Effect of *H. pertussis* on Sensitivity of Mice to Cold Stress.* (22893)

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The injection of *Hemophilus pertussis* cells into mice of certain strains has been shown to produce pronounced changes in their sensitivity to histamine(1-3) and in their ability to become anaphylactically sensitive to various antigens(4-7). Adrenalectomized mice, like pertussis-treated mice, become highly susceptible to histamine(8) and to anaphylaxis(9). Adrenalectomized mice are more susceptible to stress than normal mice, and pertussis-treated mice have been found more sensitive to stresses such as x-rays(10) and low atmospheric pressures(11). These striking similarities between adrenalectomized and pertussis-treated mice have led to the inference that pertussis produces its effect mainly by interfering with adrenal function(11,12,13). This view is strengthened further by the fact that pertussis-treated mice can be protected by adrenal steroids (cortisone, hydrocortisone) against both histamine†(14,15) and anaphylaxis.† However, there is as yet no conclusive data that limits the explanation of the observed phenomena to variation in adrenal output. In fact, Gauthier *et al.*(16) have recently questioned the role of the adrenals in the development of histamine sensitivity in pertussis-treated mice.

The present report deals with the sensitivity of pertussis-treated and adrenalectomized mice to cold stress, and relates these observations to work previously reported.

Materials and methods. Swiss-Webster female mice, weighing from 14-16 g and purchased from Tumblebrook Farms, New York (T. F. mice), were used. The pertussis cells were prepared as previously described(3). Two billion cells contained in 0.2 ml were injected intraperitoneally into each mouse. Four days following administration of pertussis cells the mice were submitted to cold stress. For this purpose, each mouse was placed in an individual tin can with a wire top, and held in a cold room at 1-2°C for a period of 8 hours or longer. In mice requiring adrenalectomies, these were performed as previously described(8) and the mice were used not later than 24 hours after removal of the glands. When used, cortisone and hydrocortisone were suspended in saline and administered intraperitoneally 16 hours prior to cold stress. The experimental results were evaluated statistically by the methods of Wilcoxon(17) and Krushal and Wallis(18).

Results. Susceptibility of H. pertussis-treated mice and normal mice to cold stress. Twenty mice, injected with 2 billion *H. pertussis* cells, were exposed to cold stress 4 days following injection of cells. At the same time a group of 20 normal, untreated mice were placed in the cold to serve as controls. The results obtained conclusively demonstrated that pertussis-treated mice were more susceptible to cold stress than were normal mice. All but one of the treated mice died within 3 hours at 1-2°C while none of the normal mice died during this period. Even after 8 hours under conditions of this experiment 4 normal mice were still living. The difference between these 2 groups was shown to be highly significant ($P<0.001$). This experiment has been repeated several times with essentially the same results as may be seen in succeeding experiments (Table I, Groups I vs. IV).

Effect of adrenalectomy and sham adrenalectomy on cold stress. Adrenalectomized mice also become highly sensitive to cold stress. This is illustrated in the following experiment where in addition, the effect of sham adrenalectomy of normal and pertus-

* We wish to express our appreciation to Mr. J. L. Ciminera for statistical analysis of our experimental results, and to Dr. W. F. Verwey for his valuable suggestions and criticisms in preparing this manuscript.

† Unpublished observations, Munoz, J. and Schuchardt, L. F.; Munoz, J., and Baer, J.; Munoz, J., and Peck, H. M.

TABLE I. Effect of Adrenalectomy and Sham Adrenalectomy on Cold Stress.

Group No.	Treatment I	II	III	IV	V
Adrenalectomized	—*	+	—	—	—
Sham adrenalectomized	—	—	+	—	+
Pertussis	—	—	—	+	+
Time of death at 1-2°C (hr)	No. dead†				
1	0	0	0	0	0
2	0	0	0	1	2
3	0	2	1	7	5
4	0	2	0	2	3
5	0	3	1		
6	0	1	0		
7	3	2	1		
8	0		0		
>8	7		7		

* + = Received treatment. — = Did not receive treatment.

