The in vitro influence of retinol on the markers of gingival Langerhans cells (LC) was investigated using an organ culture system. Retinol at a dose of 5 \( \mu \text{g/ml} \) produced an increase in the density of T6-positive cells within the epithelium which peaked during the first 24 h of culture. LC HLA-DR and ATPase markers were maintained for the same period, while all markers were depressed after 72 h. These effects were not seen in explants cultured in conventional or alcohol-enriched media, in which all markers were lost in an exponential fashion. In addition to modulation of LC markers, retinol treatment also prolonged the expression of HLA-DR antigens by gingival keratinocytes. These findings, together with the augmented production of interleukin-1-like activity by retinol-treated gingival organ cultures suggest that low doses of retinol may alter immune reactions within epithelia via stimulation of both keratinocytes and LC.

It is well established that the principal function of vitamin A (retinol) and its esters (retinoids) is to regulate the differentiation and maintain the integrity of several epithelia. Retinoids exert profound effects of keratinocyte differentiation both in vivo and in vitro [1-3] and, in addition to their frequently described antikeratinizing effects, may stimulate keratinocytes when used at low concentrations (< 3 \( \times 10^{-8} \text{ M} \)) [3]. Specifically, the promotion of keratinocyte growth and concurrent inhibition of stratification and keratinization in cell culture have been attributed to the low concentration of retinol present in culture media [3,4]. In vivo stimulation of keratinocytes by retinoids may alter immune responses within a variety of epithelia, specifically via modulation of the secretion of epidermal cell-derived thymocyte activating factor (ETAF). This potent cytokine is capable of amplifying a diverse number of proliferative and inflammatory processes and closely resembles the macrophage product interleukin-1 (IL-1) [5,6]. Since ETAF may have an important regulatory role in numerous inflammatory and proliferative skin disorders [7], it was of interest to examine the influence of retinol on the in vitro production of thymocyte activating factors by epithelial cells. While such activity may be produced by both keratinocytes and dendritic Langerhans cells (LC) [8], in the present study no attempt was made to differentiate between the cytokines IL-1 and ETAF.

In addition to their effects on keratinocytes, there is early evidence that retinoids may alter the expression of the various membrane markers which identify epidermal LC. Häftek et al [9] observed an increase in the expression of T6 antigens by LC (mean explant wet weight 9.5 mg) from patients undergoing retinoid therapy suggestive of a metabolic activation by the drug. While the relationship between the expression of LC markers and the functional capacity of the cells is at present unclear, a modulation of LC function by retinoids would be of significance in view of the central role of these cells in initiation of cutaneous immune responses (reviewed in [11]). Accordingly it was of interest to also examine the influence of retinol on the expression of LC markers—membrane-bound ATPase and the HLA-DR and T6 antigens. For this purpose a gingival organ culture model was utilized. Previous studies [12,13] have detailed the behavior of LC markers within this culture system, and demonstrated that modulation of LC antigens may be induced by such agents as bacterial endotoxin.

### MATERIALS AND METHODS

#### Tissues

Human gingival tissue was obtained from 22 patients undergoing periodontal surgery. Extensive presurgical preparation, including scaling of the adjacent teeth, ensured that the tissue taken was minimally inflamed. The tissue was immediately placed in Hanks' balanced salt solution (HBSS) (Commonwealth Serum Laboratories, Melbourne) containing 50 IU/ml penicillin and 50 µg/ml streptomycin, and set up in organ culture within 40 min of its removal from the mouth.

#### Gingival Organ Culture

The culture method used has been previously described [12]. Briefly, tissue samples were washed in 3 changes of HBSS, sectioned with a scalpel to give pieces 1.5 mm cubed, and weighed in sterile tubes. Where necessary, explants were adjusted to give weights within the 5-15 mg range (mean explant wet weight 9.5 mg). The explants were then transferred, connective tissue side down, onto stainless steel grids in 25-well culture dishes (Sterilin Ltd., Teddington, U.K.). Culture media (see below) were added to the level of the grids and the cultures maintained at 37°C in a humidified atmosphere of 5% CO\(_2\) in air for up to 72 h. Culture media were not replaced during the culture period. Preincubation controls were prepared in the manner described above, but were immediately removed from the culture dishes before the addition of the culture medium.

