

TABLE I. Mortality of Pertussis-Treated Mice Following Challenge with Histamine and Betazole.

	Control mice		Pertussis-treated	
	Histamine	Betazole	Histamine	Betazole
LD ₅₀	.807 mg/g*	.86 mg/g	.027 mg/g*†	.72 mg/g†

* The difference between these values is highly statistically significant, $p = <.001$.

† The difference between these values is highly statistically significant, $p = <.001$.

Challenge dose given on 4th day subsequent to pertussis-sensitization and deaths recorded after 24 hr.

tussis-sensitized mouse is about $80\times$ that for histamine. Death following challenge is probably the result of a toxic effect other than a histamine-like shock. Betazole is considered to possess a negligible histamine-like action.

1. Kirsner, J. B., Ford, H., J. Lab. Clin. Med., 1955, v46, 307.

2. Rosiere, C. E., Grossman, M. I., Science, 1951, v113, 651.

3. Lee, H. M., Jones, R. G., J. Pharmacol. Exp. Therap., 1949, v95, 71.

4. Parfentjev, I. A., Goodline, M. A., *ibid.*, 1948, v92, 411.

5. Finney, D. J., Probit Analysis, Cambridge Univ. Press, Cambridge, 1952.

6. Munoz, J., Schuchardt, L. F., J. Allergy, 1953, v24, 330.

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Initial Dietary Influences on Antibody Absorption in Newborn Puppies.* (31227)

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The newborn animal can absorb ingested antibody for only a short period after birth. The factors which regulate this period of intestinal absorption of intact protein molecules are not well understood, although the subject of considerable research in several species(1, 2,3,4). Earlier work had indicated the possibility that the initiation of ingestion and digestion of food, particularly proteins, was linked to this limited absorption of antibodies (5,6). The present study was undertaken to investigate the influence of dietary protein on antibody absorption in newborn puppies.

The appearance of specific antibody in the

blood after the antibody was fed is assurance of intestinal absorption, but contrarily the absence of the specific antibody in the blood after being fed poses the problem of whether or not this antibody was destroyed or altered after it passed intact through the intestinal epithelium. The progress of fluorescently labeled antibodies was followed in order to eliminate doubts of this kind.

Methods. Antibody absorption of puppies allowed to nurse was compared to that of puppies fed a sucrose-control diet, a balanced but synthetic diet, and to puppies not fed at all. Some puppies were naturally whelped and suckled. Surgically obtained, colostrum-free puppies were kept in groups of 2 or 3 in a room maintained between 24 and 29°C. All puppies were weighed and at various times after birth a randomly selected puppy was fed a single dose of the antibody at a rate of 3 ml/100 g. Blood samples were taken 12 to 15 hours later and analyzed.

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The source of antibody was pooled blood serum from dogs previously hyperimmunized against *Salmonella pullorum*. It was given *via* stomach tube at birth or at least 4 hours after the last feeding. Blood was collected by either jugular or cardiac puncture. After these samples clotted at 22°C, the serum was collected. Most samples were frozen and stored until titrated with a tube agglutination procedure. The antigen for the agglutination procedure was a phenolized suspension of *Salmonella pullorum*. After addition of antigen to the serum dilutions, the tubes were incubated in a waterbath at 52°C for 4 hours and then for 12-16 hours at room temperature.

In some trials, beta and gamma globulins of canine origin were conjugated with fluorescein dye(7) and 3-5 ml of the conjugate was fed *via* stomach tube. Prior to usage, the fluorescent preparation was stirred in Sephadex 0-25[†] and centrifuged to remove any dissociated fluorescein. One to three hours later the puppy was euthanized with sodium pentobarbital and the intestinal tract removed. Sections were prepared using standard frozen tissue techniques and examined microscopically with ultraviolet illumination.

The protein diet was formulated from a commercial bitch milk replacer[§] to yield 12.5% protein solution. Ingredients of the dry diet included soybean, cottonseed, and peanut oils, dried skimmed milk, alkaline caseinate, and dried egg yolk with vitamin and mineral additives. Manufacturer's guaranteed analysis of the dry product is as follows: crude protein, min. 33%; crude fat, min. 40%; crude fiber, none; moisture, max. 5%.

The sucrose-control diet consisted of 10 g of sucrose dissolved in 100 ml of .85% NaCl aqueous solution.

A total of 135 puppies were studied: 66 on the protein diet from 1 to 60 hours of age; 42 puppies on the sucrose-control diet from 10 to 84 hours of age, 12 puppies on the starvation diet from 34 to 43 hours of age

and 15 puppies on the natural diet 24-72 hours.

All feeding, except the suckling of the natural diet, was *via* stomach tube in order that a known quantity was ingested. Puppies were fed every 4-6 hours at the rate of 1-2 ml of liquid diet per 100 g body weight or until mild abdominal distension was noted. Starved puppies were not fed or watered. All dietary materials were fed at room temperature.

Results. There was considerable variation in the quantity of antibody absorbed between litters of the same age; however, similarly treated puppies within a litter exhibited little variation in antibody absorption. The influence of dietary proteins on antibody absorption is represented in Fig. 1. Statistical analysis in a randomized complete block design using littermate comparison did not indicate a significant difference in antibody titers of protein and sucrose-control fed puppies.

