with a small volume of Tyrode (4 °C) saline, which was then discarded. About 1 ml of fresh saline was added and RPE cells were gently brushed loose from the surface of the eye cup with a 1/2″ camel-hair brush. The detached cell suspension was removed by Pasteur pipette. The brushing and collecting of RPE cells was repeated three times. Before protein determination and enzymatic assay, RPE cells were centrifuged at 7000 × g for 10 min, resuspended in 0.05 M citrate phosphate buffer, pH 5, (3 ml/eye), and homogenized with a Brinkman PT10/35 polytron (tissue grinder/homogenizer) for 30 s.

In the second method (Method 2), the RPE cell layer and underlying choroid were removed together from the eye cup. Before analyses, the RPE/choroid was vigorously vortexed for 1 min in 3 ml of citrate phosphate. The remaining RPE cells, together with the underlying choroidal layer, were subsequently removed. The cell suspension was then homogenized using a Polytron for 30 s.

RPE histology. RPE cells were placed on a microscope slide and examined under an Olympus Model BM compound microscope with an oil immersion lens (magnification 15 × 100).

Protein determination. Proteins were quantitated by the method of Lowry, using bovine serum albumin as standard[4].

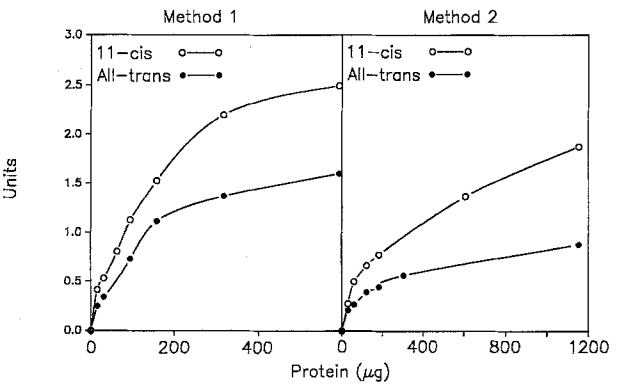
Retinyl ester hydrolase (REH) assay. Enzymatic activity was monitored by an REH assay. RPE cells collected from (1) and (2) were placed in 3 ml of sodium 0.05 M citrate-phosphate, pH 5. Tritiated retinyl palmitate was obtained by reacting 11-*cis* or all-*trans* retinol, with the symmetric anhydride of tritiated palmitic acid[5]. 10 µl of substrate was dissolved in ethanol and added to 190 µl of sample and the mixture was incubated in a shaker bath for 1 h at 37 °C. 3 ml of extraction solvent (1.41 methanol: 1.25 chloroform: 1.00 heptane) and 1 ml of 0.05 M potassium carbonate buffer, pH 10, were added. The mixture was then vortexed and centrifuged at 1000 × g for 15 min. 1 ml of the upper aqueous phase was removed from each sample and radioactivity was measured in a liquid scintillation counter. All assays were carried out in triplicate. One unit of activity refers to the formation of 1 pmole product per s.

Results

Microscopic examinations show that patches of RPE cells were recovered using both of these methods. Hexagonally shaped RPE cells were observed in single layers with intact melanin granules and other cell inclusions. Most cells remained unperturbed and small structures which resembled oil droplets were observed in the surrounding saline. Cell suspensions from (1), in comparison to those from (2), appeared to contain a larger number of intact cells. In addition, RPE cells in suspensions prepared by (1) tended to be aggregates with larger cell patches than those prepared by (2). Non-pigmented cells were not present in our preparations.

Table 1. Total RPE proteins (mg/RPE) determined using the method of Lowry et al.[4]

Method 1	Method 2
1.0	15.7
2.8	14.3
2.6	17.3
1.6	13.4
1.2	14.0
1.9	18.2
$\bar{X} = 1.85 \pm 0.7$	15.48 ± 1.9



Retinyl ester hydrolase activities in methods 1 and 2.

