

Rat Immunoglobulins in Serum and Secretions: Comparison of IgM, IgA and IgG in Serum, Colostrum, Milk and Saliva of Protein Malnourished and Normal Rats¹ (38699)

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It has been reported that protein malnourished children demonstrate lower levels of serum immunoglobulin G (IgG) (1, 2) and of secretory IgA (s-IgA) in nasal washings (3) than normally-nourished children. This decreased synthesis of Ig could help explain the increased susceptibility to infection observed in malnourished children (4). However, other investigators have reported that protein malnourished children produce normal or even greater than normal levels of Igs (5-9). These conflicting reports on the levels and production of Igs in malnourished children lend themselves to the limitations of human studies where protein supplementation (6) and clinical and sub-clinical infections (7-9) during the testing period could play an important role in determining the effect of malnutrition on the humoral immune system.

At the same time, other investigators employing experimental animals, where diet and infection are more easily controlled, have generally agreed that protein malnutrition results in decreased Ig production (10) with concomitant induction of less specific antibody (10-12). Additional studies have reported that protein malnourished rat offspring exhibit overall retarded growth (13, 14) which could also involve irreversible damage to lymphoid tissue in the developing animal (15).

This study was undertaken to determine the effect of protein malnourishment on Ig levels in colostrum and milk of rat dams and in the serum and saliva of their developing offspring. A difference in Ig levels in normal and malnourished offspring could

help explain the well documented increased susceptibility to infection associated with malnutrition (4).

Materials and Methods. Animals and diets. Timed pregnant COBS/CD outbred albino rats were obtained from Charles River Laboratories (Wilmington, MA). Upon arrival, dams were divided into two equal groups and fed either low protein (8% casein) or normal protein (25% casein) defined diet (14). All rat pups were weaned at 19 days of age and fed a high protein diet.

Collection of colostrum and milk from rat dams and serum and saliva from rat pups. The method employed for collection of rat colostrum and milk has been previously reported (16). Briefly, all rat dams were uniformly milked with a milking device at two to five day intervals from day of delivery until pups were weaned. Colostrum and milk samples were stored at -20° until assayed.

As previously described (16) rat pups were bled by cardiac puncture from day of birth until sacrifice. Pilocarpine stimulated whole saliva was collected from rats at approximately five day intervals from 20 to 45 days of age (16). All serum and saliva samples were stored at -20° until assayed.

Quantitation of colostrum, milk, serum and saliva immunoglobulins and salivary proteins. Immunoglobulins in colostrum, milk, serum and saliva were quantitated by radial immunodiffusion employing purified anti-rat α , γ and μ (16). Salivary protein levels were determined by the turbidimetric tannin micromethod (17) employing purified human IgG as standard.

Experimental design. The overall experimental design is outlined in Fig. 1. Pregnant rat dams were randomly assigned into two groups and fed either a low pro-

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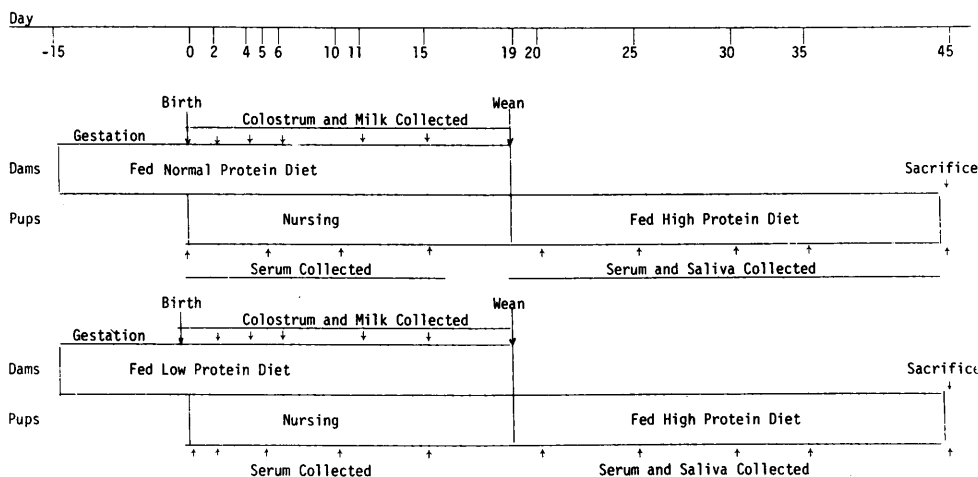


