the intravenous injection of 5 mg per kg was between 2% and 4%, normal values for the monkey. A second possibility that could have accounted for the failure of inactivation was accessory venous drainage of the spleen by one or more routes that circumvented the liver. This possibility was examined in 2 of the animals at the time of removal of the pellet by injection 0.25 cc of bromsulphalein (50 mg/cc) into the spleen after clamping the splenic vein. Five minutes later a sample of peripheral venous blood was drawn and tested for the dye. None was found.

Clearly, sufficient amounts of estrogen passed from the spleen into the general circulation to elicit 4 of the characteristic responses ordinarily evoked by estrogens in the ovariectomized monkey: reddening of the sex skin, modification of the vaginal smear, growth and edema of the endometrium, and estrogen-deprivation menstruation. Moreover, the estrogen was absorbed from intrasplenic pellets of a size that provoke no responses in ovariectomized rats. In at least 2 of the animals venous drainage of the spleen by routes other than that through the liver was inadequate to transport detectable amounts of dye into the general circulation. It seems reasonable to presume, therefore, that the estrogen absorbed from the intrasplenic pellets passed through the liver. It is not known, of course, whether derangement of the ability of the liver to prevent estrogen's reaching the general circulation would exist in the presence of normal liver function as judged by other tests. In any case, the administration of B vitamins did not modify the responses given to the estrogen absorbed from the intrasplenic pellet.

It does not follow from these observations that the monkey's liver is unable to inactivate estrogens *in vivo*. It is apparent, however, that estrone and estradiol were not inactivated completely. Since, on the other hand, the liver of the rat completely inactivates estrogens absorbed from intrasplenic pellets (or prevents their reaching the general circulation), it seems evident that the monkey's liver exhibits less, perhaps much less, ability to inactivate estrone and estradiol. This finding is consistent with the report[22] of slight inactivation of estrogen by human liver *in vitro* as compared with the liver of the rat.

Summary. Intrasplenic implantation of pellets of estrone and estradiol in 4 ovariectomized monkeys was followed by reddening of the sex skin and alteration of the vaginal smear as promptly and with the same intensity as in a control animal given estradiol benzoate intramuscularly. Endometrial changes in the animals were alike, and removal of the intrasplenic pellet was followed by typical estrogen-deprivation menstruation. Liver function tests revealed no hepatic damage and administration of B vitamins did not reduce the activity of the intrasplenic estrogens.

15907

Effects of DCA on Digitoxin Toxicity in Cats.

HUGH CHAPLIN, JR. (Introduced by H. B. van Dyke.)

From the Departments of Medicine and Pharmacology, College of Physicians and Surgeons, Columbia University.

It has been noted that a number of patients with Addison's disease, while apparently being satisfactorily treated with NaCl added to the diet and supplementary DCA (desoxycorticosterone acetate), developed rather unusual cardiac irregularities, which in some cases have terminated rapidly in death. These deaths, apparently cardiac in origin, have appeared to be associated with an overactive vagus,[1] and have also resembled those follow-

ing an overdosage of a digitalis glycoside.

Addisonian patients have been suspected of being hypersensitive to vagal stimuli, a suspicion which has been strengthened by the experiments of Perera,[2] who demonstrated markedly elevated responses of these patients to small doses of Mecholyl. Pharmacological analysis of the action of the digitalis glycosides has revealed them to have effects directly upon the myocardium and myocardial conduction system and to increase the heart's sensitivity to local and central vagal stimulation. Other studies, stimulated by the empirical observation of a fairly consistent bradycardia in patients with obstructive jaundice, have shown certain of the bile salts to be capable of producing cardiac irregularities and death by both vagal and direct actions upon the heart.[3,4]

In view of the structural resemblance of the adrenal cortical steroids (DCA in particular) to the digitalis glycosides, and the bile salts, it was considered desirable to investigate the possibility that DCA might potentiate the toxic effects of a digitalis glycoside.

Methods. In order to study this effect, 2 groups of healthy cats (8 cats in each group) were selected, weighing between 2 and 4 kg. The cats in Group 1 received 10 mg of DCA* in sesame oil, by subcutaneous injection, every day for 10 days (100 mg in all)—half of this group was on a normal diet, half on a diet containing about 2 g additional NaCl daily. The cats in Group 2 received no DCA, but were similarly divided as to normal and high salt diets. On the 10th day of DCA administration, the DCA-treated animals and the control animals were subjected to the standard U.S.P. digitalis assay—using a pure glycoside, digitoxin.† The assay of the DCA-treated animals was performed between 3 and 6 hours after administration of the final injection of 10 mg of DCA. The cats receiving DCA seemed to be abnormally susceptible to serious central respiratory depression by ether, and 2 animals of this group died during the induction of anesthesia. The number of doses of digitoxin (0.0025%, 1 cc/kg being injected rapidly intravenously at 5-minute intervals) and the total time required for the onset of ventricular fibrillation were recorded for all animals. EKG tracings were made in all the animals (with an initial control record and subsequent tracings at 15-minute intervals). Continuous observation of the EKG pattern was made possible by the use of the Sanborn cardioscope. Immediately following the onset of ventricular fibrillation, blood was taken under oil from the inferior vena cava for direct Na and K determinations. The adrenal glands were weighed, and histological sections were made from samples of heart, kidney, and adrenal.

