

matographed in the same manner as the toxin; this yielded a fluorescent fraction which emerged at the same position as the unknown fluorescent material. This suggests that the fluorescent product obtained from hydrolyzed plague toxin may be a degradation product of tryptophane.

Summary. A detailed elemental and amino acid analysis was performed on highly purified *Pasteurella pestis* murine toxin. Eighteen amino acids and a number of elements were identified. The high proportions of acidic amino acids found are in accordance with the previously observed isoelectric point of the toxin of 4.7(1). On a dry weight basis, 98% of the toxin molecule was accounted for by the organic analysis including ammonia and ash content.

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Electrophoretic Distribution of Serum Protein and Glycoprotein in the Tuberculous Rat, Rabbit and Guinea Pig.* (23160)

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(Introduced by M. M. Cummings)

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Serum protein distribution changes in man and experimental animals during tuberculous infection have been the subject of several investigations(1-2). Likewise, alterations of serum glycoprotein concentration in tuberculosis have been of interest(3-5).

The present study attempts to compare serum protein and glycoprotein response of the natively resistant rat and the susceptible guinea pig and rabbit following infection with tubercle bacilli. In a previous study(6) we compared the response of these species to an attenuated (BCG) strain of tubercle bacilli.

Methods. Eight male albino rats, Sprague-Dawley (avg wt 350 g), 8 male albino guinea pigs (500 g) and 6 male albino rabbits (1200 g) were inoculated intraperitoneally with 0.1 mg (wet wt) of *Mycobacterium tuberculosis*, human strain H 37Rv. Six other rabbits were

similarly injected with bovine strain (Ravenel) tubercle bacilli. All animals were bled before and 4 weeks following infection. The sera were stored at -20°C and control and experimental sera were analyzed in duplicate at the same time. Electrophoretic separation of serum protein and glycoprotein was done by a paper strip technic previously described (6), with the exception of using 8 Ma of current for electrophoresis instead of the former 10 Ma used. The electrophoretic strips for serum glycoprotein determination were stained by a method described by Roboz(7). Stained strips were scanned in the Spinco Analytrol with a green (Wratten XI) filter. Relative % distribution of glycoprotein was determined in this manner. Total (non-glucosamine) polysaccharide content of serum was measured by the method of Shetlar(8). Milligram % glycoprotein concentration of each protein fraction was determined from total polysaccharide and relative % distribution of glycoproteins

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TABLE I. Electrophoretic Distribution of Serum Protein in Rabbits, Guinea Pigs and Rats following Inoculation with Virulent Tubercle Bacilli.

Group	Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin	Total protein
	g %					
Normal rat	1.72	1.00	.89	1.71	1.18	6.53
H 37Rv	1.71	.97	1.06	1.34	1.37	6.45
“t” value	.1	.5	5.6 *	7.8 *	3.8 *	.5
Normal rabbit	3.86	.72	.36	.85	1.01	6.82
H 37Rv	3.11	.69	.47	.88	2.21	7.39
“t” value	5.7 *	.6	3.0 *	.3	7.0 *	6.2 *
Normal guinea pig	2.88	.29	1.89	.74	.45	5.99
H 37Rv	2.49	.51	1.73	.91	.88	6.55
“t” value	3.2 *	8.1 *	.8	4.6 *	7.5 *	5.6 *
Normal rabbit	3.86	.72	.36	.85	1.01	6.82
Bovine R	2.61	.60	.82	1.15	2.78	7.69
“t” value	10.8 *	2.2	5.1 *	2.9 *	10.4 *	7.1 *

* Statistically significant ($P = .01$).

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

TABLE II. Electrophoretic Distribution of Protein Bound Polysaccharide (Glycoprotein) in the Rabbit, Guinea Pig and Rat following Tuberculous Infection.

