

Discordant allergic manifestations in monozygotic twins: Genetic identity versus clinical, physiologic, and biochemical differences

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Clinical observations, blood group genotyping, and fingerprints established with a high degree of probability the monozygosity of two pairs of twins. Medical histories, skin test reactions, biochemical studies, pulmonary function tests, and exercise and methacholine challenges of these four individuals demonstrated that monozygotic twins: (1) may show comparable wheal and erythema skin reactions and clinical sensitivity to selected inhaled substances, but can vary in allergic specificity; (2) may be clinically discordant, one twin having chronic asthma and the other not, this difference not necessarily related to allergic sensitivities and to serum IgE levels; (3) do develop marked bronchoconstriction after inhalation of methacholine if they have a history of asthma, while non-asthmatic siblings tolerate much higher doses of this parasympathomimetic drug; (4) may experience significant impairment in ventilatory function after exercise if they have had persistent chronic asthma, while the unaffected twins can exercise with impunity; (5) may show enzymatic changes in leukocyte membranes consistent with the hypothesis that hypofunction of adenylyl cyclase²⁹ and hyperfunction of membrane transport ATPase³⁵ may be acquired abnormalities related to the presence and progression of asthma. Extended observations and continuous monitoring of twins discordant with respect to asthma ought to provide additional insight into the genetics, the pathogenesis, and the frequently unpredictable variability of this intermittent obstructive airway disease.

The familial incidence of asthma and other conditions commonly called atopic has been documented and questioned frequently.¹⁻⁵ In an effort to explore the possible genetic mechanisms involved, several investigators⁸⁻¹⁰ have focused on twins. They have shown that the prevalence of allergic manifestations among fraternal dizygotic twins is the same as in ordinary sibships, while identical monozygotic twins display a much higher degree of concordance with respect to asthma, atopic eczema, hay fever, etc. Most of these observations had to rely on superficial appearances and on simple historical or clinical data for ascertaining monozygosity and for determining the diagnosis.

During a 10 year period from July 1, 1960, through June 30, 1970, of 880 children with severe chronic asthma admitted to the Children's Asthma Re-

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search Institute and Hospital (CARIH) in Denver, 11 had an unaffected twin at home. By history and appearance these were considered to be fraternal twins. As discordance is not unusual in such cases, no further studies were carried out. Also during this decade, three pairs of twins were admitted to CARIH with both siblings suffering from severe asthma. These three pairs—4 boys and 2 girls—were considered to be monozygotic twins on the basis of appearance and birth records and also of blood groups and dermatoglyphics in the case of the last pair seen in 1969 to 1970. The present study was initiated when two more pairs of apparently identical twins, discordant with respect to asthma, came to our attention. Our purpose was (1) to confirm the monozygosity of the pairs, and (2) to investigate clinical, physiologic, and biochemical differences related to the presence or absence of asthma in these twins.

MATERIALS AND METHODS

The subjects were two pairs of twin sisters, 16 and 22 years old, respectively. Twin K. McC. was a patient at CARIH; her twin sister and Twins C. H. and K. H. volunteered to participate in this study for testing and subsequent continued recording of relevant data.²¹ Medical histories and hospitalization records were made available by the referring physicians and hospitals.* Additional information was obtained directly from the subjects and also, in the case of Twins K. McC. and S. McC., from their parents.

All examinations and tests were performed with the subjects' full cooperation and informed consent. The order of procedures was such as to avoid interference or carry-over effects. Interviews and physical examinations were completed first, next photographs, dermatoglyphics, x-rays, and blood samples were taken, and these were followed by the pulmonary function tests, allergy tests, and exercise and methacholine challenges within a period of 4 to 7 days.