The limiting age of antibody absorption is shown in Fig. 2. Puppies suckling the bitch did not absorb antibody after 24 hours, while absorption continued in those puppies maintained on sucrose-control diets to 36 hours of age. In no instance was antibody absorption detected after 36 hours of age, although it was investigated up to 84 hours of age.

The absorption of fluorescent labeled beta and gamma globulins occurred primarily in the jejunum and ileum. The absorption of globulin by intestinal epithelial cells was evidenced by marked intracellular fluorescence 1 to 2 hours after feeding of the labeled material. In puppies studied 2 to 3 hours after feeding, the fluorescence was located in the submucosa. Absorption of the labeled gamma globulin was detected in two 28-hour-old puppies maintained on the protein and sucrose-control diets, respectively. There was no absorption of labeled globulin in two 50-hour-old puppies maintained on the protein and sucrose-control diets, respectively, or in a 24-hour-old suckling puppy.

Discussion. The failure to demonstrate antibody absorption beyond 36 hours of age regardless of the diet indicates that the major mechanism by which this phenomenon is terminated must be an age or hormone controlled process. Our earlier work(4) as well

[†] Pharmacia Fine Chemicals, New Market, N. J.

[§] Esbilac Feed Supplement Division, Borden Co., New York.

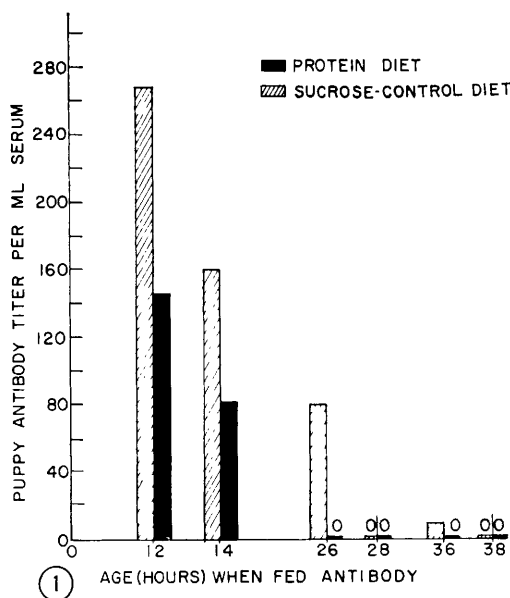


FIG. 1. Influence of dietary protein on antibody absorption in newborn puppies. (Littermate comparison.)

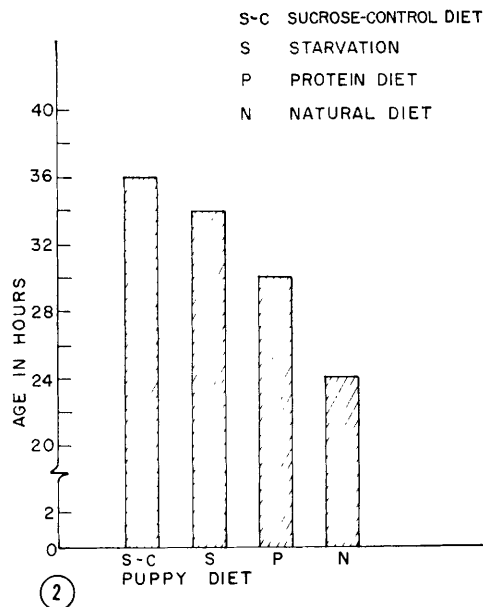


FIG. 2. Dietary influence on period of antibody absorption in newborn puppies.

as work of others(8) indicates adrenal steroids are involved in this mechanism.

The current findings are in agreement with others who found that dietary protein did not elicit the cessation of large molecule absorption in the piglet(3). Other workers have failed to demonstrate any significant dietary influence on the period of antibody absorption in newborn calves(2).

The failure to demonstrate fluorescently labeled globulin absorption by older puppies indicates that the absence of *Salmonella pullorum* antibody in the serum of the puppies fed after 36 hours of age was due to a cessation of antibody absorption as such and not due to other types of interference. The shorter period of antibody absorption in suckling puppies cannot be attributed solely to a colostral factor such as indicated by Balfour and Comline(2) for several reasons. First, the other puppies were more immature (they were obtained before birth) than the colostral puppies. This could account for the difference in cessation of absorption. Second, these puppies were deprived of the sensory gratifications involved in nursing and staying with the bitch. Since we have already shown

adrenal involvement in antibody absorption (4) and since adrenals are a well known component of behavior and stress, this deprivation may be a factor.

Summary. The ingestion of protein or other food does not appear to be a primary factor in the cessation of absorption of antibodies from the intestine of newborn puppies.

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1. Brambell, F. W. R., Biol. Rev., 1958, v33, 488.
2. Balfour, W. E., Comline, R. S., J. Physiol., 1962, v160, 234.
3. Leece, J. G., Morgan, D. O., Matrone, G., J. Nutr., 1964, v84, 43.
4. Gillette, D. D., Filkins, M. E., Am. J. Physiol., 1966, v210, 419.
5. Leece, J. G., Morgan, D. O., J. Nutr., 1962, v78, 263.
6. Payne, L. C., Marsh, C. L., *ibid.*, 1962, v76, 151.
7. Cherry, W. B., Goldman, M., Carski, T., Fluorescent antibody techniques in the diagnosis of communicable disease, U.S. Dept. H.E.W., Atlanta, Ga., 1960.
8. Halliday, R., J. Endocrinol., 1959, v18, 56.

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