parts of the tubule distal to the damaged portion was as already described for the normal tubule. The sharp and uniform localization of a necrotic portion within the proximal convolution indicates an antecedent functional differentiation in this portion which predisposed it to injury in this instance. Repair of the damaged epithelium was shown to have been active (Fig. 4, "X").

6496

Further Studies on Extracts Made from Holly.

RUSSELL A. WAUD.

From the Department of Pharmacology, University of Western Ontario Medical School, London, Canada.

The author has shown that extracts made from holly have a digitalis-like effect upon the frog's heart.[1] The present work is a continuation and a more detailed study of the same problem, and an attempt to isolate the active principle in pure form. Some species of holly contain caffeine. A number of other substances have been isolated. Pancoast[2] found the berries of American holly *(Ilex opaca)* to contain pectin, albumin, 2 crystalline principles and organic salts of potassium, calcium and magnesium. The leaves of the European holly *(Ilex aquifolium Linne)* have been more carefully examined than those of any American species. They are said to contain a bitter principle, ilicin, a yellow coloring substance, ilexanthin, and a peculiar acid, ilicic acid. According to Allen's laboratory[3] Mate' which consists of the leaves of *Ilex paraguayensis* contains 0.13% caffeine.

Three species of the Ilex (Family Aquifoliaceae) were investigated, *I. opaca* (American Holly), *Ilex aquifolium* (European Holly) and *Ilex paraguayensis*. Both the fruit and the leaves of the European and the American holly were extracted but the leaves only of the South American species were available.

Preparation of Extracts. The first extracts were prepared by macerating the drug with alcohol. When used, the greater part of the alcohol was evaporated off and replaced by saline solution. Attempts were made to obtain the substance in the pure state and if

[1] Waud, R. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 976.

[2] Pancoast, D. P., *Am. J. Pharm.*, 1856, **28**, 314.

[3] Allens Commercial Organic Analysis, 4th ed., **6**, 642.

possible crystalline form. It was not possible, however, to obtain a crystalline substance, which, when tested pharmacologically, gave the characteristic effects. The following method, however, yielded a fairly pure form of the active substance. The drug was macerated with alcohol for 12 hours; the alcohol was then evaporated off and the residue taken up in water, placed in a separatory funnel and shaken 4 or 5 times with chloroform. Here an emulsion was formed which when allowed to evaporate left a brown powder. This was further purified by solution in alcohol and passage through charcoal.

The substance thus obtained is an amorphous powder soluble in alcohol, but insoluble in chloroform, as was proven by the following experiments: Holly berries in No. 20 powder were macerated with chloroform, filtered and allowed to evaporate spontaneously. The yellowish amorphous residue was then taken up in saline and allowed to perfuse through the frog's heart. The results were negative. Also the purified active substance was extracted with chloroform for several hours and then filtered. It was found that the active substance was not contained in the chloroform soluble portion, but remained behind. This would lead one to believe that the separation by shaking with chloroform is brought about by absorption rather than by solution. The murexide reaction is negative. After boiling with acid and subsequent neutralization the subjection of the solution to Benedict's test for sugar showed no evidence of reduction.

Attempts to isolate the active principle by the methods employed in the separation of Ouabain yielded negative results.

Pharmacodynamic Action. The pharmacology of the substance was investigated by (1) the one hour frog method for the standardization of digitalis bodies, (2) the perfused frog's heart, (3) the isolated rabbit's heart, (4) blood pressure in the rabbit, (5) the isolated uterus. The general effects of the drug were found to be very similar to those of digitalis bodies and when compared with the Canadian standard digitalis on the same frogs, 4 gm. of the crude drug was found to be equal in activity to 1 gm. of digitalis leaves.

For perfusion of the frog's heart a cannula was placed in the inferior vena cava. This was attached to a 3-way stopcock which in turn was connected with 2 aspirator bottles, one containing Ringer's solution and the other Ringer's solution plus the drug. The aortic output was determined by collecting and measuring the amount of fluid expelled from the aorta per unit of time. A perfu-

sion pressure of 45 mm. was maintained throughout the experiments. The contractions were recorded on a drum with a time marker and signal magnet for recording of the time of changing solutions.