A Stead-Wells 9 liter spirometer (W. E. Collins, Inc., Braintree, Mass.) was used for measurements of the sitting timed vital capacity (VC), forced expiratory volume (FEV₁), and other aspects of airway dynamics not detailed herein. Airway resistance (R_{aw}), total lung capacity (TLC), residual volume (RV), and functional residual capacity (FRC) were calculated from oscillographic tracings obtained with a variable-pressure body plethysmograph system (Electro/Med, No. 3000, Houston, Texas). Standard methods for calculating the results and for estimating normal values were used.^{11, 12} Sequential measurements of peak expiratory flow (PEF) rates for the exercise and methacholine challenges were read directly on the Wright peak flowmeter (Air-Med Ltd., Harlow, England). The exercise tests were carried out on a Warren Collins treadmill (P-2000) with variable speed and grade. Each exercise period was of 6 minutes' duration so that a steady state would be obtained (respiratory exchange ratio, $R \leq 1.0$). Expired gas collections were not included. The treadmill speed was adjusted for comfortable walking at 0° grade. In both sets of twins this was 2.5 miles per hour (m.p.h.). All other levels of exercise were performed at this speed. The asthmatic twin was exercised first. Spirometries were done 10 minutes after the end of each exercise period. Another ten minutes of rest were allowed before the grade was increased. The increase in grade was adjusted qualitatively on the basis of spirometric findings of the previous exercise period. In the case of the McC. twins, exercise was terminated at 8°; and in the case of the H. twins, it was continued to 10°. Exercise tolerances on the nonasthmatic twins were done second at duplicate grades. The methacholine (methyl beta choline, Mecholyl, Merck & Co., Inc.) inhalation tests employed serial dilutions of 0.1, 0.5, 1.0, and 2.5 per cent. Repeated inhalations were taken with a Vaponefrin (USV Pharmaceuticals Co., Inc., New York, N. Y.) nebulizer, operated by the patient so that the solution

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was aerosolized under 10 pounds per square inch pressure during successive inspirations only. The results were monitored by continuous auscultation of the chest and repeated PEF measurements every 1 to 15 breaths. Allergy skin tests were performed with the scratch method, using a Sterneedle triple-puncture device (Panray Div., Ormont Drug & Chem. Co., Inc., Englewood, N. J.) and concentrated antigens (5 per cent solutions), and also endodermally with extracts containing 500 PNU per milliliter, 0.02 ml. injected in the skin of the forearm. Immunoglobulin E was determined with the radioimmunoassay technique described by Ishizaka and associates,¹⁰ Adenyl cyclase and adenosine triphosphatase (ATPase) activities associated with leukocyte membranes (prepared by sedimenting homogenates at 5000 g, 0°, for 20 minutes) were assayed by the methods of Krishna and associates,¹⁴ and Marsh,¹⁵ respectively. Our experience with leukocyte membrane adenyl cyclase suggests that the activity of the enzyme varies over a considerable range, depending on the method of homogenizing the cells and sedimenting the membrane fraction. Therefore, the comparative experiments were performed identically, using blood drawn at the same time from the twins. Care was taken that no drugs were taken for 24 hours prior to the experiment, and no coffee or tea was ingested on the day of the experiment. Under these conditions reproducibility among replicate tubes is good, standard error of the mean being less than 5 per cent of the enzyme units. Dermatoglyphic patterns were obtained and analyzed by Dr. Wm. C. Gentry, Jr., of the Department of Dermatology, University of Colorado School of Medicine, using standard methods.^{16,17} Blood group genotyping was performed at the Belle Bonfils Memorial Blood Bank, University of Colorado Medical Center.

Case histories

Pair 1. Twins K. McC. and S. McC. were born in Ohio, the second (K) and third (S) of the five daughters in this family. The obstetric records contained the diagnosis of "single ovum" twins at birth. There was no history of asthma in the family. The mother had a history of sinusitis, the oldest sister had had eczema since infancy and continued having seasonal (ragweed) hay fever. The two younger sisters were well. The twins always shared a room at home and were in the same classroom at school through the eighth grade.

Twin K. McC. was reportedly well until the age of 5 years when, in the spring of 1959, she had her first attack of asthma associated with what was called a "bronchial infection." The frequency and severity of asthma increased rapidly. Since the age of 10, she had required treatment with corticosteroids in addition to the adrenergic bronchodilators and also had taken repeated courses of antibiotics. Factors noted to precipitate attacks of asthma included infections, foods (chocolate, nuts), weather changes, house dust, exertion, emotional upsets, and contact with animals, namely, horses and cats. The latter also caused "swelling of the eyes." Hyposensitization therapy for 9 years with dust, mold, grass, and ragweed extracts resulted in no improvement. She was admitted to CARH on November 10, 1969.

