Production of Peptone Shock in Mice Following Administration of *H. pertussis* Vaccine. (23116)

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Previous studies(1,2,3) in this laboratory have shown that brucella endotoxin is lethal for mice within 24 hours after injection. Mice were protected against endotoxin with cortisone and chlorpromazine. It was also demonstrated that the sensitivity of mice to endotoxin could be enhanced by pertussis vaccine. Further investigations(4,5) in larger animals revealed that endotoxin caused shock with the pooling of venous blood. This shock simulated that following the injection of peptone, histamine, trypsin and other agents(6).

The purpose of the present investigation was to compare the action of peptone with endotoxin when injected into mice. Of particular interest was a study of the enhancing effect of pertussis vaccine on peptone, as well as the protection against peptone offered by cortisone, chlorpromazine and an antihistaminic agent.

Materials and methods. Groups of ABC male and female mice, and Swiss-Webster female mice, weighing 20-30 g were employed.* A 30% suspension by volume in distilled water of proteose-peptone (Difco) was used. The suspension was autoclaved after constitution and stored at 4°C. A standardized *Hemophilus pertussis* vaccine (Lederle) was administered to the mice. The antihistaminic was diphenhydramine hydrochloride (Benadryl), and fresh saline suspensions of cortisone (Merck) and chlorpromazine were injected. Unless otherwise stated, all materials were administered intraperitoneally.

Results. 1. *Enhancing effect of H. pertussis vaccine on lethality of peptone.* All populations of mice used in these studies were resistant to doses of peptone up to 150 mg. However, when mice were given 0.2 ml of pertussis vaccine 6 days prior to receiving peptone, the peptone was lethal. The results of a typical experiment are as follows: To each of 20 Swiss-Webster female mice 150 mg of peptone was administered without any demonstrable ill effects. However, when 0.2 ml of pertussis vaccine was given to each of 20 mice 6 days prior to the peptone, 18 out of the 20 mice died within 24 hours.

Increased susceptibility of the mice to peptone could be detected as early as one day after the administration of pertussis vaccine, increasing to a maximum sensitivity within 5-6 days, and then disappearing in 21-28 days. While death within 24 hours was used as the criterion for susceptibility to peptone, most of the mice died within a few hours after the injection of peptone, many within 30 minutes. Just before death the mice exhibited respiratory difficulty like that usually observed after the injection of histamine. The animals quickly became inactive with ruffling of the fur. Respiratory difficulty was manifested by the animals rubbing their noses with their paws; slowing and deepening of the respirations; inspiratory difficulty; and a progressive respiratory failure. At necropsy, serous fluid was present in the peritoneal cavity. The lungs were distended, but collapsed when the thorax was opened. No gross hemorrhagic areas were noted in the lungs. The heart continued to beat after respiration had ceased.

The foregoing phenomenon of increased susceptibility was also demonstrated in ABC female and male mice, and in Ace F_1 female mice.

2. *Protection by an antihistaminic agent against increased susceptibility to peptone.* As seen in Table I, 4 groups of Swiss-Webster female mice were used. A control group was given 150 mg of peptone alone; a second group was pretreated with 0.2 ml of pertussis vaccine, and then given 150 mg of peptone 6 days later; a third group was treated similarly as the second group, except that 3½ hours and 10 minutes before, as well as 3

* The ABC mice were obtained from Dr. John Bittner, Division of Cancer Biology, University of Minn.

TABLE I. Protective Effect of Benadryl in Swiss-Webster Mice Sensitized to Peptone by Prior Treatment with Pertussis Vaccine.

No. of mice	Treatment	Outcome*
20	Peptone alone	0/20
20	Pertussis vaccine + peptone	18/20
20	*Idem* + Benadryl	0/20
10	Peptone + Benadryl	0/10

* Numerator denotes No. of deaths. Denominator denotes total No. of animals.

hours after the peptone injection, 50 μg of Benadryl was administered; a fourth group was given the combination of peptone and Benadryl without prior treatment with pertussis vaccine.

It will be noted that no deaths occurred in the mice treated with Benadryl. It was of interest that in the Benadryl-protected mice, the animals appeared inactive and the fur was ruffled shortly after the injection of peptone, but they soon recovered completely.

3. *Protection by chlorpromazine against increased susceptibility to peptone.* An experiment similar to that described with Benadryl was carried out, except that an attempt was made to protect sensitized Swiss-Webster female mice with chlorpromazine. When 10 sensitized mice were given 50 μg of chlorpromazine 2 hours before, 15 minutes before, and 30 minutes after the injection of 150 mg peptone, none of the mice died. Nine of 16 control mice died within 24 hours. Chlorpromazine appeared to offer the same degree of protection as Benadryl.

4. *Protection by cortisone against increased susceptibility to peptone.* A similar group of experiments was designed to test the protective action of cortisone. When 10 mice were sensitized with pertussis vaccine as described, and then given 150 mg of peptone, death was prevented by treating the animals with one intramuscular injection of 0.5 mg cortisone 6 hours prior to the peptone. Seven of 10 mice expired within 24 hours after the injection of peptone. Under these circumstances, cortisone protected the mice similar to Benadryl and chlorpromazine.

