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Failure of the Liver of the Monkey to Inactivate Estrogens *in vivo*.*

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On the strength of numerous reports that the liver inactivates estrogens or prevents their reaching the systemic circulation in the rat,[1-8] guinea pig,[9,10] rabbit,[5,11] and dog,[12-14] it is often presumed that this is a universal hepatic action. For example, the testicular atrophy, gynecomastia, and changes in hair distribution frequently seen in men with cirrhosis of the liver,[15-17] the elevated level of urinary estrogens in men with infectious hepatitis[18] and hepatic cirrhosis,[19] as well as menstrual disturbances[20] and poor postpartum involution of the uterus[21] which may be relieved by treatment with the vitamin B complex have all been attributed to the failure of the liver in these patients to inactivate estrogens. As compared with that of the rat, however, human liver is reported[22] to exhibit much less capacity to inactivate estrogens *in vitro*, and the ability of the liver to inactivate estrogens *in vivo* has apparently not been examined directly in man or any of the primates. Accordingly, the capacity of the liver of the monkey to inactivate estrogens *in vivo* has been tested by implanting pellets in the spleen as has been done in lower animals. Since the venous drainage of the spleen is into the liver, estrogen absorbed from intrasplenic pellets is exposed to the action of the liver before reaching its target organs and, in the animals listed above, exerted none of its characteristic actions.

Five ovariectomized monkeys, *Macacus rhesus*, were employed. The diet consisted of Purina "Laboratory Chow" fed *ad libitum* and supplemented twice weekly with cabbage. On this regime the animals grow well and reproduce. At the end of a preliminary period of at least 2 weeks, during which daily vaginal smears stained by the method of de

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1 Zondek, B., *Lancet*, 1934, **2**, 356.

2 Golden, J. B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

3 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

4 Biskind, G. R., *Endocrinology*, 1941, **28**, 894.

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

6 Pincus, G., and Martin, D. W., *Endocrinology*, 1940, **27**, 838.

7 Segaloff, A., *Endocrinology*, 1943, **33**, 209.

8 Schiller, J., *Endocrinology*, 1945, **36**, 7.

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

10 Lipschutz, A., Becker, C., Mello, P. F., and Riesco, A., *Science*, 1945, **101**, 410.

11 Engel, P., *Endocrinology*, 1944, **35**, 70.

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

13 Cantarow, A., Rakoff, A. E., Paschkis, K. E., Hansen, L. P., and Walkling, A. A., *Endocrinology*, 1942, **31**, 515.

14 Pearlman, W. H., Paschkis, K. E., Rakoff, A. E., Cantarow, A., Walkling, A. A., and Hansen, L. E., *Endocrinology*, 1945, **36**, 284.

