moved with the rubber tube in the vertical position since the rubber will distend slightly due to the pressure of the mercury column. When the remaining rubber tube is completely filled with mercury a metal obturator is inserted and several turns of thread applied tightly around both the obturator and the distal terminal.

Application of electrodes. The splanchnic nerves are exposed through bilateral dorsal incisions. The nerves are carefully dissected free of surrounding tissue. An electrode is placed around each nerve and the obturator tied in place. Two or three turns of linen ligature around the rubber tube and through adjacent fascia holds the electrode in position so that the course of the nerve is altered as little as possible. The mercury lead is then brought through the incision by a circuitous route and finally through the skin approximately two inches from the spine at about the level of the last rib. Anchoring the leads to bony structures and fascia at several points aids in securing the electrode in the proper position and prevents any pull directly on the nerve. Small flexible wire leads connect the mercury leads to a terminal block on the gravel screen saddle used to protect the leads (Fig. 9). The animal is tied to one end of a rectangular cage which allows him to lie down or stand at will but prevents turning completely around, thus preventing destruction of the leads to the stimulator.

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17205. Urinary Excretion of Digitoxin in the Rat.*

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Heretofore it has not been possible to measure the actual renal excretion of digitalis glycosides. However, by means of the embryonic duck heart method, we have been able to measure in a quantitative fashion minute amounts of digitoxin (Sandoz) in the 24-hour urine volumes of the rat.

Methods. The 24-hour urine sample of a rat is concentrated to 10 ml volume by boiling. Three grams of infusorial earth are added and the mixture dried at 80° *in vacuo*. The dried residue is broken up with a glass rod, 50 ml of U.S.P. grade chloroform is added, and the mixture is shaken for one hour in a mechanical shaker. The chloroform extract is separated by filtration through paper. The residue on the paper is washed 3 times with fresh 10 ml portions of chloroform, the solvent being pressed out of the residue after each washing. The washings are combined with the filtrate, the extract is concentrated to a small volume by boiling, and is then dried at 80°C *in vacuo*. The dried extract is taken up in 1 ml of ethanol by shaking for one hour. Ten ml of water is added to the alcohol and the solution is concentrated to 1 ml by boiling. Another 10 ml of water is added and the solution again concentrated to 1 ml. This concentrated solution is diluted to a final volume of 60 ml by the addition of 59 ml of Tyrode's solution (without glucose). Embryonic duck hearts are exposed to this latter solution and the time of "digitalis effect"[1] observed and the quantity of digitoxin estimated according to previously described methods.[2] It thus was found possible to detect as well as measure as little as one microgram of digitoxin added to the 24-hour urine sample of the rat. A quantitative differentia-

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1 Friedman, M., and Bine, R., Jr., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 162.

2 Bine, R., Jr., and Friedman, M., *Am. J. Med. Sci.*, 1948, **216**, 534.

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.