Tissues were utilized as follows: the first 18 patients were divided into 6 groups of 3 patients, with each group representing one of the culture times—6, 12, 18, 24, 48, or 72, so as to provide triplicate determinations. At least 4 explants were obtained from each individual patient: preincubation control, standard-treated, alcohol-treated, and vitamin A-treated. Tissue from a 19th patient provided data for 3-h incubation period, while organ cultures from the remaining patients were used to determine culture viability for each of the 3 treatments and to investigate the effects of indomethacin on the organ cultures.

#### Culture Media

The standard culture medium consisted of Medium 199 (Commonwealth Serum Laboratories, Melbourne) supplemented with 50 IU/ml penicillin, 50 µg/ml streptomycin, 10% heat inactivated fetal calf serum...
(FCS) (Flow Laboratories Australasia, Sydney), and 2 mM glutamine. The same batch of FCS was used in all experiments, and was previously evaluated for its ability to support organ and cell cultures. The endotoxin content of this FCS, as measured in the Limulus assay (M. A. Bioproducts, Walkerville, Maryland) was 50 endotoxin units/ml. Pure crystalline retinol (Sigma Chemical Co., St. Louis, Missouri) was dissolved in absolute ethanol to give a stock solution of 0.5 mg/ml which was stored in the dark at 4°C. Aliquots of this solution were added to standard medium just prior to use to give a final concentration of 5 µg/ml, a dose similar to that utilized in previous studies [14,*]. An equivalent amount of ethanol (5 µg/ml) was added to control treatment cultures. The retinol content of both Medium 199 and the FCS (0.1 µg/ml [15], 0.001 µg/ml [4], respectively) was considered sufficiently small that delipidizing of these components was considered unnecessary. For each explant, a control well containing an equivalent culture medium but devoid of tissue was set up. These control wells were harvested at the termination of individual organ cultures. In order to eliminate possible suppressive effects of prostaglandins, cultures from 2 patients were also treated with indomethacin (final concentration 4 µg/ml) or with an equivalent volume of the ethanol solvent.

Culture Viability

Epithelial cell viability was assessed prior to incubation and at several incubation times for each of the 3 treatments used. Epithelial sheets were prepared according to the method of Scalatta and MacCallum [16]. The sheets were then incubated in 1% trypsin in OCT saline in liquid nitrogen. Culture supernatants were divided into 2 portions, one dissolved in absolute ethanol to give a stock solution of 100 µg/ml and the other dialyzed against PBS, pH 7.2, and filter sterilized (0.22 µm, Millipore Corp., Bedford, Massachusetts) before storage.

Six micrometer-thick cryostat sections were cut from each block, numbered to facilitate serial staining, air-dried, and then fixed and processed for either histochemistry or immunofluorescence.

Tissue Processing

On termination of each organ culture, the tissues were embedded in OCT compound (Lab-Tek Products, Naperville, Illinois) and quenched in liquid nitrogen. Culture supernatants were divided into 2 portions, one portion being immediately stored in liquid nitrogen, while the remaining portion was dialyzed against 1000 vol of phosphate-buffered saline (PBS, pH 7.2) and filter sterilized (0.22 µm, Millipore Corp., Bedford, Massachusetts) before storage.

Six micrometer-thick cryostat sections were cut from each block, numbered to facilitate serial staining, air-dried, and then fixed and processed for either histochemistry or immunofluorescence.

Histochemistry

Fixation for ATPase histochemistry was a modification of that previously described [17] in that the sections were fixed in formal sucrose at 4°C for 20 min. Ten sections from each block were stained for ATPase using a lead precipitation technique as previously described [12] and lightly counterstained with hematoxylin. Known positive sections of human gingiva and tonsil served as positive controls, while negative controls were obtained by incubating sections in ATP-free buffer. Heat-inactivated sections were used to determine the extent of nonspecific sulfide binding. Several formal sucrose-fixed sections from each block were also stained with hematoxylin and eosin according to conventional techniques.

Surface Antigen Analysis

Tissue preparation and staining using immunofluorescence was carried out according to the method of Poultter et al. [18]. Briefly, air-dried cryostat sections were fixed in chloroform-acetone for 5 min at room temperature, then wrapped in cling film and stored at −20°C until used. Following washing in PBS, they were incubated for 30 min at room temperature with the respective antibody or antisera in a humidified chamber. All washes were carried out in PBS and finally the sections were mounted in an antifade mountant (AF-1, Citifluor, London) and viewed using a Zeiss fluorescence microscope with epi-illumination.

