

This section publishes the works presented at the scientific meetings organised by Autonomic or Regional Allergy and Clinical Immunology Societies. In this issue the Section contains the communications to the first two main subjects of the Annual Meeting of the Aragonese Society of Allergology, held in Formigal in February 1999. Papers corresponding to this Section which have been published previous by in other sections of this Journal, as well as those published in other journals, appear only as references.

Meeting Point

ANNUAL MEETING OF THE ARAGONESE SOCIETY OF ALLERGOLOGY

Third Main Subject: Occupational asthma in bakers: role of enzymes

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OCCUPATIONAL ASTHMA IN BAKERS: ROLE OF ENZYMES

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Cereals remain, even today, the main component of the human diet worldwide. From them an enormous variety of foodstuffs are derived that provide carbohydrates, proteins and group B vitamins.

Since Man first began availing himself of these grain varieties, back in the Prehistory, he also became conscious that they might cause disease, and particularly respiratory symptoms. For this reason, in ancient Rome the slaves who were employed in milling and in the bakeries used face masks for their own protection^{1,2}. The first known scientific description of baker's asthma was reported in 1713 by the "Father of Occupational Medicine", Bernardino Ramazzini³. However, the dust of cereals is not the only sensitizing agent in this context. A large number of natural contaminants and of additives that may be present in the grain itself or in the flour derived from it actually also act as sensitizing allergens able to cause disease.

Among the "natural contaminants" the following deserve mention: fungi and yeasts (*Alternaria tenuis*, *Aspergillus fumigatus*), grain weevils (*Sitophilus granarius*), and storage mites (*Acarus siro*, *Lepidoglyphus destructor*, *Glycyphagus domesticus* and *Tyrophagus putrescentiae*). These are the contaminants that are most often present in the grain, and they are the major causes of "silo worker's asthma".

A number of additives are added to wheat flour in order to improve its rheologic properties and thus improve the quality and modify the aspect of breads and pastries, fantasy pastries, etc. Furthermore, with the help of those additives the baking process is shortened and rendered more economical.

Pride of place among these additives corresponds to carbohydrate-hydrolysing enzymes, particularly the fungal α -amylase derived from *Aspergillus oryzae*⁴, but also to other glycolytic enzymes such as the cellulase and hemicellulase from *Aspergillus niger*^{5,6}.

Moreover, patients have also been reported with baker's asthma induced by non-cereal flours, which are added to the former in order to improve or modify the final product. The best-known example is soybean flour, in which the main allergenic determinants are also enzymes: soybean trypsin inhibitor (SBTI) and lipo-oxidase.

Also the flour of buckwheat or "Saracen wheat" (*Fagopyrum sculentum*), which is used in the preparation of *crêpes*⁸ and the flour of the vetch⁹, occasionally used by man for the preparation of a form of porridge, may induce occupational asthma.

It is quite obvious that there are many substances that are currently components of "cereal" flours that may behave as allergens. A given batch of flour will thus evidence a particular "allergenic profile" that is determined by the duration and conditions of storage of the grain it has been prepared from and also by the later manipulation and processing it will be subjected to.

Among the allergens in flour, enzymes probably represent the most interesting group, for a number of reasons. Firstly, because this group, collectively considered, is the one that most frequently induces sensitization. Secondly, because enzymes represent an allergen group with an extensive distribution and which constitutes a part in most of the known antigens.

For instance, Der p I and Der f I are glycoproteins with a molecular weight of 24,000 Da and with an amino acid sequence that is quite similar to that of papain. Der p III and Der f III are 29 kDa proteases, and group IV of the *Dermatophagoides* antigens is constituted by enzymes from the digestive tract of the mites, with molecular weights about 60 kDa.

The main allergenic fractions from the urine, salivary secretions and sweat of animals contain proteases. Acid phosphatases represent a highly important allergenic component in pollens, and phospholipases are enzymes constituting the major antigenic determinants in *Hymenoptera venoms*¹⁰.

Knowledge of the enzymes as allergens is thus fundamentally important to a better understanding of Allergology; in this context, as in so many others, research on occupational asthma has contributed "a considerable proportion of the material" to the files and archives related to this knowledge.

The decision of the Aragonese Society of Allergy to include this subject as the third Main Subject in its present Annual Meeting is thus more than amply justified.

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IMPORTANCE OF ENZYMES IN OCCUPATIONAL ASTHMA

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Introduction

Among the many known aetiological agents for occupational asthma, enzymes represent one of the most important groups.

Further to their high sensitizing power comes their increasingly striking frequency in the human environment. Further to this, their high molecular weight renders them a perfect model for studying the -IgE-mediated- hypersensitivity mechanisms involved in the pathogenesis of occupational asthma.

The known levels of sensitization prevalence among exposed subjects greatly exceed those accepted for conventional allergens. In parallel, the presence of allergic symptoms -rhinitis or asthma- among exposed and sensitized workers is also strikingly high.

On the other hand, there is at present growing interest in the study and knowledge of the enzymatic activity of common allergens inducing allergic phenomena. Some of the major allergens of mites, pollens

and animal excreta, among others, have been characterised as enzymes.

The possibility of sensitization to enzymes goes well beyond the occupational environment. Their widespread use, not only at the industrial level (the pharmaceutical and drug industries), leads to other modalities of exposure possibly being responsible for sensitization and for the apparition of clinical symptoms. There have thus been reports of anaphylaxis caused by the ingestion of papain-containing meat, or of conjunctivitis triggered by contact lens cleaning fluids also containing papain. And we should not forget the anaphylactic episodes induced by the use of chymopapain in the management of discal hernia.

In the context here to be discussed, the inhalation pathway is the most important one. Yet, as allergologists, we should not forget that sensitizations may arise through other pathways of allergen access: digestive, cutaneous or percutaneous.

A number of enzymes have been reported as responsible for inducing occupational asthma episodes: trypsin, bromelain, papain, peptinase, pepsin, cellulase, flaviastase, lipase, α -amylase, subtilysines, xylanase, etc.¹. The most important ones either because of their frequency or because of their having been more extensively studied in our country are discussed below.

Table I. The most frequent uses of papain, and the possible sources of exposure

Domestic

Bathing salts.
Softener in meat products.
Some cosmetics contain papain.

Drugs

Antidyspeptics.
Topical treatment of skin ulcers and for debridement of necrotic tissues.
Therapeutic measure for the management of meat impactation in the oesophagus.
Chymopapain has been used in the management of discal hernia.

Occupational

Cosmetology industries.
Alimentary industries.
Pharmaceutical industries.
Biochemistry, haematology and immunology laboratories.
Beer brewing industry.

Origin of enzymes and their more frequent uses

The origin of the enzymes in our environment is quite variegated. Some of them are derived from plants, such as papain and bromelain, which are derived respectively from the papaya fruit and the pineapple. Others are derived from yeasts and moulds, such as α -amylase, which is derived from *Aspergillus oryzae*, or cellulase from *Aspergillus niger*. Yet others are obtained from bacteria, the best known being those obtained from *Bacillus subtilis* (subtilysines A and B). Animal organs (pig pancreas) are the source for trypsin, α -amylase, lipase, etc.

The uses of enzymes are legion, and in some instances –such as papain, for example–, quite heterogeneous and disparate (Table I). There are however two main sources of occupational exposure responsible for the induction of occupational asthma episodes, although they are by far not the only ones: the pharmaceutical industry and the alimentary industry, where these enzymes are used as constituents of some drugs and as additives, conditioners or improvers of bread. However, we should not forget the use of enzymes in the detergent industry, and the important role of detergents as triggering agents in occupational asthma in workers exposed to biologic detergents.

Enzymes derived from *B. subtilis*

From this microorganism proteolytic enzymes are obtained which generically receive the name of subtilysines, among which alcalase and amaxatase evidence enzymatic activity within wide pH and temperature ranges. Because of their ability to clear protein stains they are adequate for use in the so-called biologic detergents.

Two main groups of enzymes have been described among the subtilysines, the Type A and Type B subtilysines; all of them are heat-stable alkaline peptidases. However, in the enzymatic preparations marketed in the biologic detergents there are other enzymes, such as amylase. In the '60s, the use of such biologic detergents was enormous both in Europe and in the USA, and up to 70% of the detergents in use contained these enzymes.

Shortly after the beginning of their use, the first reports of sensitization to these enzymes appeared in the United Kingdom. The first one, by Flindt², was published in 1969 and described 20 workers in a detergent industry who were sensitized to these enzymes. In that same year, Pepys³ reported that 21% of the exposed workers in one industry were sensitized, and that the latency period had

been six months. Because he had detected precipitins in the sera of these patients, he described the pulmonary symptoms as Type III reactions (extrinsic allergic alveolitis), but this finding has not been confirmed later. There were also reports of asthma induced by these enzymes in persons using biologic detergents at home.

These publications caused public and medical concern, to the point of causing heated debates between industrial producers and physicians; this led to the institution of important preventive measures. The encapsulation of the enzymes in order to reduce their ability to generate dust led to a very considerable reduction in the number of workers sensitized and with clinical manifestations of bronchial asthma.

In this particular case it can be stated that the implementation of correct environmental control measures and the modification of the production systems have been enough for rendering rather unimportant a problem which had been of utmost medical importance.

Enzymes of vegetable origin: papain and bromelain

Papain

Papain is a proteolytic enzyme derived from *Carica papaya* with a molecular weight of 23,000 Daltons. Because of its widespread use and its high sensitizing ability it is beyond doubt one of the most important enzymes in the category of causative agents of occupational asthma.

Its uses and applications are quite numerous (Table I), so that sensitizations may occur outside the occupational environment. There have thus been reports of anaphylaxis caused by the ingestion of hamburgers to which papain had been added as a meat softener, or of severe conjunctivitis due to the use of papain for cleaning contact lenses; even its use as a clarifier for beer has caused allergic reactions upon the ingestion of that beverage.

However, the most important source of exposure is the pharmaceutical industry. Papain is used as a powder in a number of medicinal preparations, and the inhalational pathway is the most important one for sensitization.

Although there are reports of papain sensitization dating back over 50 years into the past^{4,5}, it was at the end of the '70s when important international publications first appeared highlighting the significant aetiological role of papain in occupational asthma and the high sensitizing power of this enzyme, and all of these publications stressed the pathogenetic involvement of an IgE-

mediated mechanism. The high prevalence of sensitization among the exposed workers was also confirmed.

Tarlo⁶, Baur^{7,8}, Novey⁹ and Flindt¹⁰ were the authors of the first studies to appear, and they were all coincident in that papain sensitization among exposed workers could be as high as 30-40% of the cases¹¹.

The Spanish contribution to the knowledge of papain-induced occupational asthma has not been small. Back in the '80s the first Spanish publications appeared, followed by further ones in later years. Our own group¹²⁻¹⁴ at that time carried out an epidemiological study in a pharmaceutical manufacturing plant in which drugs were manufactured containing a number of enzymes, papain among them. Among the 83 workers participating in the study the skin tests with papain were positive in 26 (31%), and the specific IgE levels were significantly higher in the exposed group than in the group of workers used as a control; among those exposed there were also highly significant differences between those with positive and those with negative skin tests. The inhalational challenge tests performed induced immediate and in some cases dual responses.

Other Spanish investigators have also made equally important contributions¹⁵, confirming the high sensitizing power of this enzyme.

Bromelain

Bromelain is a further one among the vegetable-origin enzymes implicated as aetiological agents in occupational asthma. This enzyme is obtained from *Ananas comosus* (the American pineapple); like papain it has proteolytic activity, and its molecular weight is 33,000 Daltons. Its use as a component in dyspepsia preparations leads to the pharmaceutical industry being the main source of exposure.

The first report of occupational asthma induced by bromelain was published in 1978¹⁶. This report demonstrated that an IgE-mediated response was responsible for the clinical manifestations. The first Spanish publications appeared a few years later¹².

Baur⁷ demonstrated the existence of cross-reactivity between papain and bromelain, and this fact was later confirmed by our own group¹³. This observation confers upon papain and bromelain highly interesting immunologic peculiarities.

In our previously cited study^{12,13}, 18 of the participant workers had positive skin tests and specific IgE to bromelain, yet none of them were exposed to this enzyme, but to papain.

Among our patients, the inhalational challenge tests induced immediate responses, and in some cases dual ones.

Mycogenic enzymes: cellulase and α -amylase

Cellulase

This is an enzyme with various origins, although it is mainly derived from fungi: *Aspergillus niger* and *Trichoderma viride*. It is used in the pharmaceutical industry as a digestive assistance preparation, and in the alimentary industry as an additive or improver in bakery products or for the separation of protein fractions or components in other foodstuffs.

Our own group was the first one to report the aetiologic role of this enzyme in occupational asthma¹⁷. The reported cases were two workers from the pharmaceutical industry who were exposed to dust of several enzymes; it was demonstrated that they were sensitized to cellulase (positive skin tests and specific IgE), and the specific bronchial challenge tests induced immediate responses in both cases. This investigation also demonstrated one fact that we consider to be important: the existence of cross-reactivity between the enzyme (cellulase) and the producing fungus (*A. niger*).

A few years later reports were published on the influence of this enzyme in baker's asthma¹⁸⁻²¹. Between 5 and 24% of the patients with baker's asthma are sensitized to enzymes of fungal origin¹⁸, and 10% among a group of 247 symptomatic bakers had a positive RAST with hemicellulase derived from *Aspergillus niger*²².

These data, and those from other later reports, stress the increasingly important role of this enzyme as an aetiologic agent in occupational asthma.

α -Amylase

Among all the known enzymes, α -amylase is perhaps the most important one in the allergologic context. Its high sensitizing potential and its widespread use in both the pharmaceutical and –most particularly– the alimentary industry, where it is increasingly used as an improver in bakery products, favours the apparition of sensitizations. The number of reports published over the last few years is quite high.

This enzyme has quite diverse sources: animal organs, bacteria, and fungi. The best known variety is doubtless that derived from *Aspergillus oryzae*, with a molecular weight of 51,000 Daltons. It is a glycolytic

enzyme that catalyses the hydrolysis of the 1-4 glycoside groups in carbohydrates.

In the pharmaceutical industry it is used because of its antidyspeptic and antiinflammatory activities, and in the bakery industry it finds widespread use as an improver in bread and bakery products.

Flindt¹⁰, in 1979, was the first one to report occupational asthma caused by α -amylase. He based his diagnosis upon the anamnesis and upon the results of the skin tests. A few years later, Wiessman and Baur²³ reported sensitization to α -amylase of pancreatic origin among a group of exposed workers, and our own group²⁴ presented a report of two workers from the pharmaceutical industry with occupational asthma due to α -amylase from *Aspergillus oryzae*. An IgE-mediated mechanism was demonstrated in both these cases, and the bronchial challenge tests were positive. Some years later we published the results of a study performed on 83 workers from the pharmaceutical industry with enzyme exposure; 26 of them had positive skin tests and specific IgE to α -amylase²⁵. The bronchial challenge tests performed on 14 workers were positive in all cases. Further contributions have recently appeared²⁶ regarding sensitization to various α -amylase preparations.

In the alimentary industry the important role of this enzyme in baker's asthma began to be recognised through the works of Baur^{27,28} and Birnbaum²⁹. Many reports have in recent years confirmed these findings and the importance of α -amylase as an aetiologic agent in baker's asthma.

The Spanish contribution to this field over the last years has been not only numerous but also important. Blanco Carmona and co-workers³⁰ reported the case of a patient with baker's asthma in which α -amylase was the responsible agent. Quirce and co-workers²¹ published a more extensive series of workers exposed to cereal flours, in whom they confirmed the existence of sensitization to enzymes, α -amylase and cellulase of fungal origin. Blanco Carmona et al.³¹ later reported a series of seven cases of occupational asthma in a bakery environment due to sensitization to α -amylase from *A. oryzae*. Further Spanish investigators have published interesting contributions to this field^{32,33}.

