Hypoproteinemia in Mice after Treatment with Histamine-Sensitizing Factor from *Bordetella pertussis* (34020)

R. K. BERGMAN AND J. MUNOZ

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana 59840

The histamine-sensitizing factor in *Bordetella pertussis* has a remarkable capacity to induce in certain mouse strains a high susceptibility to the lethal effects of histamine and/or serotonin, anaphylaxis, bradykinin, X-rays, and other noxae (1–7). Accompanying this effect is an altered glucose metabolism expressed by a hypoglycemia (8, 9) that lasts for up to 3 weeks (10). Intravenous (iv) administration of relatively large quantities of glucose (500 mg/kg) produces little if any elevation of blood glucose levels in *B. pertussis*-treated mice, whereas in normal mice a pronounced rise is observed (11). *In vivo* and *in vitro* alterations in the metabolic pattern of fatty acids have also been reported in *B. pertussis*-treated mice (12).

In the course of studying histamine shock in *B. pertussis*-treated mice we noted that a hypoproteinemia developed in these mice. This paper describes these findings.

Materials and Methods. Mice. Male and female mice of the Rocky Mountain Laboratory (RML) strain and of the CFW strain (Carworth Farms, New City, New York) raised in our laboratory were used. The mice were housed in glass jars (5 mice/jar) with beet pulp bedding and had feed (Purina Laboratory Chow) and water *ad libitum*.

Histamine-sensitizing factor from B. pertussis. An alkaline saline extract (SE) was prepared as previously described (13). SE dissolved in 0.2 ml physiological saline was given intravenously in the desired quantity. Control mice received only saline.

Histamine challenge. Five-tenths milligram histamine base[given as histamine diphosphate (Nutritional Biochemical Corp.)] in 0.2 ml physiological saline was administered intraperitoneally. Deaths were recorded 2–3 hr later.

Determination of total serum proteins or plasma proteins and hematocrit values. Total plasma proteins or total serum proteins (TSP) were determined by either the specific-gravity technique, described by Lowry and Hunter (14), or by the biuret method as given by Annino (15). A cathetometer was used to measure the height of the droplets in the density-gradient technique. The majority of protein determinations were performed on sera, but some of the initial work was done with plasma. Blood from the infraorbital sinus was collected with a heparinized microhematocrit tube or with a plain Caraway microtube (Clay-Adams Co.). The plasma or serum was separated by centrifugation and then assayed for total protein. Hematocrit values were determined in an Adams Autocrit centrifuge (Clay-Adams Co.). Blood samples were centrifuged for 5 min at 12,500 rpm (15,500 rcf) and hematocrit values are expressed as milliliters of packed cells/100 ml whole blood.

Electrophoresis of mouse serum proteins. Albumin and globulin fractions of mouse sera were separated by cellulose acetate electrophoresis. Three microliter samples were applied in a narrow band across the center of Sepraphore III (Gelman) strip 1 × 6¾ in. Electrophoretic separation was achieved in Veronal buffer, pH 8.6, ionic strength 0.075, with a Vokam power supply set to provide 100 V for 75 min. The strips were then stained with ponceau S (500 mg in 100 ml of 5% trichloroacetic acid) and rinsed in three changes of 5% acetic acid. The albumin and the globulin fractions were cut from the strips and eluted with 2 ml of 0.1 *N* NaOH in test tubes. The optical density of each eluate was determined in a spectrophotometer at 530 mμ. In order to estimate the quantity of albumin in the mouse serum samples, a solution of purified mouse serum albumin