Antibodies

HLA-DR antigens were detected using a mouse monoclonal antibody RF1 (a gift from Professor G. Janossy, Royal Free Hospital, London). LC were detected using NA1/34 (a gift from Professor G. Janossy, Royal Free Hospital, London), a monoclonal antibody to human thymocytes which also reacts with the T6 surface antigen of intraepithelial LC [19,20]. Staining for each surface antigen involved a 2-layer technique incorporating a fluorescein isothiocyanate FITC-conjugated goat antimouse immunoglobulin as the second layer (Dako Immunohemochiences, Glostrup, Denmark) at a 1:50 dilution.

The dilution used was previously determined by titration to optimize specific staining with minimal background florescence. Sections of reactive tonsil were incubated at the same time as the gingival tissue to provide positive controls. Sections incubated with PBS in place of the first-layer monoclonal antibodies served as negative controls.

Cell Counting Procedure

All sections were assigned random numbers and examined without the knowledge of which patients, treatments, or time intervals were represented. The average number of positive cells contained within a standard area of oral epithelium was determined as previously described [12,13] with 15–20 counts per LC marker being performed. Ten sections per block were counted for ATPase activity, while 3 pairs of serial sections were evaluated for expression of HLA-DR and T6 antigens.

Data from individual explants was then expressed as a percentage of the count for the relevant preincubation control explant, thereby removing possible effects of small variations among individual patients. Percentage counts for the 3 patients per time were then used to calculate means and standard errors. Finally, data were analyzed using a two-tailed Student's t-test. Data for unsupplemented (standard) cultures were also scrutinized according to the previously described model for the behavior of LC markers in gingival organ culture.

IL-1 Standard

IL-1-containing supernatants from cultured murine macrophages were prepared according to the method of Kido et al. [21], modified as described. Female C3H/HEJ (H-2b) mice, 8–10 weeks old, were injected i.p. with 2 ml of HBSS containing antibiotics as previously detailed. Peritoneal cells were washed 3 times and resuspended in Medium 199 containing antibiotics and 10% FCS at a cell density of 3.5 x 10^6/ml. One-milliliter aliquots were allowed to adhere to multwell plastic plates, 24 x 17 mm (Limbro, Hamden, Connecticut) in a humidified incubator with 5% CO2 at 37°C. After 2 h, the nonadherent cells were removed by washing thoroughly with HBSS. Adherent cells were further incubated in aliquots of 1 ml of Medium 199 + antibiotics + 10% FCS for 20 h [22]. Supernatants were centrifuged at 8400 g for 10 min, sterile filtered (0.22 µm), aliquotted, and frozen at −20°C until use. This IL-1 standard was used to calibrate all thymocyte proliferation assays.

IL-1 Biosassay

IL-1 activity was assayed by measuring tritiated thymidine incorporation into mouse thymocytes with a microculture system [22]. Female C3H/HEJ (H-2b) mice were obtained from the Central Animal Breeding Facility, University of Queensland, Brisbane, Australia, and were all 8–10 weeks old at the start of individual assays. Briefly, thymi were removed aseptically and pressed through a stainless steel mesh to obtain a single cell suspension. The cells were washed by centrifugation and adjusted to a final density of 5 x 10^5/ml. Thymocytes were cultured in 96-well microculture trays (Disposable Products, Sydney) in RPMI 1640 (Flow) containing 5% FCS, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 2.5 x 10^-5 M 2-mercaptoethanol. Each well contained 50 µl of the cell suspension, 25 µl of phytomembrane (PHA-P), 5 µl (4 µg) of the IL-1 standard, at serial dilutions. Since preliminary experiments had repeatedly demonstrated no difference between dialyzed and complete culture supernatants, nondialyzed supernatants were utilized in the bioassay system. Thymocyte cultures were incubated in a humidified CO2 incubator for 72 h, the last 6 h in the presence of 0.5 µCi tritiated thymidine (Amersham Corp., Arlington Heights, Illinois). Cells were collected on glass fiber filter paper using a semiautomatic cell harvester (Micromash, Dynatech, Singapore) and samples counted in a Beckman LS2800 scintillation counter (Beckman Instruments, Fullerton, California). All cultures were performed in quadruplicate. Thymocyte proliferative activity was expressed as units of activity using established methods [23,24]. The standard IL-1-containing supernatant was arbitrarily assigned an activity of 100 U of IL-1/ml, and was tested in decreasing dilutions in each experiment along with the experimental samples.

