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Two lines of guinea pigs sensitive and nonsensitive to chemical mediators and anaphylaxis

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We produced by pedigree breeding two lines of guinea pigs having, respectively, bronchial walls sensitive and nonsensitive to chemical mediators and pilocarpine. However, the sensitivity of the salivary gland and the skin tissue showed almost no difference between the two lines, while the vasculature and ileum showed slight differences. This sensitivity was not increased in either line even by intensive sensitization with egg albumin. Moreover, both active and passive anaphylaxis were more severe in the sensitive than in the nonsensitive line. These findings probably indicate not only that the sensitivity of the bronchial walls to the above chemicals is essentially of an inborn nature but also that this sensitivity is a necessary prerequisite for the occurrence of anaphylaxis. Since the above chemicals have parasympathomimetic or parasympathomimetic drug-like action, it may be suggested that the genetic background of sensitivity of the bronchial walls is to be ascribed to the irritability of the bronchial vagi.

The susceptibility of guinea pigs to anaphylaxis differs not only with their strain¹ but also with the time, the day, and the season.²⁻⁵ The former difference is related to genetic factors, whereas the latter is related to environmental factors.

Stone and associates¹ described the difference of this susceptibility among strains to a genetic difference in the quantity of histamine present in the lung. However, this cannot fully explain individual differences in this susceptibility in the same strain.⁶ Since the susceptibility of guinea pigs to anaphylaxis has a close relationship to irritability of the bronchial vagi, and since the irritability of this nerve also changes considerably with environmental factors,^{4, 5, 7-9} one of us (Y. Takino) attached much importance to the irritability of the bronchial vagi in the occurrence of anaphylaxis. A very similar conclusion was reported earlier by Takino senior¹⁰⁻¹² in respect to bronchial asthma. However, it is not

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TABLE I. Guinea pigs sensitive and nonsensitive to acetylcholine obtained by closed breeding for 10 successive generations

Line	Sensitivity							
	Sensitive				Nonsensitive			
	Yes		No		Yes		No	
	M	F	M	F	M	F	M	F
P	1	2					1	1
F-1	0	3	2	2	1	1	1	0
F-2	1	4	3	2	1	1	3	0
F-3	3	2	1	2	0	3	1	2
F-4	2	2	0	1	2	1	4	3
F-5	5	3	1	2	5	0	3	5
F-6	6	6	1	1	3	5	7	6
F-7	7	3	0	4	2	2	2	7
F-8	3	6	0	1	0	0	4	2
F-9	4	5	1	0	0	1	2	6
F-10	10	7	2	0	7	3	11	16

M = male; F = female.

yet clear whether irritability of the bronchial vagi in guinea pigs and asthmatic patients is essentially congenital or acquired. To solve this problem, pedigree breeding was used for about five years in two lines of guinea pigs with bronchial walls sensitive and nonsensitive to acetylcholine, respectively.¹³ Since the animals did not reproduce well, it was difficult to obtain pure lines.

With the two lines of animals thus obtained, we first studied whether hypersensitivity to chemical mediators can be localized only to the bronchial walls by pedigree breeding of sensitive guinea pigs, then whether this hypersensitivity can be further increased by sensitization in the sensitive line, and finally whether hypersensitivity can be acquired by sensitization in nonsensitive animals. For these purposes, we examined the action of chemical mediators and pilocarpine upon the bronchial walls, the action of acetylcholine upon the heart, tracheo-bronchial and ileum strips, and the skin capillaries, the action of pilocarpine upon the salivary gland, and the intensity of bronchospasm due to active and passive anaphylaxis.

MATERIALS OBTAINED BY PEDIGREE BREEDING

The animals were raised in a breeding case kept at a constant temperature ($25^{\circ} \pm 2^{\circ}$ C.) in a constant-humidity (55 ± 5 per cent) room. The animals were placed in a 10 liter glass container with an atomized aqueous solution of acetylcholine or histamine blown in by our⁵ technique in order to select those with sensitivity of the bronchial walls. In the beginning, animals were selected according to the difference in the intensity of bronchospasm due to inhalation of 2.5 per cent aqueous histamine solution. Since, however, the guinea pigs of the sensitive line were often killed by histamine, this chemical was replaced by a 2.5 per cent aqueous solution of acetylcholine. Three guinea pigs sensitive to acetylcholine and histamine (2 females, one male) and 2 not sensitive to acetylcholine and histamine were selected after 4 generations had descended from the original 10 females and 7 males obtained commercially.

Starting from these two groups of animals (Table I), further selections were made by determining the time interval before the animals in the glass container fell with dyspnea caused by inhaled acetylcholine (2.5 per cent). The time duration of standing dyspnea may be regarded as an expression of sensitivity of the bronchial walls or irritability of the vagal bronchoconstrictor nerve. After the fifth generation, guinea pigs were selected as sensitive

animals when they fell with dyspnea within 3 minutes; guinea pigs selected as nonsensitive animals when they remained standing for 5 minutes, at which time this particular experiment was terminated.