FIG. 1. Experimental design employed to determine the level of Igs in colostrum and milk of rat dams fed low or normal protein diets and in serum and saliva of their offspring.

tein or normal protein defined diet during gestation and lactation. Colostrum and milk was collected from all dams on days 0, 2, 4, 6, 11, 15 and 19 of lactation. On the day of birth (day 0), all pups from each group were pooled and reassigned eight pups/litter. Excess pups were exsanguinated by cardiac puncture and the individual serum samples assayed for immunoglobulin levels. Individual pup serum samples were collected from three pups from each litter on days 2, 5, 10 and 15. On day 19, all pups were weaned and fed a high protein diet. Saliva and additional serum samples were collected on days 20, 25, 30 and 35. On day 45 (sacrifice) saliva and serum was collected from all rats.

Results. Rat colostrum and milk immunoglobulin levels. As shown in Table I, the level of IgA in colostrum collected on day 0 was lower in malnourished dams (0.48 mg/ml) than in dams fed normal protein diet (1.15 mg/ml). The level of IgA increased in both groups and by day 4, no significant difference was observed in milk IgA levels from malnourished and normally-nourished dams. Furthermore, by day 6, the level of milk IgA in both groups of dams had decreased to one half the level observed in early milk. This lower concentration of IgA remained constant throughout the remainder of lactation.

Although no significant difference was observed in milk IgA levels, the level of

TABLE I. CONCENTRATION OF IMMUNOGLOBULIN A IN COLOSTRUM AND MILK FROM RATS DAMS FED NORMAL OR LOW PROTEIN DIET.

Day of lactation	Group	
	Normal	Protein malnourished
0	1.15 ± 0.24 ^a	0.48 ± 0.12
2	1.26 ± 0.31	1.04 ± 0.28
4	1.42 ± 0.34	1.85 ± 0.39
6	0.75 ± 0.16	0.70 ± 0.14
11	0.66 ± 0.05	0.76 ± 0.20
15	0.65 ± 0.07	0.70 ± 0.09
19	0.59 ± 0.03	0.64 ± 0.03

^a Expressed as milligrams of immunoglobulin per milliliter of samples ± standard error. Each value represents the mean of six females.

IgG_{2a} in milk from malnourished rats was significantly lower (approximately two- to threefold less) than the level in normal rats throughout the entire period of lactation (Fig. 2). The level of IgG_{2a} increased in both groups, however, after day 6, the level remained constant throughout the remainder of lactation. No IgM could be detected in colostrum or milk from either malnourished or normal rats at any interval tested.

Immunoglobulin levels in pup serum. As shown in Table II, serum IgA was first detected in 20-day old normal rats and 25-day old malnourished animals. However, by 35 days of age, no significant differ-

ence was observed in serum IgA levels from either group.

Immunoglobulin M was first demonstrated in pup serum on day 10 in both malnourished and normal pups (Table II). Between 15 and 45 days of age, the level of this serum Ig in both groups of rats increased steadily and no significant difference could be discerned between malnourished or normal rats.

