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INVOLVEMENT OF A HEAT-STABLE AND HEAT-LABILE COMPONENT OF *BORDETELLA PERTUSSIS* IN THE DEPRESSION OF THE MURINE HEPATIC MIXED-FUNCTION OXIDASE SYSTEM*

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Abstract—Experiments were conducted to determine whether the decrease in ethylmorphine *N*-demethylase and aniline hydroxylase activities and in the levels of cytochrome P-450 observed after injection of *Bordetella pertussis* to mice was related to an activity of the well-characterized 80°-heat-labile bacterial component (HSF) which causes an increased sensitivity to histamine. Temporal studies over 10–15 days following *B. pertussis* inoculation of mice suggested a possible correlation between the development of histamine sensitivity and the decrease in both *in vivo* and *in vitro* activities of the hepatic mixed-function oxidase system. Treatment of mice with unheated or 56°-heated vaccine produced a decrease in microsomal drug-metabolizing enzyme activity and an increased sensitivity to histamine at both 24 hr and 5 days after injection. In contrast, mice injected with an 80°-heated vaccine did not show an increased sensitivity to histamine at either time point or a decrease in drug-metabolizing activity at 5 days. There was, however, a significant loss of microsomal enzyme activity determined at 24 hr post-injection of the 80°-heated vaccine. Injection of *B. pertussis* into a strain of mice insensitive to HSF activity was shown to produce a decrease in the drug-metabolizing activity at both 24 hr and 5 days without the concomitant increase in histamine sensitivity. On the other hand, injection of partially purified HSF into a susceptible strain of mice produced histamine sensitization without the loss of hepatic enzyme activity. These studies suggest that HSF *per se* is not the bacterial component responsible for the reduction in the hepatic microsomal enzyme activity. The results would indicate that two separate bacterial components may be involved. One is stable to heat of 80° and may cause the acute (24 hr) loss of activity. The second is labile to heat between 56 and 80° and may be responsible for the prolonged (5–10 days) loss of enzyme activity. The identity of the heat-labile component is unknown but the former, heat-stable component may be bacterial endotoxin. This conclusion is based on the similarity between the transient reduction in drug-metabolizing activity produced by injection of 500 μg/kg of endotoxin and that produced by the 80°-heated vaccine.

Certain strains of mice and rats develop a marked sensitivity to histamine, serotonin, bradykinin, slow-reacting substance of anaphylaxis [1–3] and, at least in one strain of mice, to acetylcholine when inoculated with live or killed *Bordetella pertussis* cells. Of the above substances, the exquisite hypersensitivity to histamine has been the most extensively investigated and has been shown to be caused by a heat-labile constituent of the bacterial cell wall, the so-called "histamine-sensitizing factor" (HSF) [1–3]. The increased sensitivity of mice to histamine appears within 24–48 hr after the injection of *B. pertussis*, reaches a peak between day 3 and day 5, and after about 30 days returns to normal.

Recently, we reported a previously unobserved effect of *B. pertussis* inoculation of mice of altering the activity of the hepatic drug-metabolizing enzyme system [4, 5]. Five days after *B. pertussis* administration, i.e. the time of peak sensitivity to histamine, a marked diminution was seen in the activity and levels of the components of the mixed-function oxidase system. Renton and Mannering [6] have also shown that administration of *B. pertussis* daily for 3 days to rats causes a depression of hepatic cytochrome P-450-dependent mono-oxygenase systems. In addition, Renton [7] reported that a 4-fold increase in phenytoin half-life was observed 24 hr after the administration of *B. pertussis* to rats. Also, microsomes from *B. pertussis*-treated rats had a decreased rate of hydroxylation of phenytoin and a decreased level of cytochrome P-450. The present communication further explores this effect of pertussis administration on hepatic drug-oxidation reactions.

Studies were conducted with killed *B. pertussis* cells to determine whether the decrease in drug-metabolizing activity could be attributed to HSF itself or to other bacterial components. The results indicate that the decrease in drug-oxidation after pertussis administration is related not to the activity of HSF but to a heat-stable and another heat-labile constituent of the bacterial cell. The identity of the latter constituent is unknown, while the former may be identical to the endotoxin component of the gram-negative organism. A preliminary report of these investigations has been published [8].

