Histamine-sensitizing Factors from Microbial Agents, with Special Reference to *Bordetella pertussis*

J. MUNOZ AND R. K. BERGMAN

National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana 59840

INTRODUCTION

Scope

The physiological mechanisms by which various hypersensitivity phenomena manifest themselves are poorly understood. Presumably, the various types of hypersensitivity reactions result from either a systemic or localized release of pharmacological agents. The specific pharmacologic agents involved in allergic or hypersensitivity reactions are known with some certainty for only a few animal species. Histamine is of great importance in anaphylaxis of the guinea pig and rabbit, and serotonin has been implicated in the mouse and in the rat. Other pharmacological agents have also been implicated in hypersensitivity reactions, such as the "slow reacting substance," bradykinin, acetylcholine, and others less well known. Histamine, by far, has been best studied. It is a potent, naturally occurring amine known to produce vasodilatation, promote capillary permeability, and induce bronchoconstriction. Marked differences in susceptibility to histamine exist among animal species. Guinea pigs and rabbits are highly susceptible to the lethal effect of histamine, whereas mice and rats have a remarkable tolerance to it (Table 1). It has been known for several years that the tolerance of at least some strains of rats and mice can be greatly diminished by administering certain bacterial substances. An understanding of how these substances sensitize animals to histamine would increase our knowledge of hypersensitivity in general. This review attempts to summarize the present knowledge of bacterial substances which have been reported to enhance sensitivity of animals to histamine.

Most of the observations in this field have been made with cells from smooth cultures of *Bordetella pertussis* or with extracts therefrom. The literature dealing with other microorganisms consists mainly of isolated observations on histamine sensitization produced in animals. Little or no work has been reported on the mode of action and the purification of the sensitizing substances from microorganisms other than *B. pertussis*. This review covers various aspects of the histamine sensitization phenomenon, and discusses the relationship of this sensitization to other phenomena associated with the treatment of animals with histamine-sensitizing materials. The nature of the substances producing sensitization and the various postulated mechanisms of action of these intriguing substances will be also discussed.

Reviews have appeared which cover various aspects of this topic (71, 100, 107, 152, 154, 205, 217, 223). The present review will emphasize recent advances and topics not well covered previously.

Historical Notes

Eldering (45) was the first to report that treatment with fractions from *B. pertussis* increased the susceptibility of mice to intraperitoneal challenge with living cells of *B. pertussis*. Similar phenomena were observed later with whole-cell vaccine as the sensitizing agent and a variety of microorganisms as infectious agents (11, 44, 180, 182). Ospeck and Roberts (179) described another type of sensitization produced by *B. pertussis* extracts when they observed that mice immunized with crude toxoid, prepared from culture filtrates, often died of shock after challenge with the

TABLE 1. *Toxicity of histamine for common laboratory animals*

Species	Toxicity[a]	References
Mouse	344	102
	>362	94
	800	19
	844	119
Rat	>544	124
	565	—[b]
Rabbit	2.3	226
Guinea pig	0.9	226

[a] Toxicity = LD_{50} of histamine given intraperitoneally, expressed as milligrams of histamine base per kilogram of body weight.

[b] J. Munoz, *unpublished data.*

toxin. This reaction was most probably an anaphylactic shock, although it was not recognized as such by Ospeck and Roberts. Parfentjev et al. (186–188) observed a similar phenomenon when they found that mice pretreated with pertussis vaccine died after receiving a denatured nucleoprotein isolated from *B. pertussis*. This sensitivity was considered to have an immunological basis, but it was not clearly recognized as being associated with anaphylactic shock until Malkiel and Hargis described their observations on the increased susceptibility of *B. pertussis*-treated mice and rats to actively induced anaphylaxis (121–124). These observations have been confirmed and extended by various workers employing a variety of antigens (100, 154, 205, 223). Furthermore, *B. pertussis*-treated mice were also seen to become more susceptible to passively induced anaphylaxis (156, 169, 206; J. Munoz, L. F. Schuhardt, and W. F. Verwey, Federation Proc. **13**:507, 1954). The observations of Parfentjev et al. (186–188) that *B. pertussis*-treated mice become sensitive to a denatured nucleoprotein preparation from *B. pertussis* led these workers to test the sensitivity of *B. pertussis*-treated mice to histamine. They found that pertussis vaccine increased the susceptibility of their mice to histamine up to 100-fold (185). This observation was soon confirmed (79, 94, 102, 202, 229). Ten years after Parfentjev and Goodline's (185) discovery of histamine sensitization in mice, it was shown that some strains of mice also become hypersensitive to serotonin after treatment with pertussis vaccine (89, 98, 150, 205). This observation was considered to be of further significance because serotonin had been implicated in mouse anaphylaxis (55, 67, 232).

The above observations established at least three recognizable and seemingly distinct effects produced by killed *B. pertussis* cells in mice: (i) an increase in susceptibility to infection, (ii) an increase in susceptibility to anaphylaxis, and (iii) an increase in susceptibility to histamine and serotonin. We should mention that an increase in resistance to infection (44, 191) and to tumor growth (129) has also been observed under certain conditions in mice treated with *B. pertussis*. This resistance-inducing factor is probably similar to endotoxin from other gram-negative bacteria and different from the factor responsible for increased susceptibility to histamine, serotonin, and perhaps to other agents. Ample confirmatory evidence regarding the effect of the histamine-sensitizing factor (HSF) from *B. pertussis* is now found in the literature (71, 100, 119, 152, 154, 205, 217, 223).

Pertussis vaccine produces many other changes in mice, which may or may not be due to the same substance that produces histamine sensitization. It is not known whether these changes are produced by a common mechanism or whether they develop from a variety of effects produced by pertussis vaccine.

HISTAMINE-SENSITIZING PROPERTIES OF VARIOUS BACTERIA

The criterion for histamine sensitization that has been used almost exclusively is the increased lethal effect of histamine in mice or rats treated with histamine-sensitizing factors. A few workers have employed reactivity of the skin (215, 216) or symptoms of shock induced by this amine (106) to measure increased sensitivity to histamine.

Histamine is ordinarily given as a salt, such as the diphosphate or dihydrochloride, but doses are frequently expressed in terms of the base. In all of our work, as in this review, doses of histamine are expressed as histamine base.

Most bacterial vaccines that have been tested do not sensitize mice to histamine (Table 2). Sensitization has been reported with killed *Brucella abortus* cells (123, 125), although some have failed to demonstrate sensitivity with heat-killed vaccine from this organism (1). We have tested Merthiolate-killed and acetone-dried strain 19 *B. abortus* cells and obtained erratic sensitization when mice were challenged 4 days later with 1 mg of histamine, but no demonstrable sensitization to a 0.5-mg challenge. With the larger challenge, we also have obtained some indication of increased sensitivity after treatment with Merthiolate-killed cells from strains of *B. bronchiseptica*, *B. parapertussis*, and *Escherichia coli* O111. None of these produced increased

TABLE 2. *Bacteria and bacterial products that have been tested for histamine-sensitizing properties in mice*

Agent	Dose	Sensitization[a]	References
	Cells per mouse $(\times 10^9)$		
Bacterial cells:			
Bordetella pertussis (Phase I)	0.8–32	Excellent	119
B. pertussis (Phase IV)	30	None	80, 94
B. parapertussis	16	None	119
B. bronchiseptica	32	None	119
Brucella abortus strain 19	1.2–2	Variable	1, 123, —[c]
Haemophilus influenzae	8.75–40	None	123
Pasteurella pestis (live cultures)	?[b]	Weak	106
P. pestis toxic autolysates	?	Weak	106
Escherichia coli	?	None	94
Shigella dysenteriae	?	None	94
Salmonella typhosa	15	None	185
Neisseria catarrhalis	0.6	None	123
Typhoid-paratyphoid vaccine (triple vaccine)	0.7	Variable	94, 123
Diplococcus pneumoniae	?	None	80
Cholera vaccine	1	None	123
	μg/mouse		
Endotoxin from:			
Bordetella pertussis	100–2,100	Variable	130, 200, —[c]
B. bronchiseptica	200	Variable	130, —[c]
E. coli O8	100–200	None	130
Salmonella typhosa	27–729	Variable	130, 200
S. enteritidis	5–25	None	—[c]
S. abortus equi	100–200	None	130
Brucella abortus	200	Weak	125, 130
Serratia marcescens	200	Weak	130
Pseudomonas (Piromen)	200	None	130

[a] The evaluation of sensitization is given only as a general comparison. Excellent means consistent and high mortality reported by all workers; variable means erratic and high mortality not usually obtained; weak means low mortality after histamine challenge; none means no sensitization observed.