Twin S. McC. was reported to have had fewer respiratory infections and fewer school absences than her twin sister. She showed symptoms of allergic rhinitis at age 12, and she reported having had "hay fever" and "sinusitis" which were worse in the spring and summer. She had received oral antihistaminics almost daily for three years up to the time (March 24, 1970) she came to Denver. Hyposensitization injections with dust, mold, and ragweed antigens had been given for nearly three years with some apparent benefit. Twin S. McC. reported wheezing only once, about the age of 13, when both twins had a "cold." Interestingly, her twin, K. McC., had no asthma with that same illness, but only marked nasal congestion. Exposure to cats had caused Twin S. McC. to have watery and swollen eyes and sneezing.

Pair 2. Twins K. H. and C. H. were born in California, first (C) and second (K) in a family of four girls. Their father had wheezing episodes as a child and only allergic rhinitis as an adult. The mother and sisters had no asthma. One sister was reported to have had a chronic morning cough, and another sister possible gastrointestinal allergy in infancy and later allergic rhinitis.

TABLE I. Blood genotypes of twins

Blood group	K. McC. (A ₁)	S. McC. (A ₁)	K. H. (O)	C. H. (O)
Rh system:				
D	Pos.	Pos.	Neg.	Neg.
C	Pos.	Pos.	Neg.	Neg.
C ^w	Neg.	Neg.	—	—
E	Neg.	Neg.	Neg.	Neg.
c	Pos.	Pos.	Pos.	Pos.
e	Pos.	Pos.	Pos.	Pos.
M	Pos.	Pos.	Pos.	Pos.
N	Pos.	Pos.	Neg.	Neg.
S	Pos.	Pos.	Pos.	Pos.
s	Pos.	Pos.	Neg.	Neg.
P	Pos.	Pos.	Neg.	Neg.
K	Neg.	Neg.	Neg.	Neg.
Fy ^a	Pos.	Pos.	Pos.	Pos.
Jk ^a	Pos.	Pos.	Pos.	Pos.
Jk ^b	Neg.	Neg.	Neg.	Neg.
Le ^a	Neg.	Neg.	Neg.	Neg.
Le ^b	Neg.	Neg.	Pos.	Pos.

Twin K. H. was in good general health with the usual mild childhood illnesses until the age of 3 years when she developed her first attack of asthma following an acute febrile respiratory illness. Asthma "snowballed" to age 7 and remained severe between the ages of 7 and 14 years. Cyanosis accompanied severe attacks, which required frequent injections of epinephrine and repeated courses of corticosteroids for control. Elimination diets and hyposensitization injections (molds and epidermals) were ineffective. A move to Maryland at age 16 was associated with improvement in the winter but still "miserable" summers. Since the age of 18, attending college in Switzerland and California, she has had very little asthma, requiring only occasional (about one a week) oral bronchodilator tablets and no other treatment for asthma.

Twin C. H. reported no unusual or serious medical problems and no clinical allergic reactions except for "itchy and watery eyes" after contact with rabbits (in the laboratory). In the course of a common "cold" she found herself wheezing, an experience which frightened her initially but which did not last long. She had received no antiallergic treatments.

The twins shared a room in their home until they were 17 years old and attended the same school until college age.

Neither pair of twins provided any data suggesting that psychological factors^{16, 17} could account for the medical differences noted.

RESULTS

Initial physical examination ascertained the closely similar appearance of the twin sisters in both pairs. Twin K. McC. was heavier than her sister—possibly a consequence of steroid therapy—and Twin K. H. was shorter than her sister, C. H. At the time of the study, Twin K. McC. was still taking prednisone, 10 mg. every other day, but all other bronchodilator drugs were stopped. The other three subjects did not take any medication before or during the week of this investigation. None of the four subjects had any symptoms or signs of asthma, eczema, or rhinitis when first examined for this study.

Blood group genotyping revealed identical results for the two pairs of twins (Table I). Dermatoglyphic analyses revealed that 10 out of 10 finger patterns

