A more plausible explanation for the increase in cyclopropane-adrenalin tachycardia in the hyperthyroid state and the decrease in the hypothyroid state seems to be that the thyroid hormone sensitizes the heart to adrenalin. A cardiac accelerator response to adrenalin greater than the control has been demonstrated when cats,[6] rabbits,[7] and terrapins[8] are in the hyperthyroid state. Thyrotoxic rabbit and dog hearts were reported as more susceptible to irregularities than normal controls.[9] An increased cardiovascular reaction to adrenalin is the basis for a clinical test for hyperthyroidism.[10]

Aumann and Youmans[11] recently demonstrated that the thyroid hormone sensitizes the excitatory adrenergic but not the inhibitory adrenergic neuro-effector division of the autonomic nervous system. The present work on cyclopropane-adrenalin tachycardia also seems to indicate that the thyroid sensitizes the excitatory adrenergic neuro-effector system.

Summary. Following thyroidectomy the duration of cyclopropane-adrenalin tachycardia was decreased in the dog. In the hyperthyroid state the period of tachycardia was longer than in the control.

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13677 P

Excretion of Estrogen in Bile.

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There is considerable evidence that the liver plays an important part in the metabolism of estrogens. This evidence may be summarized as follows: (a) incubation of liver slices with estrogens *in vitro* results in marked diminution in estrogenic activity;[1-7] (b) ex-

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8 Lewis, J. K., and McEachern, D., *Bull. Johns Hopkins Hosp.*, 1931, **48**, 228.

9 Rosenblum, H., Hahn, R. G., and Levine, S. A., *Arch. Int. Med.*, 1933, **51**, 279.

10 Goetsch, E., *Penn. Med. J.*, 1920, **23**, 431.

11 Aumann, K. W., and Youmans, W. B., *Am. J. Physiol.*, 1940, **131**, 394.

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traction of minced tissues after injection of estrogens results in recovery of only a small proportion of the quantity injected;[8, 9] (c) little or no estrogenic effect can be detected when ovarian tissue or estrogen pellets are implanted in the portal field;[10–13] (d) high levels of urinary excretion of free estrogen have been reported in patients with cirrhosis of the liver;[14] (e) the hypothesis of inactivation of estrogens by the liver has been supported by studies of the effects of endogenous and exogenous estrogens in normal animals and in animals with liver damage induced by hepatotoxic agents.[6, 15–17]

The chemical similarity between natural estrogens and bile acids and the fact that the latter, like the former, disappear rapidly from the blood and urine following intravenous injection in normal animals, but less rapidly in the presence of liver damage, suggested the possibility that estrogens, like bile acids, may be removed from the blood by the liver and subsequently undergo an enterohepatic circulation. The presence of estrogen in the bile after administration of estrone, estradiol and diethylstilbestrol has been reported by Stamler[18] and Dingemanse and Tyslowitz.[19]

Longwell and McKee,[20] employing bile-fistula dogs, found a maximum 72-hour excretion of 8% of the estrogenic activity of 1 mg of subcutaneously injected estrone, practically all of this being in the form of non-ketone estrogen, presumably estradiol. The

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2 Zondek, B., *Skand. Arch. Physiol.*, 1934, **70**, 133.

3 Israel, S. L., Meranze, D. R., and Johnston, C. G., *Am. J. Med. Sci.*, 1937, **194**, 835.

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5 Heller, C. G., *Endocrinology*, 1940, **26**, 619.

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7 Engel, P., *Endocrinology*, 1941, **29**, 290.

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11 Biskind, G. R., and Mark, J., *Bull. Johns Hopk. Hosp.*, 1939, **65**, 212.

12 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 259.

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14 Glass, S. J., Edmondson, H. A., and Soll, S. N., *Endocrinology*, 1940, **27**, 749.

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17 Segaloff, A., and Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 33.

18 Stamler, C. M., *Bull. de Biol. et de Méd. Exp.*, 1937, **3**, 31.

19 Dingemanse, E., and Tyslowitz, R., *Endocrinology*, 1941, **28**, 450.

20 Longwell, B. B., and McKee, F. S., *J. Biol. Chem.*, 1942, **142**, 757.

marked discrepancy between their findings and those reported here may be due to the relatively small dose employed by them and to the relatively slow absorption following subcutaneous injection of estrone in oil.

