

Alteration of Plasma Electrophoretic Patterns and Anaphylactic Reactions in Protein Depleted Dogs.* (22784)

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In 1939 one of us (P.F.H.) reported a means of demonstrating the limitation of hemoglobin production through protein depletion(1). Briefly this method consisted in the forcing of hemoglobin production by maintaining the dog at an anemic level by repeated bleeding, accompanying this by the intravenous administration of colloidal iron in quantities more than equal to the amount of iron removed as hemoglobin, and at the same time maintaining the animal on a diet highly restricted in its protein content. The dog has a propensity to manufacture hemoglobin at the expense of most other body proteins and, as a result, a rapid depletion of protein reserves was achieved. Preliminary studies at the time suggested that the ability of the animal to form antibodies was probably impaired as judged by the fact that the administration of a shocking dose of horse serum 2 weeks after injection of sensitizing doses did not result in a typical anaphylactic reaction. Resumption of the protein depletion work has recently been undertaken with the specific intent to prepare animals which may be suitable for homo-grafts of various tissues. The dog is usually regarded as an unsatisfactory animal for studies of the anaphylactic response, at least insofar as it can be compared with the guinea pig. Nevertheless, we have continued to use this animal for these studies for several reasons, namely: 1. the dog is more amenable to changes in diet than most experimental animals. 2. phlebotomy is considerably facilitated in the dog as compared to small animals and, 3. results of this type of study are much more easily translatable to human studies than in the cases of some of the more widely used small experimental ani-

mals. Although skin-graft type of experimentation could probably be equally as well carried out with any of the animals, such studies as survival of homologous kidney transplants are much more practicable in the dog.

We wish to present some preliminary studies relative to depletion of the plasma protein constituents of animals under such a regime. Electrophoretic patterns have been determined on these animals and are now being used as criteria to indicate whether or not the animal has been sufficiently depleted of the protein fractions to warrant an anaphylactic test. The absence or modification of the anaphylactic reaction in such depleted animals is in turn used as an indicator of the animal's readiness for the above-mentioned surgical procedures. Such studies show that, although slight to moderate reduction of the total plasma proteins occurred, marked alteration in the plasma protein makeup was clearly demonstrated.

Methods. Eight litter-mate hounds were used for depletion studies in this series. The animals were adults (over 1 year of age) and weighed from 11.7-13.6 kg initially. They were dewormed, vaccinated against distemper, and maintained on a diet of commercial dog food for 4 months prior to use to assure their status of health and to establish a relatively constant weight, blood picture, etc. During the experimental period they were fed a diet consisting of 300 g of bananas, 100 g of canned salmon, 120 g of Karo syrup, 40 ml of cod liver oil and 1 g of McCollum-Simmonds salt mixture daily. In our early studies the animals maintained or gained weight during the experimental procedure, whereas the animals cited in Table I lost weight throughout the experiment and became quite emaciated. The only obvious difference in the two experimental periods resided in the lowering of the intake of protein during the last 6 weeks of

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TABLE I. Anaphylaxis in Control Animals.

	Normal	Exp.
Hypernea	8/11	2/8
Salivation	8/11	4/8
Extension	5/11	6/8
Retching	8/11	4/8
Urination	11/11	3/8
Defecation	10/11	3/8
Vomiting	8/11	4/8
Collapse	5/11	1/8
Bleeding tendency	7/11	1/8
Hematuria (gross)	4/11	0/8
Other signs	0/11	1/8
Death	4/11	1/8

the experimental period in our present group, the amount of salmon muscle received in the daily diet having been reduced from 100 to 10 g. It is probable that such an alteration in protein intake may have been quite critical at this stage. Anemia was initially induced by bleeding an estimated $\frac{1}{4}$ of the blood volume (assuming 72 ml blood per kg) on each of 3 successive days. The animals were then rested for 1 day and the same amount of blood was withdrawn on the 5th day. Subsequent bleeding was such as to maintain an hematocrit value of 20-30%. Iron was administered in the form of the saccharated oxide, "Feojectin"[†] in quantities which were determined by previous phlebotomy to ensure that an excess existed in stores at all times. No attempt was made to quantitate the amount of hemoglobin removed but rather an estimation was made on a basis of total blood withdrawn and the hematocrit value. The depletion period lasted for 6 months. Electrophoretic studies were carried out on a portable apparatus made by the American Instrument Co., Silver Springs, Md. The plasmas were diluted with an equal volume of diethylbarbiturate buffer of pH 8.6 and an ionic strength of 0.1. The diluted plasmas were dialyzed against the buffer solution for two hours at 1-1.5°C after which the cell was filled. The duration of all runs was 90-120 minutes. Sensitization to the horse serum was accomplished by intravenous administration of 5 ml of horse serum and an additional 5 ml subcutaneously. At the end of 3 weeks