[b] ? = not given.

[c] R. Ross, J. Munoz, and R. K. Bergman, *unpublished data*.

sensitivity to a 0.5-mg challenge of histamine, if judged by its lethal effects (R. Ross and J. Munoz, *unpublished data*). These results seem to confirm the failure reported by others to demonstrate marked histamine sensitization by *B. parapertussis, B. bronchiseptica, Haemophilus influenza* (119, 123), *E. coli, Shigella dysenteriae* (94), *Salmonella typhosa* (123, 185), *S. paratyphi, Neisseria catarrhalis,* cholera vaccine, influenza virus vaccine (123), *Diplococcus pneumoniae* [80], and even phase IV *B. pertussis* (80, 94). When endotoxins extracted from many of these organisms were tested for their ability to produce histamine sensitization, however, some sensitization in mice was obtained (130, 196). This sensitization was inferior to that produced by *B. pertussis,* and, in addition, large amounts of endotoxin were needed, in many cases approaching the lethal dose of these lipopolysaccharides (Table 2). Malkiel and Hargis (130), for example, found that the intraperitoneal doses (100 to 200 μg) which induced histamine sensitivity were in-

variably lethal when given intravenously. Pieroni et al. (196) showed that the sensitization produced by endotoxin from *S. typhosa* was of low grade, a dose response was not obtained, and, in various experiments, doses from 37 to as much as 2,100 μg failed to produce histamine sensitization. If their materials were active endotoxin preparations, all doses employed should have produced marked toxic reactions which would confuse the interpretation of results. In our hands, 1 to 25 μg of purified endotoxins from *E. coli* or *S. enteritidis* and crude endotoxins from various *B. pertussis* strains have failed to show histamine-sensitizing effects in CFW female mice (J. Munoz, R. Ross, and R. K. Bergman, *unpublished data*). These doses of endotoxin are in excess of the amount needed to produce most of the biological activities recognized for these lipopolysaccharides (pyrogenicity, chick embryo toxicity, production of Shwartzman reaction, etc.). Some endotoxin preparations from *B. pertussis* have failed to give histamine sensitization in doses as high as 1,000

μg/mouse (J. Munoz, R. Ross, and R. K. Bergman, *unpublished data*).

Kratinov and Maksimenko (106) reported that subcutaneously administered avirulent but toxic cells of *Pasteurella pestis*, in numbers that killed two-thirds of the mice inoculated, sensitized the survivors to histamine. Toxic autolysates of these cultures also produced similar sensitization in mice, rats, and guinea pigs. The degree of sensitization produced in mice and rats by these agents was from 5 to 35 times that of normal animals when shock symptoms were used as a criterion of sensitization; in guinea pigs, it was increased 7 to 15 times. The number of animals employed was small, and the sensitization was of a low order. For these reasons, it is difficult to determine the significance of this work. It has also been reported that mice bearing sarcoma 180 tumor become more sensitive to histamine (183).

At best, the sensitivity to histamine produced by agents other than *B. pertussis* has been weak and inconsistent.

NATURE OF THE HISTAMINE-SENSITIZING FACTOR (HSF) FROM *BORDETELLA PERTUSSIS*

The active principle of *B. pertussis* is located in, or at least associated with, the cell wall (26, 27, 164, 227, 242), because cell wall preparations contain greater activity on a weight basis than do other fractions. Some workers have questioned that HSF is located in the cell wall (138), but their results can be explained by contamination of other cell fractions with solubilized HSF. It is not known whether HSF forms an integral part of the cell wall structure or is just associated with it. By serological tests, HSF has not been identified with any of the agglutinogens (9, 10, 46–49, 209, 219), but various workers believe that HSF is identical to the mouse-protective antigen (86, 111, 161, 195). Others feel that HSF and mouse-protective antigen are two distinct substances (43, 170, 218, 227).

Soluble preparations of HSF have been obtained from culture supernatant fluids (74, 118, 119, 172), from sonically disrupted cells (51, 119, 239), from cells dissolved with sodium deoxycholate (17, 42, 237), from cells treated with lysozyme (15, 16, 139, 211), by extraction of cells with a mixture of thiourea-urea and formamide (76, 118), by autolysis (119), and from acetone-dried cells extracted with alkaline saline (160, 161). These soluble preparations have supplied materials for further purification of HSF. Niwa (172) obtained a high degree of purification by precipitating HSF from culture supernatant fluids with zinc acetate at pH 6 to 6.2. The precipitate was extracted with 20% Na2HPO4·12H2O and then

dialyzed against water at pH 6.2 (adjusted with 0.1 M acetic acid). The precipitate that developed during dialysis contained the HSF. This precipitate was dissolved in 0.1 M phosphate buffer at pH 8 and again dialyzed against water for 24 hr at 4 C. The precipitated material was highly active, and contained the highest specific activity [2,170 histamine-sensitizing doses (HSD50) per mg, or an HSD50 of 0.46 μg per mouse] of all fractions reported by Niwa (172). This preparation had both HSF and protective activity (M. Niwa, *personal communication*). Many lots of our crude alkaline saline extracts have an activity (HSD50 of about 0.6 μg or less) comparable to that of Niwa's purified material (159). Thus, it seems that the actual activity of HSF is greater than reported. Unfortunately, as the substance is further purified, it becomes somewhat insoluble and its specific activity does not increase appreciably. We have obtained purified preparations from saline extracts by starch-block electrophoresis at pH 6.2. These purified active fractions contained mainly one antigenic component, as determined by agar-gel diffusion tests employing an antiserum which demonstrated at least 12 different antigens in the starting material. This purified HSF also protected mice challenged intracerebrally with *B. pertussis* (160, 161). Pieroni et al. (195) have prepared active materials from acetone-extracted cells disrupted in a Waring Blendor in the presence of glass micro-beads. The extracts were centrifuged at 37,000 × g for 3 hr and then precipitated with ammonium sulfate at 35% saturation. The HSF was in the precipitate, which contained only a small percentage (6% or less) of the nitrogen found in the starting material. Although no critical tests for purity were reported, their preparations were active in doses from 3.7 to 7.1 μg of N per mouse, representing at least 25 to 48 μg of dry weight (assuming all to be protein). Thus, their preparations were 1 to 2.5% as active as Niwa's preparation or our crude saline extracts. Our experience with ammonium sulfate precipitation has indicated that the precipitate obtained at 35% saturation is heavily contaminated with other cellular antigens.

The ammonium sulfate-precipitated materials contain not only HSF but also protective activity, and indeed many of the activities found in the whole cell (195, 197, 198, 200). Levine and Pieroni (111) have strongly suggested that all of these activities are due to the same substance. Although this is probable from some of the results that have been obtained (160, 161, 195, 197–200), it is still premature to assign most of the reactions observed with whole cells, or with relatively crude extracts, to a single molecular entity.

Recently, two groups have published observations regarding the separation of HSF from protective activity. Both claimed to have separated the two activities, one by sucrose density gradient centrifugation of soluble starting materials (218), and the other by centrifugation at 4 C for 2 hr at 60,000 × g of sodium deoxycholate lysates of *B. pertussis* (170). Sato and Nagase (218) stated that no histamine-sensitizing activity was found in fractions giving full protection. The HSF activity was found in a fraction with a sedimentation coefficient of 4S, and the protective activity was present in a 22S fraction. The work of Nagel (170) also shows that HSF and protective antigen are separable. These results support previous claims that HSF and protective antigen are two different substances (43, 74, 210, 227).

Recently, we have found that magnesium sulfate in low concentrations aids in precipitating HSF from dialyzed saline extracts at pH 5.6 (J. Munoz and B. M. Hestekin, Federation Proc., p. 267, 1968). The precipitate contains all of the activity and only 10 to 20% of the total material found in saline extracts from acetone-dried cells. Unfortunately, this precipitate is rather insoluble and still contains at least three to five antigens. Again, both HSF and protective activities, as well as the anaphylactogenic activity, are found in this material. It has only traces of endotoxin, and has relatively low toxicity for mice, rabbits, and chick embryos.