The influence of retinol and ethanol on thymocyte proliferation was investigated by adding enriched (5 µg/ml) culture media to the thymi.
mocyte culture system (50 µl/well), in the absence of PHA. The effects of medium 199 with or without 10% FCS were similarly evaluated.

RESULTS

Expression of LC Markers in Preincubation Controls

Explants utilized as preincubation controls exhibited evidence of mild chronic inflammatory changes, including the presence of focal aggregations of mononuclear cells in the lamina propria. The immunohistology of the tissues utilized in this study has been detailed in a separate communication [24]. Briefly, ATPase activity within the gingival epithelium was confined to suprabasal dendritic cells which were shown in serial sections to bear HLA-DR and T6 antigens. ATPase activity within the connective tissue was localized to blood vessels and an antigen-presenting subpopulation of macrophages [25]. In all cases the density of ATPase of positive cells within the epithelium was much less than that detected by either of the immunofluorescence techniques. Reactivity for T6 was confined to occasional connective tissue cells (T6+, HLA-DR+) and dendritic epithelial cells, with the expression of HLA-DR antigens being variable in the latter. The expression of the various markers in preincubation control tissue from 19 patients was as follows (means ± SE): T6 = 144.1 ± 17.4, HLA-DR = 76.5 ± 3.2, ATPase = 47.6 ± 10.9 positive cells per mm². Expressed as a percentage of T6-positive cells, the percentage reactivity for HLA-DR and ATPase was 53% and 33%, respectively.

In addition to their presence on T6-positive cells, HLA-DR antigens were also detected on keratinocytes in all sections examined. In most sections the staining involved the full thickness of the epithelium, and predominantly involved the cell membrane. This pattern allowed differentiation between HLA-DR-positive keratinocytes and LC. No keratinocyte staining was seen in control sections in which the first-layer antisera had been omitted.

In Vitro Modulation of LC Markers by Retinol

Explants incubated in standard media demonstrated an exponential loss of each of the 3 LC markers (Fig 1), with loss of the HLA-DR antigen preceding that of T6 and ATPase. Despite the previously mentioned variation in the number of positive cells prior to incubation, the percentage changes in each marker were not significantly different, with the single exception of the absence of HLA-DR at 48 and 72 h.

The kinetics of LC markers incubated in standard media did not differ significantly from that previously described [12]. Explants cultured in solvent control (ethanol-enriched) media demonstrated a decrease in each marker which closely resembled that occurring in standard group cultures (Fig 2). The only significant difference between these two treatments was a more pronounced decrease in ATPase expression in ethanol-enriched cultures at the 6-h time period.

The expression of ATPase, HLA-DR, and T6 by LC in cultures treated with retinol (5 µg/ml) differed markedly from both standard and solvent cultures (Fig 3). ATPase expression was maintained for the first 24 h of culture, an effect significant at all incubation periods greater than 6 h (p < 0.005). The expression of HLA-DR was maintained in a similar fashion,
however the percentage decrease was more marked at intermediate culture periods than was the case with ATPase.

The expression of T6 within the epithelium was markedly altered by retinol treatment, such that during the first 30 h of culture, the density of T6-positive cells exceeded that prior to incubation. This change was highly significant ($p < 0.005$) when compared to both standard and ethanol treatments, and also in relation to the preincubation T6 counts. In addition, epithelial cells positive for T6 at the 6- to 18-h period exhibited a marked dendritic configuration (Figs 4, 5). This dendritic morphology was not maintained beyond 24 h of culture.

**In Vitro Effect of Retinol on Keratinocyte HLA-DR Expression**

Expression of HLA-DR by keratinocytes was not a feature of cultures incubated in standard or ethanol-enriched media beyond 6 h. In contrast, HLA-DR reactivity was retained by retinol-treated keratinocytes for 48 h, albeit somewhat diminished at the later culture times. Expression of HLA-DR by keratinocytes or connective tissue cells was not increased in any of the treatment groups.

