intracellular phase of most tissue cells, a wash-out of an extracellular pool of hyperosmotic sodium concentration appears indicated, *viz.*, from the vasa recta and adjoining medullary interstitial spaces. However, this could be supplemented by more complete removal of intraluminal sodium, and perhaps more complete removal of sodium in transit through those cells of the nephron active in sodium reabsorption. However, except in the loop of Henle, this should not be greatly in excess of that in the arterial plasma.

In the absence of quantitative data on renal plasma flow, further speculation on the mechanism of wash-out is unwarranted. For this reason also, no attempt has been made to calculate the rate of restoration of osmolality during the post-transfusion recovery period, where errors would be greater because of the greater volume of plasma flow. It is of considerable interest that the kidneys in the present series showed signs of recovery of concentrating power even as the animals as a whole often gave indication of passing into irreversible shock.

Summary. The influence of hemorrhagic shock on renal clearance of PAH, creatinine, osmolar constituents, and sodium was examined in relation to changes in the kidney's ability to concentrate urine. By comparison of renal venous and arterial concentrations of osmolality and sodium, and relating these to changes in C_{CR}, C_{PAH}, and urine volume, evidence was supplied which appeared to support the hypothesis that the loss of concentrating power was ascribable to wash-out of the papillary zone of hyperosmolarity.

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Effect of Hypercholesterolemia on Digoxin Tolerance. (27874)

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The effect of a marked elevation of serum cholesterol on digitalis tolerance was determined by performing digitalis bioassays in normocholesterolemic and hypercholesterolemic male rabbits.

Method and materials. The control animals consisted of 21 male adult New Zealand albino rabbits, average weight 3048 g (range 2607-3647). Seventeen male rabbits of similar age, breed, nutrition and weight (average 3127 g with a range of 2840-3820) composed the hypercholesterolemic group. Hypercholesterolemia was produced by feeding a diet containing 1% cholesterol for from one to 8 weeks. This diet was prepared by mixing 10 g of cholesterol dissolved in 50 g soy bean oil with 1,000 g Purina rabbit pellets.

Sodium pentobarbital (Nembutal), 65 mg was injected intraperitoneally and was followed in 10 minutes by light ether anesthesia. Blood was obtained for cholesterol(1) and potassium(2) determinations. Digoxin,*

* Lanoxin, Burroughs Wellcome & Co., Tuckahoe, N. Y.

0.8 mg per kilo per hour was infused intravenously in 5% aqueous glucose solution at one cc per minute with a constant rate infusion pump *via* the central ear vein. Continuous electrocardiographic monitoring was accomplished throughout the bioassay, utilizing implanted needle electrodes. The end point for the digoxin bioassay was the absence of all electrical activity for one minute.

The animals were selected at random in order to assure that the investigator performing the bioassay would be uninformed whether any particular rabbit was in the control or hypercholesterolemic group. An autopsy was performed on each animal to verify the absence of gross disease. Atheromata were noted and observations made on the condition of the liver.

Results. The lethal dose of digoxin in the 21 control rabbits required an average of 94.7 minutes (range 51-130) to administer at the standard infusion rate. The lethal dose in the 17 hypercholesterolemic rabbits required an average of 118.3 minutes (range 76-166). This difference is statistically significant ($0.001 < P < 0.01$). The average time of onset of the initial digitalis induced arrhythmia in the control group was 81.6 minutes (range 44-117), as compared to 96.8 minutes (range 40-141) in the cholesterol group. This is barely significant ($0.1 > P > 0.05$).

Multiple cardiac arrhythmias were noted as the digitalis cardiotoxicity progressed. These included nodal tachycardia, A-V dissociation, premature ventricular contractions, and ventricular tachycardia. All animals expired with cardiac arrest. Ventricular fibrillation was not observed.

The mean serum cholesterol in the control rabbits was 57 mg % (range 36-90) and in the hypercholesterolemic group the mean serum cholesterol was 1395 mg % (range 316-2840). The serum potassium in the control group averaged 5.2 meq/l (range 4.3-6.3); in the hypercholesterolemic rabbits, the mean serum potassium concentration was 5.1 meq/l (range 4.1-5.9).