GENERAL PROPERTIES OF HSF FROM *B. PERTUSSIS*

The activity of HSF is destroyed by heat (80 C for 0.5 hr) (8, 90, 97, 119, 162, 172), by Formalin (88, 91, 136, 162), and by some proteolytic enzymes such as trypsin [Sutherland (227) did not obtain destruction by trypsin], Pronase, and a protease from *Bacillus subtilis* (82, 107, 138, 195). Deoxyribonuclease, ribonuclease (237), lysozyme (15, 16, 139, 211), lipase, cellulase, and amylase (238) do not destroy it.

The activity is always found in fractions containing high concentrations of nitrogen (160, 161, 170, 195), and our saline extract preparations and MgSO$_4$-precipitated material have an almost full complement of amino acids, except cysteine and methionine (J. Munoz and B. Hestekin, Federation Proc., p. 267, 1968). The absence of these two amino acids has also been noted by Hiramatsu (82). HSF is only slowly inactivated by NaIO$_4$, suggesting that HSF does not depend on carbohydrate for its activity (82, 238, 239). The purest fractions so far obtained also contain a considerable amount of lipid. For this reason, it is suspected that HSF may be a lipoprotein.

HSF appears to be polydispersed in 0.15 M saline solutions of crude extracts, since under centrifugal force the active material does not sediment uniformly (82). Some HSF sediments quickly, but as much as 16 hr at 125,000 × g is required to sediment all the activity (J. Munoz, *unpublished data*). At higher purity, the sedimentation pattern of HSF could change completely, because it becomes less soluble in saline. Purified HSF preparations made by MgSO$_4$ precipitation are rather insoluble, although they can be solubilized in the presence of sodium deoxycholate or sodium lauryl sulfate. The latter is more efficient, but it also causes faster deterioration of the HSF activity.

The purified HSF retains a certain amount of toxicity, although this is low and requires about 1,000 μg given intraperitoneally to kill mice and the same amount given intravenously to kill rabbits (152; J. Munoz and B. M. Hestekin, Federation Proc. 27:267, 1968). Purified preparations contain little or no endotoxin, since they fail to elicit a Shwartzman reaction, to produce fever in rabbits, or to kill chick embryos in doses which are much higher than those of endotoxin required

TABLE 3. *Some differences between MgSO$_4$-precipitated HSF and purified endotoxin from gram-negative bacteria*

Property	HSF	Endotoxin
Chemical nature	Protein + lipid	Carbohydrate + lipid
Stability to heat	Easily destroyed	Resistant
Dose required to induce pronounced histamine sensitivity	1–5 μg	100–1,000 μg[a]
Elicitation of Shwartzman reaction	>1,000 μg	0.08 μg
Pyrogenicity for rabbits (FI$_{40}$)[b]	160 μg	0.1–0.4 μg
Chick embryo LD$_{50}$	7.6 μg	0.005 μg

[a] Sensitization produced is not regular even at these doses.

[b] FI$_{40}$ = dose producing an area, under the fever curve, above normal temperature, of 40 cm^2, when plotted on arithmetic graph paper with a scale such that 1 C and 1 hr = 1 inch (140).

to produce these phenomena (Table 3). Gel-dif-
fusion tests have also confirmed the very low
content of endotoxin.

HSF in crude saline extracts from acetone-dried
cells is stable in frozen or lyophilized form.
Lyophilized preparations made in our laboratory
have retained their full activity for at least 4 years.
HSF has previously been found to remain active
for 14 years in cell suspensions containing 1:
10,000 Merthiolate (93; W. F. Verwey, L. F.
Schuchardt, and J. L. Ciminera, Bacteriol.
Proc., p. 80, 1957). High alkalinity (pH 10 to 11)
or high acidity (pH 1 to 2) destroys the activity
(Niwa, personal communication).

HSF is antigenic, and neutralizing antibodies
can be produced (118, 119). However, most anti-
sera to B. pertussis cells seem to have little or no
antibody to HSF. In most cases, antisera fail to
neutralize the activity of soluble preparations of
HSF, although they neutralize the activity of
whole cells (J. Munoz, Federation Proc. 23:404,
1964). Moreover, some workers have reported
that whole cells treated with antiserum and subse-
quently washed still sensitize mice to histamine
(38, 193). Recently, we have produced antisera
with purified HSF, which react specifically with
it and neutralize its activity. The neutralization
observed in many laboratories with most hyper-
immune sera to B. pertussis might have been due
to antibodies to surface antigens which coat the
bacterial cells and thus render them more easily
phagocytized and perhaps prevent the active
material from reaching the sites in which the
HSF acts.

Our purest preparations of HSF have always
exhibited not only histamine- and serotonin-
sensitizing properties, but also protective activity
against intracerebral challenge with B. pertussis.
They have also had the anaphylactogenic effect,
the adjuvant effect, and have promoted the in-
creased disappearance of Evans blue from the
circulation. For these reasons, we have suspected,
as did Levine and Pieroni (111), that these
activities reside in the same molecule. Further
purification of HSF is required to settle this point.

The only other substances that, to our knowl-
edge, have been characterized and that have been
reported to produce histamine sensitization are
the endotoxins of gram-negative organisms. Since
the nature of these substances has been exten-
sively studied and it is still doubtful whether or
not endotoxin has a primary histamine-sensitiz-
ing action, they will not be discussed in this
review. Those interested in learning about the
methods of purification and characterization of
endotoxins should consult the various reviews
and symposia on these interesting substances
(e.g., 108).

CHARACTERISTICS OF SENSITIZATION TO HISTAMINE

Animal species differ widely in their sensitivity
to the lethal effects of histamine (Table 1). Mice
and rats are highly resistant, whereas guinea pigs
and rabbits are highly susceptible. The effects of
the histamine-sensitizing substance from B.
pertussis have been demonstrated mainly in
animals that are highly resistant to doses of
histamine, because death has been used as the
main criterion of sensitization. Mice become 50
to 100 times more sensitive after treatment with
HSF; the increase is of a lower order in rats (32,
124, 185). Other animals have not been thor-
oughly investigated with respect to histamine
sensitization by HSF. Guinea pigs and rabbits
may become, if anything, more resistant to
histamine after injection of B. pertussis vaccine
(119, 226). One report (106) indicates that guinea
pigs, rats, and mice become more susceptible
to histamine after injection of avirulent P. pestis
cells or cell autolysates, but, as stated above, this
work was not extensive and confirmation is re-
quired. Sanyal (215) observed an increased skin
sensitivity to histamine in children with whooping
cough. Mathov (133) reported a slight increase in
skin sensitivity to histamine in children vac-
cinated with pertussis vaccine, but the observed
increase was not statistically significant.

Marked differences in the susceptibility of
mouse strains to the sensitizing effects of HSF
have been reported (Table 4). Some strains,
mainly those derived from the Swiss-Webster
line, are highly susceptible to the histamine-
sensitizing effects of HSF, but many other
strains, including various inbred strains, are
rather resistant (23, 154). Recent work in our
laboratory (24), however, indicates that HSF
produces an effect in most mouse strains (inbred
or noninbred), when sensitivity is tested with a
combination of histamine and serotonin (Table
4). Some strains, such as the SW-55 (85) and the
CFW mice (C. W. Fishel, personal communica-
tion), have been found normally sensitive to a
combination of serotonin and histamine. Some
strains of mice become sensitive to serotonin and
not to histamine (23, 150), indicating that the
importance of these two amines varies in differ-
ent mouse strains. Marked differences in sus-
ceptibility to sensitization to histamine after
administration of HSF have also been noted in
the same strain of mouse raised in different
laboratories (J. Munoz and R. K. Bergman,
unpublished data).