† Significance of difference: I *vs* II, $P < 0.001$; I *vs* III, not significant; I *vs* IV, $P < 0.001$; II *vs* IV, $P < 0.01$; IV *vs* V, not significant.

sis-treated mice was studied. Fifty mice, divided into groups of 10, were used. The mice of Group I were normal, untreated controls. Those of Group II were adrenalectomized, Group III were sham adrenalectomized, Group IV were treated with pertussis 4 days before cold stress, Group V were treated with pertussis 4 days before and sham operated the day before cold stress. The 5 groups were then submitted to cold stress. From the results given in Table I it can be seen that the adrenalectomized mice are more susceptible to cold than normal mice (Groups I and II), that the sham operated "normal" animals behave like the normal controls (Groups I and III), and that the sensitivity to cold of pertussis-treated mice is not affected by sham adrenalectomy (Groups IV and V). In addition, the results also show that pertussis-treated mice are more sensitive to cold than adrenalectomized mice (Groups II and IV).

Since the similarity of pertussis-treated mice to adrenalectomized mice is striking, it was of interest to investigate the effect of adrenal steroids on the cold sensitivity developed in mice treated with *H. pertussis*.

Effect of cortisone on pertussis-treated and adrenalectomized mice submitted to cold stress. Fifty mice, divided into groups of 10, were used. Two groups received pertussis, 2 groups were adrenalectomized, and 1 group was kept as normal controls. One group of each of the pertussis-treated and adrenalectomized mice received an intraperitoneal injection of 4 mg of cortisone per mouse 16 hours before cold stress, while the other groups received no treatment. The results given in Table II indicate that cortisone protected both pertussis-treated mice and adrenalectomized mice from cold stress, and that adrenalectomized mice appeared to have been protected better than pertussis-treated mice (Groups I and III).

Effect of hydrocortisone on cold sensitivity of pertussis-treated intact and adrenalectomized mice. It was of interest to know if the combination of pertussis treatment and adrenalectomy would increase the susceptibility of mice to cold stress more than either treatment alone. It was also important to know if mice receiving both treatments could be fully protected with adrenal steroids. In this experiment, hydrocortisone was used because it has been found to be more effective than cortisone. Twenty mice were used in each of 7 different groups. The mice in Group I received 2 billion *H. pertussis* cells 4 days

TABLE II. Effect of Cortisone on Susceptibility of *H. pertussis* Injected Mice and Adrenalectomized Mice to Cold Stress.

Group No.	Treatment I	II	III	IV	V
Pertussis inj.	+*	+	—	—	—
Adrenalectomized	—	—	+	+	—
mg cortisone/mouse	4	—	4	—	—
Time of death at 1-2°C (hr)	No. dead†				
1	0	0	0	0	0
2	0	0	0	0	0
3	0	4	0	1	1
4	1	4	0	1	1
5	2	2	0	0	2
6	1		1	4	0
7	2		1	1	1
8	1		1	0	0
>8	3		7	2	5

* + = Received treatment. — = Did not receive treatment.

† Significance of difference: I *vs* II, $P < 0.001$; I *vs* III, not significant; I *vs* V, not significant; II *vs* IV, $P = 0.006$; II *vs* V, $P < 0.01$; III *vs* IV, $P = 0.013$; III *vs* V, not significant; IV *vs* V, not significant.

TABLE III. Effect of Hydrocortisone on Sensitivity to Cold of Pertussis Injected and Adrenalectomized Mice.

Group No.	Treatment I	II	III	IV	V	VI	VII
Pertussis inj.	+*	+	—	—	+	+	—
Adrenalectomized	—	—	+	+	+	+	—
mg hydrocortisone per mouse	—	4	—	4	—	4	—
Time of death at 1-2°C (hr)	No. dead†						
1	0	0	1	0	1	0	0
2	2	0	6	0	17	5	2
3	10	4	3	1	1	6	0
4	5	6	2	3	1	4	2
5	3	3	5	1		2	0
6		2	3	3		1	1
7		1		1		0	0
8		0		0		0	3
>8		4		11		2	12

* + = Received treatment. — = Did not receive treatment.