**In Vitro Effect of Retinol on the Production of IL-1 Activity**

Supernatants from control wells which contained media but not tissue did not contain IL-1 activity, irrespective of treatment or incubation time. Organ culture supernatants from standard and ethanol-treated cultures contained IL-1 activity at all sample times, this activity being minimal at 48 h and maximal at 72 h (Fig 6). There were no significant differences between standard and alcohol treatment groups at any time period. Supernatants from organ cultures treated with retinol demonstrated marked IL-1 activity in the initial 24 h of culture, with a peak activity occurring from 12-18 h. Minimal IL-1 activity was seen at 48 h, with an increase in IL-1 activity occurring in the final 24 h of incubation.

**Effect of Culture Supplements on Thymocyte Activation**

In order to exclude stimulation of thymocyte mitogenesis by non-IL-1 factors, the effect of various organ culture components on thymocyte proliferation was evaluated. The following results are from a typical experiment in which 50 µl of supernatant was tested in the absence of PHA (mean cpm ± SE): Unsupplemented Medium 199 = 622 ± 62; Medium 199 + FCS = 651 ± 69; Medium 199 + FCS + ethanol 5 µg/ml = 636 ± 71; Medium 199 + FCS + ethanol 5 µg/ml + retinol 5 µg/ml = 692 ± 77. There were no significant differences among these data (two-tailed t-test).

**Effect of Indomethacin on IL-1 Activity**

Organ cultures were treated with indomethacin in order to abrogate any possible production of prostaglandins by gingival organ cultures. The following data are from a typical experiment in which 50 µl of supernatant from 6-h cultures was assayed in the presence of PHA (mean cpm ± SE). Supernatants before dialysis: standard culture 4095 ± 538, ethanol-enriched culture 3952 ± 491, indomethacin-enriched culture 4327 ± 288. Following dialysis, the respective cpm were 4319 ± 444, 4167 ± 347, and 4166 ± 271. There were no significant differences either between individual treatment groups or between dialyzed and nondialyzed samples.

**Epithelial Cell Viability**

Viability of gingival epithelial cell (GEC) suspensions prepared from preincubation controls was 90%. GEC viability in the standard culture system was 82% at 24 h, 76% at 48 h, and 56% at 72 h. Standard errors were routinely less than 5%.
In all cases, the most rapid decline in viability occurred after 48 h. While recruitment of T6(+) cells from the connective tissue cannot be excluded in the present study, the paucity of these cells in the connective tissue of preincubation explants suggests that other mechanisms are operative. Differentiation of connective tissue macrophages into LC or indeterminate cells is one mechanism whereby the number of T6(+) cells in the connective tissue, and therefore available for recruitment, would be supplemented. Retinoid-induced differentiation of histiocytes into dermal macrophages has been reported [10], however, this phenomenon was observed in psoriatic epidermis following a 3-week administration of an aromatic retinoid (RO 10-9359). Accordingly, it is difficult to draw parallels to the present in vitro investigation. It is tempting to speculate that differentiation of precursor cells into T6(+) cells is occurring in either the epithelium or connective tissue, in view of the kinetics of the response observed viz the rapidity of increase in T6. In such an instance, the achievement of a constant number of T6(+) GEC during the 12- to 24-h period would reflect differentiation of the limited pool of precursor cells present in the original tissue. Further ultrastructural studies are required in order to determine the mechanisms underlying the increase in T6(+) GEC report in this study. While the Birbeck granule has been accepted as the definitive marker of the LC [29,30], the functional significance of this marker is questionable [31], and accordingly the hypothesis that retinol is capable of stimulating LC precursors is difficult to establish using ultrastructural criteria alone.

The maintenance of HLA-DR and ATPase activity by dendritic GEC under the influence of retinol suggests that this substance is capable of modulation of the immune responsiveness of these cells. While T6(+)/HLA-DR(+)/LC are considered to represent an activated subset of LC [13,32], the significance of ATPase expression by LC has been questioned [30]. With respect to the former, while an increase in HLA-DR expression may follow in vitro stimulation of gingival LC [13], the percent positivity of HLA-DR(+)/LC seen prior to incubation suggests that two distinct subsets of LC were originally present in the tissue. The 53% HLA-DR positivity reported in this study closely reflects that documented for normal skin [32]. Since the expression of HLA-DR and ATPase did not track that of T6 in retinol-treated cultures, it is suggested that the T6(+) cells which had appeared during the first 24 h of culture were positive for T6 alone, and that the observed maintenance of HLA-DR and ATPase reflects a mild stimulation of the original LC population which was HLA-DR(+). Because of the nature of the present study, it was not possible to follow the fate of individual LC and accordingly such a proposal must remain entirely speculative.

