

Sensitization to anaphylaxis and to some of its pharmacological mediators by blockade of the beta adrenergic receptors

Robert G. Townley, M.D.,* Ignatius L. Trapani, Ph.D.,
and Andor Szentivanyi, M.D., Denver, Colo.

Strain differences are described in the hypersensitivity to anaphylaxis, and to some of its pharmacologic mediators, produced by pertussis or blockade of the beta-adrenergic receptors. Thus, pertussis did not produce histamine hypersensitivity in CF₁ but did in CFW mice; in contrast, pertussis produced methacholine hypersensitivity in CF₁ but not in CFW mice. Blockade of beta receptors with dichloroisoproterenol (DCI), Nethalide, or MJ-1999 sensitized both CFW and CF₁ mice to histamine, but the hypersensitivity was greater in CFW, followed by CF₁, and finally by nonobese siblings of "obese-hyperglycemic" mice. This relationship was paralleled by that of the natural histamine-sensitivity of the three strains. A bimodal response to histamine was found in untreated CFW mice, but only after beta blockade in CF₁ mice. In general, MJ-1999 was the most and DCI the least potent sensitizer, with Nethalide showing intermediate activity. MJ-1999 produced hypersensitivity to the amines in Hartley, but not in Trapani-Campbell, guinea pigs. Both blocked strains could be sensitized, however, to anaphylaxis. Interventions conceivably resulting in additive blockade of beta or simultaneous stimulation of alpha receptors could break strain resistance to sensitization. Conversely, protection of susceptible strains was obtained through procedures known to protect beta receptors against development of a blockade, or to by-pass or eliminate an already-established blockade. While alpha blockers did not show sensitizing activity, such properties of beta blockers were shown to be unrelated to some of their nonspecific (i.e., histamine-releasing) effects. Likewise, no correlation was found between the antihistaminic-antiserotonin and the protective activities of alpha blockers against pertussis-induced hypersensitivity. These findings were interpreted to mean that the sensitizing activity of the beta adrenergic blocking agents is due to their ability to inhibit the beta pharmacological actions of the adrenergic neurotransmitters or those of their exogenous counterparts.

From the Departments of Asthma-Allergy and Experimental Immunology, National Jewish Hospital, Denver, and the Department of Pharmacology, University of Colorado School of Medicine, Denver, Colo.

Supported by United States Public Health Service Research Support Grant FR-05474-03-5 and by Grant AI 07426-01 from the National Institute of Allergy and Infectious Diseases.

Received for publication July 29, 1966.

*Present address: Chief, Section of Allergy, Department of Medicine, Creighton University School of Medicine, Omaha, Neb.

In contrast to anaphylaxis, diseases of atopic allergy (bronchial asthma, allergic rhinitis, certain forms of urticaria, atopic dermatitis, and so forth) appear to involve a quantitatively and qualitatively abnormal reactivity to their immunologically released pharmacologic mediators as well as abnormal immunological mechanisms for the release of these mediators.^{1, 4-6} In recent years the *B. pertussis* vaccinated mouse has been used extensively as an experimental model for the investigation of these atopic mechanisms.^{2-9, 24, 25} It has been found that the peculiar pharmacological and metabolic responses of such mice can be reproduced in normal CFW mice through the pharmacological blockade of beta-adrenergic receptors produced by dichloroisoproterenol (DCI) or Nethalide.

Among the pertussis-induced responses which have been duplicated by beta blockade in normal mice has been the production of an increased sensitivity to histamine and serotonin⁷⁻⁹ following pretreatment with DCI and/or Nethalide. One of the newer beta blockers, MJ-1999 (4-[2-isopropylamino-1-hydroxyethyl] methane sulfonanilide HCl), was also shown to be capable of inducing hypersensitivity to histamine, even in a strain of mice (CF₁) not susceptible to pertussis sensitization.¹⁰ The sensitizing capacity of MJ-1999 also applies to guinea pigs with respect to mecholyl and exogenously administered or immunologically released histamine.¹⁰

This report describes strain differences in the production of the aforementioned pharmacological hypersensitivity and presents further evidence that beta-adrenergic blockade can also sensitize to anaphylaxis. It also offers additional information in support of the beta-adrenergic specificity of the sensitizing activity of various adrenergic blocking agents.

MATERIALS AND METHODS

Animals

Female CFW and CF₁ mice weighing 18 to 22 grams and 20 to 24 grams, respectively, at the time of testing, were obtained from a single commercial breeder.* Obese-hyperglycemic mice and their nonobese siblings were obtained†; groups of 10 to 25 mice were housed in metal cages and maintained on pellets and water ad libitum. Female albino Hartley and Trapani-Campbell guinea pigs (National Jewish Hospital at Denver) weighing 500 to 800 and 700 to 1,000 grams, respectively, were employed.

Drugs

Histamine and serotonin (5-hydroxytryptamine) were obtained from Nutritional Biochemical Corporation in the forms of diphosphate and creatinine sulfate, respectively. Commercially available preparations of epinephrine (Adrenalin Chloride) norepinephrine (Levophed bitartrate), and theophylline ethylenediamine (Aminophylline) were used. The adrenergic blocking agents, MJ-1999 (4-[2-isopropylamino-1-hydroxyethyl] methane sulfonanilide HCl), and dibenamine (Dibenamine hydrochloride, Lot 00870) were obtained.‡ Nethalide

*Carworth Farms, New York, N. Y.

†Supplied by R. B. Jackson Memorial Laboratories, Bar Harbor, Maine.

‡Through the courtesy of Dr. P. M. Lish (Mead Johnson Company) and Eli Lilly and Company, respectively.

(Alderlin, pronethalol), dichloroisoproterenol (DCI), and phenoxybenzamine hydrochloride (Dibenzylin) were purchased. Compound P-286 (N, N-di-isopropyl-N'-isoamyl-N'-diethylaminoethylurea HCl) was supplied.*

The drugs from commercial stock solutions were dissolved and/or diluted to the appropriate concentration with physiologic, sterile, nonpyrogenic saline immediately before each experiment.

The doses of histamine are expressed in terms of free base, and those of the other amines, as well as the various blocking agents, as salts.

Pertussis vaccine

A suspension of killed *B. pertussis* organisms. Lot 764645, was supplied.† This suspension was diluted 1:2 in sterile nonpyrogenic, physiologic saline, and 0.5 ml. was injected intraperitoneally. Five days were allowed to elapse between sensitization and the experiments outlined below.