We should not leave unmentioned the highly important contributions to the field of baker's asthma by Dr. Alicia Armentia^{34,35}, who has investigated the role of these enzymes and of an α -amylase inhibitor as important agents in the aetiology of this condition.

It is possible that some Spanish contributions have been omitted from this bibliographic review, and I present my excuses to those concerned.

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OCCUPATIONAL RESPIRATORY ALLERGY TO α -AMYLASE IN BAKERS

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Introduction

Occupational respiratory allergy among workers in the bakery industry and associated activities is quite prevalent and may affect up to 10-20% of those profes-

nals¹⁻³. Although no epidemiological data have been published, we suspect that baker's asthma is one of the most frequent occupational respiratory diseases in Spain. Most of the bakers with occupational asthma are sensitized to wheat flour and to storage mites; however, over the last few years we have seen a growing importance of some flour additives, particularly α -amylase, as a cause of rhinitis and asthma. Fungal α -amylase (1,4- α -D-glucanase-glucanohydrolase), usually derived from *Aspergillus oryzae*, is a 52-kD glycoprotein that catalyses the hydrolysis of internal α -(1,4)-glycosidic bonds to simpler polysaccharides. It is commonly and routinely added to wheat flour, in a mg-to-kg-flour proportion, for accelerating the panification process and improving the quality of the resulting bread.

The first cases of occupational asthma due to α -amylase allergy were reported by Flindt⁴ in workers of the pharmaceutical industry; he based his diagnosis on the anamnesis and on skin tests. Some years later, Wiessman and Baur⁵ demonstrated that pancreatic α -amylase was responsible for the respiratory allergy symptoms in a group of workers exposed to pancreatic extracts through an IgE-mediated immediate hypersensitivity mechanism. This finding was later confirmed by Aiken *et al.*⁶. The first description of allergy to enzymes in bakers with occupational asthma was published by Baur *et al.*⁷, who demonstrated a 34% prevalence of α -amylase sensitization through *in vitro* and *in vivo* tests among symptomatic workers. In Spain, the first case of occupational asthma due to α -amylase and demonstrated by means of skin tests, specific IgE and bronchial challenge

tests was reported in 1991 in a baker⁸. One year later, Losada *et al.*⁹ demonstrated α -amylase sensitization through skin tests and specific IgE in 26 among 83 (31%) workers exposed to α -amylase powder in a pharmaceutical manufacturing plant. The bronchial challenge tests with α -amylase were positive in 6 of the 14 cases tested, and only an immediate response pattern was observed. In that same year, another Spanish group¹⁰ elegantly demonstrated type I hypersensitivity to α -amylase and cellulase in five bakers through skin tests, histamine release tests and reverse enzyme-linked immunoassay (REIA) for specific IgE. The bronchial challenge tests were specific with α -amylase, cellulase or both, with immediate or dual asthmatic responses. The REIA inhibition study did not reveal cross-reactivity between these two enzymes, but there was cross-reactivity between α -amylase and *Aspergillus oryzae*.

We here report the results of a clinical study performed on seven bakers exposed to the dust of wheat flour and its additives, among them fungal α -amylase, who consulted because of symptoms of rhinitis and asthma related to their occupational activity.

Material and methods

Patients

In the period between November 1994 and June 1998 seven bakers were studied at our Allergy Service because of respiratory symptoms related to their work. Their ages ranged between 22 and 54 years (mean, 31.6

Table 1. Clinical and functional features of the seven patients

Patient	1	2	3	4	5	6	7
Age (years)	35	54	22	26	29	31	24
Sex	M	M	F	M	M	F	M
Occupation	Baker	Baker	Baker	Baker	Baker	Baker	Baker
Symptoms	R-A	R-A	R-A	R-A	R-A	R-A	R-A
Atopy	+	-	+	-	+	+	-
FEV ₁ (%)	53	55	116	100	104	84	101
BDT (%)	38	61	Neg.	12	Neg.	Neg.	Neg.
PC-20 metacolina (mg/ml)	NP	NP	0,23	NP	0,74	5	1,1

R-A = rhinitis and asthma; NP = not performed; BDT = bronchodilator test; PC-20 = PC-20 in the nonspecific methacholine test (mg/ml).

years); four of them were males and three females. The clinical features of the patients are presented in Table I. All of them evidenced increasingly severe episodes of cough, dyspnoea, chest tightness, wheezing, nasal blockage, watery rhinorrhoea and sneezing bouts, with a latency period ranging between several months and over 20 years. The occupational pattern was also typical in all cases, with symptomatic improvement up to being almost completely free of symptoms during holidays and work absence periods. In patients 1 and 2 the symptoms became chronic and severe, requiring emergency medical treatment on several occasions.

Besides being exposed to wheat flour, all our patients also manipulated further foodstuffs used as flour additives, all of them containing α -amylase (*T-500*®, *Puratos*® and *MP-Vital Extra G*®). The causal relationship between the inhalation of powdery material derived from the flour and its additives and the immediate apparition of symptoms was evident. Few minutes after the exposure the patients developed acute symptoms of rhinoconjunctivitis, cough, dyspnoea and wheezing, which often worsened toward the end of the working day. Patients 2 and 4 had been previously studied at other Centres, with negative skin tests and specific IgE to storage mites and cereal flours. Despite the fact that an aetiological diagnosis of occupational respiratory allergy, none of our patients had quit their work; the reason given was an unstable labour situation. They were repeatedly counselled, as an alternative measure, to pay the utmost attention to hygienic measures and precautions at their workplaces, avoiding exposure whenever possible, installing efficient ventilation-extraction systems and giving permanent antiinflammatory and bronchodilator drug therapy.

Preparation of the antigenic extract

Pure α -amylase derived from *Aspergillus oryzae* was supplied by Merck-Igoda, S. A. (Barcelona, Spain) as a fine powder. The allergenic extract was prepared by dissolving 2 g α -amylase in 20 ml 0.9% isotonic saline at room temperature. After manual stirring for 60 minutes, the solution was filtered through filter paper and then dialysed against isotonic saline for 24 hours. Finally, it was filtered through a 0.22 μ m Millipore filter (Millipore Corp., Bedford, Mass., USA) for sterilisation. The final concentration of the solution was 10% (w/v); this was considered as the undiluted mother extract. Part of this extract was then glycerinated for skin tests, and

the remainder was diluted in isotonic saline for the bronchial challenge tests.

Skin tests

The skin tests, using the prick technique¹¹, were performed with the above extract. Histamine phosphate (10 mg/ml) and isotonic normal saline (0.9%) were used as positive and negative controls, respectively. The responses to the tests were read after 15 minutes, measuring the largest diameter of the elicited wheal. A reaction was considered to be positive when the wheal was equal to or greater than 3 mm in diameter and accompanied by erythema, in the presence of a negative prick test with the diluent. Ten non-exposed atopic subjects who served as controls were tested with the highest concentration of the α -amylase extract (10% w/v). Skin tests were also performed with a panel of common airborne and occupational allergens (Alergia e Inmunología Abelló, Madrid, Spain) which included pollens (grasses, olive tree and shrubs), mites (*Dermatophagoides* spp., *Lepidoglyphus*, *Tyrophagus*, *Acarus siro*, *Gohieria*, *Euroglyphus*, *Glycyphagus* and *Blomia*), cat and dog danders, moulds (*Alternaria*, *Cladosporium* and *Aspergillus fumigatus*) and cereal flours (wheat, barley, rye, oat and maize).

Specific IgE quantitation

The concentration of α -amylase-specific IgE antibodies was determined by the FEIA-CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions.

Bronchial challenge tests

The non-specific inhalational bronchial challenge tests with methacholine were performed using the technique described by Cockcroft *et al.*¹² with some modifications¹³. In order to demonstrate the presence of bronchial asthma through the routine bronchodynamic tests (bronchodilator or bronchoconstrictor), this test was only performed when the foregoing bronchodilator test had been negative. The aerosol particles were generated through continuous nebulisation using a Hudson model 1720 nebuliser with a 7.5 l/min activating airflow and a 0.276 ml/min output.

The specific bronchial challenge test (SBCT) was performed with the dilution of the α -amylase extract that had previously elicited positive skin tests. End-point titration skin tests were initially performed with sequential one-half dilutions of the undiluted mother

extract (1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640 and 1/1280 w/v, respectively) in order to establish the safe initial dose for the SBCT. Baseline lung function tests were performed prior to the inhalation of the antigen in order to establish the baseline FEV₁, FVC and PEFR values, followed by a control inhalational challenge test with normal saline. The patients then inhaled the aerosolised allergenic extract, increasing concentrations being administered in different days using the previously stated nebuliser and procedure; the inhalational challenge was performed with the patient breathing at his normal tidal volume, through a mouth-piece and with nasal occlusion, for 2 minutes. The FEV₁ and FVC were measured 5, 10, 15, 20, 30 and 60 minutes after the SBCT. A drop in the FEV₁ equal to or greater than 20% as compared to the baseline value was considered representative of a positive immediate asthmatic response. The patients were then instructed to monitor their PFR at hourly intervals for 24 hours (leaving out the sleep period) in order to detect an eventual late response, which was considered to be positive when the PEFR evidenced a $\geq 25\%$ drop as compared to the baseline value.

At the time of carrying out the SBCT's, all patients were under leave of absence from work, under good clinical and functional control, and without any medication that might have interfered in the test results. Four non-exposed atopic subjects with mild to moderate bronchial asthma submitted to SBCT with the maximum concentration of the α -amylase allergenic extract (10% w/v) as controls.

Results

Skin tests

All the bakers evidenced positive responses to the skin tests with α -amylase antigenic extract. Three of them also evidenced positive skin tests with wheat flour, and one with storage mites. Table II shows the results of the skin tests. The skin tests with the highest concentration of the α -amylase extract (10% w/v) were negative in the control subjects, thus ruling out a possible irritative mechanism induced by the allergenic extract.

Specific IgE quantitation

Specific IgE antibodies to fungal α -amylase were detected in all the patients' sera. The total and α -amylase-specific IgE concentrations are shown in Table III.

Bronchial challenge tests

The nonspecific methacholine bronchial challenge tests disclosed varying degrees of bronchial hyperreactivity in those patients in whom it was performed (0.23, 0.74, 5.00 and 1.10 mg/ml, respectively, in patients Nos. 3, 5, 6 and 7). As already stated, however, the aim of this test was to confirm the diagnosis of bronchial asthma in those patients in whom the lung function tests had been normal and the bronchodilator tests had been negative.

After the SBCT with α -amylase, five patients evidenced an isolated immediate asthmatic response and a further two a dual one. Figure 1 depicts one case of immediate response. The SBCT with the highest concentra-

Table II. Results of the prick tests with α -amylase (greatest diameter of the wheal, mm)

Patient	1	2	3	4	5	6	7
α -amilasa (1/10 w/v)	9	6	8	6	10	12	12

Table III. Total and α -amylase-specific IgE (kU/l)

Patient	1	2	3	4	5	6	7
Total IgE (IU/ml)	480	41	166	221	538	281	315
Specific IgE (α -amylase)	18,5	45,3	9,47	1,35	2,72	1,50	63,6

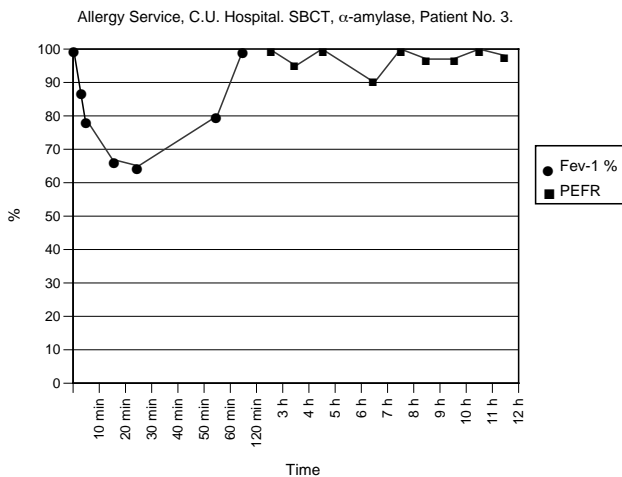


Fig. 1. Demonstration of an immediate asthmatic response after SBCT with fungal α -amylase at a 1/80 W/v concentration (in patient No. 3).

tion of the α -amylase extract (10% w/v) was negative in the control asthmatics, thus confirming the specificity of the test.

Discussion

Over many years, bakers' asthma was considered to be a form of allergic asthma mediated by IgE antibodies directed specifically against cereal flour allergens¹⁴. However, the roster of causative allergens has grown with the passage of time, and at the present time up to seven different groups of occupational allergens have been described¹⁵ which are able to induce occupational respiratory allergy in bakers and in associated occupations. Besides the already-mentioned cereal flours, soybean flour¹⁶, buckwheat flour¹⁷, storage mites¹⁸, grain weevils¹⁹, moulds of the genera *Alternaria* and *Aspergillus*¹⁵ and enzymes derived from *Aspergillus* spp.^{10,20} have also been identified as causative antigens in IgE-mediated occupational respiratory allergy. Over the last 20 years, the manufacturing processes for bread and similar products have evolved considerably. This is to a great extent due to the general use of improving additives added to wheat flour in order to accelerate bread production and to improve the quality of the bread. Among these additives, α -amylase from *Aspergillus oryzae* is the most important one²¹. The exposure is usually occupational, through the inhalation of the powdered enzyme, in workers of

the pharmaceutical industry⁹, in laboratory workers⁶ and in bakers²³.

We have presented the results of a study of seven bakers with symptoms of bronchial asthma and rhinitis in relation to their occupational activity, all of them exposed to α -amylase. Four of these workers were also sensitized to other common and/or occupational airborne allergens (flours and storage mites), and they also evidenced high total serum IgE concentrations. Atopy thus appears to play an important role in the development of IgE-mediated α -amylase sensitization, a common observation with high-molecular weight occupational allergens²². However, three of our patients evidenced monosensitization to α -amylase. Although we have not measured the environmental exposure levels in the work environments of our patients, studies have been recently published demonstrating a strong positive correlation between the exposure levels (ng α -amylase/m³) and the probability of sensitization and later development of occupational asthma²³⁻²⁵. The positive skin tests with α -amylase in our seven patients, together with the positive specific bronchial challenge tests and specific IgE quantitations demonstrate that an immunologic IgE-mediated immediate hypersensitivity mechanism was responsible for the respiratory symptoms. The negativity of the skin tests and of the specific bronchial challenge tests in the non-exposed atopic controls demonstrates the specificity of these findings. Immunologic studies with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and later immunoblotting using a pool of sera from allergic patients have identified a 52-kDa glycoprotein band with specific IgE-binding capacity^{7,10}. This important occupational airborne allergen has recently been isolated and identified as α -amylase. In accordance with the nomenclature of the International Union of Immunology Societies (IUIS), it has been suggested that this antigen be named "*Aspergillus oryzae* α -amylase", or Asp o 2²⁶.

In conclusion, it is clear that, besides cereals and other specific occupational airborne allergens, the fungal enzyme α -amylase (Asp o 2) is a common cause of occupational respiratory allergy among bakers and confectioners. On the other hand, the positivity of the skin prick tests and of the specific IgE suggest the sensitization of the patient and the degree of sensitization, but only the specific bronchial challenge test with α -amylase can provide the definitive diagnosis.

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IMMUNOCHEMICAL CHARACTERISTICS OF α -AMYLASE SENSITIZATION

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Amylases were the first enzymes to be added to bread dough, and they are still today the object of considerable interest in the research of enzymes used in bakery¹. Three sources of amylases have been used up to now: malt flour (the amylase from malt flour was historically the first one to be used), fungi (*Aspergillus oryzae*), and bacteria (*Bacillus subtilis*).

