

PEPTONE SHOCK IN THE PERTUSSIS-SENSITIZED MOUSE

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IT is now well established that the susceptibility of white mice to the lethal effects of histamine and serotonin is enhanced by the intraperitoneal injection of *Hemophilus pertussis* phase I vaccine.¹⁻³ This effect is not believed to be a result of general debilitation for such mice do not show a heightened response to tuberculin,⁴ epinephrine,⁵ carbaminocholine,⁵ Compound 48/80,⁶ heterologous sera or proteins,⁴ etc. In addition to these, our preliminary studies indicated that the pertussis-inoculated mouse was not sensitive to the intravenous administration of the specific peptone, Bacto-Peptone.

Intravenously injected peptone in the dog, rabbit, guinea pig, etc., results in a state of vascular collapse resembling histamine shock and is believed to be a consequence of the actual liberation of endogenous histamine in those species.⁷ Since the mouse is not only incapable of storing and, hence, releasing appreciable amounts of histamine⁸ but is also inherently relatively insensitive to histamine,¹ the above results with peptone were not entirely unexpected. However, following these observations, a report⁹ appeared which indicated that prior treatment with pertussis did indeed create a state of heightened response to peptone. Because the results with Bacto-Peptone could be confirmed in this laboratory, the subsequent studies were carried out with a series of peptones.

MATERIALS AND METHODS

Female, Swiss-Webster white mice of about 18 to 20 grams in weight, obtained from Tumblebrook Farm, were used in these experiments. In so far as possible the technical procedures which were followed were those of Hunder and Spink.⁹ Each mouse received an intraperitoneal injection of 0.5 ml. of a standardized *H. pertussis* phase I vaccine containing 6×10^9 organisms. A 30 per cent suspension in distilled water of each of the following peptone preparations was autoclaved and kept at 4° C. prior to use: (a) *Peptonum Siccum* nach, Witte, (b) Bacto-Peptone (Difco), (c) Proteose-Peptone (Difco), (d) Proteose-Peptone No. 2 (Difco), (e) Proteose-Peptone No. 3 (Difco), (f) Bacto-Protone (Difco), (g) Neopeptone (Difco), (h) Bacto-Tryptone (Difco), (i) Tryptose (Difco), (j) bacteriological peptone (Nutritional Biochemicals), (k)

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peptone "I" (Nutritional Biochemicals), and (1) peptone "S" (Nutritional Biochemicals). On the sixth day subsequent to the inoculation with pertussis vaccine, each mouse received 0.5 ml. of one of the above suspensions given intraperitoneally. About 150 mg. of peptone was thus injected.

RESULTS

Following the challenge inoculation all animals showed some signs of reaction such as lethargy, shallow and/or forced respirations, ruffling of the fur, edema of the paws and snout, rubbing of the snout, scratching of the skin, cyanosis, etc. Convulsions, if they occurred, were indicative of eventual death although not all animals that died exhibited this response. These described reactions, which are typical for mice in anaphylaxis, are also seen following histamine and serotonin administration. Death of the mouse within twenty-four hours after the challenge inoculation was chosen as the criterion for profound susceptibility to peptone; all other reactions were disregarded. These mortality results, number of animals dead/number of animals challenged, are shown in Table I. Our initial observations, that pretreatment of the mouse with pertussis vaccine did not result in an altered sensitivity to Bacto-Peptone,

TABLE I. PEPTONE SHOCK IN PERTUSSIS-SENSITIZED MICE

PEPTONE 150 MG. (INTRAPERITONEALLY)	MORTALITY RATE	
	CONTROL (UNTREATED)	<i>H. pertussis</i> (6 DAYS PRIOR)
Bacto-Peptone	0/20	0/29*
Bacteriological peptone (Nutritional Biochemicals)	0/8	3/10
Witte's peptone	1/15	9/25
Neopeptone	5/10	10/13
Bacto-Tryptone	1/10	11/14
Proteose-Peptone No. 3	0/10	11/13
Proteose-Peptone	3/22	29/31
Tryptose	3/6	6/10
Peptone "S"	3/6	7/10
Proteose-Peptone No. 2	8/10	11/13
Bacto-Protone	8/10	12/14
Peptone "I"	5/6	6/10

*Mortality rate = number dead/number injected.

were confirmed. On the other hand, sensitization to Proteose-Peptone did occur, as reported previously.⁹ Of the other peptones studied, Proteose-Peptone No. 3 and Bacto-Tryptone were also found to be markedly lethal to pertussis-treated animals. Several of the peptone preparations such as peptone "I," peptone "S," Neopeptone, and Bacto-Protone were toxic to control animals in the amounts given so that no conclusions could be drawn on the effects of prior vaccination with pertussis. Witte's peptone, bacteriological peptone (Nutritional Biochemicals), and Neopeptone gave intermediate results, i.e., less than 50 per cent mortality in the pertussis-vaccinated mice.