In the first generation of the sensitive line (F1), there were no sensitive males. Thus 3 sensitive females and 2 nonsensitive males were crossed. Similarly, in the first generation of the nonsensitive line (F1), nonsensitive females were not born. Therefore, one nonsensitive male and one sensitive female were crossed. In the second generation of the nonsensitive line (F2), nonsensitive females were not born. One of 3 nonsensitive males and one sensitive female were crossed. After the third generation, the required animals were born. Thus after eight to ten generations of breeding in closed colonies, 35 guinea pigs sensitive to acetylcholine and 41 not sensitive to acetylcholine were available. The selected animals, grouped into sensitive or nonsensitive lines, were picked at random for the experiments. Since there were not enough animals to carry out all the experiments as planned, some were used more than once in different experiments (exclusive of sensitization) on different days at intervals of at least 7 days.

METHODS

The inhalation method was used for studying the sensitivity of the bronchial walls of guinea pigs. For the tracheobronchial wall study, the smooth muscle was cut spirally according to the method of Levis and Koessler,¹⁶ but for the study of the intestinal tract, the ileum was used according to the routine method. The strips were investigated according to the Magnus method, i.e., each strip was suspended in a bath holding 50 ml. of Ringer-Locke solution, and the contraction of these strips was recorded in the usual manner. To measure saliva production caused by pilocarpine quantitatively, previously weighed pieces of cotton were inserted into the mouth of each guinea pig to absorb the saliva. The pieces of cotton were taken from the mouth and replaced with a fresh piece every 5 minutes. Each piece was weighed.⁹ In studying the vascular response to chemical mediators, the fall of systolic arterial pressure due to intravenous injection of acetylcholine and histamine was determined. For this purpose, the carotid artery was cannulated, and the carotid pressure was recorded with a mercury manometer. The acetylcholine and histamine solutions were injected into either the jugular or penile vein¹⁵ under anesthesia with chlorase, 50 mg. per kilogram of body weight. For the study of the sensitivity of the skin capillaries, 10 μ g or 1.0 μ g of acetylcholine in saline was intradermally injected immediately after intravenous injection of 0.5 per cent Evans blue dye in saline according to the technique of the passive cutaneous anaphylaxis (PCA) reaction. For active sensitization of guinea pigs, Grove's¹⁴ method was used; for passive sensitization, 2.0 ml. of rabbit anti-egg albumin serum (precipitin titer of 1:160) was administered intra-abdominally. Twenty-four hours after passive sensitization, challenging antigen (i.e., 1.0 mg. egg albumin per milliliter of Tyrode's solution) was given by means of inhalation for 9 minutes.

RESULTS

Action of chemical mediators on the bronchial walls of sensitive and nonsensitive guinea pigs

To evaluate the reproducibility of the bronchial response to the aerosol 2.5 per cent acetylcholine, 12 sensitive and 10 nonsensitive guinea pigs were challenged in the same manner 5 times on 5 different days at intervals of at least 7 days. The mean and standard deviation of the duration of time that the sensitive animals remained standing before collapsing was 1 minute 49 seconds $\pm$ 21 seconds. Nonsensitive animals remained standing for 5 minutes, at which time the procedure was terminated. In the same way the time duration of standing dyspnea caused by aerosol histamine (0.5 per cent), serotonin (2.0 per cent), and pilocarpine (10.0 per cent) was compared for the two guinea pig lines. The

TABLE II. Mean and standard deviation of the duration of time of standing dyspnea due to aerosol histamine, serotonin, and pilocarpine

Inhaled chemicals	Concentration (%)	Sensitive line				Nonsensitive line	
		No. of animals	Mean value		Standard deviation (sec.)	No. of animals	Mean value (min.)
			Min.	Sec.			
Histamine	0.5	8	2	34	± 40	8	> 5
Serotonin	2.0	6	2	28	± 10	6	> 5
Pilocarpine	10.0	11	1	49	± 9	11	> 5

TABLE III. Intensity of dyspnea due to intrabronchial injection of 0.02 per cent bradykinin in guinea pigs

Sensitive line				Nonsensitive line			
No.	Sex	Body weight (Gm.)	Intensity of dyspnea	No.	Sex	Body weight (Gm.)	Intensity of dyspnea
1	M	920	++	1	M	1,000	÷
2	M	760	+	2	M	660	-
3	M	760	++	3	M	990	-
4	M	700	+	4	M	930	÷
5	M	750	++	5	M	680	+
6	M	800	+	6	M	750	-
7	M	800	+	7	M	700	+
8	M	800	+	8	M	790	-
9	M	740	+	9	M	500	÷
10	M	740	+				

-, None; ÷, very slight; +, slight; ++, moderate; +++, strong.

means and standard deviations in the sensitive line were 2 minutes 34 seconds ± 40 seconds, 2 minutes 28 seconds ± 10 seconds, and 1 minute 49 seconds ± 9 seconds, respectively, whereas they were over 5 minutes in the nonsensitive line (Table II).

In the case of bradykinin, 0.05 ml. of 0.2 mg. bradykinin per milliliter of saline solution was injected intrabronchially. The intensity of dyspnea caused by bradykinin in the sensitive line was stronger than that in the nonsensitive line (Table III).

From the above findings it may be concluded that guinea pigs sensitive to acetylcholine are also sensitive to pilocarpine, histamine, serotonin, and also bradykinin, whereas animals not sensitive to acetylcholine also are not sensitive to these other chemicals.