These enzymes were initially used for the production of fermentable sugars. Starch, the main component of cereal flours, contains 75% amylopectin and 25% amylose. α -amylase acts on these constituents by randomly breaking down the 1,4-glycosidic bonds (with the exception of the terminal ones), producing maltose and most importantly (and mainly) dextrins. β -amylase acts on the latter by sequentially hydrolysing the terminal 1,4-glycosidic bonds, thus releasing maltose. This is the fermented by yeast producing gas release (CO₂,

in an amount directly dependent on the volume of the dough).

Cereal flours contain sufficient amounts of β -amylase. However, the amount of α -amylase is quite variable, and depends on variations and climatologic influences on each harvest. Because of this, the amount present must be corrected (usually through addition) at the flour-processing plants.

On the other hand, and already at the bakery, α -amylase is used as a constituent of bread improvers. These compounds add to the bread dough enzymes such as pentonases, hemicellulases, xylanases, glycoamylases and aminoglycosidases, and greater proportions of α -amylases, together with proteases, lipooxygenases, etc. The fact that the activity of α -amylase disappears at temperatures between 70 and 75°C prevents an excessive breakdown of starch, which would lead to the bread having a lumpy dough. Also, there are no dangers in its dosification, as the bread is baked at temperatures in excess of 200°C. Because of this, and even though α -amylase is added in a 0.01% proportion to the amount of flour, it is difficult to know precisely the amount of the enzyme that may be present in bakery products. The fact that the α -amylase from malt flour becomes inactivated at higher temperatures, and also that it leads to a quicker initiation of fermentation and adds protease activities that are not readily controlled has led to its being abandoned.

A further advantage of using flours with an adequate level of α -amylase is that this leads to a better roasting of the bread crust and a better aroma (also dependent on the presence of maltose), and also to a delay in the hardening of the bread, one of the main subjects of research and investigation in bakery procedures and processes at the present time.

So-called white bread, when prepared without additives and stored at room temperature, hardens within two and one half days. In this context, the behaviour of amylases of different sources is also different. The α -amylases from malt and fungi increase the half-life of bread to about five days, although the final hardening is similar. The bacterial α -amylase, however, not only increases the storage half-life, but also halves the final hardening. Nevertheless, if the amount of bacterial α -amylase used is excessive, the texture of the bread becomes rubbery after a couple of days' storage. There are two possible explanations

for this phenomenon. Firstly, the thermal stability of the bacterial enzymes allows some of them to survive the baking process and continue hydrolysing starch during storage; secondly, the dextrins formed through the action of bacterial α -amylase, with a high (25 to 35) degree of polymerisation, are humectant and inherently rubbery and tacky. Because of this, other carbohydrases are being investigated in search of a better control of bread hardening: amyloglycosidase, amyloglycosidase plus α -amylase, pullulanase plus α -amylase, and exo- α -amylase. In the industrial bakeries in our area the enzyme used is α -amylase from *Bacillus subtilis* that has been genetically modified with a strain of *Bacillus stearothermophilus*, with the result of increasing the storage half-life of the bakery products without their turning rubbery and tacky and also without affecting the manipulative properties of the dough.

From the foregoing it can be deduced that the α -amylase predominantly used in the bread industry is the α -amylase from fungi, and it is this α -amylase that shall be the focus of our further discussion.

Fungal α -amylase (1,4- α -glucan-glucane-hydrolyase) is a glycoprotein with 478 aminoacid residues². Its tridimensional structure has three dominions, and its conformation is stabilised by four disulfide bonds. The presence of calcium is required for its activity; two calcium-binding regions are located close to the zones of enzymatic activity³.

α -Amylase contains epitopes which are recognised mainly by B-cells, and it has been demonstrated that enzymatic digestion by trypsin and Arg-C endoprotease does not inhibit its IgE-binding capacity⁴. Baur has shown that the component that is almost exclusively bound to IgE in asthmatics with sensitization to this enzyme corresponds to a 53-kDa band in SDS-PAGE, with pI 4.0 in the isoelectrofocusing experiments⁵.

Considering that an 18-kDa allergen from the genus *Aspergillus* had been previously characterised and designated Asp f 1, Baur has proposed that, following the nomenclature of the International Union of Immunological Societies, this new and important allergen be designated "Asp o 2"⁵.

Our own series of occupational asthma due to α -amylase sensitization presently includes 15 patients, 8 males and 7 females, with ages ranging between 27 and 57 years (mean age, 33.5 years). Most

of them work in family-owned bakeries of small size, which are usually poorly ventilated and are often located close to the dwelling.

In all these patients, the diagnosis was based on a suggestive clinical history, positive (3+/4+) skin test with α -amylase, positive (\geq Class 2) specific IgE (Pharmacia CAP System, Pharmacia, Uppsala, Sweden) and a positive specific bronchial challenge test. Fourteen patients have evidenced in this test an immediate response pattern, and only one a dual response. In the assessment of the pulmonary involvement according to the 1993 criteria of the American Thoracic Society, 14 cases (one of which was the one with a dual response) had Grade I involvement, and only one was Grade II. After three years' follow-up, and without any of the patients having quit work, the levels of involvement and deterioration had not changed⁶.

None of our patients reported any problems upon the ingestion of bread or of other foodstuffs in which α -amylase is used. However, Kanny⁷ has reported one case (a female) with baker's asthma who had a 21% drop in the PEF_R after a double-blind oral challenge with cooked α -amylase (100°C for 30 minutes). Baur⁸ has reported one case of "baker's asthma" with exclusively nasal symptoms and increase of the nasal airflow resistances in the rhinomanometry after ingestion of bread with α -amylase. This same investigator asks himself if α -amylase might not be considered an ingestional antigen: among eleven bakers studied he observed a weak response to pre-heated α -amylase (200°C) in only one, and deduced that heat does not completely abolish the IgE-binding capacity of this enzyme. In that same paper, he suggests that further work is required for assessing the residual allergenicity or the apparition of new allergenic determinants after baking⁹.

In the light of these published reports of patients with baker's asthma and α -amylase sensitization who had evidenced adverse reactions after eating bread, we decided to investigate what happened with this allergen when being subjected to baking temperatures.

For this investigation we had the help of seven bakers, five males and two females with ages ranging between 27 and 53 years (mean age, 35 years), who were sensitized to α -amylase from *Aspergillus oryzae*.

For the preparation of the allergenic extracts we

used *Aspergillus oryzae* α -amylase (Arkady ADM Ibérica, Barcelona, Spain), samples of which were cooked at 100 and 200°C for 20 minutes. Three working samples were prepared: raw α -amylase (A), α -amylase cooked at 100°C (B), and α -amylase cooked at 200°C (C). 5-Gram samples of each were incubated in 25 ml phosphate-buffered saline (PBS) for 18 hours; the solutions were then filtered through filter paper and again through 0.22-mm Millipore filters. The final concentration of the extracts (w/v) was 2 mg/ml. Aliquots of the extracts were then frozen until used in the studies.

Skin tests were performed in triplicate using the prick technique on the volar aspect of the forearm, using Dome-Hollister lancets. The dimensions of the wheals were calculated using the mean value of the greatest diameter and the one perpendicular to it, expressed in mm. The results were compared to those of control prick tests with 10 mg/ml histamine (positive) and PBS (negative).

SDS-PAGE and SDS-PAGE immunoblotting was carried out with the three extracts and the seven patients' sera; the results of these studies are discussed below.

The skin tests were positive in all seven patients with the raw (A) α -amylase extract. The positivity persisted in all of them when testing the extract of α -amylase pre-cooked at 100°C (B), although in four cases the wheal elicited by this extract was smaller than the previous one. However, the tests performed with the extract of α -amylase pre-cooked at 200°C (C) were negative in all seven patients.

SDS-PAGE

Upon electrophoresis in the presence of SDS, the raw α -amylase sample (A) revealed a main protein band (48.3 kDa) representing 95% in weight of the sample. Under the same conditions, sample B (α -amylase pre-cooked at 100°C for 20 minutes) evidenced one single protein band (50 kDa) representing 100% of the sample weight. In the study of sample C (α -amylase pre-cooked at 200°C for 20 minutes) no protein bands were observed.

SDS-PAGE immunoblotting

For the SDS-PAGE immunoblotting, the three SDS-PAGE samples were incubated with the patients' se-

ra. The IgE in the patients' sera bound to the 48.3 kDa and the 50 kDa proteins present in the A and B samples, while no IgE binding to the membrane was seen with sample C.

Even though this study was performed on only a small group of cases, it appears to again demonstrate that the allergenic activity of the enzyme is destroyed upon being subjected to the usual baking temperatures, as evidenced in the skin tests. Furthermore, the protein becomes degraded *in toto*, as shown by the SDS-PAGE results, and is most probably reduced to very small peptides lacking any allergenic character.

In conclusion, we may state that, firstly, the dominant allergen in the α -amylase enzyme is a glycoprotein with a molecular weight of approximately 50 kDa. Secondly, its accepted denomination is "Asp o 2". Thirdly, it contains epitopes which are mainly recognised by B-cells. Fourthly, α -amylase does not appear to behave as an allergen of interest upon ingestion after being subjected to the usual baking temperatures.

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ALLERGY TO CEREAL α -AMYLASE INHIBITORS

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The so-called cereal grains include wheat, maize, rice, barley, sorghum, oat, millet and rye. All these, collectively, represent 72% of the dietary protein in the World.

Wheat, barley and rye are closely related grain (grass) species belonging to the tribe *Triticeae*. A number of different proteins enter into the composition of their seeds: water-soluble albumins, saline-soluble globulins, water-alcohol-soluble prolamines, and insoluble glutenines¹⁻⁴. The soluble proteins represent up to 25% of the protein contents in the seeds, and a number of studies have confirmed their importance in IgE-mediated responses, both because of their role in inhalational sensitizations^{5,12} and because of their role in atopic dermatitis¹³. The former is collectively known as "baker's disease" or "baker's asthma". It is a condition that gives rise to considerable economic and legal problems, quite besides its severity and difficult management when the patient cannot avoid inhaling the causative grain dusts.

Cereal workers (farmers, millers, fodder packagers) and those working in the baking and confectionery environments are exposed to a number of antigenic stimuli^{2,4}. In our area they mostly work in small family-owned enterprises or in silos and granaries, with the worst imaginable ventilation. Although they are aware of the personal protection measures, they usually do not apply them because of the inconveniences they cause in an environment in which they must carry out considerable efforts under high environmental temperatures. In the case of the dough improvers, solid preparations are being increasingly used instead of powders, and this leads to less particle dispersion. However, this is not possible in the case of flours. Because of the high prevalence of this type of asthma in our region (25% of the individuals exposed suffer occupational asthma), we have been trying since several years ago to isolate, purify and assess the allergenicity of cereals in an effort to find appropriate measures for the environmental and therapeutic control of this condition⁵⁻¹⁸.

We must bear in mind that flour is a complex ecosystem in which a number of elements are represen-



Fig. 1. High-resolution bidimensional electrophoresis of a wheat flour extract, showing (arrows) the most reactive proteins. From Posch et al. (Electrophoresis 1995; 16: 1115-9) and Weiss et al. (Electrophoresis 1993; 14: 804-16).

ted: vegetable elements such as cereal pollens, proteins from the grain endospermium, spores and fungi; a number of additives such as α -amylase, and arthropodal allergens from grain parasites, cockroaches and mites (mainly storage mites, which are themselves potent allergens)^{19,20}. Episodes of asthma caused by the ingestion of cereals contaminated with storage mites have been recently reported²¹. How then are we to identify the allergens that are causative of the allergic symptoms?

The electrophoretic techniques for protein isolation are better than other methods such as chromatography, as they have a better resolution and are able to resolve the complex protein composition of flour in the endeavour to characterise its possible allergens². Posch *et al.*, using high-resolution protein electrophoresis in combination with protein analysis, detected some 700 soluble

proteins in wheat (Electrophoresis 1993; 18:804-816); 70 of these proteins were able to detect IgE, mainly those in the 14-18, 27, 37, 55 and 70-kDa regions. Using amino-acid sequencing, they found homologies in α -amylase-inhibiting proteases (14-18 kDa), acyl-CoA oxidase (26 kDa) and fructose biphosphate aldolase (37 kDa) from wheat, maize and barley (Fig. 1). However, these interesting studies do not fully clarify which proteins might be clinically relevant. We therefore attempted to separate and isolate them so that, once purified and isolated in diagnostic extracts, they may be used for assessment of their reactivity *in vivo*.

To this purpose, we first used a pool of 35 sera from patients with baker's asthma with Class 4 RAST to wheat and barley flour. Our first results allowed us to establish that the most reactive allergens were saline-soluble proteins of 12-15 kDa molecular weight belonging to a single family that includes several heterologous α -amylase inhibitors, with a high level of presence in the cereals and with potential participation in the cereals' defence mechanisms against infesting storage pests¹⁴. Thus, the major allergen in barley was able to inhibit the α -amylase from *Tenebrio molitor*, a common parasite of this cereal⁶. A number of members of this inhibitor family were recognised by our patients' specific IgE; however, they had widely diverging allergenic capacity. Figure 2 shows the bidimensional electrophoresis and the chromatogram of the fractionation through high-resolution reverse liquid chromatography (HPLC) of the tetrameric inhibitor from wheat. We would like to call attention to the positions of the most reactive protein (CM16*), marked by an arrow in the bidimensional map and in the elution profile. The CM16 and CM16* proteins have the same molecular weights, but their immunoblot behaviour against sera from reactive patients is

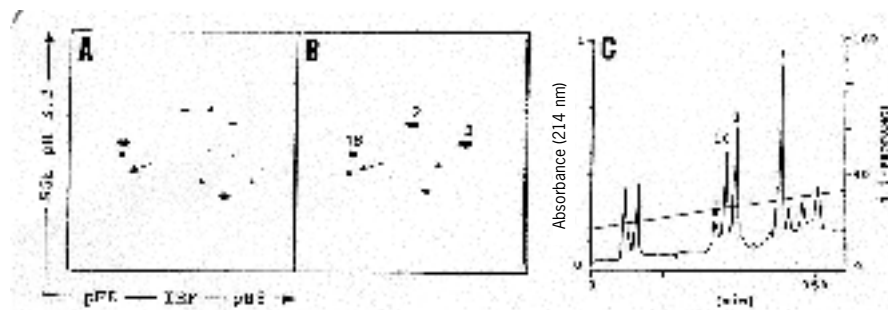


Fig. 2. Isolation of the CM16* protein from wheat dough: bidimensional electrophoresis (IEF x SGE) of the source preparation of the inhibitor (A) and of the gel-filtrated 60-kDa fraction containing tetrameric inhibitors from *Triticum turgidum*, *Senatore Capelli* (pasta wheat). HPLC fractionation of the tetrameric inhibitor (Fig. 2B). The position of the CM16* component is shown (arrows) in the two bidimensional maps and in the HPLC elution profile. Also shown are the positions of the WTAI-CM2 tetrameric inhibitor (2) and of CM16 (16) and CM3B (3).

