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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7. Synthetic *N*-acetyl neuraminic acid, 99-percent pure, was obtained from General Biochemicals, Chagrin Falls, Ohio.
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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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12. Unpublished observations. We have found by bioassay that pituitary glands from 60-day-old male rats contain approximately ten times as much FSH as comparable female glands; by comparison, total sialic acid values were only about 35 percent higher in male pituitaries than those reported here for females. It is important to point out that most of the pituitary sialic acid, as measured in this study, apparently is not contained in the gonadotrophic hormones. Thus, our calculations, based on extraction and purification data in the literature (for ovine hormones), indicate that normally no more than 1 to 3 percent of the total pituitary sialic acid would be contained in FSH molecules.
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

Table 1. Relative percentages of mouse serum proteins separated by agar electrophoresis (means ± S.D.).

No. of mice	Sex	Albumin*	Globulins			
			α-1†	α-2‡	β§	γ‖
			Strain A. SW			
13	M	57.80 ± 1.93	4.50 ± 0.40	12.04 ± 0.27	15.32 ± 0.57	10.32 ± 0.43
13	F	63.73 ± 0.70	2.05 ± 0.14	8.70 ± 0.25	14.04 ± 0.20	11.00 ± 0.72
			Strain C57BL/10–H–2^d			
11	M	67.28 ± 1.74	8.08 ± 0.52	10.72 ± 0.37	10.03 ± 0.74	3.89 ± 0.63
11	F	71.37 ± 2.61	2.32 ± 0.27	8.67 ± 0.73	10.62 ± 0.98	7.12 ± 1.21
			Strain P1			
13	M	61.15 ± 1.21	6.94 ± 0.54	13.03 ± 0.55	13.19 ± 0.52	5.70 ± 0.76
13	F	65.98 ± 2.76	1.96 ± 0.23	11.35 ± 1.01	14.58 ± 1.36	6.14 ± 0.88
			Strain B10.Sn			
12	M	60.38 ± 1.58	5.25 ± 0.53	13.45 ± 0.57	13.78 ± 0.51	7.14 ± 0.58
12	F	64.53 ± 2.27	1.80 ± 0.21	11.21 ± 1.06	13.09 ± 0.72	8.93 ± 1.01
			Strain A.CA			
12	M	59.80 ± 1.83	4.21 ± 0.42	11.66 ± 0.64	16.43 ± 1.20	8.21 ± 0.95
12	F	60.96 ± 1.36	1.79 ± 0.09	10.20 ± 0.47	16.77 ± 0.90	10.25 ± 1.84
			Strain R III			
8	M	66.08 ± 2.50	4.33 ± 0.72	12.03 ± 0.52	11.91 ± 0.65	5.28 ± 0.99
7	F	68.84 ± 2.40	2.46 ± 0.40	9.29 ± 0.90	12.41 ± 0.72	6.97 ± 0.67

* $p = <.01$ for both sexes of strain A.SW. † $p = <.001$ for all strains except R III, where $p = <0.05$. ‡ $p = <.001$ for strain A.SW; $<.05$ for strain C57BL/10-*H*-2^a; $<.02$ for strain R III. § $p = <.05$ for strain A.SW. ‖ $p = <.05$ for strain C57BL/10-*H*-2^d.

and two-dimensional electrophoresis are reported.

Serum was obtained from male and female mice, 75 to 90 days old, of C57BL/10-*H*-2^d, B10.Sn, A.SW, A.CA, R III, and P1 inbred strains. Agar electrophoresis was performed with agar strips (20 × 1.4 × 0.1 cm) prepared by allowing 2.5 ml of hot 1-percent Difco Noble agar in barbital buffer (0.05 μ, *p*H 8.6) to solidify on glass slides. Sample holes, 0.2 cm in diameter, were made in each strip, 6 cm from the cathode end. The slides were placed in a Grassmann horizontal electrophoresis tank containing the barbital buffer. Samples of 0.003 ml were placed in the prepared holes and immediately afterward a potential gradient of 4 volt/cm was applied for 5 hours between the ends of the agar strips. The strips were then treated as recommended by Uriel and Grabar (*7*). After staining with amido black 10 B and eluting with 0.02*N* NaOH, the concentrations of the protein fractions were determined by spectrophotometric readings at 620 mμ. The serum fractions were named albumin, α-1, α-2, β-, and γ-globulins, in analogy with the protein fractions of human serum. The albumin was associated with a faster moving component, which because of its small size and poor separation, was difficult to measure accurately. This fraction was included with the albumin in our results. Two-dimensional electrophoresis patterns were obtained by an agar hydrolyzed-starch method (*8*).

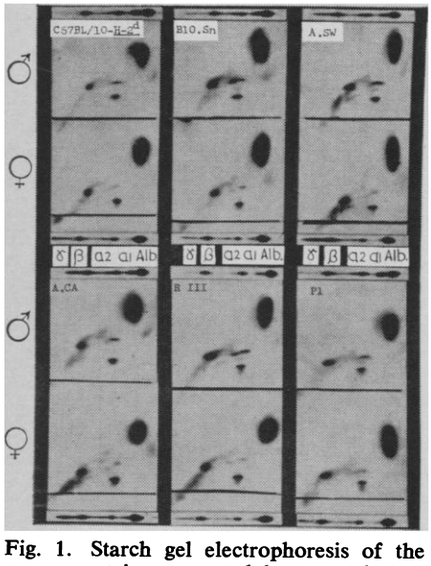

Fig. 1. Starch gel electrophoresis of the serum proteins separated by agar electrophoresis in different inbred strains of mice. The agar strips from the first electrophoresis were inserted into the gel slots and a second electrophoresis was performed at right angles to the first. The agar-strip controls are included to show correspondence of the Tiselius zones with fractions obtained in the starch gels.

The results of the agar electrophoresis, which are summarized in Table 1, showed that in the strains studied, there was a considerable difference between males and females in the relative concentrations of several protein zones.

Figure 1 shows two-dimensional electrophoresis patterns of individual male and female serum samples from each different strain; serum patterns similar to those shown were found in each of six other individuals of the same sex and strain. Although strain differences appear, a characteristic sex-associated pattern is very apparent and permits differentiation between males and females in all of the strains studied. Sex-associated differences are clearly observed in the α-1 and α-2 globulin components. Alpha-1 globulin, almost absent in female serums, appears in males as a semicircular cap above and to the left of albumin. In the starch gel, α-2 globulin resolves into three major components, the relative levels of which differ depending on the sex of the mouse. These results disagree with those of Cons *et al.* for mice of the Cal A strain (*6*), since no protein fraction was found to be absent in the serum of any male mouse of the strains studied by us.

The influence of sex has been demonstrated not only in serum proteins but also in proteins of rat liver (*9*) and of mouse and rat urine (*10*) so that it would be worthwhile to investigate the effect of sex upon other proteins of the animal organism. Experiments to elucidate the mechanism of the serum protein differentiation between males and females are now under way.

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