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Early Permeability Effects of Estradiol on Castrate Rat Uterus.* (25673)

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The view that the primary mechanism of action of various hormones at a cellular level may involve regulation of permeability processes in target cells is being investigated in this laboratory. We previously studied cellular permeability effects of ACTH upon the adrenal(1) and have reported certain similarities between this action of ACTH and that of insulin on muscle fibers(2,3). In the present work we examined the early effects of estradiol-17β upon permeability processes in castrate uterus (using C^{14} labeled "non-utilizable" sugars, α-aminoisobutyrate (AIB), and Na^{22}) to determine whether the primary action of estrogen might be related to increasing substrate entry into the cell. Alterations in carbohydrate metabolism in isolated uteri following estrogen administration *in vivo,* have been ascribed to increased transfer of glucose into the cell(4). The effect of estrogen to increase amino acid incorporation into uterine protein under similar conditions(5) might likewise be considered as the result of increased amino acid transport, inasmuch as Noall *et al.*(6) have shown that estrogens administered 20 hours previously specifically increase accumulation of AIB in the uterus. Our results indicate that estradiol-17β does not increase uterine permeability to substrates at a time when changes in uterine metabolism of carbohydrate and protein are evident.

Methods. Sprague-Dawley rats (200-250 g), ovariectomized 4-7 weeks previously, were functionally nephrectomized at time of experiment to maintain relatively constant blood levels of radiotracers(1). In most cases they were adrenalectomized 48 hours prior to experimentation to remove possible inhibition by corticoids (liberated during stress of kidney ligation) of uterine response to estrogen(4,7). After ligation of both kidney pedicles under

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Avertin anesthesia, one of the following tracer substances was injected subcutaneously: inulin-C^{14} (carboxyl label); sucrose-C^{14} (uniformly labeled); D-xylose-1-C^{14}; α-aminoisobutyric acid-1-C^{14} or Na^{22}. Ninety minutes after tracer injection, plasma from aortic blood was obtained and at the same time uterus and certain other tissues were excised, weighed and prepared for radioactive assay as described elsewhere(1). *Uteri were slit longitudinally and blotted between filter paper in standard manner before weighing to remove luminal fluid.* Effects of estradiol-17β were determined 1.5 and 6 hours after single injection (10 μg in 1 ml saline-PO_4 into tail vein). For the 1.5 hour interval the hormone was given immediately prior to radioactive tracer. In those cases where estradiol was given for 6 hours, the hormone was administered 4.5 hours before kidney ligation and tracer injection.

Results obtained in functionally nephrectomized, adrenalectomized, castrate rats are shown in the Table; essentially similar results were obtained when adrenals and/or kidneys were not removed. Estradiol produced the expected increase in wet weight and water content of the uterus after 6 hours, but no significant changes were detected at 1.5 hours. The Table also shows percent volume of distribution (counts/g wet tissue/counts/ml plasma) of various tracer substances studied in uterine tissue, with the italicized values in the Table representing volume of distribution of radiotracers in total tissue water. No change was seen 1.5 hours after estradiol administration in distribution of sucrose, Na^{22}, D-xylose or AIB; either in terms of wet weight or total tissue water. The inulin space was significantly increased 90 min after estrogen, but was not further increased at 6 hours. Six hours after estrogen, there was a small but significant increase in distribution of D-xylose and AIB over the control level, which was not accompanied by significant change in sucrose or Na^{22} spaces.

Discussion. To assess the significance of the results in the Table in terms of entry into cell water, it is necessary to have a measure of extracellular space. Inulin, sucrose (and Na to a lesser extent) are excluded from the

TABLE I. Effect of Estradiol-17β on Weight and Water Content of Castrate Rat Uterus and Volume of Distribution of Radiotracers.*

