creases in the plasma concentration of the vitamin. However, data furnished by concurrent observations[11] on the amount of ascorbic acid *excreted* by the subjects of this study, showed that with the greater intake of the vitamin, more was retained over a 12-hour period.

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Effect of Protein Depletion on Plasma Proteins in the Dog Measured by Electrophoretic Analysis.

BACON F. CHOW, JAMES B. ALLISON, WILLIAM H. COLE, AND ROBERT D. SEELEY.

From the Division of Protein Chemistry of the Squibb Institute for Medical Research, and the Bureau of Biological Research, Rutgers University, New Brunswick, N.J.

Electrophoresis has become a valuable tool for studies of the effect on plasma proteins of disease and other abnormalities in man or experimental animals. Immune sera[1] and sera of patients suffering from diseases such as multiple myelomatosis,[2] pneumonia, peritonitis, acute lymphatic leukemia, etc.[3,4] show electrophoretic patterns differing from the normal. Although most diseases produce a rise in α globulin content of the sera, a rise in β_2 and γ fractions but not in α globulin has been reported in rheumatic fever.[4] In many of these studies the alteration of plasma proteins may have been caused in part by protein depletion as well as by other metabolic shifts attributed to disease. There have been no reports published on the effect of protein depletion alone on the plasma electrophoretic pattern although there is need for such data on the plasma of normal and experimental animals, particularly the dog. In this animal, which has been used extensively in studies on hypoproteinemia and plasma protein regeneration, it has been demonstrated by chemical precipitation methods[5–7] that protein depletion results in a loss of albumin greater than that of the other plasma proteins. In the following are presented the results of studies of the electrophoretic patterns of plasma of dogs before and after depletion, both by protein-free feeding and by plasmapheresis.

Methods. The dogs were depleted of protein by a protein-free diet[8] for 6 to 10 days, after which plasmapheresis was performed for 2 to 5 days. During these days, one-quarter of the blood volume of the dog was removed daily, the cells being returned suspended in saline. This period of plasmapheresis was followed by protein-free feeding alone until the plasma protein concentration was relatively constant at 4.0 to 4.5 g per 100 ml of plasma.

The plasma was analyzed for total nitrogen and non-protein nitrogen by the micro Kjeldahl method. The technic of Howe (1921) as modified by Robinson, Price, and Hogden,[9]

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TABLE I.
Composition of Plasma Protein of Dogs Before and After Plasmaphoresis and Protein-Free Feeding.

Dog No.	Wt, kg	Plasma proteins, g/100 ml	Plasma vol., ml	Plasma protein fractions, %				Total circulating plasma proteins, g			Albumin/Globulin		Treatment
					Globulins				Globulins				
				Albumin U = 6.4	α_1 U = 5.2	α_2 U = 4.6	γ U = 1.1	Albumin	α	γ	Howe	Electrophoretic	
28	8.9	5.41	564	44	13	12	8	13.4	7.6	2.4	2.56	0.79	control
	8.8	4.20	460	37	32	—	8	7.1	6.2	1.5	0.86	0.54	depleted
31	5.8	5.96	431	42	6	9	9	10.8	3.9	2.3	1.32	0.72	control
	5.9	3.84	352	27	32	5	9	3.7	5.0	1.2	1.21	0.37	depleted
33	7.9	5.79	443	40	7	8	5	10.2	3.8	1.3	1.60	0.66	control
	7.3	4.32	415	34	15	10	9	6.1	4.5	1.6	1.30	0.52	depleted
52	9.5	6.36	442	29	13	8	12	8.2	5.9	3.4	0.77	0.41	control
	9.0	5.15	388	24	19	9	14	4.8	5.6	2.8	0.60	0.32	depleted
54	12.5	5.35	519	45	16	—	9	12.5	4.4	2.5	1.05	0.82	control
	11.1	4.10	559	21	18	11	11	4.8	6.7	2.5	0.58	0.26	depleted
61	12.3	5.67	566	43	9	11	8	13.8	6.4	2.6	1.26	0.75	control
	11.0	4.40	498	16	20	16	10	3.5	7.9	2.2	0.56	0.19	depleted

