

From the Department of Paediatrics,
University Medical School, Szeged, Hungary (Director: Domokos Boda)

INVESTIGATION OF THE CROSS REACTION OF COW'S HAIR AND MILK ANTIGEN IN BRONCHIAL PROVOCATION

By

P. OSVÁTH, L. MURÁNYI, L. ENDRE & G. HARSÁNYI

In infancy and childhood the most important protein source is cow's milk. Among those susceptible, temporary milk allergy manifested in eczema, urticaria and spasmodic bronchitis, is frequent before the age of three (8, 10, 18). It was demonstrated in our previous investigations (12, 17, 19) that cow's hair extracts contain bovine serum albumin and alpha-globulin in addition to two protein fractions with molecular weight 20,000. Thus, there is a common antigen in milk and cow's hair. It is possible that a reagin which induces milk allergy in infancy through enteral sensitization, also sensitizes the organism to cow's hair introduced by inhalation at a later age. However, as the two small-molecular fractions of the hair extract are different from the serum proteins, the production of cross allergy *in vivo* is not inevitable despite the identity of the other two components (17). The importance of immunological investigations, therefore, had to be ascertained with *in vivo* observations.

MATERIAL AND METHODS

The children examined were 3-14 years of age. The results of skin reactions to cow's hair extract (Pasteur Institute, Paris) were compared with the histories of atopic dermatitis and urticaria during infancy of 176 asthmatic patients,

using computer-evaluated punched-cards. Inhalative provocation was carried out in nine patients having asthmatic symptoms regularly after consumption of pasteurized milk, indicating that they undoubtedly had respiratory allergy to milk at the time of examination. Provocation was also carried out in four children not allergic to milk but exhibiting a positive skin reaction to cow's hair extract. Bronchospastic symptoms appeared during the skin test in one further milk-allergic child and in one giving a skin reaction only to cow's hair. Both of these patients participated in scarification tests performed with cow's hair fractions separated and concentrated by DEAE chromatography (12).

The provocation was performed by inhalation of cow's hair antigen in sprays diluted 1/10,000, 1/1,000 ad 1/100, respectively, for five minutes. The bronchospasm was recorded by means of a pneumotachograph (Godart) using it for the measurement of integrated volumes (VC, FEV, FEV_{1.0}). This examination was only performed in children more than seven years old (7, 9, 21) and was carried out before, and 15 minutes, 6 hours and 24 hours after provocation. In all patients but one the expiratory capnogram (Capnograph, Godart) was recorded during and after provocation at the times mentioned above.

Our previous examinations (14, 15) showed that the slopes of the ascending line and plateau of the curve of CO₂ (capnogram) in the expiratory air reliably indicate the induction of bronchospasm, even before the age of seven years. The state of the gas-exchange is given by the quotient of the base-line projections of the plateau and ascending line (T_2/T_1). If the fall in the quotient exceeded 40 per cent, the provocation was evaluated as positive. The clinical severity of the asthmatic state was evaluated by a slightly modified (16) Engström classification (7): Grade V is the most severe asthmatic crisis, grade III a medium asthmatic attack and grade II a markedly elongated expiration without resting dyspnoea.

TABLE 1

Relation between Skin Reaction to Cow's Hair Extract and Cutaneous Symptoms Observed after Introduction of Cow's Milk during Infancy.

Skin reaction to 100 Norm. U. cow's hair	Anamnesis data		No cutaneous symptoms	
	Eczema	Urticaria		
+++	12 (30 %)	9 (22 %)	19 (48 %)	40
++	15 (28 %)	13 (25 %)	25 (47 %)	53
Negative	11 (13 %)	17 (21 %)	55 (66 %)	83
$p < 0.01$		$t = 7.787$		176

MILK ALLERGY. PROVOCATION: COW'S HAIR EXTRACT

K.T. ♂ (3y)

Dg: Asthma bronchiale

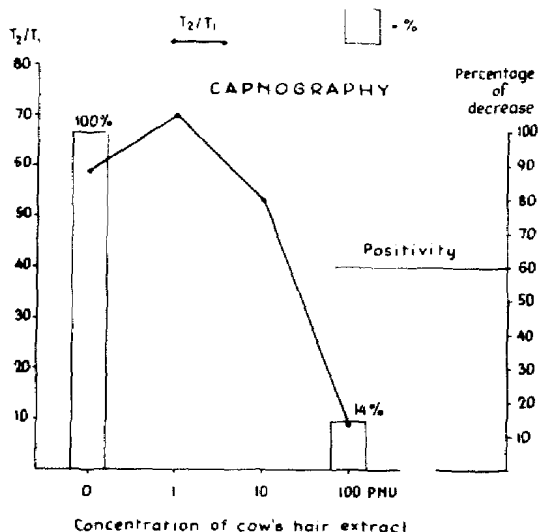


Fig. 1.

