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1. Christensen, L. R., and MacLeod, C. M., *J. Gen. Physiol.*, 1945, v28, 559.

2. Lewis, J. H., and Ferguson, J. H., *J. Clin. Invest.*, 1950, v29, 1059.

3. Geiger, W. B., *J. Immunol.*, 1952, v69, 597.

4. Müllertz, S., and Lassen, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1953, v82, 364.

5. Troll, W., *et al.*, *J. Biol. Chem.*, 1954, v208, 85.

6. Troll, W., and Sherry, S., *ibid.*, 1955, v213, 881.

7. Kline, D., *ibid.*, 1953, v204, 949.

8. Christensen, L. R., and Smith, D. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v74, 840.

9. Werbin, H., and Palm, A., *J. Am. Chem. Soc.*, 1951, v73, 1382.

10. Hagan, J., *et al.*, *PROC. SOC. EXP. BIOL. AND MED.*, 1956, v92, 627.

11. Ablondi *et al.*, *ibid.*, 1956, v93, 414.

12. Jackson, H. D., *et al.*, *ibid.*, 1954, v86, 827.

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Sensitivity of Pertussis-Inoculated Mice to Serotonin.* (23166)

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It is well known that pertussis vaccine increases susceptibility of mice to histamine(1) and to active(2) and passive(3,4) anaphylaxis. It hardly seems likely that the concomitant sensitivity of pertussis-inoculated mice to histamine and to anaphylaxis is coincidental. However, some evidence has been presented against the importance of histamine in mouse anaphylaxis(5). Recently, Fink demonstrated that the anaphylactic contraction of isolated mouse uterus is inhibited by lysergic acid diethylamide (LSD) and reserpine(5). It was also shown that the egg-white sensitized and normal mouse uterus is 1000 times more sensitive to serotonin than to histamine. In view of the aforementioned experiments, the sensitivity of pertussis-inoculated mice to serotonin was determined.

Materials and methods. Male 20 to 27 g Tumblebrook Farm (TF) Swiss Webster mice and male 17 to 21 g Swiss Webster mice from the National Laboratory Animals Co. (NLA) were inoculated i.p. with 0.1 ml of *Hemophilus pertussis* vaccine (48.8 billion organisms/ml). Four days later (when the peak of histamine sensitivity is known to occur) pertussis-inoculated mice as well as uninoculated control animals were challenged

i.p. with various doses of histamine dihydrochloride or serotonin creatinine sulfate. In the experiment involving pertussis-inoculated and uninoculated NLA mice 5 dosage groups containing 8 mice per group were employed in determining the LD₅₀ of either histamine or serotonin. An exception to this procedure occurred in the uninoculated mice challenged with histamine, where only 1 group of 8 mice was challenged with a single dose of histamine. Since all animals survived, the LD₅₀ was expressed as > the dose injected. In the experiment involving pertussis-inoculated and uninoculated TF mice 3 dosage groups consisting of 6 mice per group were utilized in each instance in determining the LD₅₀ of each drug. Total number of animals used in both experiments was 200. Deaths were noted for a period of 24 hours. Median lethal doses were calculated by the method of Litchfield and Wilcoxon(6) and are expressed in terms of mg/kg of base.

Results. The data in Table I demonstrate that administration of pertussis vaccine greatly increases susceptibility of the mouse to the lethal effects of both serotonin and histamine. The LD₅₀ and 19/20 confidence limits of serotonin were 7.9 (5.4–11.4) mg/kg in pertussis-inoculated TF mice and 364 (251–528) mg/kg in uninoculated TF ani-

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TABLE I. LD₅₀ of Serotonin and Histamine in Pertussis-Inoculated and Uninoculated Mice.

Mice	LD ₅₀ and 19/20 confidence limits of:	
	Histamine dihydrochloride† (mg/kg base)	Serotonin creatinine sulfate†*
Pertussis-inoculated (TF)	3.0 (2.1- 4.7)‡	7.9 (5.4- 11.4)§
Uninoculated (TF)	915 (822 -1071)‡	364 (251 -518)§
Pertussis-inoculated (NLA)	8.0 (5.0- 12.9)	12.6 (5.4- 28.4)
Uninoculated (NLA)	> 620	603 (568 -639)

* Creatinine itself (1000 mg/kg) was not lethal to pertussis-inoculated or uninoculated mice.

† Both materials were dissolved in distilled H₂O.

‡ Difference between these values is statistically significant (p = .05).

§ *Idem*

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mals. The LD₅₀ and 19/20 confidence limits of histamine were 3.0 (2.1–4.7) mg/kg in pertussis-injected TF mice and 915 (822–1071) mg/kg in uninoculated TF controls. The LD₅₀ and 19/20 confidence limits of serotonin were 12.6 (5.4–28.4) mg/kg in pertussis-inoculated NLA mice and 603 (568–639) mg/kg in uninoculated NLA animals. The LD₅₀ and 19/20 confidence limits of histamine were 8.0 (5.0–12.9) mg/kg in pertussis-inoculated NLA mice; the LD₅₀ of histamine in uninoculated NLA controls was >620 mg/kg. Since no overlapping of the confidence limits occurred, the difference between the LD₅₀ doses of these drugs in pertussis-inoculated and uninoculated mice can be considered significant at the p = .05 level.

Discussion. It should be pointed out that the pertussis-inoculated mouse is not a generally weakened animal more susceptible to all sorts of stressor agents(3). Its altered reactivity is apparently selective. While it is more sensitive than normal mice to lethal effects of histamine(1) serotonin, anaphylaxis(3,4) Gram negative vaccines(3) and endotoxins(7,8) it is no more susceptible than normal animals to lethal effects of carbachol(3), methacholine, epinephrine and heat stress(9). It is quite probable that both histamine and serotonin are of importance in mouse anaphylaxis. It has recently

been demonstrated that edema produced in the rat by ovomucoid and other agents which damage mast cells is mediated by a combination of serotonin and histamine or closely related substances(10).

Summary. Pertussis vaccine sensitizes mice to the lethal effects of serotonin. The LD₅₀ of serotonin in pertussis-inoculated NLA mice was 12.6 mg/kg as compared with 602 mg/kg in uninoculated NLA controls. The LD₅₀ of serotonin in pertussis-inoculated TF mice was 7.9 mg/kg as compared with 364 mg/kg in uninoculated TF animals.

1. Parfentjev, I. A., and Goodline, M. A., *J. Pharm. Exp. Therap.*, 1948, v92, 411.
2. Malkiel, S., and Hargis, B. J., *Proc. Soc. Exp. Biol. and Med.*, 1952, v80, 122.
3. Kind, L. S., *J. Immunol.*, 1953, v70, 411.
4. Pittman, M., and Germuth, F. G., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1954, v87, 424.
5. Fink, M. A., *ibid.*, 1956, v92, 673.
6. Litchfield, J. T., Jr. and Wilcoxon, F., *J. Pharmacol. and Exp. Therap.*, 1949, v96, 99.
7. Parfentjev, I. A., *Yale J. Biol. and Med.*, 1954, v27, 46.
8. Abernathy, R. S., and Spink, W. W., *J. Immunol.*, 1956, v77, 418.
9. Loew, E. R., and Woodman, E., *Am. J. Physiol.*, 1956, v187, 615.
10. Rowley, D. A., and Benditt, E. P., *J. Exp. Med.*, 1956, v103, 399.

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