PROTEIN COMPOSITION AND IMMUNOGENIC PROPERTIES OF MILK FROM DOGS AT DIFFERENT PERIODS OF LACTATION

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The protein content is maximal in the colostrum before the puppies are fed, and this colostrum possesses marked agglutinating and opsonizing properties against Shigella sonnei. During the first days of feeding, the protein content, especially γ-globulins, in the colostrum falls to reach a minimum in the mature milk. The agglutinin titer falls at the same time, accompanied by a decrease in the opsonizing properties of the milk.

Natural feeding is an important factor influencing the formation of immunity in early ontogenesis [1-3,5,6]. A study of the immunologic indices of the blood in puppies born after a normal pregnancy showed a rapid increase during the period of colostrum feeding (from the 1st to the 4th day) followed by a decrease with a change to feeding on mature milk (from the 8th to the 21st day).

To determine the relationship between the level of natural immunity and the character of feeding of puppies, the protein composition and agglutinating and opsonizing properties of the milk were studied at different periods of lactation. Cells of Shigella sonnei and Escherichia coli serotype O-Sh-V4 were used as antigens..

EXPERIMENTAL METHOD

The protein content in the milk was determined by Lowry's method [4]. The composition of the protein fractions were studied by electrophoresis of a milk protein preparation (MPP) in agar. To obtain the MPP the milk was freed from fat by centrifugation at 3000 rpm and lyophilized. The dry residue was dissolved in distilled water in a volume 2.5 and 5 times less than the initial volume of colostrum and mature milk. The agglutinin titer in the MPP was determined by plate agglutination with microscopic evaluation of the result. To determine the opsonizing properties of the milk, blood cells of newborn puppies were washed three times with a 1% solution of normal rabbit serum in physiological saline. The serum was first freed from normal antibodies by Castellani's method and inactivated at 56°. The washed blood cells were added to a suspension of the bacteria (2 billion cells/ml), MPP, and physiological saline. Instead of MPP, physiological saline was added to the control tubes. The tubes were incubated for 30 min by the usual method and the phagocytic index (PI) and intensity of phagocytosis (IP) calculated in percent.

EXPERIMENTAL RESULTS

The highest protein content (9.9 ± 0.4 g%) was found in the colostrum before the puppies were fed. Electrophoretic investigation of the colostrum revealed six protein fractions, as follows: albumin 2.71 ± 0.2 g%, α_1-globulins 1.32 ± 0.12 g%, α_2-globulins 1.70 ± 0.14 g%, β-globulins 2.01 ± 0.06 g%, γ_a-globulins 2.02 ± 0.1 g%, and γ_b-globulins 0.17 ± 0.02 g%. During the 2-4 days immediately following the beginning of feeding the total protein level fell by almost half (5.6 ± 0.2 g%). The content of β- and, in particular, γ_a-globulins fell at the same time (0.78 ± 0.05 and 0.25 ± 0.03 g%, respectively). From the 8th to the 21st day, with a slight increase in the total protein content (6.1 ± 0.1-6.3 ± 0.3 g%), the content of these globulins fell still further (0.66 ± 0.05 and 0.17 ± 0.02 g%). While the globulin level fell, the albumin content steadily increased to reach 3.48 ± 0.16 g% by the 21st day.

The study of the agglutinating action of MPP revealed the presence of antibodies against S. sonnei in the colostrum and the mature milk of the dogs. The agglutinin titer in the colostrum before the puppies were fed varied from 1 :640 to 1 :2560, while in the mature milk it fell appreciably to reach 1 :160-1 :640

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TABLE 1. Effect of MPP of Dogs on Phagocytic Activity of Leukocytes (M ± m)

Days after beginning of feeding of puppies	Indices of phagocytosis			
	S. sonnei		E. coli O-Sh-V4	
	PI	IP	PI	IP
Before beginning of feeding (8)	6,5±0,07	94,9±1,0	1,3±0,3	3,0±0,7
2—3-rd (8)	2,4±0,1	54,8±4,0	1,3±0,06	5,5±1,3
8—12-th (14)	1,6±0,05	16,0±1,5	1,2±0,1	3,5±1,4
21-st (16)	1,6±0,1	11,2±1,6	1,2±0,06	4,0±0,7

Note. Number of tests shown in parentheses.

on the 12th-21st day. Agglutinins against E. coli were not found either in the colostrum or in the mature milk.

The results of treatment of puppies' leukocytes with MPP are summarized in Table 1.

The phagocytic activity of the leukocytes against E. coli differed only slightly from phagocytosis of washed leukocytes in the control series. The specificity of the opsonizing action of MPP on S. sonnei cells was evidently due to normal antibodies.

These investigations thus demonstrated the presence of a well-marked connection between the protein composition and immunogenic properties of dogs' milk.

These findings, indicating changes in the protein composition of the milk during lactation, explain the relationship observed previously between the character of feeding and the immunologic properties of the blood in growing puppies.

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