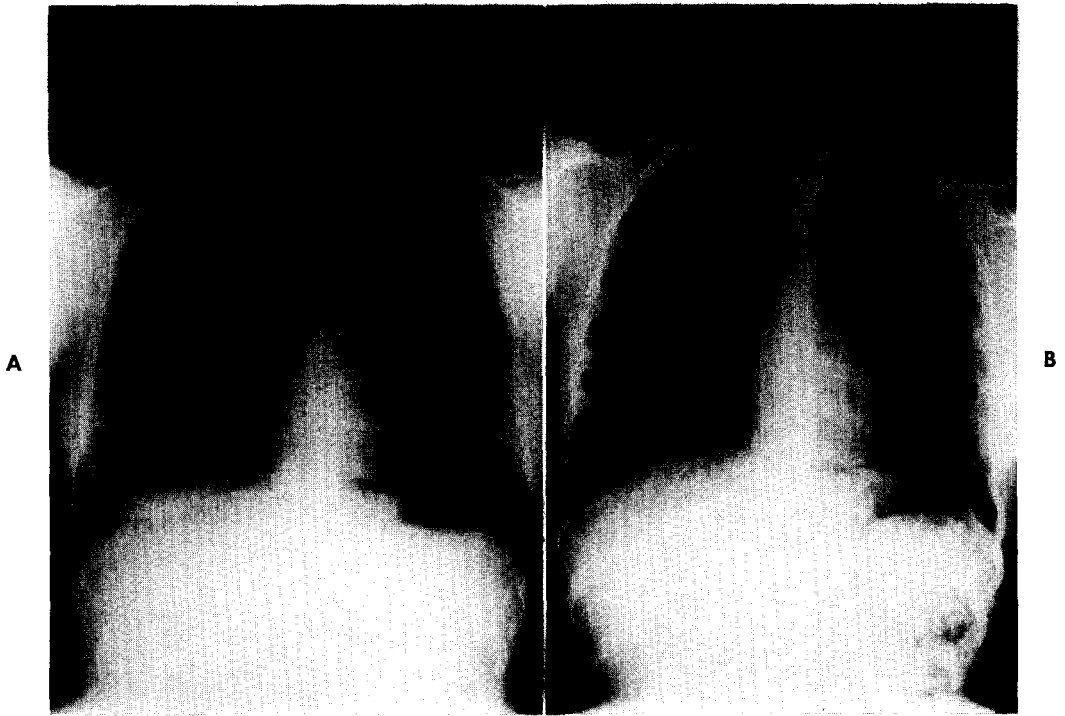


FIG. 1. X-ray films taken the same day to show a relatively mild hyperinflation of chest of Twin K. McC. (A) compared with that of her twin sister (B).

of Twins K. McC. and S. McC. were identical. The finger ridge counts also were identical. Twins K. H. and C. H. had 9 out of 10 finger patterns identical. The difference in total finger ridge counts was 18. The palmar patterns were identical. These findings were consistent with monozygosity.^{6, 8}

Chest x-rays, posteroanterior and lateral, showed a relatively mild (considering the duration and severity of asthma) hyperinflation of the lungs with increased translucency in the films of the twins with asthma, more noticeable in the case of Twin K. McC. (Fig. 1).

Blood eosinophil counts per cubic millimeter were: Twin K. McC., 342 (8 per cent), Twin S. McC., 375 (9 per cent), Twin K. H., 594 (9 per cent), and Twin C. H., 270 (4 per cent). Serum immunoglobulin E levels were within the range considered normal, at 125 ng. per milliliter for Twins K. McC. and for S. McC., 165 ng. per milliliter for Twin K. H. and under 125 ng. per milliliter for Twin C. H. All four subjects showed immediate-type reactions to skin testing with a number of antigens. Endodermal tests generally gave stronger reactions than scratch tests. Twins K. McC. and S. McC. were negative to timothy and *Alternaria* by scratch but positive endodermally, while Twins K. H. and C. H. reacted only with mild erythema by scratch but with sizable wheals and erythema after endodermal testing. The latter were considered more significant and, for the sake of clarity, are exclusively shown in Table II. Although the reactions in

TABLE II. Endodermal skin tests to selected allergens in two pairs of identical twins

Allergen	Pair 1				Pair 2			
	K. McC.		S. McC.		K. H.		C. H.	
	Wheal	Erythema	Wheal	Erythema	Wheal	Erythema	Wheal	Erythema
Pollen								
Grasses								
Timothy	5	20	8	25	0	0	0	0
Bermuda	NT	NT	NT	NT	5	20	6	20
Weeds								
Giant ragweed	5	15	9	25	7	20	3	20
Common sage	NT	NT	NT	NT	5	20	6	25
Molds								
Alternaria	7	20	6	25	10	15	13	25
Hormodendrum	NT	NT	NT	NT	7	15	0	0
Epidermals								
Cat	11	40	7	25	3	10	3	10
Dog	13	35	5	15	5	10	0	0
Horse	8	25	0	0	0	0	0	0
Rabbit	NT	NT	NT	NT	5	10	0	0
Feathers								
Chicken	6	15	10	25	0	0	0	0
Wool	0	0	0	0	5	8	0	0
House dust	10	30	9	30	7	25	7	25
Histamine	10	30	7	20	7	30	5	30
Buffered saline	0	0	0	0	0	0	0	0

