Studies on Mechanism of Ascites Produced by Pertussis Vaccine.* (26860)

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Bordetella pertussis vaccine produces a cutaneous hypersensitivity reaction following paw injections(1) and ascites following intraperitoneal injections(2) in the rat. In both cases, the reaction is greatly augmented by prior sensitization with the same vaccine. Prior sensitization also enhances the lethal effects of *B. pertussis* vaccine in mice; the sensitizing ability but not the lethal capacity is destroyed by heat(3,4). The heat stability of the lethal factor has suggested a possible relation to endotoxin(3). In view of these data, it was of interest to determine the effects of heat on the ability of *B. pertussis* vaccine to produce ascites, and to investigate the mechanism of the reaction. The following experiments on the properties of heated *vs.* unheated vaccines and on passive transfer provide no evidence for participation of endotoxin in the genesis of ascites, and in fact, give additional evidence of the immunological nature of this phenomenon.

Methods. Female Wistar-derived rats (Hemlock Hollow Farms), 180-220 g, were fed Purina Laboratory Chow and tap water *ad libitum*. Six-tenths ml *B. pertussis* vaccine‡ containing 36 billion killed phase I organisms was diluted to 3.0 ml with sterile non-pyrogenic saline and injected intraperitoneally through a short-bevelled 21-gauge needle. Heating was accomplished before dilution by suspending the vial in a boiling water bath for one hour. Dose and dilution were the same for sensitizing and challenge injections and for heated and unheated vaccines. Ascitic fluid was measured by draining widely opened peritoneal cavities of exsanguinated rats into graduated cylinders. Omentum, mediastinal and renal lymph nodes and spleen were studied histologically.

Sensitization for passive transfer was accomplished in 3 ways. Ten donors of serum were sensitized with pertussis vaccine according to the method of Rowley *et al.*(1) for donors, and were bled from the heart. Seven donors of mediastinal lymph nodes were given 2 intraperitoneal injections of 3.0 ml diluted pertussis vaccine at an interval of 19 days and were sacrificed 5 days later. Five donors of superficial lymph nodes (popliteal, axillary, elbow) were sensitized intracutaneously in the 4 paws and at 4 flank sites with 1.2 ml of a 1:1 water-in-oil emulsion of undiluted pertussis vaccine and Freund's complete adjuvant; 19 days later they were given 3.0 ml of diluted vaccine intraperitoneally and were sacrificed 5 days later. Lymph node donors were killed by a blow to the head and dissected under sterile conditions. The nodes were minced, suspended in Ringer's solution by cycling between 2 syringes connected by a double-hubbed 18-gauge needle and passed through a sieve. Suspensions from each donor varied in volume from 2.0 to 4.2 ml and were always injected into only one recipient. As controls, several suspensions were heated 20 minutes in a 48°C water bath before being injected(5).

Results. Effects of heating pertussis vaccine. Rats were sensitized and challenged intraperitoneally 12 days later as outlined in Table I. Two injections of unheated pertussis vaccine caused severe ascites in rats sacrificed 5 days after challenge. Two injections of heated vaccine caused no ascites. Cross tests showed that heat destroyed the ability of pertussis vaccine either to sensitize or to elicit ascites. When heated vaccine was followed by unheated vaccine and when unheated vaccine was followed by heated vaccine, rats had no ascites 5 days after chal-

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TABLE I. Effect of Heat on Ability of Pertussis Vaccine to Produce Ascites.

Pertussis vaccine		Ascitic fluid (ml)	
1st inj.	2nd inj.	5 days after challenge	1 day after challenge
Unheated	Unheated	26, 24, 22, 19, 6.0, 0	5.3, 4.6, 3.8, 3.2, 1.8
Heated	Heated	0, 0, 0, 0, 0	
"	Unheated	1.0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0
Unheated	Heated	10.0, 0, 0, 0, 0, 0	
" *	" *	0, 0, 0, 0, 0, 0	

* Interval between injections prolonged to 19 days to assure complete subsidence of response to first inj. All other intervals were 12 days.

lenge except for a single anomalous result in each group (Table I).

The first was the occurrence of 1.0 ml of ascitic fluid in one rat pretreated with heated vaccine and challenged with unheated vaccine. This small volume 5 days after challenge could be due to the second injection alone(2). To confirm this, the experiment was repeated but rats were sacrificed 24 hours after challenge, that is, before the challenge injection alone could produce ascites(2). Two injections of unheated vaccine caused ascites but heated vaccine followed by unheated vaccine produced no fluid. Therefore, the anomalous result noted above could not be attributed to sensitization by heated vaccine.

The second aberrant result was found in one rat that had 10.0 ml ascitic fluid after sensitization with unheated pertussis vaccine followed 12 days later by challenge with heated vaccine. This could be due to an exceptionally prolonged and severe response to unheated vaccine used for the first injection. This possibility was confirmed by observation of rats sensitized with unheated vaccine. Twelve days after sensitization an occasional animal showed a swollen abdomen indicative of ascites. This disappeared by 19 days, at which time 6 rats were challenged with heated vaccine. At sacrifice 5 days later these rats had no fluid while ascites was present in all control rats challenged with unheated vaccine. Therefore the aberrant result provided no evidence that heated vaccine could produce ascites in sensitized rats.

