Endotoxin-Detoxifying Capacity of Reticulo-Endothelial System in Normal and Pertussis-Treated Mice.* (29563)

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Pertussis vaccine greatly increases the susceptibility of mice to death from endotoxin (1). The reason for this is obscure. Recently we identified a protein fraction in mammalian spleen that quickly detoxifies endotoxin(2). The detoxifying power of this fraction is substantially increased in rabbits made tolerant to endotoxin(3), and decreased or lost altogether in rabbits made hypersensitive to endotoxin by a brief exposure to hemorrhagic shock(2). The data reported herewith show that the hypersensitivity of the pertussis-treated mouse to endotoxin can also be explained in terms of the potency of this detoxifying principle.

Procedure and results. Five-week-old female mice (Charles River Laboratories) received 0.4 ml of pertussis vaccine (Lederle) intraperitoneally, and were challenged by the same route 6 days later with *A. aerogenes* endotoxin suspended in saline and sterilized by millipore filtration. The MLD_{100} of this endotoxin, within the following 96 hours, was 200 γ in normal mice, and 6.25 γ in pertussis-treated mice.

The detoxifying fraction of spleen or liver from normal mice and mice given pertussis vaccine 6 days previously was prepared according to a method described elsewhere(2). One ml aliquots of this preparation, each representing one gram (wet weight) of spleen or liver, were incubated with varying amounts of the endotoxin for one hour at 37°C. The reaction mixture was then injected intraperitoneally into pertussis-treated mice.

The effect of cortico-steroid hormones on the lethality of endotoxin in pertussis-treated mice was also determined by comparing the mortality rate with and without intraperitoneal injection of 2.0 mg of hydrocortisone 2 hours before challenge.

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TABLE I. Lethality in Pertussis-Treated Mice of Endotoxin Reacted *in vitro* with Extracts of Spleen and Liver from Normal and Pertussis-Treated Mice.

Dose of endotoxin, μg	Splenic extract from		Liver extract from	
	Normal mice	Pertussis-treated mice	Normal mice	Pertussis-treated mice
200	10/10	—	—	—
100	9/10	—	—	—
50	6/10	10/10	9/10	10/10
25	2/10	10/10	4/10	10/10
12.5	0/10	10/10	0/10	8/10
6.2*	0/10	8/10	0/10	6/10
3.1	0/10	4/10	0/10	0/10

* MLD_{100} in pertussis-treated mice.

Table I shows that the potency of the detoxifying protein from normal liver is nearly as high as it is in normal spleen. It also shows that pertussis vaccine causes nearly a 13-fold reduction in the potency of this protein.

Table II shows that hydrocortisone protects the pertussis-treated animal in spite of the fact that the potency of the detoxifying enzyme is greatly reduced (Table III).

Comment. The increased susceptibility to endotoxin produced by pertussis vaccine has been investigated extensively. Such changes as have been observed in the metabolism of amine oxidases(4) and steroids(5), and in the susceptibility to histamine(6), serotonin

TABLE II. Mortality from Endotoxin in Pertussis-Treated Mice. Effect of pretreatment with hydrocortisone.

Dose of endotoxin, μg	Mortality	
	No hydrocortisone	Hydrocortisone*
50	5/5	2/5
25	5/5	0/5
12.5	5/5	0/5
6.2	4/5	0/5
3.1	3/5	0/5
1.6	0/5	0/5

* 2 mg hydrocortisone intraperitoneally 2 hr before challenge with endotoxin.

TABLE III. Lethality in Pertussis-Treated Mice of Endotoxin Reacted *in vitro* with Extracts of Spleen from Pertussis-Treated Mice Pretreated with Hydrocortisone.

Dose of endotoxin, μg	Not given hydrocortisone	Given hydrocortisone*
50	10/10	10/10
25	9/10	10/10
12.5	10/10	9/10
6.2	8/10	7/10
3.1	5/10	4/10
1.6	0/10	0/10

* 2 mg hydrocortisone intraperitoneally 2 hr before exsanguination.

(7) and anaphylactic shock(8), do not explain it. This study suggests that hypersensitivity is a result of the loss of endotoxin-detoxifying activity in the RES. This view is confirmed by the further observation that the recovery of normal resistance to endotoxin is concurrent with recovery of normal detoxifying power of the spleen and liver, which occurs about 6 weeks after injecting the vaccine.

In the light of other data showing that the lethal action of endotoxin is mediated *via* the nervous system(9), the anti-endotoxic effect of hydrocortisone appears to be achieved by blocking the interaction of endotoxin with nerve tissue. This hypothesis is being studied.

Conclusion. Pertussis vaccine produces hypersensitivity to endotoxin in mice by reducing the potency of the endotoxin-detoxifying component in the reticulo-endothelial system.

1. Kind, L. S., *J. Immunol.*, 1953, v70, 411.
2. Smith, E. E., Rutenburg, S. H., Rutenburg, A. M., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 781.
3. Rutenburg, S. H., Rutenburg, A. M., Smith, E. E., Fine, J., *On the nature of tolerance of endotoxin.* Symposium on endotoxin, Rutgers Univ. Press, 1964.
4. Kind, L. S., Woods, E. F., PROC. SOC. EXP. BIOL. AND MED., 1954, v84, 601.
5. Kind, L. S., *J. Allergy,* 1953, v24, 52.
6. Parfentjev, I. A., Goodline, M. A., *J. Pharmacol. Exp. Therap.,* 1948, v92, 411.
7. Munoz, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 200.
8. Malkiel, S., Hargis, B. J., *ibid.,* 1952, v80, 122.
9. Palmerio, C., Zetterstrom, B. E. M., Shammash, J., Euchbaum, E., Frank, E. D., Fine, J., *N. Engl. J. Med.,* 1963, v269, 709.

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Supersensitivity to Barium in the Denervated Nictitating Membrane of the Spinal Cat.* (29564)

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Recent research with the nictitating membrane of the cat indicates that the supersensitivity produced in that organ by denervation involves two or more independent mechanisms(1-3). The supersensitivity which follows decentralization or reserpine is moderate in degree and relatively nonspecific since the sensitivity is increased to an equal degree to both norepinephrine and acetylcholine(3,4). The mechanism involved in this nonspecific supersensitivity apparently is identical to one of the mechanisms of denervation supersensitivity(3). The purpose of this communication is to demonstrate that this nonspecific supersensitivity is extended to a third substance, barium, in addition to norepinephrine and acetylcholine.

Methods. Cats of 1.5 to 3.0 kg body weight and of either sex were used. The cats were pithed under ether anesthesia using the spinal method of Burn(5). Following this operation the animals were maintained on artificial respiration and ether discontinued. The responses of the right nictitating membrane were recorded isometrically with a

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