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A COMPARATIVE CHEMICAL AND CLINICAL INVESTIGATION OF HOUSE DUST EXTRACTS FROM ALPINE AND LOWLAND REGIONS

By

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INTRODUCTION

It has been known for a long time that patients with allergic bronchial asthma or atopic dermatitis benefit from a stay in the high mountains. Several explanations have been forwarded in the past for the favourable effect of the mountain climate on manifest atopic disease. At the present time, it seems reasonably well-established that several different factors – direct and indirect – cooperate in creating the environmental conditions which ultimately effect the rapid relief of clinical symptoms. Reference can be made here to the low relative humidity of mountain air registered at Davos throughout the year, and to the extensive ultraviolet radiation as important physical factors (13, 14). Also, the low mould and – possibly – mite counts help creating an atmosphere of diminished asthmato-genic potential (9, 10, 16).

One further factor has attracted the attention of several investigators. It has been speculated that, in the high mountains, the dust gathering indoors might contain an appreciably lower proportion of a universal "house dust allergen" than comparable dust samples collected in the low countries (e.g. Holland). *Van Geuns* (11) and, more recently, *Milner et al.* (12) have in actual fact been able to show that allergenic extracts of Swiss house dust and of Dutch or British samples – pre-

pared on an equal weight (of dry dust) per volume (of extractant) basis — differ quantitatively, though not qualitatively. However, because of the notorious inadequacy of the methods of preparation and standardization of dust extracts, the question remains open whether the low potency of the Swiss extracts is to be attributed to an unappreciable allergen content of the dust, or whether this dust simply contains just as much potential allergen, which has either *become inactivated* or *has never been active*. With this in mind, we have conducted a few experiments which permit a specification of this point.

MATERIALS AND METHODS

As delineated elsewhere (6), the *potency* of an allergenic extract is the mathematical product of two independent parameters, i.e. the *specific activity* of the allergen molecules and their *chemical concentration*. For a meaningful comparison of two allergenic house dust extracts, these two factors must be assessed separately. In this paper, one unit of *relative* specific activity is arbitrarily defined as being associated with the minimal amount of freeze-dried substance, in mg, which will yield a $1+$ cutaneous reaction (wheal 5–10 mm with associated erythema) on average in a group of clinically house dust sensitive patients (1). Relative specific activities can then be expressed in units per mg. The chemical concentration may be determined on a freeze-dried basis, on a nitrogen basis, or otherwise; the first method has been adopted here.

Samples of house dust were collected during the month of September 1966 from 5 different dwellings at Utrecht (codes A through E) and 3 individual houses at Davos-Platz (codes F through H); dust from the Asthma Clinic at Davos was also obtained (code K). Careful records were taken to document some relevant conditions of each house and of its inhabitants. The batches of house dust were weighed, and extracted at ambient temperature with a dilute phosphate buffer pH 6.9, containing 0.5 per cent phenol (1). After filtration on kieselguhr, each extract was worked up individually into 5 major subfractions according to the modified purification schedule for house dust allergen described by Berrens, Morris & Versie (3): the method is shown schematically in Fig. 1.

Aliquots of each subfraction were withheld at each stage of purification for the determination (by lyophilization) of yields and recoveries, and for chemical analyses; whenever this proved feasible, these fractions were also assayed for allergenic potency by direct intradermal test in groups of at least 5 dust-sensitive atopic patients. Solutions for skin-testing were prepared on a weight (of soluble nondialysable substance) per volume (of solvent) basis. This permitted the computation, at different stages of purification, of the yield of soluble ma-

