dextrose and water was infused through an intravenous catheter at a rate of 5 cc per minute. After a near-maximal diuresis was established, urines were collected for three 20-minute control periods. Three subjects then ingested 50 g of galactose dissolved in water. An additional 20 g of galactose was taken orally at the beginning of the next two 20-minute periods and 10 g was taken orally at the beginning of the last period, a total of 100 g. Urines were collected during an initial 20-minute period allowed for intestinal absorption and during the next 3 test periods. The results of 3 control studies in which no galactose was given have been published(1).

Measurements were made on each urine for volume, osmolality, sodium, potassium, calcium, magnesium, chloride, phosphate, lactate, citrate, total organic acid, pH, inulin and PAH. Bloods were drawn at the midpoint of each urine collection and measure-

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ments were made for osmolality, sodium, potassium, calcium, magnesium, chloride, phosphate, lactate, citrate, inulin and PAH. Methodology has been described(1).

Results. The results are shown in Table I and Fig. 1. Control results and results following ingestion of alcohol have been reported(1). Ingestion of galactose resulted in increased urinary excretions of magnesium and calcium and a decreased urinary excretion of potassium similar to results following alcohol ingestion(1). Urinary excretion of lactate increased slightly in 2 subjects and greatly in a third. Essentially no changes were observed in urinary citrate and total organic acid or in blood citrate and lactate levels. In addition to the results shown in Table I, urinary pH's and phosphates showed no changes and urinary chlorides closely paralleled urinary sodium excretions. Inulin and PAH clearance showed mean increases of 5 and 8% respectively between control and post ingestion studies.

TABLE I. Effect of Galactose Ingestion on Urinary Excretion of Electrolytes and Organic Acids.

Subject, date, conditions	Volume, cc/min	Total solute, μosm/min	Na^+, μeq/min	K^+, μeq/min	Ca^{++}, μeq/min	Mg^{++}, μeq/min	Total organic acid, μeq/min	Lactate, μg/min	Citrate, μg/min	C_{inulin}, cc/min
L.H. 4/3/62										
Control	10.6	767	139	90	18.1	6.6	64	200	—	119
Post-ingestion	15.2	1081	181	64	32.3	18.1	75	429	—	126
M.S. 5/16/62										
Control	10.3	743	124	33	6.2	8.9	124	287	230	113
Post-ingestion	14.3	871	152	24	13.8	15.7	126	327	259	117
L.D.H. 7/6/62										
Control	15.0	694	168	45	7.3	5.9	101	92	282	98
Post-ingestion	17.7	985	204	34	14.4	12.7	103	123	271	106
Mean values										
Control	12.0	735	144	56	10.5	7.1	96	193	256	110
Post-ingestion	15.7	949	179	41	20.6	15.5	101	293	265	116
% change	+31	+33	+24	-27	+96	+118	+5	+52	+4	+5
Mean serum levels										
Control	—	271	136	3.8	4.8*	1.66	—	13	1.2*	—
Post-ingestion	—	270	135	3.9	5.0	1.77	—	14	1.1	—

* Based on two studies.

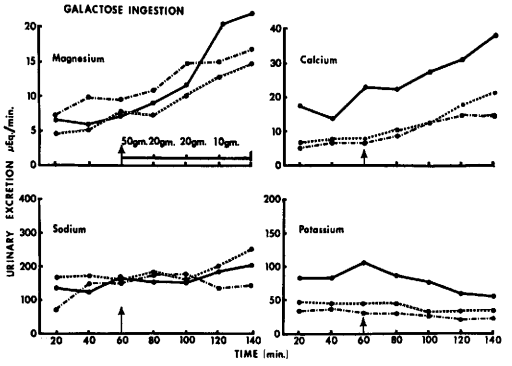

FIG. 1. Effect of ingested galactose on urinary magnesium, calcium, sodium, and potassium excretion in 3 normal males.

Discussion. Some factor common to both alcohol and galactose ingestion probably is present to explain the similar increases in urinary magnesium and calcium and decrease in potassium excretions observed. Alcohol and galactose are both oxidatively metabolized through the citric acid cycle utilizing the DPN-flavoprotein-cytochrome enzymes for hydrogen transport in initial and subsequent steps. The rate of metabolism of alcohol is apparently limited by the rate of reoxidation of DPNH(5,6). An increase in DPNH:DPN ratio has been observed in liver slices following incubation with ethanol(6). Since the kidneys also possess this ability to oxidize alcohol(7), a similar increase in this ratio undoubtedly occurs in this organ. In the metabolism of galactose, the conversion of UDP-galactose to UDP-glucose requires an epimerase and reduction of DPN. Alcohol interferes with the metabolism of galactose pre-

sumably by competing with galactose for available DPN in this step(8,9).