MATERIALS AND METHODS

Animals. Female CD_1 and CDF_1 mice (Carworth Farms, Portage, MI), weighing 18–20 g, were used throughout the study. The animals were fed standard laboratory diet and water *ad lib.*

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Bacterial products. Commercially available *Bordetella pertussis* vaccine (Eli Lilly & Co., Indianapolis, IN) was diluted 1:1 and injected intraperitoneally (i.p.) in a volume of 0.35 ml, representing 7×10^9 killed pertussis cells per mouse. *Escherichia coli* endotoxin 026:B6 (Difco Laboratories, Detroit, MI) was dissolved in saline and injected i.p. in a volume of 0.2 ml at a dose of either 50 or 500 μg/kg body wt. Histamine-sensitizing factor (HSF), extracted and partially purified from *B. pertussis* cells [9, 10], was obtained from Dr. Samuel B. Lehrer of Tulane University. The mice received an i.p. injection equivalent to 50 times the intravenous 50 per cent sensitizing dose (SD_{50}). Sterile, pyrogen-free isotonic saline was used as diluent for all injected substances as well as for administration to control animals.

In vivo *determination of histamine hypersensitivy and pentobarbital sleep-time.* The presence of histamine hypersensitivity was assessed by the incidence of death during a 60-min time period following the i.p. injection of 70 mg/kg of histamine. An effect of vaccine administration on the *in vivo* disposition of drugs was determined from the duration of pentobarbital-induced narcosis. Sodium pentobarbital (75 mg/kg) was administered i.p., and sleep-time was defined as the interval between the loss and the recovery of the righting reflex.

In vitro *assays.* The levels and activity of the hepatic drug-metabolizing enzymes were determined using pooled microsomal preparations from three to five animals per group. The microsomal fraction was prepared as described previously [5]. Aminopyrine and ethylmorphine *N*-demethylase activities were determined according to the method of Nash as described by Anders and Mannering [11]. Aniline hydroxylase activity was measured by the method of Imai *et al.* [12]. Cytochrome P-450 was calculated from the dithionite difference spectrum between 450 and 490 nm according to the method of Omura and Sato [13]. Cytochrome b_5 was determined from the difference in absorption between 424 and 410 nm produced by the addition of a few mg of NADH to the microsomal suspension of the sample cuvette. Extincition coefficients used for cytochrome P-450 and cytochrome b_5 were 91 $cm^{-1}\,mM^{-1}$ and 185 $cm^{-1}\,mM^{-1}$, respectively. NADPH-cytochrome *c* reductase activity was measured according to the method of Williams and Kamin [14] as reported previously [15].

RESULTS

The data in Table 1 show that the effect of *B. pertussis* vaccination was to produce an increased sensitivity to exogenously administered histamine and to prolong the pentobarbital-induced narcosis. The latter is customarily regarded as an *in vivo* correlate of hepatic drug-oxidation activity. In agreement with previous reports [1–3], histamine sensitization was observed within 24 hr, reached a peak at about 5 days, and was still appreciable 10–15 days following injection of the killed bacterial cells. Similarly, *B. pertussis*-treated animals also showed a significant prolongation of the pentobarbital sleep-time which persisted for at least 5 days. No significant difference between control and pertussis-treated animals was observed in the onset of narcosis (data not shown), suggesting that treatment of mice with the vaccine did not alter the central nervous system sensitivity to the barbiturate.

The time course of the effect of pertussis inoculation on the variables of the microsomal mixed-function oxidase system is shown in Figs. 1 and 2. A significant decrease in the rates of *N*-demethylation of aminopyrine and ethylmorphine and of hydroxylation of aniline was noted at 24 hr after vaccination. Of these three activities, ethylmorphine *N*-demethylation was the most affected by the pertussis treatment and aniline hydroxylation the least affected. Aniline hydroxylase activity remained between 65 and 75 per cent of control value for up to 5 days post-vaccination. Ethylmorphine *N*-demethylase activity was depressed to as low as 45 per cent on day 1 and began to return to the control value after day 3. On day 5, enzyme activity against each of these three substrates was approximately 65 per cent of control levels and, from day 5 to day 15, each showed a parallel return toward control values. However, each of these activities was significantly less than control activity in mice killed 15 days after pertussis treatment.