Capnography of patient KT. Explanation: See text.

RESULTS

A comparison of the skin reactions of asthmatic patients to cow's hair extracts with the cutaneous symptoms in infancy described in the case record (Table 1) showed that previous eczema was significantly more frequent in the anamnesis in the case of positivity ($p < 0.01$, $t = 7.787$), whereas urticaria occurred in the same ratio in positive and negative groups.

As the clinical significance of the skin test may be of restricted value, inhalative provocation using cow's hair extract was carried out in nine children who were undoubtedly

TABLE
Respiratory Provocation of Milk-Allergic
 (10.000)

Name, sex, age			Skin reaction Milk protein	Cow's hair (100 PNU)
1.	T. K.	boy 3 years	+++	++++
2.	A. B.	boy 6 years	+	++
3.	C. T.	boy 5 years	o	+++
4.	I. T.	boy 4 years	BSA+++	++++
5.	S. D.	boy 8 years	o	++++
6.	E. C.	girl 5 years	BSA+	+++
7.	Z. G.	boy 13 years	BSA++	+++
8.	I. P.	girl 5 years	BSA+++	+++
9.	M. B.	boy 4 years	BSG++	+++
10.	J. Sz.	boy 12 years		
				Non-allergic
11.	L. G.	boy 14 years	o	++++
12.	G. G.	boy 13 years	o	+++
13.	J. D.	boy 10 years	o	+++
14.	G. L.	boy 5 years	o	+
15.	Z. L.	boy 12 years	o	++++

milk-sensitive and reacted to milk consumption with asthmatic symptoms.

K. T., a boy of 3 (Fig. 1), was treated for several months in various institutions because of a lasting bronchospasm. After being admitted to this institution, the symptoms ceased if milk was eliminated from the food, but when milk was reintroduced, urticaria and asthmatic dyspnoea developed. The

²
Children with Cow's Hair Extract
 PNU/ml)

Acetyl- choline sensitivity	Respiratory provocation (10,000 PNU/ml) Early reaction		Clin.	Late reaction	Cow-shed
	Spirometry	Capno- graphy T ₂ /T ₁			
800	—	58-8+	III	o	none
	Tiff:				
—	56-46 p. c.	25-16±	o	o	none
600	—	31-24±	I	III	none
—	—	—	II	o	yes
	Provocation with 100				
200	PNU extract	29-12+	II	o	yes
500	—	51-62—	o	II	yes
	Tiff:				
—	74-65 p. c.	26-12+	o	II	none
800	—	52-55—	o	o	none
—	—	30-36—	o	o	none
	Tiff:				
400	92-96 p. c.	65-64	o	o	yes
to milk					
2000	VC:	36-19+	II	o	yes
200	3.6-2.5 L	—	II	o	yes
4000	—	70-74—	o	o	yes
	Tiff:				
600	69-67 p. c.	71-75—	o	o	none
	Tiff:				
400	83-86 p. c.	94-82—	o	o	none

cow's hair extract, administered by inhalation in a dilution of 10,000, elicited a considerable bronchospasm and a corresponding change in the capnogram. This child did not stay or live in the neighbourhood of a cattle-shed.

L. Gy., a boy of 14 (Fig. 2), suffered from an extended eczema during infancy, but at the time of examination, no symptoms were provoked by milk consumption. He had lived in the vicinity of a cow-shed. There was a strongly positive skin reaction to cow's hair extract, and the bronchospasm could be provoked by inhalation too.

MILK ALLERGY DURING INFANCY. PROVOCATION: COW'S HAIR EXTRACT

Gy. L. ♂ (14 y.) Dg: Asthma bronchiale

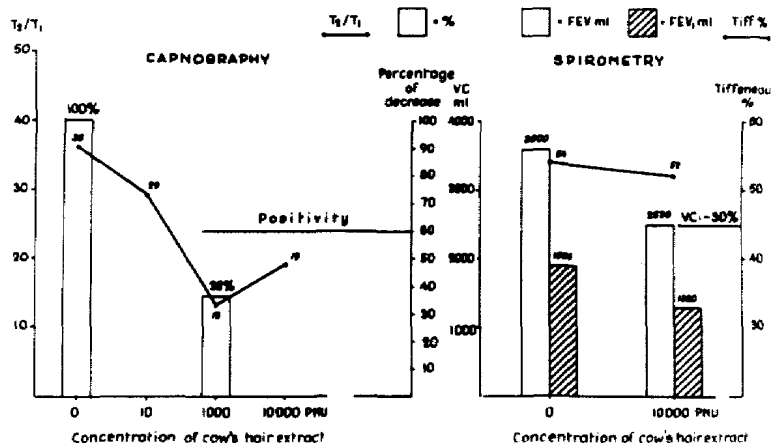


Fig. 2.