Inhalation challenge

Matched (sex, weight, age, strain, and housing) pairs of guinea pigs were exposed in a 17 liter plexiglass chamber to separate aerosols of histamine, serotonin, methacholine, or antigens 30 minutes after one of the pair had received a beta-adrenergic blocking agent. The same Vaponephrin nebulizer was used for all studies and was activated at the same pressure, so that 5 ml. of solution was aerosolized in 10 minutes. Respiration was recorded with a pneumotachograph by means of a mercury-in-elastic strain gauge tied around the animal's chest and connected to a Parks' impedance plethysmograph (Fig. 1). This simple method was designed to circumvent the anesthesia and tracheotomy often required with apparatuses for measuring bronchial air flow or airway resistance. When an animal showed signs of severe dyspnea, cough, or cyanosis, it was immediately removed from the chamber and given Isuprel by inhalation (hand nebulizer) and was thus saved for rechallenge in a crossover study performed 7 days later.

Production of anaphylaxis

For passive anaphylaxis, Hartley or Trapani-Campbell guinea pigs were given intravenous injections of rabbit gamma globulin containing antiovine serum albumin antibodies (anti-BSA) of a predetermined antibody nitrogen concentration (40 to 400 gamma antibody nitrogen per kilogram body weight). Twenty-four hours later, they were challenged with an aerosol of one per cent BSA. Responses were determined by measuring the average time of onset of severe dyspnea.

Sensitization of guinea pigs for active anaphylaxis was produced by three intraperitoneal injections of 50 mg. (0.5 ml.) horse dander extract (Hollister-Stier) at 4 day intervals. Twenty-one days after the last injection, the animals were challenged with an aerosol (10 mg. per milliliter) of the same extract. Most of the studies were done by the crossover method so that each guinea pig served as a test animal and as its own control.

*By courtesy of Dr. L. C. Weaver of Pitman-Moore, Indianapolis, Ind.

†By Eli Lilly and Company.

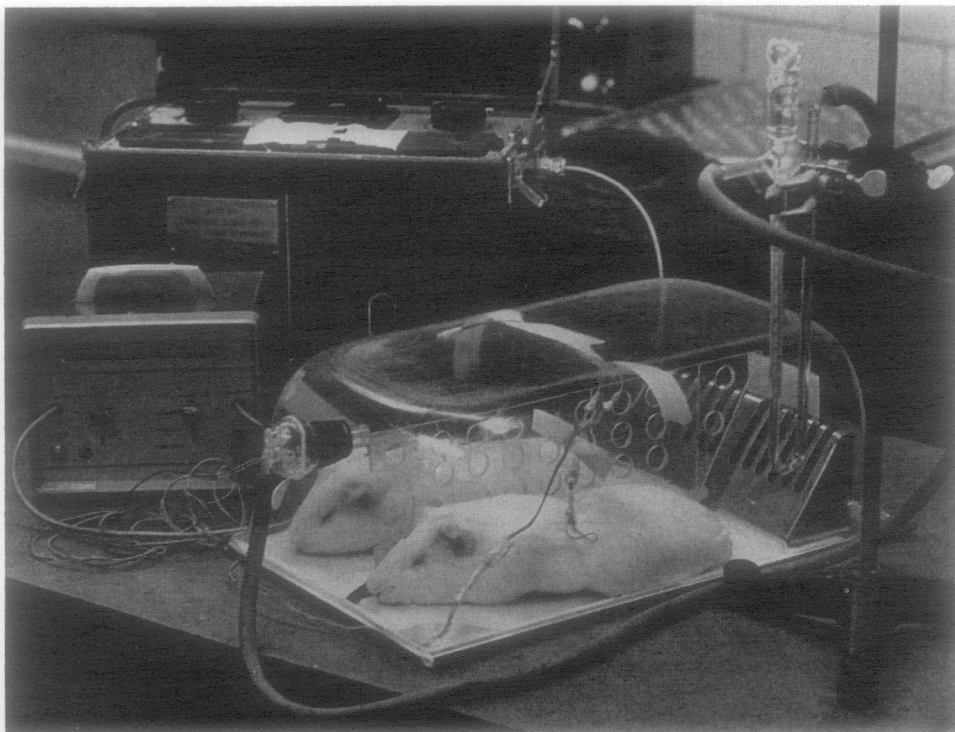


Fig. 1

Guinea pig challenge chamber and apparatus. Respiration is recorded with a pneumotachograph by means of a mercury-in-elastic strain gauge tied around the animal's chest and connected to a Parks impedance plethysmograph.

Tissue analyses

The paired adrenal glands or lungs were assayed for epinephrine and norepinephrine content by adopting the simplified extraction procedure of Bertler, Carlsson and Rosengren.¹¹ In principle, this procedure consisted of homogenization of the tissues in 0.4N perchloric acid, treatment with potassium carbonate, and subsequent removal of potassium perchlorate. In the eluates the 2 amines were oxidized to adrenochrome and noradrenochrome and subsequently rearranged in alkali to adrenolutine and noradrenolutine. Differential estimation of the fluorophores was then performed in the Aminco-Bowman spectrophotofluorometer by measuring the fluorescence intensity at different activation wavelengths, i.e., 410 and 455 $m\mu$ while readings were made at a fluorescent wavelength of 540 $m\mu$.

For histamine measurements, the procedure of Shore, Burkhalter, and Cohn¹² was used involving an extraction of histamine into butanol from alkalinized perchloric acid tissue extracts, return to an aqueous phase, and condensation with O-phthalaldehyde to form a strongly fluorescent product which is then measured in the Aminco-Bowman spectrophotofluorometer.

Calculations

Means and the standard errors were calculated by standard procedures. The significance of differences between means was estimated by Student's *t* test.

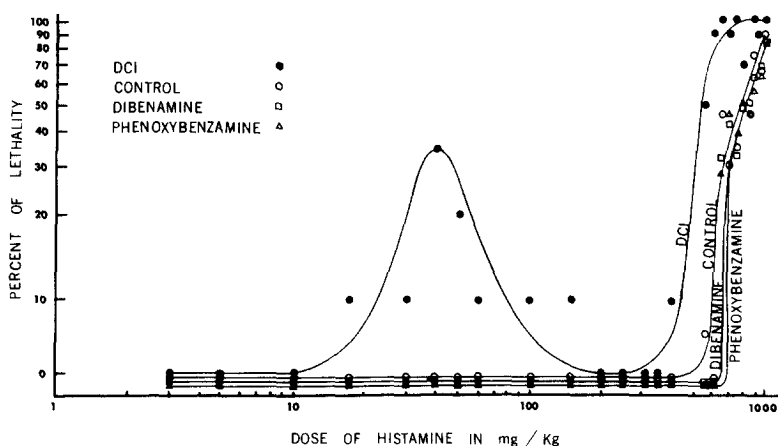
Results

In the mouse experiments, three strains of mice (CFW, CF₁, and the so-called "obese-hyperglycemic"), three beta-adrenergic agents (DCI, Nethalide, and MJ-1999), and two alpha-adrenergic blocking agents (dibenamine and phenoxybenzamine) were used. Unless otherwise stated, 20 mg. per kilogram was adopted as the standard dose of all blockers and was administered intraperitoneally 30 minutes before the test agent.