Action of acetylcholine upon tracheobronchial and ileum strips in sensitive and nonsensitive guinea pigs

The sensitivity of tracheobronchial strips in 6 sensitive and 6 nonsensitive guinea pigs was compared. For this purpose the intensity of contraction of these strips caused by 0.01 mg. of acetylcholine in saline and its relation to various weights (equivalent to 1.0, 1.5, 2.0, and 2.5 Gm.) previously put on the lever (Fig. 1) was investigated. When a 1.0 Gm. weight was placed on the lever, all

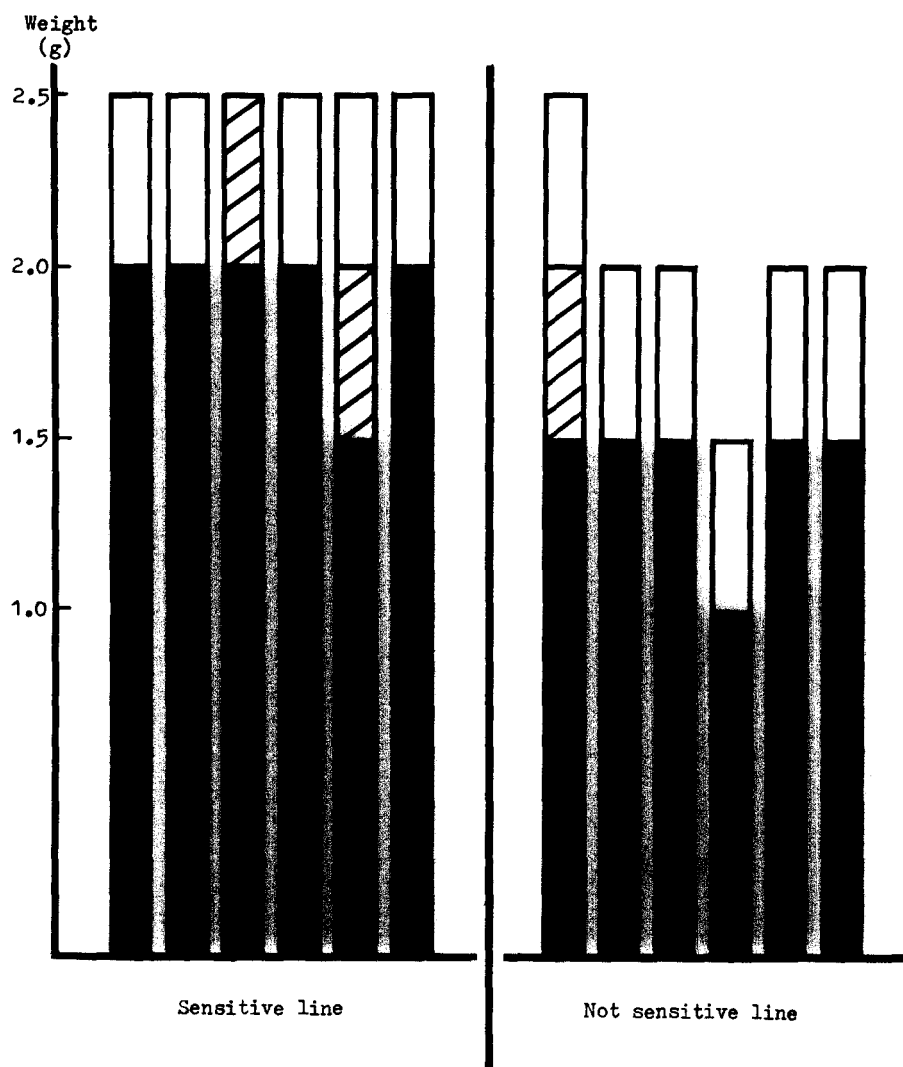


FIG. 1. Comparison of contraction of tracheobronchial strips due to acetylcholine between sensitive and nonsensitive guinea pigs. Dosage: 0.01 mg. of acetylcholine per 50 ml. of Ringer-Locke solution. Strong contraction; moderate contraction; no contraction.

strips in both groups were strongly contracted by acetylcholine. When a 1.5 Gm. weight was put on the lever, all strips were strongly contracted except one in the nonsensitive group, which showed no contraction. When a 2.0 Gm. weight was placed on the lever, 5 of the 6 strips of the sensitive group were still strongly contracted by acetylcholine; the other demonstrated a slight contraction. Only one strip in the nonsensitive group showed a slight contraction.

If a strong contraction of the strips is expressed as a positive reaction and a slight contraction or no contraction as a negative reaction, Chart I shows the

CHART I

	Weight (Gm.)			Total
	2.0	1.5	1.0	
Sensitive line	5	1	0	6
Nonsensitive line	0	5	1	6
Total	5	6	1	12
$\chi^2 = 8.64; p < 0.05$				