Whole-cell vaccines given intraperitoneally
produce an increasing sensitization to histamine
during the first 4 days. At this time, sensitization

TABLE 4. *Susceptibility of mouse strains to sensitizing effects of B. pertussis cells or cell extracts*

Strain	Breeder	Sensitization[a] to			References
		Histamine	Serotonin	Serotonin + histamine	
Non-inbred					
CFW[b]	Carworth Farms	+	+		59, 183, 195
CF-1[c]	Carworth Farms	−	+		150, 165, 198
BF[b]	Beverly Farms	+			165
TF[b]	Tumblebrook Farms	+	+		97, 98, 125
					150, 165
SD₁[b]	Sharp and Dohme	+			165
SD₂	Sharp and Dohme	−			165
RML (young)	Rocky Mtn. Lab.	−	−	+	19, 24
RML (old)	Rocky Mtn. Lab.	+			19
NIH[b]	Natl. Insts. Health	+			23
GP[b]	Natl. Insts. Health	±		+	23
Swiss-Webster	Taconic Farms	+			66
NLA[b]	Natl. Lab. Animal Co.	+	+		98
Prinston	Millerton Farm	−			183
NIH-BS[b]	Natl. Insts. Health	+			226
C58, F-1	Millerton Farm	−			184
Inbred					
AAF₁	Natl. Insts. Health	−		+	23
CDF₁	Natl. Insts. Health	−		+	23
CAF₁	Natl. Insts. Health	−		+	23
C3H[fb]/HeN	Natl. Insts. Health	−		+	23
AL/N	Natl. Insts. Health	−		+	23
C57L/N	Natl. Insts. Health	+		+	23
STR/1N	Natl. Insts. Health	−	+	+	23
BRSUNT/N	Natl. Insts. Health	−		+	23
C57BL/10ScN	Natl. Insts. Health	−		+	23
DBA/ZN	Natl. Insts. Health	−	−	+	23
C57BL/6N	Natl. Insts. Health	−	−	+	23
Balb/C AnN	Natl. Insts. Health	−	+	+	23
C3H/HeN	Natl. Insts. Health	−	−	−	23
A/HeN	Natl. Insts. Health	−	−	+	23
STR/N	Natl. Insts. Health		−		23
AKR/N	Natl. Insts. Health		±		23
AKR	Millerton Farms	+			183
Isko	Not given	+	+		89
LAF₁	Not given	±	+		127

[a] Symbols: + = susceptible to sensitization; − = not susceptible to sensitization; ± = questionable sensitization; blank = not tested.

[b] Known to originate from Swiss-Webster strain.

[c] A substrain of CF₁ raised in the University of Montreal has been found susceptible to histamine sensitization by Guerault (75).

reaches its peak and then gradually decreases for the next 3 to 4 weeks, after which little sensitivity to 0.5 mg of histamine is demonstrable (119, 169). Sensitivities to serotonin (150) and to endotoxin (101) follow similar time courses (Fig. 1). When the LD₅₀ of histamine is determined in mice sensitized intravenously with soluble preparations of HSF, mice become highly sensitive within 90 min after administration of HSF; sensitivity increases slightly during the next 24 to 48 hr, and remains at a high level for 3 to 4 weeks thereafter.

After the 30th day, sensitivity declines, but it can still be detected in HSF-treated CFW mice 80 days later (159) (Fig. 2). These results indicate either that the substance responsible for producing histamine sensitization is not rapidly metabolized or that its effects are long lasting. It should be re-emphasized that, although marked differences in sensitization to histamine have been found among mouse strains, most seem to become sensitive to mixtures of histamine and serotonin (23). It should also be noted that strains that
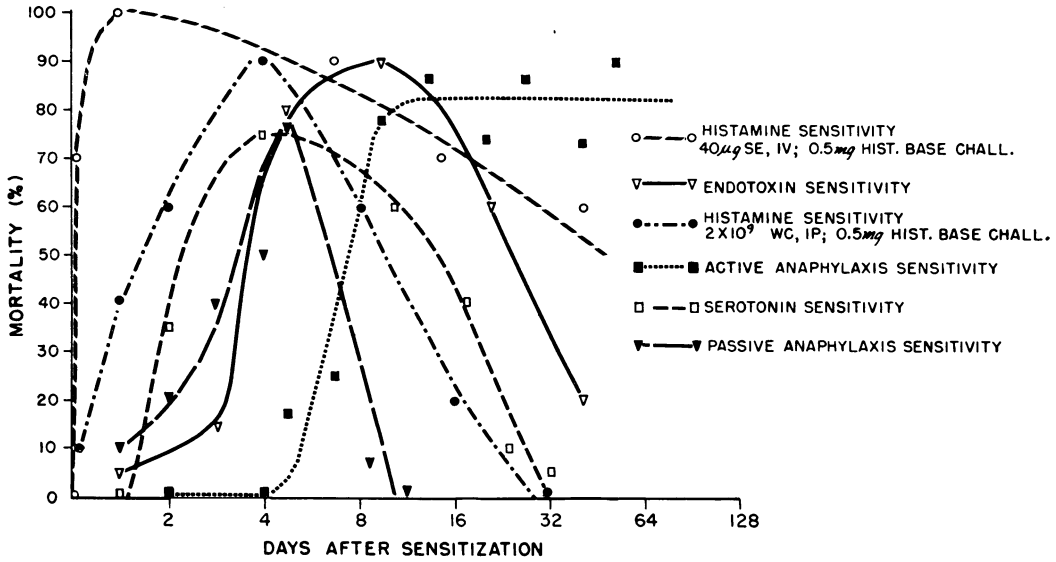
FIG. 1. *Time course of sensitivity to histamine, serotonin, endotoxin, active anaphylaxis, and passive anaphylaxis with heterologous antiserum, in mice treated with B. pertussis. Data for this figure were obtained from experiments in which a single challenge dose of the substance involved was given and were derived from the following sources: for histamine (151, 159); for serotonin (150); for endotoxin (101); for active anaphylaxis (153; J. Munoz, unpublished data); and for passive anaphylaxis (169; J. Munoz, unpublished data). Note that the curve for active anaphylaxis differs from all others in that once sensitivity is established it remains unchanged for a long period of time; all other sensitivities decrease with time. Passive anaphylaxis sensitivity falls very rapidly, while histamine, serotonin, and endotoxin sensitivities do so more gradually. As noted in the text, when LD_{50} of histamine is determined, sensitivity to this drug can be detected for at least 80 days after administration of HSF (159).*
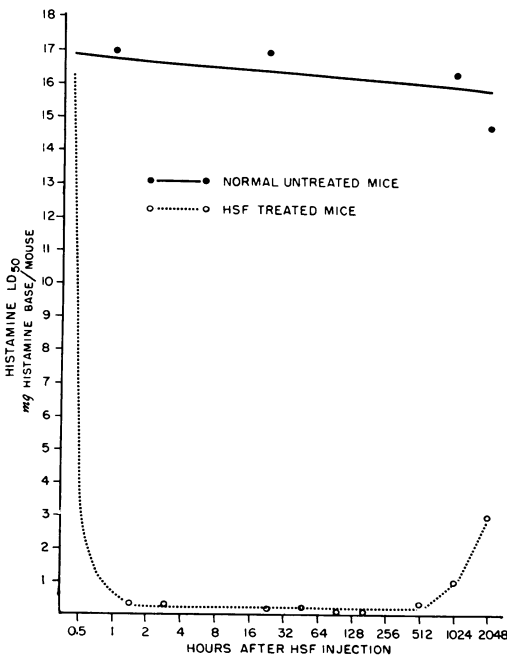

FIG. 2. *Onset and persistence of histamine sensitivity in CFW mice treated with alkaline-saline extract of B. pertussis given intravenously. Data for this figure taken from reference 159.*

have been considered resistant to histamine sensitization are somewhat sensitive to histamine when histamine LD_{50} values are determined (19). HSF produces sensitization to histamine plus serotonin challenge in most strains; only a few strains become sensitive to histamine or serotonin given alone (Table 4).

Within mouse strains that become sensitive to histamine, the female mouse is usually found to be more sensitive than the male to histamine and to sensitization by *B. pertussis* vaccine (119, 202). This difference in response between sexes was not found by Kind (94) in individual experiments, but our experience, like that of Pittman (202) and that of Maitland et al. (119), indicates that female mice, on a statistical basis, are more susceptible to the action of HSF. In many individual experiments, this difference is not apparent and the trend may even be reversed. Perhaps the difference in susceptibility is actually due to the stress produced by fighting among adult male mice.

Age can be an important factor in histamine-sensitization studies with HSF. In certain strains of mice, such as the CFW, age does not seem to play a marked role, but in other strains it is very important (19, 94, 165). Young (3- to 7-week-old) Rocky Mountain Laboratory (RML) mice, after treatment with HSF, do not become highly

sensitive to histamine, but older mice (over 7 weeks of age) do (19). Mice of some strains usually become more sensitive to histamine with age (184).

Housing conditions and diet have an influence on the sensitization phenomenon (154); animals kept in isolation do not become as sensitive as those kept in groups of 5 to 10 animals (154). Isolation stress and acute stress have also been found to affect anaphylaxis in mice (212; P. E. Treadwell et al., Federation Proc. **18**:602, 1959).

