Electrophoresis of Serum Proteins and Selected Enzymes in Males, Non-Pregnant, Pregnant, and Lactating Female Mice.* (28523)

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Antigens are transferred from maternal serum to the mouse ovarian oocyte(1), to oviducal ootids, and probably to cleavage stage embryos(2). Transfer to the ovarian oocyte is selective as to molecular species. Thus, native serum antigens are transferred to mouse ovarian oocytes of all classes; bovine plasma albumin is transferred only to oocytes in tertiary follicles, while rabbit globulins are not detectable in mouse ovarian oocytes(1). Transfer to oviducal embryos is selective as to egg or embryonic *stage*, although it is not yet known whether there is selective *molecular* transfer. In the oviduct, there is no transfer of native serum antigens to unfertilized or fertilized oocytes surrounded by follicle cells but, after follicle cell dispersal, serum antigens appear in the ootid(2). Data about yolk sac or placental transfer of maternal serum antigens in the mouse and rat are not available; such transfer does occur in the rabbit(3). Utilization by oocytes, embryos, or fetuses of the molecules transferred from maternal serum has not been investigated extensively.

Differences between maternal and fetal and between male and female serum protein components have been reported for rat(4,5), hamster(6), and human(7,8,9,10); at least 8 enzymes were detected in electrophoretic studies of male mouse serum(11). Although there have been extensive clinical investigations of human serum enzymes and proteins (12), little is known about the significance of their changes during pregnancy and lactation.

Partial characterization by electrophoresis of male and female mouse serum proteins and of selected enzymes is reported here. Further experiments may allow a more accurate definition of the molecular and stage selectivity of the transfer phenomena described above, and an understanding of the embryonic utilization of the transferred molecules may become possible.

Materials and methods. Serum from each of 193 adult mice of the Cal A strain† was examined individually by starch gel electrophoresis. Categories included male sera, female sera from animals at various stages of the estrous cycle (proestrous, estrous, metestrous, diestrous), at 4, 8, 12, 16, or 19 days of pregnancy (4P, 8P, etc.), and a 4, 10, or 21 days of lactation (4L, 10L, etc.). At least 6 animals in each category were used for each protein or enzyme component studied.

Blood (and whatever pleural fluid was present) was pipetted from the thoracic cavity of ether anesthetized mice whose inferior vena cava had been cut. After centrifugation, sera were frozen for electrophoretic analysis. Upon thawing, samples were diluted 50% with the borate buffer used in preparation of starch gels; 50 μl serum samples were used. The vertical starch gel electrophoresis method of Smithies was used(13); gels were run for 16 hours at room temperature with a voltage gradient of 135 volts (5 v/cm). Gels were stained for protein with Amido-Black 10B. Using appropriate substrates(11), gels were examined for esterase (alpha-naphthyl acetate), cholinesterase (6-bromo-2-naphthyl carbonaphthoxy choline iodide), cytochrome oxidase (alpha naphthol and dimethylparaphenylenediamine), alkaline phosphatase (sodium alpha naphthyl acid phosphate, pH 8.8), acid phosphatase (sodium alpha naphthyl acid phosphate, pH 5.0), and aminopeptidase (L-leucyl-beta-naphthylamide HCl) activity.

The color density of the stained bands was graded visually in arbitrary units, one (lightest) to 4 (darkest), to estimate the levels of protein or enzyme concentration in each band.

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† Obtained from Cancer Research Genetics Laboratory in 1958; originally A/Crgl/2.