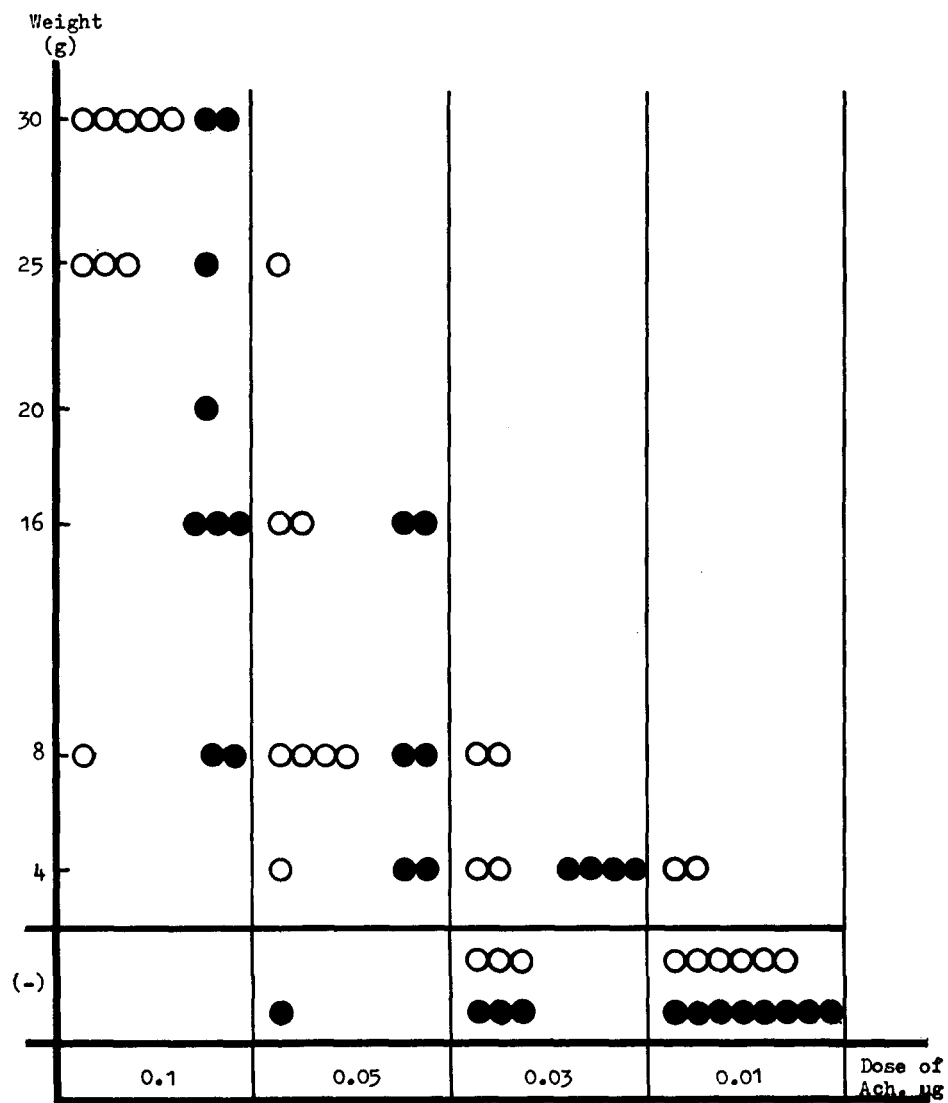


FIG. 2. Comparison of action of acetylcholine upon ileum strips between guinea pigs sensitive and not sensitive to acetylcholine. ○ Sensitive; ● not sensitive.

CHART II

	Weight (Gm.)					Total
	8	16	20	25	30	
Sensitive line	1	0	0	3	5	9
Nonsensitive line	2	3	1	1	2	9
Total	3	3	1	4	7	18

$\chi^2 = 6.6; p > 0.05$

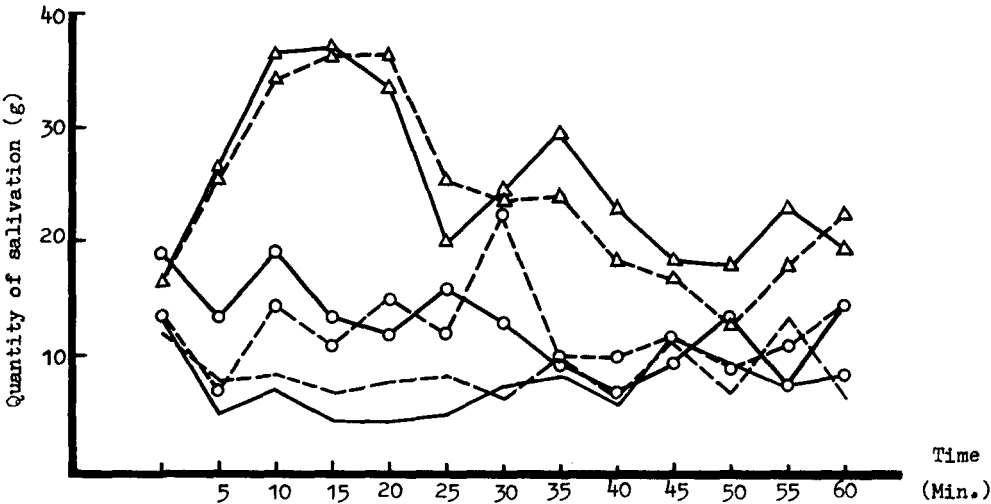


FIG. 3. Average results of comparison of salivation due to pilocarpine between 6 sensitive and 6 nonsensitive guinea pigs. — Sensitive; ---- not sensitive. Dosage: Δ 6 μ g of pilocarpine per 100 Gm. of body weight; $\circ$ 2 μ g of pilocarpine per 100 Gm. of body weight; — control.

maximal weight permitting a positive response. Thus a significant difference was found between the groups regarding sensitivity of isolated tracheobronchial walls to acetylcholine.