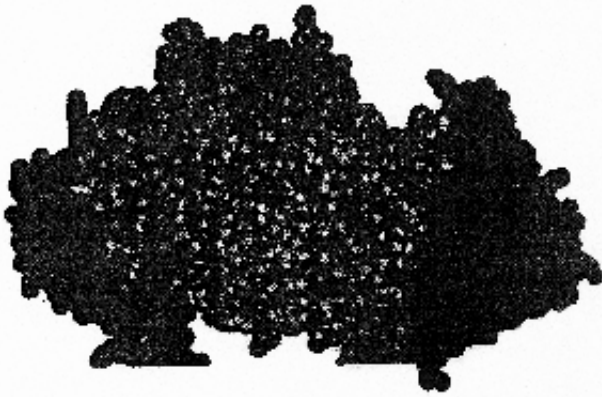


Fig. 3. Molecular structure of *Aspergillus oryzae* α -amylase, highlighting its three domains (Baur et al., Reference 25).

quite different. The same occurred with the CMb and CMb* components from barley. After a number of attempts to find out what was the difference between these proteins as regards their allergenicity (aminoacid sequencing, peptide excision, glycan detection tests) it was shown that the particularity rendering these proteins reactive was the binding to complex xylose and fucose glycans, and that these glycans acted as IgE-binding epitopes⁹. This explained why our cereal workers or bakers mainly reacted to glycosylated purified proteins from barley and wheat, while their skin tests and RASTs with commercial cereal extracts were negative. However, the inhalational challenge tests with these purified proteins were positive, demonstrating their importance as aetiological agents in occupational asthma²². We have recently shown that the complex glycans from other invertebrates and plants have similar epitopes⁹. In this way, the IgE antibodies from our bakers were able to recognise glycoproteins from coleoptera, from pulses and, curiously enough, from bee venom (Fig. 3). This might be the explanation for the curious finding of cases of anaphylaxis after a first sting by these insects, without a prior latency period or sensitization. In this same context, the α -amylase from *Aspergillus oryzae*, Asp o 2, is also a glycoprotein with 478 aminoacyl residues and with three structural domains (Fig. 4).

Recent studies by Sandiford *et al.*¹² have demonstrated that persons sensitized to soluble proteins from wheat are able to generate also specific IgE against the insoluble proteins. Their Western Blot experiences have led to the conclusion that gliadins and glutenins with molecular weights similar to those of the enzyme inhibi-

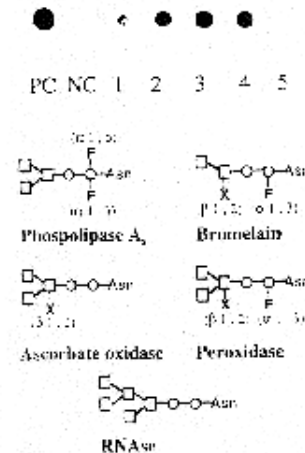


Fig. 4. Glycoprotein immunodetection with hypersensitive sera. Samples: (1) bee venom phospholipase A₂; (2) pineapple bromelain; (3) cucumber ascorbate oxidase; (4) horseradish peroxidase; (5) bovine pancreatic ribonuclease. The membranes were treated with the hypersensitive sera and then with labelled anti-IgE. The wheat allergen CM16* and its non-glycosylated form (CM16) were included as positive (PC) and negative (NC) controls, respectively. The structure of the glycans in these proteins is graphically depicted: L: mannose; O: GlcNAc; X: xylose; F: fucose.

tors are also important allergens (apparently because they share interreactive epitopes with the soluble proteins), so that also they might induce asthma. These results suggest that the number of allergens involved in the development of cereal hypersensitivity is higher than previously believed.

Nevertheless, the albumins and globulins from cereal grains appear to be the most important proteins in the triggering of immediate hypersensitivity reactions²⁻⁵. A number of investigators have confirmed the presence of specific IgE to albumins and globulins in the sera of children with cereal-induced food allergy and with baker's asthma, but not in patients with coeliac sprue^{3,22}. However, while the inhalatory sensitization to cereal flours is one of the better-studied forms of occupational allergy, there are but few investigations of the allergens involved in allergic reactions after the ingestion of these proteins²³. We have been able to show that patients with allergic symptoms after the ingestion of Coca-Cola and Cola Cao might be sensitized to cereals, and particularly to rye proteins in the cases with asthma and to barley proteins in those with anaphylaxis²⁴.

In summary, we believe that the study of the allergenicity of cereal proteins may be highly important, and this importance is mainly based in the fact that baker's

Table I. Characterised allergens in wheat flour

Substance	Molecular weight kDa	Purity	Test	Patients with positive prick test	Reference
α-Amylase inhibitors					
<i>Heterotetramer subunits:</i>					
WTAI-CM2	14	e.p. ¹	Skin prick test	11/31	Armentia et al. 1993
WTAI-CM3B	14	e.p. ¹	Skin prick test	11/31	Armentia et al. 1993
WTAI-CM16	16	e.p. ¹	Skin prick test	7/31	Armentia et al. 1993
WTAI-CM16*	16	e.p. ¹	Skin prick test	14/31	Armentia et al. 1993
<i>Homodimer subunits:</i>					
WDAI-1	15	e.p. ¹	Skin prick test	5/31	Armentia et al. 1993
WDAI-1 and/or		e.p. ²	Immunoblot	4/8	Fränken et al. 1994
WDAI-2		e.p. ¹	2D-Immunoblot	Sera pool	Posch et al. 1995
		e.p. ²	EAST	13/25	Walsh et al. 1989
<i>Monomer:</i>					
WMAI-1	14		Skin prick test	9/31	Armentia et al. 1993
Agglutinin					
<i>WGA homodimer subunits</i>					
	17	e.p. ²	RAST	5/9	Sutton et al. 1984
Acyl-CoA oxidase					
(rice homologue)	27	e.p. ¹	2D-Immunoblot	Sera pool	Posch et al. 1995
Fructose biphosphate aldolase					
(rice homologue)	37	e.p.	2D-Immunoblot	Sera pool	Weiss et al. 1997

* = glycosylated subunit; e.p. = electrophoretically pure; ¹one single point in bidimensional electrophoresis; ²one single band in SDS-PAGE.

asthma is the most prevalent occupational respiratory allergy in many countries. According to Baur²⁵, 1800 bakers request compensation because of this condition every year in Germany. In his series of 405 bakers, 60% evidenced sensitization to wheat flour, 57% to rye flour and 27% to α -amylase (Asp o 2). Among the purified allergens causing baker's asthma, a number of saline-soluble proteins stand out as the major allergens: proteins from the endospermium of the cereal grains with molecular weights between 12 and 15 kDa and belonging to the same family of α -amylase and trypsin inhibitors^{1,5-10} (Table I).

These proteins appear to be involved in the defence mechanisms of plants through the inhibition of the digestive α -amylases of different parasites, thus preventing their degradation. A number of observations concur with this consideration, some of them from the field of pest studies and some from the studies of inhibitor specificities against enzymes from a number of insects: the insects that feed on wheat endospermium show very high α -amylase levels^{26,27}; the major allergen in barley is a specific inhibitor for *Tenebrio molitor* α -amylase (*T. molitor* is a common parasite of this cereal⁶), while the inhibitors from rye restrict their activity

to *Omoplus lepturoides* and evidence no activity at all against *T. molitor*.

After the characterisation, isolation and purification of the wheat α -amylase inhibitors involved in baker's disease, our analyses of the allergenic role of proteins from certain foodstuffs (Coca Cola, Cola Cao, certain malted soluble coffee preparations) have pointed out their usefulness in the diagnosis of occult cereal allergy, and lead to the conclusion that one single protein may be reactive through both sensitization pathways. These findings, together with the already-quoted ones of Sandiford¹² and Baur²⁵, might define their behaviour as a multigenic expression of the family of α -amylase inhibitors in different cereals. Thus, the various species in the Tribe *Triticeae* might be considered to represent different manifestations of a single genome, and sensitization to their various proteins might explain the variability in the clinical findings and in the therapeutic response in patients with sensitizations to different cereal flours²⁶.

Finally, and as a practical application of our own studies and of those of other authors, the following suggestions for clinical application may be proposed:

The optimal purification and isolation of the major allergens for use in *in vitro* and *in vivo* tests might be highly important in the diagnosis of these diseases.

The knowledge of these proteins and of their biologic activities in humans might constitute the basis for genetic manipulation of the plants for the cultivation of less-allergenic species that are also more resistant to pests.

The reduction of the allergenicity of these plants through genetic manipulation might lead to the synthesis of less-sensitizing drugs, which represents a serious limitation of present-day plant biotechnology.

Acknowledgements

I am indebted to Dr. Rosa Sánchez Monge, to Dr. Gloria García Casado and to Dr. Gabriel Salcedo, excellent persons at both the scientific and the human levels, for the opportunity to work with them. I should also like to express my acknowledgement to all the members of the Allergy and Biochemistry Sections of the "Rio Hortega" University Hospital in Valladolid, for the same reason.

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CROSS REACTIVITY BETWEEN ENZYMES

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Enzymes behave as potent inhalational allergens in the occupational environment and are able to sensitize a large proportion of the workers that manipulate them. Whether this great sensitization capacity is related to their enzymatic activity or simply to the fact that they are glycoprotein macromolecules able to trigger an IgE-mediated immunological response has long been a subject of debate.

A number of additives are used in the manufacture of bread, which are added to the dough with the aim to improve and accelerate the panification process.

Table I. Characteristics of the enzyme additives used in bakery processes

- Mixtures of a number of products
- Scarcely purified industrial enzymes
- Contain impurities
- Unknown composition
- Similar fungal origin
Aspergillus oryzae
Aspergillus niger
- Allergenic potential

Table II. Main allergens responsible for asthma in bakers

- Cereals (wheat and rye): albumins, globulins, gliadins, glutenins, prolamines
- Soybean: trypsin inhibitor, lipo-oxidase
- Fungal enzymes:
 α -amylase (*Aspergillus oryzae*)
Hemicellulase and cellulase (*Aspergillus niger*)
Xylanase (*A. niger*)
Glycoamylase (*A. niger*)
- Storage mites
- Bread yeast (*Sacharomyces cerevisiae*)
- Moulds (*alternaria*, *aspergillus*)
- Grain weevil (*Sitophilus granarius*)

Table III. α -amylase: characteristics of allergologic interest

- Glycolytic enzyme usually derived from *Aspergillus oryzae* (Asp o 2).
- Sensitizes 20-32% of symptomatic bakers.
- Partially thermolabile.
- May induce symptoms upon ingestion.
- Glycoprotein with 478 aminoacid residues.
- Continuous sequential epitopes.
- Exposure/sensitization ratio: 0,25 ng/m³.

Among these additives, glycolytic fungal enzymes are one of the major groups. And yet, the enzymatic additives used are scarcely purified industrial products, so that a number of factors must be kept in mind when a possible sensitization is studied. These factors are summarised in Table I.

Bakers' and confectioners' asthma may be caused not only by cereal flour, but also by any of the additives or contaminants present in those flour and summarised in Table II. The sensitization to a number of

enzymes with glycolytic activity derived from *Aspergillus* poses questions about the possible cross reactivity between them, but also between these enzymes and the fungi producing them and, last but not least, between the fungal enzymes and the natural ones to be found in cereal grains.

Cross reactivity between *Aspergillus*-Derived enzymes

The glycolytic enzymes α -amylase and cellulase are additives that are commonly used in the panification process for accelerating the fermentation of the dough. By hydrolysing the starch in the grain flour to fermentable sugars, they continuously generate a substrate that may be used by the yeast. The usual origin of such amylases has been the flour of malted barley, but they are currently derived from microorganisms such as fungi and bacteria. The main characteristics of the fungal α -amylase enzyme are shown in Table III.

In a study of five bakers with α -amylase sensitization, four of whom were also sensitized to cellulase, we investigated the possible cross reactivity between these two fungal enzymes and between the enzymes and the fungi producing them, respectively *A. oryzae* and *A. niger*¹. We found that there was no cross reactivity between α -amylase and cellulase. However, a degree of antigenic community existed between α -amylase and *A. oryzae*, as a 60% inhibition of the α -amylase ELISA (REIA) was achieved with high concentrations of the *A. oryzae* extract. This suggested that α -amylase might represent a relevant allergen in this fungus. A discrete level of cross reactivity was also observed between cellulase and *A. niger*, although in this case the degree of inhibition of the cellulase ELISA with this fungus did not exceed 30%. In a previous study in two patients with occupational asthma who were sensitized to cellulase we had observed moderate cross reactivity between the enzyme and *A. niger*, while there was no cross reactivity between α -amylase and cellulase².

However, the ingestion of bakery products containing α -amylase and hemicellulase did not cause any problem in 17 patients sensitized to *Aspergillus* spp.³.

A very recent study investigating enzyme sensitization in a group of 171 bakers with respiratory allergic symptoms⁴ revealed a 22.8% frequency of sensitization to α -amylase, followed by cellulase (12.9%),

xylanase (10.5%) and glycoamylase (7.6%). This study confirmed that there was no cross reactivity between α -amylase and cellulase or xylanase. However, the cellulase and xylanase extracts, which contained at least six different proteins each, did show cross reactivity in the 80% level⁴. In this same study it was observed that glycoamylase, another glycolytic enzyme from *A. niger* used in bakery, inhibited the xylanase and cellulase RAST by about 50%, suggesting that common epitopes are shared by these enzymes. The authors showed that the main allergenic component in xylanase was a 105 kDa protein with a 5 isoelectric point, which was identified as the β -xylosidase enzyme from *A. niger*; this enzyme was given the designation Asp n 14⁴.

Tarvainen *et al.*⁵ had previously described four workers in biotechnology laboratories who evidenced occupational asthma and contact urticaria after exposure to cellulase or xylanase (a type of hemicellulase that cleaves xylane). These four patients had positive RAST both to cellulase and to xylanase. RAST inhibition studies showed that xylanase inhibited the cellulase RAST by 92%, indicating a high level of cross reactivity between these two enzymes. A primary sensitization to cellulase or hemicellulase will therefore be very difficult to identify because of the high degree of antigenic community between these two enzymes.

Cross reactivity between the natural amylases in cereals and the fungal amylase

A further problem that arises is the possible cross reactivity between the amylases naturally present in cereal grains and in their flours and the fungal amylases used as additives. In our previously discussed study¹ we found that wheat flour caused slight inhibition of the fungal α -amylase RAST, dose-dependently and up to a maximum of 40%. This was interpreted as probably due to a low level of cross reactivity between the natural cereal amylases and the fungal α -amylase, or perhaps to the possible presence of fungal contaminants in the wheat flour.

This aspect was later studied and confirmed by Sandiford *et al.*⁴, who used the sera from 30 patients with inhalational sensitization to wheat flour and compared the IgE levels (RAST) to natural barley α -amyla-

se and β -amylase to those to the fungal α -amylase. Using RAST inhibition studies they demonstrated a low level of cross reactivity between the natural amylases and the fungal α -amylase (in the order of 30 to 40%). They concluded that the natural α -amylase and β -amylase are important allergens in patients with respiratory allergy to cereal flours, but that there is almost no cross reactivity between these enzymes and the fungal α -amylase.

Cross reactivity between bromelain and papain

Bromelain is a mixture of very similar proteolytic enzymes derived from the stalk and the fruit of tropical pineapple (*Ananas comosus*). The two first cases of occupational asthma caused by bromelain were described in 1978⁷. Baur and Frühmann⁸ later reported a 58-year-old female who had been working in a pharmaceutical laboratory for ten years. In her work she was environmentally exposed to papain and bromelain, and she became sensitized to both enzymes. The patient had positive skin tests, RAST and specific bronchial challenge tests with both enzymes. The bromelain RAST could be inhibited with pineapple juice, but this agent did not inhibit the papain RAST. An oral challenge test with pineapple induced abdominal pain and bronchospasm within 30-40 minutes. A positive RAST to bromelain was observed in five out of six patients sensitized to papain, and also in 8 out of 60 control asthmatics not exposed to proteases. Furthermore, bronchial challenge tests with bromelain were carried out in two of the papain-sensitized patients, with positive results; however, the challenge test with bromelain was negative in three of the control patients with positive RAST.

