of intestinal absorption of B_{12}, calculated in terms of parenteral equivalents, is more suitable for comparison than figures of hepatic uptake of B_{12} obtained in various individuals. The shape, size and position of the liver and the crystal of the scintillation counter influence the hepatic count. Calculation of efficiency of intestinal absorption of B_{12} in terms of parenteral equivalents disposes of these technically interfering elements. For clinical purposes, however, such as differentiation of macrocytic anemias, or detection of asymptomatic forms of pernicious anemia or sprue, calculation of parenteral equivalents of intestinal absorption has no advantages. Here the simplified, 48-hour technic of measuring hepatic uptake of radioactivity following oral administration of $Co^{60}B_{12}$, alone or with intrinsic factor, is entirely adequate and satisfactory(14).

Summary and conclusions. Hepatic uptake of radioactivity following oral administration of a tracer dose of $Co^{60}B_{12}$ alone and with intrinsic factor preparation was compared with that after intramuscular injection of the same dose of $Co^{60}B_{12}$ on 31 individuals with various diseases. Efficiency of intestinal absorption and effect of intrinsic factor preparation was quantitated in this way and was expressed in terms of parenteral equivalents of intestinal absorption.

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Received April 23, 1957. P.S.E.B.M., 1957, v95.

Effect of *H. pertussis* on Sensitivity of Mice to Serotonin. (23209)

J. MUNOZ (Introduced by W. F. Verwey)
(With technical assistance of M. A. Greenwald)
Merck Institute for Therapeutic Research, Rahway, N. J.

It is well known that injection of *Hemophilus pertussis* cells to mice of certain strains produces a marked increase in histamine sensitivity(1,2,3) as well as an increase in ability of mice to become anaphylactically sensitive (4,5). Since histamine plays an important role in anaphylaxis of guinea pigs and other animals as well as man(6), it was tempting to conclude that pertussis increased susceptibility of mice to anaphylaxis mainly by increasing their susceptibility to histamine(7). This, however, does not explain why some strains do not become sensitive to histamine after pertussis injection(3,11) while becoming highly susceptible to anaphylaxis(11). Moreover, the state of histamine hypersensitivity in susceptible strains disappears much sooner than that of anaphylactic sensitivity.

TABLE I. Effect of *H. pertussis* Injection on Toxicity of Serotonin to Mice.

Serotonin, mg/kg	Results: TF mice, Normal D/T*	TF mice, Normal % D	TF mice, Pertussis treated D/T	TF mice, Pertussis treated % D	CF mice, Normal D/T	CF mice, Normal % D	CF mice, Pertussis treated D/T	CF mice, Pertussis treated % D
1.17	0/15	0	0/15	0	0/15	0	0/15	0
2.34	"	0	9/35	25.3	"	0	3/14	21.2
4.68	"	0	24/33	72.7	"	0	6/15	40.0
9.37	"	0	24/34	70.5	"	0	10/15	66.6
18.75	"	0	20/33	60.6	"	0	9/14	64.2
37.50	"	0	22/33	66.7	2/15	13.3	12/14	85.6
75.00	1/15	6.6	18/35	51.4	0/15	0	13/15	86.6
150.00	13/15	86.7	11/15	73.4	9/15	60	10/15	66.6

* D/T = Deaths/Total mice inj.

For these reasons, it appears that in anaphylaxis of pertussis treated mice, hypersensitivity to circulating histamine does not play an important part. Other mechanisms must then be sought. The role that serotonin (5-hydroxytryptamine) may play in anaphylaxis has been suggested by various investigators. In guinea pigs serotonin can produce symptoms that are similar to those observed in anaphylaxis(8), and it is released *in vitro*, together with histamine, from platelets by an antigen-antibody reaction(9). Fink(10) found that serotonin inhibitors [d-lysergic acid diethylamide (LSD) and reserpine] prevent the Shultz-Dale reaction. For these reasons, it was of interest to investigate the effects of *H. pertussis* on sensitivity of mice to serotonin.