20 ml of horse serum were introduced by vein for anaphylactic test. Following administration of the shocking dose the dog was immediately untied and placed on the floor for observation. Record was kept of subsequent symptoms such as stiffening of legs, excessive salivation, bleeding tendencies, gagging, retching, vomiting, scratching, defecation and collapse. A group of 11 stock animals in apparent healthy condition was sensitized to horse serum at the same time as the test animals, using the same material. When shocking doses of horse serum were administered to this group for comparison with the experimental animals, one dog showed a minimal response inasmuch as there were no symptoms except those of excessive itching as shown by the animal scratching himself vigorously for a prolonged period. The other controls exhibited the usual symptoms such as extension of limbs, retching, collapse, etc. (Table I). Two died within 12 hours. One of the depleted dogs was extremely weak and emaciated at time of the test shock, being barely able to stand alone. Administration of the shocking dose was followed by death without

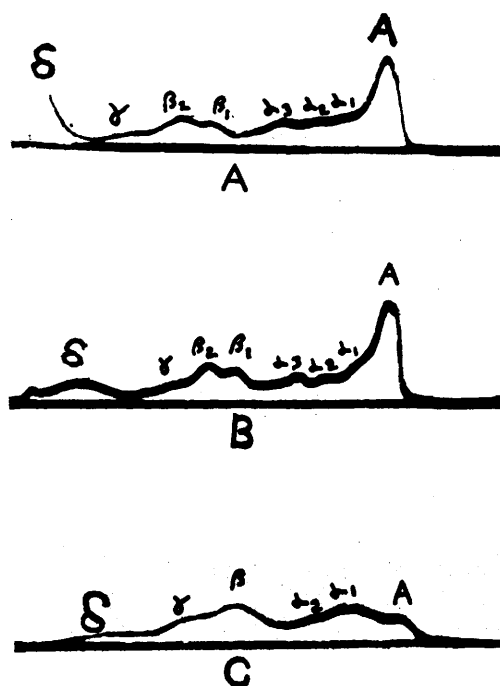


FIG. 1.

[†] Supplied in generous amounts through the courtesy of Smith, Kline & French Co.

TABLE II. Total Plasma Proteins and Electrophoretic Fractions of Plasma ($\pm$ S.E.), in 7 Dogs per Series.

Condition of dog	Total proteins*	A/G	Electrophoretic fractions (%)						
			Alb.	Alpha-1	Alpha-2	Alpha-3	Beta-1	Beta-2	Gamma
Normal	6.34	.85	45.9	10.3	7.7	6.9	10.4	13.3	4.8
	$\pm .24$	$\pm .05$	± 1.41	± 1.07	± 1.16	± 1.06	$\pm .45$	$\pm .39$	$\pm .30$
"	5.17	.81	44.0	10.7	9.7	6.9	11.7	16.3	5.0
	$\pm .14$	$\pm .09$	± 2.60	± 1.00	± 1.37	± 1.85	$\pm .52$	$\pm .46$	$\pm .18$
Depleted†	4.86	.12	10.5	29.7	12.5	—	37.7 $\pm$ 1.21		9.4
	$\pm .15$	$\pm .01$	$\pm .65$	$\pm .37$	± 1.19				$\pm .98$

* Determined by Semi-Micro-Kjeldahl method.

† Two dogs died during depletion regime.

the common signs of anaphylaxis within a few minutes. In the others the usual manifestations were less severe or absent and they all survived. This phase of the study will be described at length in another communication.

Results. Plasma electrophoretic patterns of the depleted animals are represented by that of dog 54-97 in Fig. 1 which is quite typical of the group.

Table II shows the plasma electrophoretic studies on these 7 litter mate hounds. In each case patterns were run at time of acquisition of the dog and again 5 months later, during which interim the animals were maintained on Purina dog chow and observed closely for abnormalities and for the establishment of baseline hematologic studies. Immediately after the second series of electrophoretic studies the litter was placed on the depletion regime for 6 months. At the end of this time another electrophoretic pattern was determined on each animal. It can be noted that there were only moderate alterations in the *total plasma proteins* with an average reduction of about 20%. This relatively small decrease in total protein content was in contrast to the marked change in the A/G ratio which was decreased from an average of 0.83 under normal conditions to 0.12 in the depleted state or a lowering of approximately 85%. The percentage of albumin ranged from 40-50% in the normal state, while in the depleted state it was reduced to values ranging from 9-13%. In each instance there was a marked increase in total α -globulin. In no case were there 3 alpha globulin peaks found

in the dogs following depletion as were found in the normal state. The combined beta-1 and beta-2 globulins were increased consistently in each plasma sample following depletion, the increase amounting to about 1.5 times the value found in the normal state. The gamma globulin values were approximately doubled in the depleted state. This fraction is generally believed to contain most of the antibodies, partial depletion of which by this technic we have reason to feel is accomplished. It is known that the gamma globulin fraction as determined by electrophoresis consists of several components and so obviously a marked decrease in one element with which we are critically concerned may be entirely masked by a concomitant increase in several of the other contributing components. On the other hand it is possible that the antibody with which we are concerned exists in the β_2 -globulin fraction.

Summary. 1) Dogs were depleted of body proteins by forced hemoglobin production superimposed on a low protein diet. Electrophoretic patterns indicated markedly decreased albumin values and A/G ratios in spite of relatively little change in total plasma proteins. In all instances there were marked increases in total α , β and γ globulins. 2) Anaphylactic reactions following immunization to horse serum were diminished or absent in the depleted animals as contrasted to controls.

1. Hahn, P. F., and Whipple, G. H., *J. Exp. Med.*, 1939, v69, 315.

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