

vegetables, and sunflower seeds than in those sensitized to foods of animal origin (2). Allergy to sunflower seeds has been documented in food-allergic patients from Mediterranean countries (3), and sunflower pollen sensitization has been reported as an occupational allergy (4). The case presented here is different in that the patient in our report did not have any food allergy, and the clinical circumstances of presentation were unique.

Increased concentrations of specific IgE in our patient against other members of the Compositae family of vegetables were consistent with the observation of cross-reactivity between sunflower and other Compositae pollen, including mugwort and dandelion, as shown by RAST and immunoblotting-inhibition experiments (5). Thus, the production of IgE against additional members of the Compositae family supports the validity of our conclusions.

Eating sunflower seeds is common in Israel as well as in other Mediterranean countries. The seeds are usually bought unpeeled, cleaned, and then peeled and eaten. However, commercially available packages of peeled sunflower seeds are not always clean enough and apparently may contain sunflower pollen. The case further shows that ingestion of such preparations of peeled seeds can cause systemic allergic symptoms in some patients.

Therefore, we recommend the following two measures to improve the safety of the public:

- 1) Care should be taken to remove the flowering heads during the sunflower harvest.
- 2) Certain "natural" food products may be contaminated with pollen, and allergic subjects should be warned of such a possibility.

Key words: food allergy; sunflower.

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A simplified measurement of protein in natural latex

LATEX allergy causes several clinical manifestations which are now recognized as significant contributors to morbidity and mortality during medical and surgical procedures (1). The results of protein assays are established as an acceptable criterion with which to rate the potential allergenicity of current natural rubber products (2), and this explains the need for a sensitive, rapid, and convenient protein assay in these samples.

Colorimetric assays are not suitable to measure proteins in latex, since interfering substances have to be removed. The modified bicinchoninic acid (BCA) assay precipitates proteins with sodium deoxycholate (DOC) and trichloroacetic acid (TCA) (3). Still,

Description of a simplified method of protein measurement, avoiding protein precipitation, with bicinchoninic acid as color reagent in natural latex.

latex rubber particles coagulate in acid media and therefore could interfere by scattering light, giving erroneous results. Furthermore, when only micrograms of protein are to be analyzed, DOC is not efficient as co-precipitant of TCA when detergents are present (2), and

they are necessary for particle-bound protein extraction.

Some 25% of proteins in natural latex are particle-bound. Therefore, a protein assay for latex must include solubilization of particle-bound proteins; for this purpose, sodium dodecyl sulfate (SDS) and Triton X-100 have been shown to be effective by

electrophoretic analysis (3). The present study shows that double centrifugation efficiently removes deproteinized rubber particles, making the protein precipitation step in the BCA assay unnecessary.

For comparison of results, the measurement was performed in three ways:

- 1) by the Siler & Cornish (3) procedure for extracting proteins with SDS and by precipitation
- 2) by extracting proteins with Triton X-100 and by precipitation
- 3) by extracting proteins with Triton X-100 but without precipitation.

The study of the method validation parameters and results obtained in five different samples are included.

■ **Materials.** SDS, DOC, Triton X-100, and BCA were purchased from Sigma (St Louis, MO, USA). All other reagents were from Scharlau (Barcelona, Spain). Commercial high-ammoniated latex (HA cream) was provided by Tecnilatex SA.

■ **Procedures.** The whole assay was performed in polypropylene Eppendorf tubes of 1.5 ml.

■ **Method 1.** Proteins were solubilized by diluting 100 µl of latex in 900 µl of 1% SDS in water. Samples were gently mixed, and, after 1 h, they were centrifuged at 12 000 g for 10 min. The bottom layer was carefully transferred to another tube, avoiding disturbance of the rubber cream, and a new centrifugation was carried out. From the last

bottom layer, a 500-µl aliquot was brought up to 2.5 ml with distilled water. A volume of 100 µl of this solution containing the proteins was brought to 400 µl with water, 50 µl of DOC (1.5 mg/ml) was added, and the mixture was allowed to stand at room temperature for 10 min. Then 50 µl of TCA (72% in water) was added. After 10 min at 4°C, the mixture was centrifuged at 12 000 g. The supernatant was discarded, and 100 µl of SDS (5% in 0.1 M NaOH) was added to solubilize the protein pellet. This was followed by the addition of 1.25 ml BCA assay solution, and, after 1 h of incubation at 60°C, absorbances were measured at 562 nm.

■ **Method 2.** This was the same procedure as described in method 1, except that 100 µl of latex was initially treated with 1% Triton X-100 (10%) instead of SDS.

■ **Method 3.** Measurement of protein was done directly, without protein precipitation. Proteins were solubilized by diluting 100 µl of latex in 900 µl of 1% Triton X-100 (10%). Samples were double centrifuged as above, and from the last bottom layer, a 500-µl aliquot was brought up to 2.5 ml with distilled water. Volumes of 100 µl of water and 1.25 ml of BCA reagent were added to 100 µl of this solution containing the proteins, and samples were incubated for 1 h at 60°C.

Protein concentrations were determined with a standard curve prepared with BSA (0–30.5 µg) from a stock solution of 1.5 mg/ml diluted 1:10 that was processed with or without protein precipitation, as described in each method for the corresponding samples.

■ **Validation.** Standards linearity was verified by analysis of two replications of 0, 25, 50, 100, 150, and 200 µl of the stock protein-diluted solution. Sample linearity was tested in two replications of 25, 50, 100, 150, and 200 µl of diluted solution. Intra- and interassay precision was determined by processing two six-sample series of 100 µl each on different days. Accuracy was evaluated by processing two five-aliquot series of 50 µl of sample solution, to which volumes of 25, 50, 75, 100, and 150 µl of diluted stock solution were added in methods 1 and 2, and 12.5, 25, 50, 100, and 150 µl of the same solution in method 3.