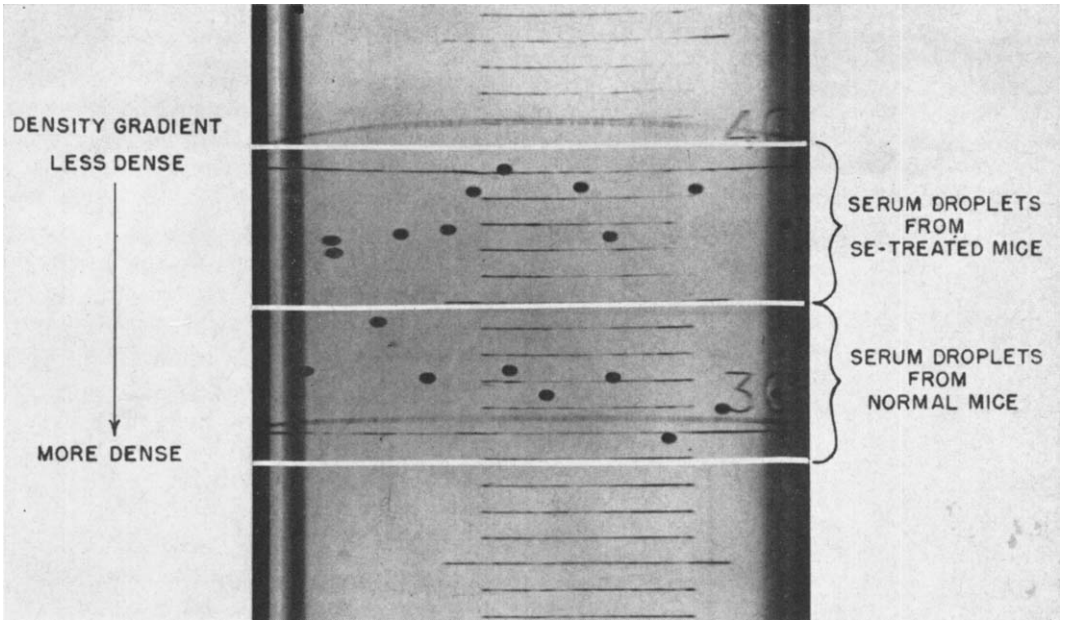

FIG. 1. Distribution of serum droplets in a density gradient showing distinct separation of serum droplets in the gradient dependent on whether they came from normal or from SE-treated mice.

(Pentex) containing 45 mg albumin/ml was run as a standard in exactly the same manner as the serum samples.

Surgical methods. Bilateral adrenalectomy was performed as previously described (16) and adrenal demedullation by the method of Ingle and Griffith (17).

Statistical analysis. The significance of differences between mean values of serum protein analyses and hematocrit values of normal and SE-treated mice was determined by Student's t test (18).

Results. Serum or plasma from SE-treated mice was of lower specific gravity than that from normal mice (Fig. 1). When specific gravities were converted to protein values, the average for the SE-treated mice was about 1 g/100 ml plasma lower than that for the normal mice (Table I). Subsequent experiments confirmed these results.

Since specific gravity determinations could conceivably be affected by factors other than changes in protein, TSP determinations were performed by the biuret method. This method confirmed that SE-treated mice have a significant hypoproteinemia when compared

TABLE I. Total Plasma Protein Values Determined by a Specific Gravity Method on Bloods Obtained from Normal and SE-Treated Mice.[a]

Normal mice		SE-treated mice	
Mouse no.	Total plasma protein (g/100 ml plasma)	Mouse no.	Total plasma protein (g/100 ml plasma)
1	6.13	1	4.98
2	6.45	2	5.20
3	6.00	3	5.06
4	5.99	4	5.01
5	6.22	5	5.50
6	6.17	6	5.42
7	6.59	7	5.13
8	6.18	8	5.35
9	6.15	9	5.35
10	6.13	10	4.86
Mean	6.20		5.19
SE of mean	.06		.07
t (normal vs. treated mice)		11.3	
p		<.001	

[a] Normal mice received saline and SE-treated mice received 10 μg SE/mouse. Blood samples were obtained the following day.

TABLE II. Total Serum Protein Values Determined by a Biuret Method on Bloods Obtained from Normal and SE-Treated Mice.[a]

Normal mice		SE-treated mice	
Mouse no.	Total serum protein (g/100 ml serum)	Mouse no.	Total serum protein (g/100 ml serum)
1	6.14	1	4.91
2	5.78	2	4.98
3	5.80	3	5.14
4	6.03	4	4.75
5	5.80	5	5.50
6	5.43	6	5.00
7	5.96	7	4.96
8	5.91	8	5.59
9	6.03	9	4.73
10	5.46	10	5.09
Mean	5.83		5.06
SE of mean	.07		.09
t (normal vs. treated mice)		6.59	
p		<.001	

[a] Normal mice received saline and SE-treated mice received 20 μg SE/mouse. Blood samples were obtained the following day.

to normal mice (Table II). Numerous experiments with CFW and RML mice have given similar results. The serum protein level of SE-treated mice was usually between 0.5 and 1.0 g/100 ml less than that of normal mice.

