Sex Difference in Lipid Content of Adrenal Glands in Mice. (26345)

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Sex differences in adrenal cortical sudanophilia(1) and in eosinophil counts(2) have been observed in mice. While dissecting adrenals of several inbred strains of mice it was noted that female adrenals appeared grossly larger, and more yellow and opaque than those of males which were somewhat translucent. A study was therefore undertaken to characterize further the adrenal cortical sex difference in lipids by histochemical and chemical means. Eosinophil counts were also done in an attempt to obtain simultaneous physiologic data.

Materials and methods. Eleven male and 11 female virgin 7 months old STR/N mice were maintained for 7 months after weaning on sawdust in plastic cages at 26.5 ± 1.0°C. They received Purina Laboratory chow with water *ad libitum*. Between 2 and 3 p. m. male and female mice were alternately sacrificed with ether and exsanguinated from the right ventricle of the heart with heparinized syringes. Eosinophil counts were made in a Levy counting chamber using the diluting fluid of Speirs(3). The adrenals were weighed individually on a 50 mg Roller-Smith precision balance. One adrenal of each animal was fixed in 10% formalin, frozen sections were made and one slide of each was stained with hematoxylin and eosin (H & E) and a second with oil red O(4).

The other adrenal was frozen immediately and set aside for tissue analysis. The protein was estimated following micro-Kjeldahl digestion and analysis for nitrogen with Vanselow's modification of Nessler's solution(5). Total lipid was determined by the potassium dichromate method as described by Bragdon (6). Phospholipid was estimated by determination of phosphorus by Fiske-Subbarow method as outlined by Umbreit, Burris and Stauffer(7). A modification of Zak's ferric chloride(8) method was used to determine cholesterol after hydrolysis and conversion to its digitonide.

Results. Plates I and II show typical female and male adrenal gland stained with oil red O. The zona fasciculata of the female mice is stained intensely with oil red O over its entire extent. In general, the zona fasciculata of the male stained less intensely. The inner half as well as columns and patches of fasciculata takes little or no stain. Individual cortical cells of the female fasciculata tend to have larger numbers and more intensely staining round droplets which fill the cytoplasm. The reticularis stains only in occasional clumps particularly in its inner zone in both males and females. Glomerulosa of both male and female mice are stained less intensely than fasciculata, but stained somewhat more heavily in females than in males. Sections stained with H & E reveal more cortical cytoplasmic pallor, which corresponds to lipid positive areas, in females than in males. Similar observations were made on several other inbred strains (BALB/c, C57/BL and DBA/2JN).

Results of the chemical analysis and eosinophil counts are shown in Table I. Adrenal weights for the female mice were greater than those of the males even though body weights of males were heavier than of females. Total lipid content of female adrenals was twice that of the male, while total lipid concentration was slightly though significantly higher in the females. Much larger cholesterol content and concentration were found in female adrenals than in the males. In the female adrenal, total cholesterol represented twice as much of total lipid than in the male. Phospholipid content, but not concentration, was significantly higher in females. Protein concentration was significantly lower in the female adrenal, by an amount proportional to the higher total lipid content.

Discussion. Higher lipid content in female

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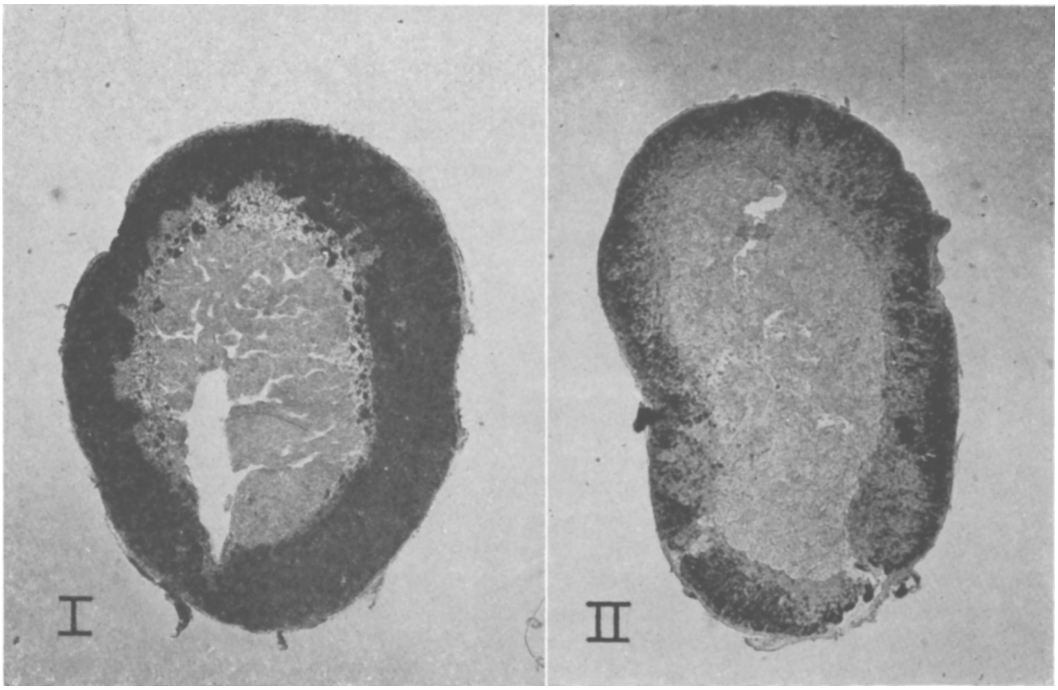