Table 1. Effects of *B. pertussis* inoculation on the development of histamine hypersensitivity and the duration of pentobarbital sleep-time of CD_1 mice

	Control		Pertussis-treated	
Time after vaccination	Histamine sensitivity*	Pentobarbital sleep-time (min)	Histamine sensitivity*	Pentobarbital sleep-time (min)
6 hr	0/54	—	0/54	—
1 day	0/59	62 ± 7†	27/59	192 ± 8†‡
3 days	0/44	56 ± 6	36/44	157 ± 6‡
5 days	2/64	82 ± 10	61/64	180 ± 10‡
10 days	0/44	86 ± 14	24/44	118 ± 13
15 days	0/40	—	21/40	—

* Values represent the number of animals dead 60 min following an i.p. injection of 70 mg/kg histamine relative to total number of animals tested at each time period.

† Values represent the mean ± S.E. of five animals per group.

‡ Significantly different from control value, $P < 0.01$.

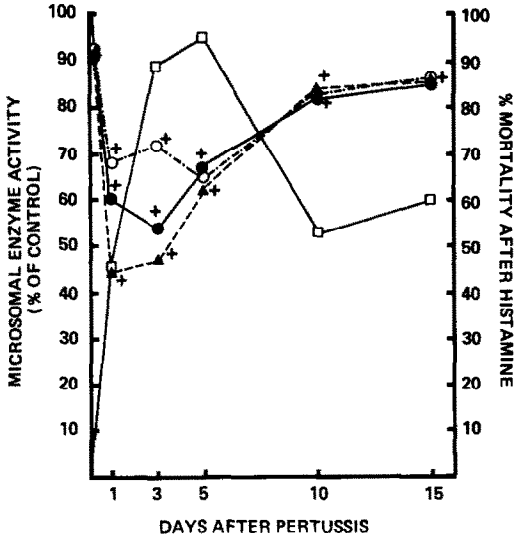

Fig. 1. Histamine sensitivity (□——□) and the rate of microsomal metabolism of ethylmorphine (▲——▲), aminopyrine (●——●) and aniline (○——·——○) at various times after a single i.p. injection of *B. pertussis* vaccine (7×10^9 cells/mouse). Enzyme activities from four to six experiments at each time point are expressed as the percentage of the corresponding control value. Histamine sensitivity is expressed as the percentage of pertussis-inoculated mice killed relative to the number challenged with an i.p. administration of 70 mg/kg histamine. One hundred per cent control values: aminopyrine *N*-demethylase activity, 0.859 ± 0.029 μmole HCHO formed/mg protein/hr; ethylmorphine *N*-demethylase activity, 0.867 ± 0.044 μmole HCHO formed/mg protein/hr; and aniline hydroxylase activity, 52.82 ± 1.11 nmoles *p*-aminophenol formed/mg protein/hr. The cross (+) indicates significantly different from control value, $P < 0.05$.