The levels of serum IgG_{2a} in malnourished and normal rats from day 0 to 45 days of age is presented in Fig. 3. At all intervals tested, malnourished rats had significantly lower levels of IgG_{2a} than normal animals. Both groups of animals demonstrated increasing levels of IgG_{2a} during lactation and reached peak titre between 15 and 20 days of age in both malnourished (3.96 mg/ml) and normal (6.36 mg/ml) pups. Following weaning, both groups of animals demonstrated a rapid decrease in serum

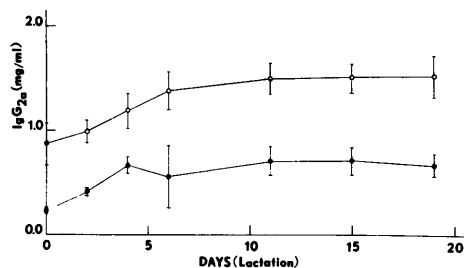


FIG. 2. Levels of IgG_{2a} in rat colostrum and milk during lactation: dams fed normal protein diet, —○—; dams fed low protein diet, —●—.

IgG_{2a} levels until day 35; by 45 days of age increasing levels of this serum Ig were again observed. However, the level in malnourished rats (2.36 mg/ml) was still approximately twofold less than that observed in normal rats (4.11 mg/ml).

Immunoglobulin levels in pup saliva. Since the concentration of salivary Igs is dependent on the final volume of saliva collected (16), the levels of IgA and IgG_{2a} in saliva of normal and malnourished rats is expressed as μ g of Ig/mg of protein (Table III). Immunoglobulin A was first observed in 25-day old malnourished and normal rats. No significant difference in the level of this Ig could be discerned between the two groups of rats at any interval tested. However, the levels of salivary IgA demonstrated a 2-fold decrease between days 30 and 35. This later concentration has been previously reported (16) as the normal adult rat level of salivary IgA.

With the exception of day 20, no significant difference was observed in the level of salivary IgG_{2a} in normal and malnourished rats. The level of this Ig remained constant throughout the entire test period.

Discussion. Although the levels of Igs have been reported to be lower in protein malnourished rats (10–12), it has not been clearly documented as to the effect of protein malnutrition on the Ig synthetic tissue during the early stages of growth and development. In this series of experiments rat dams were maintained on defined normal

TABLE II. LEVELS OF SERUM IgA AND IgM IN NORMAL AND PROTEIN MALNOURISHED RAT OFFSPRING.

Immuno- globulin	Group	Age (Days)							
		0–5 ^a	10	15	20	25	30	35	45
IgA	Normal	0	0	0	0.05 ^b ±0.01	0.06 ±0.02	0.19 ±0.08	0.12 ±0.01	0.19 ±0.01
	Protein malnourished	0	0	0	0	0.05 ±0.02	0.09 ±0.01	0.16 ±0.12	0.09 ±0.01
IgM	Normal	0	0.085 ±0.02	0.27 ±0.01	0.36 ±0.01	0.39 ±0.02	0.47 ±0.03	0.56 ±0.05	0.87 ±0.08
	Protein malnourished	0	0.012 ±0.012	0.312 ±0.015	0.384 ±0.02	0.45 ±0.06	0.67 ±0.13	0.63 ±0.13	0.89 ±0.09

^a No IgA or IgM was detected.

^b Expressed as milligrams of immunoglobulin per milliliter of sample \pm standard error. Each value represents the mean of eight pups.