Both CFW and CF₁ mice can be sensitized to histamine by beta-adrenergic blockade (Table I). Although CF₁ mice are not sensitized by *B. pertussis* vaccine to histamine,¹³ a 200 fold increase in sensitivity to histamine is produced with MJ-1999. A bimodal response to histamine can be shown in untreated CFW mice, but only after beta-adrenergic blockade in CF₁ mice (see also Fig. 2). In general, MJ-1999 seems to be the most potent sensitizer, followed by Nethalide and DCI (Table V), and the degree of histamine hypersensitivity produced appears to be greater in CFW mice, followed by CF₁, and finally by the non-obese siblings of the obese-hyperglycemic mice. This relationship is apparently paralleled by that of the natural histamine sensitivity of the three strains (compare Tables I, IV, and V).

Another strain difference (Table II) is that CF₁ mice can be sensitized to methacholine by both a pharmacologically established beta-adrenergic blockade and pertussis sensitization (i.e., a condition currently considered to be mechanistically similar). Interest in these features lies in the fact that pertussis does not produce histamine hypersensitivity in CF₁ mice, but does in CFW mice; in contrast, pertussis produces methacholine hypersensitivity in CF₁ mice, but not in CFW mice.

Under certain conditions, protection against the histamine-sensitizing effect of beta-adrenergic blockade or pertussis vaccination could be achieved by the administration of catecholamines, aminophylline, and alpha-adrenergic blocking agents, or by an inherited obese-hyperglycemic condition of the animal. Thus, by decreasing the amount of the blocker and increasing that of the catecholamine, a significant degree of protection can be obtained (Table III). For instance, administration of 5 mg. per kilogram of MJ-1999 followed 15 minutes later by 2.5 mg. per kilogram of epinephrine, reduced the lethality from 95 to 40 per cent. Substituting 3 mg. per kilogram isoproterenol for epinephrine, lethality was reduced from the same original level (95 per cent) to 20 per cent. Order of administration as well as the time relationship between blocker and amine were critical for protection. Thus, 50 per cent of the animals were protected by 2 mg. per kilogram isoproterenol if blockade preceded amine administration. No protection was observable, however, when the order of administration was reversed or when amine and blocker were applied simultaneously. Based on the small number of observations available, norepinephrine did not appear to provide any protection. Conversely, 50 mg. per kilogram aminophylline

**Fig. 2**

Dose-response curves of *histamine* in normal, alpha (*dibenamine*, *phenoxybenzamine*), and beta (*DCI*) adrenergically blocked CFW mice. Note that only beta blockade sensitizes to *histamine*.

Table I. Comparison of effects of three beta-adrenergic blocking agents* on the dose response to *histamine*† in CFW and CF₁ mice

Group	Dose of histamine (mg./Kg.)																		
	1	4	5	8	10	13	15	17	30	60	100	200	240	250	300	350	400	800	900
CFW	0†	0	2	0	20	15	30	40	47	55	40	20	27	5	0	10	10	75	100
CFW + DCI			37	53	75	70	65	67	82	88	93			65	73	65	65	95	100
CFW + Nethalide		30		45	55		80	85	85	90	85	90	70	70	60	50	40	100	100
CFW + MJ-1999	10	40	40	70	100	30	100	50	90	100	100	90	70	100	90	60	30	100	90
CF ₁		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	65
CF ₁ + DCI			0		0	10	10	10	10	10	10	0		0	0	0	10	70	90
CF ₁ + Nethalide		0	20	40			40		85	75	10		0	0			5		100
CF ₁ + MJ-1999		13	33				80		10			0	0	0					100

*Dose of all 3 blocking agents (20 mg. per kilogram) given 30 minutes before histamine.

†Dose of histamine is expressed in terms of the base.

‡Per cent lethality; each lethality figure was obtained on 15 to 65 mice.

Table II. The effect of beta-adrenergic blockade* or B. Pertussis† on the sensitivity of CF₁ mice to methacholine

	Dose of methacholine (mg./Kg.)						
	8	16	32	64	120	240	480
Methacholine	0†	0	0	—	70	60	100
Methacholine + MJ-1999	0	30	56	80	100	100	100
Methacholine + per-tussis	—	—	11	11	89	89	—

*20 mg. per kilogram MJ-1999 intraperitoneally 30 minutes before challenge.

†Pertussis sensitization 5 days prior to testing.

‡Per cent lethality; each lethality figure was obtained on 9 to 20 mice.

Table III. *Effect of catecholamines on the histamine-sensitizing activity of beta-adrenergic blockade in CFW mice*

Catecholamine	Catechol- amine dose (mg./Kg.)	MJ-1999 dose (mg./ Kg.)	Histamine, 32 mg./Kg.* intraperitoneally				
			Cat- echol- amine	MJ-1999	Cat- echol- amine mixed with MJ-1999	MJ-1999 first, cat- echol- amine 15 min- utes later	Catechol- amine first, MJ-1999 15 min- utes later
Epinephrine	2.00	0	0†				
	0.00	5		95			
	0.00	20		100			
	0.10	20				90	
	0.25	20				90	
	0.50	20				100	
	0.63	20			100	100	100
	1.00	20				80	
	1.25	20			100	80	80
	1.50	20			80	100	80
	2.00	20			100	80	100
	2.00	10				70	
	2.00	5				60	
	2.50	5				40	
	3.00	5				50	
Isoproterenol	2.00	0	0				
	0.00	5		95			
	0.00	10		100			
	0.50	20				100	
	1.00	20				80	
	1.50	20			100	80	100
	2.00	10			100	50	100
	2.00	5				60	
	3.00	5				20	
Norepinephrine	5.00	5				80	
		5		80			

*Histamine 32 mg. per kilogram 30 minutes after MJ-1999.

†Per cent lethality; each lethality figure was obtained on 4 to 20 mice.

Table IV. *The effect of aminophylline on the histamine sensitizing activity of beta-adrenergic blockade in CFW mice*

MJ-1999 (mg./Kg.)	Lethality after histamine, 32 mg./Kg. intraperitoneally	
	Control	Aminophylline* (mg./Kg.)
0.00	0/10†	
0.01	0/10	
0.1	7/10	
0.2	7/10	10 6/10†
0.2		25 1/10
1.0	10/10	50 0/10
2.0		50 1/10
3.0		50 0/10
4.0	8/10	50 7/10
4.0		100 1/10
5.0	9/10	100 9/10
5.0		200 6/10

*Aminophylline injected intraperitoneally 10 minutes after MJ-1999 and 20 minutes before histamine.