The sensitivity of mice to histamine is affected by still other factors, the identity of which is not well known. During the past year, we tested the normal sensitivity of CFW female mice (6 to 7 weeks old, 22 to 24 g body weight) approximately 4 days after they arrived at our laboratory from New York. The results of this survey are illustrated in Fig. 3. It is clear that these mice vary markedly from shipment to shipment in their sensitivity to 0.5 mg of histamine, when they are tested after the stress of shipment. Since dehydration may increase the sensitivity to histamine (208), we have studied the role of dehydration, but we have obtained conflicting results and cannot state that dehydration was responsible for the high sensitivity observed in some lots of mice. It may be that adrenal function is an important consideration in the variability of mice, but this factor has not yet been studied.

The toxicity of *B. pertussis* cells affects sensitization. Toxic vaccines sensitize mice less than nontoxic vaccines (203). Toxic extracts, heated at 55 C for 0.5 hr to reduce their toxicity, are more effective than unheated extracts in producing histamine sensitivity in mice (J. Munoz, *unpublished data*).

The physical form in which HSF is given and route of administration are other important factors. By the subcutaneous route, poor histamine sensitization is obtained with freshly made intact cell vaccines (132, 154; J. Munoz and R. K. Bergman, *unpublished data*), but with soluble HSF preparations sensitization is obtained (Fig. 4). The route of choice with whole cells or soluble extracts is the intravenous route (119, 159). Some workers have also found the intracranial route to be highly effective (87). Intranasal infection with *B. pertussis* also induces histamine sensitization (204).

Treatment of mice with many different substances (aspirin, propylene glycol, etc.) during the period between sensitization with *B. pertussis* cells and challenge with histamine changes the dose response to histamine from one in which increasing doses of histamine produce correspondingly increased mortality, to one in which greater mortality is obtained at lower doses (0.2 to 2 mg of histamine) than at relatively higher doses (4 to 8 mg) (166). This phenomenon is as yet unexplained, but might be due to a "triggering" action of a critical concentration of histamine on epinephrine release. Mild stress may similarly modify the dose response to histamine. On the other hand, severe and prolonged stress may, by producing adrenal exhaustion, make mice more sensitive to histamine.

Injection of Formalin-treated extract from *B. pertussis*, given some days before the sensitizing dose of pertussis vaccine, inhibits sensitization to histamine by a second active dose of cells (136). With active soluble preparations of HSF, this inhibition by a previous injection does not occur (159). The adjuvant and the anaphylaxis promoting effects of *B. pertussis* vaccine are, however, inhibited by a previous dose of the same vaccine
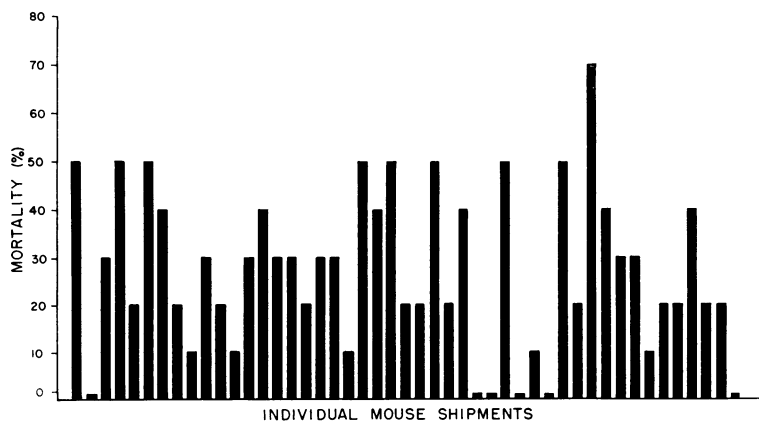

FIG. 3. *Variation in sensitivity to 0.5 mg of histamine given intraperitoneally to different shipments of CFW female mice received at Rocky Mountain Laboratory from New York. Four days after arrival, animals were challenged in groups of 10. Testing covered a period of 11 months from March 1967 through January 1968. No correlation between season of year and mortality was evident (R. K. Bergman, unpublished data).*

(104). As stated above, it is not clear whether the neutralization observed by most workers employing whole cell vaccines to sensitize mice is due to specific antibodies to HSF or to antibodies to other surface antigens of the *B. pertussis* cell.

FIG. 4. *Effect of route of administration and type of HSF preparation on histamine sensitization of CFW female mice. The doses of sensitizing material in the upper part of the figure correspond to those indicated in the lower portion. IV = intravenous sensitization; IP = intraperitoneal sensitization; IM = intramuscular sensitization; SC = subcutaneous sensitization; ID = intradermal sensitization. Data for this figure obtained from J. Munoz (unpublished data).*

Species other than the mouse that have been shown to be susceptible to the histamine-sensitizing activity of HSF are the rat (124) and the chicken (A. Guérault and M. Quevillon, Bacteriol. Proc., p. 52, 1965), but sensitization has not been as striking as with susceptible mouse strains.

In summary, it appears that the factors which affect histamine sensitization are: (i) species and strain of animal, (ii) various forms of stress, and (iii) agents that prevent HSF from reaching its site of action. The importance of these factors will become clearer when the mode of action of HSF is discussed.

VARIOUS CHANGES OBSERVED AND OTHER PHENOMENA ASSOCIATED WITH HISTAMINE SENSITIZATION IN MICE

Histamine sensitization in mice by HSF from *B. pertussis* is accompanied by a number of other phenomena (Tables 5 and 6). In Kind's review on this topic, the following phenomena in mice treated with pertussis vaccine were described: increased sensitivity to histamine, serotonin, anaphylaxis, infection, endotoxins, X irradiation, reduced atmospheric pressure, cold stress, peptone shock, and pollen extracts. To this list, others can now be added, such as increased sensitivity to bradykinin, to a combination of histamine and serotonin, to a neurotoxin from *S. typhimurium*, to epidemic typhus toxin, and to methacholine (Tables 5 and 6). This list, we are sure, could be extended to many other agents. By this we do not mean that *B. pertussis* cells change the susceptibility of mice to all agents, but rather
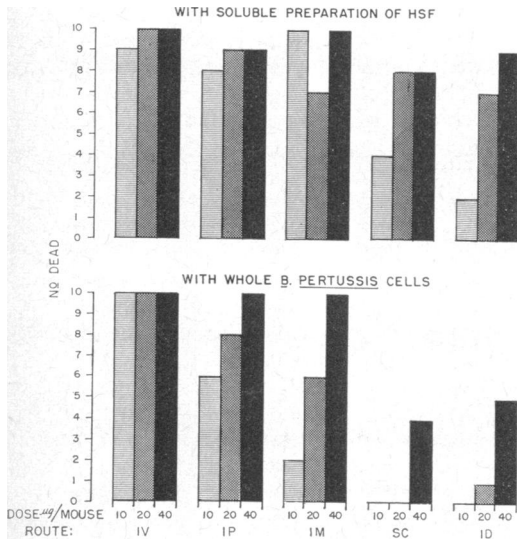
TABLE 5. *Hypersensitivity to various agents induced by B. pertussis whole cells or cell extracts*

Hypersensitivity to	Sensitizing material	Activity destroyed by 80 C for 0.5 hr[a]	References
Histamine	WC or Ext[b]	+	79, 94, 102, 185, 202, 229
Serotonin	WC or Ext	+	89, 98, 150, 205
Serotonin + histamine	Ext	+	23
Bradykinin	WC		131
Endotoxin	WC or Ext	+	1, 34, 37, 101, 137, 181, 200
Neurotoxin from *Salmonella typhimurium*	WC		207
Epidemic typhus toxin	Ext		—[c]
Peptone shock	WC or Ext	+	84, 110, 128, 198
Pollen extract	WC		103
Active anaphylaxis	WC or Ext	+	110, 121–124, 153, 188
Passive anaphylaxis	WC or Ext	+	156, 169, 206, —[d]
Anoxia	WC		102
Cold stress	WC		168
X irradiation	WC	+	110, 205, 213
Methacholine	WC		233

[a] + = activity destroyed.
[b] WC = killed whole cells; Ext = extract or purified HSF; blank = not known.
[c] E. J. Bell, *unpublished data.*
[d] J. Munoz, L. F. Schuhardt, and W. F. Verwey, Federation Proc. **13**:507, 1954.

