

taken in this study, but, histologically, the submaxillary gland appeared the most drastically affected by hypophysectomy.

Summary. 1) A histochemical study of location and concentration of RNA in the major salivary glands was made in animals which had been subjected to various experimental procedures. It was noted that only the acinar portion of the salivary gland exhibited RNA. None was found in the duct portion of any of the 3 major salivary glands. In the hypophysectomized rat RNA is strikingly reduced in the acini of all major salivary glands. Injection of cortisone does not appreciably affect this condition whereas thyroxine and testosterone in combination restore the RNA to normal concentration and location. 2) A progressive loss of thyroid function produced by injecting graded doses of I^{131} resulted in progressive loss of cyto-

plasmic RNA. This effect was obtained in submaxillary and sublingual glands. The parotid gland RNA was essentially unaffected by thyroidectomy.

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Effect of Estrogens on Myocardial Sensitivity to Toxic Effects of Digoxin.* (22999)

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In experiments previously reported at the Federation meetings, 1955, it was found that a single dose of 0.15 mg of digoxin/kg body weight, given intravenously to dogs under pentobarbital anesthesia, produced an arrhythmia characterized by complete A-V dissociation and persistent idioventricular tachycardia. The interval between administration of the glycoside and onset of idioventricular rhythm varied from 5 to more than 30 minutes. It was of considerable interest to us that female animals, without exception, exhibited the greatest delay in onset of ar-

rhythmia. This observation suggested that susceptibility to this particular manifestation of digitalis intoxication might be influenced by estrogenic hormones. To investigate this possibility, a new series of experiments was instituted, in which the time interval was measured with the greatest possible accuracy.

That such an influence exists is shown by data presented below, which includes experiments on normal males, normal females in anestrus, normal females in natural estrus, castrate females and castrate females treated with estrogenic drugs.

Materials and methods. Forty-four healthy, adult, mongrel dogs of both sexes, weighing 7 to 20 kg, were used. Of the 44, seven were normal males, 4 were normal females in anestrus, 3 were normal females in natural estrus and 30 were castrate females. The castrate females were spayed by technic which included both bilateral oophorectomy

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The digoxin used was given to us by Burroughs Wellcome & Co., (U.S.A.). The estradiol was synthesized in the laboratory of Dr. Max N. Huffman.

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TABLE I. Influence of Estrogens on Susceptibility to Digoxin Intoxication.

No. animals	Avg time for onset of arrhythmia (min.)	Range (min.)	Fate
4 normal ♀, anestrus	26.0	23- 31	All survived.
7 normal ♂	13.6	6- 17	Ventricular fibrillation; 5 dogs died; one treated, survived; one exp. termi- nated early.
13 castrate ♀, estrogen treated	31.7	23- 45	All survived.
17 castrate ♀	14.3	5- 22	Ventricular fibrillation; all died.
3 normal ♀, natural estrus	71.6	45-120	All survived.

and hysterectomy. The castrate animals were divided into 2 groups. Seventeen served as controls and the remaining 13 were treated with one of 3 estrogenic substances injected intramuscularly according to the following dosage schedule: Estrone 2 mg/day; Estradiol 2 mg/day, Diethylstilbestrol 10 mg/day. Experiments on estrogen-treated castrate female dogs were performed when the animals were in peak estrus as proved by vaginal smear technic. Acute experiments were carried out in the same manner on all animals. Food was withheld for 18 to 24 hours prior to experiment. Water was allowed *ad libitum* during the fasting period. The animal was anesthetized with pentobarbital sodium, 30 mg/kg body weight, and connected to a Cathode-Ray Electrocardiograph for continuous observation of electrocardiogram. Lead aVf was routinely employed. After normal electrocardiogram had been observed, digoxin was given intravenously at 0.15 mg/kg of body weight, and time of administration recorded. Time was again recorded when digoxin-induced idioventricular rhythm had become fully established. In the discussion and the Table we refer to this critical endpoint as the "onset" of arrhythmia. Animals in which ventricular fibrillation did not occur during observation period of 3 to 4 hours, are referred to as having survived, whether or not they were killed to terminate the experiment or saved for later use.

Results. The results of experiments are summarized in Table I. It can be seen that for 4 normal female dogs in anestrus, the average time required for onset of arrhythmia was 26 minutes, and not one fatality occurred. On the other hand, the average time of onset

of arrhythmia in 7 normal males was 13.6 minutes, and 5 of the 7 died of ventricular fibrillation. One experiment was terminated for technical reasons shortly after onset of arrhythmia, and one animal survived after treatment with potassium ethylenediamine tetraacetate according to our previous description (*Federation Proceedings*, 1955).