Cellulose acetate electrophoresis of serum samples from normal and SE-treated mice and quantitation by integration of the areas under the curves of densitometer tracings indicated that the albumin fraction was the main serum protein affected (Fig. 2). Quantitation of the serum albumin and globulin fractions by elution of the protein bands from the cellulose acetate strips and determining the optical density at 530 mμ confirmed that it was the serum albumin which was depressed. The average albumin concentration was 4.11 g/100 ml serum for 10 normal mice, but only 3.15 g/100 ml serum in SE-treated mice, a difference of 0.96 g/100 ml serum (Table III). For the globulin determinations, the mean optical density $\pm$ standard error of the mean for the ten normal mice was 0.202 $\pm$ 0.007 and for the ten SE-treated mice, 0.201 $\pm$ 0.010.

The onset of the hypoproteinemia occurred within 1 day after administration of SE; at 4 days the protein values were increasing and by the Day 10–14 the values had returned to normal.

In several experiments, hematocrit values were determined concomitantly with TSP values to ascertain if there was a parallel reduction in red blood cell concentration after SE administration. In two cases there was a significant drop in hematocrit values, but this was not a consistent finding in a number of other experiments, in which the differences were not significant (Table IV).

Heating the SE preparation (97° for 30 min), which destroys most of the ability of SE to produce histamine hypersensitivity (19), also destroyed its ability to produce a drop in TSP (Table V). The TSP of adrenalectomized mice was depressed about 0.9 g/100 ml serum from that of untreated con-

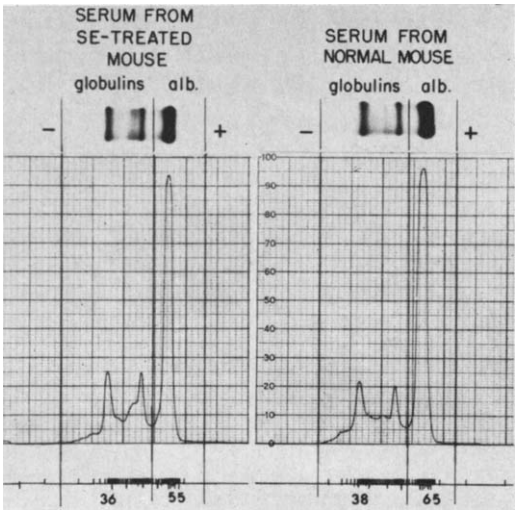

FIG. 2. Electrophoretic separation of mouse serum samples on cellulose acetate strips. Their respective densitometer tracings with arbitrary integration values are also given. Comparison of the arbitrary values for the areas under the globulin and albumin (alb.) portions of the curves shows quantitative differences. The amount of globulin in each serum was similar (36 vs. 38), but less albumin was present in the serum from a SE-treated mouse than that of a normal mouse (55 vs. 65).

TABLE III. Depressed Serum Albumin Levels in SE-Treated Mice as Determined by Cellulose Acetate Electrophoresis.

Normal mice		SE-treated mice	
Mouse no.	Serum albumin (g/100 ml serum)	Mouse no.	Serum albumin (g/100 ml serum)
1	3.53	1	2.65
2	3.86	2	3.09
3	3.35	3	3.28
4	3.51	4	2.66
5	4.29	5	3.19
6	5.19	6	3.78
7	3.79	7	2.70
8	4.68	8	3.71
9	5.49	9	2.42
10	3.44	10	4.02
Mean	4.11		3.15
SE of mean	.245		.175
t (normal vs. treated mice)		3.225	
p		<.005	

trol mice, but "sham operated" mice also had a hypoproteinemia amounting to about 0.6 g/100 ml serum below that of the untreated controls (Table V).

CFW mice which had been bilaterally adrenal-demedullated and then allowed 30 days to recover from the operation did not have any significant hypoproteinemia, but at this time they were hypersensitive to histamine (Table VI).

The hypoproteinemia phenomenon is not a

TABLE V. Total Serum Protein Values of Mice which Received Heated Saline Extract or Were Adrenalectomized.

Treatment	TSP[a] (g/100 ml serum)
Effect of heating saline extract (SE)	
Controls (no SE given)	6.0
SE heated at 97° for 30 min	5.9
SE not heated	4.9
Effect of adrenalectomy	
No operation	6.3
"Sham" adrenalectomy	5.7
Bilateral adrenalectomy	5.4

[a] Averages for 10 mice/group.

