# Pregnancy-Associated Serum Protein in Mink[1] (35280)

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(Introduced by S. C. Madden)

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Electrophoretic and immunoelectrophoretic studies of mink serum proteins showed that they were basically similar to human serum proteins, although differences in mobility and relative concentration were noted (1). While we were examining the serums of mink for elevated levels of immunoglobulin found in Aleutian disease (2), a new serum protein was found in pregnant mink. This pregnancy-associated serum protein is reported below.

*Materials and Methods.* Mink (*Mustela vison*) of the 3 major genotypes were caged individually and fed a diet consisting of 25% fish, 25% poultry scrap, 15% fortified cereal (Kellogg's), 10% pork liver, 10% nutria or horse meat, 5% beef tripe, and 10% added water. All mink used in this study were free of detectable diseases.

Serum protein electrophoresis and immunoelectrophoresis on cellulose acetate or paper was done with commercially available equipment. Immunoelectrophoresis in agar or agarose was done by the method of Scheidegger (3). Preparative electrophoresis used Pevikon as the supporting medium (4). All buffers were barbital, pH 8.6 $\mu$ 0.04 to 0.10. Analytical ultracentrifugation was done in a Beckman Model E machine at 59,780 rpm, using both Schlieren and absorption optics. Serum protein concentration was determined by the biuret method of Dittebrandt (5), and the pregnancy protein concentration was determined by elution of Ponceau S stained cellulose acetate strips with spectrophotometric determination of the eluted dye.

Antiserums to whole pregnant and nonpregnant female mink serums were made in rabbits by injecting 4 ml of complete Freund's adjuvant containing a 1:20 dilution of serum 6 times at 3-week intervals. Rabbit antiserums to mink IgG, fibrin, $\beta_{1C}$ globulin and albumin were prepared as previously described (2, 6).

Serum was obtained during the last third of pregnancy from 3 humans, 1 baboon, 6 dogs, 8 cats, 5 rabbits, 8 hamsters, and 8 mice, electrophoresced on cellulose acetate, and examined for a protein of similar mobility to that found in mink. Serum from nonpregnant females of the same species was used as a control.

*Results.* The serum of pregnant mink revealed a previously unrecognized protein, designated component 1, when serum was electrophoresed using cellulose acetate as the supporting medium. This new component is shown in Fig. 1B, while nonpregnant female and male mink serums are shown for comparison in Fig. 1A and D. This pregnancy protein was of slower mobility than gamma globulin in barbital buffer, pH 8.6 $\mu$ 0.075, and was not seen when paper was used as the supporting medium for electrophoresis. The diffusely migrating component 2 seen in the pregnant mink serum was also found in nonpregnant female and male mink serum, but at too low a concentration to be photographed.

The mean duration of pregnancy in mink is 50 days, with a usual range of 45 to 60 days. Ranch mink are mated twice at 1-week

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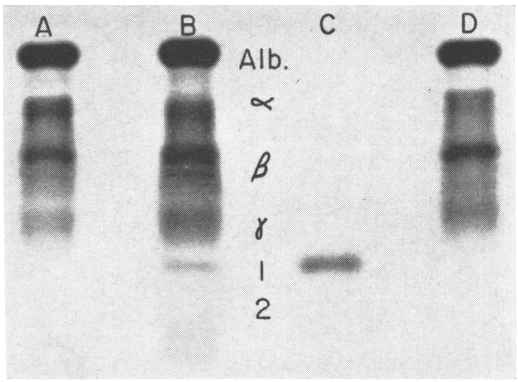


FIG. 1. Cellulose acetate electrophoresis of mink serum proteins: (A) nonpregnant female mink (0.25 μl); (B) pregnant mink (0.5 μl); (C) isolated pregnancy protein (0.75 μl); and (D) male mink (0.25 μl). Anode is at the top. The unique and homogeneous pregnancy protein is denoted by 1, while 2 indicates an electrophoretically heterogeneous protein increased in concentration during pregnancy, but also present in nonpregnant female or male mink.

intervals; and most, if not all, offspring are the result of the second mating. Serum protein electrophoresis was done at weekly intervals on 39 female mink from the time of mating until 2 weeks after parturition, and the pregnancy protein was usually first seen 3 weeks after the second mating, and was always present 4 weeks after the second mating. The serum concentration of pregnancy protein reached a maximum of 50 mg/100 ml 7 to 10 days before parturition and became undetectable 5 to 8 days following parturition. At the maximum level, the pregnancy protein represented 0.8% of the total serum proteins. The mean total serum protein of the pregnant mink was 6.8 g/100 ml, and this value did not change during the course of pregnancy. The pregnancy protein was not found in 200 female mink which were not mated or which did not become pregnant after mating, or in 200 male mink, and was not present in the serum of 40 fetal or newborn mink. Of special interest, a mink with a pseudopregnancy identified at the time of expected parturition had a high level of component 1 in its serum. Histologic examination of the uterus of this mink failed to reveal placental or fetal tissue.

When agarose or cellulose acetate was used as the supporting medium for immunoelectrophoresis, the pregnancy protein reacted with antibody to whole pregnant mink serum, but did not react with antibody to nonpregnant female mink serum. When various types of agar were used, no reaction between the pregnancy protein and antibody was seen. Antibody to the pregnancy protein was not removed from the antiserum by addition of equal volumes of male mink serum or nonpregnant female mink serum, but was completely removed by addition of 20 μl of pregnant mink serum/ml of antiserum. Monospecific antiserums to mink IgG, fibrin, $B_{1C}$ globulin and albumin did not react with the pregnancy protein. The $\alpha_f$ fetoprotein (7) was identified as a globulin of $\alpha$ mobility in fetal mink serum, and was found in low concentration in the serum of the pregnant female. The diffuse protein band designated 2 in Fig. 1B was immunogenic in rabbits immunized with either whole pregnant or nonpregnant female mink serum. Absorption showed that pregnant female mink serum contained about 4 times the amount of component 2 found in nonpregnant female or male mink serum.

The mink pregnancy protein was readily purified by preparative electrophoresis of pseudoglobulin (serum proteins soluble in phosphate buffer, pH 5.4, $\gamma/2$ 0.02). The slow moving component 2 was removed in the euglobulin fraction. The isolated pregnancy protein shown in Fig. 1C was electrophoretically and ultracentrifugally homogeneous with $S_{20,w}$ 1.6 at a concentration of 0.7 mg/ml.

Identical electrophoretic techniques using serum from the pregnant human, baboon, dog, cat, rabbit, hamster, and mouse failed to show a pregnancy protein similar in mobility to that found in mink.

*Discussion.* The mink pregnancy protein apparently is made by the dam, since it was found in a mink with pseudopregnancy, and could not be detected in fetal or newborn mink serum. It thus differs from $\alpha_f$ fetoprotein in distribution as well as in electrophoretic mobility (7). Immunologic studies indicate that the pregnancy protein is unique, and not a normal serum protein altered in electrophoretic mobility during pregnancy. The fail-

ure to detect this protein when agar or paper supporting media were used suggests that the relatively basic protein is bound to these media. To our knowledge, a protein of this type has not been found in mammals, but new serum proteins associated with egg-laying have been observed in several classes of vertebrates and in arthropods (8, 9). The function, if any, of this pregnancy-associated serum protein of mink is presently unknown.

*Summary.* A new serum protein was found in pregnant mink. The protein is more basic than immunoglobulin, is 1.6S, and appears to be made by the dam.

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