In an enlarged follow-up of that study⁹, Baur investigated a group of four bakers sensitized to wheat and rye flour and a group of 25 pollen-sensitive asthmatics. One of the bakers and seven of the pollenosis patients had positive RAST to bromelain, and three of the pollenosis patients had positive RAST to papain. In the RAST inhibition studies using the sera of the bakers and of the pollenosis patients the authors observed homologous inhibition of the bromelain and papain RAST with the corresponding allergen, and also RAST inhibition between papain and bromelain, although in the latter case the level of the inhibitor

was highly variable from one patient to another. Using the serum of the patient who was clinically sensitized to papain and bromelain and pollen extracts as inhibitors, a certain degree of inhibition (mild to moderate) of the papain RAST was seen (grass pollens 9-63%, birch 3-63%, wheat 9-74%), and also of the bromelain RAST (grass pollens 15-86%, birch 9-69%, wheat 13-89%, rye 6-90%). In a further experiment, the RAST to grass pollens and to bromelain could be inhibited in almost all cases with bromelain, but only in a few cases and to a lesser extent with papain.

A high level of cross reactivity has also been reported between papain and chymopapain, an enzyme that is widely used for carrying out chymonucleolysis in patients with intervertebral disc hernia and which might induce sensitization and severe anaphylactic reactions in patients sensitized to papain¹⁰.

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Fourth Main Subject: Prognostic factors in childhood asthma

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EVOLUTION OF CHILDHOOD ASTHMA

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Introduction

Three main criteria should constitute the basis for the determination of the natural history of any disease. Firstly, a precise definition allowing the investigator to identify those subjects who have that disease and to rule out other diseases that course with similar symptoms. Secondly, the identification of risk factors that may help suggest the course of the disease from its beginning up to its remission or the eventual demise of the patient. Thirdly –and lastly– an assessment of the effects of the therapy on the course of the disease and on its resolution.

Concept of childhood asthma

The clinical manifestations of asthma are not specific; thus, this concept encompasses a constellation of symptoms rather than a specific disease. The inclusion into its definition of the pathogenetic mechanisms has rendered the definition of asthma more specific in the present times. However, pathologic studies of asthma in children are scarce, so that the definition of the disease in this particular context is still a subject of debate.

Bronchial asthma is a chronic inflammatory disease of the airways, in which a number of cells intervene, but particularly mast cells, eosinophils, T lymphocytes, neutrophils and epithelial cells of the airway. In susceptible individuals, this inflammation induces episodes of wheezing, dyspnoea, chest tightness and cough, predominantly at night and/or in the early morning hours. These symptoms are associated to a variable airflow restriction, which can revert spontaneously or under medical therapy. The inflammation also induces a coincident enhan-

cement of the airway response to a number of diverse stimuli.

The above is very much a working description, and encompasses clinical, pathological and pathophysiological criteria. The aspects more obviously stressed are those of chronicity and inflammation. However, the concept of cell-mediated inflammation has not been demonstrated and documented because of the lack of morphologic studies in the mild and moderate forms of childhood asthma, so that this mechanism cannot be included in the definition even though all the data suggest it.

The definition of asthma in children under three years of age is particularly difficult, as wheezing in children in this age group are mainly due to mechanical factors associated to the size of the airway. Over the last ten years there has been heated conceptual and terminological debate regarding the description of the episodic wheezing occurring in early childhood, which are quite often triggered by a viral infection. Descriptive terms such as *spastic (or spasmodic) bronchitis*, *descending airway catarrh*, *asthmatform bronchitis*, *wheezy bronchitis*, *dyspnoeic bronchitis*, *infectious asthma*, *postbronchiolitis wheezing* and *postbronchiolitis syndrome* have all been used, implying a causality in the episode that can not always be demonstrated. Later on these terms were superseded by others such as "bronchial hyperreactivity", which however denotes not a diagnosis but a characteristic or particular condition of the bronchi. Other English-speaking authors have proposed the use of the descriptive designation of "Lower Respiratory Illness" (LRI) with or without wheezing. Finally, in an attempt to globalise the concept, it was decided to call this condition simply *asthma*.

In the case of young children, the International Paediatric Asthma Consensus Group in 1992 decided to use the denomination *asthma* for that chronic airway disease comprising recurrent episodes of cough and/or dyspnoea that revert spontaneously or under medication, after having ruled out other conditions². At present it is accepted that any child having had three or more wheezing episodes should be considered to be asthmatic regardless of his or her age, although other possible congenital or acquired conditions coursing with cough and/or wheezing must first be ruled out.

Among the diseases that course with wheezing during childhood and which should be ruled out in children presenting with clinical features suggestive of asthma are bronchiolitis, diphtheria, tuberculosis, gastroesophageal reflux, bronchopulmonary dysplasia, foreign body

aspiration, cystic fibrosis, tracheo-oesophageal fistula, abnormalities and malformations of the greater thoracic blood vessels, tracheomalacia, obliterative bronchiolitis and many others.

Pathophysiologic factors

Childhood asthma is a heterogeneous condition with substantial variations in presentation, severity and clinical evolution, and the patient's age is a modulating factor for all these parameters. From the clinical point of view, the condition in the school-age child and in the adolescent is the same as that in the adult, but in the young child further factors intervene that may condition the clinical features.

A number of these factors refer to the structure of the airway: greater airway resistance because of the smaller airway size and cross section, irregular muscle distribution, lesser elastic recoil, smaller number of collateral channels, increased secretions and poor clearing mechanics. Further factors are derived from the thoracic structure: weak thoracic musculature, poor diaphragmatic mechanics. Finally, there are general factors: patient's immunity in a development stage, infections, fever, dehydration, etc.

All these factors lead to the fact that in the young child the accumulation of secretions is predominant over the wheezing, readily causing mucus plugs, atelectasis and false pneumonias, overinfection and air trapping.

Epidemiology

Asthma is the chronic respiratory disease that is most frequent in infancy and childhood. About 7 to 15% of the paediatric population suffers asthma, and the prevalence is increasing³. This increasing prevalence of asthma in childhood can be attributed to a number of factors. The term "asthma" is at present used for what was earlier called "asthmatic bronchitis", "wheezy bronchitis", etc. A further factor to be considered is the better diagnosis of the condition. Nevertheless there appears to be occurring a true increase in the prevalence of asthma because of the increasing incidence of risk factors such as atopy, familial tobacco smoking, lifestyle habits, etc.

Magnus and Jaakkola⁴ have analysed the results of 16 prevalence studies, all of them using the method of repeated cross-sectional survey. Despite the fact that the results of most of these studies suggest an increase in the prevalence of asthma, the authors remain sceptical; they ascribe this increased prevalence to a possible over-

Table I. Factors that may influence the evolution of childhood asthma

-
- Gender.
 - Atopy.
 - Familial and genetic factors.
 - Respiratory infections.
 - Environmental pollution.
 - Indoor allergens.
 - Initial severity of asthma.
 - Altered pulmonary function.
-

ruse of the diagnosis of asthma. It is still unclear if the increase reported in many studies is real, or if it rather reflects changes in the diagnostic criteria used.

Factors that may influence the evolution of asthma

A number of factors have been put forward that may influence the evolution of asthma in early childhood (Table I).

Gender: A number of studies have reported that males are at a greater risk of developing asthma before the age of 14 years⁵, while females are at greater risk in adult life⁶. However, the gender does not appear to affect the evolutive course of the disease⁷. Weiss et al.⁸ have reported that males have a greater incidence of asthma, while females evidence greater alteration of the pulmonary function.

Atopy: Among patients between 3 and 14 years of age, sensitization to some allergen detected by positive skin tests has been significantly associated to clinical asthma⁹. Increased total serum IgE levels are also associated to the prevalence of asthma over time¹⁰. Children with eczema and positive skin tests usually have more severe asthma¹¹, and the persistence of eczema has been associated to the persistence of asthma⁷. Atopy is associated both to the persistence of wheezing in infants¹² and to a greater risk of early- or late-onset childhood asthma.

Familial and genetic factors: A potential genetic component exists in asthma. This leads to the clustering of asthmatic patients in the same family and among twins. There has been recent confirmation of the importance in this context of the chromosome regions 5q, 6q, 11q, 12q and 14q, and further regions have been suggested¹³. The presence of asthma in the mother represents the most important genetic burden.

Respiratory infections: Respiratory infections often cause wheezing in children, both in asthmatics and in

non-asthmatics. Derangements of the pulmonary function have been demonstrated in children admitted to hospital because of respiratory infections, as well as increased bronchial reactivity after such infections¹⁴. A number of studies suggest that a history of bronchiolitis or croup in the early months of life represents a risk factor for increased bronchial reactivity five years later¹⁵. However, the precise role of viral respiratory infections as a cause of asthma is still debated.

Severity of asthma: Some reports concur in that the more severe the asthma is during childhood, the less likely it is to disappear in adult life.

Environmental pollution: Environmental pollutants, such as nitrous oxide and ozone, have been demonstrated to increase the effects of allergens, possibly because they increase bronchial reactivity. A number of studies suggest that teenagers are more sensitive to environmental changes than both adults and younger children.

Indoor allergens: There is clear evidence that the degree of exposure to household mites may influence the incidence of asthma¹². The levels of mites in dwellings may predict the development of asthma. Children with high levels of mite allergens in their homes tend to an earlier beginning of asthma, and they will persist with asthma if exposure to the allergens to which they are sensitized is not prevented.

Passive smoking: Tobacco smoke exposure non-specifically increases bronchial reactivity, possible through increasing the degree of bronchial inflammation. Active tobacco smoking in the mother increases the risk of developing asthma and of asthmatic exacerbations in the child. Intrauterine exposure to tobacco smoking may affect the bronchial reactivity and induce early derangements in lung function in the newborn¹⁶. Parental smoking considerably increases the risk for a diagnosis of asthma.

Wilson¹⁷ has suggested a profile of the possible risk factors influencing lower respiratory illness with wheezing in children according to the age of the patients. According to this proposed model, in the period between birth and the age of four years, and with prevalence peaking by the age of 12 months, recurring wheezing would be related to mechanical alterations of the airway, genetic factors and tobacco smoking in the mother. Between that age and that of five to six years, the wheezing episodes would frequently correlate with viral infections; from that age onwards, the importance of viral infections as triggering factors for asthmatic episodes would be much less. Finally, from the age of one year and in increasing pro-

portion, atopic asthma would be the most important and frequent cause of wheezing in children.

Evolution and prognosis

In 1993, the Allergy Service of the "Niño Jesús" Hospital in Madrid participated in a multicentre study of asthma in children under three years of age¹⁸. In that study data were collected on 145 patients aged less than three years with bronchial asthma. The first wheezing episode occurred before the age of 12 months in 70% of the cases; however, over 80% of these patients first attended the Allergy outpatient clinic for this reason when they were between 13 and 36 months old. A diagnosis of allergic asthma was established in 27 of these 145 patients (18.6%) on the basis of positive skin tests to airborne allergens and/or foodstuffs. In over 80% of the cases the asthma episodes were associated to airway infection, without any significant difference between the allergic and non-allergic children. In this study, the severity of asthma was assessed using criteria based on the frequency and severity of the asthmatic episodes and on the intercritical symptoms.

At the time of the first consultation, 52% of the children evidenced moderate asthma, 26% severe asthma and 22% mild asthma. Sixty per cent of these children had asthmatic episodes one or twice monthly, and 30% of the patients had required between 1 and 7 admissions because of asthma. Both the "allergic" and the "non-allergic" patients had favourable and similar evolution. At the time of the first consultation 26% of the patients had severe asthma; however, after three to four years of follow-up (60% of the children were over six years of age) none of the patients had asthmatic manifestations classed as "severe". It is noteworthy that 19 of the 27 children with a diagnosis of allergy (70%) still attend the Allergy outpatient clinic after three years, while only 25 of the 119 children in whom no allergic sensitization had been detected (21%) still came to the clinic for the periodic revisions.

This was a retrospective study, with all the difficulties and disadvantages this entails, but still conclusions may be derived from it that are similar to those of other studies. Atopic children persist with asthma, and those in whom the asthmatic episodes had been triggered by infections exclusively usually show a favourable evolution with resolution of their asthma.

The study carried out in Tucson (Arizona, USA) by

Martínez *et al.*¹⁹ in 1995 is the most frequently cited one in the literature. This was a prospective evolutive study of 1,246 children who were controlled at the ages of three and six years. This study included "point zero" data, that is data from the time before the respiratory disease first appeared. The data considered were the umbilical cord blood IgE, the total serum IgE at 9 months and 6 years of age, the pulmonary function prior to the first apparition of the lower airway involvement and at age 6, and the skin tests performed at age 6. The parents of the children also completed a questionnaire.

According to the results of this study, 48.5% of the children had evidenced at least one wheezing episode by the age of six years. Wheezing episodes had been recorded before the age of three years, but not by age 6, in 19.9% of the cases. Conversely, 15% had not had wheezing prior to age 3 but had asthma at age 6, and 13.7% had had wheezing before they were 3 and persisted with asthma at age 6. Thus, 40.7% of the children with asthma before the age of 3 years persisted with wheezing episodes at the age of 6 years. Those children who had had wheezing episodes before they were 3, but not when they were 6 years of age, evidenced reduced pulmonary function (length-adjusted maximal expiratory flow at Functional Residual Capacity, or V_{\max} FRC) both before one year of age and at the age of 6 years. They also had a greater probability to have smoking (but not asthmatic) mothers, and they did not have increased IgE levels in cord blood or at the age of 9 months, nor positive skin tests.

The children who had begun having wheezing episodes in the earlier years of life and continued having them at the age of 6 years had a greater probability to have mothers with a history of asthma, increased serum IgE levels at the age of 9 months with normal pulmonary function during the first year of life, and reduced pulmonary function with increased serum IgE levels by the age of 6 years. These children with persisting wheezing were the ones with the lowest pulmonary function in all groups. Those in whom asthma had first appeared at a later age had an increased probability of having positive skin tests, without increased IgE levels during the early months of life. This study by Martínez *et al.* showed that one-third of all children aged 3 or less than 3 years had lower respiratory illness with wheezing, but almost 60% of these children did not have any wheezing episodes at the age of 6 years.

The natural history of asthma in children has been addressed in other prospective studies, and their results

lead to a better understanding of its evolution²⁰⁻²⁷ (Table II).

Park *et al.*²² studied a cohort of 11,465 children at birth and at the ages of 5 and 10 years in the United Kingdom. Among those children who had at least one episode of wheezing before the age of 5 years, only 20% had one or more episodes by age 9. The number of episodes before the age of 5 was a predictive factor for the prognosis by the age of 10. Among the children with a diagnosis of asthma at the age of five years, 50% did not have asthma when they were ten years of age.

McNicol and Williams⁶ published a study of children aged 7 years in 1964, with a 21-year follow-up. By the time they were 28, 43% of the patients who had had wheezing at the age of 7 years did not have clinical features of asthma, but 32% still had wheezing at least once a week²⁹. Further studies were begun in the United Kingdom in 1958^{25,28,30} in which the patients were monitored over 33 years. The parents of the participants were interviewed when the children were 7, 11 and 16 years of age, and then the participants themselves were interviewed at the ages of 23 and 33 years. Among the children with a history of asthma or wheezing before the age of 7 years, only 10% had wheezing when they were 23. However, in the interviews when they were 33, 27% of the subjects reported having had wheezing in the immediately previous years. The subjects who reported wheezing at the age of 33 had 10% less FEV₁ than the control subjects.

Other, earlier studies^{5,6,11,31-34} (Table III) have yielded similar results. Generally, all studies concur in that at least 30% of the children aged less than 3 years have had wheezing at some time in their lives. In at least 50% of the cases these children are asymptomatic by the age of 6 years. Among the children who have asthma at the age of 7-8 years, almost 50% will also be asymptomatic by the time they reach adulthood. Most of the studies also suggest that children over 6 years of age who have altered pulmonary function when in a stable phase tend to persist with asthma when they become adults.