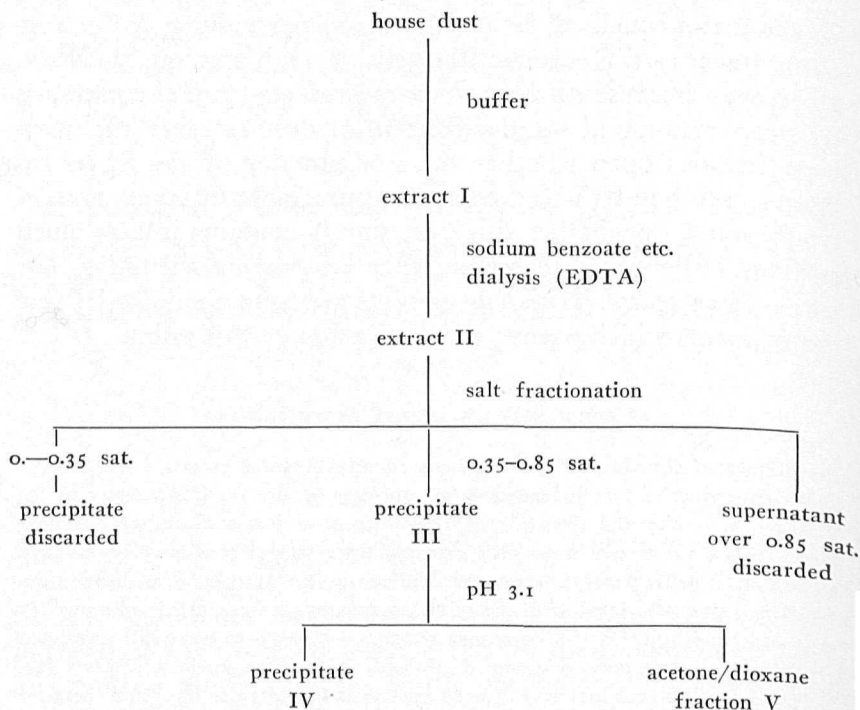


Fig. 1.

Schematic representation of fractionation schedule for house dust extracts according to *Berrens, Morris & Versie* (3), and featuring the code numbers I $\rightarrow$ V employed in the present investigation.

terial, the mean allergenic potency in units per mg of soluble substance and, therefore, the total allergenic potential for each kg of dry dust extracted (i.e. by multiplication).

Procedures for the analytical determination of nitrogen, total hexoses, hexuronic acids, and optical properties (ultraviolet absorption spectra, fluorescence emission) have been described in previous communications (1, 2, 3, 4, 5). Immunoelectrophoresis was performed in agar gel at pH 8.5 by the micromethod of *Scheidegger* (15), using polyvalent rabbit anti human serum as antibody source (Behringwerke, Marburg, Germany).

RESULTS

In order to allow a comparison with previous results, the yields and specific allergenic activities of the direct, unfrac-

TABLE 1
Composition and properties of dialysed and lyophilized direct extracts (I) from different Dutch and Swiss house dust samples.

Sample	Fraction	Yield mg/kg	Percent nitrogen	Percent* total hexose/ pentose	Percent† hexuronic acid	E ₂₆₀ /E ₂₈₀ uncorr. pH 2.0	Fluoresc.§ intensity (460 nm)	Spec. act., units/mg	Total activity, units/kg × 10 ⁻⁶
Dutch	A I	4800	7.7	31.4	9.1	0.74	16.5	3500	16.6
Dutch	B I	2000	2.9	21.8	4.3	0.69	14.6	5000	10.0
Dutch	C I	1600	3.7	17.6	5.2	0.70	14.4	25000	40.0
Dutch	D I	3000	3.1	19.3	5.2	0.71	15.8	7500	22.5
Dutch	E I	5800	10.1	12.3	n.d.	0.64	13.6	4000	23.2
Swiss	F I	2400	5.7	19.4	5.2	0.82	11.5	<100	2.0
Swiss	G I	3400	6.9	14.7	6.4	0.75	16.0	<100	2.0
Swiss	H I	3600	6.3	25.4	n.d.	0.69	13.0	1500	5.4
Swiss	K I	1140	3.0	53.7	n.d.	0.68	13.7	<100	2.0

* Determined with orcinol-sulphuric acid, galactose as standard.

† Determined with carbazole, glucuronic acid as standard.

§ Excitation: 365 nm; measured in 0.01 percent concentration in 0.01 N HCl.