DISCUSSION

The similarity observed between anaphylaxis and peptone shock in certain species is reflected by the toxins or biologically active mediators released, that

is, histamine, serotonin, heparin, bradykinin, etc., and, perhaps, other slowly reacting substances.⁷ The mouse (and rat), however, presents a unique situation in that this animal is relatively insensitive to the lethal effects of exogenous histamine and serotonin, per se, or to the endogenous release of these and other substances following anaphylactic and peptone shocks. Although the resistance to these agents is markedly decreased by prior sensitization with pertussis vaccine, it is not likely that fatal anaphylaxis or peptone shock is the result, solely, of the release of these mediators. The following aspects should be considered: The mouse does not have a store of readily available histamine and it has been said⁸ that, even if all the stored histamine could be released simultaneously, it would not be of sufficient quantity to be lethal. Pretreatment with *H. pertussis* phase I vaccine diminishes the resistance of mice to histamine by about fiftyfold.¹ Even under these circumstances, the LD₁₀₀ for a 20 gram mouse is relatively high, being approximately 200 γ of histamine as the base inoculated intraperitoneally.⁴ In addition, it should be emphasized that the potent histamine-releasing agent, Compound 48/80, apparently is not capable of mobilizing at once the LD₁₀₀ of histamine, for Compound 48/80 is not lethal even to pertussis-treated mice.⁶

The differences in activity of the various peptone preparations is not unexpected. Peptones are usually prepared by the enzymatic degradation of proteins from different sources. The type of enzyme, protein substrate, length of digestion, temperature, etc., required for the process characterizes the peptone. The hydrolysis which results from enzymatic digestion in the preparation of peptone may be accompanied by decarboxylation and result in the formation of toxic amines. Indeed, it is assumed that histamine-like substances (if not histamine itself) are usually present in amounts varying with the different preparations of peptone.¹⁰ The histamine content as base/gram* for several peptones utilized in this study was as follows: Proteose-Peptone, 180 γ ; Witte's peptone 136 γ ; and Bacto-Peptone 16 γ , respectively. As might be expected, the peptone containing the greatest amount of histamine (Proteose-Peptone) displayed the greatest relative reactivity in the pertussis-sensitized mouse, i.e., a high mortality rate was obtained (see Table II). Witte's peptone, containing an intermediate amount of histamine, was only moderately lethal while Bacto-Peptone, with the least quantity of histamine, was not at all lethal to pertussis-treated mice. The maximum quantity of histamine delivered to the mouse in 150 mg. of Proteose-Peptone is only about one tenth the intraperitoneally injected LD₁₀₀ for the histamine-sensitive mouse.

TABLE II. RELATIONSHIP OF HISTAMINE AND SEROTONIN CONTENT TO MORTALITY

PEPTONE	HISTAMINE (γ /GM.)	SEROTONIN (γ /GM.)	MORTALITY IN PERTUSSIS- VACCINATED MICE
Bacto-Peptone	16	<10	0/29
Witte's peptone	136	<10	9/25
Proteose-Peptone	180	<10	29/31

*We are indebted to Dr. Floyd C. McIntire of Abbott Laboratories, North Chicago, Ill., for the determinations of histamine.

The pertussis-inoculated mouse also exhibits an increased sensitivity to 5-hydroxytryptamine (serotonin)^{3, 4}; the content of 5-hydroxyindole for the above three peptones was found to be $< 10 \gamma$ per gram each,* indicating that the contained serotonin was not an important factor in the death of the animal.

Peptone is reported as causing mast cell disruption in the mouse.¹¹ Since mouse tissues are very rich in mast cells, being present in loose connective tissue and along the lymphatic channels, small blood vessels and nerves of the lung, mesentery, intestine, etc., destruction of many of these cells would release appreciable quantities of serotonin as well as histamine, heparin, etc. The LD_{50} of serotonin for the pertussis-inoculated animal is about 2 to 6 γ per gram of mouse.^{2, 3} A lethal quantity of serotonin from disrupted mast cells would be reflected by the presence of its metabolic products such as urinary 5-hydroxyindole acetic acid. However, none of the latter was found in urine collected from a group of mice over a 24-hour period after peptone administration. It is probably unlikely, therefore, that the mechanism of death from peptone is a result of the serotonin released from mast cells.

It is apparent from the above observations that there is no one mechanism which can account for the lethal effects of peptone in the pertussis-sensitized mouse. In addition to the toxic substances, possibly administered together with the peptone, and the mediators released by its stimulation one must take cognizance of the unknown factors present in peptone, perhaps, capable of causing the release of or activation of a lethal factor. The enzyme systems need be considered in this regard. Rather than considering a single entity, it is more than likely that the circumstances leading to a fatal outcome are an accumulation of several of these factors. The mouse deserves special consideration because of its inherent resistance to histamine and serotonin and the resemblance of the shock state whether due to histamine, serotonin, peptone, or anaphylaxis.

SUMMARY

The susceptibility of pertussis-sensitized mice to fatal shock varies with each peptone preparation. The peptone containing the greatest amount of histamine produced the highest mortality rate. It was confirmed that Bacto-Peptone, with relatively little histamine content, was without appreciable effect. The mechanism of death is probably neither the exogenous histamine administered nor the histamine or serotonin engendered but a combination of these and other factors, still unknown.

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