FIG. 1. Adrenal gland of female STR/N mouse, 7 mo old, ×30. Frozen section, oil red O stain. Note intense staining of majority of cortex with less staining of zona reticularis and glomerulosa.

FIG. 2. Adrenal gland of male STR/N mouse, 7 mo old, ×30. Frozen section, oil red O stain. There is less intense cortical staining than in female adrenal. Patchy areas of zona fasciculata are devoid of stain, particularly in inner portion.

mouse adrenal cortex, particularly in the zona fasciculata, have been observed using oil red O. Part of the differences in lipid content have been further shown in Table I to be due to cholesterol, a key precursor of the steroid hormones(9). Whitehead(1) showed that lipid as characterized by Sudan III staining was relatively more abundant in female adrenals cortex than in male at 150 days in mice, and that cholesterol staining (Schultz Reaction) followed the same pattern as the Sudan III stain(10). The protein and phospholipids, though their concentrations are lower in the female adrenals, are

TABLE I. Lipid and Protein of Adrenal Gland and Eosinophil Counts of 7 Months Old Male and Female STR/N Mice.

	Male	Female	p
Body wt, g	32.6 ± .66 *	23.1 ± .82	<.01
Adrenal wt, mg	4.16 ± .207	7.25 ± .260	<.01
mg/100 g body wt	12.7 ± .50	31.5 ± .99	<.01
Lipid, total, mg	1.08 ± .51	2.22 ± .127	<.01
mg/100 mg adrenal	26.3 ± 1.10	30.7 ± 1.47	<.05
Cholesterol, mg	.173± .132	.747± .459	<.01
mg/100 mg adrenal	4.19 ± .299	10.23 ± .547	<.01
mg/100 mg lipid	16.1 ± .92	34.4 ± 1.40	<.01
Phospholipid, mg	.128± .0093	.210± .166	<.01
mg/100 mg adrenal	3.09 ± .179	2.79 ± .250	>.05
Protein, mg	.88 ± .093	1.19 ± .064	<.02
mg/100 mg adrenal	21.5 ± 2.24	16.4 ± .84	<.05
Eosinophil count, cells/mm³	161 ±50.5	80.6 ±19.8	>.05

* Mean ± stand. error.

present in sufficient amounts in the total adrenals to imply greater amount of cellular material in the female adrenals.

Halberg(2) showed that eosinophil counts of female mice were lower at all times of the day than those of males. The present findings are in agreement with this observation. Adrenal cortical steroids are known to depress eosinophil counts(11). This suggests that steroid activity may be higher in female than in male mice. The finding of more cholesterol in the female mouse adrenal than in the male suggests that female mice may have higher stores of steroid precursors and possibly of steroids themselves in the cortex. The zona fasciculata is thought to elaborate glucocorticoids which are most effective in depressing circulating eosinophil levels(11). It is of interest that the sharpest differences between male and female adrenal glands exist in the zona fasciculata.

Summary. The zona fasciculata and to some extent the zona glomerulosa of the adrenals of female STR/N mouse stained more intensely and uniformly for lipid with oil red O than those of the males. The female adrenal was considerably larger than that of the male although body weights of the male were larger than the female. The absolute amounts of total lipid, cholesterol and phospholipid in the adrenals in female mice were higher than in males. Eosinophil count of female mice was lower, but not significantly so, than male counts.

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Antigenicity of Cholesterol Induced Chicken Atheroma.* (26346)

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These experiments were undertaken to determine whether or not the lipid or lipoprotein complex comprising the central mass of an experimentally produced arterial atheroma could stimulate specific antibody formation, and to clarify the serologic characteristics of such an antibody. It was also planned to investigate the *in vivo* effects of such anti-atheroma antibody on lesions of experimental atherosclerosis. Antigenic properties of experimental atheroma and normal intima were to be compared.

Methods and materials. The chicken was chosen as the experimental animal. The necessary dietary conditions for atheroma induction in the chicken have been well defined and are easily reproducible(1).

Eight-week-old HYLINE leghorn cockerels (Corn Belt Hatcheries, Joliet, Ill.) were

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