†Number dead over number inoculated.

was capable of completely abolishing the histamine-sensitizing effect of the beta-adrenergic blockade produced in CFW mice by 1 mg. per kilogram of MJ-1999, i.e., by a dose which caused 100 per cent lethality to 32 mg. per kilogram of histamine administered intraperitoneally (Table IV).

Apparently, complete protection against sensitization by beta blockade can also be seen in the "obese-hyperglycemic" mice (Table V). This protection occurs, furthermore, against an amount of histamine which is 100 per cent lethal in identically prepared CFW mice. The peculiar metabolic aberration of these animals appears to be responsible for this high degree of protection, since 42 per cent of their nonobese siblings succumbed to histamine challenge under the same experimental conditions.

Table V. *The effect of the "obese-hyperglycemic" syndrome on the histamine-sensitizing* activity of beta-adrenergic blockade in mice*

Mouse strain	Beta-adrenergic blocking agent†			
	MJ-1999 (%)	Nethalide (%)	DCI (%)	Control (%)
CFW	100	80	70	30
CF ₁	80	40	10	0
OB‡	0	—	—	0
OB§	42	—	—	0

*A standard dose of 15 mg. per kilogram histamine base intraperitoneally was employed 30 minutes following the administration of the blocker.

†Dose for each blocker, 20 mg. per kilogram. Lethality percentages based on 20 or more mice per group.

‡OB, obese hyperglycemic.

§OB, nonobese siblings.

Table VI. *Histamine and serotonin resistance of pertussis-sensitized* CFW mice following pretreatment† with two alpha-adrenergic blocking agents*

Blocking agent	Dose (mg./Kg.)	Histamine (260 mg./Kg.)	Serotonin (260 mg./Kg.)
Dibenamine	250	0‡	0‡
	200	0	0
	150	10	0
	100	33	0
	50	33	0
	20	41	0
	10	100	0
	0	100	100
Phenoxybenzamine (Dibenzylamine)	250	0	0
	200	0	0
	150	0	0
	100	10	0
	50	19	0
	20	38	0
	10	62	0
	0	100	100

*Pertussis sensitization 5 days prior to testing.

†Blockers were administered 30 minutes before testing.

‡Per cent lethality; each lethality figure was obtained on 25 mice.

To explore further the beta-adrenergic specificity of the sensitizing activity of the commercially available beta blockers, two different approaches were used. In the first, the effects of various doses of two, in many respects pharmacologically not comparable, alpha blockers (dibenamine and phenoxybenzamine) were examined on the sensitizing action of pertussis to histamine and serotonin in CFW mice (Table VI). A dibenamine dose of 200 mg. per kilogram conferred complete protection against a 260 mg. per kilogram dose of either amine. Decreasing the dose of dibenamine gradually to 20 mg. per kilogram reduced markedly the protection against histamine but not against serotonin. Lowering the dibenamine concentration to 10 mg. per kilogram abolished entirely the protective effect against histamine, but still provided complete protection against serotonin. The effects of the second alpha blocker (phenoxybenzamine) were very closely similar.

In the second approach, these two alpha blockers were compared with one beta blocker (DCI) in their effects on the response of normal CF₁ mice to increasing doses of histamine (Fig. 2). The responses of the untreated controls describe a curve which closely overlaps those of the two alpha blockers. By sharp contrast, beta blockade resulted in a marked sensitization to histamine producing a bimodal dose-response curve.

In addition, the histamine-sensitizing activity of beta blockade produced by DCI does not appear to be accompanied by a depletion of tissue histamine in CFW mice. This is evidenced by the normal histamine concentrations in 8 representative tissues of CFW mice found under experimental conditions most frequently used for sensitizing mice to the pharmacologic mediators (Table VII).

Marked strain differences in susceptibility to sensitization by beta-adrenergic blockade have also been observed in the guinea pig studies. Following blockade with MJ-1999, Hartley guinea pigs showed a significant increase in sensitivity to 0.1 mg. per milliliter aerosols of histamine, methacholine, and serotonin (Table VIII). Pneumotachographs of a beta-adrenergically blocked Hartley guinea pig which died after 3 minutes of exposure to methacholine are shown in Fig. 3. In contrast, Trapani-Campbell strain guinea pigs did not show an increased sensitivity to any of these amines under the same experimental conditions (Table VIII).

However, when beta-adrenergic blockade with MJ-1999 was reinforced by the simultaneous administration of methoxamine, an agent possessing potent alpha-receptor stimulating properties (c.f. discussion), this relatively resistant strain of guinea pigs (T.C.) did show an increased sensitivity to both histamine and methacholine (Table IX). A similar break in strain resistance to histamine could also be produced in Trapani-Campbell guinea pigs with a combined blockade of the adrenal-medullary synapse by P-286 and the beta-adrenergic receptors by MJ-1999. However, some indication of the higher susceptibility of the Hartley strain to sensitization by beta blockade could still be noted, when the higher lethality and occurrence of protracted shocks to histamine is compared with the behavior of the Trapani-Campbell guinea pigs (Table X). Nevertheless, that under appropriate conditions the Trapani-Campbell strain can be made susceptible to the sensitizing effect of beta blockade is implied again by the results

Table VII. Tissue histamine levels* in normal and beta-adrenergically blocked CFW mice

Tissue	Normal	DCI†
Brain	0.39±0.02‡	0.43±0.06
Liver	0.92±0.03	0.81±0.08
Kidney	1.33±0.07	1.12±0.08
Lung	1.73±0.18	1.54±0.20
Heart	2.23±0.13	2.61±0.18
Spleen	2.42±0.11	2.11±0.14
Small intestine	1.80±0.10	1.67±0.16
Large intestine	2.45±0.10	2.73±0.19
Stomach	9.62±0.44	9.05±0.63
Skin	35.50±0.60	38.72±2.23

*Expressed as µg histamine per gram tissue.

†Animals were killed, and tissues were removed for analysis 30 minutes after the intraperitoneal administration of 20 mg. per kilogram DCI.

‡Values represent the means and standard errors of 6 to 9 determinations; each determination was performed on aliquots of pooled homogenates of the respective tissues derived from at least 8 mice.