While the present study did not permit differentiation between direct effects of retinol on LC and secondary effects such as increased lymphokine production, some insight into the mechanisms underlying the increase in T6(+) LC may be gained by comparing the effects of retinol with those of lypo-polsaccharide (LPS). We have recently demonstrated [13] that treatment of gingival organ cultures with LPS stimulates both LC and keratinocyte HLA-DR expression while causing no increase in T6(+) LC. Since lymphokines (interferons) have been implicated in the former effect [13,25], the lack of increase in T6(+) LC in the present investigation and the marked increase in T6(+) LC in the present study taken together would exclude involvement of interferons in the modulation of LC T6 expression. Further studies are required to more precisely evaluate the effects of both retinoids and interferons on LC markers.

With respect to keratinocytes, the maintenance of HLA-DR expression by these cells induced by retinol may simply reflect a stimulation of this population. Keratinocytes have the capacity to synthesize HLA-DR antigens given appropriate immunologic stimuli [33], and in the culture system described such an influence may be provided by bacterial products such as endotoxin from Escherichia coli [13]. However, since endotoxin concentrations in the culture media used in the present study are well below the apparent threshold for such stimulation (2.0 µg/ml [13]), this mechanism alone could not account for the observed effects. A more probable explanation implicates gamma interferon, a product of T lymphocytes [34], in the induction and maintenance of HLA-DR expression by gingival keratinocytes. Lymphocyte populations within the gingiva have been implicated in the induction of keratinocyte HLA-DR expression in chronic gingivitis [25], and accordingly stimulation of such T lymphocytes by retinol [35], leading to an increased production of gamma interferon as the most likely mechanism responsible for a continued synthesis of HLA-DR by keratinocytes. A similar scheme has been recently demonstrated for cultured normal human keratinocytes [36].

In relation to the IL-1 bioassay, thymocyte mitogenesis was not influenced by the presence of retinol or the solvent ethanol. Taken together with data indicating no significant presence of interfering factors such as endotoxins and prostaglandins, these findings indicate that the assay used was specific for IL-1. Furthermore, that dialysis of supernatants failed to alter their IL-1 activity suggests that no low-molecular-weight M, < 12,000) inhibitors of IL-1 were produced by the gingival organ cultures.

The increased production of IL-1 activity by retinol-treated culture suggests that the cells responsible for the synthesis and release of IL-1 and related factors are stimulated by retinol. While the capability of LC to generate IL-1 has been recently demonstrated [8], it appears that their capacity to do so does not greatly exceed that of keratinocytes, and in view of the relative paucity of LC in the gingival epithelium, it is reasonable to conclude that a sizable portion of the IL-1 activity has been generated by other cell types. Since connective tissue cells degenerate more rapidly than their epithelial counterparts in gingival organ culture [12], it is unlikely that macrophages or other resident connective tissue cells are entirely responsible for IL-1 production. In view of the maintained epithelial cell viability during the initial 48 h of culture, non-LC gingival epithelial cells (i.e., keratinocytes) are implicated as an important source of IL-1 activity (putatively ETAF) in the culture system described. Such an hypothesis is in accord with the finding that levels of supernatant IL-1 activity increased during the 48- to 72-h culture period, an interval during which keratinocyte viability diminishes rapidly and during which IL-1 membrane leakage of performed ETAF would be most likely to occur. Accordingly, the augmentation of IL-1/ETAF production induced by retinol may represent a stimulation of keratinocytes in response to low doses of retinol [3].

Further studies are required to determine the cellular source of IL-1 related factors in gingival explants. The recent development of a monoclonal antibody to IL-1 [37] may facilitate this task, and allow more detailed analysis of interactions between lymphokines and the cells responsible for their production.
duction. That retinol affects both these agents provides further evidence that retinoids have the capacity to influence cell populations that reside within and subjacent to a variety of epithelia.

REFERENCES