TABLE I. Liver Glycogen of Fasted and Non-Fasted Rats Given Cortisone Acetate.

Daily dose cortisone, mg	Hr post-feeding	No. rats	Days on cortisone	Avg liver glycogen, % of liver wt
5	24	6	7	7.17
	4	2	6⅙	9.23
10	24	6	7	8.06
	6	4	6¼	9.97
20	24	8	7	9.06
	6	7	6¼	8.92

this time. In the remaining animals the livers were removed 24 hours after feeding. Although the numbers of animals are small, the data (Table I) do suffice to show that the peak level of liver glycogen in rats treated with large doses of cortisone acetate is about as high in the fasted as in the fed rat. The highest individual value was 11.84% glycogen in the liver of a fed rat given 10 mg of cortisone acetate daily for 7 days.

Discussion. Liver glycogen is very high in rats having steroid diabetes induced by cortisone acetate. At each higher dose there is an increase in both the amounts of urinary glucose and of liver glycogen. However, the slopes of the dose-response curves for urinary glucose and for liver glycogen are not parallel and at any given dosage level the correlations between the 2 values for individual animals are low. We have not measured these 2 indices of altered carbohydrate metabolism under identical conditions. Urinary glucose is excreted following each feeding and the urine is sugar-free by the end of a 24-hour fast. The deposition of liver glycogen is cyclic. If it were important to study more closely the interrelationship between these 2 indices it would be necessary to measure them simultaneously and at frequent intervals following feeding and during fasting.

Summary. Normal male rats were force-fed a medium carbohydrate diet. Steroid diabetes was induced by the administration of cortisone acetate in doses of 2, 5, 10, and 20 mg/rat/day for 7 days. The level of liver glycogen reached very high values in both fed and fasted animals. The glycogen level was proportional to the dose of cortisone acetate and was correlated with the level of urinary glucose.

1. Mering, J. von, and Minkowski, O., *Arch. f. exp. path. u. pharmakol.*, 1889, v26, 371.
2. Morita, Y., and Orten, J. M., *Am. J. Physiol.*, 1950, v161, 545.
3. Ingle, D. J., Beary, D. F., and Purmalis, A., *Endocrinology*, 1953, v52, 403.
4. Shaffer, P. A., and Williams, R. D., *J. Biol. Chem.*, 1935, v111, 707.
5. Peterson, R., and Rose, D., *Canad. J. Technol.*, 1951, v29, 317.

Received September 21, 1953. P.S.E.B.M., 1953, v84.

Excretion of Urinary Mucoprotein Following Parathyroid Extract in Rats.* (20639)

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Following the administration of parathyroid extract to rats, the levels of serum mucoprotein rise(1). When doses of 1000 or more units of the hormone are given over a short period of time, striking changes are seen in the kidneys. The tubules become filled with a water-soluble, alcohol-insoluble material which gives a positive periodic acid-leucofuchsin reaction, and mucoprotein granules appear in tubular cells. These observations suggested that in such animals the urinary mucoprotein levels also might be enhanced.

* This work was supported in part by grants from the Department of the Army, Surgeon General's Office, from the American Cancer Society, recommended by the Committee on Growth of the National Research Council, and from the Graduate School, University of Illinois.

TABLE I. Urinary Mucoprotein in Rats: Amount of urinary mucoprotein excreted before and after injection of 1000 units of parathyroid extract (Parathormone, Lilly), expressed as mg of mannose-galactose standard.

	Animal No.												
	1	2	3	4	5	6	7	8	9	10	11	12	Mean
72 hr pre-inj.	1.37	.29	.78	.35	1.44*	1.11	.85	1.43	.63	.76	.82	1.92	1.08
72 hr post-inj.	2.52	2.00	2.25	.52	3.87*	2.63	2.47	3.66	2.83	3.52	4.52	1.35	2.84

* Urine collections were made for 48-hr period only.

A mucoprotein has been demonstrated in normal human urine by Tamm and Horsfall (2,3). Their method of precipitating this substance at a concentration of 0.58 M NaCl was applied to rat urines collected before and after the administration of parathyroid extract.

Methods. Twelve young adult female rats (Sprague-Dawley) were housed in individual metabolism cages for the collection of urine without fecal contamination. Urines were collected for a 3-day control period. The animals were fed outside their cages during a one-hour daily period to avoid contamination of the urine samples with food, and water was given *ad lib.* A few days after the control period, animals were injected with 1000 units of parathyroid extract† in 2 divided doses over 6 hours. Urine collections were begun after the last injection and continued for a 3-day period interrupted by daily feeding periods of one hour. Urine collections were taken daily for mucoprotein separation. *Separation and determination of urinary mucoproteins.* Each daily sample of urine was filtered and brought to a final volume of 50 ml by washing the filter paper and residue with distilled water. To the filtrate was added 1.8 g NaCl, to give a concentration of 0.58 M, and the mixture was placed in the refrigerator overnight, when mucoprotein flocculated; the mixture was centrifugalized and the supernatant discarded. The sediment was dissolved in 6 ml of 5% Na_2CO_3 to which a few drops of normal NaOH had been added. After standing for one hour, an aliquot was taken for estimation of the carbohydrate content using the orcinol reagent and a standard containing equal amounts of mannose and galactose.

