

methods are applied for determining its partial or total amino acid sequence. These studies, which require relatively large sample amounts, may at present even be obviated by the study of the DNA, which may yield the allergen's deduced sequence⁴. The study of this sequence then allows the localisation within it of the areas or subsequences that are antigenic for the T and B lymphocytes⁵.

Function studies, cross-reactivity and classification into families

Once the molecular structure is known, it becomes possible to derive data from similar molecules, which may or may not have been already reported as allergens but about which there is information available regarding function and characteristics. This novel concept requires that the molecule under study have a high degree of sequence homology, which will then allow us to group that particular allergen within a family in which members from various plant or animal species are progressively assembled; one such family is, for instance, that of the prophyllins. This grouping also allows us to recognise possible cross-reactivities between members of the same family. However, the most salient result of this grouping is that it has begun to provide explanations for hitherto unexplainable cross-reactions. For instance, the family of the "pathogenesis-related proteins" encompasses a vast number of plant proteins, the mission of which appears to be the protection of plants against aggressions. These proteins are widely distributed throughout the plank kingdom, and the more relevant allergens presently known include pollens (such as that of the birch tree), fruits (e. g., the apple) and other vegetables, among which the celery and the carrot are among the foremost. Cross-reactivity between them had long been suspected, but only the biochemical basis of their molecular structure has allowed us to demonstrate this hypothesis⁷. This explains how cross-reactivities may occur across the phylogenetic barriers.

Allergen isolation and purification

Logically, these many conceptual changes and transformations occurring in recent times have been to a great extent determined by the ease with which allergen DNA cloning studies and directed mutagenesis induction studies can be performed today, and by the easy availability of considerable amounts of allergens produced by genetic engineering procedures. The number of recombinant allergens currently used in in vitro studies is already quite important, and this leads to thinking that within a relatively short time such allergens may and will be massively used

in two large areas of application: as diagnostic test reagents, and as therapeutic resources for use in immunotherapy⁸. This immunotherapy may then be performed either through the traditional method of administering increasing doses of the allergen, or alternatively, using formulations quite different from those presently in use and which are currently in the design process, such as modified allergens that are devoid of side effects.

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OPTIMISATION OF ALLERGENIC MITE EXTRACTS

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The biological raw materials used in the production of allergenic extracts are in general of a great variability, less in the case of pollens but quite high for mites. Al-

though this variability may be minimised by biological standardisation methods it is evident that the production of mite cultures through controlled and reproducible procedures will provide consistent and high-quality extracts. The present study establishes how, once other parameters such as culture medium, temperature and humidity have been settled, the culturing period for mites of the genus *Dermatophagoides* is highly determinant of the resulting allergenic expression, both quantitatively (higher number of allergens and major allergens concentration), and qualitatively (greater in vitro and in vivo allergenic reactivity and enzymatic activity).

Key words: Allergenic extracts. Optimisation. Standardisation. Mites. *Dermatophagoides*.

OPTIMIZACIÓN DE LOS EXTRACTOS ALERGÉNICOS DE ÁCAROS

Los materiales biológicos utilizados como materias primas en la obtención de extractos alergénicos están sujetos a una alta variabilidad, que es menos acusada en el caso de los pólenes pero notable en lo referente a ácaros. Aunque dicha variabilidad pueda minimizarse mediante procesos de estandarización biológica, es evidente que la consecución de cultivos de ácaros mediante sistemáticas controladas y reproducibles rendirá extractos consistentes y de alta calidad. En el presente estudio se demuestra cómo el tiempo de cultivo de ácaros del género *Dermatophagoides* es altamente determinante -una vez fijados otros parámetros como el medio de crecimiento, la temperatura y el grado de humedad- de la expresión alérgica resul-

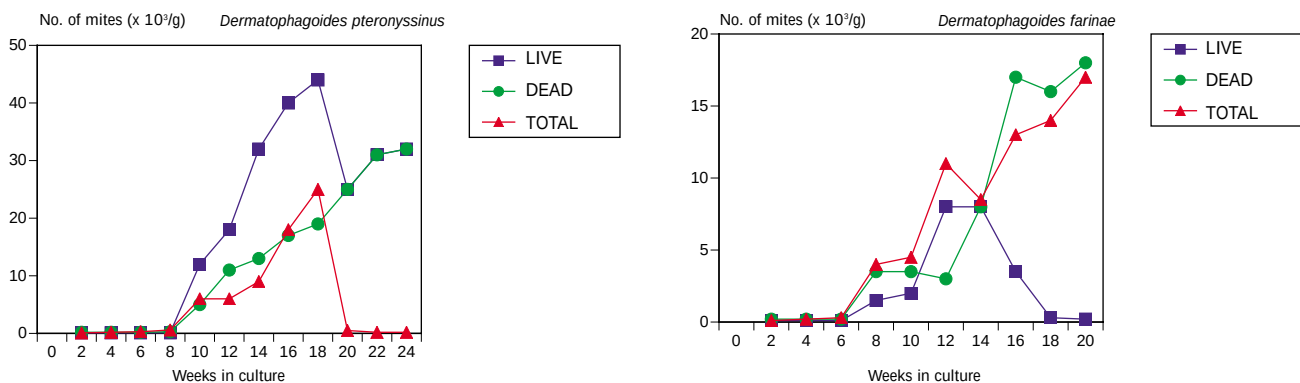


Fig. 1. Growth curves.