TABLE 6. *Physiological changes produced by B. pertussis cells or cell extracts*

Physiological change	Material given	Activity destroyed by 80 C for 0.5 hr[a]	References
Increased susceptibility to infection	WC[b]		11, 44, 45, 180, 182
Increased resistance to infection[c]	WC		44, 191
Increased resistance to sarcoma S-180[c]	WC		129
Increased growth of lymphomas	WC		62, 83
Increased antibody production to various antigens	WC or Ext		61, 99, 126, 153, 199
Increased production of mast cell sensitizing antibody and skin fixing antibody	WC or Ext		28, 145–149
Increased active cutaneous hypersensitivity in rats	WC		214
Leucocytosis	WC or Ext	+	141–144, —[d]
Enhancement of EAE	WC	+	109, 112, 117, 224
Induction of hyperacute EAE	WC or Ext	+	113–115
Development of renal lesions when given with kidney extracts	WC		29
Increased levels of histidine decarboxylase[c]	WC		222, —[e]
Increased level of some lysosomal enzymes in liver	WC or Ext		—[f]
Increased insulin level in blood	WC or Ext		—[g]
Hypoglycemia	WC or Ext		190, 226
Decreased serum albumin	Ext		—[h]
Increased disappearance of Evans blue from blood	WC or Ext		151

[a] + = activity destroyed at 80 C for 0.5 hr.
[b] WC = killed whole cells; Ext = extracts or purified HSF; blank = not known.
[c] These effects may be due to endotoxin in whole cell vaccines.
[d] I. A. Parfentjev and E. E. Manuelidis, Federation Proc. 15:607, 1956.
[e] A. Szentivanyi, S. Katsh, and B. McGarry, Federation Proc. 27:268, 1968.
[f] P. E. Treadwell, *personal communication.*
[g] A. Gulbenkian, *personal communication.*
[h] R. K. Bergman and J. Munoz, *in preparation.*

that they change a fundamental physiological function normally involved in protecting against the toxic effects of many substances that kill the animal through some form of shock. The isolated uterus or intestine of the *B. pertussis*-treated mouse does not show an increased reactivity to histamine or anaphylaxis (56, 163), and the skin is not more reactive to passive cutaneous anaphylaxis (157).

The *B. pertussis*-treated rat becomes more sensitive to histamine and anaphylaxis (124), to some cutaneous hypersensitivity reactions (214), and to the development of nephritis when homologous kidney extracts are given with *B. pertussis* vaccine (29). The skin sensitivity of the rat to histamine or serotonin is not modified (214). In this connection, it should be mentioned that endotoxin from gram-negative bacteria has been shown to increase the reactivity of the isolated guinea pig uterus to histamine in an entirely in vitro system (236). The endotoxin must be present in the tissue bath to exert this effect.

One prominent feature of pertussis vaccine is its ability to increase anaphylactic sensitivity of mice (121, 188). Although *B. pertussis* has the ability to increase antibody production to a given antigen (13, 14, 31, 52, 61, 72, 73, 99, 126, 153,

178, 235), this effect only partially explains the anaphylactogenic effect, because pertussis vaccine also increases the susceptibility to passive anaphylaxis. In the latter case, antibody formation is not involved, and, thus, differences in antibody titers can be minimized (156, 169, 206). The increased sensitivity to passive anaphylaxis is most pronounced in the early stages after administration of *B. pertussis*. This sensitivity seems to be increased for only about 5 days after *B. pertussis* injection (169). With actively induced anaphylaxis, the increase can be noticed from the 7th to 10th days and persists for at least 77 days (154) (Fig. 1). Sensitivity to actively induced anaphylaxis is also increased by various adjuvants (126, 153, 225) or by repeated injections of the same antigen (63). This supports the view that stimulation of antibody production is one of the important effects of *B. pertussis* in inducing active anaphylaxis. The type of antibody stimulated by *B. pertussis* may also be of importance, since Mota (145–148) and Mota and Peixoto (149) have demonstrated that *B. pertussis* stimulates the production in rats and mice of a mast cell-sensitizing antibody or a reaginic type of antibody. This antibody may play an important role in mouse anaphylaxis. The reaginic type of antibody is heat-labile and fixes

to tissues strongly so that passive cutaneous anaphylaxis (PCA) can still be demonstrated 72 hr or longer after intradermal administration (28, 147, 149). This antibody is different from the $7S\gamma_1$ globulin, which produces PCA in mice but disappears from the skin site 24 hr after its administration (147, 157, 177). This property of B. pertussis of selectively stimulating the formation of one type of antibody during the early period after immunization may partly explain the anaphylactogenic effect of B. pertussis cells in actively sensitized mice. However, it does not explain the increased sensitivity to passive anaphylaxis. In this case, the increase in susceptibility must be due to a mechanism similar to that which induces sensitization to other substances, such as histamine, serotonin, peptone, bradykinin, X ray, and endotoxin. It should be emphasized that the state of hypersensitivity to histamine or serotonin produced by HSF is not necessary for the increased sensitivity to either passive or active anaphylaxis in the mouse (154). Fatal active anaphylaxis can, for example, be observed at a time when sensitivity to the amines has greatly diminished. Fatal passive anaphylaxis cannot be observed 2 to 3 weeks after HSF administration when high sensitivity to histamine and serotonin exists.

Many other physiological changes have been observed in B. pertussis-treated mice (Table 6). One effect of B. pertussis vaccine, which may be related to the histamine-sensitizing factor, is acceleration of production of experimental allergic encephalomyelitis (EAE) in mice (109) and rats (112–115). This effect is observed when the encephalitogenic material is given in Freund's adjuvant containing B. pertussis instead of mycobacteria (117, 224, 240) or when pertussis vaccine is mixed in an aqueous suspension with the encephalitogenic antigen. Moreover, the encephalitogenic material can be given simultaneously with pertussis vaccine or several days after the administration of the vaccine (109, 112–114). It is noteworthy that adrenalectomy also makes rats more susceptible to EAE (116). The effect of pertussis vaccine on EAE in rats has been studied in some detail by Levine et al. (112–115). They have found that B. pertussis cells given with an aqueous suspension of the encephalitogenic antigen produce an accelerated disease which has a different histological character, inasmuch as many polymorphonuclear cells, edema fluid, and accumulated fibrin are present in the perivascular exudates. This form of hyperacute EAE has also been produced by partially purified HSF made in our laboratory and elsewhere (115). The activity is destroyed by heating HSF at 80 C for 0.5 hr or by treatment with Formalin. Moreover, all active fractions tested also contained the histamine-sensitizing substance. Endotoxin from E. coli, S. typhosa, or B. pertussis does not induce hyperacute EAE (115). The mechanism by which this phenomenon is produced is not yet clear.

In human infection with B. pertussis, one of the prominent features is development of a pronounced leukocytosis, accounted for by an absolute increase in the lymphocytes (64). Killed pertussis vaccine also produces lymphocytosis in man (219) and in experimental animals (7, 30, 39, 50, 53, 141, 234), although some workers have observed mainly a granulocytosis (33, 81; I. A. Parfentjev and E. E. Manuelidis, Federation Proc. 15:607, 1956). In mice, pertussis vaccine produces an increase in spleen weight (141; Parfentjev and Manuelidis, Federation Proc. 15: 607, 1956), a decrease in thymus and lymph node weight, and a marked lymphocytosis (141). Pertussis vaccine also has an accelerating effect on the growth of lymphomas in mice (62, 83).

Fichtelius and Hassler (54) observed that injection of pertussis vaccine into adrenalectomized rats, maintained on mineralocorticoids, stimulated production of lymphocytes at a faster rate than in adrenalectomized unvaccinated controls. The increase in lymphocytes is first noticed 1 day after intravenous inoculation of pertussis vaccine, and maximal response is reached 4 days later (141). Leukocyte and lymphocyte populations return to normal by the 14th day. The number of polymorphonuclear leukocytes also increases after B. pertussis treatment, but the most important change, by far, occurs in the small lymphocytes. The number of large lymphocytes and monocytes does not change significantly. The intravenous route of treatment is the most effective, followed by the intraperitoneal route; the subcutaneous route is ineffective in producing lymphocytosis (141). The time course and the effect of route of injection of B. pertussis cells are strikingly similar to the histamine-sensitization phenomenon in that maximal sensitization is reached at about 4 days, and subcutaneous injection of whole-cell vaccines is also ineffective (119, 132; J. Munoz, Federation Proc. 23:404, 1964). The substance producing the leukocytosis is heat-labile and is found in soluble alkaline saline extracts (C. Clausen, unpublished data). Some of the properties of the lymphocytosis factor are similar to HSF, and the factor does not appear to be related to endotoxin. Since the increase in lymphocytes is accompanied by a depletion of small lymphocytes from lymph nodes and thymus, with no obvious increase in multiplication of the lymphocytes, Morse and Riester (143, 144) believe that the lymphocytosis is due to shifting of the lymphocyte circulation. Splenec-

tomy or thymectomy does not prevent the lymphocytic response after B. pertussis vaccine, but active and passive immunization of mice against B. pertussis prevents or markedly reduces the leukocytic response (141). Mice rendered lymphocytopenic by X irradiation or by administration of hydrocortisone still show lymphocytosis and granulocytosis (142). Histamine sensitization in mice does not require the presence of leukocytosis, since histamine sensitivity lasts beyond the period of leukocytosis, and antileukocyte serum that markedly reduces leukocytosis does not affect histamine sensitivity (C. Clausen, unpublished data). These observations do not show that the HSF and the leukocyte-promoting substances are different, but rather that the two phenomena are independent of each other.