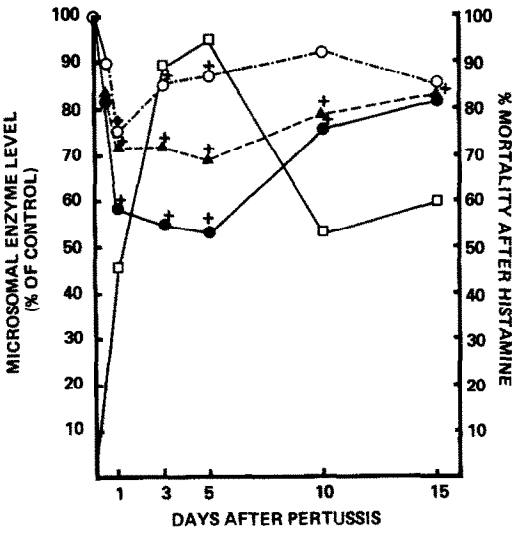

Fig. 2. Histamine sensitivity (□——□) and levels of cytochrome P-450 (●——●), cytochrome b_5 (▲——▲) and NADPH-cytochrome *c* reductase activity (○——·——○) at various times after a single i.p. injection of *B. pertussis* vaccine (7×10^9 cells/mouse). Enzyme levels from four to six experiments at each time point are expressed as the percentage of the corresponding control value. One hundred per cent control values: cytochrome P-450, 1.21 ± 0.04 nmoles/mg protein; cytochrome b_5, 0.46 ± 0.01 nmole/mg protein; NADPH-cytochrome *c* reductase activity, 188 ± 4 nmoles cytochrome *c* reduced/mg protein/min. Histamine sensitivity is expressed as in Fig. 1. The cross (+) indicates significantly different from control value, $P < 0.05$.

Of the three microsomal electron transport components studied the level of cytochrome P-450 changed the most markedly (Fig. 2). The level of this hemoprotein in microsomal preparations from pertussis-treated mice was from 52 to 58 per cent of the control level for up to 5 days after vaccination and was 80 per cent of control after 15 days. Cytochrome b_5 concentration, although not as markedly affected, decreased during the same time intervals

Table 2. Effects of heated and unheated *B. pertussis* vaccine on histamine sensitivity and ethylmorphine *N*-demethylation and aniline hydroxylation activity*

Treatment and time after injection	Histamine sensitivity (No. dead/No. tested)	Ethylmorphine *N*-demethylase activity (μmoles HCHO/hr/mg protein)	Aniline hydroxylase activity (nmoles *p*AP/hr/mg protein)
Control			
1 Day	0/25	0.555 ± 0.062†	50.25 ± 3.22†
5 Days	2/30	0.620 ± 0.070	51.59 ± 2.32
Unheated vaccine			
1 Day	6/25	0.224 ± 0.034‡	36.07 ± 2.23‡
5 Days	29/30	0.338 ± 0.027‡	30.41 ± 2.21‡
56°-Heated vaccine			
1 Day	2/10	0.189 ± 0.045‡	36.09 ± 1.22‡
5 Days	9/10	0.276 ± 0.028‡	37.32 ± 2.10‡
80°-Heated vaccine			
1 Day	1/25	0.241 ± 0.018§	36.69 ± 1.17§
5 Days	4/30	0.530 ± 0.058	46.91 ± 3.66

* Vaccine was diluted 1:1 with sterile physiological saline. Separate aliquots were injected either unheated or after heating at 56 and 80° for 30 min.

† Values are means ± S.E. for three to six experiments.

‡ Significantly different from control value, $P < 0.01$.

§ Significantly different from control value, $P < 0.05$.

Table 3. Effects of heated and unheated *B. pertussis* vaccine on levels of cytochrome P-450 and cytochrome b_5 and on NADPH-cytochrome *c* reductase activity*

Treatment and time after injection	Cytochrome P-450 (nmoles/mg)	Cytochrome b_5 (nmoles/mg)	NADPH-cytochrome *c* reductase (nmoles reduced/mg/min)
Control			
1 Day	1.11 ± 0.18†	0.49 ± 0.02	199 ± 9
5 Days	1.09 ± 0.07	0.42 ± 0.02	187 ± 6
Unheated vaccine			
1 Day	0.67 ± 0.08‡	0.33 ± 0.02§	151 ± 10§
5 Days	0.60 ± 0.08‡	0.28 ± 0.03‡	149 ± 7§
56°-Heated vaccine			
1 Day	0.54 ± 0.04‡	0.25 ± 0.03‡	—
5 Days	0.70 ± 0.08‡	0.25 ± 0.04‡	—
80°-Heated vaccine			
1 Day	0.69 ± 0.01‡	0.32 ± 0.04§	150 ± 3§
5 Days	1.02 ± 0.07	0.38 ± 0.03	173 ± 9

* Vaccine was prepared as described in Table 2.
† Values are means ± S.E. of three to six experiments.
‡ Significantly different from control value, $P < 0.01$.
§ Significantly different from control value, $P < 0.05$.

following pertussis administration. The NADPH–cytochrome *c* reductase decreased to 75 per cent of control level 24 hr after pertussis administration and by 3 days after treatment was reduced 15 per cent in activity. The reductase activity remained at this decreased level for up to 15 days.

