

CORRESPONDENCE

Of mites and men

To the Editor:

The recent contribution to your JOURNAL on "The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house-dust allergen" by Voorhorst and his colleagues¹ must have come as a surprise to those of your readers engaged in active research in the field of allergen composition and structure. Though the approach is certainly original in that mites were not yet on the extensive list of materials and organisms accused of producing the specific house-dust allergen, the evidence presented in the article cited above should fail to convince even the casual reader. To begin with, the pitfalls inherent in drawing conclusions from apparent correlations between factors not necessarily causally related and, at any rate, without mathematical justification should be evident to anyone even faintly acquainted with the capriciousness of the laws of statistics and with the complexity of factors involved in house-dust allergy. Seemingly significant correlations similar to the one between the number of mites in samples of house dust and the allergenic potency of these dust extracts can, in fact, easily be found for relationships between extract potency and temperature (or humidity), growth of molds, or, for that matter, degree of chemical decomposition of the dust substrate. Secondly, incrimination of mites or of mite components as producers of house-dust allergen is totally irreconcilable with everything known to date of the chemistry of highly purified house-dust allergens, including the data of the senior author himself.²

It seems quite clear that, to prove that the allergens supposedly produced by mites and those in house-dust extracts are the same, it will have to be demonstrated unequivocally that purified and homogeneous allergens isolated from both sources are, chemically and biologically, indistinguishable; this is indeed going to be a formidable task. Unfortunately anyone wishing to embark on such a project is denied the opportunity of even a preliminary experimental setup for the simple reason that one searches in vain for a methods section giving detailed information on how to prepare these mite extracts. The isolation of dead mite bodies described in the addendum to the paper may of course be interesting from the biological point of view, but it does not teach us how to prepare an active extract, even if the allergens had not already been extracted by the aqueous lactic acid or chemically modified by the boiling procedure. The article suffers some essential shortcomings because quite a few important data have been omitted from the text, such as: what would be the wet weight of these mites and on what basis could the extracts possibly be standardized?

For the benefit of those of your readers puzzled by such technicalities, attention should perhaps be drawn to the recent doctoral thesis of one of the junior authors,³ which does contain some valuable further information. From that manuscript it may be learned that these house-dust mites are cultured on a nutrient medium consisting of a mixture of powdered human dandruff and dried yeast cells; also, some idea is given of the quantities of mite material involved, in that the wet weight of one thousand of these mites is estimated at about 1 mg. In a typical experiment, 75 mites (representing 75 μ g wet weight) were being transferred (?) to a jar containing some glass powder only, and the whole lot was extracted after 3 weeks of storage at 25° C. and 95 per cent humidity. It is not surprising that this experiment led to confusing results and, therefore, to quote from p. 54 of the

manuscript: "It was concluded that for the preparation of extracts containing high concentrations of allergen produced by *D. pteronyssinus*, it would be far more profitable to use full-grown mite cultures instead of clean mites. Extracts of full-grown cultures of *D. pteronyssinus* were tested in 43 patients, and the skin reactions compared to those obtained with the standard house-dust extract." These data have, in fact, been incorporated in Fig. 10 in the recent article in your JOURNAL. The salient point of course is that, to quote again: "in these full-grown cultures about 2,500 specimens of *D. pteronyssinus* are found per 250 mg. nutrient medium in a cultivation cup." The nutrient medium (i.e., human dandruff, not counting the yeast powder) has long been known to contain a powerful skin-reactive allergen, and a constant yield of 0.4 to 0.6 per cent of purified human dandruff allergen may consistently be extracted from the dry powder^{4, 5}; the yield of crude allergen is five times higher. This means that if the total mite-nutrient medium extract is 0.0001 per cent (by weight) with respect to the mites, it still is 0.01 per cent with respect to the dandruff material. Assuming that 1 per cent of the wet weight (2.5 mg.) of the mites in the example above is soluble and allergenic, and that the mite bodies are not water repellent, the extract contains 0.01 μg per milliliter at best of the proposed mite allergen, but it also encompasses at least 0.5 μg per milliliter of the allergenically potent human dandruff allergen (and five times more of the crude allergen) extracted from 250 mg. of dandruff, regardless again of soluble yeast-cell constituents and potential allergens conceivably generated by the interaction of the nutrient components during storage under the conditions chosen. The clinical results recorded in the article by Voorhorst and colleagues have, in consequence, been obtained with an allergenic extract containing an at least fifty times more active human dandruff allergen than the optimal quantity of crude allergen that could possibly have been extracted from the mites. Purified human dandruff allergen gives positive skin-test reactions in the majority of atopic patients in concentrations of 1.0 to 0.2 μg per milliliter; moreover, human dandruff components have been shown to constitute a considerable portion of the allergenic fractions of house dust.^{6, 7} A close correlation between skin-test results in atopic individuals with extracts of human dandruff and of house dust has repeatedly been reported in the past, and relevant diagrams illustrating this relationship and comparable to the one in Fig. 10 of Voorhorst's article have actually been published.^{5, 8} The calculations given above may serve to demonstrate that there is no obvious justification for substituting the term "Dermatophagoides" for human dandruff allergen in such diagrams, and it is quite embarrassing to note that neither manuscript of the above authors contains any reference to the crucial control experiment which should have checked the allergenic potency of an extract of the nutrient medium *alone*.