TABLE IV. Hematocrit Values of Normal and SE-Treated Mice in Experiments which Always Resulted in Hypoproteinemia of SE-Treated Mice.

Experiment no.	Group	Number of mice	Mean ± SEM (packed cells/100 ml)	t value	Probability
I	Controls	10	50.8 ± .39	8.131	<.001
	SE-treated	10	46.6 ± .33		
II	Controls	10	51.2 ± .54	2.069	NS
	SE-treated	10	49.6 ± .56		
III	Controls	8	50.6 ± .71	.104	NS
	SE-treated	8	50.5 ± .65		
IV	Controls	10	48.4 ± 1.31	.529	NS
	SE-treated	10	49.2 ± .76		
V	Controls	10	49.0 ± .80	2.431	<.05
	SE-treated	10	45.8 ± 1.04		
VI	Controls	10	47.2 ± 2.17	.502	NS
	SE-treated	10	48.5 ± 1.43		
VII	Controls	10	47.6 ± .58	.297	NS
	SE-treated	10	47.3 ± .83		
VIII	Controls	10	48.9 ± 1.27	0	NS
	SE-treated	10	48.9 ± .64		
IX	Controls	10	50.1 ± .48	.556	NS
	SE-treated	9	50.6 ± .78		

TABLE VI. Histamine Sensitivity and Total Serum Protein (TSP) Levels in Adrenal-Demedullated and Endotoxin-Treated Mice.

Treatment	TSP[a] (g/100 ml serum)	Histamine sensitivity (D/T)[b]
Effect of adrenal demedullation		
"Sham" operated	6.7	1/10
Bilateral demedullation	6.6	8/10
Effect of endotoxin treatment		
Controls (no endotoxin)	6.7	0/10
Endotoxin, 10 μg, iv	5.6	1/10

[a] Average for 10 mice/group.
[b] D/T = deaths/number of mice tested.

specific response to SE treatment, since CFW mice receiving an intravenous dose of 10 μg of purified endotoxin from *Salmonella enteritidis* developed hypoproteinemia within 24 hr equal to that produced by SE treatment. Mice treated with this endotoxin preparation were not hypersensitive to histamine (Table VI).

The hypoproteinemia produced by SE could not be reverted by aministration of 5–10 mg of purified human or mouse albumin (Pentex) given 40 min prior to collecting the serum sample. Furthermore, 0.5 ml of straight or two-fold concentrated mouse serum given intravenously to SE-treated mice immediately after histamine challenge failed to protect against shock.

Discussion. The hypoproteinemia in mice after treatment with SE appears to be primarily due to a loss of albumin from the blood. That it is not due to a significant movement of protein-free fluid into the vascular fluid is indicated by the lack of a concomitant decrease in hematocrit values or a lowering of serum globulin values. These results are somewhat analogous to those reported by House and Baker (20) on the effects of Compound 48/80 and endotoxin on the plasma proteins in splenectomized dogs. Their studies were of a more acute nature, but they found that decreases in the plasma protein concentration after administering either Compound 48/80 or endotoxin could not be explained simply on the basis of protein-free fluid moving into the vascular system.

The significance of the hypoproteinemia which occurs in mice after treatment with SE from *B. pertussis* is not clear. Since the serum albumin pool serves to regulate fluid balance in the body, a decrease in albumin concentration was thought to explain the greater susceptibility of SE-treated mice to death from histamine shock. This seemed especially plausible since our previously published work on the mechanism of death in mice undergoing "shock" had proved that a pronounced hemoconcentration occurred and that blood volume expanders were beneficial in protecting mice from death (21). However, there does not appear to be a cause and effect relationship between histamine hypersensitivity and hypoproteinemia. Onset and duration of hypoproteinemia (1–14 days) do not occur so rapidly nor last so long as do those for histamine sensitivity in SE-treated mice (22). The inability of exogenously administered mouse serum proteins to protect against fatal histamine shock indicates that hypoproteinemia is not directly involved in the shock syndrome. Furthermore, mice from which the adrenal medulla had been removed 30 days before challenge were sensitive to histamine, but had normal serum protein levels. Endotoxin treatment of mice, on the other hand, produced hypoproteinemia but failed to produce histamine sensitivity.