Since the time course for prolongation of pentobarbital sleep-time and the depression of the hepatic microsomal enzyme-dependent components seemed to parallel the development of histamine sensitivity (Table 1, and Figs. 1 and 2), experiments were designed to determine whether the two responses were elicitied by the same bacterial component, namely HSF. These studies utilized the established heat-lability of HSF, the resistance of certain strains of mice to HSF activity and, finally, the administration to mice of a partially purified preparation of HSF obtained from *B. pertussis* organisms.

The histamine-sensitizing activity of *B. pertussis* has been shown previously to be destroyed by heating the vaccine at 80° for 30 min, whereas the other heat-labile activities attributed to the heat-labile toxins (HLT) of the organism are destroyed by heating the vaccine at 56° [1]. Table 2 compares the effects of injection into mice of the non-heated vaccine or of vaccines heated at 56° or 80° on the *N*-demethylation of ethylmorphine and the hydroxylation of aniline by hepatic microsomal enzymes as well as the development of histamine hypersensitivity at 24 hr and 5 days after inoculation. It can be seen that animals receiving the unheated or the 56°-heated vaccine showed histamine sensitivity at both 24 hr and 5 days, whereas histamine sensitivity was significantly attenuated in the animals inoculated with the 80°-heated vaccine. However, a differential effect on the drug-metabolizing enzymes was observed between the two heated and the unheated vaccines at the two time points. Injection of either of the heated or the unheated vaccines produced comparable decreases in the enzyme activities determined 24 hr after vaccination. But, 5 days after vaccination, animals injected with the unheated or the 56°-heated vaccine showed a persistent and significant decrease in microsomal enzyme activity, whereas enzyme activity of the microsomal preparations from animals inoculated with the 80°-heated vaccine was not significantly different from control values.

Table 3 shows the effects of the heat-treated and the unheated vaccines on the levels of cytochrome P-450, cytochrome b_5 and NADPH-cytochrome *c* reductase. One day after vaccination with the various preparations, the level of each of these microsomal electron transport components was markedly decreased. At 5 days after vaccine inoculation, the levels of these components were diminished in those animals that had received the unheated or 56°-heated vaccine. Animals injected with the 80°-heated preparation had values that were not significantly different from control values.

These results suggested that the depression of drug-metabolizing enzyme activity seen at 24 hr after vaccination was not associated with the 80°-heat-labile HSF, while the prolonged effect observed 5 days later might be related to HSF activity. Therefore, a study was initiated with a strain of mice (CDF_1) which does not develop increased sensitivity to histamine following pertussis vaccination [2]. As shown in Table 4, an increase in histamine sensitivity was not seen 5 days after administration of *B. pertussis* to female CDF_1 mice, despite the fact that these animals showed a marked decrease in microsomal enzyme activity. This decrease in enzyme activity was comparable to that produced by pertussis administration to the CD_1 strain, i.e. histamine-sensitive mice. Although these results implied that the observed decrease in drug-metabolizing activity was probably not related to HSF, it was considered possible that the two effects, i.e. histamine sensitivity and decreased drug metabolism activity, might be

Table 4. Effects of *B. pertussis* vaccination on the hepatic drug-metabolizing enzymes in a strain of mice resistant to development of histamine hypersensitivity*

Treatment	Histamine sensitivity (No. dead/No. tested)	Ethylmorphine *N*-demethylase activity (μmoles HCHO/hr/mg protein)	Aniline hydroxylase activity (nmoles *p*AP/hr/mg protein)	Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)
Control	0/40	0.657 ± 0.012	58.02 ± 3.88	0.92 ± 0.04	0.49 ± 0.50
Pertussis vaccine	0/40	0.375 ± 0.036†	40.44 ± 1.06‡	0.57 ± 0.05†	0.38 ± 0.03‡

* CDF_1 female mice were injected with *B. pertussis* vaccine (7×10^9 cells/mouse). Five days after vaccination, groups of ten mice were challenged with 70 mg/kg histamine base. The livers from an additional group of three mice were pooled and microsomal drug-metabolizing activity was determined. Values represent the means ± S.E. of four experiments.