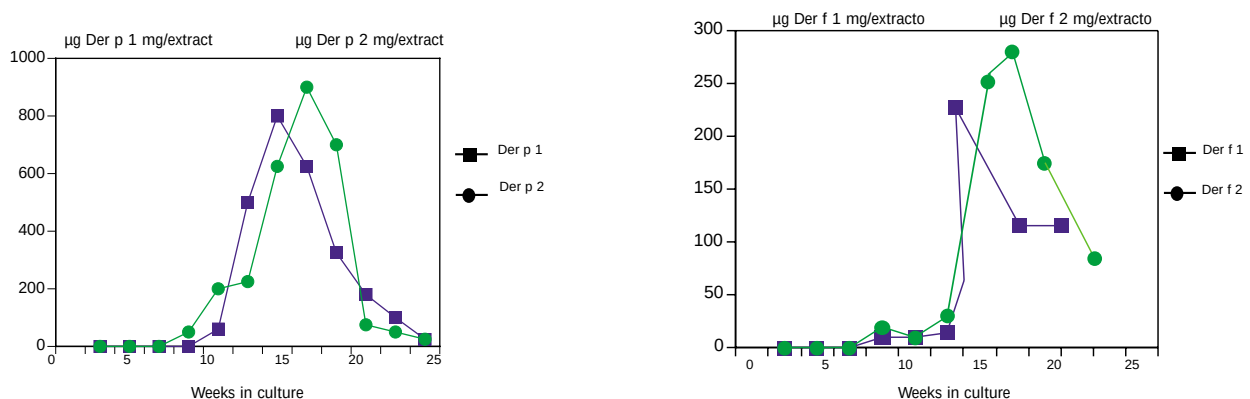


Fig. 2. Expression of major allergens of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in culture.

tante, tanto en cantidad (mayor número de alérgenos, mayor concentración de alérgenos mayores) como en calidad (mayor reactividad alérgica in vitro e in vivo y mayor nivel de actividad enzimática).

Palabras clave: Extractos alérgicos. Optimización. Normalización. Ácaros. *Dermatophagoides*.

Ever since the first descriptions, back in the 1960's, of the role of mites of the genus *Dermatophagoides* as triggering agents of respiratory allergic conditions through their presence in house dust, considerable research efforts have focused on this airborne allergen, leading to an exhaustive knowledge of its major and minor allergens as regards location, sensitisation prevalence, molecular parameters and -through the application of molecular biology techniques- physiologic function and structural similarity to other known proteins. Table I presents an update of this knowledge¹.

While the Group 1 and Group 2 allergens are those most relevant for the *Dermatophagoides*-sensitised patients, the tropomyosin molecule (Group 10) is the one most frequently involved in cross-reactions to other allergenic sources^{2,3}. And yet, its IgE-binding prevalence remains controversial, with reports of levels as divergent as 80% for Der f 10⁴ and 5% for Der p 10⁵.

Despite this high level of knowledge at the ultrastructural level, very few studies have been reported that focus on the ways and means for achieving *Dermatophagoides* mites cultures that may yield the greatest and most diversified allergen expression and thus lead to the production of extract preparations that are optimal for the diagnosis and therapy of allergic diseases caused by this ubiquitous airborne allergen. This is the aim and purpose of the present study.

Table I. Main characteristics of the *Dermatophagoides pteronyssinus* and *D. farinae*

Allergen	Prevalence of IgE antibodies (%)	Molecular mass (kDa)	Biologic function
Group 1	80-90	25	Cysteine-protease
Group 2	> 90	14	Epididymal protein
Group 3	~60	28-30	Trypsin
Der p 4	40	60	Amylase
Group 5	40	14	Unknown
Group 6	40	25	Chymotrypsin (cysteine-protease)
Group 7	50	22	Unknown
Der p 8	40	26	Glutathione-S-transferase
Der p 9	>60	30	Serine-protease
Group 10	5-80	36	Tropomyosin

As a brief introduction, it should be remembered that the mites are arthropods belonging to the class Arachnida and, within it, to the sub-class Acari. The mites responsible for respiratory allergic conditions are included in the order Astigmata, to which the *Pyroglyphidae* family belongs. It is in this family where the clinically most important genera are included: *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*.

