

by cortisone treatment. Comparison of the E.E.E. virus growth in both cortisone-treated and untreated animals showed no effect of cortisone on rate of virus proliferation. The effect of this neurotropic virus infection on rate of phosphate transfer from blood to brain acid-soluble phosphates appeared to be due to inflammation, rather than to a specific metabolic requirement for virus synthesis.

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Electrophoretic Distribution of Serum Proteins in Rabbit, Guinea Pig and Rat Following BCG Administration. (22388)

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Pulmonary tuberculosis in humans has been shown to produce an increase in the alpha-1, alpha-2, and gamma globulin concentration of the serum protein (1-4). Electrophoretic studies of tuberculous guinea pig and rabbit sera revealed an increased total protein and gamma globulin with a decrease in the albumin concentration (5-7).

In an attempt further to understand biochemical factors of host resistance to tuberculosis, a study of serum protein composition in natively resistant (rat) and susceptible (guinea pig and rabbit) animals was undertaken. It also seemed important to study the effect of an immunizing strain (BCG) on the serum protein pattern of susceptible and resistant animals.

Methods. Eight male albino rabbits (avg wt 1100), 8 male albino guinea pigs (550 g) and 8 male albino rats (360 g, Sprague-Dawley) were fasted for 16 hours and bled by cardiac puncture to obtain normal sera. The

sera were separated and stored at -20°C until chemical and physical determinations could be made. The animals were then inoculated intraperitoneally with a suspension of tubercle bacilli (BCG)*. The rabbits received 6.0 mg; the guinea pigs, 3.0 mg; and the rats, 2.0 mg (wet wt) of the organism suspended in 1.0 ml of saline. The rabbits and guinea pigs were maintained on a diet of rabbit chow pellets (containing no antibiotics) and the rats were fed laboratory Purina chow (no antibiotics). All diets were supplemented with greens and water *ad libitum*. Four weeks following the inoculation of BCG, all of the above animals were again fasted for 16 hours and bled by cardiac puncture. The sera were stored at -20°C . The analyses on control and experimental sera were performed in duplicate at the same time. Electrophoresis of the serum protein was done on

* Culture obtained from Dr. S. R. Rosenthal, Tice Laboratory, University of Illinois, Chicago.

TABLE I. Total Serum Protein Concentrations in Rabbit, Guinea Pig, and Rat following BCG.

Group	Serum total protein g/100 ml	S.D.	"t"
Normal rabbit	7.20	.11	
BCG "	7.67	.21	5.6*
Normal guinea pig	5.68	.09	
BCG "	6.35	.27	4.6*
Normal rat	6.71	.15	
BCG "	6.67	.13	.6

* Statistically significant ($P = 0.01$).

S.D. = Stand. dev. from mean.

filter paper using the Durrum(10) type, Spinco, Model R paper electrophoresis apparatus. Whatman 3 MM paper strips $1\frac{1}{4} \times 11\frac{1}{2}$ inches were used. All electrophoretic separations were done at room temperature at a constant current (10 Ma) in pH 8.6 veronal buffer (ionic strength 0.075) for 16 hours. The voltage varied with conditions of evaporation within the system. A volume of 0.01 ml of serum was applied with a mechanical applicator on a line 25 mm wide at the apex of the paper strip. At the end of 16 hours of migration the protein was fixed to the paper strips by oven drying at 120°C for 30 minutes. The strips were then stained with bromphenol blue (0.1% in 5% zinc-sulfate-5% acetic acid solution) for 16 hours, rinsed in 5% acetic acid and oven dried for 15 minutes. The protein composition and distribution of the serum was determined by scanning the papers in the Spinco Analytrol Photometer-Computer. Total serum protein was determined by the biuret method of Weichselbaum(11). Absolute values for the pro-

tein fractions were computed from the relative distribution and the total protein values. The significance of the deviations from the normal means were computed by the "t" test (12).

Results. The summaries and statistical analysis of the distribution and concentration of serum proteins before and after BCG administration in the rabbit, guinea pig, and rat are presented in Tables I and II.