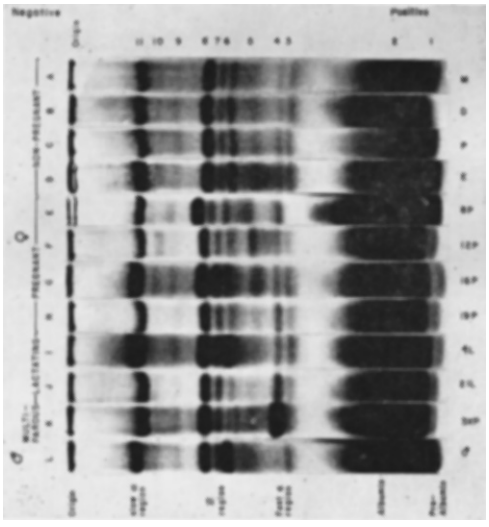


FIG. 1. Electrophoretic patterns of mouse serum protein. A. Non-pregnant female in Metestrous (M); B. Diestrous (D); C. Proestrous (P); D. Estrous (E). E. Pregnant female at the 8th day of gestation (8P); F. 12th day of gestation (12P); G. 16th day of gestation (16P); H. 19th day of gestation (19P). I. Lactating female 4 days post partum (4L); J. 21 days post partum (21L). K. Non-lactating diestrous female which had borne 3 litters (3×P). L. Male (♂).

Serum components were numbered "1" through "11" in order of decreasing mobility. Since mouse serum components have not been characterized chemically, mouse constituents may not be homologous with human components migrating at the same rate in starch gel electrophoresis. For convenience, however, mouse serum components were grouped according to the nomenclature of Smithies for human serum(13) as follows: slow and fast pre-albumin (component "1"), albumin ("2"), fast alpha-globulins ("3" and "4"), beta-globulins ("5," "6," "7," and "8"), and slow alpha-globulins ("9," "10," and "11").

Results and discussion. Protein. Total protein averaged 51 mg/ml with a range of 49 mg/ml to 56.8 mg/ml in sera from all categories. Quantitative and qualitative differences in particular serum protein constituents were observed between male and female mice and during pregnancy and lactation (Fig. 1, A-L).

Eleven protein components were present in sera from all categories of mice (Fig. 1, A-K) except for male sera which had 10 components (Fig. 1, L). Components "1" and "2" in the pre-albumin and albumin regions and components "9" and "10" in the slow alpha region varied in concentration from individual to individual rather than from category to category and are omitted from Fig. 2. Component "3" (Fig. 2A) was at higher concentration than at the estrous stage of the estrous cycle in 16P animals and was lower in 12P and 19P animals; it was higher in 4L than in 21L females. Component "4" (Fig. 2A) had higher concentration during pregnancy and lactation, was heavily concentrated in sera from multiparous females, and was absent in male sera.

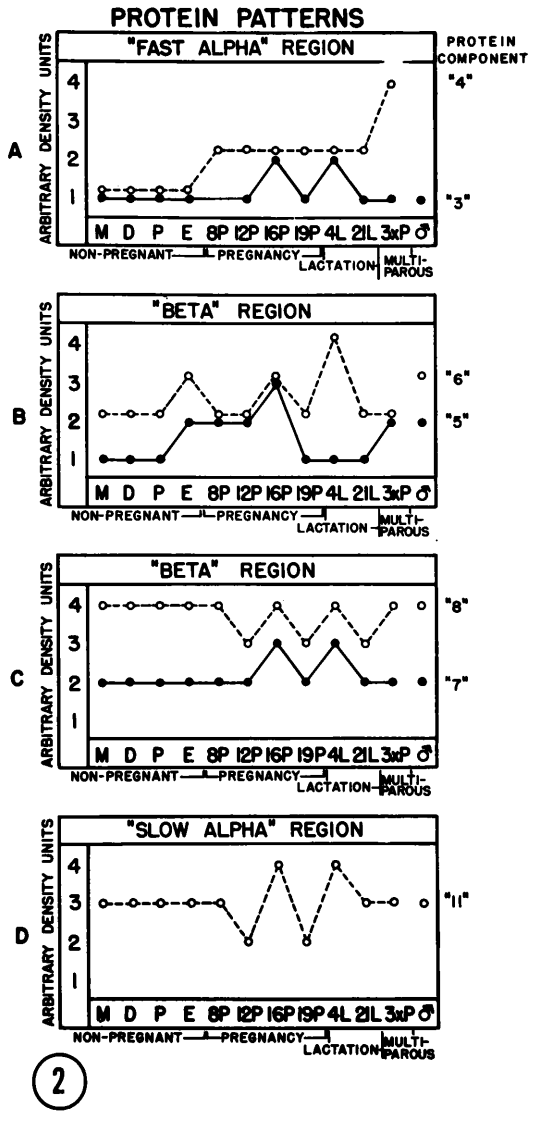
Component "5" (Fig. 2B) did not differ from estrous levels in early pregnancy but was lower in 19P, 4L and 21L; the level was higher at 16P. Component "6" (Fig. 2B) had lower concentration than at estrus in all categories except 16P, 4L and male. Component "7" (Fig. 2C) had greater concentration than at estrus in 16P and 4L. Component "8" (Fig. 2C) had lower than estrous concentrations at 12P, 19P, and 21L. Component "11" (Fig. 2D) had higher concentration in 16P and 4L animals, and lower concentration in 12P and 19P animals.

