sults given in Table I show that both these fractions contained gonadotrophin, which is similar to the results obtained by Catchpole[8] who reported that a major part of the gonadotrophin of sheep pituitary glands is found in the large granule fraction.

The large granule fraction C plus the small granule fraction F of glands from both normal and castrate animals contained more than 50% of the total gonadotrophin. The major part of the remaining hormone is found in the soluble fraction I, which may be accounted for either on the basis of the presence of small granules which were not removed by centrifugation, or by the solubility of the hormone in the sucrose. The greater part of the gonadotrophin was removed from the granule fractions C and F by extraction with 0.9% sodium chloride solution to give fractions E and H, respectively. The insoluble fractions (D and G) that remained after extraction contained little gonadotrophin. Whether the gonadotrophin was extracted from the granules or whether granules containing gonadotrophin were dissolved is not clear from present results. Further work is necessary to determine whether the hormone is associated with the mitochondria or with granules that are recovered with the mitochondria.

Conclusions cannot be made as to whether the FSH and LH activities are differentially distributed between the different fractions since all the active fractions produced follicles and corpora lutea.

Summary. Approximately 60% of the succinoxidase activity of rat pituitary glands were found to be associated with the large granule fraction, and more than 50% of the gonadotrophin of these glands was associated with two granule fractions isolated from sucrose homogenates by differential centrifugation. The supernatant fraction from which the granules were removed contained the remaining gonadotrophic activity. The gonadotrophin can be extracted from the isolated granules with isotonic sodium chloride solution.

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[8] Catchpole, H. R., Abstract, *Fed. Proc.*, 1948, **7**, 19.

17207. Urinary Excretion of Amino-acids During Alloxan-induced Diabetes in Rats.*

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The experiments reported here were undertaken to determine whether the metabolic disturbances of alloxan-induced diabetes in rats, with and without insulin-control, would entail an abnormal excretion of amino-acids in the urine.

Paper chromatographic methods developed by Consden and Martin[1] and adapted to urine analysis by Dent[2-4] have greatly facilitated the qualitative detection of amino-acids. Several investigators applying these methods have gathered much new information on the abnormal amino-acidurias found in the Fanconi syndrome,[2,3] acute yellow atrophy of the liver,[2] and in progressive muscular dystrophy.[5] Results of studies on these unrelated disorders indicate that the patterns of urinary

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[1] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 1944, **38**, 224.

[2] Dent, C. E., Conference on Metabolic Aspects of Convalescence, Josiah Macy, Jr. Foundation, 1946, Nov. 12-13, 126.

[3] Dent, C. E., *Biochem. J.*, 1947, **41**, 240.

[4] Dent, C. E., *Biochem. J.*, 1948, **43**, 169.

[5] Ames, S. R., and Risley, H. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 131.

TABLE I.
Urinary Excretion of Amino-acid N and Glucose, and Balance N During Periods of Control and Insulin Insufficiency.*

Period	Balance N, mg	Urine amino-acid N		Urine glucose, g
		mg	% of total urine N	
Normal control	+57.3	.961	.6	0
Diabetic + insulin	+14.4	1.24	.5	.869
Diabetic no insulin	—126.	1.29	.6	2.25

* The values represent the average of 9-24 hr collections, 3 from each of 3 rats.

TABLE II.
Amino-acids and Related Substances in Urines of Rats, Demonstrated by Paper Chromatography.

Amino-acids	No. of 24 hr urine samples containing the respective amino-acids		
	Normal control (9 samples)	Alloxan-diabetes + insulin (9 samples)	Alloxan-diabetes without insulin (9 samples)
Aspartic acid	6	5	2
Glutamic "	9	9	9
Glycine	9	9	8
Alanine	9	9	9
Valine	3	3	1
Leucine	3	2	1
Taurine	6	9	9
Glutamine	9	7	8
Unidentified polypeptide	5	5	5

excretion of amino-acids may vary considerably in conditions that involve disturbances of nitrogen metabolism in the body and alterations in renal function. It seemed of interest to determine whether ketonemic acidosis in the diabetic rat, with greatly increased cellular catabolism and losses of nitrogen from the body, would be characterized by significant changes in amino-aciduria.