The administration of BCG resulted in a significant elevation in total protein and gamma globulin in the rabbit and guinea pig but not in the rat. The guinea pig revealed an increase and the rat a decrease in the concentration of alpha-1 globulin. There was a significant decrease in the relative, but not the absolute, concentration of albumin in the rabbit and guinea pig. Significant alterations in the other serum protein components were not observed.

Discussion. The increased total protein concentration in the guinea pig and rabbit following BCG immunization is of interest in light of a report(6) demonstrating a similar protein increase in the tuberculous guinea pig. Similarly, elevation of the gamma globulin has been noted in the tuberculous guinea pig and rabbit(8,9). The possible relationship of the alteration in the alpha-1 globulin composition in the guinea pig and rat to mechanisms of allergy, immunity, or host resistance is not known at this time. The absence of changes in the gamma globulin fraction of rat serum following the inoculation

TABLE II. Electrophoretic Distribution of Serum Protein in Rabbit, Guinea Pig and Rat following BCG.

Group	Albumin		Alpha-1 globulin		Alpha-2 globulin		Alpha-3 globulin		Beta globulin		Gamma globulin	
	Rel. %	g %	Rel. %	g %	Rel. %	g %	Rel. %	g %	Rel. %	g %	Rel. %	g %
Normal rabbit	57.22	4.11	8.73	.63	6.21	.45			14.58	1.06	13.22	.95
BCG "	48.67	3.73	7.85	.60	5.60	.43			14.47	1.11	23.42	1.80
"t" values	6.10*	2.65	1.80	.75	1.50	.57			.20	.94	12.00*	13.90*
Normal G. pig	40.19	2.28	6.45	.36	23.70	1.36	8.75	.50	12.26	.69	8.66	.49
BCG "	34.32	2.19	7.40	.47	22.53	1.44	8.22	.52	11.97	.76	15.54	.98
"t" values	5.70*	1.18	2.30	2.94*	1.50	1.23	1.00	.58	.40	1.62	9.00*	6.80*
Normal rat	28.89	1.94	16.22	1.09	12.11	.81	5.02	.34	22.00	1.48	15.71	1.06
BCG "	29.70	1.98	14.68	.98	11.87	.79	4.34	.29	22.08	1.48	17.11	1.13
"t" values	1.20	.69	4.00*	4.40*	.60	2.50	1.90	2.00	.20	.00	2.10	1.20

* Statistically significant ($P = 0.01$).

Rel. % = Percent distribution of total protein. g % = Absolute value for grams of protein/100 ml serum based on total protein.

of an antigen such as BCG is not surprising in view of the fact that this species is known to respond only slightly, or not at all, to most antigenic or allergenic stimuli. It is possible that the unusual lack of response of the rat to BCG immunization is another reflection of its peculiar biological behavior and may be in some way related to its native resistance to infection.

The increase in gamma globulin during BCG immunization in the rabbit and guinea pig follows the classical relation of an increase in gamma globulin with the production of antibody(13,14). The extra-normal T component sometimes seen to occur as a result of certain antigenic stimulation(15) was not observed in this study.

The comparative protein response of the natively resistant rat and susceptible rabbit and guinea pig to tuberculous infection is now under investigation in this laboratory.

Summary. Total serum protein concentration and electrophoretic distribution of the serum protein components of the rabbit, guinea pig, and rat were determined by a paper electrophoretic technic. The serum protein was again analyzed 4 weeks after the animals were inoculated with an attenuated strain (BCG) of tubercle bacilli. Statistically significant increases in the total protein and gamma globulin occurred in the rabbit and guinea pig, but not in the rat. A decrease in the alpha-1 globulin in the rat and an increase of this fraction in the guinea pig

was observed following BCG administration. Significant alterations in the other protein fractions were not observed in any of the 3 species of animals.

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Oxidation of Lecithin and Sphingomyelin by Tissue Preparations.* (22389)

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The metabolic functions of the phospholipids are not clearly understood at the pres-

ent time. Liver(1-3) and liver mitochondria (3) are known to contain dehydrogenases which act on purified phospholipids. However, the ability of other tissues to attack phospholipids has not been tested. Accordingly in the present experiments the phos-

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