Capnography and spirometry of patient L. Gy. Explanation: See text.

To summarize the respiratory provocation data (Table 2), of the ten milk-allergic patients, four reacted to the cow's hair extract with an early, one with a mild early and a serious late, and two with a late bronchospasm, after six and twenty-four hours. In three patients, the provocation was negative. At the time of investigation a positive provocation to cow's hair inhalation was observed in two of the children not sensitive to milk. One of these was the patient L. Gy. mentioned above, who had eczema in infancy; the other had never been sensitive to milk. This child gave a positive skin reaction to fraction I obtained by DEAE chromatography of the cow's hair extract (12), *i. e.* to the protein of molecular weight 20.000, characteristic of the hair extract, but absent from the serum. In this child respiratory tract symptoms were also provoked by the same component, and he had been living in the neighbourhood of a cow-shed.

DISCUSSION

Our statistical investigations indicate that children giving a positive skin reaction to cow's hair have suffered from eczema in infancy with significantly higher frequency than asthmatic patients with a negative reaction.

In the same patients a similar correlation was not found between a positive skin reaction to other extracts (house dust, feather, candida, fungi) and eczematous anamnesis. These results are similar to those of *Tuft et al.* (20): If parenteral sensitization was carried out on rabbits with bovine serum albumin followed later by the same material given in aerosol, allergic bronchitis and alveolitis developed.

In the respiratory provocation tests ten children were examined in whom dyspnoea developed after milk-consumption. In seven of these, the inhalation of cow's hair extract was followed by bronchospasm. This is a higher ratio than the 30-60 per cent positive correlation to the skin test with the identical antigen, as described in the literature (1, 4, 11). *Christiaens et al.* (5) observed bronchospasm in only 20 per cent of cases showing a positive skin reaction to cow's hair after inhalation of the same allergen extract. In the case of milk allergy, on the other hand, only some proteins, (*i. e.* the serum proteins: albumin, alpha-globulin) can give a cross reaction to the cow's hair extract, while beta-lactoglobulin has a different antigenic structure (16, 17).

The high ratio of positivity in the provocation tests on milk-allergic children shows the importance of ingestive sensitization in cow's hair asthma (3). The serum proteins are contained in fraction III (6) from the DEAE chromatography of cow's hair extract. As the protein-bound hexose content of the same component is high and it is yellow, the proteins in the cow's hair extract might also contain lysin-hexose bindings (2, 3) which increase its allergen activity. The UV spectrum of the cow's hair fractions also suggests this possibility (6). Nevertheless a child living in the vicinity of a cow-shed may be sensitized exclusively to the small-molecular

component of cow's hair whose antigenicity is different from that of serum proteins, as it was shown in one of our patients. The immunological investigations and the connection between the positive cow's hair skin test and the eczematous anamnesis equally suggest that in consequence of the common component, the antigens inducing enteral milk allergy in infancy also sensitize to cow's hair introduced by inhalation into the organism.

SUMMARY

The biological cross reactions of milk and cow's hair extract were examined in asthmatic children allergic to milk. Patients with positive skin reaction to cow's hair extract had suffered from eczema in infancy significantly more often. Seven of ten children suffering from asthmatic dyspnoea due to milk consumption also reacted with bronchospasm to cow's hair extract administered in inhalative provocation. It is concluded that the reagins responsible for the transitory milk-induced bronchospasm in infancy often cause asthmatic dyspnoea after inhalation of cow's hair.

