TABLE II. Subcellular Localization of P^{32} in Liver.

Fraction	Counts/min./Mg N	% total counts
A. $NaH_2P^{32}O_4$		
Cell debris I	753	12.8
II	1,102	18.7
Nuclei	1,465	24.9
Mitochondria	1,554	26.4
Supernatant	1,011	17.2
B. Trimetaphosphate		
Cell debris I	811	13.9
II	652	11.2
Nuclei	1,519	26.1
Mitochondria	1,618	27.7
Supernatant	1,229	21.1
C. Polymetaphosphate		
Cell debris I	409	14.4
II	365	12.8
Nuclei	750	26.4
Mitochondria	765	26.9
Supernatant	551	19.4

$P^{32}O_4$ as a P^{32} donor to bone, the extent depending upon experimental conditions. In particular, polymetaphosphate has a longer body retention and a higher bone-soft tissue ratio. The equivalent localization of P^{32} from $NaH_2P^{32}O_4$, trimetaphosphate and polymetaphosphate into the nuclei and mitochondria of liver cells is offered as evidence that the metaphosphates are enzymatically broken down to phosphates before incorporation.

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1. Friedell, H. L., Storaasli, J. P., *Am. J. Roent.*, 1950, v64, 559.

2. Maxfield, J. R., Jr., Maxfield, J. G. S., Maxfield, W. S., *Southern Med. J.*, 1958, v51, 320.

3. Kaplan, E., Miree, J., Jr., Hirsh, E., Fields, T., *Abst. 5th Ann. Meet., Soc. Nuc. Med.*, Los Angeles, Calif., June 1958; *Internat. J. Appl. Rad. and Isotopes*, in press.

4. Kitasato, T., *Biochem. Z.*, 1928, v197, 257.

5. ———, *ibid.*, 1928, v201, 206.

6. Herrman, H., Perlman, G., *Nature*, 1937, v140, 807.

7. Briggs, D. R., *J. Biol. Chem.*, 1940, v134, 261.

8. Lamm, O., *Ark. Kemi Min. Geol.*, 1944, v17A, No. 25.

9. Jones, L. T., *Ind. Eng. Chem. Anal.*, 1942, v14, 536.

10. Schreier, K., Noller, H. G., *Naturwiss*, 1957, v44, 517.

11. Schneider, W. C., Hogeboom, G. H., *J. Biol. Chem.*, 1950, v183, 123.

12. Franceen, C. C., McLean, R., *Am. J. Cancer*, 1935, v24, 299.

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Electrophoretic Studies of Bovine Serum. II. Concurrent Hypoglobulinemia and Natural Infections of Eperythrozoonosis.* (24521)

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Although data have been accumulated on pathogenesis of eperythrozoonosis together with descriptions of the causative agent (1-4), no work, thus far, has been directed toward physicochemical characterization of alterations manifested in the host. During the present investigations, in which bovine anaplasmosis was studied primarily, it was occasionally observed that stained blood smears showed microscopic evidence of eperythrozoonosis prior to experimental infection with anaplasmosis. This report presents results of serum protein changes concurrent with natural infections of eperythrozoonosis in calves.

Materials and methods. Cattle: In our investigation a total of 30 dairy calves of mixed breeds, ranging in age from 3 weeks to 6 months, were studied. They were housed and fed as previously described(5). Of this num-

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TABLE I. Electrophoretic Analyses of Serums from Splenectomized Dairy Calves Naturally Infected with Eperythrozoonosis after Convalescence from Surgery.