Largest diameter in millimeters, 10 to 15 min. after application of extract: 0 = negative; wheal 1 $\geq$ 10 mm. = 3 to 4+; NT = not tested.

each pair of twins were quite similar, a few noteworthy differences were observed, such as Twin K. McC.'s reaction to horse epithelium, and Twin K. H.'s reaction to Hormodendrum, rabbit hair, and lamb's wool.

Initial pulmonary function measurements on all subjects (Table III) were within the range of normal, a finding consistent with the clinical absence of symptoms. Of course, the clinical diagnosis of asthma could not be founded on such single measurements but was based on history and on subsequent tests repeated daily.²¹

Only one of the four subjects (K. McC.) responded to the three successive 6 minute walks on the treadmill with asthma, a drop in VC and FEV₁ and an increase in R_{AW}, RV/TLC, and FRC/TLC (Table III and Fig. 2). The maximum work load was comparable for all four girls, differing only moderately in relation to their weight: at 8° it was 3,360 Kg. M. for Twin K. McC., 2,790 for Twin S. McC., 3,220 for Twin K. H., and 3,150 for Twin C. H. Tachycardia ranging from 150 to 168 was noted following the third effort on all subjects. An inhalation of isoproterenol aerosol relieved Twin K. McC.'s exercise-induced asthma promptly.

Both subjects with a history of asthma (K. McC. and K. H.) experienced a rapid bronchoconstriction after metacholine inhalations: Twin K. McC. after only 6 inhalations of 0.1 per cent methacholine and Twin K. H. after inhaling

TABLE III. Pulmonary function of twins before and after exercise and methacholine inhalations

A. Pair 1								
	K. McC.: height, 155 cm., weight, 60 Kg.				S. McC.: height, 155 cm., weight, 50 Kg.			
	VC (L.)	FEV ₁ (L./sec.)	FEV ₁ /VC (%)	PEFR (L./min.)	VC (L.)	FEV ₁ (L./sec.)	FEV ₁ /VC (%)	PEFR (L./min.)
Predicted values	3.30	2.52	75	380	2.95	2.45	75	380
Per cent predicted								
Exercise								
Resting values	84	79	71	—	90	94	86	—
6 min., 2.5 m.p.h., 4°	77	58	58	—	—	—	—	—
6 min., 2.5 m.p.h., 8°	62	34	42	—	102	94	76	—
Methacholine								
Control	87	76	67	79	106	101	79	90
0.08 mg.	57	46	61	37	—	—	—	—
0.10 mg.	—	—	—	—	—	—	—	83
0.80 mg.	—	—	—	—	—	—	—	73
3.30 mg.	—	—	—	—	99	57	48	68

B. Pair 2								
	K. H.: height, 155 cm., weight, 46 Kg.				C. H.: height, 157 cm., weight, 45 Kg.			
	VC (L.)	FEV ₁ (L./sec.)	FEV ₁ /VC (%)	PEFR (L./min.)	VC (L.)	FEV ₁ (L./sec.)	FEV ₁ /VC (%)	PEFR (L./min.)
Predicted values	3.02	2.50	75	375	3.12	2.59	75	390
Per cent predicted								
Exercise								
Resting values	98	104	87	—	90	102	94	—
6 min., 2.5 m.p.h., 4°	96	97	84	—	92	108	97	—
6 min., 2.5 m.p.h., 10°	92	82	74	—	95	108	94	—
Methacholine								
Control	95	85	74	104	100	107	89	105
0.10 mg.	—	—	—	77	—	—	—	94
0.20 mg.	69	32	39	21	—	—	—	90
3.30 mg.	—	—	—	—	97	93	80	90