Discussion. It has been shown experimentally that digitalis, a plant sterol, may have both an estrogenic(3) and a corticosteroidal(4) effect. The estrogenic effect has been demonstrated by the positive uterotrophic action of digoxin in castrated female rats. The microscopic appearance of the endometrium following digoxin administration is similar to that produced by exogenous estrogens.

It has also been suggested that at least in the experimental animal(5,6) there is a significant difference between males and females in tolerance to digitalis. This appears, in part, to be estrogen dependent since an increase in digitalis tolerance has been demonstrated in castrated female dogs treated with exogenous estrogens(5,6). The estrogenic steroid providing the most resistance to digitalis proved to be a synthetic compound which insofar as its effects on vaginal smears were concerned, was non-estrogenic(6).

The corticosteroidal action of digitalis was recently studied by Vernikos-Danellis and Marks(4) with observations of pituitary suppression and adrenal hypofunction following administration of digitoxin. Further evidence has been provided by Zwemer *et al.*(7) who demonstrated that strophanthin, like adrenal cortical hormone, afforded some protection against both potassium intoxication and insulin (hypoglycemic) convulsions. In the adrenalectomized mouse, however, strophanthin cannot sustain life(8).

To date there have been no clinical observations in man relating serum cholesterol levels to digitalis tolerance. Both digitalis and cholesterol share the cyclopentaperhydrophenanthrene ring. However, all cardiac aglycones have in common an unsaturated lactone ring at C-17 and a hydroxyl group at C-14; both are missing in the cholesterol molecule.

Experimental studies by Karaulow(9) in 1911 with the isolated perfused frog heart indicated that addition of cholesterol to certain glycosides would reverse their cardiotoxic effects. However, this occurred only with the saponins, digitonin and helleborein. With the cardiac glycosides, strophanthin and a preparation of folia digitalis, no alteration in cardiotoxicity was observed on addi-

tion of cholesterol. The toxicity of digitalin, the water soluble portion of *Digitalis purpurea,* was also unchanged by cholesterol. Absence of a purified solution of digitoxin prevented Karaulow from making observations on the interaction of digitoxin and cholesterol.

Explanations for the increased tolerance to digoxin in the hypercholesterolemic rabbit are not clear. Several points must be considered.

There is a definite increase in fat content of the rabbit liver at the end of 2-4 weeks of cholesterol feeding. This might be associated with failure of the fatty liver to detoxify estrogens and in a sense provide some "estrogen protection" from digitalis cardiotoxicity. Paradoxically, however, there is evidence that poor liver function would predispose to digitalis cardiotoxicity as a consequence of inadequate digitalis degradation (10).

A second possibility is that cholesterol, itself, may exhibit an estrogenic effect since both compounds have a somewhat similar chemical structure. However, there is no experimental evidence to substantiate this.

Another consideration might be that hypercholesterolemia *per se* alters certain factors known to affect tolerance to digitalis. The most commonly associated electrolyte abnormality coexisting with and predisposing to cardiotoxicity is a low serum potassium concentration. In the present study there was no difference in serum potassium levels in control and experimental groups. Total body potassium was not determined.

Cholesterol might selectively compete with digitalis in tissue binding and as a result increase tolerance to digitalis. Cosmides and co-workers(11) demonstrated that lactones similar in chemical structure to the unsaturated butyrolactone portion of the glycoside molecule can protect against digitoxin poisoning. It was postulated that these lactones, tetrahydrofurfuryl alcohol and γ-butyrolactone may compete with the unsaturated butyrolactone of the cardiac glycoside in tissue binding.

Summary. A significant increase in digitalis tolerance was observed in hypercholesterolemic male rabbits when compared to the normocholesterolemic male rabbit. The explanation, however, is not clear but may be related to the chemical similarity of cholesterol to digitalis.

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