Enzymes. As with protein, both quantitative and qualitative differences in serum enzymes were observed according to sex, or stage of pregnancy or lactation.

Esterase. Ten components with esterase activity were present in all categories of sera (Fig. 3, A-D). One or more components was associated with each protein region. Two components migrated more rapidly than albumin and were probably associated with slow and fast pre-albumin (Fig. 3A). During 8P, 19P and 4L, esterase activity was higher than in estrus in the fast pre-albumin component; there was lower concentration in the albumin region at 10L (Fig. 3A). There was no clear separation of components "3" and "4" in the fast alpha region; this complex was slightly greater in concentration about the middle of gestation and at 21L than in estrus (Fig. 3B). Three esterase components were present in the beta region (Fig. 3C); changes in concentration of each component during pregnancy apparently occurred independently of the other components. Component "5" was

at a concentration greater than the estrous level during pregnancy (Fig. 3C). Components "9," "10," and "11" apparently had unchanging esterase activity in all categories examined (Fig. 3D).

Cholinesterase. Cholinesterase activity was associated with 6 serum protein components (Fig. 4, A-D). Concentration of cholinesterase activity in the albumin region was the same in all categories of sera (Fig. 4A). The single component in the fast alpha region, associated with either protein component "3" or "4," was in lower concentration than estrous levels during most of pregnancy and lactation (Fig. 4B). Two of the 3 components in the beta region, associated with protein components "5" and "8," were present in all categories of sera. One component, however, corresponding to protein component "6," was absent from sera of animals nearly to term and in sera from all lactating animals (Fig. 4C). The single cholinesterase component in the slow alpha region was lowest in concentration during the last half



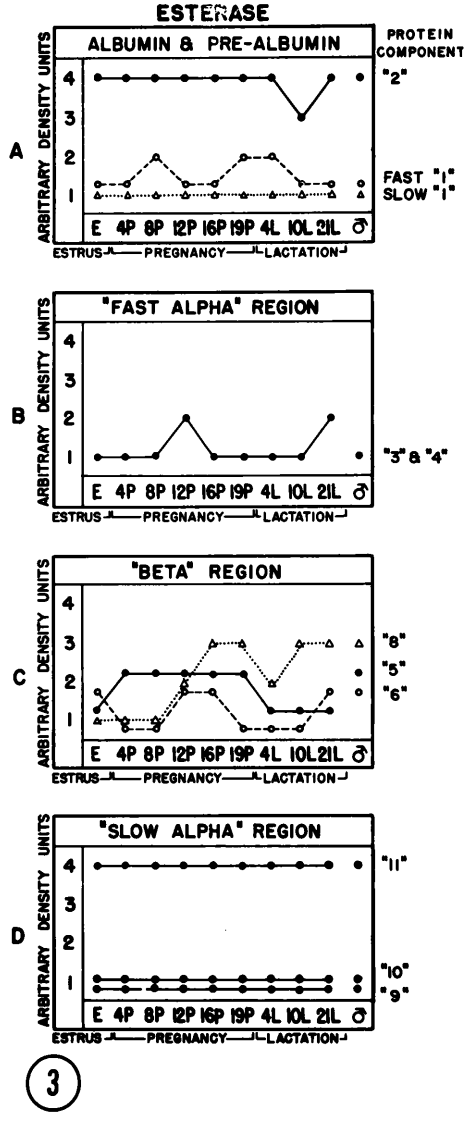
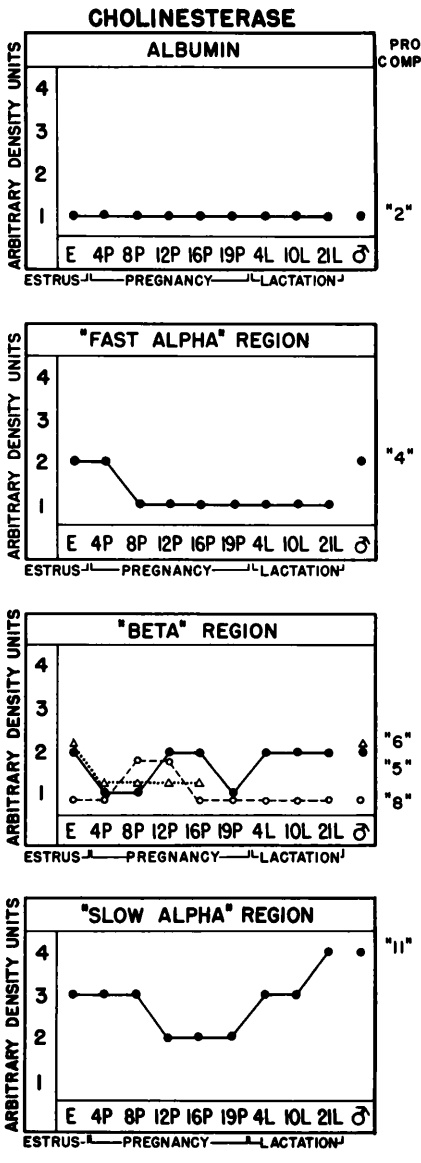