Days (bleeding intervals)	A/G*	GG	TG	TSP	EP
0	.48	2.64	5.55	8.23	—
3	.53	2.12	4.61	7.03	—
7	.45	2.18	5.02	7.27	—
10	.57	1.97	4.43	6.95	—
17	.53	2.22	5.08	7.76	—
24	.58	2.05	4.53	7.16	—
29	.77	2.27	4.26	7.52	—
31	.59	1.55	4.36	6.95	—
36	ND	ND	ND	ND	+
38	.75	1.46	3.61	6.33	+
49	.97	.62	2.94	5.78	+
56	ND	ND	ND	ND	+
60	.77	.70	3.43	6.08	—
63	ND	ND	ND	ND	+
66	.72	.89	3.62	6.21	—
70	ND	ND	ND	ND	+
73	.96	.80	3.22	6.31	—
80	.80	1.12	3.57	6.42	—
87	.64	1.91	4.29	7.03	—
94	.60	2.06	4.37	6.97	—
100	.49	2.44	5.30	7.91	—
115	.61	2.87	4.84	7.80	—
122	.57	2.65	4.64	7.29	—

* A/G = albumin/globulin ratio.

GG = gamma globulin
TG = total globulins
TSP = total serum protein
} as g/100 ml serum.

EP = appearance of eperythrozoonosis on stained blood smears.

ND = not done.

ber 17 animals, which had been splenectomized, and 3 others which were intact, showed evidence of eperythrozoonosis. The remaining 10 calves, 4 of which were splenectomized and 6 which were intact did not develop eperythrozoonosis. Splenectomy was performed to increase susceptibility to experimental infections of anaplasmosis(6). Results shown on alterations in serum proteins in all splenectomized calves are for the period after recovery from surgery. It has been reported that the serum proteins return to normal levels after several weeks convalescence (5). *Serum proteins:* Sera were separated by paper electrophoresis in barbital buffer, pH 8.6, 0.075 ionic strength. The paper strips were subsequently dyed with bromphenol blue(7) and scanned with a commercial densitometer-integrator for relative percentages of serum protein fractions. Absolute protein values on each serum fraction were determined after protein nitrogen determinations of whole serum were made using a semi-micro nesslerization technic.

Results. Since no satisfactory immunologic or serologic test was available for study of eperythrozoonosis with which results might be related to antibody, or to a degree of immunity or susceptibility, the pertinent information derived from a serum protein analysis was an indirect evaluation of the disease process. The conventional methods commonly used have considered changes in gamma globulins (GG), total globulins (TG), and total serum proteins (TSP) based on g/100 ml of serum. To a lesser extent the albumin/globulin (A/G) ratio, based on relative concentrations of these fractions, varies widely even in normal animals and does not indicate a true picture of serum protein changes that occur. A low A/G ratio with a high TSP may approximate the same figures as a high A/G ratio and a lower TSP.

After convalescence from splenectomy, as evidenced by return of normal pre-surgical serum protein levels(5) in 17 calves, severe

TABLE II. Electrophoretic Analyses of Serums from Non-splenectomized Dairy Calves Naturally Infected with Eperythrozoonosis.

Days (bleeding intervals)	A/G*	GG	TG	TSP	EP
0	.39	2.28	4.84	6.72	—
3	.48	2.05	4.62	6.84	—
7	.41	2.29	4.87	6.88	—
10	.34	2.34	4.93	6.61	—
17	.34	1.41	4.22	5.65	—
24	.46	1.80	4.42	6.44	—
29	.76	1.83	3.84	6.74	+
31	.44	1.92	4.72	6.80	—
38	.81	.72	3.23	5.84	+
49	.90	.43	2.95	5.59	+
60	ND	ND	ND	ND	+
66	.81	1.29	3.41	6.16	+
73	.61	1.64	4.13	6.65	—
80	.59	2.47	4.27	6.80	—
87	.53	2.35	4.47	6.84	—
94	.58	2.54	4.28	6.78	—
100	.54	2.37	5.21	8.01	—
115	.67	2.18	4.16	6.95	—
121	.73	1.81	3.67	6.33	+

* A/G = albumin/globulin ratio.

GG = gamma globulin
TG = total globulins
TSP = total serum protein
} as g/100 ml serum.

EP = appearance of eperythrozoonosis on stained blood smears.

