37.34°C, possibly allowing more "activating" material to diffuse into the medium, nor the presence of calcium in the incubation medium which would partially inhibit the respiration of brain tissue was sufficient to allow a stimulation to be observed in the presence of added substrates.

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## Blood Non-Protein Sulfhydryl Levels in States of Acidosis.* (28886)

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Though extensively studied, the relationship of sulfhydryl (SH) moieties to diabetes mellitus remains obscure. The finding of Illig (1), that blood glutathione (GSH) levels in human diabetes are inversely related to degree of ketosis rather than to glycemia, and of Nath(2), that intravenous administration of acetoacetate in rabbits causes a prompt decline in blood GSH levels implicate ketone bodies and an altered fat metabolism in the SH-diabetes relationship. This paper is concerned with the question: are these blood SH changes due to an altered acid-base state, or are they related to the ketone body level? If the latter is the case, is the reduction in SH level due to chemical combination of SH compounds with ketones? This paper reports studies on rats, rabbits, and human subjects during starvation, or on rats in metabolic acidosis and alkalosis, and by *in vitro* experiments.

*Methods and materials.* Protein sulfhydryl (PSH) and non-protein sulfhydryl (NPSH) concentrations were determined by the amperometric technique of Benesch and Benesch (3). Ethylenediaminetetraacetate (EDTA) was used to prevent heavy metal reaction with NPSH. Blood proteins were precipitated with 20% sulfosalicylic acid. Acetone, acetoacetic acid, and total acetone bodies in blood and aqueous solution were determined by the method of Thin and Robertson(4). Standard solutions of acetone covering the expected range of values for each experiment were simultaneously determined as a control. pH determinations were made on blood taken by heart puncture under ether anesthesia. The anesthesia apparently produced a respiratory acidosis in all normal rats. This artifact augmented any induced acidosis, and mitigated any induced alkalosis. If the normal control, though indicating an acidosis, was the standard of reference, the abnormal state was clearly definable. Nitrogen was determined by the micro-Kjeldahl, and glucose by the Somogyi-Nelson techniques. The hematocrit was determined by the Wintrobe method. Acidosis was induced in rats by feeding $NH_4Cl$; 0.5 g/kg/day for 5 days; alkalosis by feeding $NaHCO_3$, 1 g/kg/day for 5 days. The control rats were pair-fed.

*Results. In vitro* studies. To explore the chemical combination of SH and ketone bodies, GSH was incubated with acetone in

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TABLE I. Effect of Starvation on Blood Acetone Body and NPSH Levels.

| | Rat | Rabbit | Human |
|---|---|---|---|
| Acetone body, control | 89 ± 4* | 97 ± 1 | 1.0 ± .5 |
| Acetone body, starvation | 94 ± 2 | 98 ± .4 | 9.4 ± 4 |
| NPSH, control | 105 ± 4† | 116 ± 3 | 76 ± 6 |
| NPSH, starvation | 113 ± 5 | 106 ± 2 | 81 ± 5 |
| Hematocrit, control | | | 52 ± 1 |
| Hematocrit, starvation | | | 55 ± 1 |
| No. of exp. | 6 | 6 | 11 |

* Avg ± S.E. mg/100 cm³.
† Avg ± S.E. μM/100 ml blood.

varying concentrations at 37°C in aqueous solution. Acetone was likewise incubated with rat whole blood, and rat whole blood made hyperglycemic with dextrose. The blood was then analyzed for acetone and GSH. In aqueous solution the recovery of GSH in concentrations ranging from 100 mg to 600 mg/100 ml averaged 97%, while recovery of acetone in concentrations ranging from 10 to 100 mg/100 ml was 95% (70 experiments). Addition of acetone to whole blood did not alter the blood non-protein sulfhydryl (NPSH) level and recovery of acetone was 99% (24 experiments). The same results were obtained when dextrose, 200 mg/100 ml, was added to the blood, with or without added GSH (16 experiments).

*In vivo* studies. To study the effect of increased ketone bodies in conditions other than diabetes, rats, rabbits, and humans were starved for 72 hours. The results are shown in Table I. Starvation produced no ketosis in the rat or rabbit and no significant change in the blood NPSH. In human subjects a moderate ketosis was associated with an unchanged blood NPSH.

The results of the production of metabolic acidosis by $NH_4Cl$ and metabolic alkalosis by $NaHCO_3$ are shown in Table II. Other tissues in addition to blood were investigated. Here again an altered acid base balance, as such, produces no change in NPSH or in total sulfhydryl (TSH), that is, protein SH plus NPSH.

Attempts were made to produce respiratory acidosis and alkalosis in rats by subjecting the animals to an atmosphere of 8% $CO_2$ in air and 10% $O_2$ in nitrogen for one hour (4 animals each). No effect on NPSH or ketone bodies was found.

*Discussion.* In the concentrations used, acetone in aqueous solution does not form any compound with GSH which is not freely reversible. This finding is confirmed by the chemical studies of Schubert(5), who showed that ketones, as acetone, acetophenol, diacetyl, do not combine with thioglycolic acid, and that aldehydes and pyruvic acid form compounds with cysteine and thioglycolic acid which are readily dissociated. Under physiological conditions mercaptol formation is not an important form of SH when acetone bodies are present.

The ambulatory diabetic patients with positive test for urinary acetoacetic acid, as reported by Illig *et al*(1) did show a modest but significant decrease in blood GSH when compared with controls. However, normal starving human subjects with elevated blood ketone bodies and strongly positive urinary ketone tests, showed no change in blood NPSH. These findings are in agreement with those of Stock and Currence(6) on fasting diabetics. From the studies on human ketosis in starvation it appears that any effect of diabetes on SH is not related directly to a rise in ketone body levels as a result of fat mobilization and degradation. Moreover, a

TABLE II. Effect of Metabolic Acidosis and Alkalosis on Blood and Tissue NPSH and TSH, and Blood Acetone Levels in Rats.

| | Control | Acidosis‖ | Alkalosis¶ |
|---|---|---|---|
| pH | 7.31±.03* | 7.18±1.3 | 7.39±.02 |
| Blood acetone† | .5 ±.5 | 2.0 ±1 | .5 ±.4 |
| " TSH‡ | 23.8 ±.8 | 24.1 ± .6 | 24.1 ±.4 |
| " NPSH | .7 ±.01 | .8 ± .1 | .9 ±.1 |
| Liver TSH§ | 15.3 ±.4 | 17.3 ± .7 | 16.0 ±.3 |
| " NPSH | 5.9 ±.3 | 6.4 ± .4 | 6.6 ±.2 |
| Kidney TSH§ | 11.9 ±.2 | 12.3 ± .5 | 11.1 ±.6 |
| " NPSH | 4.3 ±.2 | 4.5 ± .8 | 3.5 ±.3 |

* All figures avg ± S.E.—6 animals per group.
† mg/100 cm³.
‡ μM/ml.
§ μM/g.
‖ Fed $NH_4Cl$ 0.5 g/kg/day for 5 days.
¶ Fed $NaHCO_3$ 1 g/kg/day for 5 days.

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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