Conclusions

There are a number of questions that may be posed: is the asthma of the young child (infant and up to school age) the same disease entity as that of the school child and the adolescent? Does this correspond to the pattern of adult asthma? What is the short- and long-term prognosis of childhood asthma? Which would be

Table II. Studies on the natural history of childhood asthma

Study	Population	Follow-up	Findings
Kelly 1988	401 7-year-old children	Up to age 28	– Those with initially severe symptoms persist with asthma
Park 1986	11,486 newborn in the U.K.	Up to age 10	– 80% of those with wheezing before age 5 were asymptomatic by age 10 – 50% of those with asthma at age 5 were asymptomatic by age 10
Sporik 1991	67 at risk for atopy, U.K.	At ages 5 and 11	– Asthma at age <2 yrs (21 yrs) at age 11 76% no clinical symptoms, 63% no BHR – Asthma at age >2 yrs (21 yrs) at age 11 17 (80%) with asthma; 12 (57%)
Godden 1994	455 9-to-15-year-olds, Scotland	25 years	Persistence if altered lung function or BHR
Martínez 1995	1,246 niños <3-year-olds, USA	Up to age 6	– 40% asthma persists; children of asthmatic mothers, high IgE
Strachan 1996	1,335 newborn, U.K.	Up to age 35	– 302 (22%) asthma at age 16; 40% of these asthma at age 35
Kokkonen 1993	108 de 15-year-olds, Sweden	Up to age 20-24	– 28% asymptomatic – 22% weekly symptoms – 48% BHR
Roorda 1994	406 de 8-12 year-olds, Netherlands	Mean follow-up 14 yrs, to age (mean) 25	– HRB ↓ with time FEV ₁ ↑ with time

Table III. Clinical course of childhood asthma

Study	Population	Follow-up	Asymptom.	Symptom.	Died
Rackeman 1952	688 n. <13 yrs.	20 years	66%	31%	1.5%
Dees 1957	236 n. <14 yrs.	5 years	44%	56%	1%
Ogilvie 1962	1000 children	11 years	48%	45%	7%
Buffum 1966	518 children	10 years	41%	58%	1%
Blair 1977	244 <12 yrs.	20 years	27%	70%	1%
McNicol 1973	314 7-yr-olds	7 years	48%	52%	–
Schachter 1984	25 7-17-yr-olds	7 years	72%	28%	–

the most adequate therapy? Can therapy modify the prognosis?

According to the concept of asthma this is a chronic disease that, as do all other chronic diseases, may fluctuate in severity over time with periods of remission. Respiratory infections and an altered pulmonary function are important factors during infancy and early childhood, and the prognosis at this age is more benign than that in older children. In the latter atopy and sensitization to airborne allergens usually play a more significant role, which is closely associated to the development of persistent asthma. For all these reasons, it is readily deduced that age is a conditionant in the clinical features and evolution of asthma.

The observation that asthma tends to cluster in families has led to carrying out a number of genetic studies, which are gradually clarifying the genetic component in this disease. Also environmental influences may have a considerable importance in the persistence of asthma.

Considering all the above, it must be admitted that asthma beginning during childhood has a considerable probability of persisting into and during adult life. Many adult asthmatics report having had their first episode during childhood. The truth is that the natural history of bronchial asthma in each particular case is rather unforeseeable. While the importance of the risk factors for the

development of asthma during childhood is still not sufficiently clear, the identification of "at risk" children appears to be fundamental for applying to them enhanced therapeutic and preventive strategies. How, why and for how long we are to treat asthma appearing during the early years of life, or even whether the therapy should be aimed at "remission" or at "prevention" of the inflammatory process by using pharmacotherapeutic and/or environmental strategies are questions that are still very much open to debate.

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PROGNOSTIC FACTORS IN CHILDHOOD ASTHMA. ROLE OF INFECTIONS

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Over the last 30 years, evidence has accumulated relating chronic bronchial conditions in the adult with the antecedent of thoracic symptoms during childhood due to respiratory tract infections. This has led to the incorporation of the antecedent feature of respiratory tract infections during childhood to the list of risk factors intervening in the pathogenesis of chronic obstructive pulmonary disease and bronchial asthma.

One of the infectious processes of childhood that has been receiving increasing attention over the last years is that of acute infantile bronchiolitis. This disease evidences a number of interesting features: it is a very frequent condition; it affects very young children in whom it often causes respiratory failure, and it also often is the precursor of repetitive wheezing episodes during childhood. Its correlation with bronchial asthma is hotly debated, as it is quite difficult to establish a differential diagnosis between these two entities. This is mainly due to the lack of universally accepted diagnostic criteria for bronchiolitis and to the irregular response in infants with wheezing show to bronchodilator and steroid therapy.

A number of prospective follow-up studies have been carried out on children with an antecedent of bronchiolitis, and their results have been surprisingly uniform considering the discrepancies in the diagnosis of the condition. These studies demonstrate the frequent apparition of repetitive wheezing episodes that in most cases disappear with the passage of time, of a poorer lung function with frequent derangement of the parameters reflecting the state of the peripheral airways, and of high levels of bronchial hyperreactivity.

In order to assess the behaviour of this condition in our environment, we have retrospectively studied up a group of children who had required hospital admission because of a clinical picture suggestive of acute bronchiolitis. To this purpose, we reviewed the clinical histories of 1117 children with families residing in Saragossa,

who had been admitted to the "Miguel Servet" Hospital and the University Hospital of this city in the period between 1973 and 1978 because of respiratory tract infection. Among these clinical files, we selected those of 330 patients that strictly fulfilled McConnochie's criteria for the diagnosis of bronchiolitis (Table I); the mean age at the time of admission had been 18 weeks. It was possible to establish contact with the families of 134 of these children, and 72% of them (97 children) accepted participating in this study.

The follow-up study was carried out in two phases: one during the adolescence period of the patients, and the second one at the beginning of their adult life.

At the time of the first assessment the study group comprised 57 boys and 40 girls with a mean age of 12 years. In the course of this assessment a clinical history was recorded with the help of one of the parents, with particular emphasis in the detection of wheezing episodes; also at this time a baseline spirometry, a methacholine non-specific bronchial hyperreactivity test (according to Cookson et al.) and skin tests with a panel of 13 airborne allergens that are common in our environment were performed.

Almost one-half of the boys and girls with an antecedent of bronchiolitis (42%) reported having had wheezing episodes after recovering from their bronchiolitides; these episodes had usually been triggered by respiratory tract infections.

The results of the baseline spirometries were compared with normal result tables that had been established at the same Pneumology Laboratory on the basis of a sample of the general children population of Saragossa. The children with bronchiolitis antecedents evidenced a poorer lung function as compared to their predicted theoretical values. Specifically, the children who had had bronchiolitis evidenced significantly lower values for Peak Expiratory Flow Rate (PEFR), Maximal Flow Rate

Table I. McConnochie's diagnostic criteria for acute bronchiolitis in the infants

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- Age < 24 months.
 - Absence of previous respiratory processes that might have affected the lower respiratory tract.
 - Signs of viral respiratory disease: coryza, otitis media or fever.
 - Expiratory wheezing with acute onset.
 - With or without signs of respiratory distress, pneumonia or atopy.
-

Table II. Respiratory function at age 12 years after a prior episode of acute bronchiolitis

	Bronchiolitis (B) (N=97) (X±SD)	Significance level P vs Predicted	Bronchiolitis without subsequent wheezing (BNW) (N=57) (X±SD)	Significance level BMW vs. predicted	Predicted values (X±SD)
FVC (L)	3.3±0.8	NS	3.4±0.8	NS	3.3±0.7
FEV ₁ (L)	2.9±0.7	NS	3.0±0.7	NS	3.1±0.7
FEF ₂₅₋₇₅ (L)	3.5±1.0	<0.05	3.6±0.9	NS	3.8±1.0
PEFR (L.s ⁻¹)	5.6±1.4	<0.05	5.7±1.5	NS	6.0±1.4
MEF ₅₀ (L.s ⁻¹)	3.4±0.9	<0.0001	3.4±0.9	<0.0001	5.1±1.3

B = complete group of children with antecedents of bronchiolitis.

BNW = complete group of children with antecedents of bronchiolitis who thereafter remained asymptomatic.

between 25 and 75% of the Forced Vital Capacity (FEF₂₅₋₇₅) and Maximal Expiratory Flow Rate at 50% of the Forced Vital Capacity (MEF₅₀). When only the group of children denying wheezing episodes after bronchiolitis was considered, so as to avoid the inclusion of asthmatics, it was seen that the MEF₅₀ was still significantly lower in these subjects (Table II).

As our study was retrospective and the methacholine bronchial hyperreactivity (BHR) test evidences a high sensitivity, the results of this test might be altered if among the volunteer controls there had been an "auto-selection" with a trend to include an excess proportion of subjects with "respiratory problems". As it was impossible to guarantee that the control group in this test represent a true sample of the general population, it was decided to admit to this group only subjects who had never had wheezing, so as to be able to compare it with that of the children remaining asymptomatic after their bron-

chiolitides. The only difference between these two groups would thus be the antecedent of bronchiolitis.

When the BHR levels were compared between the subjects with an antecedent of bronchiolitis and the control group it was seen that the former had a greater level of BHR. However, when the children who had evidenced later episodes of wheezing were excluded from the study, the BHR levels continued to be significantly higher in the group of children with bronchiolitis antecedents (Table III).

The results of this first phase of our study led us to the conclusion that the antecedent of hospital admission because of acute bronchiolitis is associated to a poorer lung function and to a higher level of bronchial hyperreactivity during the adolescence period, even when these children had not developed clinical features suggestive of bronchial asthma up to this time.

We could thus confirm a generally accepted obser-

Table III. Bronchial hiperreactivity at age 12 after prior episode of acute bronchiolitis

	Bronchiolitis* (N=80) N (%)	Bronchiolitis with no subsequent wheezing episodes** (N=49) N (%)	Control subjects (N=27) N (%)
Severe	–	–	–
Moderate	19 (23.7)	7 (14.2)	–
Mild	44 (55.0)	27 (55.2)	7 (25.9)
Negative	17 (21.3)	15 (30.6)	20 (74.1)

*Significance of the difference between the complete group of children with antecedents of bronchiolitis and the control group: P<0.0001.

**Significance of the difference between the complete group of children with antecedent of bronchiolitis who then remained asymptomatic and the control group: P<0.001.

Table IV. Lung function at age 12 years and current bronchial asthma among the children with antecedents of acute infantile bronchiolitis (AB)

Parameters (% of predicted)	Current asthma (26% of the AB group) (X ± SD)	No current asthma (74% of the AB group) (X ± SD)
FEV ₁ /FVC (%)*	84 ± 8	88 ± 5
FEV ₁ (L)*	90 ± 17	98 ± 13
FEF ₂₅₋₇₅ (L)	86 ± 31	96 ± 18
MEF ₅₀ (L · s ⁻¹)**	54 ± 23	70 ± 15

* P < 0.05

** P < 0.005

vation regarding the repercussions of acute bronchiolitis in infancy: this condition involves a great risk of developing repetitive wheezing episodes, and also of developing changes in lung function consisting of increased bronchial hyperreactivity and poorer expiratory flow rates. In the light of these and similar results, a large number of investigators have pointed out the suggestion that bronchiolitis represents a risk factor for the later development of asthma during childhood, and even may itself represent a first episode of bronchial asthma. However, and because of the favourable clinical evolution of these children, with frequent remission of the sibilances as the child grows, a number of different explanations have been put forward for the findings observed in the follow-up, and the repercussion of this childhood condition during the adult life of the individual remains uncertain.

With the aim of ascertaining some of the hypothe-

ses considered regarding the significance of the findings in those children, we are presently carrying out the second assessment of our cases, performing the same tests as in the first assessment.

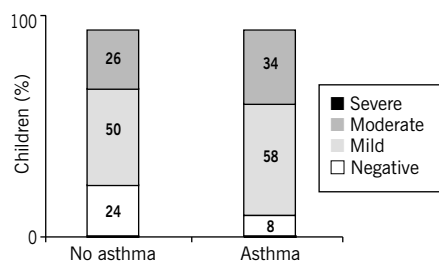
Seventy-one of the subjects from the original group (73%) participate in this second phase of the study. Seventeen subjects could not be located, and a further nine refused participation or did not come to their appointment. Thus, the study group is now formed by 36 males and 35 females with a mean age of 21 years, that is at the beginning of their adult life. This age period is particularly interesting as it coincides with one of the periods in life with the lowest incidence of bronchial asthma; furthermore, a number of epidemiologic studies have demonstrated that the highest levels in lung function are achieved during this period.

Among the subjects participating in the second phase of our study, 19 (27%) have been classed as asthmatics. Symptomatic BHR could be demonstrated in 17 of them (defined as having had wheezing sometime in the last 12 months and having a high level of bronchial hyperreactivity either in the methacholine test [11 subjects] or in the bronchodilator test [6 subjects]). Two subjects evidencing typical clinical features of asthma, a FEV₁ <80% of their predicted value and required permanent bronchodilator and steroid therapy were also included in this group although they had negative bronchodilator tests.

Significance of the post-bronchiolitis functional impairment

In asthmatic subjects, a certain degree of bronchial obstruction may persist at the level of the peripheral airways even during the periods of clinical remission. This functional impairment might be due to a subclinical persistence of bronchial inflammation, or it might be a consequence of the airway remodelling caused by that inflammation.

On the other hand, a number of follow-up studies in children with bronchial asthma have revealed a correlation between the lung function level at the time of diagnosis and the later clinical evolution. It is thus more difficult for the clinical symptoms to disappear in those children in whom a greater previous functional impairment had been detected. In this context, our subjects with prior bronchiolitis antecedents have evidenced a behaviour that is similar to that of the asthmatic patients, so that also we have observed a significant correlation



AB = Children with antecedents of acute bronchiolitis.
p = NS

Fig. 1. Bronchial hyperreactivity at age 12 years and current (at age 21 years) bronchial asthma in the AB group.

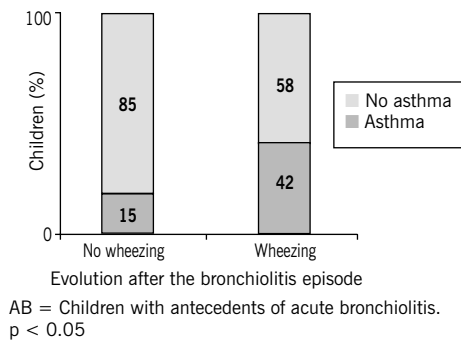


Fig. 2. Bronchial hyperreactivity at age 12 years and current (at age 21 years) bronchial asthma in the AB group.

between their pulmonary function at age 12 years and the current presence of asthma (Table IV).

However, this functional impairment may not have been due only to the development of overt bronchial asthma. Exceptionally, bronchiolitis itself can induce irreversible changes in the airways (bronchiectasies, obliterating bronchiolitis) that may find expression in functional deficiencies at a later date. Nevertheless, none of these conditions was detected in our study group, and the eventual post-bronchiolitis symptoms always appeared episodically.

Another possibility is that the "post-bronchiolitis" functional impairment may even have preceded the bronchiolitis itself, and thus not be really its consequence. As a matter of fact, and as already demonstrated in the epidemiologic studies carried out by the Tucson group, those children who tend to present wheezing du-

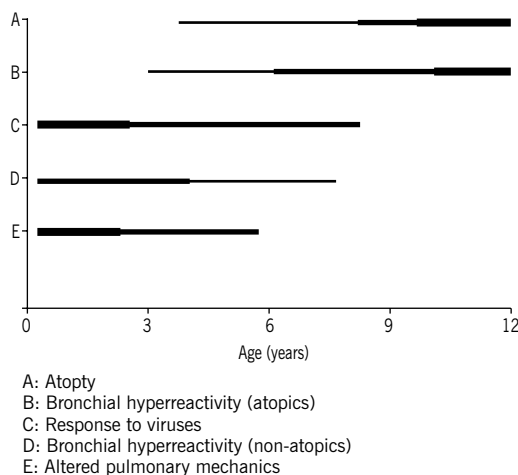


Fig. 3. Risk factors for the apparition of childhood wheezing and ages at which they preferently incide.

ring respiratory tract infections already had a poor lung function.