† Parathormone generously supplied by Eli Lilly Co., Indianapolis, Ind.

Results. (Table I). During a 72-hour control period, rats excreted a total of 0.35-1.92 mg (mean: 1.08 mg) mucoprotein carbohydrate determined as mannose-galactose. Using each animal as its own control, an elevation was found in 11 out of 12 animals following parathyroid extract. For a 72-hour period following injection, the excretion was 0.52-4.52 mg (mean: 2.84 mg). The difference was highly significant.

Discussion. Current opinion is divided as to the origin and significance of the plasma mucoproteins. The problem has been recently discussed in some detail(4). The origin of the urinary mucoproteins has, so far as we know, been scarcely considered, although the consensus is that the urinary proteins are excreted through the glomeruli(5). We have inclined to the view that increases in plasma mucoproteins in certain specific situations might be related to the formation of water soluble fractions from the ground substance of connective tissues(6-9,1). Comparable connective tissue changes may underlie even some of the so-called non-specific situations where blood mucoproteins become increased in amount, and a similar explanation would then apply to them. Following parathyroid hormone injection, the following events are noted: dissolution of the bone matrix and the formation of water soluble glycoprotein moieties as demonstrated by direct chemical analysis of frozen-dried tissues; increase in plasma mucoproteins; kidney changes suggesting excretion (and possible reabsorption) of mucoproteins; finally, as shown here, a doubling or trebling of the urinary mucoprotein output. While a part of the normal complement of urinary mucoprotein may be derived from the urinary tract, the above sequence appears to associate at least the additional mucoprotein excreted with

events occurring at some point proximal to the urinary tract. To define a causal relationship will entail a greatly expanded knowledge of the tissue mucoproteins and their breakdown products. Meanwhile this study suggests that quantitative urinary mucoprotein studies might profitably be undertaken in a variety of diseases involving connective tissues, including especially the acute and chronic bone dystrophies.

Summary. Control rats excrete, in a 72-hour period, 0.35-1.92 mg (mean: 1.08 mg) urinary mucoprotein determined as mannose-galactose standard. Following injection of 1000 units of parathyroid hormone, mucoprotein excretion increased significantly to 0.52-4.52 mg (mean: 2.84 mg). This increase is correlated in time with a dissolution of bone matrix, with an increase in plasma mucoprotein, and with the appearance of mucoprotein granules in kidney tubule cells and mucoprotein-containing casts in tubules.

1. Engel, M. B., *A. M. A. Arch. Path.*, 1952, v53, 339.

2. Tamm, I., and Horsfall, F. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 108.

3. ———, *J. Exp. Med.*, 1952, v95, 71.

4. Winzler, R. J., *Advances in Cancer Research*, 1953, v1, 506, Academic Press, New York City, N. Y.

5. Rigas, D. A., and Heller, C. G., *J. Clin. Invest.*, 1951, v30, 853.

6. Gersh, I., and Catchpole, H. R., *Am. J. Anat.*, 1950, v85, 457.

7. Catchpole, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 221.

8. Pirani, C. L., and Catchpole, H. R., *A. M. A. Arch. Path.*, 1951, v51, 597.

9. Catchpole, H. R., and Engel, M. B., *Fed. Proc.*, 1953, v12, 24.

Received September 21, 1953. P.S.E.B.M., 1953, v84.

Use of an Organic Chelating Agent in Histochemical Study of Alkaline Phosphatase Activation.* (20640)

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Ethylene diamine tetraacetic acid (EDTA), by virtue of its capacity to form stable chelate complexes with many bivalent metals, will inhibit a number of enzymes *in vitro* presumably through competition with the enzyme for the activating metallic component(1-4). Histochemical application of this property was suggested by the solubility of the chelates formed, and alkaline phosphatase was chosen as a test enzyme for several reasons. 1) It has an ionically available activating metal (magnesium). 2) The enzyme has been extensively studied *in vitro* and considerable data has been accumulated with respect to its activation and inhibition. It provided, therefore, a reasonably satisfactory baseline for the method. 3) At least one component of the system is relatively resistant to alcohol fixation and paraffin embedding. 4) It can be readily demonstrated by 2 different histochemical technics which give identical localizations. 5) Alkaline phosphatase (phosphomonoesterase I) is part of an isodynamic enzyme system and there is reason to suspect that it may actually represent a group of enzymes with a similar pH optimum rather than an entity. There was a possibility, therefore, that these might be separable histochemically on the basis of their potential metallic activators and anionic inhibitors as had been demonstrated *in vitro* in the case of members of the system having different pH optima(5,6).

Method. Rabbit kidney which contains large amounts of alkaline phosphatase in characteristic distribution was used as a test tissue. Thin pieces of fresh kidney were fixed in cold 80% alcohol for 24 hours, dehydrated in cold

* This work was supported in part by a grant from the U. S. Public Health Service and by the John R. Stark Memorial Fund.