FIG. 2. Relative concentrations of protein components.
FIG. 3. Relative concentrations of esterase components.

of pregnancy; during lactation, however, it increased above estrous levels (Fig. 4D). Levels of cholinesterase activity in male sera were the same as at estrus except that male sera concentrations were higher in the component in the slow alpha regions (Fig. 4D).

Cytochrome oxidase (Fig. 5A). Cytochrome oxidase activity was seen in the albumin and pre-albumin regions for some animals. The single component with consistent cytochrome oxidase activity, associated with protein component "8," was at the same low level in non-pregnant and pregnant females and in male sera. A slightly higher level was



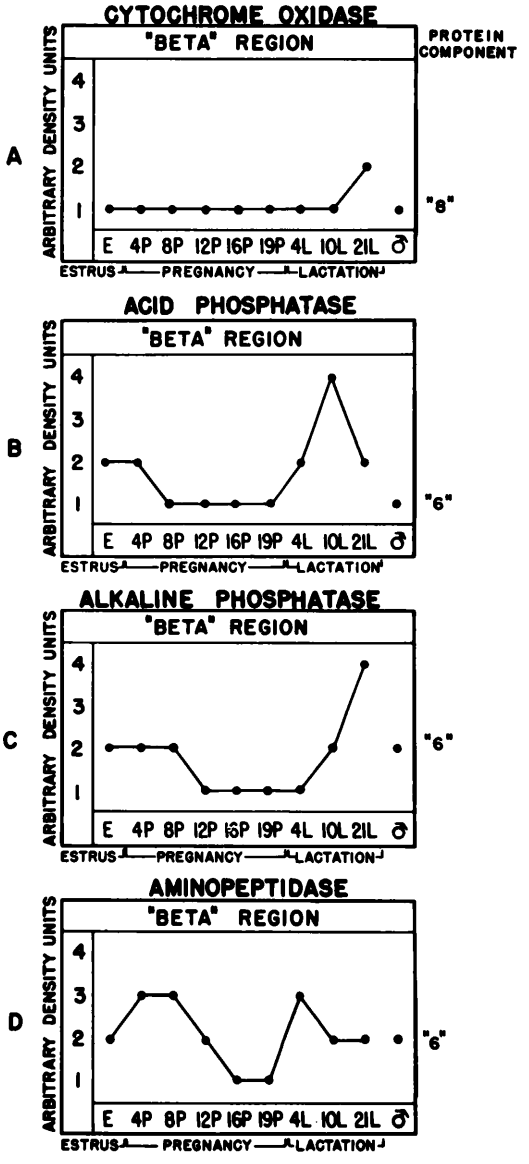


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FIG. 4. Relative concentrations of cholinesterase components.