Table VIII. Bronchial sensitivity to serotonin, histamine, and methacholine in beta-adrenergically blocked guinea pigs

Challenge agent	Aerosol concentration (mg./ml.)	Guinea pigs	Average time of onset of dyspnea in minutes		
			MJ-1999 (20 mg./Kg. intramuscularly)	Crossover control	P
Serotonin	0.1	20 Hartley	8.9 (0)*	>25.8 (8)	<0.005
Serotonin	0.1	9 T.C.†	>24.7 (5)	>26.6 (7)	N.S.‡
Serotonin	0.15	16 T.C.†	>19.6 (8)	>22.9 (10)	N.S.
Histamine	0.1	20 Hartley	6.5 (0)	13.0 (0)	<0.01
Histamine	0.1	20 T.C.†	7.2 (0)	7.5 (0)	N.S.
Methacholine	0.1	20 Hartley	5.4 (0)	12.0 (0)	<0.01
Methacholine	0.1	12 T.C.†	>16.9 (4)	>13.6 (1)	N.S.

*Numbers in parentheses represent guinea pigs with no reaction after 30 minutes of challenge.

†Trapani-Campbell strain.

‡N. S., not significant.

Table IX. Histamine and methacholine challenges in Trapani-Campbell guinea pigs following the administration of methoxamine* and/or MJ-1999†

Challenge	Average time of onset of dyspnea in minutes				
	Methoxamine and MJ-1999	Methoxamine	MJ-1999	Control	p
Histamine (0.1 mg./ml.)	5.6	11.2	11.7	10.8	<0.025
Methacholine (0.1 mg./ml.)	6.7	11.8	13.3	11.0	<0.050

*Dose, 5 mg. per kilogram intraperitoneally.

†Dose, 20 mg. per kilogram intraperitoneally.

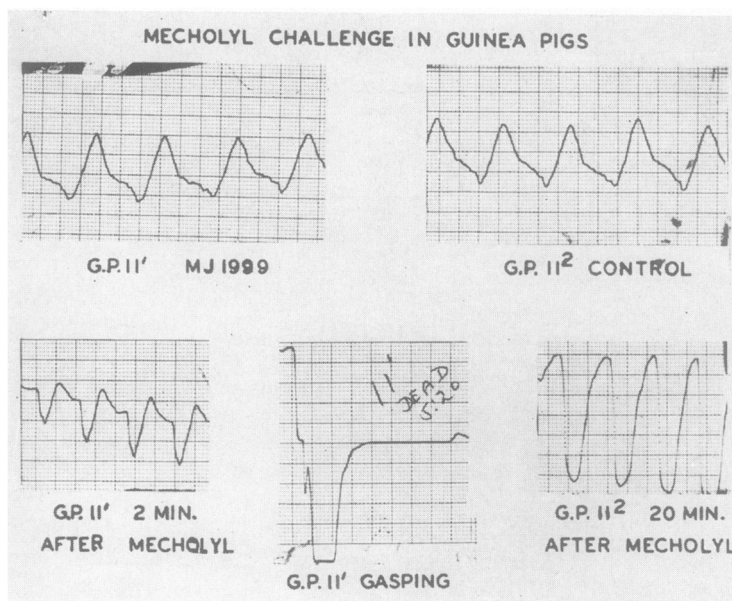


Fig. 3

Pneumotachographs of two *guinea pigs* challenged with methacholine (*Mecholyl*). Guinea pig *II'* received *MJ-1999* (20 mg. per kilogram) 30 minutes before challenge and died after 3 minutes of exposure.

presented in Table XI. They show that the protection of Trapani-Campbell guinea pigs by catecholamines against histamine or methacholine is abolished in the presence of a beta-adrenergic blockade produced by MJ-1999.

These studies showed so far that, under appropriate experimental conditions, several strains of mice and guinea pigs can be sensitized to representative members of the pharmacologic mediators of antigen-antibody responses, when these agents are administered exogenously. The remainder of the findings indicate that the same applies to the immunologically mobilized, i.e., endogenously released, mediators as well.

Thus, in active anaphylaxis following aerosol challenge with horse dander extract, the onset of dyspnea occurred in an average of 45.2 minutes, with 4 of the 8 Hartley guinea pigs not reacting even after 60 minutes, i.e., remaining completely asymptomatic during the observation period. In a crossover study after beta-adrenergic blockade, the average time of onset of anaphylaxis was 10.7 minutes and all 8 guinea pigs reacted in 18 minutes or less (Table XII). Pneumotachographs of such a beta-adrenergically blocked anaphylactic guinea pig and those of its crossover control are presented in Fig. 4.

In 16 Trapani-Campbell guinea pigs which had been passively sensitized with rabbit anti-BSA gamma globulin 24 hours prior to aerosol challenge with BSA, the average time of onset of anaphylaxis was 29 minutes and 7 did not react after a total of 60 minutes' exposure. In an identical set of experiments in another 16 guinea pigs of this same strain, but blocked beta-

Table X. *A crossover study on the effect of histamine and methacholine aerosols blockade of the adrenal-medullary synapse and that of the beta-adrenergic receptors*

<i>Guinea pigs</i>	<i>Challenge agent</i>	<i>P-286 (20 mg./Kg.)</i>
12 Trapani-Campbell	Methacholine	19.8 B* 15.0 C*
10 Trapani-Campbell	Histamine	23.0 B 13.6 C
20 Hartley	Histamine	

*B represents the blocker; C represents the chamber control exposed only to challenge agent.

†Four animals died within minutes following removal from chamber.

‡The upper and lower values refer to significance between experimental group and chamber or

§Thirteen animals died and 3 developed protracted shock following removal from chamber.

Table XI. *Abolition of catecholamine protection in histamine or methacholine challenged Trapani-Campbell guinea pigs by beta-adrenergic blockade*

<i>Challenge agent</i>	<i>Aerosol concentration (mg./ml.)</i>	<i>Number of guinea pigs</i>	<i>Average time of onset of dyspnea (minutes)</i>	
			<i>MJ-1999 (20 mg./ml.)</i>	<i>Crossover control</i>
Isoproterenol + Histamine	0.1	20	7.8 (1)*	> 30 (20)
Epinephrine + Histamine	0.1	16	12.7 (0)	> 30 (16)
Norepinephrine + Histamine	0.1	20	8.9 (0)	> 26 (15)
Isoproterenol + Mecholyl	0.15	20	> 16.9 (3)	> 30 (20)

*Numbers in parentheses represent guinea pigs that did not react after 30 minutes.

adrenergically, the average time of onset of anaphylaxis was only 3.9 minutes and all animals reacted. In these beta-adrenergically blocked guinea pigs not only was the time of onset more rapid, but the degree of anaphylaxis was more severe and 9 of the 16 animals died. This occurred in spite of their immediate removal from the chamber and the administration of isoproterenol at the onset of severe dyspnea (Table XIII). Postmortem examination of these guinea pigs showed typical changes of acute anaphylaxis with markedly overdistended lungs. Essentially the same results were obtained in 18 passively sensitized Hartley guinea pigs with the average onset of anaphylaxis in the control and beta-blocked groups being 24.0 and 4.4 minutes, respectively. In addition, there were 3 nonreactors in the control group even after 30 minutes of exposure.