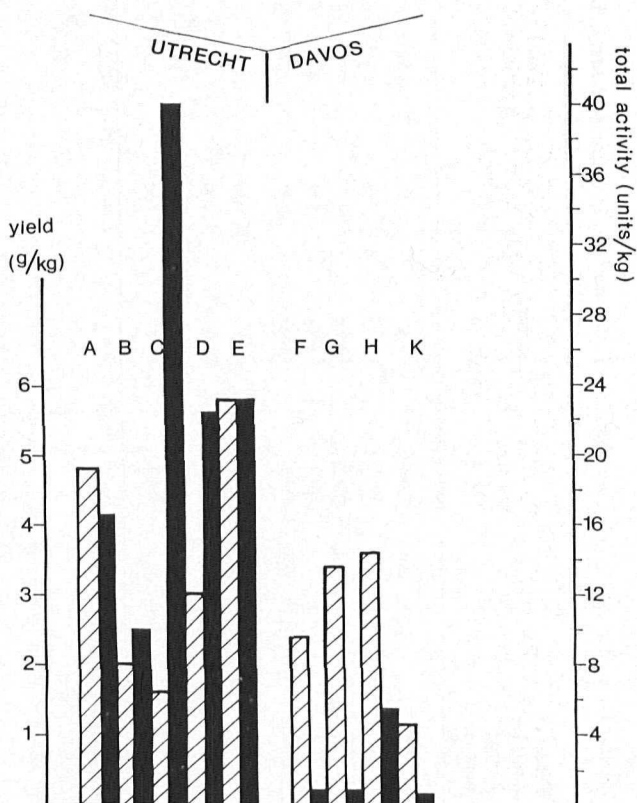


Fig. 2.

Block diagram illustrating the yield (in g/kg dry dust) of direct extracts (fractions I) from different batches of house dust collected at Utrecht and Davos (hatched columns). The black columns represent the calculated total allergenic potential (in units/kg dry dust) of the individual samples; these figures were obtained by multiplication (yields in mg/kg $\times$ specific skin-reactivities in units/kg as indicated in Table 1).

tionated extracts (I) were investigated in greatest detail. The results have been listed in Table 1. Contemplation of these data immediately reveals that the yield of total extractable material does not vary significantly among the Dutch and Swiss dust samples. Likewise, the analytical compositions fail

to disclose conspicuous disparities. The ultraviolet absorption spectra of the various I-fractions featured identical characteristics, as shown by the E_{305}/E_{280} - ratios documented in Table 1. No marked differences were noted among the fluorescence spectra, nor in the intensity of fluorescence emission from UV-irradiated solutions under standard conditions. However, fractions I from the Dutch samples were considerably more allergenic per mg than their Swiss counterparts. Consequently, the total allergenic potential (i.e. the yield in mg/kg $\times$ the specific activity per mg of fractions I) of the latter was significantly less than that of the former. This is illustrated by the overall block diagram of Fig. 2.

Interesting differences were noted in the immunoelectrophoretic patterns of the various I-fractions. As shown in Fig. 3 a, elevated concentrations of human serum albumin were especially encountered in the Dutch dust extracts, though lesser amounts of the same protein occurred in the Swiss extracts as well (Fig. 3 b). The quantitative differences could be traced to some overt characteristics of the individual houses (and inhabitants), as indicated in the legend to Fig. 3.

More particularly, the elevated titer of human albumin and other proteins in the extracts C and E were attributed to the fact that these houses were inhabited by patients with very severe atopic dermatitis of long duration. It has been shown that extracts of the skin scales shed by man - containing the powerful human dandruff allergen - feature high levels of (partly denatured) human serum albumin (4). Conversely, it can be expected that the desquamated skin flakes occurring in house dust contribute materially to the allergenic potential of the dust samples (1, 3, 6).

The observation of insignificant differences in the yield and analytical composition of soluble material which should embrace all the potential house dust allergens, was found true for the other preparations secured by fractionation (Fig. 1). This is exemplified by the collection of data in Table 2.