Similarly, the sensitivity of ileum strips between 9 other sensitive and 9 nonsensitive guinea pigs was compared (Fig. 2). The relationship between the intensity of contraction of strips due to various concentrations of acetylcholine (0.01, 0.03, 0.05, and 0.1 μ g in saline) and the various weights (equivalent to 0.4 to 3.0 Gm.) previously put on the lever was studied. The frequency of contraction of the strips that could balance each weight in each experiment is larger in the sensitive group than in the nonsensitive one. However, as Fig. 2 shows, it is apparent that the differences between the 2 groups are not significant except possibly at the 0.1 μ g dosage level. Statistical analysis of these latter results reveals the data shown in Chart II. Thus, a significant difference was not found between the two groups of animals at any dosage level.

The differences in the sensitivity of the tracheobronchial strips between the sensitive and nonsensitive groups are greater than the differences in the sensitivity of ileum strips between the groups.

Action of pilocarpine upon salivation in sensitive and nonsensitive guinea pigs

Six sensitive and six nonsensitive guinea pigs were investigated by our⁹ method in different experiments on different days. Salivation in untreated animals showed little difference between the two groups. A week later they were given a subcutaneous injection of 2 μ g of pilocarpine in saline per 100 Gm. of body weight. Similarly, 2 weeks later they were given 6 μ g of pilocarpine in saline per 100 Gm. of body weight. The salivation thus produced showed no significant difference between the two groups (Fig. 3).

Action of acetylcholine and histamine upon arterial pressure in sensitive and nonsensitive guinea pigs

Since strong bronchospasm caused by acetylcholine or histamine more or less influences arterial pressure,¹⁷ only 0.02 or 0.05 μ g of acetylcholine and 0.1 or 0.5 μ g of histamine in saline were injected either into the jugular or the penile vein to bring about a slight or moderate fall in arterial pressure. No statistically significant difference was found between the sensitive and nonsensitive animals, though the sensitive group tended to respond less to 0.02 μ g of acetylcholine (Fig. 4).

Action of acetylcholine upon cutaneous blood vessels in sensitive and nonsensitive guinea pigs

The degree of exudation caused by acetylcholine in sensitive and nonsensitive guinea pig lines was compared on the basis of the size of colored areas developing 30 minutes after the intradermal injection of acetylcholine following intravenous Evans blue dye. No significant difference was found between the two groups (Fig. 5).

Relationship between sensitization of guinea pigs to antigen and reactivity of the bronchial walls

Our former investigations^{4, 5} suggested that the reactivity of the bronchial walls of guinea pigs obtained commercially is not affected by sensitization with egg albumin. To reconfirm this finding, a further study was made of guinea pigs sensitive and nonsensitive to acetylcholine. For this purpose, the duration of standing dyspnea caused by inhaled acetylcholine (2.5 per cent) was measured twice on different days before sensitization and 21 days after intensive sensitization to egg albumin by Grove's¹⁴ method. No differences were found in the length of standing dyspnea caused by inhaled acetylcholine (2.5 per cent) before or after sensitization (Table IV). Thus, sensitivity of the bronchial walls of the sensitive animals was not further increased even by intensive sensitization. Likewise, observations on 12 nonsensitive guinea pigs showed no statistically significant change in bronchial wall reactivity following sensitization to egg