(0.1 mg./ml.) in Hartley and Trapani-Campbell strain guinea pigs with a combined

Average time of onset of dyspnea in minutes			
MJ-1999 (20 mg./Kg.)	MJ-1999 plus P-286 (20 mg./Kg.)	Crossover control	n
17.3 B	19.0 B	13.6	N.S.
16.6 C	16.3 C		N.S.
14.0 B	7.3 B†	13.6	<0.01 ‡
11.8 C	13.0 C		<0.005‡
	4.1§	7.0	0.005

crossover controls, respectively.

Table XII. A crossover study of active anaphylaxis to horse dander* in beta-adrenergically blocked Hartley guinea pigs

Guinea pig code	Time of onset of dyspnea (minutes)	
	MJ-1999 (20 mg./Kg.)†	Crossover control
8 ¹	4	49
8 ²	7	60‡
5	5	50‡
4	5	3
9 ¹	16	60‡
9 ²	18	60‡
14 ¹	13	16
14 ²	18	60‡
Average	10.7 minutes	>45.2 minutes

*Animals were challenged with an aerosol of horse dander extract (Hollister-Stier, 10 mg. per milliliter) 21 days after the third intraperitoneal sensitizing injection of 50 mg. extract given in a volume of 0.5 ml. at 4 day intervals.

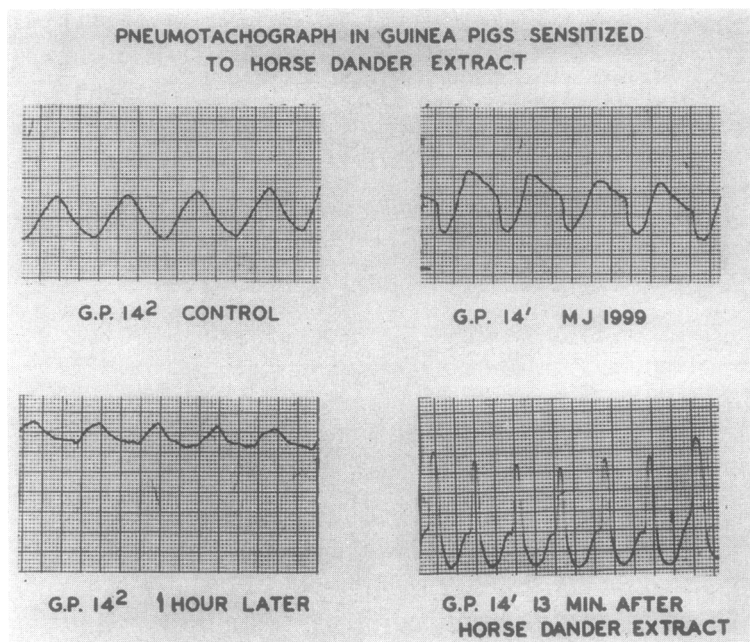
†Administered intramuscularly 30 minutes prior to challenge.

‡Challenge terminated (no symptoms).

Finally, no significant difference was found in the adrenal and pulmonary catecholamine or pulmonary histamine contents of normal, beta-adrenergically blocked, histamine-, or antigen-challenged guinea pigs (Table XIV).

DISCUSSION

One of the major obstacles impeding progress in our understanding of the nature of the pharmacological and immunological peculiarities of atopy has

**Fig. 4**

Pneumotachographs of two guinea pigs challenged with horse dander extract. Guinea pig 14' received MJ-1999 (20 mg. per kilogram) 30 minutes before challenge and developed severe dyspnea after 13 minutes of exposure.

Table XIII. *Passive anaphylaxis* to bovine serum albumin in beta-adrenergically blocked Trapani-Campbell guinea pigs*

Rabbit anti-BSA γ -globulin (γ AbN/Kg.)	Experiment	Time of onset of dyspnea (minutes)	
		MJ-1999 (20 mg./Kg.)	Cage control
40	I	17	60†
400	II	12	60†
		3‡	64
		3‡	60†
		3‡	3
100	III	2	2
		2	60†
		2‡	60†
200	IV	2	5
		2	7
		2‡	5
		3‡	6
		1/2	4
		1‡	3
		5‡	60†
		3‡	5
Average		3.9	>29.0

*Rabbit anti-BSA intravenously 24 hours before challenge with 1 per cent BSA aerosol.

†On termination of exposure still no symptoms detectable.

‡Dead within minutes after removal from chamber.

Table XIV. Mean catecholamine* and histamine concentrations in the adrenal glands and lungs of beta-adrenergically blocked, histamine-, and antigen-challenged guinea pigs

Group	Aerosol concentration (mg./ml.)	Number of guinea pigs	Adrenal glands		Lung
			Epinephrine (μ g/Gm. w.w.†)	Norepinephrine (μ g/Gm. w.w.)	Histamine (μ g/Gm. w.w.)
Control		10	220.03 $\pm$ 8.98	14.34 $\pm$ 5.10	3.06 $\pm$ 1.22
Histamine	0.1	10	211.94 $\pm$ 21.29	12.62 $\pm$ 2.15	
MJ-1999†		5			3.48 $\pm$ 0.78
Histamine + MJ-1999	0.1	10	207.10 $\pm$ 20.08	16.60 $\pm$ 4.59	
BSA§	10.0	9	241.80 $\pm$ 20.96	17.92 $\pm$ 7.34	
BSA	10.0	9	207.98 $\pm$ 26.53	15.79 $\pm$ 6.86	
BSA + MJ-1999	10.0	9	246.32 $\pm$ 27.28	15.52 $\pm$ 4.32	

*Pulmonary epinephrine and norepinephrine concentrations in all groups were $<2.5 \times 10^{-8}$ gram.

†Abbreviation w.w. = weight per weight.

‡Dose, 20 mg. per kilogram intramuscularly 30 minutes before killing.

