tion could be made between urine samples containing different quantities of added digitoxin.

Ten albino rats (average weight 165 g) were given 100 μg of digitoxin per kilogram of body weight by intravenous injection, and their urine was collected for 24 hours. The urine samples of 5 of these rats were also collected on the second and third day following injection. Twelve rats (average weight 223 g) were given 1000 μg of digitoxin per kilogram of body weight and similar urine collections were made during the first 24 hours. Urine collections of eight of these rats also were made on the second and third days following injection. All urine samples were extracted and tested on the duck hearts as described above.

Results. The injection of a moderately large amount of digitoxin (*i.e.*, 100 μg/kilo) into 10 rats was not followed by the detectable appearance of digitoxin in the 24-hour samples of urine of any of the rats either 24, 48, or 72 hours after the injection. In view of the fact that each rat actually received approximately 16.5 μg of digitoxin, absence of as much as one microgram of digitoxin in the urine indicated that little or no digitoxin was excreted by the kidneys of these rats.

The urine samples, however, collected during the first 24 hours in the 12 rats who had received 10 times the above amount of digitoxin (1000 μg/kilo) contained an average of 6 μg digitoxin (Range, 4 to 10 μg). However, none of the urine samples collected during the second or third days after injection contained a detectable amount of digitoxin (*i.e.*, less than 1 μg).

Thus, even in these rats which had received a relatively tremendous amount of digitoxin (average, 223 μg) the average renal excretion of only 6 μg (less than 3% of the administered dose) again indicates that the renal excretion of digitoxin in the rat is negligible. These results, however, are not to be construed as evidence for the negligible excretion of other cardiac glycosides in the rat. Moreover, these results may not be valid for other species in view of the fact that the rat appears to react anomalously to the administration of digitoxin.

Conclusion. A method for the quantitative determination of minute amounts of digitoxin in urine is described. The renal excretion of digitoxin is negligible in the rat.

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17206. Gonadotrophic Activity of Granules Isolated from Rat Pituitary Glands.*

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The respiratory enzyme systems, cytochrome oxidase and succinoxidase, have been shown to be associated to a large extent with a large granule or mitochondrial fraction isolated by differential centrifugation of alkaline water homogenates (Schneider[1]), and saline extracts (Hogeboom, Claude and Hotchkiss[2]) of rat liver. Subsequently morphologically intact mitochondria were isolated by differential centrifugation of 0.88 M sucrose homogenates of rat liver and kidney (Hogeboom, Schneider and Pallade[3,4]). On

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1 Schneider, W. C., *J. Biol. Chem.*, 1946, **165**, 585.

2 Hogeboom, G. H., Claude, A., and Hotchkiss, R. D., *J. Biol. Chem.*, 1946, **165**, 615.

3 Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 320.

4 Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.*, 1948, **172**, 619.

the basis of these results it seemed that it might be possible to isolate granule fractions from fresh pituitary tissue, and study them not only from the standpoint of their enzyme activity but also from the standpoint of whether gonadotrophic hormone is associated with them. Results of preliminary experiments using rat pituitary gland are given in this report.

Experimental. The pituitary glands were obtained from 4 groups of 50 to 60 normal adult rats (Holtzman strain) and one group of 35 adult rats castrated for 3 months. The animals were killed by decapitation. The glands were removed immediately, dissected free of the pars nervosa, and the adenohypophyses were placed on moist hard filter paper in a Stender dish which was kept on ice until the collection was completed. The glands were weighed and placed in a sharp pointed homogenizing tube that contained 0.4 ml of cold 0.88 M sucrose. The tube was kept in an ice bath at all times except during homogenization. The glands were finely homogenized in 19 volumes of 0.88 M sucrose which was the concentration of sucrose used by Hogeboom, Schneider and Pallade.[3,4]

The pituitary homogenates were fractionated by differential centrifugation in the multispeed head of an International refrigerated centrifuge kept at 2°C. The method of fractionation as to time and speed of centrifugation is similar to that reported by Schneider[5] for the isolation of mitochondria from isotonic sucrose (8.5 g sucrose per 100 ml) homogenates of rat liver. The liver mitochondria were washed with isotonic sucrose and they were shown to contain the major portion of the succinoxidase activity of the homogenates. It should be pointed out that the granule fractions isolated from the pituitary homogenates were washed with 0.9% NaCl and this resulted in the separation of the gonadotrophin from these fractions. The method of fractionation is as follows:

1. The whole homogenate designated as *A* was centrifuged at approximately 500 × g for 10 minutes. The opaque extract was removed and the residue was washed twice by suspending in 0.88 M sucrose and centrifuging in the same way. The washings were combined with the above sucrose extract. The residue which consisted of cell nuclei and other insoluble material was designated as *B*.