15 Edmondson, H. A., Glass, S. J., and Soll, S. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 97.

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

17 Morrione, J. G., *Arch. Path.*, 1944, **37**, 39.

18 Gilder, H., and Hoagland, C. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 62.

19 Glass, S. J., Edmondson, H. A., and Soll, S. N., *J. Clin. Endocrinol.*, 1944, **4**, 54.

20 Biskind, M. S., Biskind, G. R., and Biskind, L. H., *Surg. Gynec. Obst.*, 1944, **78**, 49.

21 Biskind, L. H., and Biskind, M. S., *West. J. Surg. Obst. Gyn.*, 1944, **52**, 266.

22 Twombly, G. H., and Taylor, H. C., *Cancer Research*, 1942, **2**, 811.

Allende, Shorr, and Hartman[23] were examined and the condition of the sex skin was recorded daily, a compressed pellet of estrogen was introduced into the spleen in a subcapsular position. In 2 animals the estrogen was estrone and in 2 animals estradiol. The pellets of estrone weighed 4.0 and 9.0 mg, the pellets of estradiol weighed 4.0 and 11.0 mg. In the hope of relieving any dietary deficiency in the vitamin B complex, which is reported[24] to decrease inactivation of estrogen by the rat's liver, 2 animals were daily given subcutaneously an aqueous solution of members of the B complex for a period of 10 days preceding the implantation of the pellet of estrogen and throughout the interval that the pellet (in one animal estrone and in the other estradiol) was in place. The daily supplement was 1 mg thiamine hydrochloride, 5 mg nicotinic acid, 1 mg riboflavin, 1 mg pyridoxine hydrochloride, 3 mg calcium pantothenate, 50 mg choline chloride.[25] Instead of an intrasplenic pellet of estrogen, the 5th monkey was given a daily intramuscular injection of 16.6 μg of estradiol benzoate dissolved in sesame oil.

The 5 animals responded in essentially the same manner. The sex skin reddened to the same extent and with the same promptness in the animals bearing intrasplenic pellets as in the animal given intramuscular injections. The vaginal smears prior to treatment in all of the animals were typically those of ovariectomized monkeys in being scanty and composed of leucocytes and detritus. Within 3 days after the introduction of the intrasplenic pellet of estrogen the vaginal smear had increased in amount and within 5 days was composed almost or entirely of cornified epithelial cells of types IIIb and IV.[23] The identity of the estrogen or the size of the pellet made no obvious difference in the intensity and promptness of either of the 2 responses, nor were the responses to the intrasplenic pellets in any way different from those of the animal given subcutaneous injections. The administration of the B vitamins was without evident influence.

After 9, 15 or 18 days the pellets were removed. Two animals were thereafter given no further treatment; each exhibited a typical estrogen-deprivation menstruation of 5 days' duration beginning 7 days after removal of the pellet. The other 2 animals were given a daily intramuscular injection of progesterone beginning on the day of removal of the pellet. The daily dose of progesterone in one animal was 0.5 mg, an amount that fails to inhibit estrogen-deprivation bleeding indefinitely.[26] This animal began menstruating 15 days later. The second animal was given 1.0 mg of progesterone daily for 20 days at which time a uterine biopsy was taken; 3 days after the last injection of progesterone menstruation started. The animal given the intramuscular injections of estradiol benzoate was so treated for 12 days; thereafter a daily intramuscular injection of 1.0 mg of progesterone was substituted. A uterine biopsy was taken on the 23rd day of the latter course of injections. The 2 biopsy specimens were alike in exhibiting a premenstrual endometrium with the typical glandular change, moderate edema, and large, vesicular stromal nuclei with distinct nucleoli and large chromatin particles.[27]

Inasmuch as the failure of the liver to inactivate the estrogen absorbed from the intrasplenic pellets might have been the result of deficient liver function (despite the administration of B vitamins to 2 of the animals), hepatic function was examined at the time of removal of the pellets by the bromsulphalein test[28] and the cephalin-cholesterol flocculation test.[29] The latter test was negative at 24 and 48 hours. The retention of bromsulphalein 30 minutes after

[23] de Allende, I. L. C., Shorr, E., and Hartman, C. G., *Carnegie Contrib. Embryol.*, 1945, **31**, 1.

[24] Biskind, M. S., and Biskind, G. R., *Endocrinology*, 1942, **31**, 109.

[25] Waisman, H. A., Rasmussen, A. F., Jr., Elvehjem, C. A., and Clark, P. F., *J. Nutrition*, 1943, **26**, 205.

[26] Hisaw, F. L., and Greep, R. O., *Endocrinology*, 1938, **23**, 1.

[27] Pfeiffer, C. A., and Hooker, C. W., *Yale J. Biol. and Med.*, 1944, **17**, 249.

[28] Rosenthal, S. M., and White, E. C., *J. A. M. A.*, 1925, **84**, 1112.

[29] Bodansky, A., *J. Biol. Chem.*, 1937, **120**, 167.

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.

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Effects of DCA on Digitoxin Toxicity in Cats.

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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-