

EFFECTS OF EXPERIMENTAL DISTURBANCE OF PREGNANCY ON PROTEIN COMPOSITION AND IMMUNOGENIC PROPERTIES OF THE MILK IN DOGS

V. I. Ermolov

UDC 618.3-092.9-07 :618.19-008.8-07

Disturbance of the normal course of pregnancy in dogs as a result of stress-induced neurosis causes changes in lactation and in the protein composition of the milk. The total protein in mature milk is reduced, while the albumin level is lowered and the γ -globulin level raised in the colostrum and mature milk. The agglutinating and opsonizing power of the milk against Shigella sonnei is substantially reduced.

Previous investigations [3, 4] have shown that during normal pregnancy the colostrum of dogs has the highest content of antibodies and is one of the sources of the increased natural immunity of newborn puppies.

To study how the character of the course of pregnancy influences the immunogenic properties of the milk, a study was made of the protein composition and the agglutinating and opsonizing properties of the milk in dogs with experimental disturbances of pregnancy. Shigella sonnei cells were used as antigen.

EXPERIMENTAL METHOD

The protein concentration in the milk was determined by Lowry's method [7]. The composition of the protein fractions was studied by electrophoresis of a milk protein preparation (MPP) in agar. The MPP was obtained by defatting the milk by centrifugation at 3000 rpm and lyophilization. The dry residue was dissolved in distilled water, so that the original volumes of colostrum and mature milk were reduced by 2.5 and 5 times respectively. The agglutinin content and opsonizing properties of the milk were determined as described previously [4]. The opsonizing action of the MPP on leukocytes was characterized by the phagocytic index (PI) and intensity of phagocytosis (IP) in per cent. The normal course of pregnancy was disturbed by I. A. Arshavskii's method, consisting of the induction of neurosis by exposure to stressors (the amplified sound of an automobile horn and an alternating electric current (20 V), applied for 30 and 60 sec with intervals of 30 sec over a period of 1 h). The action of the stressors was repeated from four to eight times, at intervals of 3-4 days, starting from the fifth week of pregnancy.

EXPERIMENTAL RESULTS

During the disturbance of pregnancy by exposure to stressors, changes occurred in the animals' behavioral responses, in the form of general depression and refusal to eat. The state of homeostasis of the dogs acquired the features of acidosis, accompanied by a periodic increase in the titratable acidity and ammonia concentration and a decrease in the titratable alkalinity and pH of the urine. Meanwhile the protein concentration in the urine was increased. Four of the 10 dogs went into labor prematurely (by 3-4 days). Pregnancy in one animal terminated by birth of stillborn fetuses 15 days before full term. Nearly all dogs showed delay in the secretion of colostrum during the first hours after birth of the puppies. On the following days, lactation was appreciably reduced in three animals. The highest protein concentration ($10.1 \pm 1.1\%$) occurred in the colostrum before the puppies were fed. Electrophoretic investigation of the

Institute of Obstetrics and Pediatrics, Ministry of Health of the RSFSR, Rostov-on-Don. (Presented by Academician V. V. Parin.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 71, No. 1, pp. 16-18, January 1971. Original article submitted March 23, 1970.

©1970 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00.

TABLE 1. Effect of MPP from Dogs on Phagocytic Activity of Leukocytes ($M \pm m$)

Day from beginning of feeding puppies	Experimental animals			Normal animals		
	number of tests	PI	IP	number of tests	PI	IP
Before beginning of feeding	8	2.8 ± 0.4	52.1 ± 9.2	8	6.5 ± 0.07	94.9 ± 1.0
2nd-3rd	9	1.7 ± 0.3	28.1 ± 6.0	8	2.4 ± 0.1	54.8 ± 4.0
8th-12th	9	1.4 ± 0.2	7.9 ± 1.9	14	1.6 ± 0.05	16.0 ± 1.5
21st	8	1.1 ± 0.03	4.4 ± 0.9	16	1.6 ± 0.1	11.2 ± 1.6

colostrum revealed six protein fractions: albumins 1.83 ± 0.18 g%, α_1 globulins 1.37 ± 0.13 g%, α_2 globulins 1.57 ± 0.23 g%, β globulins 2.25 ± 0.39 g%, γ_a globulins 2.73 ± 0.38 g%, and γ_b globulins 0.33 ± 0.02 g%. The albumin level in the experimental animals was significantly lower than in dogs with a normal course of pregnancy (2.71 ± 0.2 g%). In the 2-4 days immediately after the puppies began to feed, the total protein level fell by half to 5.2 ± 0.3 g%. Meanwhile the concentrations of albumins and β and γ_a globulins fell (1.40 ± 0.10 , 0.67 ± 0.04 , and 0.56 ± 0.04 g% respectively). In this period the albumin level was lower, and the γ_a globulin level higher, than in the control dogs (2.63 ± 0.08 and 0.25 ± 0.03 g% respectively). From the eighth to the 21st day the total protein level showed no significant change, and was between 4.9 ± 0.3 and 5.2 ± 0.3 g%. The albumin concentration (between 1.79 ± 0.07 and 1.74 ± 0.07 g%), as before, was lower and the γ_a globulin level higher (between 0.27 ± 0.04 and 0.27 ± 0.02 g%) than in healthy dogs ($P < 0.02$).

The titers of agglutinins in the MPP varied during the first 8-12 days from 1:20 to 1:640 (averaging from 1:291 to 1:332). By the 21st day they had fallen to 1:20 to 1:320 (mean 1:96) and were lower than in corresponding preparations from normal animals. The effects of treatment with MPP on the leukocytic activity of the puppies are summarized in Table 1.

These results show that the MPPs of the experimental dogs have reduced opsonizing properties.

The change in protein composition and immunogenic properties of the dogs' milk was accompanied by a disturbance of physical development, by weakening of the general resistance, and by an increased tendency toward autoinfection in the newborn puppies. Of the 42 puppies, 21 died during the first 10 days from enteritis, pneumonia, and other pathological conditions. Of the 20 puppies which survived until the 10th day, only six had doubled their birth weight. During the first 2-3 weeks the phagocytic activity of the circulating leukocytes of the puppies against *Shigella sonnei* was considerably reduced.

These experiments thus suggest that the disturbance of lactation and the changes in protein composition and immunogenic properties of the dogs' milk were dependent on the disturbance of the normal course of pregnancy due to stress-induced neurosis, inhibiting the central component of the mechanism for the nervous regulation of pregnancy [1, 5]. This resulted in changes in the hormone-producing activity of the adenohypophysis and in the development of the mammary gland during pregnancy, a weakening of reflex communications between the hypothalamo-hypophyseal region and the mammary glands during lactation, and changes in the synthetic, secretory, and motor functions of the mammary glands [2, 6].

LITERATURE CITED

1. I. A. Arshavskii, Outlines of Age Physiology [in Russian], Moscow-Leningrad (1967), p. 16, 96.
2. I. A. Baryshnikov, in: The Neuro-Hormonal Regulation of Lactation [in Russian], Moscow-Leningrad (1966), p. 3.
3. V. I. Ermolov, Byull. Éksperim. Biol. i Med., No. 1, 57 (1969).
4. V. I. Ermolov, Byull. Éksperim. Biol. i Med., No. 2, 56 (1969).
5. L. A. Pronin, in: Problems in General and Age Physiology and Pathology [in Russian], Moscow (1959), p. 199.
6. G. B. Tverskoi, in: The Neuro-Hormonal Regulation of Lactation [in Russian], Moscow-Leningrad (1966), p. 25.
7. O. Lowry, N. Rosenbrough, et al., J. Biol. Chem., 193, 265 (1951).