When perfused in a ratio of 1-3000 to 1-1000 solution the frog's heart showed after a time an increase in the size of the beat, the systole being more complete while relaxation in diastole is unchanged or slightly increased; as perfusion proceeded the contraction in systole becomes more complete and the diastolic relaxation was lessened until the ventricle stopped in systolic standstill. If the drug is not washed out at this point the heart remains in the contracted condition. However, if the drug is replaced by pure Ringer's solution there is some relaxation. In the contracted condition the sinus continues to beat for several hours while the auricle is widely dilated. With the proper concentration of the drug the heart would continue to beat for hours with a considerable increase in the height of contraction and aortic output over that which existed in the first hour of perfusion before the drug was added.

If the concentration of the drug is too high the therapeutic stage is absent and the heart almost immediately goes into systolic standstill from which it does not recover.

The rabbit's heart was perfused in an apparatus similar to that designed by Eyster and Loevenhart,[4] the only important change being that the water bath was kept in motion by means of an electric windshield wiper to which 2 paddles were attached. This has the advantage over the ordinary stirrer in that there is less noise and vibration and the agitation is more complete. When perfused by the above method the rabbit's heart showed essentially the same changes as the frog's heart.

In the blood pressure experiments the rabbit was arranged for injection of the drug and the recording of blood pressure in the usual method. With comparatively large doses there were no appreciable changes in the blood pressure, nor were any other effects on the animal noted.

In the uterine experiments one horn was removed from a freshly killed guinea pig and suspended in a muscle tube filled with saline with the proper amounts of potassium, calcium, sodium bicarbonate and dextrose. The solution was maintained at 37° C. and freely oxygenated. The addition of varying amounts of the drug to the saline bath containing the uterine strip produced no evidence of oxytocic activity.

[4] Eyster, J. A. E., and Loevenhart, A. S., *J. Pharm. Exp. Ther.*, 1913, **5**, 57.

As caffeine has been found in some species of Ilex, and as it is known to have a definite effect upon the heart, the question arises as to whether this effect is due to caffeine. It does not seem possible for the following reasons: (1) The substance considered here does not respond to any of the ordinary chemical tests used for the detection of the Zanthine compounds. (2) When caffeine is extracted from some species of Ilex and perfused through the frog's heart the effect produced differs definitely from that obtained with the substance studied in this work. (3) Species of the Ilex in which no caffeine can be demonstrated show the characteristic effect.

Summary. The pharmacological action and method of preparation of a substance obtained from various species of Ilex are described. The action of the drug resembles very closely that of the digitalis bodies. It produces first an increase in the amplitude of the heart by increased relaxation and increased systolic contraction, followed by decreased relaxation in diastole with slowing and finally systolic standstill. All attempts to obtain the substance in crystalline form were unsuccessful. The possibility of the substance being caffeine is considered.

6497

Effect of Peritoneal Lavage in Acute Uremia.

E. v. HAAM AND A. FINE.

From the Department of Pathology, Charity Hospital, and the Medical Center, Louisiana State University, New Orleans.

Ganter,[1] Landsberger and Gnoinski,[2] Rosenak and Siwon[3] report favorable results of peritoneal lavage in nephrectomized rabbits and dogs. The end products of nitrogen metabolism dialyze into the fluid and can be removed with it. Bliss and his coworkers[4] report that the survival period of nephrectomized dogs treated with peritoneal lavage is from 13 to 16 days as compared with the survival period of untreated dogs, from 2 to 3 days. The experiments re-

[1] Ganter, G., *Muenchen Med. Wchnschr.*, 1923, **70**, 1478.

[2] Landsberger, M., Gnoinski, H., *Compt. rend. Soc. de Biol.*, 1925, **93**, 787.

[3] Rosenak, St., Siwon, P., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1926, **39**, 391.

[4] Bliss, S., Kastler, A. O., Nadler, S. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1078.