1 ml. of 0.1 per cent and 3 whiffs of 0.2 per cent methacholine within a 15 to 20 minute period. They inhaled an estimated 0.08 and 0.2 mg. of methacholine (Fig. 3), respectively, which in both cases produced a precipitous drop in PEF rates. The monitoring of the test with PEF made it possible to interrupt the procedure promptly; consequently, only mild wheezing occurred and no dyspnea was noted. The effect was very transient and seemed to subside in 5 to 10 minutes. Twins S. McC. and C. H. showed only a slight drop in PEF rate after more than one hour of testing and after more than 50 inhalations of 2.5 per cent methacholine (total 1 ml.). Spirographic and plethysmographic measurements on these two nonasthmatic twins suggested that after inhaling about 30

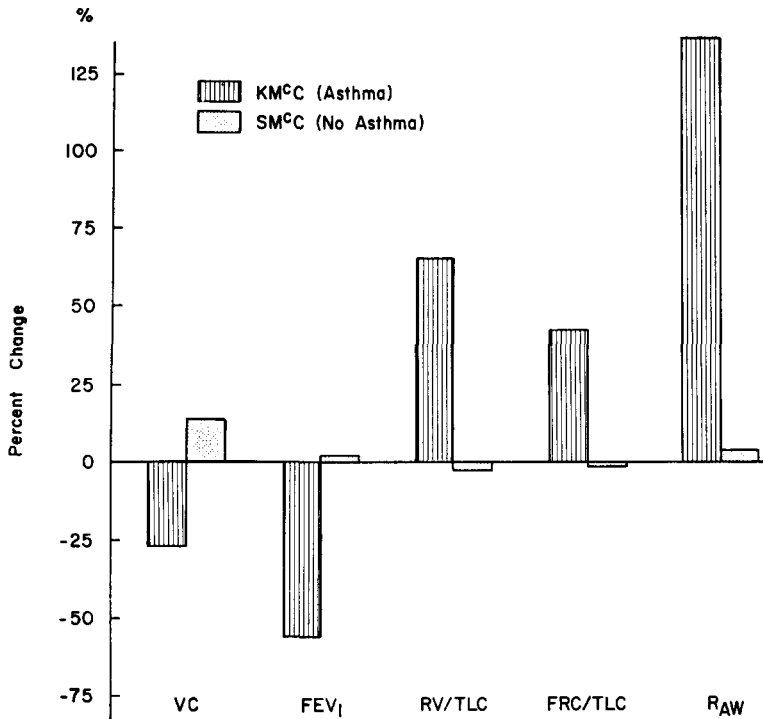


FIG. 2. Effect of exercise on pulmonary function of monozygotic twins. Measurements after 6 minutes at 2.5 miles per hour, 4 degrees, and another 6 minutes at 2.5 miles per hour, 8 degrees, on the treadmill compared to initial values.

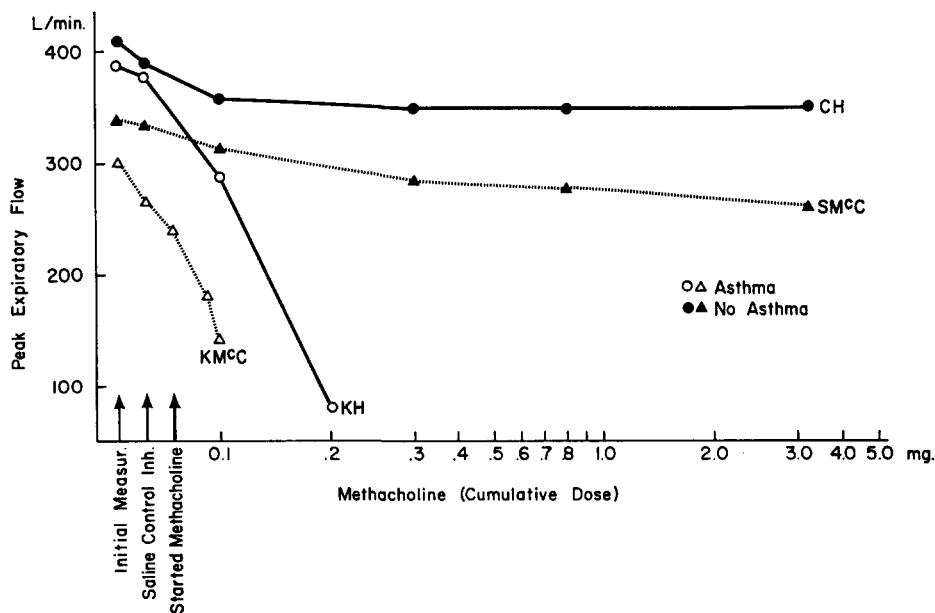


FIG. 3. Effect of methacholine on peak expiratory flow rates of twins.