Methods. Male Rockland Farm rats weighing approximately 250 grams were fed the diet mixture of Cox and Imboden.[6] The diet was thoroughly mixed to ensure uniformity of composition, especially with regard to the nitrogen content of aliquots. Adequate supplements of vitamins A and D were given at weekly intervals. The animals were placed in individual metabolism cages designed to permit measurement of food intake and for separate collection of the urine and feces. Urine specimens were collected under toluol and preserved by freezing. Alloxan (Bios) was administered as a single dose, 50 mg per kg body weight, in 2.5% aqueous solution, injected intravenously after the rats were fasted 12 hours.

Total nitrogen determinations on diet, urine, and feces were performed by the micro-Kjeldahl method. Urea nitrogen was determined by the manometric method of Van Slyke and Kugel.[7] Amino-acid nitrogen was determined by the ninhydrin, carbon-dioxide method of Van Slyke, MacFadyen, and Hamilton.[8] The Nelson modification[9] of the Somogyi method was employed for the determination of glucose in blood and urine. Qualitative tests for acetone in the urine were made with a powdered mixture of sodium nitroprusside, sodium carbonate, and ammonium sulfate (Denco test). Paper chromatographic methods as described by Dent[2,4]

6 Cox, W. M., and Imboden, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 443.

7 Van Slyke, D. D., and Kugel, V. H., *J. Biol. Chem.*, 1933, **102**, 489.

8 Van Slyke, D. D., MacFadyen, D. A., and Hamilton, P. B., *J. Biol. Chem.*, 1943, **150**, 251.

9 Nelson, N., *J. Biol. Chem.*, 1944, **153**, 375.

were used to detect and identify individual amino-acids in all urines.

Experimental. The results summarized here were obtained on 3 rats, each studied in 3 metabolic periods of 3 or more days: I, in normal state during preliminary control periods; II, in the diabetic state following the injection of alloxan, treated with adequate doses of insulin, and III, after withholding insulin. Severely diabetic states were induced in every animal, as indicated by the rapidity of the development of acetonuria, acidosis and death within 3 to 5 days when insulin was withheld. The preliminary period I included three consecutive 24-hour collections of urine. After the administration of alloxan the ensuing diabetic state was controlled by daily injections of 1 to 3 units of protamine zinc insulin, the doses being adjusted to maintain the body weight constant and to keep the urine acetone-free. After the establishment of satisfactory control, 24-hour collections of urine were resumed and continued through periods II and III, until the animal died.

Data from 3 rats are presented in Table I. The individual amino-acids identified by the chromatographic method in the urines of the 3 animals in respective periods are listed collectively in Table II.

Discussion. During periods of severe and fatal acidosis in the alloxan-diabetic rat, deprived of insulin, the pattern of excretion of amino-acids in the urine remained essentially unchanged, compared with periods of control. The ratio of amino-acid nitrogen to total nitrogen excreted in the urines remained relatively constant, independent of the development of ketosis, acidosis, and increased excretion of total nitrogen. These facts suggest the conclusion that although protein catabolism is increased in alloxan-induced diabetes during periods of insulin insufficiency, normal metabolic pathways are utilized, and amino-acids are efficiently conserved by the kidney. This is in accord with the results of studies on human diabetics as reported by Hall.[10]

Summary. Nitrogen balance studies on 3 male rats during periods of normal control, of alloxan-induced diabetes with insulin therapy, and of fatal acidosis after withholding insulin, are reported.

The pattern of excretion of urinary amino-acids, and the ratio of total amino-acid nitrogen to total urinary nitrogen remained essentially unchanged throughout the 3 periods, including the terminal stage with greatly increased losses of total nitrogen.

[10] Hall, D. A., *Biochem. J. Proceedings*, 1948, **43**, lvii.

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17208. Lack of Identity of Hyaluronidase Inhibitor and Certain Mucoproteins in Blood Serum.*

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Winzler and co-workers[1,2] observed that elevations of plasma mucoprotein levels occur in patients with cancer and pneumonia. Prinzmetal *et al.*[3] have noted a similar increase in patients with myocardial infarctions.

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[1] Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, 1948, **27**, 609.

[2] Winzler, R. J., and Smyth, I. M., *J. Clin. Invest.*, 1948, **27**, 617.

[3] Simpkin, B., Bergman, H. C., and Prinzmetal, M., *Am. J. Med.*, 1949, in press.