Significance of the presence of post-bronchiolitis bronchial hyperreactivity

Bronchial hyperreactivity (BHR) is a typical feature of asthma. Its presence in this disease is so frequent that it is even contemplated in its definition. The finding of high BHR levels in subjects with antecedents of bronchiolitis lends support to the existence of an association between acute bronchiolitis and the development of bronchial asthma; however, it is difficult to establish a prognosis on the presence of post-bronchiolitis BHR in subjects who deny having symptoms of asthma. Actually, the studies carried out on the general population in an attempt to demonstrate the possibility that subjects with high BHR levels but who are asymptomatic may have a greater propension to the development of bronchial asthma have also led to heated debate, so that while some results appear to confirm this possibility yet others do not.

In our own study we have seen that while those subjects with an antecedent of bronchiolitis and a present diagnosis of asthma had higher BHR levels than those who had not developed asthma, these differences do not achieve statistical significance (Fig. 1). Furthermore, BHR might also have been facilitated in these children by the existence of previous bronchial obstruction, so that, as the airway resistance is directly proportional to the fourth power of the radius of the bronchial lumen ($R_{aw} = r^4$), slight reductions of the bronchial lumen might facilitate bronchoconstriction during the methacholine test, thus yielding higher BHR levels as a result.

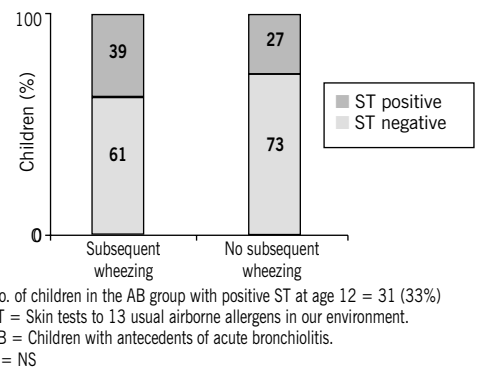


Fig. 4. Skin tests (ST) at age 12 years and presence of wheezing episodes at this age after having had bronchiolitis.

Significance of the post-bronchiolitis wheezing episodes

The possibility that the post-bronchiolitis wheezing episodes might represent the development of bronchial asthma is strongly supported by the fact that even though bronchiolitis is a process that does not generally respond to the usual therapy for bronchial asthma, the subsequent wheezing episodes do usually evidence a good response to bronchodilator and steroid therapy. Furthermore, our own results reveal a significant relationship between the appearance of post-bronchiolitis wheezing episodes during the first 12 years of life and the current presence of asthma (Fig. 2), suggesting that those wheezing episodes represent a prognostic factor for the development or persistence of asthma in the adult.

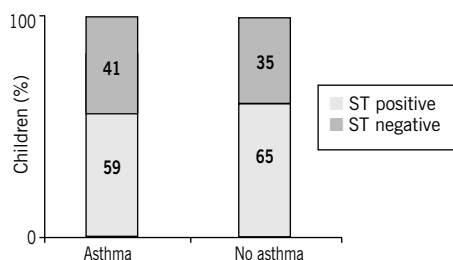
Nevertheless, a considerable fraction of these subjects will become asymptomatic before they reach adolescence, and further possibilities justifying the presence of wheezing must therefore be considered. One of those possibilities that bears consideration is that both the bronchiolitis and the apparition of later wheezing episodes may be caused by a heterogeneous group of proces-

ses that would only exert their action on the individual during a definite period of time, after which period the subject would not evidence respiratory problems again. This has led to the concept of the "infantile wheezing phenotype". A number of different phenotypes may be observed within the group of subjects who will develop wheezing during childhood (Fig. 3). During this period of life there would thus exist a series of risk factors for the development of wheezing that might exert their actions during varying and different time periods, and it would then be the degree of susceptibility of the children to these risk factors that leads to the apparition of wheezing episodes at some time during their childhood. Thus, for instance, while viral infections might constitute a risk factor for the development of wheezing during the early years of life and lose their transcendence as the child grows, atopy would achieve a greater relevance in the causation of respiratory symptoms at later ages during childhood.

Significance of atopy in the post-bronchiolitis wheezing episodes

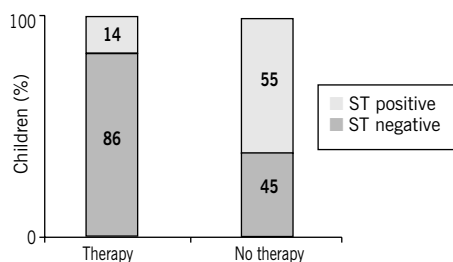
On the basis of multiple epidemiologic studies performed on the general population, the existence of an evident correlation between the symptoms of asthma and the presence of atopy, so that the role of the latter in the pathogenesis of bronchial asthma is no longer open to discussion. However, when the presence of atopy was studied in our group of children with bronchiolitis antecedents we were unable to detect any correlation between the presence of positive skin tests and the presence or absence of post-bronchiolitis wheezing episodes in the assessment performed at the age of 12 years (Fig. 4), nor between the positive skin tests and the presence of asthma (symptomatic BHR) when both were assessed at age 21 (present assessment) (Fig. 5). Nevertheless, when the requirement for bronchodilator or steroid therapy was considered in the group of current asthmatics with bronchiolitis antecedents, six out of the seven subjects in this group who had required this therapy in the 12 months preceding the second assessment were atopics (Fig. 6). As the amount of therapy an asthmatic requires for the control of his symptoms represents a measure of the degree of severity of his disease, this finding suggests that those subjects with antecedents of bronchiolitis who develop bronchial asthma will have a worse evolution when the presence of underlying atopy is demonstrated.

Thus, our results suggest that the conclusions from



No. of children in the AB group with positive ST at age 12 = 32 (49%).
ST = Skin tests to 13 usual airborne allergens in our environment.
AB = Children with antecedents of acute bronchiolitis.
p = NS

Fig. 5. Skin tests (ST) and current (at age 21) asthma in the AB group.



ST = Skin tests to 13 usual airborne allergens in our environment.
AB = Children with antecedents of acute bronchiolitis.
p < 0.05

Fig. 6. Skin tests (ST) and requirement for bronchodilator or steroid therapy (in the 12 months preceding the assessment carried out at age 21) in the AB group.

epidemiologic studies carried out on the general population that relate the symptoms of bronchial asthma and atopy may not be necessarily applicable to other study groups, in which respiratory tract infections may be relevant for the development of such symptoms. Our study thus confirms the existence of multiple factors responsible for the development of wheezing episodes during childhood

Conclusions

In summary, the follow-up of our study group of children with an antecedent of hospital admission because of acute bronchiolitis has disclosed the frequent apparition of wheezing episodes at later times during childhood (42% of the studied group) and a poor lung function at the beginning of adolescence. Both findings appear to represent risk factors for the development/persistence of bronchial asthma in adult life.

On the other hand, our results also confirm the existence of multiple factors responsible for the episodes of childhood wheezing.

PREVENTIVE EFFICACY OF IMMUNOTHERAPY

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Allergy and its manifestations constitute the phenotypic expression of genotypically predisposed individuals after contact with critical amounts of allergens, conditioned by other, nonspecific environmental factors¹. Keeping this in mind, it is easily understood that all preventive efforts have three possible levels of intervention²:

- Tertiary Level: by avoiding the apparition of symptoms in patients who have already developed an allergic disease.
- Secondary Level: by avoiding the development of an allergic disease in individuals who have already developed an IgE-mediated sensitization.
- Primary Level: by avoiding the development of an IgE-mediated sensitization, especially in individuals who are at risk.

Consequently, the possible preventive efficacy of Immunotherapy must needs be considered in relation to these three levels.

Tertiary-level preventive effect of immunotherapy

The efficacy of immunotherapy in the control of symptoms in patients affected by allergic disease has been extensively proven since Noon published his first study, back in 1911. Quite recently, two reviews have categorically confirmed this concept. One of these review studies is the meta-analysis published by Abramson, which demonstrates that patients treated with immunotherapy have a threefold greater possibility of clinical improvement as compared to those receiving placebo³. The second one is the WHO Position Paper, which underscores and confirms the efficacy of immunotherapy in the management of allergic rhinoconjunctivitis, allergic asthma and allergic sensitization to Hymenoptera venoms⁴.

Secondary-level preventive effect of immunotherapy

The prevention of the development of clinical allergic disease in already sensitized individuals constitutes a further possible effect of immunotherapy. The evidence regarding this level is scarce, although the PAT (*Preventive Allergy Treatment*) trial, underway since already five years in northern European countries, shows that children with allergic rhinitis have significantly less probability to develop bronchial asthma in the future if they receive specific immunotherapy during at least three years⁵.

This aim at secondary-level prevention would also be that of "booster" immunotherapy, which aims at preventing recurrences in patients in whom adequate control has been achieved with previous immunotherapy⁶.

Primary-level preventive effect of immunotherapy

Preventing a person at risk from becoming sensitized is the paradigmatic preventive aspiration. Although there are no studies in humans that might warrant the use of immunotherapy with this aim, a number of experimental evidences allow speculation with the idea of the future possibility of immunointervention at early ages, before the allergic sensitization has occurred or has become consolidated, with the aim to redirect the immune response along non-pathogenic pathways. We know today that during the foetal life there is physiologically a remarkable Th2 polarisation with considerable release of anti-Th1 factors (IL-4, IL-10, PGE₂, etc.), the aim of which is to buffer Th1 responses that are toxic for the placenta⁷. For a variable period after birth, and largely as a consequence of the environmental microbial stimulation (be it patho-

genic or saprophytic), a change occurs in this Th2 responses towards Th1 ones to inhaled or alimentary allergens⁸⁻¹⁰. The release of Th1 cytokines will then protect the organism against the potentially pathogenic Th2 reactivity, and successive exposures to the allergen will consolidate the protective Th1 memory¹¹. It thus appears that the underlying differential phenomenon in atopic patients would be a defective Th1 response that would be unable to brake and revert those Th2 responses that were to a large extent physiological during the foetal/neonatal period^{7,12-17}. There is however a number of evidences that document some further important aspects in this context:

- The age at which a child receives an antigenic stimulus, the route through which this stimulus is received and the antigenic burden represent crucial factors in the development or absence of it of allergic responses to that stimulus^{8,13,18}.

- The Western life-style (which includes the elimination of certain endemic infections) favours the development of allergic diseases¹⁹⁻²¹.

- A number of bacterial, viral or parasitic infections, either naturally-occurring or vaccination-induced, may non-specifically stimulate a certain degree of protection against the development of allergic diseases^{9,22-25}.

- Furthermore, in some cases, the resolution of a chronic infection may trigger an enhancement of allergic responses²².

- In contraposition, allergic patients may develop weaker cell-mediated Th1 responses to bacterial antigens^{12,26}.

- Also, recent studies show that immunotherapy induces a Th2 → Th1 shift²⁷⁻³¹.

- Further to this, a recent study by the Montpellier group has shown that monoallergic (monospecific) immunotherapy has a non-specific preventive effect on the development of sensitizations to new allergens³².

All these considerations suggest that it would be possible to act on the immune system during the first few months of extrauterine life with the aim to stimulate Th1 responses before a more or less solid shift towards the Th2 pathway has occurred. From the theoretical point of view, therefore, it is not far-fetched to think that, in the future, some form of immunointervention at very early ages will be possible in populations at allergic risk^{7,11,33-35}:

- **Immunoprophylactic vaccines**, with the aim to induce a Th2 → Th1 shift, for instance through the intranasal administration of allergenic extracts.

- **Immunostimulatory vaccines**, with the aim of accelerating the transition from the foetal pattern (Th2 predominance) to the adult one (Th1 predominance). This

might be achieved through a manipulation of the intestinal flora, or considering the possibility of reintroducing BCG vaccination in newborn infants at allergic risk, etc.

- **Oral tolerance**, aiming at reproducing with inhalable allergens what often occurs with alimentary ones (in atopics and non-atopics). The oral administration of high doses of inhalable airborne allergens is an ever less theoretical possibility since current cloning techniques would render feasible the production of large amounts of recombinant allergens.

However, a number of questions does not allow the above possibilities to go at present beyond the realm of merely theoretic consideration.

- Ethical problems condition the possibility of carrying out clinical studies in that context in newborns and infants.

- Would it be preferable to use immunoprophylactic vaccines containing a pool of specific allergens, or to use non-specific immunostimulation techniques, or both?

- If immunoprophylactic vaccines are used, would it suffice to use a pool of standard allergens (*e. g.*, mites, *Alternaria*, cat dander, grass pollens), or would the vaccine have to be prepared according to the particular child's environment?

- What would be the amount of allergen to be administered? How often? For how long?

- How long is the "window" period? Would a "point of no return" exist, up to which the immunointervention would be effective but beyond which it would be useless?

- Would the whole population be amenable to this type of procedures, so that an "Allergic Vaccine" would be incorporated into the standard Immunisation Calendar, or would such procedures be restricted to the children at allergic risk? In the latter case, how are we to define precisely what is "a child at allergic risk"?

- Might it be convenient to enhance such vaccines with some vehicle that would stimulate Th1 responses?

The time when these and many other questions may have their proper answer may not be very far away. When that happens, the preventive use of Immunotherapy will move from the realm of simple theoretical lubrication to that of well-founded reality.

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EVOLUTION OF CHILDHOOD ASTHMA. INFLUENCE OF DRUG THERAPY

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Introduction

Most of the studies published in recent years assess the prevalence of asthma to be about 5 to 10%. All of

those studies agree in that both the prevalence and the mortality of the disease are increasing in spite of the advances in the knowledge of its pathogenesis and of having available continuously improving diagnostic and therapeutic resources for combating it.

Some authors currently postulate early therapy of asthmatic children with inhaled corticosteroids in an attempt to improve their evolution^{1,2}.

The present paper reviews the recent literature regarding the natural history of childhood asthma³⁻¹⁷ (Table I) and the possibilities of modifying it through drug therapy. It also presents the preliminary results of a prospective study being carried out at our Allergy Unit for the assessment of the evolution of children with persistent moderate and severe asthma diagnosed between 1995 and 2000 at our Centre and their evolution over ten years. The data presented are those available after the first three years.

Natural history of asthma

The idea is quite prevalent that an asthmatic child will become free of his asthma when he or she attains puberty; the epidemiologic evidence, however, is less optimistic.

The methodology of the studies reviewed is quite heterogeneous, so that their results must be analysed with care. The aim common to all of them is to analyse the evolution of asthma; however, a number of these studies refer to children with asthma diagnosed at an age below three years, while others encompass children aged between 5 and 15 years at diagnosis. The follow-up age is also quite variable; some of the studies follow their patients up into adolescence, and others go on till the age of 20, 30 or even 40 years.

Some of these studies also analyse the factors that might be associated to the persistence of asthma into adulthood, while others assess the evolution of the patients according to the therapy indicated.

From the overall analysis of these studies it can be concluded that the evolution of asthma is quite variable, depending fundamentally on the age at which the clinical symptoms began. Wheezing during the first year of life does not constitute a prognostic predictor for persistent or more severe asthma in the following years. Quite to the contrary, if the asthmatic symptoms (wheezing and dyspnoea) begin before age three the prognosis is generally good, and symptoms will persist only in 15 to 24% of the children^{3-6,11,12,16}.

However, the prognosis of asthma in this group of early-onset patients again varies depending on the presence of familial or personal antecedents of atopy, in which case up to 80% of the cases will persist with asthma, or on the absence of such antecedents: only 20% of the non-atopic children will have their asthma persist into adolescence or into adult life^{3,4,12,16}.

In this group of children with early-onset asthma, the most frequent triggering factor for wheezing are viral respiratory tract infections (influenza and respiratory syncytial virus). Although wheezing can also be associated to allergen exposure, atopy has at this age rather a prognostic than a diagnostic significance.