TABLE IV. Leukocyte enzymes

Enzyme	No asthma		Asthma		Ratio		P	
					C. H./ K. H.	S. McC./ K. McC.	C. H. vs. K. H.	S. McC. vs. K. McC.
	C. H.	S. McC.	K. H.	K. McC.				
Adenyl cyclase*								
Basal	111	164	71	125	1.56	1.31	< 0.01	< 0.05
Isoproterenol	155	234	115	149	1.35	1.57	< 0.01	< 0.01
Increase with isoproterenol (%)	40†	43†	62†	11‡	0.65	3.90		
ATPase§								
Medium								
Na, K	0.31	0.53	0.89	0.60	0.35	0.88	< 0.01	< 0.01
Na, K, Mg	1.31	3.33	1.95	3.90	0.67	0.85	< 0.01	< 0.01

*Adenyl cyclase units are counts per minute found in ^3H -cyclic AMP formed from ^3H -ATP per milligram of protein per two hours at 37° .

†p < 0.01, indicating a significant stimulation by isoproterenol.

‡p < 0.10, indicating no significant stimulation by isoproterenol.

§ATPase units are n moles phosphate formed from ATP per milligram of protein per ten minutes at 37° .

times more methacholine than did their sisters they might have shown a borderline response, but they certainly did not show the pronounced sensitivity to parasympathetic stimulation so typical of asthma.^{22, 23}

Adenyl cyclase, the enzyme which acts as the beta-adrenergic receptor in most tissues,³⁴ was assayed in the membrane portion of leukocytes. As shown in Table IV, basal activity was significantly higher for Twin C. H. than for Twin K. H. Isoproterenol (10^{-4}M) stimulated the activity for both H. twins. Although the percentage stimulation for Twin K. H. was higher than that for Twin C. H., total enzyme activity remained higher for Twin C. H. Basal activity for Twin S. McC. was slightly higher than that for Twin K. McC., and isoproterenol provided a significant increase for Twin S. McC. However, the small increase for Twin K. McC. was not statistically significant. This is of note since Twin K. McC. was the more severely asthmatic patient and required admission to CARIH.

Adenosine triphosphatase activity was measured in leukocyte membranes in the presence of sodium and potassium cations, either with or without added magnesium. In all comparisons the leukocytes from the asthmatic subjects provided greater activity than those from the nonasthmatic subjects. In the presence of magnesium, the enzyme activity increased about fivefold for all but Twin K. H., who had already quite high activity in the absence of magnesium.

DISCUSSION

The concordance and discordance of asthma and atopy in monozygotic twins have been surveyed in various populations and under diverse circumstances.^{3-10, 25-28}

Two facts about monozygotic twins appear impressive: (1) Concordance is significantly high, i.e., asthma occurs more frequently among both twins in a monozygotic pair than among dizygotic twins or ordinary sibships.^{8, 9, 26, 27} (2)

Conversely, discordance does exist, and in many pairs of monozygotic twins asthma affects only one of the two individuals.^{10, 22, 25, 28}

Schade²⁸ tabulated reports published between 1935 and 1962 and found that 59 per cent of monozygotic twins but only 12 per cent of dizygotic twins were concordant for atopy. The possible preferential publication of concordant findings was brought up by Pfandler,²⁹ who used these data to estimate the genetic significance of concordance numbers. In a survey of 13,992 twins in Sweden, Lubs⁷ found the concordance number (defined as the number of concordant pairs in relation to the number of pairs in whom one or both the partners show the symptoms) significantly ($p < 0.001$) higher in monozygotic than in dizygotic twins for asthma (19.0 versus 4.8), hay fever (21.4 versus 13.7), and eczema (15.5 versus 4.0). The possible genetic inferences from these observations and also from an analysis of family histories of 756 children at CARIH are outlined in a separate article.³³

The main purpose of the present communication is to document the differences of monozygotic twins in relation to the clinical and laboratory manifestations of asthma. The procedures employed established with a high degree of probability the genetic identity of these pairs.^{17, 18} It seemed impractical—and an added imposition on our volunteer subjects—to extend the study to include tissue transplantations, etc., in order to establish monozygosity with absolute certainty. The study preferentially attempted to ascertain the circumstances and the biological responses related to the discordance of asthma in these twins.