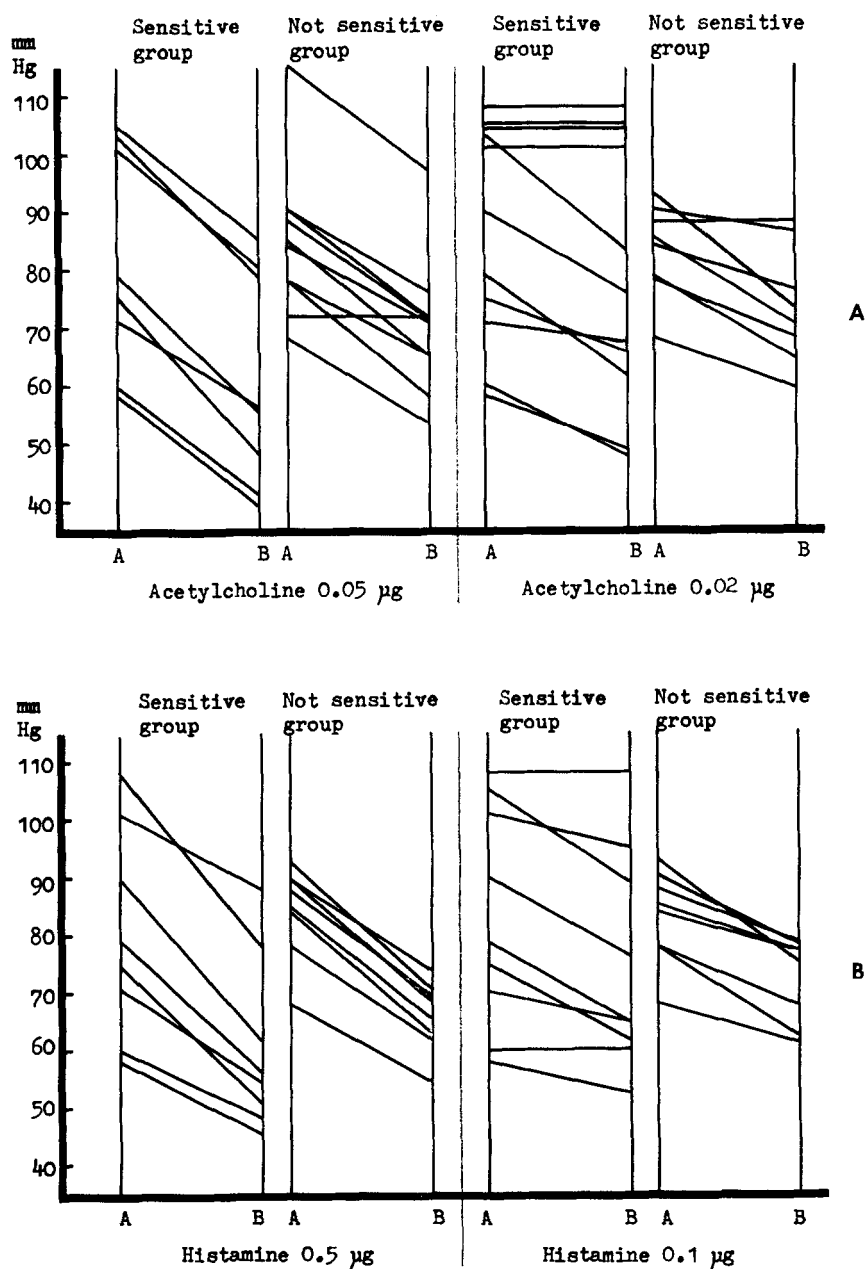


FIG. 4. Comparison of acetylcholine and histamine upon arterial pressure in sensitive and nonsensitive guinea pigs. A, Before injection; B, after injection.

albumin (Table V). In these experiments, the concentration of inhaled acetylcholine was increased to 4 per cent to shorten the time before the animals fell with dyspnea.