Considering all the above, it is suggested that there are at least two different groups of infants with asthma. In one of them the asthmatic symptoms are mainly associated to the lesser airway diameter, with no bronchial hyperreactivity; this group has a good prognosis. In the other group there is a greater prevalence of allergic markers (coexistence of atopic dermatitis, food allergy, positive tests with airborne allergens, familial history of atopy, high IgE levels) and of bronchial hyperreactivity; the prognosis for this group is worse, unless specific therapy based on a correct aetiologic diagnosis is instituted^{4,12}. The problem is that during the early years of life, the clinical differentiation of these two groups is well nigh impossible.

The results are completely different when the evolution of children diagnosed beyond the age of three years is analysed. In this group, the persistence of asthma into adulthood ranges between 60 and 80% of the cases^{4,8,9,13-16}, and atopy is in all the studies the main cause associated to persistence of childhood asthma into adult age^{7,9,11,12,16,17}.

In this group of older children (<5 years of age at diagnosis), the main feature associated to their asthma is allergy, both as regards the symptoms at onset and considering its evolution and its persistence into adult age^{4,7,16,17}.

Based on all these studies, we have prepared a hypothetical mathematic model of the natural history of asthma from birth to adult age (Fig. 1).

Drug therapy and evolution of asthma

Some authors have suggested that the early introduction of inhaled steroid therapy into the treatment of children with asthma might be beneficial in improving the natural evolution of the disease^{1,2}.

Table I. Natural history of childhood asthma

Year	Age at the beginning (years)	Age at the end (years)	Persist with asthma			
Martin AJ	1980	7	21	80%	Atopics	Non-atopics
Sporik R.*	1991	< 2	11	24%	86%	14%
(¹¹)	(¹¹)	> 2	11	81%		
Croner S.	1992	< 3	11	15%	Persistent asthma	Intermittent asthma
Wennergren	1992	< 2	5	47%	33%	14%
Bernice A	1992	< 7	16	18%		
Ruurd J.R.	1993	8-12	25	76%	22%	33%
Kokkonen J.	1993	5-15	20-24	72%	50%	22%
David J	1994	5-15	30-40	61%	34%	25%
Lewis S	1995	< 5	16	15%		
Martínez F.	1995	< 3	6	40%		
Ulrik C.S.	1995	5-15	15-25	86%		
Köning P.*	1996	2-16	18-32	54%		
Oswald H.*	1997	< 7	35	35% BO	70% asthma	90% severe asthma

It is difficult to establish what "early antiinflammatory treatment" means in the context of childhood asthma.

If "early antiinflammatory treatment" is understood to mean that in any asthmatic child primary prevention measures must be instituted (adequate environmental control), the first and most important antiinflammatory treatment of asthma in the words of the GINA²¹, together with treatment with non-steroidal antiinflammatory agents, we fully endorse this concept.

If what we mean is that in any child with persistent asthma (mild, moderate or severe) who has not responded to adequate environmental control measures and to prophylactic therapy with non-steroidal antiinflammatory drugs therapy should be instituted with inhaled steroids, attempting to achieve good compliance to that medication, again we must fully endorse this opinion²⁰⁻²².

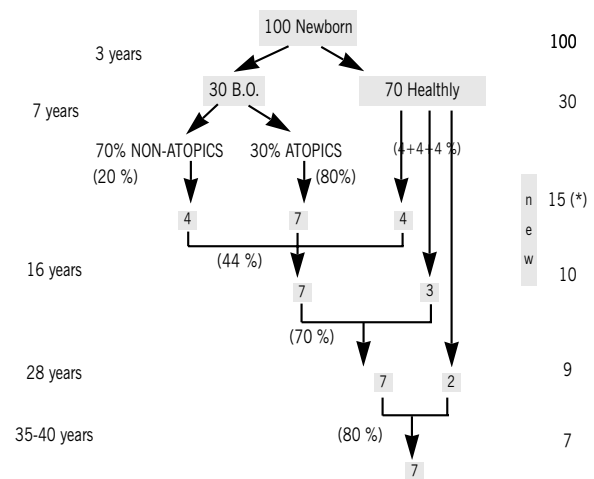
However, if what is meant is that any asthmatic child, regardless of his or her age, of the severity of his or her symptoms and of the aetiology of his or her asthma, must be managed from the beginning with inhaled steroids as first-line drug therapy, leaving aside the remainder of the therapeutic guidelines, then it is rather difficult to agree with this concept.

In the first place, there is as yet no prospective study that warrants such a procedure. Quite to the contrary, there is a host of warnings and of studies that counsel caution and prudence²⁰⁻²².

In the second place, inhaled corticosteroids have

been demonstrated to be quite useful drugs for the control of the symptoms and of the lung function in asthmatic patients, but it is also well known that the suppression of this therapy after a number of years of continuous usage leads to the patients again developing the same symptoms they had prior to its institution¹⁸. It is also a well-known fact that inhaled steroids are not completely devoid of adverse side effects²⁰.

In the third and last place, it has been demonstrated that children with episodic or intermittent asthma who had never received inhaled steroids have had a favoura-



* Note: if the asthmatic manifestations are SEVERE, 90% will continue with asthma into adult life. Out of 100 newborn, 39% evidence wheezing at some time but only 7 will persist with bronchial asthma.

Fig. 1. Natural history of childhood asthma: hypothetical theoretical model.

Table II. Varying proportions of allergic sensitizations according to the severity of asthma

Group A (Persistent asthma)	Group B (Infrequent episodic asthma)
• Mites 13/18	• Mites 21/25
• Pollens 11/18	• Pollens 5/25
• Moulds 10/18	• Moulds 6/25
• Danders 10/18	• Danders 3/25

ble evolution regarding both their clinical symptoms and their pulmonary function^{15,19}.

Thus, the statement that in the management of childhood asthma therapy with inhaled steroids should be instituted as early as possible has at present no support in the literature.

With the aim to clarify this and other open questions in the context of childhood asthma, our Unit initiated a prospective study three years ago; the following represents an exposition of the preliminary results of this study.

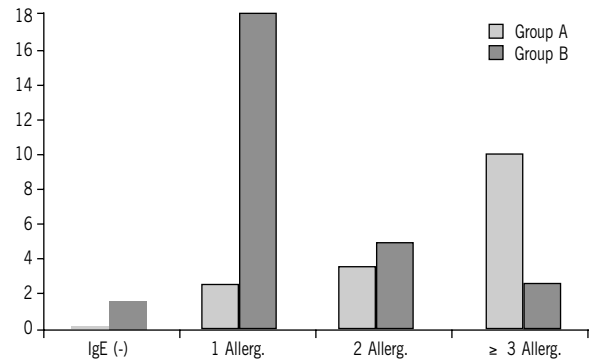
Personal experience

This prospective study was begun in 1995 with the aim to follow up during ten years the evolution of all our childhood patients with persistent severe or moderate asthma (GINA criteria²¹), comparing them to a control group of children with infrequent episodic asthma²⁰.

The study population comprised all the children

Table III. Assessment of the severity of asthma

Score	Episodes /yr	Wheezing episodes/month	Lung function	Medication
0	0	0	FEV ₁ >80% TBD(-)	None
1	≤ 5	≤ 2	(")	(")
2	(")	(")	(")	DSCG
3	(")	(")	(")	Inhaled steroids
4	(")	(")	(")	Inhaled steroids
5	(")	(")	(")	Inhaled steroids
6	> 5	> 2	FEV ₁ < 80% or TBD(+)	DSCG
7	(")	(")	(")	Budesone, > 800µg
8	(")	(")	(")	Systemic steroids



Group A: patients with persistent moderate or severe asthma.
Group B: patients with infrequent episodic asthma.

Fig. 2. Varying degrees of sensitization according to the severity of asthma.

with a first-time diagnosis of persistent moderate or severe asthma, and one out of every five of those diagnosed of episodic infrequent asthma.

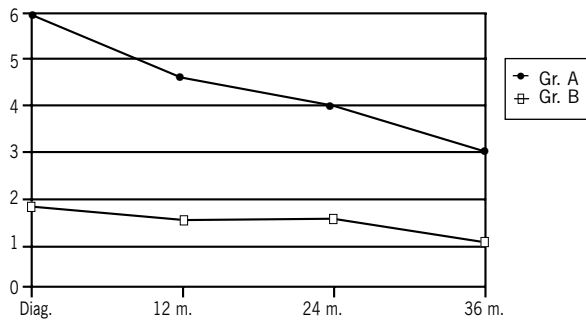
In our health care catchment area, with about 2,500 births every year, an average of 112 new asthmatic children are diagnosed yearly. Some 10% of them fulfil the criteria for persistent moderate or severe asthma, so that we have calculated a period of five years for having a study population of ca. 50 children with persistent moderate/severe asthma who can be followed during 10-15 years.

We report here the preliminary results of the 3-year follow-up of the first 18 children selected and included in Group A (persistent moderate/severe asthma) and of the 25 children selected and included in Group B (control group, infrequent episodic asthma).

A full aetiologic allergologic diagnostic work-up is carried out in all cases, and in all cases therapeutic measures are instituted with particular emphasis on the importance of compliance with the environmental control measures prescribed.

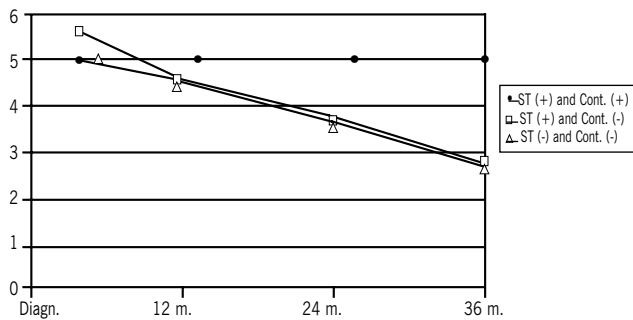
All the children with persistent asthma began inhaled steroid therapy, and at the successive review visits we have always attempted to maintain them on the lowest possible dosages that would keep them clinically stable, with normal baseline lung function and with a negative bronchodilator test. None of the children in the control group have received inhaled steroid therapy.

The mean age is 9.2 years (range, 5 – 14 years) in Group A, and 8.6 years in Group B; in both groups there is a predominance of the male gender (12 M, 6 F in Group A; 16 M, 9 F in Group B).



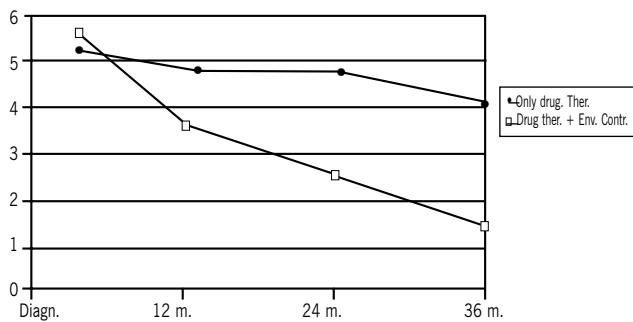
Group A: persistent moderate or severa asthma.
Group B: infrequent episodic asthma.

Fig. 3. Evolution of the severity of asthma.



Cont. (+): contact with pets continues.
Cont. (-): contact with pets suspended.

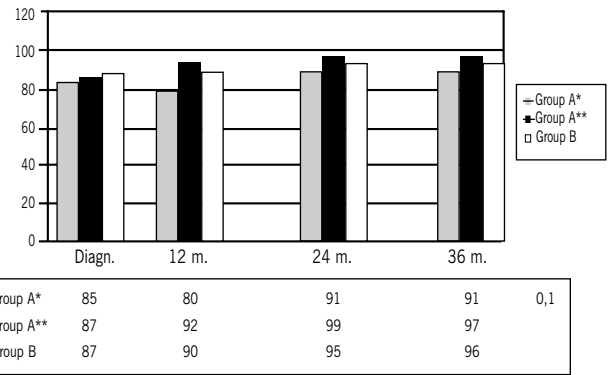
Fig. 4. Evolution of the severity of asthma in patients sensitized to animal danders.



Group A: severe asthma

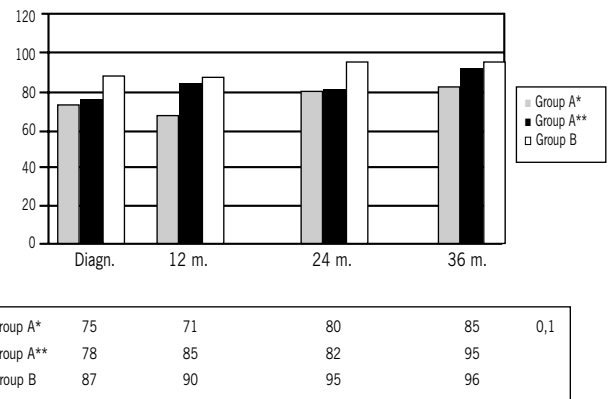
Fig. 5. Evolution of asthma with and without adequate environmental control.

There are in both groups a high proportion of allergic antecedents, both familial (88% in Group A, 76% in the control [B] group) and personal (72% and 80%, respectively).



* With inh. steroids
** Without inh. steroids

Fig. 6. Evolution of the FEV₁.



* With inh. steroids
** Without inh. steroids

Fig. 7. Evolution of the FEF₂₅₋₇₅.

An allergic aetiology could be demonstrated in 94% of our asthmatic children, both in those with severe and in those with mild asthma.

One important difference between the two study groups refers to the degree of sensitization. While 55% of the children with severe asthma were polysensitized (to three or more groups of allergens), only 8% of the children with mild asthma have such multiple hypersensitivities. Also, sensitization to moulds and to animal danders is fivefold higher in the children with severe asthma than in the control group (Table II).

The follow-up protocol contemplates four visits every year for the children with severe and two for those with mild asthma. At each review visit, baseline lung function tests and a bronchodilator test were performed on each patient, and the therapy was adjusted according to the severity of the disease graded by the assessment scores in Table III.

Results

Both study groups (severe and mild disease) have evidenced a favourable evolution (Fig. 3).

Among the children initially with persistent moderate or persistent severe asthma in whom adequate environmental control measures and/or hyposensitization could be implemented, 100% have been able to abandon the initial inhaled steroid therapy (43% are without any maintenance therapy and 57% on cromones) (Fig. 3), with a lung function that is completely normal and comparable to that of the patients in the control group, who also suffered no deterioration (Figs. 4 and 5).

On the contrary, all the children with persistent moderate or severe asthma who were unable or unwilling to implement adequate environmental control measures have continued to require inhaled steroid in order to maintain control of their disease. Globally and as a group, their lung function may still be considered to be normal (after three years' evolution and follow-up), but the mid-expiratory flow rates are already beginning to be clearly worse than those of the children with mild asthma or of those with persistent asthma who have implemented environmental control measures and are not receiving inhaled steroids (Figs. 6 and 7).

Conclusions

The conclusions to be derived both from the studies reviewed and from our own experience are as follows:

1. Only 15-20% of the children with very-early-onset asthma (onset before the age of 3 years) will persist with symptoms into adolescence.
2. Among the children whose first symptoms of asthma begin beyond the age of three years, 60-70% will persist with symptoms in adult life.
3. In the two aforementioned groups of asthmatic children, atopy is the most important factor related to the persistence of asthma in adulthood.
4. Inhaled steroid therapy improves the symptoms and the lung function in children with persistent moderate or severe asthma, but do not appear to influence the disease's evolution. When this therapy is abandoned, the symptoms reappear with the same intensity as before.
5. The lung function of the children with mild episodic asthma (not receiving inhaled steroids) and of those with persistent moderate or severe asthma in whom the inhaled steroids could be suspended after the

implementation of adequate environmental control measures and/or immunotherapy has always remained normal.

6. Prevention, understood as an adequate implementation of environmental control measures and/or hyposensitizing treatment when indicated, is the only way to favourably influence the evolution of childhood asthma.

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