ND = not done.

decreases in amounts of globulins were observed when infections of eperythrozoonosis became established. The A/G ratio increased at this time. In many cases the decreases in weights of globulins amounted to less than ¼ of previous normal values at a time when eperythrozoonosis was not detected in erythrocytes (Table I). The results obtained from 3 non-splenectomized calves also showing eperythrozoonosis were strikingly similar (Table II) in that globulins decreased, while A/G ratio increased as soon as the organism appeared in the blood.

Levels of serum proteins for 4 uninfected splenectomized calves and 6 uninfected intact calves are shown in Tables III and IV. These animals did not show decreases in globulins and increases in A/G ratio as were observed in animals naturally infected with eperythrozoonosis. The protein values were considered to be within normal range(5).

Discussion. The outstanding fact that was immediately evident was the marked decrease in serum globulins, especially gamma globulin, in calves infected with eperythrozoonosis under natural management. These changes

TABLE III. Electrophoretic Analyses of Serums from Uninfected Splenectomized Dairy Calves after Convalescence from Surgery.

Days (bleeding intervals)	A/G*	GG	TG	TSP	EP
0	.56	2.44	5.04	7.84	—
3	.61	2.16	4.89	7.86	—
7	.62	2.11	4.72	7.63	—
10	.47	2.32	4.28	6.27	—
17	.46	2.01	5.03	7.35	—
24	.57	2.29	4.84	7.59	—
31	.49	2.20	5.12	7.63	—
38	.52	2.03	4.75	7.23	—
47	.57	2.47	4.52	7.11	—
49	.39	2.49	4.49	6.25	—
59	.42	2.46	4.93	7.01	—
66	.55	1.85	4.00	6.19	—
72	.55	1.94	4.64	7.20	—
79	.52	2.00	4.76	7.23	—
86	.46	2.03	4.85	7.08	—
92	.56	1.99	4.79	7.48	—
100	.60	2.50	4.81	7.38	—
114	.54	2.06	4.17	6.40	—
119	.57	2.04	4.33	6.78	—

* A/G = albumin/globulin ratio.

GG = gamma globulin
TG = total globulins
TSP = total serum protein
} as g/100 ml serum.

EP = appearance of eperythrozoonosis on stained blood smears.

TABLE IV. Electrophoretic Analyses of Serums from Uninfected Intact Dairy Calves.

Days (bleeding intervals)	A/G*	GG	TG	TSP	EP
0	.55	1.89	4.59	7.09	—
7	.56	1.95	5.18	8.10	—
10	.63	1.97	4.45	7.27	—
17	.56	1.99	4.79	7.48	—
21	.62	1.93	4.48	7.26	—
28	.64	1.85	4.55	7.44	—
31	.61	1.96	4.26	6.84	—
38	.62	2.02	4.40	7.12	—
47	.55	2.04	4.30	6.65	—
49	.43	2.01	4.45	6.38	—
60	.48	2.05	4.62	6.84	—
65	.46	1.99	4.14	6.03	—
72	.54	2.18	4.43	6.80	—
80	.61	2.09	4.22	6.78	—
87	.64	1.94	4.15	6.82	—
100	.59	2.22	4.17	6.63	—

* A/G = albumin/globulin ratio.

GG = gamma globulin
TG = total globulins
TSP = total serum protein
} as g/100 ml serum.

EP = appearance of eperythrozoonosis on stained blood smears.

are contrary to popular schools of thought concerning alterations produced in serum proteins during infectious disease processes. It is common to recognize increases in globulins in bacterial diseases(8,9) but little or no deviation from the normal in most virus diseases (10,11) is anticipated.

Hypogammaglobulinemia has been reported in humans(12,13). In one case, where there was an almost complete absence of gamma globulin, TSP values were also low(13). Surprisingly, however, the patient had not become ill with any serious disease until she was 20 years of age. In another report(12) several patients, showing recurrent severe bacterial infections, exhibited an almost complete lack of serum gamma globulin, but in these instances TSP concentrations were normal.