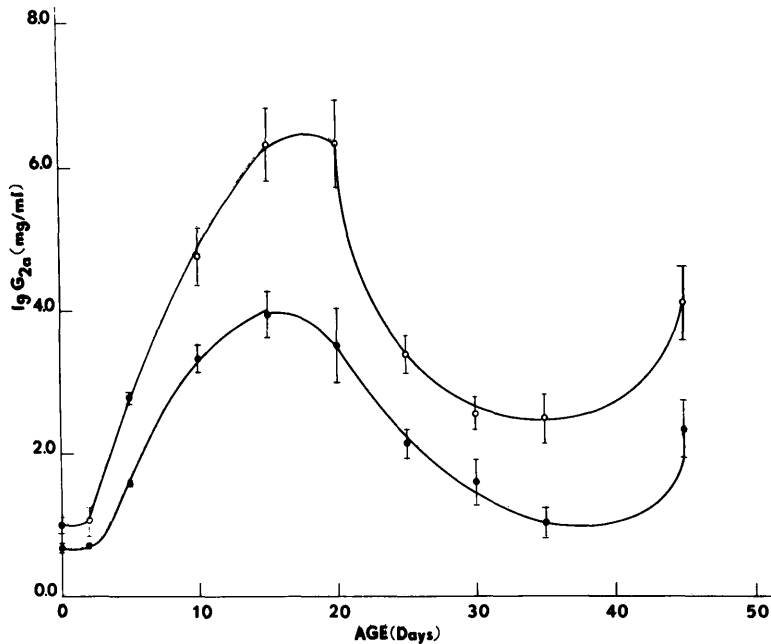


FIG. 3. Levels of serum IgG_{2a} in rat pups: pups derived from dams fed normal protein diet, —○—; pups derived from dams fed low protein diet, —●—.

TABLE III. IMMUNOGLOBULIN LEVELS IN SALIVA OF YOUNG RATS.

Immunoglobulin ^a	Group	Age				
		20	25	30	35	45
IgA	Normal	0	100 ^b ± 40	170 ± 60	40 ± 10	60 ± 20
	Protein malnourished	0	130 ± 20	120 ± 20	60 ± 10	55 ± 25
IgG _{2a}	Normal	70 ± 20	40 ± 10	30 ± 20	50 ± 20	50 ± 10
	Protein malnourished	20 ± 5	60 ± 40	30 ± 20	50 ± 10	55 ± 15

^a IgM was not detected in pup saliva at any of the intervals tested.

^b Expressed as micrograms of immunoglobulins per milligram of protein. Each value is the mean of eight pups ± standard error.

or low protein diet during gestation and lactation. As previously reported (14) protein malnourished rat offspring exhibited retarded growth as compared to normal animals, however, both groups of offspring demonstrated overall good health.

During the early stages of development rat offspring acquire serum Igs by passive transfer from their mother (18) and from newly synthesized Igs in the developing offspring itself. In these studies protein deprivation appeared to have no effect on the level of IgA in the colostrum and milk of mothers or in serum and saliva of their

offspring. In addition, IgA was not observed in the serum or saliva of young rats until the animals were between 20 and 25 days of age. These findings were in agreement with the reports of others, where it was found that little or no IgA was transferred across the gut of young rats (19). This would suggest that IgA in serum and saliva was newly synthesized.

Along the same lines, the level of serum IgM in young rats was not affected by dietary protein restriction. This serum Ig was first observed in 10 day old normal and protein malnourished rats and steadily in-

creased throughout the experimental test period. As previously reported (16) the level of serum IgM was approximately 90% of the adult level by 45-days of age.

The level of IgG_{2a} in colostrum and milk of malnourished dams was approximately twofold lower than normal animals throughout the entire period of lactation. Furthermore, a twofold difference in the level of serum IgG_{2a} was observed in their offspring. These findings are in agreement with the reports of others (1, 2) that protein malnutrition results in decreased levels of serum IgG. However, the level of serum IgG_{2a} in both groups of offspring increased during nursing, markedly decreased following weaning and began to increase between 35 and 45 days of age. These results confirm and extend the report that increasing levels of Ig are transferred across the gut during nursing (20). Further, active synthesis of this serum Ig would account for the increase after day 35.

The observed decrease in IgG_{2a} in colostrum and milk in rat mothers fed low protein diet and in serum of their offspring could help explain the well documented overall lowered resistance to infection associated with malnutrition (4).

Summary. Immunoglobulin levels in colostrum and milk of normal and protein malnourished rat dams and in serum and saliva of their offspring were quantitated by radial immunodiffusion employing purified anti-rat α , γ and μ . In general, no significant difference was observed in the level of colostral IgA in malnourished as compared to normal rat dams or in the level of serum IgA and IgM and salivary IgA and IgG_{2a} of their offspring. On the other hand, malnourished mothers demonstrated approximately twofold lower levels of colostral IgG_{2a} than normal mothers throughout the entire period of lactation. In addition, at every interval tested, the level of IgG was approximately 1.5- to 2-fold less in malnourished as compared to normal offspring.

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