A number of metabolic changes have been observed in mice treated with pertussis vaccine (Table 6). HSF-treated mice exhibit a marked hypoglycemia (190, 226), and some investigators have thought that sensitization to histamine and other agents is a consequence of the hypoglycemia (71). Alloxan diabetes induced in HSF-treated mice protects them from histamine death, and the protective effect is diminished by insulin treatment (65, 230, 231; O. H. Ganley and H. J. Robinson, Federation Proc. 18:392, 1959). It has also been noticed that the blood levels of insulin are increased in HSF-treated mice (A. Gulbenkian and also C. W. Fishel, personal communications). Pertussis vaccine-treated mice show an altered glucose tolerance which is manifested by failure of an intravenous dose of 0.5 mg of glucose per g of body weight to produce a rise in blood glucose (58). Normal mice similarly treated show a pronounced rise. Pertussis vaccine-treated mice and rats also fail to show the hyperglycemic response after release of endogenous epinephrine or after treatment with exogenous epinephrine (58, 77, 228). Moreover, epinephrine administered to HSF-treated mice does not reduce the tissue uptake of glucose (58). Pertussis-treated mice also fail to respond to catecholamines with an elevated level of lactic acid and free fatty acids, although the effect of catecholamines on liver glycogen is not reduced (92). All these effects on sugar and fatty acid metabolism may be of great significance in the phenomena of sensitization induced by pertussis vaccine; however, interpretation is still difficult.

Another physiological change noticed in mice receiving HSF is a significant hypoproteinemia which persists for several days and is apparently due to a depressed albumin level. Administration of exogenous albumin or whole serum has not been effective in restoring the depressed protein

level or in protecting mice from death after histamine challenge (R. K. Bergman and J. Munoz, in preparation).

When HSF-treated mice and untreated mice are given equal doses of Evans blue dye intravenously, the HSF-treated mice have, 20 min to 4 hr later, a lower dye concentration in their serum than the controls. This was interpreted to indicate an increased capillary permeability to the dye in HSF-treated mice (151). The finding that HSF produces a hypoalbuminemia casts some doubt on this interpretation, because Evans blue dye readily combines with albumin to form a complex which remains in the circulation for a long period of time. If the capillaries are freely permeable to unbound dye and the quantity of dye given is in great excess of the binding capacity of the serum albumin, the dye concentration in the blood would primarily be related to the serum albumin concentration and not to capillary permeability.

The role which HSF may play in permeability is far from being clear, however. The uptake of ^{14}C-glucose into certain tissues and its disappearance from the blood seem to be influenced by B. pertussis treatment (58; C. W. Fishel and K. F. Keller, Federation Proc. 27:267, 1968). The serum concentration of insulin is increased (A. Gulbenkian, personal communication), indicating that permeability of tissues to glucose is increased as a result of this increase in insulin (12). The serum concentration of dextrans (molecular weight $\geq$ 80,000) administered to HSF-treated and untreated mice is somewhat analogous to the situation with Evans blue dye; HSF-treated mice always have a lower serum concentration of dextran than do the control mice (R. K. Bergman, unpublished data).

Some experiments also indicate that a change in the intercellular substance may occur after HSF treatment, because carbon particles diffuse more widely from an intracutaneous inoculation site in pertussis-treated animals than in untreated mice (189). A similar phenomenon has been observed in B. pertussis-treated rats (40).

Presently, we are inclined to believe that the HSF may in some way block the mouse's normal protective responses to vascular changes produced by histamine or serotonin and perhaps other vasoactive substances (70). Thus, while these agents may initiate important changes in the capillary permeability of both normal or pertussis-treated mice, the effects may be fatal only in HSF-treated mice, because they cannot make the necessary vascular compensations to sustain life (see also 20, 158). It has also been observed that, in HSF-treated mice, intracutaneous injection of histamine or serotonin, or

both, produces a greater permeability change than in normal mice, as judged by Evans blue dye accumulation at the site of injection (R. K. Bergman, *unpublished data*).

MECHANISM OF ACTION OF HSF FROM *B. PERTUSSIS*

A number of hypotheses on the mechanism of action of HSF have been advanced based on experimental observations. The most important of these are presented below. It should be noted that the phenomenon of histamine sensitization does not depend on formation of antibodies to HSF (57) but on other responses still not fully understood.

One possible mode of action of HSF is that it interferes with the mechanism of destruction of histamine. This view is supported by the demonstration that histaminase activity in tissues of animals treated with pertussis vaccine is reduced (105, 134, 135, 173–176). The postulated role of depressed histaminase levels has been questioned, however, because: (i) the main mechanism of detoxifying histamine in the mouse is methylation (220), (ii) animals like the guinea pig, which do not normally become sensitive to histamine, also show a decrease in histaminase activity when treated with pertussis vaccine, and (iii) a decrease in histaminase would not explain the more generalized sensitivity of the mouse to various agents (serotonin, peptone, anaphylaxis, cold stress, X rays, etc.). For these reasons, this hypothesis does not explain the mode of action of HSF.

Another hypothesis attempts to explain the mechanism of HSF as a result of an increased production of histidine decarboxylase which, by its action on histidine, produces histamine. It was thought that this would increase the level of free histamine in the tissues and make the mouse more susceptible to exogenously administered histamine. Histidine decarboxylase is increased in mice treated with pertussis vaccine (222), but this increase occurs equally in mice that become sensitive to histamine and in mice that do not (222). Endotoxins of gram-negative bacteria also increase the level of the enzyme without producing marked sensitivity to histamine (221, 222), and recently it has been found that HSF preparations free of endotoxin do not increase histidine decarboxylase (A. Szentivanyi, S. Katsh, and B. McGarry, Federation Proc., p. 268, 1968). In view of these objections, it seems that this hypothesis does not adequately explain histamine sensitization.

Some observations on the effects of *B. pertussis* on other enzyme systems at the cellular level are suggestive, but their significance with respect to histamine sensitization is not clear, and all require further investigation. Treadwell has found that levels of certain of the lysosomal enzymes in tissue extracts of liver and intestine are altered by treatment with HSF (P. E. Treadwell, *personal communication*). Cronholm and Fishel (41) recently found that injection of cyclic 3′,5′-adenosine phosphate (3′,5′-AMP) and 5′-adenosine phosphate (5′-AMP) elicited hyperglycemia in CFW mice, and that mice that had received 5′-AMP became hypersensitive to histamine, but 3′,5′-AMP did not influence histamine sensitivity. These observations at the cellular level may well be related to the phenomenon of histamine sensitization by HSF.

The hypothesis that HSF changes permeability of capillaries and perhaps of tissues in general is still attractive (151), but further work is needed to assess its importance.

As stated above, some workers believe that the hypoglycemia produced by *B. pertussis* vaccines is responsible for the hypersensitivity phenomena (71). This may well be true for some forms of sensitivities, such as the dextran anaphylactoid edema (2, 3, 4, 5, 6, 18, 68, 69, 230, 231), but probably not for the histamine sensitization produced by HSF. In our hands, small doses of insulin which produce a marked hypoglycemia fail to induce histamine sensitization in mice (R. K. Bergman and J. Munoz, *in preparation*), and large doses of glucose or other monosaccharides fail to protect HSF-treated mice from histamine (58; Bergman and Munoz, *in preparation*). In addition, the time course of hypoglycemia does not correspond to that of histamine sensitivity (Bergman and Munoz, *in preparation*), and a short-term diabetogenic agent (D-mannoheptulose) does not protect HSF-treated mice from histamine challenge. The lack of relationship between hypoglycemia and histamine sensitivity in mice has also been noticed by Cronholm and Fishel (*to be published*). Thus, it is evident that hypoglycemia per se does not explain histamine sensitization.

