

ANAPHYLACTIC SHOCK IN THE MOUSE VACCINATED WITH HEMOPHILUS PERTUSSIS

IV. STUDIES ON THE ADRENAL GLAND

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PREVIOUS reports^{1, 2} have described a technique whereby anaphylactic shock can be elicited regularly in both the white mouse and the rat, animals which usually are difficult to sensitize. A high per cent mortality is consistently attained on challenging animals which previously have received a vaccine of *Hemophilus pertussis* Phase I organisms together with a sensitizing antigen. At the present time the mechanism of this enhancement of sensitization is not understood. It does not appear that the mechanism can be entirely accounted for by the "schlepper" effect of the cells.³ In addition, the speculation⁴ that *H. pertussis*, by its inherent toxicity, injures the adrenal gland and in effect produces an adrenalectomized, and hence a highly sensitive, animal does not seem warranted.⁵ The present report, concerning itself with endocrine and histopathologic studies of adrenals of mice sensitized thereby, attempts to establish the role of this gland in the pertussis-vaccinated animal.

PROCEDURES

Effect of Adrenalectomy on Sensitization.—Adrenalectomized female white mice of the Swiss-Webster strain were maintained throughout the course of the experiment on a 1 per cent salt solution and received, in addition, a daily subcutaneous injection of 0.1 ml. of aqueous adrenal cortical extract (ACE).^{*} These mice were divided subsequently into two groups, with Group 1 receiving 1 mg. of crystalline egg albumin intraperitoneally and Group 2 receiving 1 mg. egg albumin as a mixture with either 8×10^8 *H. pertussis* Phase I organisms or 1 ml. of a saline extract of the cells. It has been shown previously³ that the saline extract of *H. pertussis* is as effective as the intact organisms in enhancing sensitization. A third group, acting as an additional control and consisting of adrenalectomized animals injected with 1 mg. egg albumin only, did not receive the daily maintenance dose of ACE. On the tenth day subsequent to the immunization, all the animals of the three groups were challenged with an intravenous injection of 1 mg. egg albumin given in 0.1 ml. of solution.

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^{*}The biologic activity of the ACE used in this study was equivalent to approximately 0.2 mg. compound F per milliliter.

Results: The mortality results due to the anaphylactic shock can be seen in Table I. They indicate that *H. pertussis* is capable of enhancing the production of sensitivity over and above that obtained by the antigen alone in adrenalectomized mice. A 96 per cent mortality was observed for that group of animals receiving the egg albumin and *H. pertussis*, as compared to a 25 per cent mortality for the group sensitized with egg albumin only. The mortality of 41 per cent observed for that group of animals not receiving the daily injection of ACE indicates that ACE is a somewhat effective replacement therapy, as noted by the partial suppression of sensitization of the adrenalectomized mice.

TABLE I. SENSITIVITY OF ADRENALECTOMIZED MICE

INTRAVENOUS CHALLENGE	INTRAPERITONEAL SENSITIZATION		
	ACE		NO ACE
	EGG ALBUMIN ONLY	EGG ALBUMIN AND H. PERTUSSIS	EGG ALBUMIN ONLY
Mortality	25/98 (25%)	97/101 (96%)	12/29 (41%)

The above adrenalectomized mice were maintained on 1 per cent sodium chloride and 0.1 ml. adrenal cortical extract (ACE) daily. Sensitization was accomplished with 1 mg. egg albumin intraperitoneally and challenge with 1 mg. egg albumin intravenously after ten days.

Effect of *H. pertussis* on 17-Ketosteroid Excretion.—Four groups, each consisting of two young female white rats, weighing 175 to 200 grams, were arranged in metabolism cages. The total urine excreted over a seventy-two hour period was collected from each group and the 17-ketosteroids were determined and recorded as the average total milligrams excreted per twenty-four hours.* Two such seventy-two hour control pre-sensitization periods determined the base line of milligram of steroid excreted. Two groups of rats were then injected intraperitoneally with 1 ml. of a 1:10 dilution of horse serum and two groups received a suspension of 5×10^{10} *H. pertussis* organisms in the same volume of antigen. During the subsequent fifteen days, urine from each group was collected over three seventy-two hour periods and the average total milligram of 17-ketosteroid excreted per twenty-four hours was calculated. On the fifteenth day, all eight rats were challenged by the intravenous injection of 1 ml. of a 1:10 dilution of horse serum.

TABLE II. AVERAGE TOTAL MILLIGRAM STEROIDS EXCRETED PER TWENTY-FOUR HOURS FOR EACH THREE-DAY PERIOD

GROUP	PREINOCULATION		POSTINOCULATION		
	<i>Inoculated With Horse Serum</i>				
I	0.12	0.06	0.20	0.12	0.13
II	0.10	0.07	0.09	0.08	0.14
	<i>Inoculated With Horse Serum and H. Pertussis</i>				
III	0.09	0.13	0.16	0.11	0.18
IV	0.19	0.11	0.15	0.16	0.19

Each group consists of two rats, each weighing 175 to 200 grams.