Intraperitoneal injections of unheated pertussis vaccine, in addition to eliciting ascites, caused fibrinopurulent peritoneal exudate, inflammation of omentum, hyperplasia and granulomas in spleen and mediastinal and renal lymph nodes, thrombosis of lymph node sinuses, and an increase in body weight averaging 1.2 g per day greater than in untreated controls. None of these changes occurred in rats injected twice with heated vaccine.

Effect of cortisone. Twelve rats were sensitized intraperitoneally with unheated pertussis vaccine. Five of these rats received daily intramuscular injections of 20 mg/kg of cortisone acetate from day of sensitization through day 7 and 10 mg/kg from day 9 through 18. All rats were challenged intraperitoneally with unheated pertussis vaccine on day 19 and sacrificed 5 days later. Rats not treated with cortisone had 16, 16, 11, 8.0, 3.2, 1.4, and 1.0 ml of ascitic fluid (average 8.1 ml. Rats treated with cortisone had only 7.0, 4.8, 0.4, 0.1, and 0.0 ml of fluid (average 2.5 ml). This difference is statistically significant at probability level $p = .05$.

Passive transfer. Each of 6 recipients was given intraperitoneally 3.0 ml serum from actively sensitized donors, and was challenged intraperitoneally 24 hours later with 3.0 ml diluted pertussis vaccine. None had ascites 24 hours after challenge.

Seven recipients were given intraperitoneal injections of living lymph node cells from actively sensitized donors and 5 recipients were given heat-killed lymph node cells. The rats were challenged intraperitoneally with 3.0 ml diluted pertussis vaccine 24 hours later and were sacrificed one day after challenge. None of the rats treated with killed cells had ascites. In contrast, rats sensitized with living cells had 3.2, 2.7, 0.9, 0.5, 0.2, 0.2, and 0.0 ml fluid (average 1.1 ml). Ascites occurred after transfer from either mediastinal or superficial lymph nodes.

Discussion. The results indicate that the fraction(s) of the pertussis organism responsible for sensitization and production of ascites and lesions in omentum, lymph nodes, and spleen are heat-labile and are, therefore, different from the heat-stable lethal factor implicated in the experiments of Kind(3)

and Anatolii(4). This suggests that endotoxin is not involved in production of ascites. Sensitization for production of ascites was transferred passively by lymph node cells but not serum from pertussis sensitized donors. It seems likely, therefore, that the ascites phenomenon is analogous to the cutaneous hypersensitivity reaction of Rowley *et al.*(1) because in both cases there are similar responses to 1 and 2 injections of pertussis vaccine(2) and reactivity can be transferred passively with cells from sensitized donors. Cortisone decreased ascites but it is uncertain whether this was due to immunological effects, aberration of electrolyte and water metabolism, or general debility.

Summary. Rats sensitized and challenged intraperitoneally with pertussis vaccine developed severe ascites. However, pertussis vaccine that had been heated for 1 hour at 100°C neither sensitized rats for subsequent challenge nor elicited ascites in adequately sensitized rats. Unheated pertussis vaccine caused lesions in omentum, lymph nodes and spleen, and excessive gain in weight. These were not observed in animals injected with heated vaccine. Sensitization for production of ascites by pertussis vaccine was transferred passively to normal rats by intraperitoneal injection of lymph node cells but not serum obtained from sensitized rats. The results suggest that ascites elicited by pertussis vaccine resembles the delayed type of hypersensitivity and is not related to endotoxin.

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Decrease of Sialic Acid in Epiphyseal Plates of Aminoacetonitrile-Treated Rabbits.* (26861)

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Extensive lesions occur in the connective tissues of animals treated with Lathrogenic agents(1). The epiphyseal plates of young mammals are particularly vulnerable to the action of aminoacetonitriles (AAN). The biochemical nature of the lesion produced with these compounds is poorly understood.

The importance of sialic acid in the connective tissues has been recently emphasized (2,3). The content of sialic acid in the epiphyseal plates of normal and AAN-treated rabbits is here reported.

Method. Twenty 8- to 9-day-old rabbits from 3 different litters were divided into 2 groups. One group of 11 animals received a daily subcutaneous injection of 10 mg AAN during 5 days. The AAN solution was prepared dissolving AAN sulfate neutralized with sodium carbonate in saline solution and stored in a refrigerator at 5°C. Nine control animals received no injections. After 5 days the animals were sacrificed by decapitation and the epiphyseal plates from tibia, femur, and distal radius and ulna were obtained and placed in test tubes in ice. The epiphyseal plates were homogenized in a potter in 0.1 N H_2SO_4 as suggested by Svennerholm(4). The homogenate was hydrolyzed during one hour at 80°C. The hydrolyzate was filtered in a Dowex 50 column and absorbed in Dowex 2 × 8 (analytical grade). The sialic acid was eluted in 1 M acetate buffer pH 4.6 and the eluate was treated with resorcinol copper

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