† Significance of difference: I *vs* II, $P < 0.01$; I *vs* III, not significant; I *vs* V, $P < 0.001$; II *vs* IV, $P < 0.02$; II *vs* VII, $P < 0.02$; III *vs* V, $P < 0.01$; IV *vs* VII, not significant; VI *vs* VII, $P < 0.001$.

before cold stress, those of Group II received in addition to 2 billion *H. pertussis* cells 4 mg of hydrocortisone 16 hours before cold stress. Animals from Group III were adrenalectomized 24 hours before cold stress and Group IV were adrenalectomized 24 hours before and treated with 4 mg of hydrocortisone 16 hours before cold stress. The mice from Group V received 2 billion *H. pertussis* cells 4 days before and were adrenalectomized 24 hours before cold stress. Group VI was treated similarly to Group V but in addition each mouse received 4 mg of hydrocortisone 16 hours before being placed in the cold. The last group (Group VII) consisted of normal, untreated mice.

The results of this experiment are given in Table III. Here it can be seen that sensitivity to cold of pertussis-treated mice was, as previously noticed, slightly greater than that of adrenalectomized mice (Groups I and III) and that hydrocortisone, just as cortisone, protected adrenalectomized mice better than pertussis-treated mice (Groups II and IV). These observations confirm those made in the previous experiments. A highly important finding in this experiment is the fact that adrenalectomy of pertussis-treated mice renders them even more sensitive to cold stress than either treatment alone (Groups I, III and V), and that hydrocortisone, in the dose given, did not protect these mice completely (Groups VI and VII), while the same dose fully protected adrenalectomized mice (Groups IV and VII). Moreover, pertussis-treated mice are not fully protected by 4 mg of hydrocortisone (Groups II and VII). Since sham adrenalectomy does not change the sensitivity to cold of normal or pertussis-treated mice, the results indicate that pertussis may be interfering with functions other than that of the adrenal glands.

Effect of different amounts of hydrocortisone on sensitivity of pertussis-treated mice to cold stress. The observation that pertussis-treated mice were not fully protected from cold stress by hydrocortisone raised the question as to whether greater amounts of this compound could return the cold sensitivity of these mice to the normal level. To answer this question, the following experiment was performed. Seven groups of 10 mice each were used—Groups I, II, III, IV and V were given *H. pertussis* 4 days before challenge, and Groups VI and VII received no pertussis. Sixteen hours before cold stress, hydrocortisone was given to Groups II, III, IV, V, and VI; Group II received 1 mg/mouse; Group III received 2 mg; Group IV received 4 mg; Group V received 6 mg; and Group VI received 4 mg. Groups I and VII received no hydrocortisone.

The results given in Table IV show that there is a significant linear regression of mean reciprocal time on doses of hydrocortisone from 0 to 2 mg/mouse ($P < 0.01$). Larger doses of hydrocortisone up to 6 mg/mouse show no effect different than that given by 2 mg, indicating that 2 mg in this experiment gave the maximum protection to the pertussis-treated mice. Again, 2 mg or 4 mg of hydrocortisone failed to bring the pertussis-treated mice to their normal sensitivity since these 2 groups showed significantly lower survival times than the normal controls ($P < 0.05$). In this experiment, however, the difference between the normal group and that receiving 6 mg of hydrocortisone did not

TABLE IV. Susceptibility to Cold Stress of Pertussis Injected Mice Treated with Various Amounts of Hydrocortisone.

Group No.	Treatment I	II	III	IV	V	VI	VII
Pertussis inj.	+*	+	+	+	+	—	—
mg hydrocortisone per mouse	—	1	2	4	6	4	—
Time of death at 1-2°C (hr)	No. dead						
1	0	0	0	0	1	0	0
2	3	1	1	2	0	0	2
3	6	6	1	1	3	0	0
4	1	3	6	4	3	0	0
5			1	0	2	0	0
6			0	2	1	0	0
7			1	0	0	0	2
8				1	1	0	1
8.5					0	0	2
>8.5					1	10	3
Mean reciprocal time†	0.375	0.325	0.268	0.279	0.277	—	0.165

* + = Received treatment. — = Did not receive treatment.