A possible role of the reticuloendothelial system (RES) in histamine sensitivity of *B. pertussis*-treated mice is suggested by the observation that RES "blockade" by colloidal particles protects mice against the effects of serotonin (241; O. H. Granley, Bacteriol. Proc., p. 88, 1960). The manner by which blockade of the RES protects mice is not clear, but perhaps it is related to a depressed release from certain cells of substances that elicit or enhance the shock syndrome.

Some years ago, it was noted that adrenalectomy makes mice more sensitive to histamine, serotonin, cold stress, anaphylaxis, endotoxin shock, and other noxae (34, 78, 151, 167, 168,

194, 201; J. Munoz, L. F. Schuchardt, and W. F. Verwey, Federation Proc. **13**:507, 1954). The similarities between *B. pertussis*-treated mice and adrenalectomized mice are striking, but one exception has been recorded, in which adrenalectomy did not make mice more sensitive to histamine, whereas *B. pertussis* vaccine did (66). It was first throught that HSF acts in some way on the adrenal gland, but the histological appearance of the glands is not changed (120), and adrenal function does not seem to be affected by *B. pertussis* treatment (34, 100, 102, 120). A decrease in ascorbic acid content has been reported (192), however, and evidence of stress has been seen histologically (171). Adrenal steroids, such as cortisone and hydrocortisone, protect against histamine challenge, but the amount of these hormones required is from 1 to 4 mg per mouse (36, 95). These steroids were used as water-insoluble preparations given intraperitoneally 16 to 24 hr before challenge with histamine. Even these unphysiological doses were not 100% effective and their protective action may have been nonspecific, as is the protection afforded by RES blocking agents in anaphylaxis (241) and serotonin challenge experiments (Ganley, Bacteriol. Proc., p. 88, 1960). Antihistamine drugs and dibenzyline (an α-adrenergic blocking agent with antihistamine activity) protect against challenge with histamine (95, 96, 123). Other α-adrenergic blocking agents have failed to protect mice (J. Munoz, *unpublished data*).

The proposed importance of the steroid hormones in histamine sensitization diminished when it was observed that adrenal-demedullated mice are hypersensitive to histamine and that small doses (5 to 7.5 μg) of epinephrine given to HSF-treated mice 30 sec after challenge protects them from histamine death (21, 22). The doses of epinephrine required to protect adrenalectomized or adrenal-demedullated mice are lower than those required to protect HSF-treated normal mice. Thus, it appears that HSF somehow blocks the action of catecholamines. There also seems to be a quantitative relationship between amount of HSF employed and amount of epinephrine needed for protection (22). The work of Fishel and co-workers (58–60) strongly indicates that *B. pertussis* affects the function of epinephrine. They found that β-adrenergic blocking agents (DCI, pronethalol, propranolol) can produce histamine sensitivity in mice and that HSF blocks the hyperglycemic effect of epinephrine. Moreover, they showed that the altered pattern of glucose metabolism, which is elicited by HSF in mice, is duplicated in many respects by β-adrenergic blocking agents. From their data, there is little doubt that HSF interferes with

glucose metabolism and that this effect is similar in many respects to that of β-adrenergic blockade. Therefore, they proposed that *B. pertussis* cells either possess a β-adrenergic blocking substance or cause the animal to elaborate a substance with steric configuration complementary to the β-adrenergic receptors (60). In either case, the site normally available for the adrenergic transmitter (adrenaline and/or noradrenaline) would be blocked. When such animals are challenged with histamine or serotonin, the antagonistic effect of endogenously released catecholamines is, according to this view, blocked at the β-receptor level, leaving the α-adrenergic activities unopposed. This results in an imbalanced response of the receptors, thus producing unfavorable metabolic adjustments, as well as smooth muscle and neural responses that culminated in a reduced resistance to histamine and serotonin (60).

The effect of β-adrenergic blockade on histamine sensitivity has been confirmed (22, 233). However, we do not agree with the suggestion that death is due to an "imbalance" in the reactivity of the α- and β-receptors (25). It is possible that β-adrenergic blockade may produce a condition whereby epinephrine is incapable of protecting against the shock syndrome elicited by histamine or other noxae. Mice should then become more sensitive to any condition in which the vascular bed is affected and changes in permeability and blood volume are involved. This indeed seems to be the case. It is also unlikely that HSF stimulates the production of a β-adrenergic blocking substance by the mouse, since passive sensitization by means of serum has not been achieved. Moreover, a normal mouse joined by parabiosis to a sensitized mouse does not become sensitive to histamine (35). It is more likely that HSF itself is the substance that produces the adrenergic blockade.

CONCLUDING REMARKS

The substances that have been found to sensitize mice to histamine are HSF from *B. pertussis* and endotoxins from gram-negative bacteria. Of these, only HSF from *B. pertussis* (and perhaps *Brucella abortus*) seem to produce a primary sensitization to histamine. Endotoxins (and other toxins, such as that of *Pasteurella pestis*) seem to produce sensitivity to histamine as a result of their intrinsic toxicity, which nonspecifically sensitizes the animal to other noxious agents. This view seems reasonable on the following grounds: the amount of endotoxin required borders on the lethal dose; "sensitization" is not easily reproduced; a dose-response curve is not observed; and the mortality rate is usually low. HSF from *B. pertussis*, on the other hand, acts in

minute amounts (1 to 5 μg) without obviously stressing the animal; the response is dose-dependent, and sensitization is uniform and reproducible when test conditions are standardized. The *B. pertussis* effect seems thus to be a true primary effect. Sensitivity appears to be due to a direct blocking effect on a mechanism normally capable of counteracting the shock effects of histamine, serotonin, anaphylaxis, peptone, endotoxin, etc. This mechanism appears to be associated mainly with the adrenal medullary hormone, epinephrine. This has become increasingly clear through the work of Fishel and co-workers (58–60, 92, 228) as well as our own (21, 22, 25). HSF does not seem to interfere with production of adrenal hormones but rather to prevent the normal action of epinephrine by "blocking" the β-adrenergic receptors needed for some of the activities of epinephrine. Much support was given to this view when Fishel and co-workers showed that β-adrenergic blocking agents mimic the effect of HSF and that HSF produces many metabolic disturbances associated with β-adrenergic activity (58–60, 92, 228, 233). If HSF actually blocks the β-adrenergic receptors, this blocking effect must be long lasting, and as a result it may produce many other modifications in the animal. Thus, it is not surprising that many enzyme systems and physiological functions are changed in HSF-treated animals. In this regard, however, it should always be kept in mind that most studies have been carried out with *B. pertussis* whole cells or with complex extracts from these cells, containing substances with pronounced biological activities, such as endotoxin and heat-labile toxin. Relatively pure HSF, however, has other activities besides producing the increased susceptibility to various types of shock. Thus, highly purified preparations of HSF have the following activities: they induce protection of mice against intracerebral challenge with virulent *B. pertussis* cells (161); they induce leukocytosis (C. Clausen, *unpublished data*); they promote antibody production to other antigens given with it (155); they accelerate the induction of EAE; and they change the type of disease produced to a hyperacute type of EAE (113–115). Moreover, these purified preparations produce many of the physiological changes reported to be produced by whole cell vaccines (hypoglycemia, increased insulin in blood, etc.) (R. K. Bergman and J. Munoz, *in preparation*; A. Gulbenkian, *personal communication*). Whether HSF has a multitude of actions or whether all the observed phenomena are the result of only one fundamental effect, namely, the β-adrenergic blocking effect, is still not known. It is possible however, that a long

lasting β-adrenergic blocking effect can produce a multitude of observable changes in the animal. The final answer to this problem will not come until purification of HSF is achieved. Elucidation of the exact mechanism of action of HSF is important, because this mechanism seems to play a role in sensitivity to various pharmacological agents released during allergic reactions and suspected of playing a role in hypersensitivity reactions in man and animal (histamine, serotonin, bradykinin, etc.). HSF modifies the response to these substances in mice and possibly other animals and man; it thus may have profound effects on hypersensitivity in general. The demonstration of a possible β-adrenergic blocking activity by HSF, and the duplication of some HSF effects by β-adrenergic blocking agents, has opened an area of investigation with a wide variety of interesting problems in a field thus far much neglected by immunologists and allergists.

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