differences in catalytic properties between preparations from the two stages (*3*). Evidently the upsurge of the 3rd week reflects some reorganization in one class of alkaline phosphatase molecules, with the result that their characteristics are altered and they become more active under appropriate circumstances. The fact that the increase takes place in duodena in which mitosis has been suppressed, as by the drugs used in this study, indicates that the reorganization may occur in cells that have already moved into place on the sides of the villi.

It is unlikely that actinomycin D and puromycin exert their accelerating effect simply by increasing the output of adrenocorticoids (*2*), for these drugs also enhance the alkaline phosphatase–stimulating action of exogenous corticoids administered before the pituitary-adrenal axis is functional (*17*). Rather it appears that the three substances more directly elicit, or help to elicit, a conversion reaction that has been repressed in the infant duodenum, though the mechanism by which they act is not evident. It is tempting to argue that they may act by blocking the production of a protein that inhibits the conversion of existing alkaline phosphatase molecules to an altered state; but this explanation is brought into question by the total ineffectiveness of the aminonucleoside of puromycin in inhibiting amino acid incorporation in vitro (*18*). In the liver of the intact mouse, however, 0.15 of nucleoside per gram of body weight administered in four hourly doses reduced the incorporation of glycine-2-C^{14} into liver protein by 19 percent (*11*); possibly the much larger doses used in this study, over a longer period, did act by lowering the rate of synthesis of some protein inhibitor of the conversion of alkaline phosphatase in the duodenum.

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Sialic Acid Concentrations in the Pituitary Glands of Normal and Ovariectomized Rats

Abstract. *The concentration of sialic acid in the anterior pituitary gland of young female rats is approximately 250 micromoles per 100 grams. After ovariectomy there is a marked and persistent rise in pituitary sialic acid; this increase is probably related to the known increase in the production of gonadotrophic hormones.*

The sialic acids are an important group of compounds that are widely distributed in tissues and body fluids; they may be considered as derivatives of the 9-carbon α-keto acid, neuraminic acid, and they are known to be constituents of a wide variety of mucopolysaccharides, mucolipids, and mucoproteins (*1*).

It was recently shown that purified follicle stimulating hormone (FSH) and luteinizing hormone (LH) (*2*) of ovine or human origin contain sialic acid (*3*). Furthermore, in the case of FSH, it has been demonstrated that the release of sialic acid from the hormone preparation by incubation with neuraminidase results in an almost total loss of biological activity of the hormone (*4*). In view of these facts, it was of interest to determine the normal concentration of sialic acid in homogenates of rat pituitary glands. In addition, we have followed the changes in pituitary sialic acid which follow bilateral ovariectomy. It is, of course, well known that gonadectomy in the rat leads to a marked increase in the concentration of gonadotrophic hormones in the pituitary gland (*5*).

We used the thiobarbituric acid method of assaying sialic acid as described by Warren (*6*). A reference standard of *N*-acetyl neuraminic acid (*7*) was used and for the tissue determinations optical density readings were made at 532 and 549 mμ. Calculations were based on the use of the correction equation recommended by Warren (*6*, equation 2). Values for the pituitary assays were based on the assumption that the sialic acid was present in the form of *N*-acetyl neuraminic acid.

The female rats used in this study were of the Holtzman strain (*8*). They were divided into groups of four to six animals each and both ovaries were removed from half of them at an age of 32 days. Animals were killed by decapitation and the pituitary gland was quickly exposed by lifting up the brain. The posterior lobe was discarded and the fresh anterior lobe was weighed on a torsion balance. Assays were carried out on groups of normal animals ranging in age from 24 days to 130 days. The means and standard errors for each group were calculated and subjected to statistical analysis.

The changes in the concentration of pituitary sialic acid with increasing age and with time after bilateral ovariectomy and shown in Fig. 1. In the normal animals a gradual decline in the concentration of sialic acid occurred during the first 45 days of the study period and the curve leveled off as the rats entered the period of sexual

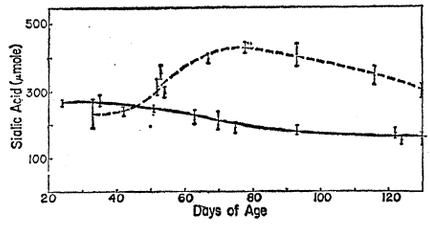

Fig. 1. Changes in the concentration of sialic acid in the pituitary gland with age and time after ovariectomy in the rat. The lower curve (solid line) shows the values for normal animals and the upper curve shows the values for ovariectomized animals. Ovaries were removed on day 32. Each point represents the mean of values from four to six animals and the vertical bars show standard errors of the means.

maturity. After ovariectomy there was a transient (and statistically insignificant) decrease followed by a dramatic rise in the level of pituitary sialic acid; this increase was statistically significant as early as 20 days after ovariectomy ($p=<.01$) and at all later times the values for operated animals were much higher than controls of equivalent ages ($p=<.001$). A gradual decline in the concentration of pituitary sialic acid began at about 50 days after ovariectomy, but even at 100 days after operation there was still a significantly higher concentration of pituitary sialic acid than in the intact controls ($p=<.001$).