Discussion. The mechanism which makes mice more susceptible to peptone after treatment with pertussis vaccine is not understood. Pertussis vaccine increases susceptibility of mice to histamine(7). In view of this it would be tempting to conclude that histamine is released by peptone in these more susceptible mice. Peptone does liberate histamine in the dog(8). In addition, the peptone death in mice simulates the respiratory failure induced by histamine, and protection is offered to the mice by those agents which are known to block histamine activity. However, it cannot be assumed on the basis of available evidence that these drugs protect the peptone-susceptible mice by a direct antihistaminic effect. Histamine is probably not the only factor involved, since it has been demonstrated in this laboratory that Swiss-Webster female mice are made more susceptible to histamine by pertussis vaccine, whereas ABC female mice remain resistant. On the other hand, increased sensitivity of both populations of mice to peptone follows administration of pertussis vaccine. The suggestion that increased sensitivity of mice to histamine due to pertussis vaccine results from altered adrenal activity remains to be proved(9).

Summary. Populations of ABC and Swiss-Webster male and female mice were found to be resistant to peptone, but an increased susceptibility to peptone with a lethal outcome could be produced by prior treatment with pertussis vaccine. Mice rendered susceptible to peptone with pertussis vaccine could be protected with the antihistaminic agent, Benadryl, with chlorpromazine and with cortisone. The possible role of histamine in the increased susceptibility of mice to peptone, produced by pertussis vaccine, has been discussed.

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Received February 18, 1957. P.S.E.B.M., 1957, v95.

Cultivation of Spirochaetes from Spinal Fluids of Multiple Sclerosis Cases and Negative Controls.* (23117)

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Since 1838 when multiple sclerosis was first recognized as a demyelinating disease, many attempts have been made to establish its etiology. There were many conflicting theories. Originally it was thought that multiple sclerosis was due to toxins that destroy the myelin sheaths; second, that it was an allergic or biochemical reaction. Buzzard(1) advanced the theory that due to resemblance of multiple sclerosis to cerebrospinal syphilis, the causative agent may be an organism of the Treponema family. He did not succeed in demonstrating the suspected organism. Steiner and Kuhn(2) found spirochaetes in brain tissues and spinal cords of patients who had died of multiple sclerosis. Adams, Blacklock and M'Cluskie(3) were able to demonstrate spirochaetes under darkfield illumination while examining fluid from lateral ventricles of monkeys and rabbits injected with material from multiple sclerosis cases. This fluid was examined several months after inoculation when the animals developed signs of illness. The spirochaetes were identical in appearance with those described by Steiner. All attempts to cultivate the spirochaetes failed. Collins and Noguchi(4) tried to grow the organism by inoculating spinal fluids from multiple sclerosis cases into Noguchi culture medium. They were unable to get positive results. In 1951, I devised a culture medium in which spinal fluids from known cases of multiple sclerosis were inoculated, and, after one to 2 weeks incubation, few spirochaetes were found under phase and darkfield illumination. Cultures from spinal fluids of healthy individuals and of patients with other neurological diseases were negative and remained sterile after one year's incubation. The original culture medium did not show many organisms per field; growth was poor. To obtain a better growth, the culture medium was modified, and subcultures from the original positive culture and fresh spinal fluid reinoculated into the modified medium showed heavy growth of spirochaetes after several days' incubation.

Methods. Stoppered test tubes are sterilized by autoclaving, then sterilized again in hot air sterilizer for one hour at 180°C. About 10 ml of spinal fluid are collected into

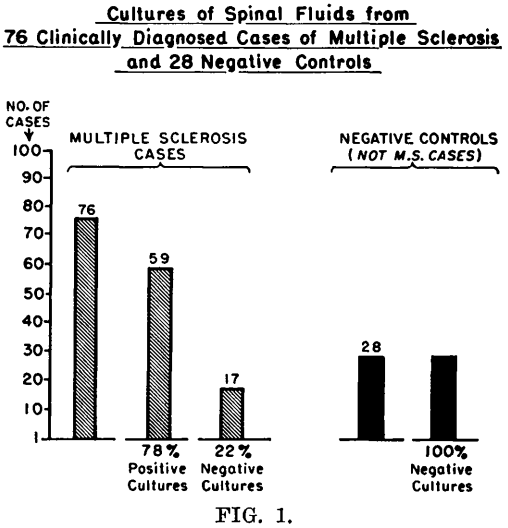

FIG. 1.

* I wish to thank Drs. M. Solis-Cohen and M. T. Moore for supplying the first spinal fluids, Dr. James C. Giuffre for promoting this research, and Dr. Harry E. Morton for his kind assistance. This research was supported through funds obtained by Dr. M. T. Moore from Harry and Rose Publiker Fund, by the Pa. Chapter of Natl. Multiple Sclerosis Soc., the Donner Foundation, and private contributions. It is receiving its main support from St. Luke's and Children's Medical Center.