† Significantly different from control, $P < 0.01$.

‡ Significantly different from control, $P < 0.05$.

Table 5. Activity of the hepatic drug-metabolizing enzymes 3 days after injection of partially purified histamine-sensitizing factor (HSF)*

Treatment	Histamine sensitivity (No. dead/No. tested)	Ethylmorphine *N*-demethylase activity (μmoles HCHO/hr/mg protein)	Aniline hydroxylase activity (nmoles *p*AP/hr/mg protein)	Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)	NADPH-cytochrome *c* reductase activity (nmoles/min/mg protein)
Control	0/20	0.556 ± 0.070	46.85 ± 4.68	0.83 ± 0.06	0.25 ± 0.02	154 ± 11
HSF	12/20	0.635 ± 0.040	51.35 ± 1.30	0.86 ± 0.08	0.29 ± 0.03	145 ± 10

* G-100 HSF was diluted with physiological saline. Each animal received 50 times the i.v. sensitizing dose. Control animals received an equivalent amount of diluted buffer solution. Values represent the means ± S.E. of four experiments; each group consisted of five animals.

Table 6. Effects of *E. coli* endotoxin on the hepatic drug-metabolizing enzyme system*

Treatment and interval post-injection	Ethylmorphine *N*-demethylase activity (μmoles HCHO/hr/mg protein)	Aniline hydroxylase activity (nmoles *p*AP/hr/mg protein)	Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)
Control				
1 Day	0.579 ± 0.057	53.18 ± 1.41	1.14 ± 0.05	0.48 ± 0.01
5 Days	0.651 ± 0.020	60.47 ± 3.80	1.31 ± 0.07	0.50 ± 0.02
Endotoxin (50 μg/kg)				
1 Day	0.461 ± 0.044	52.67 ± 2.07	1.05 ± 0.08	0.39 ± 0.04
5 Days	0.555 ± 0.064	60.39 ± 6.33	1.17 ± 0.02	0.47 ± 0.03
Endotoxin (500 μg/kg)				
1 Day	0.249 ± 0.041†	43.0 ± 1.78‡	0.73 ± 0.04†	0.33 ± 0.03‡
5 Days	0.583 ± 0.055	62.76 ± 4.98	1.21 ± 0.08	0.48 ± 0.03

* *E. coli* endotoxin 026:B6 was dissolved in sterile isotonic saline. Values represent the mean ± S.E. of three to four experiments with pooled livers of five animals per group.
† Significantly different from control, $P < 0.01$.
‡ Significantly different from control, $P < 0.05$.

different manifestations of HSF and that the resistant strain might be insensitive to one but not to both of these effects. Therefore, HSF-sensitive mice (CD_1) were injected with a partially purified preparation of HSF, the known 80°-heat-labile factor from *B. pertussis* cells. As reported in Table 5, the animals receiving this HSF preparation displayed the usual increased sensitivity to histamine, but no effect on the hepatic enzyme activities was found.

The results with the 80°-heated vaccine reported above also indicated that the early decrease in hepatic drug-metabolizing activity observed within 24 hr after vaccination was associated with a heat-stable component of the bacterial cell. Bacterial lipopolysaccharide (endotoxin) is a heat-stable component of gram-negative organisms, such as *B. pertussis*, and was, therefore, considered to be a likely candidate. Table 6 shows the effect of two doses of *E. coli* endotoxin on the hepatic drug metabolism of mice 24 hr and 5 days after injection. At 50 μg/kg endotoxin, no significant effect on drug oxidation reactions was observed. However, 500 μg/kg of *E. coli* endotoxin caused a significant depression of ethylmorphine *N*-demethylase and aniline hydroxlase activities and in the levels of cytochrome P-450 and cytochrome b_5. These effects were seen 24 hr, but not 5 days, after injection of the lipopolysaccharide. The dose of endotoxin that was estimated [16–19] to have been injected in the volume of pertussis vaccine used for sensitization throughout these studies was at least 300 μg/kg. This effect of endotoxin, therefore, was similar to that observed with the 80°-heated pertussis preparation.