Group	Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin	Total polysac- charide
	mg %					
Normal rat	28.5	39.8	25.4	35.2	34.1	165
H 37Rv	32.8	32.4	47.0	40.9	31.1	185
“t” value	.9	1.2	6.8*	1.1	.6	8.7*
Normal rabbit	15.2	16.2	18.9	17.4	20.2	88
H 37Rv	23.0	14.2	24.8	28.4	30.5	121
“t” value	5.3*	1.1	5.6*	6.1*	6.8*	26.2*
Normal guinea pig	20.1	9.7	32.2	29.3	28.5	120
H 37Rv	29.4	20.3	55.9	40.7	32.7	179
“t” value	6.3*	9.2*	5.8*	4.7*	1.3	23.2*
Normal rabbit	15.2	16.2	18.9	17.4	20.1	88
Bovine R	28.0	20.9	25.2	31.0	48.0	153
“t” value	6.2*	4.3*	4.6*	5.8*	7.1*	25.2*

* Statistically significant ($P = .01$).

mg % = Absolute mg % protein bound polysaccharide/100 ml serum, calculated from total serum polysaccharide and relative distribution in protein fractions.

by electrophoresis. The significance of deviations from normal means was computed by the "t" test(9).

Results. Distribution and concentration of serum protein and glycoprotein before and after tuberculous infection in the rat, rabbit and guinea pig are presented in Tables I and II.

Protein. A significant hyperproteinemia occurred in infected guinea pigs and rabbits, but not in rats. Comparison of individual protein components before and after tuberculosis infection reveals several types of changes with respect to animal species and type of in-

fection (Table I).

Glycoprotein. All 3 species exhibited increases in total serum polysaccharide following infection. Differential responses in the various animals were noted following tuberculous infection (Table II). Elevation of the polysaccharide content of the fractions also remained significant when expressed as milligrams polysaccharide per gram of protein.

Discussion. A previous study(6) revealed similar elevation of the total serum protein and gamma globulin in guinea pigs and rabbits, but not in rats, following BCG immunization.

The elevation in total polysaccharide appeared to parallel the degree of gross pathology in the tuberculous animals. The group showing the greatest increase in polysaccharide (rabbits infected with bovine type tubercle bacilli) also demonstrated the most extensive tuberculosis.

Increase in alpha-2 globulin glycoprotein seen in all animals following tuberculosis has also been described by Seibert(10) in clinical tuberculosis. Seibert's study suggests a possible correlation of elevated alpha-2 glycoprotein and increased tissue destruction. Our findings did not reveal this relationship in experimental tuberculosis. The greatest increase, for example, occurred in the rat, which had no gross pathology. The rabbits infected with bovine type bacilli had gross involvement of lungs, liver, spleen and kidney; whereas the rabbits infected with human type tubercle bacilli had only minimal involvement of the lungs. Despite these marked differences in gross pathology, the increase in alpha-2 glycoprotein was essentially identical in both groups. The guinea pig, with more extensive tuberculosis than the human strain infected rabbits and less pathology than the bovine type infected rabbit, had a greater alpha-2 glycoprotein increase than either of the two groups of infected rabbits.

Summary. 1. Total serum protein, polysaccharide and paper electrophoresis of protein and glycoprotein were determined before and 4 weeks following, tuberculous infection

of rats, rabbits and guinea pigs. Hyperproteinemia occurred in infected rabbits and guinea pigs. Elevation of alpha-1 globulin in the guinea pig and alpha-2 globulin in rabbits and rats was noted. Beta globulin decreased in infected rats and increased in guinea pigs, and bovine infected rabbits. Gamma globulin elevated in all species. 2. Total serum polysaccharide was elevated in all infected animals. Likewise, the alpha-2 glycoprotein was elevated in all species. Only the infected rabbit had elevated gamma globulin glycoprotein.

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Amino Acid and Carbohydrate Composition of the Mucoprotein Matrix in Various Calculi.* (23161)

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A technic for recovery of the organic matrix from calcigerous urinary calculi has been

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described(1). Preliminary studies of the amount, elemental composition and some histochemical characteristics were reported(1,2). This report is concerned with the carbohydrate and amino acid content of matrix recovered from calcium containing urinary and