These findings seem to indicate that reactivity of the bronchial walls of

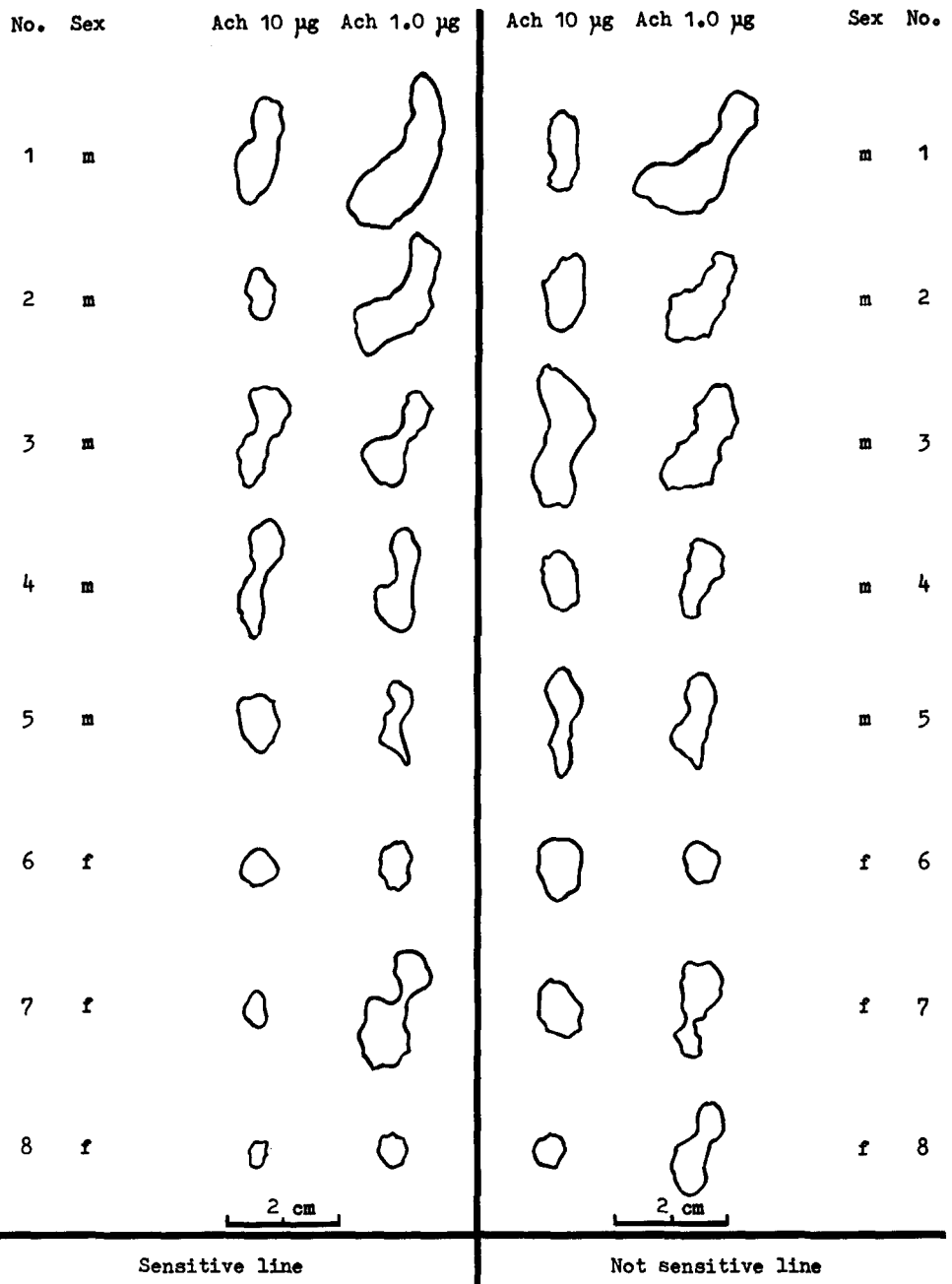


FIG. 5. Comparison of action of acetylcholine upon skin tissue between guinea pigs sensitive and not sensitive to acetylcholine.

TABLE IV. Effect of sensitization by egg albumin upon dyspnea due to inhaled acetylcholine in guinea pigs sensitive to acetylcholine

No.	Sex	Body weight (Gm.)	Time of standing dyspnea following inhaled 2.5 per cent aqueous solution of acetylcholine							
			Before sensitization				After sensitization			
			First experiment		Second experiment		First experiment		Second experiment	
			Min.	Sec.	Min.	Sec.	Min.	Sec.	Min.	Sec.
1	M	380	1	53	1	16	1	25	1	25
2	M	380	1	53	1	25	1	26	1	47
3	M	380	1	36	1	39	1	38	1	52
4	M	590	2	06	1	35	1	53	1	59
5	M	340	1	25	1	35	1	20	1	50
6	M	370	1	58	1	56	1	43	1	40
7	M	340	1	20	1	19	1	08	1	34
8	M	360	2	02	2	00	1	25	3	19
9	M	340	1	25	1	20	1	24	1	41
Means			1	44	1	33	1	29	1	54

TABLE V. Effect of sensitization by egg albumin upon dyspnea due to inhaled acetylcholine in guinea pigs not sensitive to acetylcholine

No.	Sex	Body weight (Gm.)	Time of standing dyspnea following inhaled 4.0 per cent aqueous solution of acetylcholine							
			Before sensitization				After sensitization			
			First experiment		Second experiment		First experiment		Second experiment	
			Min.	Sec.	Min.	Sec.	Min.	Sec.	Min.	Sec.
1	M	490	1	49	1	45	2	00	1	49
2	M	610	2	04	1	47	2	29	2	05
3	M	440	3	41	2	22	1	08	3	30
4	M	640	2	10	2	18	4	58	1	48
5	M	610	1	48	1	41	4	23	1	17
6	M	550	1	47	1	57	1	53	6	18
7	M	650	3	53	2	00	1	38	12	18
8	M	570	15	00	3	06	10	00	13	00
9	M	590	5	09	3	00	10	00	3	27
10	M	540	7	40	1	53	6	21	7	28
11	M	660	4	37	2	38	9	21	13	00
12	M	570	10	45	7	00	9	33	13	00

guinea pigs to acetylcholine is not the result of antigenic sensitization but is essentially of an inborn nature.

Relationship between the intensity of anaphylaxis and the sensitivity of bronchial walls

In order to study the relationship between sensitivity of the bronchial walls and intensity of anaphylaxis, the sensitive and nonsensitive guinea pigs in our study were sufficiently sensitized by 0.1 mg. of egg albumin according to Grove's method. Twenty-one days after active sensitization, challenging antigen, i.e., 1.0 mg. of egg albumin per milliliter of Tyrode's solution, was given to the animals

TABLE VI. Intensity of active anaphylaxis in guinea pigs

Sensitive line						Nonsensitive line					
No.	Sex	Body weight (Gm.)	Intensity of anaphylaxis	Time of standing dyspnea		No.	Sex	Body weight (Gm.)	Intensity of anaphylaxis	Time of standing dyspnea	
				Min.	Sec.					Min.	Sec.
1	M	510	+++	3	00	1	M	550	+	> 8	00
2*	M	510	+++	2	10	2	M	630	+	> 8	00
3	M	540	+++	1	55	3	M	620	+	> 9	00
4	M	570	+++	2	30	4	M	640	+	> 8	00
5*	M	470	+++	1	38	5	M	600	++	> 8	00
6	M	550	+++	3	45	6	M	560	++	> 8	00
7*	M	520	+++	2	15	7	M	680	+	> 8	00
8	M	500	+++	2	35	8	M	670	+	> 8	00
						9	M	600	+	> 8	00
						10	M	640	+	> 8	00
						11	M	660	+	> 8	00

+, Slight; ++, moderate; +++, strong.

*Died from anaphylaxis.

thus sensitized by inhalation until they fell with dyspnea. In this experiment the mean and standard deviation of the time duration of standing dyspnea due to inhaled egg albumin was 2 minutes 29 seconds $\pm$ 40 seconds in the sensitive line and over 8 minutes in all nonsensitive animals (Table VI).