Results. There was no appreciable difference in the total lethal dose or in the time required for onset of ventricular fibrillation between the DCA-treated animals and the control group (Table I). There was no apparent effect of the high salt diet on the final results within the groups. The variations in the values for the direct Na and K determinations were so great that no significant elevation in Na or lowering in K could be demonstrated in the serum of the DCA-treated animals. Furthermore, the histological sections of the tissues of the DCA-treated animals did not show any consistent alterations from the control group. The EKG tracings varied widely, but were similar in the 2 groups; also, no difference could be noted by observing the continuous cardioscope tracing of each cat. The average weight of the paired adrenal glands was 542 mg for 5 cats of the control group and 393 mg for 6 cats of the group receiving DCA. The significance of the difference in the means, calculated by Fisher's method of computing t, was represented by $P = 0.05$. There was no

1 Loeb, R. F., *Harvey Lectures 1941-1942*, p. 124.

2 Perera, G. A., *J. A. M. A.*, 1945, **128**, 1018.

3 Wakim, K. G., Essex, H. E., and Mann, F. C., *Am. Heart J.*, 1939, **18**, 171.

4 Meltzer, S. J., and Salant, W., *J. Exp. Med.*, 1905, **7**, 280.

* Desoxycorticosterone acetate was generously made available by Dr. F. F. Yonkman, of the CIBA Laboratory.

† Digitoxin (Lilly Research Lab. No. 405741-A) was kindly donated by Dr. K. K. Chen, of the Lilly Research Laboratories.

TABLE I.
Lethal Effect of Digitoxin Assayed by Method of U.S.P. XII.

	Control animals			DCA-treated animals		
	No.	Lethal dose of 0.0025% digitoxin, cc/kg	Total assay time, min	No.	Lethal dose of 0.0025% digitoxin, cc/kg	Total assay time, min
Normal diet	1	10	45	A	12	50
	2	12	55	B	12	55
	3	15	70	C		
	4	13	66	D	13	62
Avg		12.5	59		12.3	55.7
High salt	5	15	72	E	11	54
	6	11	53	F		
	7	13	62	G	15	73
	8	13	63	H	12	55
Avg		13.0	62.5		12.7	60.7
Avg of combined groups		12.75	60.75		12.5	58.2

significant difference between average adrenal weights if these were expressed as percentages of the body weights ($P = <0.2$ but >0.1).

Conclusion. These observations failed to support the hypothesis that DCA potentiates the toxic effects of digitoxin.

15908

Relation of Adrenal Cortex to Serum Peptidase Activity.*

HALSTED R. HOLMAN,† ABRAHAM WHITE, AND JOSEPH S. FRUTON.
From the Department of Physiological Chemistry, Yale University, New Haven, Conn.

Attention has been drawn by several investigators[1-3] to the rapid hydrolysis of *l*-leucylglycylglycine (LGG) by the sera of rabbits, swine, horses, rats and humans. The splitting of LGG was attributed by Grassmann and Heyde[1] to the action of a serum "aminopolypeptidase," but a recent study[4] has presented evidence for the participation of at least 2 enzymes in the hydrolysis of this substrate by rabbit serum, and presumably by other sera as well. One of these enzymes has been identified as a manganese-activatable leucine aminopeptidase, for which *l*-leucinamide acetate (LA) is a satisfactory substrate. The other serum enzyme which splits LGG is a peptidase with a different, but hitherto undefined, specificity. Both of these enzymes are closely related in several properties to LGG-splitting enzymes found in extracts of skin and lung,[4] intestinal mucosa,[5] muscle,[6] and in leucocytes and lymphocytes.[7] In view of the widespread distribution, in

* This study was aided by grants from the American Cancer Society (on the recommendation of the Committee on Growth of the National Research Council), and the Josiah Macy, Jr., Foundation.

† James Hudson Brown Junior Fellow 1946-1947.

1 Grassmann, W., and Heyde, W., *Z. physiol. Chem.*, 1930, **188**, 69.

2 Maschmann, E., *Biochem. Z.*, 1941, **308**, 359.

3 Weil, L., and Russell, M. A., *J. Biol. Chem.*, 1938, **126**, 245.

4 Fruton, J. S., *J. Biol. Chem.*, 1946, **166**, 721.

5 Smith, E. L., and Bergmann, M., *J. Biol. Chem.*, 1944, **153**, 627.

6 Zamecnik, P. C., Stephenson, M. L., and Cope, O., *J. Biol. Chem.*, 1945, **158**, 135.

7 Husfeldt, E., *Z. physiol. Chem.*, 1931, **194**, 137.