REFERENCES

1. *Aas, K.*: Bronchial Provocation Tests in Asthma. *Arch. Dis. Childh.* 45, 221, 1970.
2. *Berrens, L.*: An Analytic Approach to the Frequency Distribution of Positive Skin Reactions to Atopic Allergens. *Ann. Allergy* 29, 118, 1971.
3. *Bleumink, E. & Young, E.*: Identification of the Atopic Allergen in cow's Milk. *Int. Arch. Allergy* 34, 521, 1968.
4. *Chaptal, J., Jean, R., Jarrige, J. Cl., Titaut, H., Mollet, H. & Van Hai, N.*: Exploration spiromographique de l'asthme. *Arch. Franc. Péd.* 23, 851, 1966.
5. *Christiaens, L., Lelong, M., Farriaux, J. P. & Henne, J.*: Apport des tests bronchomoteurs dans l'allergie aux poils animaux chez l'enfant. *Presse Méd.* 72, 3427, 1964.
6. *Engström, I.*: Respiratory Studies in Children. XI; *Acta Paed. Scand. Suppl.* 155, 1964.
7. *Engström, I.*: Respiratory Studies in Children. XII. Serial Studies of Mechanics of Breathing Lung Volumes and Ventilatory Capacity in Provoked Asthmatic Attacks. *Acta Paed. Scand.* 53, 345, 1964.

8. *Freier, S., Kletter, B., Gery, I., Lehenthal, E. & Grifman, M.*: Intolerance to Milk Protein. *J. Pediat.* 75, 623, 1969.
9. *Geubelle, F. & Hoffmann, T.*: Diagnostische Möglichkeiten beim Asthma-Syndrom im Kindesalter. *M Schr. Kinderheilk.* 119, 233, 1971.
10. *Goldman, A. S., Anderson, D. W., Sellers, W., Saperstein, S., Kniker, W. T. & Halparn, S. R.*: Milk Allergy. *Pediatrics* 32, 425, 1963.
11. *Hajós, M. K.*: Inhalation Tests with Pneumoallergens in Bronchial Asthma. *Acta Allerg.* 23, Suppl. VIII. 1967.
12. *Havass, Z. & Osváth, P.*: Biochemische Struktur des Kuh-Haar Extraktes. *Allergie u. Immunol.* 17, 4299, 1971.
13. *Leikola, J. & Vyas, G. N.*: Human Antibodies to Ruminant IgM Concealing the Absence of IgA in Man. *J. Lab. Clin. Med.* 77, 629, 1971.
14. *Murányi, L., Osváth, P., Uhl, K. & Osváth, P.*: Continuous Registration of the CO₂ Contents in Expired Air (Capnography) in the Inhalative Provocation of Children. I. Acetylcholine Provocation of Asthmatic School-Age Children. *Acta Paed. Acad. Sci. Hung.* 10, 133, 1969.
15. *Murányi, L., Osváth, P., Uhl, K. & Butor, É.*: Continuous Registration of CO₂ Contents of Expired Air (Capnography) in the Inhalative Provocation of Children. II. Acetylcholine Provocation of Asthmatic Children under Seven Years of Age. *Acta Paed. Acad. Sci. Hung.* 10, 155, 1969.
16. *Osváth, P. & Endre, L.*: Asthma bronchialis gyermekek kezelése aluminium hydroxidhoz adszorbeált kivonatot tartalmazó (Allpyral) oltóanyaggal. (Treatment of bronchial-asthmatic children with (Allpyral) vaccine containing an extract adsorbed to aluminium hydroxide). *Orv. Hetil. (Med. Weekly)* 110, 1549, 1969.
17. *Osváth, P. & Endre, L.*: Immunanalytischer Vergleich zwischen Kuhhaar-extrakten und anderen Rinder- und Milch-proteine. II. Immunelektrophoretische Experimente. *Allergie u. Immunol.* 17, 63, 1971.
18. *Osváth, P. & Márkus, F.*: Das klinische Bild und die Behandlung der Kuhmilch-Allergie. *M Schr. Kinderheilk.* 119, 165, 1971.
19. *Osváth, P., Márkus, F., Endre, L. & Godó, B.*: Immunanalytischer Vergleich zwischen Kuhhaarextrakten und anderen Rinder- und Milch-proteine. I. Polyacrylamid- und Agargel-Diffusions Experimente. *Allergie u. Immunol.* 17, 56, 1971.
20. *Tuft, D. S., Giddens, W. E., Fairchild, G. A. & Van Arsdel, P. P.*: The Mechanism of Hypersensitivity Pneumonitis in the Rabbits. *J. Allergy Clin. Immunol.* 49, 103, 1972.
21. *Vialatte, J. & Jean, R.*: Exploration Fonctionnelle respiratoire chez l'enfant. *Entretiens de Bichat-Biol.* 645, 1965.

Author's address:

P. Osváth, C. Sc.,

Department of Pediatrics,

University of Szeged, Hungary.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.