FIG. 5. Relative concentrations of: A. Cytochrome oxidase; B. Acid phosphatase; C. Alkaline phosphatase; D. Aminopeptidase.

present by 21 days of lactation.

Acid phosphatase (Fig. 5B). One serum component, associated with protein component "6," was active for acid phosphatase. Levels of activity during approximately the last half of pregnancy were lower than at estrus. In early lactation, levels were about the same as at estrus. By the middle of lactation, levels were higher; by the end of lactation, however, levels were the same as at estrus. Concentrations in male sera were lower than estrous levels.

Alkaline phosphatase (Fig. 5C). One band, associated with protein component "6," was active for alkaline phosphatase. Level of activity was about the same during the first half of pregnancy as at estrus. During the remainder of pregnancy and early in lactation, levels were lower than at estrus. Relative concentration apparently increased and was at the highest level of activity at 21 days lactation. Activity in male sera was at the same level as in estrous females.

Aminopeptidase (Fig. 5D). The single aminopeptidase component, associated with protein component "6," had a higher activity early in pregnancy than at estrus and lower than estrous levels late in pregnancy. Levels in lactating females (except at 4L) and in males were the same as estrous levels.

Comments. Comparison of data on serum protein and enzyme constituents from different laboratories is difficult because electrophoretic methods vary and because analyses often are based on measurements of protein amounts present in serum complexes rather than of individual serum components. Nonetheless, our data agree with those studies showing quantitative and qualitative differences in various protein serum components between sex and pregnancy categories(4). The increased protein level of a component in the fast alpha region of multiparous females may be similar to the "reproduction-associated protein" of Heim(14). Relative quantities of some of the serum enzyme components varied with stage of pregnancy or lactation and differed between males and females. Quantitation by spectrophotometer is in progress.

The quantitative and qualitative variations observed may indicate differences in rates of synthesis or utilization of various components by the adult body. The variations may reflect, too, elimination of a particular constituent from the maternal body due to transfer to the egg or embryo, or retention of a constituent by slowing or cessation of transfer. These questions are under examination.

Summary. Serum proteins and selected enzymes in non-pregnant, pregnant, lactating females and in male mice were studied by starch gel electrophoresis. Esterase and cholinesterase activity were present in all protein regions; alkaline and acid phosphatase, aminopeptidase, and cytochrome oxidase activity was associated with the protein beta region. Total protein was approximately the same in all categories of sera studied, but zymograms and protein stained gels showed that the relative concentrations of certain serum components were different in different sex, pregnancy or lactation categories. The relationship between these variations and possible transfer of maternal serum molecules to the oocyte or embryo is discussed.

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1. Glass, L. E., *Develop. Biol.*, 1961, v3, 787.
2. ———, *Am. Zool.*, 1963, v3, 135.
3. Smith, A. E. S., Schechtman, A. M., *Develop. Biol.*, 1962, v4, 339.
4. Heim, W. G., *J. Embryol. Exp. Morph.*, 1961, v9, 52.
5. ———, *Am. Zool.*, 1961, v1, 359.
6. Peterson, R. P., Turbyfill, C. L., Soderwall, A. L., *Fertility and Sterility*, 1962, v13, 486.
7. Kuhns, W. J., Hyland, P. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 1.
8. Moore, D. H., DuPan, R. M., Buxton, C. L. *Am. J. Obst. and Gynec.*, 1949, v57, 312.
9. Brown, T., *J. Obst. and Gynaec. Brit. Emp.* 1954, v61, 781.
10. Brown, D. R., McGaundy, R. B., Gillie, E., Doyle, J., *Am. J. Obst.*, 1959, v77, 556.
11. Lawrence, S. H., Melnick, P. J., Weiner, H. E., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 572.
12. Ccoke, A. M., *Proc. Roy. Soc. Med.*, 1963, v56, 173.
13. Smithies, O., *Biochem. J.*, 1959, v71, 585.
14. Heim, W. G., *Nature*, 1962, v193, 491.

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