Unfortunately, it proved unfeasible to assay each and every one of the various subfractions in sufficiently large groups of

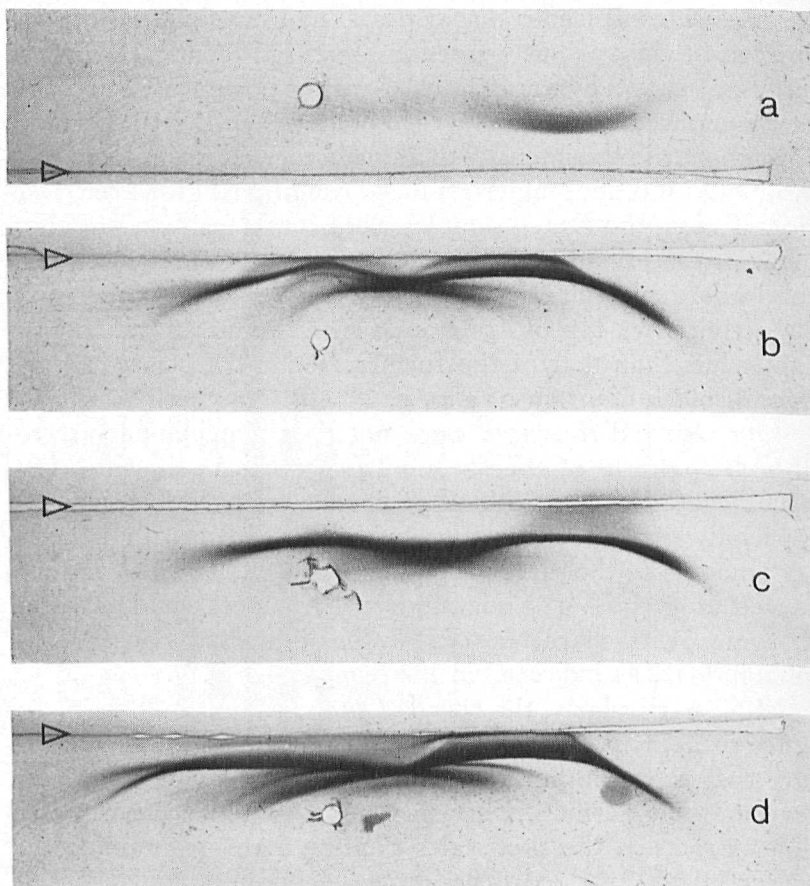


Fig. 3 a.

Immunoelectrophoresis of 5 percent solutions of dust fractions I from Utrecht samples (barbiturate buffer $\tau = 0.04$, $\text{pH} \approx 8.6$; 15 min. at 20 mA per slide). Anode on the right; rabbit antiserum to whole human serum in centre grooves for the diffusion step. Stain: Amidoblack. Samples were (a): AI, from dust collected in a new, humid house inhabited by a patient with asthma and rhinitis; (b): CI, from dust of a dry 40 year old house, inhabited by a patient with widespread recurring atopic dermatitis; (c): DI, dust sample from a less than 1 year old, damp house (patient had asthma and rhinitis); (d): EI, from dust obtained from a 40 year old damp house inhabited by an atopic patient with severe atopic dermatitis.

