

# CORRESPONDENCE

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## Cultivation of house-dust mites

To the Editor:

Many investigators in the field of Allergology have become interested in the occurrence of mites in house dust and their significance for the specific allergenic potency of house dust. All reports on this subject are in agreement with the findings of our "Leiden-group,"<sup>1</sup> and we feel happy about these confirmations. However, there is one facet in the confirming investigations about which we are rather concerned.

The studies of the mite fauna of house dust show that *Dermatophagoides pteronyssinus* is occurring most frequently, at least on the continent of Europe,<sup>2</sup> in Great Britain,<sup>3</sup> and in Japan.<sup>4</sup> Nevertheless, some of the confirming studies concerning the correlations of the reactions to skin tests and inhalation tests are done with *D. farinae* or *D. culinae*.<sup>5, 6</sup> Although it seems that *D. pteronyssinus* and *D. farinae* are sources of the same allergen, it is, in my opinion, safer to use *D. pteronyssinus* for these experiments because of its more frequent occurrence.

The reason that *D. pteronyssinus* is not used is due to the fact that people have difficulties in culturing this species in large quantities. In the reports of the "Leiden-group" it has been pointed out that human skin scales and powdered yeast are very suitable nutrient media for *D. pteronyssinus*,<sup>7</sup> and they can also be used for cultivating *D. farinae*.

It seems to me that there are two causes for the denial of our cultivation suggestions. First, most people do not know how to collect human skin scales, and, second, people think that the allergen present in human skin scales interferes with the allergen produced by the mites or even that it is the main allergen in our mite-culture extract.<sup>8, 9</sup>

The collection of human skin scales is quite simple if one finds a number of hairdressers willing to collect the hairs cut under dry conditions. From one kilo of human hair, about 10 Gm. of human skin scales can be extracted by washing and sieving the hairs in acetone.

We have pointed out that on quantitative grounds it is impossible that the skin reactions after testing with mite-culture extract are caused by the human skin-scales allergen.<sup>9</sup> The most concentrated mite-culture test fluid we use (0.001 per cent w/v) is comparable to a human skin-scales test fluid of 0.0005 per cent, if a nutrient medium consisting of equal amounts of skin scales and yeast is used. Prominent skin reactions to human skin-scales extract are seen to concentrations of 0.01 per cent, whereas skin reactions to 0.001 per cent are exceptional. We do not see why this is a weak argument.<sup>6</sup> Moreover, as preliminary experiments show, it is possible to remove the allergen from the human skin scales by extracting them in an aqueous suspension without losing the food substances for the mites.

There is another important advantage in using human skin scales as nutrient medium for *Dermatophagoides*. By injecting patients (intracutaneously for test purposes and, in the near future, subcutaneously for desensitization), one does not expose the patients to a new antigen or allergen, because everyone comes in contact with human skin scales during his life. There is no danger of causing new sensitizations. For this reason one must be very careful in preparing extracts of mites grown, for instance, on fish meal, dog meal,<sup>5</sup> or dried *Daphnia*.<sup>6</sup> Even when all precautions are taken to avoid contamination of the mites with the material of the nutrient medium, it is impossible to remove it from the mite's intestine. According to the medical workers of our group (Dr. Voorhorst and colleagues), it is clear that repeated injection of extracts containing protein-rich medium material is very dangerous.

The use of dried *Daphnia* as nutrient medium for mites, especially, has several disadvantages. The Japanese investigators seem to have been lucky in having seen no mites in their

Daphnia material.<sup>6</sup> However, from 1 Gm. of dried Daphnia material which I bought in a shop, I collected more than 500 stored-food mites (mainly *Tyrophagus*). These dried Daphnia happened to be imported into The Netherlands from China or Japan<sup>1</sup> via Denmark. The kind of food material used for the growth of Daphnia is not mentioned in the Japanese report,<sup>6</sup> but while in the shop I could clearly smell fish meal. Upon microscopic examination, the bodies of the water fleas appeared to contain large amounts of fish meal particles. Therefore, although because of lack of time I have not yet been able to prove this, I suppose that *Dermatophagoides* cultured on dried Daphnia does not eat Daphnia material itself but the fish meal. Fish meal is a relatively good nutrient medium for house-dust mites,<sup>7</sup> but for reasons mentioned we consider it too dangerous for extraction purposes.

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#### Reply

To the Editor:

With extensive microscopic studies, we could identify both living and dead *Suidasia nesbitti* in Daphnia which we have used as the nutrient of *Dermatophagoides farinae*. The number of contaminating *S. nesbitti* was so small, however, that it was considered not so important as a contaminating antigen. This was confirmed, as reported previously,<sup>1</sup> as only a few cases extremely sensitive to *D. farinae* reacted to intracutaneously injected Daphnia extract. For investigative purposes it was thought that Daphnia has a great advantage as there has been argument still that human dandruff could be the antigen to elicit skin reactions in the house-dust-sensitive subjects.<sup>2, 3</sup>

Ground Daphnia, used as the nutrient of mites, was sterilized in an oven (80° C.) for 24 hours before inoculation with *D. farinae*, which was cultured individually in tiny capillaries. After this, mites were cultured under favorable conditions in vessels (with a rubber stopper at one side and glass filter at another) containing ground Daphnia. Consequently there was no possibility of contamination by other mites during the process of the culture.

In the study of antigenicity of Daphnia, two monkeys (*Macaca mulatta*) were injected with an emulsion of equal parts of incomplete Freund's adjuvant and Daphnia extract (1:10 w/v, total nitrogen: 503 µg per milliliter) every 10 days for 3 months. Blood was obtained 3 weeks after the last injection. Skin tests were carried out by intracutaneous injection of 0.02 ml. of tenfold serial dilutions of Daphnia extract after the intravenous injection of 3 ml. of