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Results: None of the four animals sensitized by horse serum only died of anaphylactic shock, but all four rats that received *H. pertussis* in addition succumbed. This indicated that sensitization had, indeed, been effected. Table II indicates that no significant difference was noted between the sensitized and nonsensitized groups of animals with respect to the average total milligram of 17-ketosteroids excreted per twenty-four hours.

Histopathologic Studies of Adrenals of Sensitized Mice.—Three groups of mice were studied in this series. The mice of Group 1 were injected with 1 ml. egg albumin intraperitoneally, those of Group 2 received 10^{10} *H. pertussis* Phase I organisms only, whereas those of Group 3 were inoculated with a mixture. Two animals of each group were sacrificed on the second, fourth, seventh, tenth, fifteenth, twenty-first, and twenty-fifth days, respectively, following the inoculation. The adrenal glands were removed, fixed in Zenker's solution, sectioned, and stained. Individual sections were stained with either phloxine-methylene blue or methyl green-pyronine. Careful microscopic examination of the sections failed to reveal any essential differences between the adrenal glands of the three groups. Each showed normal medulla and cortex, with the latter presenting the usual three zones. The cells of each layer appeared normal and were comparable with respect to size, shape, and staining reactions.

DISCUSSION

It has been observed that either adrenalectomy⁶ or vaccination with *H. pertussis*⁷ will increase the susceptibility of mice to histamine. It has been reported also that anaphylactic sensitivity is enhanced under similar circumstances.^{1, 8} The apparent parallelism makes it tempting to suggest⁴ that the action of the toxic vaccine on the adrenals results in the enhancement of sensitivity to histamine and of antigenic sensitization. Recent studies by Gauthier, Loew, and Jenkins⁹ support the concept that enhancement to histamine is through some mechanism other than impairment of adrenal function. The results described in this report seem to indicate that dysfunction of the gland does not play a significant role in anaphylactic sensitization either.

An essential difference in the experimental procedure should be pointed out, which may account for the difference in the results of this report and that of Dougherty⁸ relative to the increase of anaphylactic sensitivity following adrenalectomy. In the latter studies, adrenalectomy was carried out *after* immunization and following an incubation period; this usually occurred just prior to anaphylactic challenge. In the present study, the mice were adrenalectomized *prior* to sensitization. Under these latter conditions, a mortality of 41 per cent was obtained by anaphylactic challenge for a group of mice inoculated with egg albumin only, while that group similarly sensitized but maintained on ACE gave a mortality of 25 per cent. ACE has the capability of acting as a replacement therapy to some degree for, although these mortality values are admittedly greater than that obtained for the intact mouse inoculated with egg albumin only (5 per cent), sensitization was somewhat suppressed by ACE in the adrenalectomized group (25 per cent versus 41 per cent mortality).

Moreover, the 96 per cent mortality, by comparison, obtained with the group of ACE-maintained, adrenalectomized mice receiving a mixture of antigen with *H. pertussis*, becomes significant and seems to be related directly to the injection of the organisms. The intact animal sensitized in similar fashion exhibits a 74 per cent mortality.³ This effect of *H. pertussis* is striking, even though the mortality values observed for the experimental groups of adrenalectomized animals are greater than that for groups of intact animals. Regeneration of adrenal rests, adventitious glands, or compound F effect need not be considered as pertinent, for these factors would rather tend to make the differences more striking by repressing even more the mortality of the control groups.

The level of urinary 17-ketosteroid excretion indicates that no significant alteration attended either inoculation of the rats with horse serum or sensitization with a mixture of the antigen and *H. pertussis*. Although these observations can be interpreted as indicating that there has been little, if any, interference with the "normal" secretory function of the adrenals and hence little serious impairment of its metabolism, nevertheless, additional tests of adrenal function would be required for supporting evidence. Little is actually known concerning the amount of toxic injury required and it may be presumed by implication that damage severe enough to alter sensitization so markedly would be reflected by an alteration in steroid excretion. Previously surgical ablation of the adrenals was required to cause such a high mortality in so drastic a measure of sensitivity as anaphylactic death in the mouse. The failure to observe any changes in cellular structure or configuration of the adrenal glands following *H. pertussis* inoculation seems to be indicative that toxic changes have not taken place at the microscopic level, at least.

The mechanism by which *H. pertussis* enhances anaphylactic sensitivity still requires further investigation. The mitigation apparently is not by a direct effect on the adrenal gland, although cortisone (in relatively large amounts) has been observed to prevent fatal anaphylactic shock in the pertussis-vaccinated² as well as intact¹⁰ mouse. One needs consider other levels of *H. pertussis* function.

SUMMARY

1. *Hemophilus pertussis* organisms have the ability of enhancing sensitization in the adrenalectomized mouse maintained on adrenal cortical extract.
2. The mechanism by which sensitization is enhanced does not seem to be a result of the direct toxic effect of the organisms on the adrenal gland.
3. The amount of 17-ketosteroids excreted in the urine is not altered by inoculating the animal with *H. pertussis*.
4. Histopathologic studies fail to reveal any changes in the cellular structure of the adrenal gland following inoculation.

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