Interval after estradiol inj., hr	Wet wt, mg	Water content, ml/g	Vol of distribution (%/g) in tissue ± S.E. and No. of rats (Italicized: Vol of distribution in total tissue water)				
			Inulin-C^{14}	Sucrose-C^{14}	Na^{22}	D-xylose-C^{14}	AIB
0	55 ± 1.5 (67)	.76 ± .003 (5)	43.8 ± 1.4 (19) *58*	57.6 ± 1.4 (30) *76*	58.4 ± 2.1 (7) *76*	69.7 ± 1.9 (32) *92*	102.8 ± 7.1 (13) *135*
1.5	56 ± 2.9 (20)	.76 ± .007 (5)	54.7† ± 1.4 (9) *72*	54.8 ± 1.5 (16) *72*	61.1 ± 3.4 (5) *80*	71.2 ± 3.6 (16) *94*	107.6 ± 13.2 (7) *142*
6.0	87 ± 4.8 (29)	.83 ± .015 (6)	55.9† ± 2.3 (6) *67*	61.1 ± 2.2 (6) *74*	56.5 ± 3.8 (4) *68*	78.9‡ ± 2.5 (9) *95*	147.8‡ ± 10.0 (8) *178*

* Dose levels: Inulin-C^{14} (New England Nuclear) 1.3 μc (0.95 mg); sucrose-C^{14} (New England Nuclear) 1.29 μc (0.17 mg); Na^{22} (Nuclear Science and Engineering Corp.) 1.0 μc (trace); D-xylose-1-C^{14} (Nat. Bureau of Standards) 1.09 μc (0.48 mg); AIB-1-C^{14} (Volk Radiochemicals) 0.71 μc (0.94 mg). Dose levels per 100 g body wt.

† Level of significance: $p < .001$.

‡ Level of significance: $p < .01$, $> .001$.

cell water of many tissues and have accordingly been widely employed as measures of the extracellular compartment of tissues. In uterus, however, all of these substances distribute in such a large fraction of the total tissue water that it is not possible to assume that they are, in fact, excluded from the cell water. In untreated castrate uterus, inulin distributes in 58% of total tissue water and likewise sucrose (and Na^{22}) distribute in a very large fraction of total uterine water (76%). Comparable values for sucrose distribution in the total water of other tissues from these same rats are 25, 19 and 15% for intestine, diaphragmatic muscle and thymus respectively. All of these tissues show the same order of wet wt. : dry wt. ratios, and furthermore the histological appearance of castrate uterus makes it difficult to visualize an extracellular space of the order suggested by inulin or sucrose. Accordingly, it would appear that in the castrate rat uterus, inulin, sucrose and Na^{22} enter into cells; the space occupied by inulin, in absence of estrogen, being somewhat less than that of sucrose or Na^{22}. Cole(8) using thiocyanate as a measure of extracellular volume in uterus, found a tissue distribution of 29%, a value lower than that indicated by inulin which would be consistent with the idea that inulin penetrates into many of the cells in castrate uterus. Pappius and Elliott(9) experienced similar difficulties in attempting to define the extracellular volume in cerebral cortex and likewise were forced to assume penetration of sucrose and Na into the cell.

Independent of whatever value is taken for extracellular volume, our results demonstrate that in absence of estrogen, D-xylose is not excluded from uterine cell water, and AIB is accumulated against an apparent concentration gradient. Where hormones do increase permeability of target cells to sugars, as in the case of insulin and ACTH, it is to be noted that barriers exist which limit the entry of D-xylose, such that the sugar is excluded from intracellular regions which are subsequently made available when hormone is present. Accordingly, the fact that estrogen has no effect on D-xylose entry in uterus is not surprising since barriers which exclude sugars do not appear to be present in this tissue. In similar fashion, estrogen has no effect on AIB transport equivalent to the early stimulation of uptake by ACTH in adrenals and insulin in muscle. The enhanced uptake of AIB which can be detected at 6 hours and is well established at 20 hours(6), appears to be one of many secondary responses of the uterus to estrogen administration. Six hours after estradiol administration, many secondary events have already taken place(5).

If the effects of estrogen on excised uterine metabolism(4,5), clearly shown one hour after intravenous injection of the hormone, were due primarily to an action of estrogen to increase substrate entry, then one would expect to see changes in sugar and amino acid permeability 1.5 hours after hormone administration. The fact that such changes do not occur, coupled with the lack of exclusion of substrates from uterine cells, indicates that estrogen *via* some primary mechanism activates enzyme systems concerned in carbohydrate and protein metabolism independently of increased substrate entry into the cell.