§Animals removed from challenge chamber and killed at the same time as their blocked partners.

||Animals remained in challenge chamber until full anaphylactic response, but not beyond 30 minutes.

been the inability to reproduce atopic diseases in man or experimental animals in contrast to the readily induced nonatopic allergic reactions (i.e., anaphylaxis) which are based on immunologic and pharmacologic mechanisms normally operative.^{2-4, 6} However, in recent years the *B. pertussis* vaccinated mouse has been used extensively as a provisional laboratory model for investigating atopic mechanisms.^{2-9, 24, 25} The possible applicability of these findings to atopy is implied by the following principal features of the pertussis-induced hypersensitive state: (a) hypersensitivity to the presently recognized pharmacological mediators of antigen-antibody responses (histamine, serotonin, acetylcholine, etc.); (b) a quantitatively enhanced (adjuvant activity) and qualitatively abnormal (production of "mast cell-sensitizing antibodies" with properties highly reminiscent of those of the atopic reagin) immune response¹³; and (c) presence of eosinophilia.¹⁴

The critical advance in these pertussis experiments has been the finding that the hypersensitivity of pertussis-sensitized mice to pharmacologic mediators may stem from a blockade or a reduced functioning of the beta-adrenergic receptors or of some of the steps in the biochemical sequence between receptor activation and adrenergic end response. Based on these and other considerations^{2-4, 6, 24, 25} Szentivanyi has suggested that the atopic abnormality common to all asthmatics might be an inherited or acquired abnormal functioning of the so-called beta-adrenergic mechanisms, regardless of what the precipitating factor(s) may be clinically (immunologic, infectious, psychic, etc.).

Thus, the main significance of the present findings is the evidence that the development of a bronchial hypersensitivity in guinea pigs may be due to a partial blockade of the beta-adrenergic receptors of the bronchial tree. Furthermore, the hypersensitivity so produced is manifested against representative mem-

bers of a class of substances that could be broadly termed the "pharmacological mediators" of antigen-antibody responses. It will be also recognized that these exogenously administered or immunologically released pharmacological mediators elicit respiratory changes in the guinea pig not unlike those in patients with bronchial asthma.

The validity of the foregoing interpretation and its possible applicability to bronchial asthma rests primarily on the beta-adrenergic specificity of the sensitizing activity of the blocking agents used. Since it is axiomatic that adrenergic blocking agents act through occupying the receptor-binding site, and prevent the catecholamine-receptor interaction, it is implicit in our discussion that the term "blockade" refers to receptor occupancy. However, blocking agents have limitations when used for the investigation of physiologic or pathologic problems since they have important actions in addition to blockade of catecholamine responses inferring a lack of absolute specificity.

Among these nonadrenergic properties, there are two which could conceivably result in their sensitizing activities. One of these is, paradoxically, the "antihistaminic," whereas the other one is the histamine-releasing activity of some of these agents.

Concerning the paradoxical "antihistaminic" activity, the degree of antagonism between histamine and antihistamines in various strains of mice depends, among other factors, on the natural resistance of the strain to histamine. It appears that the higher the natural resistance of a mouse strain to histamine, the less protection is afforded by antihistamines. In fact, in highly histamine-resistant mouse strains (e.g., C₃H/Jax) certain antihistamines, under appropriate conditions, may show a synergistic action with histamine.^{32, 33, 45} Conversely, when the histamine resistance of mice is reduced by adrenalectomy or pertussis vaccination, only protection occurs with antihistamines.^{29-31, 47, 48} Thus, on one hand, some adrenergic blocking agents are known to possess antihistaminic properties and, on the other hand, conventional antihistamines are known to increase histamine toxicity under certain conditions. Such a combination of pharmacologic properties and experimental conditions could conceivably result in a sensitizing effect of certain adrenergic-blocking agents.

The present experiments offer several lines of direct and indirect evidence against such a possibility. Thus, dissociation of histamine-sensitization properties of beta blockade and those of synergistic "antihistaminic" activity is suggested by the fact that, in the former case, susceptibility to sensitization appears to be proportional to the natural sensitivity (Tables I, IV, and V), whereas in the latter case, to the natural resistance of the mouse strain.^{32, 33, 45} This reversed relationship of the two sensitizing mechanisms to the natural reactivity to histamine is exemplified also by the behavior of the guinea pig. In this most histamine-sensitive laboratory species, the synergistic effect of antihistamines on histamine toxicity does not occur. On the contrary, the guinea pig can be protected by antihistamines to the greatest extent.⁴⁵ Nevertheless, this species, at least the Hartley strain, can be easily sensitized by beta blockade to exogenously administered or immunologically released pharmacologic mediators, including histamine (Tables VIII, X, XII, and XIII).

Also the types of interventions through which the natural resistance of a strain to sensitization by beta blockade could be overcome or, conversely, the procedures through which the sensitizing effect of beta blockade can be reduced or abolished are known to involve mechanisms, the successful operation of which could be more easily interpreted in the frame of a beta-adrenergic blockade than in that of a derailed histamine-antihistamine interplay. For instance, breaking the strain resistance (Trapani-Campbell) to sensitization by beta blockade with the complementary administration of either methoxamine or P-286, could be conceived as interventions resulting in an intensification of the already existing imbalance between the two adrenergic-receptor systems through either an additive blocking effect on the beta or a stimulation of the alpha receptors or both (Tables IX and X). This interpretation is based on the fact that, among the adrenergic receptors of the smooth muscle cells, methoxamine (beta-hydroxy-beta-[2,5-dimethoxyphenyl] isopropylamine) stimulates alpha receptors exclusively,⁴⁰ while also displaying inhibitory activities against the hyperglycemic and lipolytic effects of epinephrine.^{43, 44} Although, in terms of the dualistic receptor concept,⁴ these metabolic activities of methoxamine are not clearly classifiable at present, an essentially identical metabolic pattern of adrenergic action can also be produced by the administration of specific beta-adrenergic blocking agents.^{4, 6} Compound P-286 (N, N-di-isopropyl-N'-isoamyl-N'-diethylaminoethyl-urea HCl), in the dose range employed in the present experiments, is believed to exert no ganglioplegic action on sympathetic or parasympathetic ganglia while blocking selectively the adrenal-medullary synapse.⁴⁰⁻⁴² A selective interruption of ganglionic transmission in the adrenal medulla would be expected to make the only body store of epinephrine unavailable at the time of challenge, while firing of the postganglionic adrenergic neurons with the exclusive mobilization of norepinephrine could proceed uninterruptedly.⁴ Since norepinephrine activates mainly the alpha-adrenergic receptors, its uncompensated adrenergic activity could conceivably accentuate the already existing imbalance caused by beta blockade with MJ-1999.⁴ Likewise, procedures such as administration of aminophylline, epinephrine, and more importantly, isoproterenol (which were shown to abolish the sensitizing activity of beta blockade [Tables III and IV] and, conversely, reduction or abolition of catecholamine protection against histamine or methacholine by beta blockade [Table XI]) argue more convincingly for the beta-adrenergic rather than the paradoxical "antihistaminic" mechanism of sensitization.