Medical histories of both twins with asthma revealed that the first attack had been associated with a febrile respiratory infection. It is noteworthy that the other twin was reported not to have become ill at that time, an observation consistent with previous reports on infectious diseases among twins, which indicated that while the concordance for rubeola is 97.4 per cent for monozygotic and 94.3 per cent for dizygotic twins, for pneumonia it is only 32.3 per cent for monozygotic and 18.2 per cent for dizygotic twins.²⁹ Later in their lives both twins in both pairs reported frequent respiratory infections, and, indeed, each of the unaffected twins mentioned at least one brief episode of mild wheezing. Why the affected twins continued to have chronic and severe asthma after the initial attack and why their counterparts wheezed only once in their lives could not be explained by the information available.

All four subjects presented reliable clinical evidence of allergic sensitivities. The capacity of all four subjects to react with wheal and erythema to several allergens did not appear to determine the occurrence of asthma, unless one wishes to speculate that Twin K. McC.'s reaction to horse epithelium and Twin K. H.'s reaction to hormodendrum, wool, or rabbit hair were the determining factors. Such an assumption could not be supported by the clinical data, and neither could it explain the chronicity of the disease. The few differences in specificity seemed noteworthy, mainly in view of the identical genetic and environmental influences throughout childhood on these subjects, and did confirm previous observations.^{2, 8, 9} Serum levels of immunoglobulin E were found to be significantly below the level of 700 ng. per milliliter considered as the upper

limit of normal. The wide range of values observed in children with chronic asthma⁸ and the variability in sequential IgE determination in the same individual suggest that, quantitatively, serum IgE may bear no direct relationship to the clinical manifestations of asthma.

Initial studies of pulmonary function showed no abnormalities. The alveolar-arterial O₂ tension differences and the ratios of physiologic dead space to tidal volume (VD/Vt) recently reported to persist between attacks of asthma³¹ were not measured. The tests performed and their changes after exercise and after inhalation of metacholine reflected consistently the clinical differences between the twins with a history of asthma and their nonasthmatic siblings. Measurements of peak flow rates after every few inhalations of methacholine promptly detected early changes³⁰ and prevented overexposure to parasympathetic stimulation. The spontaneous reversibility of the methacholine-induced bronchoconstriction and the effect of isoproterenol on exercise-induced asthma were also quickly detected by PEF measurements. The spirographic tracings confirmed and amplified the information provided by peak flow rates. The trend for PEF of Twin S. McC., for example, to gradually drop after methacholine inhalations could be further documented by spirographic tracings. Thus, one might speculate that the qualitative differences (positive versus negative responses) after methacholine inhalations could, with sufficiently high doses, become more quantitative, any final differences being only in the amount of methacholine required to produce a comparable reduction in respiratory flow rates. In our studies inhalations were stopped after about one hour, as other undesirable effects of parasympathetic stimulation (urge to urinate) were noted.

The exercise tolerance tests showed a significant fall in the per cent of predicted FEV₁ (FEV₁ measured, divided by 0.83 × predicted VC) in both asthmatic patients. The fall in per cent of FEV₁ was dramatic in the case of Twin K. McC. and produced severe symptoms of asthma. The fall observed in Twin K. H. appeared physiologically significant but did not produce any symptoms.

Enzyme studies with leukocytes from the four subjects showed that adenylyl cyclase activity was higher in the twins who never had asthma or rhinitis and that it was not stimulated by isoproterenol (a beta-adrenergic agonist) in the cells of the twin who still had asthma at the time of the study. This observation is consistent with the beta-adrenergic blockade theory of asthma. Further work is in progress to determine the effects of clock hour, symptoms of asthma or other disease, range of values in populations relative to IgE levels, and various drugs on this leukocyte enzyme. Adenosine triphosphatase, on the other hand, is stimulated by norepinephrine and not by isoproterenol and thus is regarded as an alpha-adrenergic function.³⁶ Our findings may represent the result of an increased effect of circulating norepinephrine in the asthmatic patients. Alternatively, the results might be explained in terms of allosteric inhibition of ATPase by cyclic AMP as shown by Moszik³⁸ and Coffey.³⁷ Since the asthmatic patients had lower adenylyl cyclase levels, their cells would produce less cyclic AMP, decreasing the amount of ATPase inhibition.

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