A competition for available hydrogen transport enzymes necessary for tissue respiration could be the common link in these studies. Sodium reabsorption in the renal tubules appears to be dependent, at least in part, upon an intact and functioning respiratory system (or systems) as sodium reabsorption can be directly related to oxygen consumption(10, 11). Anoxia(12) and Antimycin A(13), a potent and highly specific inhibitor of aerobic glycolysis by virtue of its ability to block electron transport, will decrease sodium reabsorption. Other cation transport systems probably are similarly dependent upon an intact respiratory system.

Stop flow patterns for urinary calcium and magnesium are nearly identical, suggesting these cations share a common transport mechanism(14). The steepest reabsorption gradients occur in the distal tubule and closely approximate the sites of sodium reabsorption and potassium secretion. Furthermore, infusions of magnesium salts(15,16) or calcium salts(15,17) into man result in increased urinary excretions of calcium, magnesium and sodium and decreased urinary excretion of potassium suggesting these cation transport mechanisms are in some way interrelated. The changes in urinary cation excretion following ingestion of alcohol or galactose could be explained by an inhibitory effect on magnesium and calcium reabsorption and potassium secretion in the distal tubule. If cation transport were dependent in some way upon the availability of oxidative enzyme systems (such as the DPN-flavoprotein-cytochrome system), competition with alcohol and galactose, utilizing this same system for aerobic glycolysis, might result in a competitive inhibition of cation transport.

An alternative explanation was considered. Barker, Elkinton and Clark reported that infusions of sodium lactate were followed by increases in urinary magnesium excretions, whereas equimolar quantities of sodium bicarbonate failed to produce a similar increase (15). Ingestion of ethanol increases blood lactate levels(18, 19) raising the possibility that the increased urinary excretion of the divalent cations might be related to increased blood levels and urinary excretion of lactate. Increased complexing of divalent cations with lactate or other organic acids might increase ultrafilterable cation levels by decreasing protein binding(20,21) or might inhibit or block tubular reabsorption of the divalent cations (20,22). The observed increases in urinary excretion of lactate following ethanol and galactose ingestion appear insufficient to account for the increased urinary divalent cation excretions. Infusions of sodium lactate sufficient to produce much greater increases in urine lactate produced only small, transient increases in urinary magnesium and calcium excretion. This also fails to explain the changes seen in urine potassium excretion. The changes in urine citrate and total organic acids following ethanol and galactose were insignificant.

Summary. A prompt, significant increase in urinary excretions of magnesium and calcium and a decrease in urinary excretion of potassium follows ingestion of ethanol and galactose. The oxidative metabolism of alcohol and galactose are rate-limited by the availability of the DPN-flavoprotein-cytochrome enzymes for hydrogen transport. If the cation transport mechanisms were dependent upon this same respiratory system, a competitive inhibition of magnesium and calcium reabsorption and potassium secretion in the distal tubule might well explain our findings.

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Semi-Quantitative Immunoelectrophoresis and Its Application to the Study of Serum α-Globulins.* (28888)

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Immunoelectrophoresis has been used extensively for identification of subfractions of serum proteins and other biological materials. Quantitative analysis using immunoelectrophoresis has also been reported. Ouchterlony (1) reported a method of quantitative immunoelectrophoresis in which antigens, after electrophoretic separation, were allowed to diffuse into an agar-antiserum mixture, and the distance from the interface to the leading edge of the precipitin arc was measured. In a method reported by West *et al*(2), the antiserum was absorbed with increasing amounts of antigen, and the volume of antigen solution which resulted in nearly complete consumption of antibody was determined. Neither of these, however, is fully satisfactory for routine clinical use, owing to their relatively complicated procedures and also to their requirements for large amounts of antisera. In the present study, a simple system of criteria for a semi-quantitative interpretation of immunoelectrophoretic patterns was devised, and was used for analysis of serum α-globulins in various diseases.

Materials and methods. Serum specimens. Serum was obtained from 3 groups of normal healthy adults, each group representing a specific haptoglobin type. A total of 162 sera was tested from hospital patients of whom 30 had non-neoplastic inflammatory diseases, 37 malignant neoplastic diseases, 38 liver diseases, 22 renal diseases, and 25 other miscellaneous diseases.

Immunoelectrophoresis. Immunoelectrophoresis on microscopic slides was carried out using the method of Hirschfeld(3) who employed the discontinuous Laurell buffer system. Electrophoretic separation was continued for 120 minutes at 10 v/cm.

The identification of the orosomucoid precipitin arc was made with a rabbit anti-human orosomucoid serum. Ceruloplasmin and haptoglobin were identified with the *p*-phenylendiamine reaction of Uriel(4), and the anisidine stain of Javid(5), respectively.

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