Furthermore, the inverse antihistaminic activity as a possible mechanism of sensitization could be entertained only with respect to those adrenergic blocking agents which possess regular antihistaminic activities. With respect to the beta blockers, such a possibility has been both supported⁵⁰ and refuted.^{38, 39} In addition, the only available finding indicating such activity was based on the antagonism of Nethalide to exogenous histamine in the isolated guinea pig auricle. This tissue is among the few exceptions, however, not protected against histamine by conventional antihistamines.⁴⁹ On the other hand, even the more specific alpha-adrenergic-blocking agents, such as the 2-halogenoalkylamines (dibenamine, phenoxybenzamine, etc.) are known to possess antihistaminic,

antiserotonin, and anticholinergic activities.³⁴ Although blockade of responses to histamine and serotonin requires the same basic chemical configuration as does alpha-adrenergic-blocking activity, potency with respect to these properties may vary widely. For example, the concentration required to produce a 50 per cent reduction of the response to a standard dose of histamine was found to be more than 330 times greater for dibenamine than for phenoxybenzamine. Similarly, dibenamine was found to be 230 times less potent as a serotonin-antagonist than phenoxybenzamine.³⁴ In sharp contrast, there was no essential difference in the efficiency of the two alpha blockers in protecting pertussis-sensitized mice against histamine or serotonin (Table VI). This may be taken to mean that the protection afforded by these haloalkylamines in the pertussis-sensitized mouse was due to the alpha-adrenergic-blocking properties, and not to the antihistamine or antiserotonin activities of these agents. On the other hand, the findings presented in Fig. 2 offer clear evidence that in contrast to the potent sensitizing effect of beta blockade, neither of the two alpha blockers could sensitize the mouse to histamine.

The mechanism of sensitization by beta blockade is also not attributable to the release of histamine. This possibility was raised by *in vitro* findings indicating that histamine can be released by DCI from cells of rat peritoneal fluid,³⁵ and by Nethalide from guinea pig lung slices.³⁷ In the mouse, however, even a cursory comparison of the total body histamine content (averaging about 10 mg. per kilogram body weight) with the LD₅₀ of histamine for various strains (ranging about 750 to 1,500 mg. per kilogram) makes it rather improbable that in this species histamine release is involved to any great extent in the mechanism of sensitization by beta blockade. In the guinea pig, an analysis of the histamine-releasing properties of beta blockers indicated that these properties are unrelated to the ability of these agents to inhibit the beta pharmacological actions of epinephrine and suggested that their histamine-releasing capacity is connected rather with their catecholamine-like intrinsic action.³⁷ Indeed, both catecholamines and phenoxybenzamine have been reported to release histamine,^{4, 36} whereas these agents do not sensitize to histamine but protect against it (Tables III and VI). Finally, sensitization by beta blockade was not found to be accompanied by a reduction of tissue histamine stores in either the mouse or the guinea pig (Tables VII and XIV).

Therefore, it appears that sensitization by beta-adrenergic blocking agents is due to the ability of these substances to block the beta pharmacological actions of the adrenergic transmitters or those of their exogenously administered counterparts. In relating these findings to bronchial asthma, it is to be noted that the type of autonomic irregularities present in this disease also bears some resemblance to a functional beta receptor deficiency. This has been discussed in detail elsewhere⁴; only some of the more recent findings will be mentioned. Among these is the observation that reversal of the chronotropic cardiac effects of epinephrine, a beta receptor function,¹⁵ has been noted in a number of asthmatic individuals,⁴ as has a diminished diastolic pressure response to isoproterenol, also a beta receptor function.¹⁶ Administration of beta-adrenergic blocking agents has been reported to aggravate already existent asthmatic attacks or to provoke asthma

in asymptomatic asthmatic individuals.¹⁷ Furthermore, these agents have been shown to enhance bronchial reactivity to inhaled allergens in hay fever patients without a previous history of bronchial asthma.¹⁸ Conversely, the production of alpha-adrenergic blockade has been known to ameliorate asthmatic attacks in certain cases as well as to reduce the observed hypersensitivity to exogenously administered histamine and cholinergic agents.^{19, 20}

New light may also be shed on some unexplained aspects of the current therapy of bronchial asthma when they are reinterpreted in the frame of the beta-adrenergic theory. For instance, epinephrine which is the most commonly used drug for rapid alleviation of symptoms, often has no beneficial effect. This applies especially to the epinephrine-fast asthma cases. On the other hand, aminophylline and theophylline which are also commonly used in the treatment of asthma are frequently effective even in conditions of epinephrine-fastness.

When an attempt is made to invoke the beta-adrenergic hypothesis in accounting for the unusual status of theophylline in the antiasthmatic armamentarium, it is realized that the diverse actions of the methylxanthines make it doubtful that any single enzymatic action will adequately explain the various actions of this drug group.²¹ Nevertheless, it is tempting to ascribe its effectiveness in epinephrine fastness to its action on phosphodiesterase.²² Since in the biochemical reaction sequence believed to be associated with adrenergic action this point is beyond the receptor-activation step, theophylline's effectiveness could conceivably result from its by-passing the postulated molecular site of atopic abnormality. The protective activity of aminophylline in mice sensitized to histamine with MJ-1999 (Table IV) may support this explanation. Likewise, Blumenthal and Brody,²³ using guinea pig tracheal rings, found that the relaxation produced by epinephrine and isoproterenol was completely blocked after the tracheal preparation had been in contact with the beta-adrenergic blocking agent, pronethalol, while the effect of theophylline remained intact.

The aforementioned activity of methylxanthines in the intermediary metabolism of carbohydrates leads us to the problem of the mechanism of the sensitizing activity of beta-adrenergic blockade. Whatever the exact nature of this mechanism may prove to be, the available evidence obtained with the pertussis and beta-blocking experiments^{8, 9, 24, 25} would point to an impaired adrenergic control of the peripheral uptake of glucose. More specifically, it appears that the normal adrenergic inhibition of peripheral glucose uptake is blocked in these animals, leading to a reduction in the degree of antagonism to insulin which exists under normal circumstances. The involvement of this glycemic abnormality in the induction of the hypersensitive state is indicated by the fact that both alloxan diabetes and long-term (as well as acute) administration of glucose was found to restore the normal resistance of the pertussis-sensitized mouse to serotonin. Conversely, insulin was found to sensitize normal mice to histamine and serotonin.⁸ Furthermore, an extract of the pertussis cell which contains the so-called "histamine-sensitizing factor" was capable of reproducing the glycemic abnormality *in vitro*.²⁵ A congruent observation is that obese, hyperglycemic mice were not sensitized to histamine by MJ-1999 (Table V). Another interesting parallel between sensitivity to histamine and experimental obesity is that similar

local lesions in the hypothalamus will protect against anaphylaxis²⁶ and histamine²⁷ and will induce obesity as well.²⁸

The technical assistance of Richard Adolphson, William Selenke, Miss Linda Venske, and Mrs. Carla Gilbert is gratefully acknowledged.

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