Materials and methods. Female mice weighing from 14-16 g of either CF-1 strain (from Carworth Farms) or TF strain (Swiss Webster mice from Tumblebrook Farms) were used. Mice were injected intraperitoneally with 2,000 million killed smooth *H. pertussis* cells suspended in 0.25 ml of physiological saline solution. Mice were challenged intravenously with various doses of serotonin* 4 days after pertussis injection. The drug was dissolved in physiological saline solution. Although final results were recorded 24 hours after injection of serotonin, most deaths occurred within the first 2 hours.

Results. Effect of H. pertussis on susceptibility of mice to serotonin. Groups of TF and CF-1 mice injected with pertussis cells were challenged with various doses of serotonin. The two strains were used because it is known that the TF mice become highly sensitive to histamine after injection of pertussis, while CF-1 mice do not(3). This latter strain should not be confused with the CF-W strain that is available from the same source. The results obtained are summarized in Table I. It is clear from these results that both TF and CF-1 mice become 20-30 times more sensitive to serotonin after administration of *H. pertussis*. There is an indication that TF mice may become slightly more sensitive than CF-1, but the difference is not significant. With TF mice there is also a suggestion that the dose response curve for serotonin is a biphasic type of curve similar to that found previously with histamine(12). At 4.68 mg/kg dose of serotonin 72.7% of the animals died, while at 75 mg/kg dose only 51.4% died.

Optimal time for development of serotonin sensitivity. Groups of TF mice were given pertussis cells, and at various intervals of time thereafter were challenged with various doses of serotonin. The results obtained are given in Table II where it can be seen that a low degree of sensitivity to serotonin was ob-

TABLE II. Development of Sensitivity to Various Doses of Serotonin after *H. pertussis* injection.

Serotonin, mg/kg	Days after *H. pertussis*: 1	3	6	8	10
2.34	0/10*	4/10	4/ 9	2/10	2/10
4.68	1/10	9/10	6/10	5/10	1/10
9.37	4/10	5/10	5/10	5/10	8/10
18.75	3/10	9/10	5/10	6/10	4/ 9
37.50	3/10	4/10	7/10	3/10	8/10

* Deaths/Total mice inj.

* Purchased as creatinine sulfate salt from Nutritional Biochemicals Corp., Cleveland, O.

TABLE III. Development and Disappearance of Serotonin Sensitivity in *H. pertussis* Mice.

Group	Days after *H. pertussis*	Results, D/T
1	—*	0/20
2	1	0/20
3	2	7/20
4	4	15/20
5	11	12/20
6	18	8/20
7	25	2/20
8	32	1/20
9	—*	1/10
10	—*	0/ 9

* No pertussis. A control group of 20 mice from the same batch of animals was challenged on the same day as Group 2, and another group of 10 normal mice on the same day as Group 7 and the last control group on the same day as Group 8.

TABLE IV. Effect of Various Antigens on Development of Sensitivity to Serotonin.

Serotonin, mg/kg	Results *H. pertussis*	*S. typhosa*	*E. rhusiopathiae*	Normal
2.34	2/ 9*	0/10	0/10	0/10
4.68	7/10	"	"	"
9.37	"	"	"	"
18.75	5/ 9	"	"	"
37.50	5/10	"	"	1/10
75.00	6/10	"	"	0/10

* Deaths/Total inj.

served one day after pertussis injection. By the third day optimal sensitivity was obtained and it persisted for 10 days when the experiment was terminated. These results show that sensitization of mice to serotonin varies among individual mice just as in the case of histamine sensitization(3).

To establish how long sensitivity to serotonin lasts, another experiment was performed extending the period of observation to 32 days. One-hundred-eight TF mice were used, of which 140 received *H. pertussis* cells and 40 were kept as controls. Groups of 20 mice each were challenged at various intervals after administration of *H. pertussis* with 18 mg of serotonin per kg of body weight. From the results given in Table III, as well as those in Table II, it is clear that sensitivity to serotonin reaches its peak three to four days after *H. pertussis* injection and gradually decreases until sensitivity returns to normal by the 25th day. Groups of normal control mice challenged at the beginning and at the end of this experiment (groups 1, 9 and 10, Table III) showed no marked sensitivity to 18 mg of serotonin per kg of body weight.