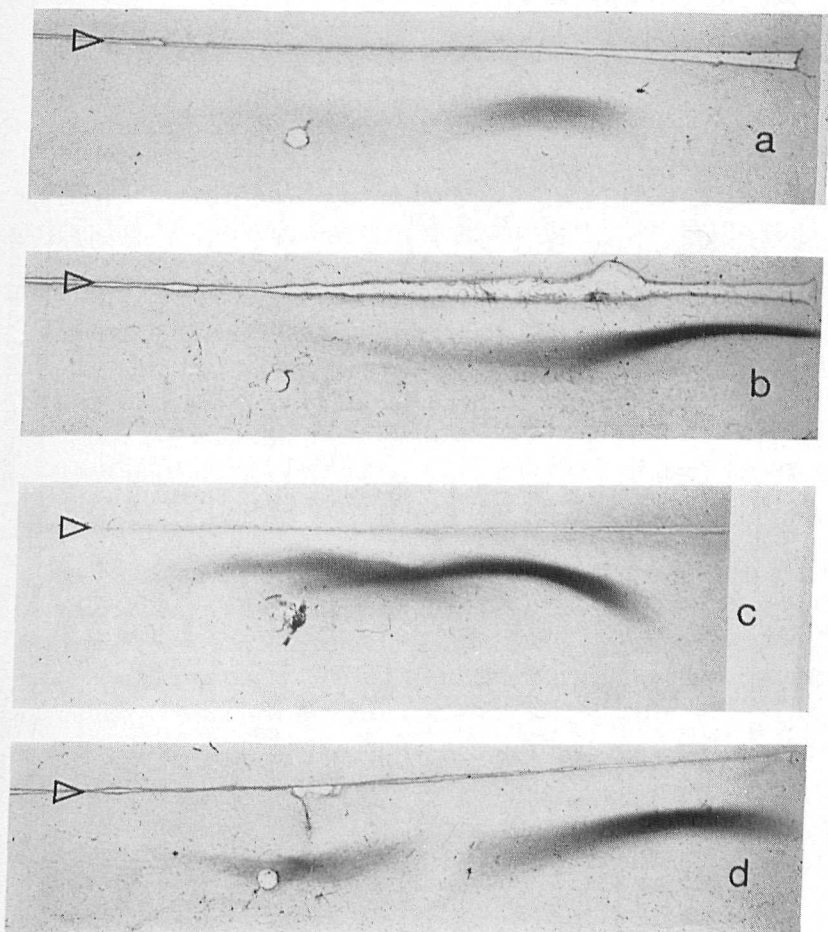


Fig. 3 b.

Immunoelectrophoresis of dust fractions I from Davos samples. Conditions as in fig. 3 a. Samples were: (a): FI, from dust collected in a very clean and dry, 55 year old spacious building, inhabited by only two people; (b): GI, from dust collected from a 10 year old house. This dust contained fragments of bird feathers, while the floors of the house were covered with thick rugs and carpets;

(c): HI, from dust collected in an inhabited, dry house of no particular characteristics; (d): KI, from dust collected in the Davos Asthma Clinic.

Note the intense albumin precipitin arcs of multiple appearance at the far anodic end in both series of immunoelectrophoretograms.

TABLE 2
Some analytical data of subfractions II and IV from Dutch and Swiss house dust extracts.

Fraction	Yield mg/kg	Percent total hexose/ pentose	Percent hexuronic acids	E 1 % 280 nm pH 2	E 1 % 305 nm pH 2	Fluoresc. intensity 460 nm *	Spec. act. units/mg	Total activity, units/kg $\times 10^{-6}$
A II	290	22.7	5.6	23.2	17.4	13.4	5000	1.5
A IV	32	10.9	7.8	63.0	47.0	10.7		
B II	737	5.5	1.6	17.6	12.8	15.0	7500	5.5
B IV	17	8.7	4.2					
C II	364	7.3	2.8	24.0	17.2	19.0		
C IV	4	8.9	3.2				1000	0.004
D II	742	3.8	1.7	15.0	10.2	15.8	2000	1.5
D IV	97	7.3	6.2	5.0	4.0	14.5		
E II	1228	4.9		16.8	10.8	19.8		
E IV	23	8.9						
F II	597	9.5	2.9	30.8	9.6	16.2	3000	1.8
F IV	96	14.1	4.8	28.8	18.8	16.4		
G II	210	10.7	4.2					
G IV	82	12.3	6.3	74.0	57.0	21.0		
H II	689	9.5		12.0	5.4	14.3		
H IV	211	7.2		16.6	9.8	10.1		
K II	461	3.9		13.4	8.4	17.4		
K IV	22	10.0		62.0	46.0	17.9	1250	0.03

* Excitation: 365 nm; measured from 0.05 percent solutions in 0.01 N HCl under standard conditions.

atopic patients. However, some of the more important fractions V were investigated in detail. These V-fractions should contain the powerful allergens previously concentrated in "fraction E" of house dust (1, 3, 6), which has hitherto defied further purification (6). As shown in Table 3, the recoveries of fractions V from batches of dust from both countries did not differ significantly. However, the V-products from the Swiss dust samples were allergenically inactive, or very nearly so, in contrast to the potent Dutch fractions V.

Here again, chemical and physical analysis failed to disclose appreciable variations between the composition and properties of the V-fractions isolated from the Swiss and Dutch samples. As an example, figure 4 reproduces the ultraviolet absorption spectra of dilute aqueous solutions of two fractions V, viz. K V from Davos (a) and C V from Utrecht house dust (6).

As reported by *Berrens & Bleumink* (2), these spectra may be valuable for appraising the degree of modification by non-enzymatic browning reactions undergone by allergen molecules. As it stands, we failed to uncover correlations between any physicochemical parameter and the specific allergenic activity of fractions from house dust investigated in the present work.