Summary. The influence of estradiol-17β on distribution of certain non-metabolizable sugars, the "non-utilizable" amino acid (AIB) and Na^{22} in uterus of functionally nephrectomized castrate rats was examined. Uterine cells did not exclude D-xylose-1-C^{14} in absence of estradiol, and no effect on uterine permeability to D-xylose-1-C^{14} or α-aminoisobutyric acid-1-C^{14} was observed 1.5 hr after hormone administration, at which time changes in uterine metabolism of carbohydrate and protein have been established.

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Selenium and Vit. E as Related to Growth and White Muscle Disease in Lambs.* (25674)

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Interest has been focused recently upon the effects of 2 dissimilar entities—selenium and Vit. E—in overcoming certain muscular dystrophies. Those dystrophies caused by feeding experimentally-compounded, Vit. E-deficient diets have been cured by addition of dietary Vit. E in rabbits(1), guinea pigs(2), mice(3), lambs(4) and chicks(5). Vit. E has also proven effective when added to dystrophogenic diets high in unsaturated fats(6). On the other hand, minute amounts of selenium have been found to protect chicks on torula yeast diets against exudative diathesis (7) and similarly-fed rats(8) and mice(3) against hepatic and multiple necroses, respectively. In Oregon, 0.1 part/million of selenium in dry matter of ewes' diet has protected lambs from white muscle disease (WMD) when their mothers were fed pre-partum on a "causative" ration(9). Some syndromes provide evidence that Vit. E and selenium may act in distinctly different ways. Thus, studies of Schwarz and associates showed that Factor 3-active selenium compounds were ineffective against rabbit muscular dystrophy on Vit. E-free diets(10) and inactive in rat resorption-gestation technic for Vit. E bioassay(11). Further, the Oregon workers showed that neither parenteral nor oral administration of Vit. E to ewes pre-partum would prevent WMD in their lambs, while oral selenium proved highly protective(9). Since previous studies suggest a growth stimulation in chicks(12,13) and in lambs(14) resulting from sub-toxic levels of selenium supplementation, it has become of interest to investigate the effects of Vit. E and selenium upon growth and dystrophy prevention simultaneously in sheep exposed to diets conducive to WMD. Additionally, it was desired to confirm and extend earlier findings as to control of WMD by administration of selenium. This paper reports on growth rate and incidence of WMD in lambs whose mothers received a dystrophogenic Ladino hay diet with and without selenium and Vit. E.

Materials and methods. Forty-eight mature, pregnant, crossbred ewes were randomly assigned to 4 lots housed in identical pens bedded with wood shavings. Lot 1 was fed the basal ration of 4 lb. Ladino clover hay and ¼ lb. ground oats per head daily. Lot 2 and Lot 3 ewes received the same basal ration; in addition the Lot 2 lambs received 1.40 mg Se, as aqueous Na_2SeO_3 inj. intramuscularly, while Lot 3 lambs were each given 2,000 IU Vit. E orally. Lot 4 ewes received the basal diet supplemented with 0.1 ppm selenium as Na_2SeO_3 calculated on basis of total ration dry matter and added to the ground oats. Diet treatments commenced about third month of gestation and continued until lambs were 6 weeks old. All groups received water and iodized salt, *ad lib.* Lambs in Lot 2 received selenium in 2 injections: the first, at 1 day of age, supplied 0.28 mg, while the second, at 14 days, supplied 1.12 mg Se. The sum of these doses was calculated to provide 0.2 ppm Se in estimated dry matter intake for the first 6 weeks of life. Vit. E was given in aqueous suspension, (Type "F-50" containing 50,000 IU Vit. E/lb, supplied by Distillation Products Industries, Rochester, N.Y.) in 2 equal doses at 1 and 4 days of age. The lambs were weighed at birth, 2 weeks and 6 weeks of

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