■ **Previous studies** in our laboratory showed that double centrifugation gave better results for precision than a single one; therefore, this was the procedure followed. Table 1 shows the validation parameters of the three methods, and, as can be seen there, the lowest inter- and intra-assay RSD was found with method 3, and the same happened

Table 1. Parameters of validation of BCA assay for protein measurement in latex

| | | Method 1 | Method 2 | Method 3 |
|---------------------|------------|---------------|---------------|---------------|
| Standards linearity | Intercept* | 0.28±0.04 | 0.33±0.04 | 0.37±0.04 |
| | Slope* | 0.035±0.003 | 0.035±0.002 | 0.041±0.003 |
| | r | 0.995 | 0.996 | 0.996 |
| Range (µg BSA/tube) | | 0–30.5 | 0–30.5 | 0–30.5 |
| Samples linearity | Intercept* | 0.29±0.05 | 0.39±0.02 | 0.41±0.05 |
| | Slope* | 0.0023±0.0004 | 0.0049±0.0001 | 0.0075±0.0002 |
| | r | 0.98 | 0.999 | 0.998 |
| Precision (µg BSA) | | | | |
| Intra-assay | Mean** | 16.58±0.02 | 17.61±0.04 | 19.084±0.006 |
| | RSD | 3.67 | 6.48 | 0.74 |
| Interassay | Mean** | 12±3 | 17.6±0.7 | 19.4±0.3 |
| | RSD | 45.08 | 6.24 | 2.59 |
| Accuracy | | | | |
| | Recovery** | 97±8 | 94±7 | 97±3 |
| | RSD | 11.5 | 9.7 | 5.1 |

*Value ± individual limit of confidence.

**Mean ± limit of confidence of mean.

with RSD for recoveries in accuracy. A deeper analysis of the results pointed to Triton X-100 as a better particle-bound protein solubilizer than SDS, because, although the recovery of standard BSA added was good for both, the values of protein content obtained in samples were quite lower when SDS was used. For investigation of whether the higher protein values obtained with method 3 were due to some interference not detected when accuracy was evaluated, the protocol was modified in the inverse direction; that is, by adding increasing quantities of sample to a fixed amount of standard (50 µl of diluted stock standard of BSA). In this case, no bias was found, the intercept just corresponding to the 50 µl BSA standard.

Five different samples of commercial high-ammoniated latex were finally analyzed for proteins by method 3, giving the following results: 9.0, 10.3, 10.6, 11.3, and 10.1 mg protein/g sample, respectively.

In view of these results, we concluded that method 3 is very suitable and convenient for routine analysis of protein in natural rubber latex, since it requires only centrifugation and commonly available, inexpensive reagents.

Key words: bicinchoninic acid; natural latex; protein measurement.

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Denture-induced local and systemic reactions to acrylate

DENTURE stomatitis is a well-known problem in denture wearers. In the overwhelming majority of cases, clinical symptoms are caused by irritation from ill-fitting dentures, combined with insufficient hygiene regimen and/or fungal infec-

tion. Allergic contact stomatitis is rare (1-2). Of 291 patients in the hospitals of the IVDK (Information Network of Departments of Dermatology) patch-tested in 1994 because of denture-associated problems, only 5.5% (n=16) were diagnosed as having allergic contact stomatitis (3). Only a few case reports about denture material-induced contact stomatitis due to acrylates have been published (2, 4-6). We report the first case, to the best of our knowledge, of combined contact stomatitis and systemic reaction due to acrylate sensitization.

A 56-year-old retired medical assistant developed painful burning sensations in the oral cavity after having a methyl methacrylate-base partial denture prosthesis fitted in the lower and upper jaws. Clinical examination showed erythema and ulcerations of the gingival mucosa at the site where the prosthesis had been inserted. No clinical evidence of candidiasis, oral lichen planus, or squamous cell carcinoma was found. In-depth examination by a dentist excluded an ill-fitting prosthesis. Swabs taken from the oral mucosa and the prosthesis were negative for candidiasis or bacterial infection. Patch tests were performed with the standard series, denture material series, and metal series recommended by the German Contact Dermatitis Research Group. The patient showed positive patch test reactions after 96 h to methyl methacrylate monomer 2% pet. and 2-hydroxypropylacrylate 0.1% pet. No reactions were shown to polymerized methyl methacrylate-containing denture materials (Table 1). No sensitization was shown to allergens of the standard or metal series. Latex sensitization could be excluded.

For confirmation of the diagnosis, the prosthesis was removed, and topical treatment with corticosteroids was performed, resulting in complete healing. Re-exposure to the prosthesis was followed by relapse of the mucosal inflammation and even development of edema of the tongue, lips, eyelids,

Table 1. Patch test results of relevant allergens

| Allergens | (pet.) | D1 | D2 | D3 | D4 |
|-------------------------|--------|----|----|----|----|
| Methyl methacrylate | 2% | - | - | ?x | + |
| 2-Hydroxypropylacrylate | 0.1% | - | - | ?x | + |
| Luxene* | As is | - | - | - | - |
| Polyan* | As is | - | - | - | - |

Patch tests were performed with denture material series, standard series (not shown) and metal series (not shown) recommended by German Contact Dermatitis Research Group. Reading was performed after 24 h (D1), 48 h (D2), 72 h (D3), and 96 h (D4): ?x: erythematous, not infiltrated test reaction, considered questionable; +: erythematous, infiltrated patch test reaction with some papules; -: negative patch test reaction.

*Polymerized denture materials with low content of acrylate resins (<1% monomeric acrylate resins when polymerized) were cut into small pieces and tested in Finn Chambers on Scanpore.