It would seem that the far-reaching conclusion arrived at by the authors to the effect that mites produce the house-dust allergen at this moment lacks the support of rigorously controlled experiments. In the face of the available evidence the introduction of mites into the subject of human allergy is decidedly questionable; also, as it stands, it does an injustice to mites.

L. Berrrens
Department of Dermatology and Allergy
University Hospital
Utrecht, The Netherlands

REFERENCES

1. Voorhorst, R., Spieksma, F. Th. M., Varekamp, H., Leupen, M. J., and Lyklema, A. W.: The House-Dust Mite (*Dermatophagoides Pteronyssinus*) and the Allergens It Produces. Identity with the House-Dust Allergen, *J. ALLERGY* 39: 325, 1967.
2. Elias, R. W., Voorhorst, R., and Van Krieken, H.: On the Purification of House-Dust Allergen, *Acta allergol.* 19: 369, 1964.
3. Spieksma, F. Th. M.: The House-Dust Mite *Dermatophagoides Pteronyssinus* (Trouesart, 1897), Producer of the House-Dust Allergen (Acari: Psoroptidae), Thesis, Leiden University, June, 1967.
4. Berrrens, L., and Young, E.: Studies on the Human Dandruff Allergen. I. The Nondia-

- lyzable Constituents of Aqueous Extracts of Normal Human Dandruff and Partial Purification of the Allergen, *Dermatologica* **128**: 3, 1964.
5. Berrens, L., Morris, J. H. and Young, E.: Studies on the Human Dandruff Allergen. III. Further Purification and Comparison With the House-Dust Allergen, *Dermatologica* **132**: 433, 1966.
 6. Berrens, L., Morris, J. H., and Versie, R.: The Complexity of House Dust, With Special Reference to the Presence of Human Dandruff Allergen, *Internat. Arch. Allergy* **27**: 129, 1967.
 7. Versie, R., and Brocteur, J.: Identification de protéines humaines dans des extraits allergéniques de poussières de maison, *Acta allergol.* **22**: 11, 1967.
 8. Hénoq, E., Bazin, J.-C., and Girard, J.: Les allergènes squames humaines et poussière de maison. Etude comparative dans l'eczéma atopique. *Rev. franc. d'allergie* **6**: 213, 1966.

Reply

To the Editor:

If Dr. Berrens, who is as much interested in the house-dust allergen problem as our group would have broken the "magic 35 miles' barrier" which evidently lies between the universities of Utrecht and Leiden, he could have been easily convinced that all the necessary control experiments and statistical calculations, which were not mentioned *expressis verbis* in our article, have nevertheless been done in abundance. Also, a number of misunderstandings could have been easily cleared up.

When we examine a house-dust sample on its allergen- and its mite-content, we do not count the number of mites first (for which process boiling in acetic acid is necessary) and then prepare a test extract from the remaining materials, in which the allergen probably would have been destroyed. Our method is to divide the dust sample into two parts, one part serving for the preparation of test extract, the other for counting the mites.

Another misunderstanding has been caused by our annotation, "0.0001 per cent extract of a mite culture."¹ This does not refer only to the mites of the culture (*not* one part of *mites* in 1,000,000 parts of extracting fluid" and, therefore, not one part of human dander to about 10,000 parts of extracting fluid). Instead the annotation "0.0001 per cent extract of a mite culture" means one part of the *whole culture*, i.e., mites plus human dander plus dried yeast powder, in 1,000,000 parts of extracting fluid, or one part of *mites* in about 100,000,000 parts of extracting fluid.

As we described* a 0.000001 per cent extract of a mite culture (containing 0.0000005 per cent human dander) still gives skin reactions in many house-dust atopic patients. For human-dander-extract skin reactions are only found with extracts diluted not more than 0.01 per cent.† It is evident that skin reactions caused by such strongly diluted mite culture extracts could not be caused by its content of human dander. For that purpose the human dander concentration in the extracts is about 20,000 times too low!

Furthermore it appeared that patients with skin reactions to human dander but *not* to house dust did not react to extracts of *Dermatophagoides* culture in the usual concentrations.‡ This fact is a strong argument in favor of our contention that the house-dust (or *Dermatophagoides*) allergen has nothing to do with the human dander allergen.§

*See pp. 54-55, reference 2.

†See p. 328, reference 1.

‡See p. 335, reference 1, and pp. 51 and 53, reference 2.

§See pp. 326 and 328, reference 1.