To exclude a possible direct toxic effect of pertussis vaccine on hepatic microsomes, aliquots of the vaccine were preincubated with microsomal fractions from control mice. The data (not shown) indicate that no diminution in enzyme activity is seen under these conditions.

DISCUSSION

Injection of *B. pertussis* cells, or their products, into experimental animals produces a variety of biological effects, including sensitization to the lethal effects of certain pharmacological agents, such as histamine and serotonin, unresponsiveness to the hyperglycemic action of epinephrine, a marked leucocytosis, and adjuvanticity with respect to both humoral antibody production and cell-mediated immunity. Previous studies have suggested that many of these diverse responses may be associated with the activity of certain components of the bacterial cell. The following substances or activities have been described so far in *B. pertussis*: (1) protective antigen (PA) ; (2) HSF; (3) leucocytosis promoting factor (LPF); (4) heat-labile toxins (HLT); (5) heat-stable endotoxin; (6) agglutinogen; and (7) hemagglutinin [1–3]. Several lines of experimental evidence suggest that the first three of these factors may be identical. Each of these is labile to heat to 80°, and attempts to separately isolate these components have so far been unsuccessful [20]. Conversely, HLT is distinguishable from the other three heat-labile activities by its destruction at 56°, a temperature that does not alter HSF, LPF or PA activities.

The purpose of the present study was to explore further the loss of hepatic drug-metabolizing activity observed in pertussis-treated animals [4–8]. The experiments were designed to determine whether the decrease in hepatic enzyme activity was related to the effect of HSF or of other bacterial components. Temporal studies following pertussis administration to mice susceptible to histamine sensitization by HSF indeed suggested the possibility that the production of histamine sensitization and the decrease in both the *in vivo* and *in vitro* drug-metabolizing activities might be correlated (Table 1, and Figs. 1 and 2). Thus, the effect of *B. pertussis* administration in producing histamine hypersensitivity, together with prolonging pentobarbital sleep-time, decreasing microsomal *N*-demethylase, hydroxylase and NADPH-cytochrome *c* reductase activities, as well as depressing cytochrome P-450 and cytochrome b_5 levels, was most marked between 24 hr and 5 days post-vaccination. Of the hepatic enzyme activities, the *N*-demethylations of ethylmorphine and aminopyrine were decreased to a much greater extent than was aniline hydroxylation. Of the microsomal electron transport components, the level of cytochrome P-450 was the most affected. NADPH-cytochrome *c* reductase activity was only marginally affected. The differential effect of pertussis vaccination on the *N*-demethylation and hydroxylation reactions may represent a selective decrease in one of the multiple cytochrome P-450 species recently shown to be present in hepatic microsomal preparations [21–23]. Pertussis administration also significantly decreased the levels of cytochrome b_5. At present the role of this hemoprotein in drug-oxidation reactions is unclear, but various investigators have suggested its participation in certain cytochrome P-450-mediated mixed-function oxidations as well as a role in the desaturation of fatty acids [24]. All of the above effects were present to a somewhat lesser, but still significant, degree 15 days post-vaccination.