It is considered likely that the increase in pituitary sialic acid which follows ovariectomy is related to the increased secretion of gonadotrophins (FSH and LH) for the following reasons. (i) Sialic acid is known to be a significant component of purified preparations of gonadotrophic hormones (*9*). (ii) Sialic acid has been demonstrated by histochemical means in certain basophiles of the cat hypophysis (*10*) and in the mucoid cells of the human hypophysis (*11*). (iii) Assays of normal male pituitary glands, which are known to be richer in gonadotrophins than those of females, showed significantly higher concentrations of sialic acid (*12*). (iv) In our study we made parallel assays of sialic acid in adrenal and submaxillary glands and found no significant changes after ovariectomy. (v) The curve of pituitary sialic acid concentrations after ovariectomy is consistent with the known changes in storage and secretion of FSH and LH after gonadectomy in the rat (*5*, *13*).

It is not possible to know exactly how much of the rise in pituitary sialic acid which was found after ovariectomy was due to increased levels of gonadotrophic hormones. However, if one assumes that 2 percent of the normal concentration (250 μmole/100 g) of sialic acid in the normal gland is contained within FSH molecules (*12*), it can be calculated that a tenfold increase in FSH would, in itself, account for one-fourth of the observed maximum rise in total sialic acid. It thus seems reasonable to suggest that the increasing levels of FSH which are known to follow ovariectomy play an important role in bringing about the increased concentrations of total pituitary sialic acid observed in this study.

Finally, it should be emphasized that analyses of ovine and human purified hormones show that FSH has a much higher content of sialic acid than does LH (*3*, *14*), and it may eventually be shown that completely pure luteinizing hormone lacks sialic acid. If this chemical difference is also found in the gonadotrophic hormones of other species, it may form a basis for the development of histochemical methods for differentiating the FSH- and LH-producing cells.

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9. Data from Gottschalk's laboratory (*4*) indicate that purified ovine FSH contains 5-percent sialic acid. Since values of less than one residue per molecule have been reported for purified LH from human and ovine sources (*3*), there would seem to be some doubt as to whether this hormone actually contains any sialic acid. In this connection, it has been demonstrated by M. Adams-Mayne and D. N. Ward (*Endocrinology*, in press) that neuraminidase does not inactivate LH.
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13. It is agreed that gonadectomy leads to an increased production and secretion of gonadotrophic hormones in the rat; however, the nature and course of these changes in exact quantitative terms (that is, actual amounts of FSH and LH contained within the gland at various time periods) is still incompletely known. From the bioassay data given in the literature for changes in the pituitary content of FSH in the ovariectomized rat (*5*), it would appear that within 3 months after ovariectomy, concentrations of FSH in the pituitary gland may increase by a factor of five- to tenfold. There is less agreement concerning the effect of ovariectomy on the concentrations of LH in the pituitary gland.
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Sex-Associated Differences in Serum Proteins of Mice

Abstract. *Agar electrophoresis of serum from mice of C57BL/10-H-2^a, B10.Sn, A.SW, A.CA, R III, and P1 inbred strains shows that the females have a lower concentration of α-1 serum globulin than the males and, in some strains, the females also have a lower concentration of α-2 and β-serum globulin. Females of the A.SW strain have a higher serum albumin concentration than males, and females of the C57BL/10-H-2^a strain have a higher γ-globulin concentration than males. Two-dimensional (agar and hydrolyzed-starch) electrophoresis gives a typical sex-associated pattern for α-1 and α-2 globulins which clearly permits recognition of male and female serums.*

In several animal species, certain serum proteins appear to be different in males and females; for example, the relative concentration of albumin in rats, as measured by moving boundary and zone electrophoresis, was found to be higher in females than in males (*1*). In cattle, males possessed less α-globulin glycoprotein and more β-globulin and γ-globulin glycoproteins than females (*2*). In toads, the separation of some of the serum components by starch gel electrophoresis has been reported to be different in the two sexes (*3*). In mice, the concentration of agglutinating antibody to chicken and sheep heteroantigens (*4*), and to human erythrocytes (*5*) was found to be higher in females; and, in addition a protein fraction has recently been described as missing in male mouse serum (Cal A strain) analyzed by starch gel electrophoresis (*6*). Sex-associated differences found in mice during the course of experiments in which normal mouse serum was examined by agar