Table 2. 11-*cis* and all-*trans* retinyl ester hydrolase activities (units/mg). The values were derived from the linear portion of the protein curves (see figure)

	Method 1	Method 2
11-*cis*	7.5	3.8
all-*trans*	5.5	2.0

Table 1 indicates that RPE cells collected from (2) yielded more than eight times more protein (mg/RPE) than that of RPE cells collected by (1). The figure shows a significant level of REH activities in the recovered RPE cell homogenate. The highest hydrolytic activity was exhibited for 11-*cis* retinyl palmitate in both (1) and (2). Table 2 indicates that both 11-*cis* and all-*trans* REH specific activities were higher in proteins collected by (1) than those collected by (2). Taking into consideration the greater amount of protein collected by (2) (see table 1), the total enzyme activity recovered from the RPE was greater in proteins recovered from method 2.

Discussion

Microscopic evaluations of RPE cells collected by the two methods employed show both yielded intact, morphologically identical RPE cells. However, (1) collected a greater amount of intact RPE cells than (2) and larger cell patches were noted in cell suspensions prepared from (1). These results suggest that the vigorous vortexing of RPE/choroid using method 2 must have disrupted more RPE cells than the gentle brushing of method 1. Nevertheless, our results show that both methods yielded intact RPE cells, although a qualitative evaluation of these 'intact' RPE cells remains to be carried out.

Our results on protein yield from (1) was in agreement with those reported in the literature (6 mg per eye in the present study compared to 8 mg per eye in Berman[6]). Moreover, Lowry protein determination showed that (2) yielded more than eight times more total protein (mg protein/RPE) than (1). This suggests that (2) has dislodged more RPE cells into the buffer than (1) therefore resulting in a higher protein yield. This appears contrary to our histological examinations that (1) yields more 'intact' RPE cells. One possible explanation is that a significant number of RPE cells collected from (2) were disrupted (i.e. not 'intact' cells) as a result of vortexing thus lowering the number of intact RPE cells from (2). The protein yield, nevertheless, suggests that (2) collected more proteins from the bovine RPE than (1).

The retinyl ester hydrolase activity has been reported in the retinal pigment epithelium of human cells[7]. In addition, the enzymatic activity was shown to be stereospecific, i.e. more active towards hydrolysis of 11-*cis* than all-*trans* substrate. In this study, our results agree with the literature that RPE protein contains active enzymes to hydrolyze both 11-*cis* and all-*trans* retinyl palmitate (table 2). Moreover, these enzymes were found to favor hydrolysis of 11-*cis* over the all-*trans* substrate. It is important to note that REH activity was found in RPE cells collected by both methods employed in this study and that RPE cells collected by (1) possessed higher specific activity for both 11-*cis* and all-*trans* retinyl palmitate yet (2) yielded more total enzyme activity.

In conclusion, both methods employed in the present study collected RPE cells of good quality. Although (1) resulted in relatively more pure RPE cell preparation, (2) yielded more RPE proteins and higher overall enzyme activity. Consequently, we suggest that the method tested in the present study (2), offers a new and convenient approach to collect more RPE protein appropriate for certain investigations such as enzyme purifications.

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Urushiol components as mediators in DNA strand scission

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Summary. Poison oak urushiol, a mixture of 3-alk(en)ylcatechol derivatives was found to mediate DNA strand scission in the presence of oxygen and with copper(II) chloride as a catalyst. The reaction is believed to occur via activated reduced oxygen produced during oxidation of the catechol into its *o*-quinone derivative.
Key words. DNA strand scission; urushiol; poison oak; 3-alk(en)ylcatechol.

The ability of plant secondary metabolites to mediate DNA strand scission has been recognized recently[1]. DNA cleaving compounds are of special interest for their possible applications in molecular biology, and in the development of novel chemotherapeutic agents[2]. Plant natural products shown to cleave DNA include quinones like daunomycin[3], and polyphenolic compounds like 5-alkylresorcinols[4]. Daunomycin cleaves DNA in the presence of a reducing agent and iron. Resorcinol derivatives require copper salts and oxidation by molecular oxygen for activity. A mechanism involving a catechol copper complex as the active species has been proposed. These results suggested that urushiol resins from poison oak (*Toxicodendron diversilobum* (L.) Kuntze) or poison ivy (*T. radicans* (T. and G.) Greene) are likely to cleave DNA as well. Urushiols are the principal cause of contact dermatitis in North America[5] and have been studied extensively for sensitizing and irritating properties, but not for antineoplastic activity. Therefore, we set out to determine if these natural products can mediate strand scission of Simian virus 40 DNA.