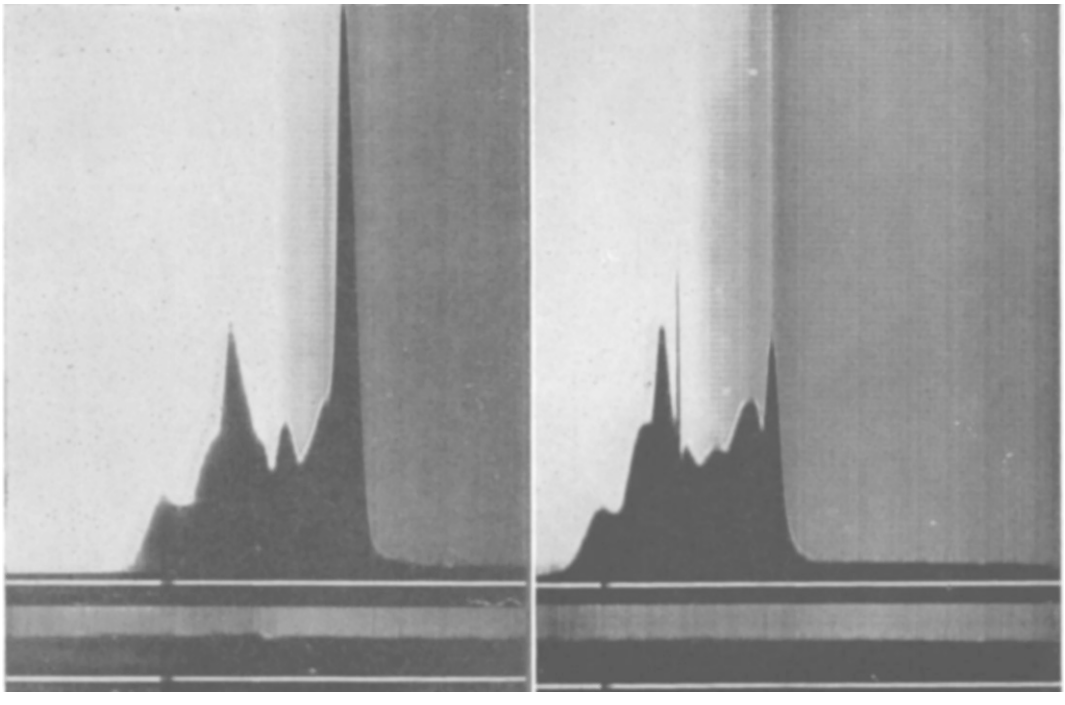

FIG. 1.
Electrophoretic pattern (descending limb) of plasma of normal (1) and protein depleted (2) dogs.

was used for the separation of the plasma proteins into an "albumin" and a "globulin" fraction and for the estimation of the ratio of the 2 fractions. Plasma volume was determined by the method of Gregersen and Stewart.[10]

For electrophoretic analysis,* 10 ml of plasma was diluted with an equal volume of 0.1 N sodium diethylbarbiturate buffer at pH 8.4 ± 0.1. The diluted plasma was dialyzed against 2 liters of the buffer for at least 24 hours in the refrigerator. Electrophoretic analysis was carried out according to the technics of Longsworth,[11] his directions and methods being followed also for the resolution of the scanning patterns. There was no difficulty in separating fractions corresponding to albumin, α globulins, and γ globulin (Fig. 1). The separation of components corresponding to β globulin fractions, however, was not clear cut. Mobility measurement of albumin, α globulins, and γ globulin, showed only slight variations, but considerable variation was found in the proteins of the β globulin group.

Results. In Fig. 1 are shown the descending electrophoretic patterns of the plasma of dog 61 kept on a protein-free diet for 30 days, with plasmaphoresis on the 10th, 11th, and 17th days. The results of electrophoretic analyses of the composition of plasma protein of 6 dogs before and after such depletion are recorded in Table I. Hypoproteinemia occurred in every dog, accompanied by reduction of plasma volume, except in dog 54. The percentage of albumin was reduced, the percentage of α globulins was increased, and the

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* The authors wish to express their appreciation for the technical assistance of Miss Shirley Quaid.

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γ globulin percentage remained essentially unchanged. The total circulating albumin decreased markedly, the γ globulin tended to decrease slightly, whereas the α globulins changed but little or increased. The maintenance of the circulating α globulins in the depleted state, even though the albumin has been markedly reduced, suggests that the replacement of α globulin in the dog is much more efficient than that of albumin.

The plasma A/G ratios determined by Howe's method were always smaller than those obtained by electrophoresis. Plasma from one normal and one depleted dog was separated into albumin and globulin fractions by Howe's technic. Electrophoretic analyses of these fractions showed that the albumin fraction of the normal dog contained 23% contaminating globulin whereas the globulin fraction contained 6% albumin. The albumin fraction of the depleted dog contained 39% contaminating globulin and the globulin fraction 3% albumin. Thus, incomplete precipitation of the globulins by the Howe method accounted for the higher A/G ratio. The decreased ratios by both methods reflected the relatively greater reduction in the albumin fraction in the depleted state.

Summary and Conclusions. Electrophoretic analyses of plasma showed that protein depletion in dogs resulted in marked decrease in circulating albumin, and slight decrease in γ globulin. In contrast to these decreases the α globulin content remained essentially unchanged or increased. The α globulins, however, always increased in concentration, which was in part due to the accompanying fall of plasma volume. The Howe method for the determination of albumin: globulin ratios resulted in higher ratios than those obtained by electrophoretic analyses, because of the contamination of globulins in the "albumin" fraction.

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The Antigenicity of *Shigella paradysenteriae* (Flexner) in Saline-in-Mineral-Oil Emulsion.*

SEYMOUR P. HALBERT, STUART MUDD, AND JOSEPH SMOLENS.

From the Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Several reports have recently demonstrated that the immune response to antigens may be markedly prolonged, and often elevated by the suspension of these antigens in the aqueous phase of a saline-in-mineral-oil emulsion.[1-3] Killed acid-fast bacilli have been included also in the oil emulsion in a number of these instances,[3,4] but Freund has demonstrated in rabbits that in some instances at least, this was not a requisite.[2] The Henles have recently obtained extremely favorable results in human subjects injected with influenza virus in saline-in-mineral-oil emulsion.[5]

In the search for a method of improving the response to *Shigella paradysenteriae* vaccines, the oil emulsion technic was investigated in mice, rabbits, and humans. Conditions were chosen for the experimental animals that

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