2. The extract was centrifuged at 8,000 × g for 20 minutes. The aqueous sucrose extract was removed. The recovered precipitate (large granule fraction) was designated as *C*, and it was washed once by suspending in 0.88 M sucrose and centrifuging. The washings were added to the sucrose extract. The large granule fraction was suspended in 0.9% NaCl and centrifuged. This treatment was repeated one time. The washed fraction was suspended in NaCl and designated as *D*, and the combined NaCl washings as *E*.

3. Fractions containing small granules were recovered from the aqueous sucrose supernatant fractions of 2 groups of normal and the group of castrate rats by centrifuging for one hour at 15,000 × g. The small granule fraction was designated as *F;* it was washed twice with 0.9% NaCl. The washed granules were designated as *G*, and the washings as *H*. The aqueous sucrose extract from which the granules were removed was designated as *I*.

The fractions obtained by the above procedure were stained by a modification of the acid fuchsin methyl green method of Bensley[6] as an aid in estimating the homogeneity of the fractions.

The above fractions were assayed for gonadotrophic activity by use of 21-day-old female rats of the Holtzman strain. The volumes of the fractions were adjusted with saline for purposes of injection. Each rat received 0.5 ml of the proper fraction on the afternoon of the first day, and 0.5 ml on the morning and afternoon of the next 4 days. Autopsy was performed on the morning afte the last injection. The ovaries were removed, dissected free of other tissues and weighed. The qualitative effect as to the presence of follicles and corpora lutea was recorded. The degree of stimulation of the uteri was also noted.

The succinoxidase activity of the whole sucrose homogenate and the large granule fraction was determined by the use of a con-

[5] Schneider, W. C., *J. Biol. Chem.*, 1948, **176**, 259.

[6] Bensley, R. R., *Am. J. Anat.*, 1911, **12**, 297.

TABLE I.
Gonadotrophic Activity of Fractions Obtained from Fresh Rat Pituitary Glands by High Speed Centrifugation.

Kind of fraction	Total amount of fraction injected, mg*	Normal rats (4 groups) Avg wt ovaries, mg	Castrate rats (1 group) Avg wt ovaries, mg
A. Whole homogenate	5		89 (3)†
	10	126 (5)†	153 (3)
	20	190 (9)	
B. Residue (nuclei and connective tissue)	20	15 (10)	18 (3)
	100	24 (2)	47 (2)
C. Large granule fraction washed once with 0.88 M sucrose	10		28 (3)
	20	63 (7)	
D. Large granule fraction washed twice with 0.9% NaCl	20	(A) 59 (3)‡	19 (3)
		(B) 16 (6)	
E. Washings from large granule fraction	20	31 (6)	33 (3)
F. Small granule fraction	10		105 (3)
	20	87 (6)	
G. Small granule fraction washed twice with 0.9% NaCl	20	15 (3)	
H. Washings from small granule fraction	10		100 (3)
	20	50 (3)	214 (3)
I. Supernatant liquid from centrifuging at 15,000 × g	10		97 (3)
	20	44 (8)	

* Amount of fraction equivalent to the milligrams of fresh tissue indicated.
† Figure in parentheses indicates number of rats used in assays.
‡ The high value for the first two groups of rats (A) is probably due to incomplete extraction of the granules since a smaller volume of sodium chloride solution was used than for the granules of the other groups of rats (B).

ventional Warburg apparatus according to the method used in this laboratory (McShan and Meyer[7]).

Results and discussion. Succinoxidase activity. The large granule fractions C obtained from two groups of normal rats contained approximately 60% of the total succinoxidase activity found in the whole homogenates. Fraction C consisted largely of mitochondria as shown by examination of stained smears. In this connection it is of interest that Schneider,[1] and Hogeboom, Claude, and Hotchkiss[2] reported that 70 and 74%, respectively, of the succinoxidase activity of rat liver is present in the large granule fraction. Hogeboom, Schneider, and Pallade[4] prepared rat liver homogenates in 0.88 M sucrose and recovered 65 to 82% of the succinoxidase activity in the mitochondria.

Gonadotrophin. The results obtained on assay of the various fractions of rat pituitary tissue are summarized in Table I. The results obtained with the whole homogenates designated as A in Table I show, as is already known, that rat pituitary glands contain a high level of gonadotrophin, and that the level of glands from castrate animals is greater than that of glands from normal rats. Very little gonadotrophic activity was retained in Fraction B, which consisted of various elements such as nuclei and connective tissue. These elements comprised a high percentage of the solid content of the glands.

The large granule fraction C consisted to a large extent of mitochondria as shown by the staining method of Bensley.[6] The small granule fraction F contained many fewer mitochondria than did fraction C. The re-

[7] McShan, W. H., and Meyer, R. K., *Arch. Biochem.*, 1946, **9**, 165.

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.