The mites of the genus *Dermatophagoides* are small in size (ranging between 300 and 350 µm in length) and live fundamentally in mattresses, carpetings and furniture upholsterings. They feed on skin scales, hairs and other materials present in common house dust, such as fibres and food residues and debris. Their growth is dependent on environmental conditions: their optimal temperature range is 20-25°C, and the optimal relative humidity range 70-80%.

The allergenic extract optimisation studies began with studies of the dynamics of *Dermatophagoides pteronyssinus* and *D. farinae* populations in culture (Fig. 1) and of their antigen expression kinetics⁶. The extracts prepared from cultures at different points in the life cycle were analysed using SDS-PAGE, immunotransfer and ELISA.

The results have identified three definite phases in this life and growth cycle: a latency phase (F1) which lasts

Table II. Biologic activity of the extracts.

Species	Growth phase	SPT (mg/ml)
<i>D. pteronyssinus</i>	F1	7,14
	F2	0,35
	F3	2,46
<i>D. farinae</i>	F1	10,80
	F2	0,55
	F3	3,20

SPT = Allergen concentration (mg/ml) inducing a wheal equivalent to that induced by 10 mg/ml histamine.

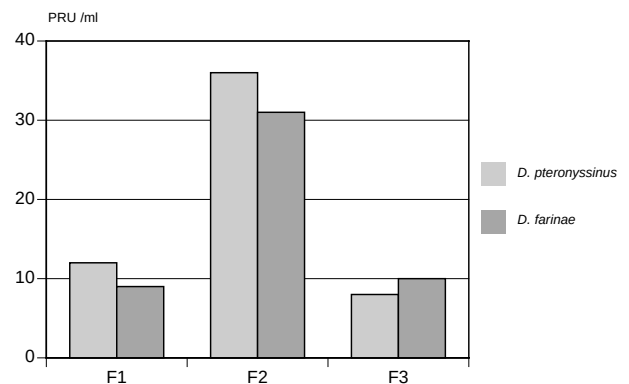


Fig. 3. Reactivity of the various extracts according to the phase in which they were expressed (RAST).

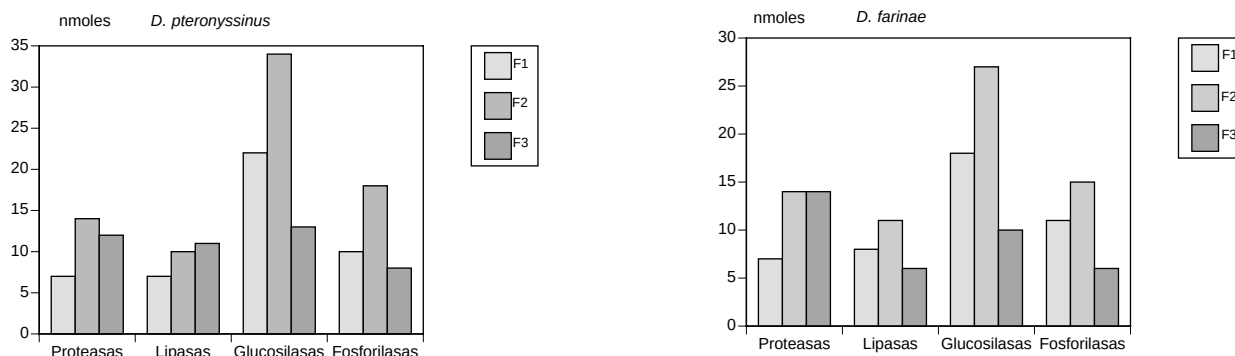


Fig. 4. Enzyme activity of the various extracts according to the phase in the growth cycle in which they were prepared.

from the 6th to the 8th week, an exponential growth phase (F2) lasting up to the 18th week for *D. pteronyssinus* and up to the 14th week for *D. farinae*, and a death phase (F3) lasting up to the 20th week.

The maximum expression of allergenic components, including that of the major allergens Der 1 and Der 2, was observed during the F2 phase (Fig. 2).

The extracts from phase F2 cultures furthermore evidenced the greatest reactivity when used as the solid phase in RAST procedures (Fig. 3)⁶ and the greatest skin reactivity in the allergic patient population included in biologic standardisation studies (Table II)⁷.

The extracts from this exponential growth phase (F2) also evidenced the greatest usefulness for in vitro cross-reactivity studies using the RAST inhibition technique⁸ and also the greatest enzymatic activity (Fig. 4)⁹. The latter reveals a greater presence of allergens, particularly considering that mite culture extracts contain a great variety of enzymes that are the mites' own and that are present both in the mite excreta and in the mite degradation products.

In conclusion, an adequate selection of the culture parameters for mite cultures crucially influences the allergenic extract finally achieved. Particular attention should be paid to the time of culture growth as this may, both by excess or by default, give rise to products with deficient allergenic presence.

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ADVERSE REACTIONS TO TETANUS TOXOID

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INSULIN DESENSITISATION

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The prevalence of allergic reactions to insulin has decreased substantially with the use of insulin of human