Untreated castrate females exhibited greatly increased sensitivity to toxic action of digoxin, indicated by average time interval of only 14.3 minutes. All 17 animals in this group died of ventricular fibrillation, the majority within one hour. The castrate female and the normal male are, therefore, almost identical with regard to their relative susceptibility to the toxic action of digoxin on cardiac rhythm.

The average interval of onset of the arrhythmia in 13 estrogen-treated castrate females was 31.7 minutes, which is not significantly different from the average for normal females in anestrus. All 13 of the estrogen-treated castrate females survived. The 3 estrogenic substances used appeared to be equally effective in restoring resistance of the castrate female animal.

Normal female dogs in natural estrus and nonpregnant, are difficult to obtain from our usual source of supply. The 3 animals of this type included in the Table show a resistance to digitalis which cannot be duplicated by estrogenic hormones alone in the castrate animal.

Summary and conclusion. The data in our study indicate that estrogenic hormones exert a protective action against the toxic effects of digoxin on the myocardium. Male dogs and castrate female dogs have been

found to be highly sensitive to toxic manifestations of a cardiac glycoside on cardiac rhythm, in comparison with normal females in anestrus, or castrate females treated with estrogenic substance. A high degree of resis-

tance to toxicity of digoxin was exhibited by the 3 normal females in natural estrus which were available.

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A Hook-like Instrument for Atraumatic Ligation of Minute Vessels.* (23000)

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The complete interruption of arterial blood flow to liver in rat presents a technical problem because of the wide anatomical variations and the very small size of vessels to be ligated. At least 3 arteries have to be tied. Chase Greene(1) describes the common hepatic artery, the gastroduodenal artery and the hepatic artery proper. Moreover, De Long(2) found a small collateral artery in

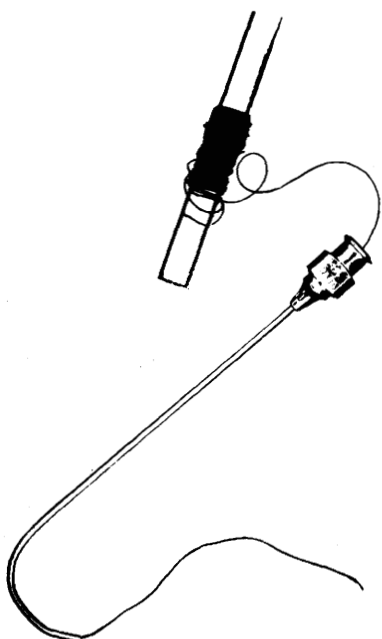


FIG. 1. Crochet hook-like instrument. Description in text.

* The author is indebted to Dr. Benito Lombardi, whose biochemical and histological studies in cirrhotic livers of rats have stimulated this work.

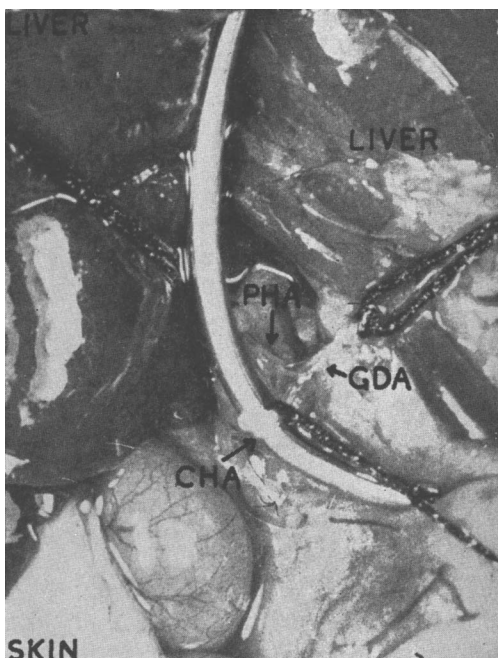


FIG. 2. First step of operation: Liver turned cranially. Gastroduodenal artery (GDA) and hepatic propria artery (PHA) are ligated. Hook passes thread around common hepatic artery (CHA). Note how large the #000 silk seems in comparison to minute size of vessels.

rats, arising from the esophageal artery and joining the intrahepatic branches of the hepatic artery proper. This artery always increased in size and became readily visible when he ligated the other three.

Having these anatomical details in mind, we were faced with the technical problem of ligating all 4 vessels with a minimum of