Experimental Observations. Studies were made of the excretion of estrogen in the bile of 2 cholecystectomized, bile-fistula female dogs, the bile draining externally into a balloon. Estrogen activity was determined by the biological assay method of Fluhmann,[21] against estrone as a standard. Conjugated estrogen was determined by preliminary boiling with HCl in a water bath at pH 1.0.

No estrogen activity was present in 24-hour specimens of bile obtained from untreated animals. The following findings were obtained following a single intravenous injection of 250,000 I.U. of estrone in 1 cc of absolute alcohol, none of the bile being refed: first 24 hours, free estrogen 100,000 I.U., total estrogen 120,000 I.U.; second 24 hours, free estrogen 88,000 I.U.; total estrogen 120,000 I.U. In another animal, following a single intravenous injection of 250,000 I.U. of estrone, aliquots of bile were taken for analysis daily for 3 days, about 90% being refed by stomach tube. The following results were obtained: first 24 hours, free estrogen 100,000 I.U.; second 24 hours, free estrogen 66,000 I.U.; third 24 hours, free estrogen 66,000 I. U. We have found, as have other observers, that the level of estrogenic activity in the blood after 3-4 hours is only about 10% of that at 10 minutes after intravenous injection, practically none being present after 24 hours. About 10% of the injected estrogen can be recovered from the urine during the first 24 hours, the quantity diminishing during the next 3 days.

The data presented here suggest that there is an enterohepatic circulation of estrogens, similar to that of bile acids. The fact that 34-48% of the quantity of estrogen administered can be recovered from the bile during the second 24 hours, a similar amount having been removed in the bile during the first 24 hours, indicates that the liver can store relatively large quantities of estrogen for at least this period of time.

Summary. Studies of the excretion of estrogen in the bile of bile-fistula dogs indicate that extremely large quantities are excreted by this route for at least 3 days following a single intravenous injection of estrone. Viewed in the light of the rapid disappearance of estrogen from the blood and its minimal excretion in the urine under such circumstances, this observation suggests the existence of an enterohepatic circulation of estrogens, similar to that of bile

[21] Fluhmann, C. F., *Endocrinology*, 1934, **18**, 705.

acids. It throws some doubt upon the hypothesis of *rapid* destruction or inactivation of estrogens by the liver in the intact animal. The liver appears to be capable of storing relatively large quantities of estrogen for at least 24 hours after administration of a single dose of estrone intravenously.

13678

Influence of Estrone upon Formation of Heterophil Antibodies.

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In a previous communication[1] we reported on the effect of the female sex hormone upon the formation of antibodies in rabbits immunized against Type I pneumococcus. Administration of estrone totaling 22 and 38 mg given before and during the process of immunization, produced a remarkable increase in specific agglutinating and protecting antibodies. The hormone alone did not elicit any specific antibody-response. Since it is known that Type I pneumococcus contains Forssman antigen (Powell and Jamieson[2]), it seemed of interest to investigate the effect of hormone-administration upon the production of heterophil antibodies, following the work of Levine, Bullowa, and Katzin.[3]

Material and Methods. Our above-mentioned sera were tested against 2% suspensions of washed human blood cells of groups AB, A, B, and O, and sheep erythrocytes. Serial dilutions of each serum were made in amounts of 0.15 cc beginning with a dilution of 1:2.5. To each diluted serum 0.1 cc of a 2% suspension of erythrocytes was added. The mixtures were shaken for 1 minute, incubated at 37°C for 2 hours and read macroscopically.

Results. Agglutinins for group A erythrocytes were demonstrable in the sera of the control animals up to a dilution of 1:2.5; for group AB, in 3 out of 6 animals up to a dilution of 1:5; for group B in the serum of only 1 rabbit to a dilution of 1:2.5. No agglutinins for group O and sheep erythrocytes could be demonstrated in any of the 6 ani-

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[2] Powell, G. H., and Jamieson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 248.

[3] Levine, P., Bullowa, J. G. M., and Katzin, E. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 617.