Effect of other bacterial cells on sensitivity of mice to serotonin. Three different bacterial suspensions were used—acetone-dryed *Salmonella typhosa* cells, *Erysipelothrix rhusiopathiae* and *H. pertussis*. One group of mice received intraperitoneally 0.25 ml of a suspension of *S. typhosa* cells containing 0.032 mg N/ml, another group received 0.25 ml of suspension of *E. rhusiopathiae* of a turbidity equivalent to 8,000 million *H. pertussis* cells/ml, and the last group received 0.25 ml of suspension of *H. pertussis* containing 8,000 million cells/ml. Four days later the mice were challenged with serotonin. The results are given in Table III. Of the 3 bacterial species used, only *H. pertussis* was capable of producing the sensitivity to serotonin.

Discussion. It has been difficult to account for the increased susceptibility to anaphylaxis induced in mice by *H. pertussis*. Although in some strains of mice the increase in susceptibility to histamine was thought to explain the greater susceptibility to anaphylaxis(7), other strains failed to sensitize to histamine(3,11) but became more susceptible to anaphylaxis after pertussis treatment (11). This observation suggested that other mechanisms, besides histamine action, may be involved in mouse anaphylaxis. Although, from the results obtained in our experiments, as well as those reported by others(8,9,10), it is tempting to suggest that pertussis cells exert their effect by increasing the sensitivity of mice to serotonin, no direct proof to support this view has been obtained. Fink's observations that LSD and reserpine inhibit specific contraction of mouse uterus *in vitro,* tend to support the serotonin hypothesis. It should be remembered, however, that both LSD and reserpine are not specific anti-serotonin drugs, and it may well be that inhibition observed by Fink was due to a property other than that of inhibiting serotonin. Although the anti-serotonin activity of LSD as well as other anti-serotonin compounds like BAS can be shown by protecting pertussis treated mice against lethal doses of serotonin, it has not

been possible with these substances to protect either mice or guinea pigs from anaphylaxis. In fact, BAS actually seems to enhance anaphylactic deaths.† For these reasons, it is premature to assume that pertussis cells exert their anaphylactic-enhancing effect by increasing the sensitivity to serotonin. It should be noted, however, that the type of death observed in pertussis-treated mice after injection of lethal doses of serotonin or histamine is grossly indistinguishable from that observed in anaphylaxis(13). Typically, after injection of serotonin mice become lethargic. Respiration becomes forced and within a few minutes signs of cyanosis are observed as evidenced by the color of snout, tail, ears and paws. The snout actually appears to be swollen. Little cyanosis is observed in non-lethal reactions. Mice usually die within 15-30 minutes giving few tonic contractions of the hind legs. Usually after these final convulsions mice continue to gasp for a short time. The similarity of death produced by these three different agents may well point to a common mechanism, and as Herxheimer(8) has suggested, death may result from a still unknown mechanism triggered by antigen-antibody reactions as well as by serotonin, histamine and perhaps other substances.

Summary. (1) Two strains of mice known to differ with respect to their ability to become sensitized to histamine, were found to become highly sensitive to serotonin after the injection of *H. pertussis* cells. (2) Some sensitivity to serotonin could be detected 16-24 hours after the administration of pertussis. Sensitivity reached its peak by the third to fourth day, and disappeared by the 25th day after pertussis injection. (3) Ability to induce sensitivity to serotonin seems to be characteristic of *H. pertussis*.

† Unpublished observations.

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Received February 15, 1957. P.S.E.B.M., 1957, v95.

Cytotoxic Effect of Nephrotoxic Serum on Rat Tissue Culture.* (23210)

CHARLOTTE T. LIU, WALLACE W. MCCRORY AND JOHN A. FLICK
(Introduced by Werner Henle)
Children's Hospital of Philadelphia, Department of Pediatrics, School of Medicine, Department of Microbiology, Graduate School of Medicine, University of Pennsylvania.

Lippman(1) reported that gamma globulin made from rabbit anti-rat-kidney serum inhibited growth in tissue culture explants of rat heart muscle and kidney as well as chick-embryo-brain, heart muscle and mesonephrons. The cytotoxicity of this serum did not appear species specific. Investigation of this problem has been renewed in our laboratory using monolayer tissue culture cells(2). This report concerns the effects of anti-rat-

* This study was supported by a research grant-in-aid from the N.I.H., U. S. Public Health Service.