Destruction of HSF activity by heating the vaccine to 80°, injection of *B. pertussis* cells into a strain of mice (CDF_1) resistant to HSF activity, and finally, injection of a partially purified HSF preparation into a sensitive strain of mice (CD_1) all resulted in observations indicating that the loss of the hepatic mixed-function oxidase system is not associated with an activity of HSF. This conclusion is based on: (1) a dissociation of effects of heating the vaccine on the development of histamine hypersensitivity and on the loss of enzyme activity, particularly 24 hr after vaccination; (2) a decrease in enzyme activity in the mouse strain resistant to the development of histamine hypersensitivity; and (3) a failure of HSF itself to affect drug-metabolizing activity while inducing increased responsiveness to histamine. The data obtained with the 80°-heated vaccine preparation also indicate that more than one bacterial component may be involved in eliciting the acute and prolonged decrease in enzymatic activity. Thus, heat treatment of the vaccine at 80° for 0.5 hr abolished the effect on cytochrome P-450 levels and associated enzyme activities seen at 5 days, but not those observed at 24 hr. Therefore, the two bacterial constituents may be tentatively referred to as one that is labile, and a second that is stable, to 80° heat. The similarity between the effects on drug-metabolizing enzymes observed following the injection of endotoxin, a known heat-stable component of gram-negative organisms including *B. pertussis*, and those following the administration of the 80°-heated vaccine suggest that the heat-stable lipopolysaccharide component of *B. pertussis* may be the agent responsible for the effects seen at 24 hr post-vaccination. On the other hand, the identity of the heat-labile constituent is presently unknown. Since LPF and PA are currently regarded to be inseparable from HSF, the only remaining *B. pertussis* activities previously described are HLT, agglutinogen and hemagglutinin. Of these, HLT can also be eliminated because heating the vaccine at 56°, which destroys HLT but not HSF, did not affect either the acute or late phase of the loss of hepatic drug-metabolizing activity. This further characterizes the heat sensitivity of the unknown agent as being between 56 and 80°.

An additional unanswered question is the mechanism through which administration of *B. pertussis* cells depresses the hepatic mixed-function oxidase system. Bissel and Hammaker [25] have reported that injection of 2 mg/kg of *E. coli* endotoxin into rats causes a loss of cytochrome P-450 as early as 4 hr after injection. Associated with the loss of the hemoprotein levels is a concomitant decrease in δ-aminolevulinic acid synthetase activity and an increase in heme oxygenase activity [26]. The former enzyme activity is the rate-limiting step in heme biosynthesis while the latter is the key enzyme in heme degradation. In addition, el Azhary and Mannering [27] have reported similar effects on heme metabolism following administration of interferon inducers to rats. In line with these observations, preliminary experiments in our laboratory with *B. pertussis* administration to mice and rats do appear to indicate that a stimulation of heme oxygenase does indeed occur at 24 hr and is still above control values at 5 days (unpublished observations).

Of further significance are the number of recent reports of various immunologically active substances, such as endotoxin [25, 28], *B. pertussis* [4–8], *Coryebacterium parvum* [29], *Bacillus Calmette-Guérin* [30], and complete Freund's adjuvant [31–33], altering the activity of the hepatic mixed-function oxidase system. Also, Renton and Mannering [6, 34] and Leeson *et al.* [35] have shown that rats injected with interferon inducers, among which many of the former agents can be included, have decreased drug-metabolizing activity. In contrast, a recent report [36] indicates that *in vitro* exposure of isolated hepatic parenchymal cells to the interferon inducer Poly I-Poly C, as well as to mouse interferon, results in the induction of cytochrome P-450. In this context, it seems pertinent to mention recent observations in our laboratory [37, 38] which show that the well-characterized *in vivo* effect of endotoxin to antagonize the glucocorticoid induction of hepatic tryptophan oxygenase [39, 40] is not due to a direct effect on the hepatic parenchymal cell but is mediated through an effect on the hepatic non-parenchymal cell fraction, presumably the Kupffer cell. These studies lend additional significance to the observation of Wooles and Munson [41] that both stimulators

and inhibitors of reticuloendothelial cell function inhibited parenchymal cell drug-oxidation reactions. It is possible, therefore, that some form of interplay exists between these two cell systems with regard to the regulation of the mixed-function oxidase and other hepatic enzymes. Similarly, the potential effect of decreased mixed-function oxidase activity in drug therapy of gram-negative sepsis and endotoxemia, as well as the use of immunostimulants such as *C. parvum* [42], in conjunction with chemotherapeutic agents needed to be metabolized to the active moiety, would appear to converge within this same range of biological possibilities.

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