Other members of both groups were passively sensitized by intra-abdominal administration of 2.0 ml. of rabbit anti-egg albumin serum (precipitin titer of 1:160). Twenty-four hours later the animals were challenged by inhalation for 9 minutes of Tyrode's solution containing 1 mg. of egg albumin per milliliter. In this experiment, 7 of the 9 sensitive guinea pigs showed severe or moderate dyspnea, while the remaining two showed dyspnea of a slight degree; three of the former died of anaphylaxis. Six of the eight in the nonsensitive group showed no dyspnea, but two showed moderate dyspnea (Table VII).

Thus, the intensity of bronchospasm caused by passive anaphylaxis was stronger in most of the sensitive guinea pigs when compared with the nonsensitive ones.

DISCUSSION

In order to confirm the localization of heightened reactivity to the bronchial walls of sensitive guinea pigs, the bronchial walls, salivary glands, cardiovascular system, intestinal tract, and cutaneous tissue of guinea pigs of sensitive and nonsensitive lines were compared. No significant differences were found between the lines except in the bronchial walls. The sensitivity of bronchial walls of the sensitive line does not seem to be caused by a milk factor, since this sensitivity was demonstrated 7 days after birth in almost all newborn animals from the sensitive line by means of inhalation of acetylcholine (unpublished). Furthermore, bronchial walls sensitive to acetylcholine and pilocarpine were also sensitive to other compounds having parasympathomimetic or parasympathomimetic drug-like action. Thus, it may be suggested that the basis of the sensitivity of bronchial walls of the sensitive animals is to be ascribed to the irritability of the

TABLE VII. Intensity of passive anaphylaxis in guinea pigs

Sensitive line						Nonsensitive line					
No.	Sex	Body weight (Gm.)	Intensity of anaphylaxis	Time of standing dyspnea		No.	Sex	Body weight (Gm.)	Intensity of anaphylaxis	Time of standing dyspnea	
				Min.	Sec.					Min.	Sec.
1*	M	820	+++	4	30	1	M	720	-	> 9	00
2*	M	800	+++	3	00	2	M	760	++	> 9	00
3	M	780	+++	3	30	3	M	680	++	> 9	00
4	M	740	+	> 9	00	4	M	780	-	> 9	00
5	M	560	+++	3	30	5	F	640	-	> 9	00
6	M	780	++	> 9	00	6	F	740	-	> 9	00
7	F	760	++	> 9	00	7	F	800	-	> 9	00
8	F	620	++	> 9	00	8	M	720	-	> 9	00
9	F	640	+	> 9	00						

-, None; +, slight; ++, moderate; +++, strong.

*Died from anaphylaxis.

bronchial vagi. This irritability also was demonstrated in the isolated tracheo-bronchial walls of the sensitive guinea pigs. This finding suggests that the irritability of the bronchial vagi of the sensitive guinea pigs is caused by an abnormality in the peripheral bronchial vagi including the regional ganglions.

In human asthmatic patients, too, the sensitivity of the organs or tissues is not uniformly high but appears confined to bronchial walls. This hypersensitivity of the bronchial walls has been demonstrated pharmacologically by inhalation of acetylcholine or histamine in asthmatic persons²⁰⁻²³ and in their relatives.²⁴ Takino senior made an early study of this problem neurologically by means of carotid sinus pressure. This test causes a pulmonary syndrome consisting of forced respiration and suffocating sensation in persons with hypersensitive bronchial walls.^{4, 9, 10-12, 25} It was regarded as an expression of hypersensitivity of the bronchial walls or hyperirritability of the bronchial vagi.^{26, 27} Mechanical stimulation of the carotid sinus should give rise almost equally to far-reaching responses of all organs or tissues supplied by the parasympathetic nervous system, but in asthmatic patients the pulmonary syndrome appears more frequently and is stronger than other carotid sinus syndromes such as slowing of the pulse rate, syncope, and bowel sounds.^{4, 9, 26, 27} The incidence of significant pulmonary syndrome is highest in asthmatic persons, fairly high among members of a family with a history of allergic or related diseases (especially in parents of asthmatic children), and the lowest in persons from families without histories of allergy.^{9, 26, 28-31} Thus it appears that hypersensitivity of the bronchial walls in asthmatic persons, as in our sensitive guinea pigs, is probably congenital. The hypersensitivity of the bronchial walls of sensitive guinea pigs was not further increased even by intensive sensitization with allergen. Furthermore, increased reactivity of the bronchial walls was not acquired by sensitization of nonsensitive animals. In human beings as well there seems to be no reliable evidence to indicate that hypersensitivity of the bronchial walls is the result of sensitization with allergens, but instead it appears to be caused by irritability

of the vagal or parasympathetic nerves which supply the diseased organs or tissues.^{4, 9} Some persons do not have hypersensitive bronchial walls despite high titers of skin-sensitizing antibodies.^{18, 19} When hypersensitivity of the bronchial walls is present, it may be further increased by contracting infectious respiratory diseases such as whooping cough, influenza, and pneumonia^{4, 26, 32} or inhaling poisonous gas, such as chlorine, formaldehyde, parathion (unpublished), and SO₂.^{33, 34} However, this hypersensitivity of the bronchial walls does not seem to be induced merely by these environmental factors in healthy persons who lack a congenital abnormality of the parasympathetic nervous system.⁴

This paper is dedicated to Dr. Masuichi Takino in celebration of the fortieth anniversary of his medical research.

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