Eperythrozoonosis has been reported to be an aggravating factor when coexisting with other diseases(3). In these instances the marked decrease in absolute weights of globulins, which contain protective substances and antibodies, may place the animal in a position of stress making it more susceptible to secondary infections.

The direct effect of eperythrozoonosis on globulin synthesis, the use of globulin changes

as possible diagnostic criteria for eperythrozoonosis and studies of the effects of this disease on production of antibody to other infectious agents are being investigated.

Summary. Results on serum protein changes in natural infections of eperythrozoonosis in calves are reported. Concurrent with microscopic evidence of infection in the blood, serum globulins decreased to an abnormally low level. This observation offers possibilities for diagnostic and clinical criteria. The implications of hypoglobulinemia in eperythrozoonosis are discussed.

1. Adler, S., Ellenbogen, V., *J. Comp. Path. Therap.*, 1934, v47, 219.

2. Jensen, R., *Louisiana Bull. No. 366*, 1943, 1.

3. Lotze, J. C., Yiengst, M. J., *N. Am. Vet.*, 1941, v22, 345.

4. Neitz, W. O., *Onderstepoort J. Vet. Sci. Anim. Ind.*, 1940, v14, 9.

5. Dimopoullos, G. T., Foote, L. E., Schrader, G. T., *Am. J. Vet. Res.*, (in press).

6. Reese, C. W., *N. Am. Vet.*, 1933, v14, 23.

7. Block, R. J., Durrum, E. L., Zweig, G., *A Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press, 1955.

8. Lynch, J. E., Stafseth, H. J., *Poultry Sci.*, 1954, v33, 54.

9. Luetscher, J. A., Jr., *Physiol., Rev.*, 1947, v27, 621.

10. Dimopoullos, G. T., Cunningham, C. H., *Am. J. Vet. Res.*, 1956, v17, 755.

11. Dimopoullos, G. T., Fellowes, O. N., *J. Immunol.*, 1958, v81, 199.

12. Bruton, O. C., Apt, L., Gitlin, D., Janeway, C. A., *Am. J. Dis. Child.*, 1952, v84, 632.

13. Reiner, M., *Med. Ann. Dist. Columb.*, 1952, v21, 11.

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Successful Skin Homografts in Mature Non-littermate Rats Treated with Fractions Containing Alpha-globulins.* (24522)

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Skin homografts are always rejected when exchanged between normal immunologically-mature non-littermates of non-inbred rats (See review by Medawar(1)). Recently, Axelrod *et al.*(2) and Fisher *et al.*(3) reported that pyridoxine-deficient Wistar and Long-Evans strain rats have retained skin homografts for over 70 days. Reporting on their non-vitamin deficient Wistar controls, these authors found 24% retaining viable skin homografts for 60 days. Referring to the common finding that no experimental treatment of normal animals has resulted in permanently retained succesful homografts, Medawar(1) concluded that in the immunologically immature animals only the nucleated cell can bring about "immunological or adaptive tolerance." Woodruff and Simpson(4) working with Wistar rats noted that nucleated cells injected into animals less than 2 weeks old can induce some degree of tolerance to subsequent skin grafts. Puza and Gombos(5) demonstrated that young dogs (11 days post-partum) could be induced, by means of repeated exchange transfusions, to accept reciprocal homografts. Successful parabiosis involves reciprocal tolerance of skin, blood and muscle tissues. A small percentage of non-inbred littermates will go into successful parabiosis; non-littermates will not unite successfully. However, Kamrin(6) achieved successful parabiotic union of non-littermate rats (pen-bred Wistar strain) by injection of alpha-globulins derived from pooled rat blood serum. The blood serum was obtained by exsanguination of ageing rats from the same colony. Enhancement of non-littermate parabiosis by treatment with alpha-globulins suggested that treatment of single animals might induce tolerance to skin homografts.

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Methods. Most alpha-globulins utilized