TABLE 3

Specific activity and total allergenic potential of some purified fractions V from Dutch and Swiss dust samples.

Sample	Fraction	Specific skin-activity, units/mg	Total activity recovered in V; units
Dutch	C V	4500	3.7×10^6
Dutch	E V	15000	14.4×10^6
Swiss	F V	<100	0
Swiss	K V	1000	0.06×10^6

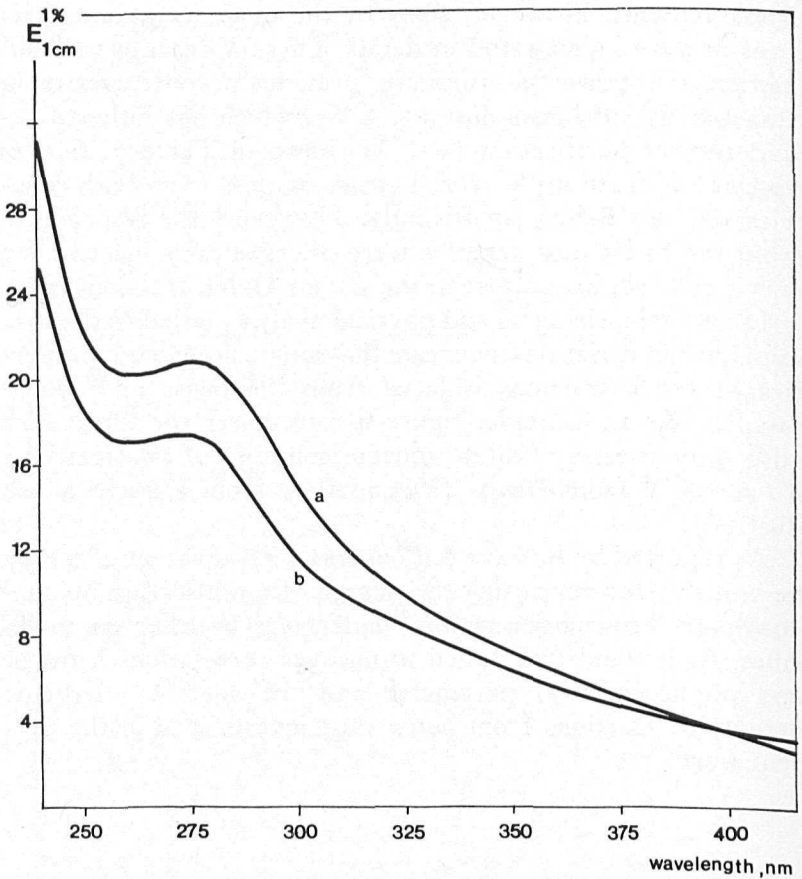


Fig. 4.

Ultraviolet absorption spectra of fractions KV from Davos (curve a) and CV from Utrecht (curve b) in 0.01 N HCl; Zeiss PMQ-H spectrophotometer.

DISCUSSION

The results of this investigation show that the dust collected from houses in a mountain village will yield a proportion of glycoprotein molecules not significantly different from that obtained from lowland dusts. However, the combined results on the direct extracts shown in Fig. 1 also clearly indicate that

these glycoproteins either never gained allergenic potency, or became inactivated by the atmospheric conditions in the high mountains. With respect to the latter, it must be pointed out that the lysine-sugar determinant – proposed to account for allergenicity (6) – is very sensitive to photo-inactivation by ultraviolet light. *Bruchhausen* and co-workers have in point of fact been able to inactivate house dust extracts by UV-irradiation (8). In a recent investigation, we have been able to confirm and extend this finding (7).

Though inactivation may be an important factor while considering the potency of *outdoor* dust, it cannot explain the abovementioned results on dust samples collected *indoors*. Hence, the most likely explanation for the low allergenic activity per mg of the Davos dust allergens studied here appears to be that the atmospheric conditions, i.e. low overall humidities and temperatures, have prevented their generation or activation. These climatic conditions are generally unfavourable for promoting the introduction of lysine-sugar determinants into the glycoprotein carrier molecules, i.e. by the Maillard-type interactions which have been proposed to account for the genesis of potent house dust allergens (6).

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