† Significance of difference: There is a significant linear regression of mean reciprocal time on doses of hydrocortisone from 0 to 2 mg/mouse ($P < 0.01$). Larger doses of hydrocortisone up to 6 mg/mouse gave no significantly different results than 2 mg/mouse.

reach statistical significance. For this reason another experiment was performed using 20 mice/group and extending the observation period to 21 hours. In this second experiment 2 mice were placed in each tin can. For this reason longer survival times were observed as compared to those of the previous experiments. Four groups of mice were used. The mice in Groups I and II were injected with pertussis 4 days before cold stress while those of Groups III and IV were not. Sixteen hours before cold stress each mouse of Groups I and III received 6 mg of hydrocortisone intraperitoneally. The results demonstrated that pertussis-treated mice receiving 6 mg of hydrocortisone do not survive as long as the normal control mice. They also confirmed the finding of the previous experiment that hydrocortisone given to normal mice prolongs significantly their survival time at 1-2°C.

Discussion. Our observations as well as those reported by other workers are consistent with the view that mice treated with *H. pertussis* cells show signs of relative adrenal insufficiency. This is supported by the fact that pertussis-treated mice are more sensitive to various types of stress just as are adrenalectomized mice, and by the fact that pertussis-treated mice, as well as adrenalectomized mice, can be protected by cortisone and hydrocortisone. In spite of this apparently marked adrenal insufficiency, however, it has not been possible to demonstrate any adrenal pathology† or a significant change in the ascorbic acid content of the adrenal glands of mice that have been injected with pertussis.† These findings, while suggestive, nevertheless do not rule out conclusivly the possibility that pertussis may produce at least a temporary decrease in the activity of the adrenal glands. Although some of the results obtained here seem to warrant the assumption that pertussis interferes with adrenal function, it is difficult to explain all of the observations made by assuming that this is the only metabolic activity effected. All of these observations indicate that pertussis-treated mice are more sensitive to cold stress than would be expected from the complete elimination of adrenal gland function by the physical removal of the glands. Therefore, they suggest that the injection of pertussis cells while possibly affecting adrenal activity also produce an effect upon some other function, or functions, in the mouse that is concerned with protection against stress.

Accessory adrenal tissue, that takes over some of the adrenal functions after adrenalectomy, has been demonstrated in some mice (19). The presence of this tissue could possibly be considered to explain the differences observed between adrenalectomized and pertussis-treated mice with respect to cold stress. Such accessory adrenal tissue, however, is usually not highly developed and adrenal function activity can seldom be demonstrated 24 hours after adrenalectomy. For this reason it is improbable that the differences observed between pertussis-treated mice and adrenalectomized mice could be explained on the presence of accessory adrenal tissue. Moreover, this view would not offer an explanation as to why cortisone and hydrocortisone given in large

amounts did not protect completely pertussis-treated mice while fully protecting adrenalectomized mice, and why adrenalectomy of pertussis-treated mice further increased their sensitivity to cold.

Recently Gauthier, *et al.*(16) have strongly questioned the role played by the adrenals in pertussis-treated mice. Their conclusion that the adrenals are not involved in the histamine sensitization produced by pertussis finds support in our previous observations that adrenalectomy of certain strains of mice does not render them sensitive to histamine (8). The same resistant mice, however, when treated with pertussis become more sensitive to cold and to anaphylaxis.† These facts suggest that pertussis may indeed have some effect on the adrenal glands but that in addition it affects other functions in the mouse body.

Summary. Injection of 2 billion *H. pertussis* cells into T. F. female mice increases susceptibility of these mice to cold stress. 2. Susceptibility to cold of pertussis-treated mice is greater than that of adrenalectomized mice. 3. Adrenalectomy of pertussis-treated mice increases susceptibility to cold stress to a greater extent than either treatment alone. 4. Sham-operated animals whether normal or pertussis-treated are neither more resistant nor more sensitive to cold stress than similarly treated but unoperated animals. 5. Cortisone and hydrocortisone protect pertussis-treated mice from cold stress but to a lesser degree than they protect adrenalectomized mice. 6. Increasing the amount of hydrocortisone from 2 to 6 mg has little effect on increasing the protective effect of this drug. 7. Hydrocortisone given to normal mice increases their resistance to cold. 8. It is suggested that while pertussis possibly affects adrenal function, it must in addition affect other functions in the mouse which are also concerned with protection against stress.

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Received August 7, 1956. P.S.E.B.M., 1957, v94.