It appears that the hypoproteinemia in SE-treated mice is another phenomenon which occurs concomitantly with histamine sensitivity, but is not responsible for it. This situation is somewhat analogous to the production of hypoglycemia by SE (10). The hypoproteinemia in *B. pertussis*–treated mice appears to be the result of some alteration in the homeostatic mechanisms which regulate the level of albumin in the serum.

Some years ago one of us (23) observed that a solution of Evans blue dye given intravenously to normal and *B. pertussis*–treated mice produced a greater concentration of the dye in the blood of normal mice than in the treated mice. This observation was interpreted to mean that *B. pertussis* increases the permeability of capillaries to Evans blue dye. In light of the present

findings, this interpretation should be re-examined. Evans blue dye readily binds to serum albumin to form a stable complex that remains in the circulation in significant amounts for many hours. If the quantity of dye administered is in excess of the binding capacity of the serum albumin, the amount of dye which remains in the blood will be directly dependent upon the serum albumin level. This does not necessarily mean that the histamine-sensitizing factor of *B. pertussis* does not affect vascular and cellular permeability, but this point requires more careful investigation.

Summary. RML and CFW mice treated with 10–20 μg of saline extract (SE) from *Bordetella pertussis* have a total serum protein (TSP) level 0.5–1.0 g/100 ml serum less than that of normal mice. The drop in TSP seems to be principally due to a lowered serum albumin. The hypoproteinemia is maximal 1 day after SE treatment and does not last beyond 10–14 days. It does not appear to be a cause and effect relationship between hypoproteinemia and histamine hypersensitivity. The hypoproteinemia phenomenon may be another manifestation of the effects produced by the histamine-sensitizing factor from *B. pertussis* on basic cellular and metabolic mechanisms in the mouse.

The authors thank Mr. Joseph Ayers for his competent technical assistance in carrying out this study.

1. Maitland, H. B., Kohn, R., and MacDonald, A. D., J. Hyg. **53**, 196 (1955).

2. Pittman, M., Federation Proc. **16**, 867 (1957).

3. Kind, L. S., Bacteriol. Rev. **22**, 173 (1958).

4. Schweinberg, H., Behringwerk-Mitt. **40**, 102 (1961).

5. Munoz, J., Bacteriol. Rev. **27**, 325 (1963).

6. Munoz, J., *in* "Bacterial Endotoxins" (M. Landy, and W. Braun, eds.), p. 460. Quinn & Boden Rahway, New Jersey (1964).

7. Munoz, J. and Bergman, R. K., Bacteriol. Rev. **32**, 103 (1968).

8. Parfentjev, I. A. and Schleyer, W. L., Arch. Biochem. **20**, 341 (1949).

9. Stronk, M. G. and Pittman, M., J. Infect. Diseases **96**, 152 (1955).

10. Bergman, R. K. and Munoz, J., Proc. Soc. Exptl. Biol. Med. **131**, 42 (1969).

11. Fishel, C. W. and Szentivanyi, A., J. Allergy **34**, 439 (1963).

12. Keller, K. F. and Fishel, C. W., J. Bacteriol. **94**, 804 (1967).

13. Munoz, J. and Hestekin, B. M., Nature **196**, 1192 (1962).

14. Lowry, O. H. and Hunter, T. H., J. Biol. Chem. **159**, 465 (1945).

15. Annino, J. S., "Clinical Chemistry: Principles and Procedures," 3rd Ed., p. 184. Little, Brown, Boston, Massachusetts (1964).

16. Munoz, J. and Schuchardt, L. F., J. Allergy **25**, 125 (1954).

17. Ingle, D. J. and Griffith, J. Q., *in* "The Rat in Laboratory Investigation" (E. J. Farris, and J. Q. Griffith, eds.), p. 444. Lippincott, Philadelphia, Pennsylvania (1949).

18. Snedecor, G. W., "Statistical Methods," Iowa State College Press, Ames, Iowa (1959).

19. Munoz, J., Bacteriol. Rev. **27**, 325 (1963).

20. House, F. C. and Baker, C. H., Am. J. Physiol. **211**, 61 (1966).

21. Bergman, R. K. and Munoz, J., J. Immunol. **95**, 1 (1965).

22. Munoz, J. and Bergman, R. K., J. Immunol. **97**, 120 (1966).

23. Munoz, J., J. Immunol. **86**, 618 (1961).

Received Feb. 17, 1969. P.S.E.B.M., 1969, Vol. 131.