Table III—Radioimmunoassay Statistics for Bromperidol Determination in Human Plasma Quality Control Samples

	Bromperidol Concentration ng/ml						
	1[a]	5[a]	10[a]	25[b]	50[b]	100[c]	150[c]
Number of assays	7	10	10	10	10	3	2
Total number of samples	22	46	46	46	46	13	8
Bromperidol concentration							
Mean ± *SD*	1.49 ± 0.32	6.38 ± 0.99	12.60 ± 1.52	27.14 ± 3.80	52.37 ± 3.89	105.35 ± 4.51	159.14 ± 3.27
n	7	10	10	10	10	3	2
Between-assay *CV*[d], %	21.2	15.6	12.1	14.0	7.4	4.3	2.1
Within-assay sample variation *CV*[e], %							
Mean ± *SD*	12.2 ± 9.4	10.3 ± 4.4	7.1 ± 4.1	7.7 ± 3.8	8.91 ± 3.8	8.2 ± 4.3	5.4 ± 0.5
n	7	10	10	10	10	3	2
Sample replicate variation *CV*[f], %							
Mean ± *SD*	9.6 ± 5.9	6.0 ± 3.8	6.0 ± 3.4	7.1 ± 4.5	8.8 ± 4.4	6.0 ± 5.1	8.1 ± 6.3
n	22	46	46	46	46	13	8

[a] Quality control plasma samples were reconstituted to original sample volume (1 mL) with RIA buffer and assayed directly by RIA method. [b] Quality control plasma samples were diluted 1:2 after reconstitution with RIA buffer prior to being assayed by RIA method. [c] Quality control plasma samples were diluted either 1:2, 1:3, or 1:9 after reconstitution with RIA buffer prior to being assayed by RIA method. [d] The mean (±*SD*) and CV of the bromperidol concentration was calculated averaging the mean bromperidol concentration of each assay for each quality control plasma concentration. [e] The mean ± *SD* of the CV observed between samples within an assay was calculated averaging the mean CV between samples of each assay for each quality control plasma concentration. [f] The mean ± *SD* of the CV of the replicates of all samples assayed for each quality control plasma concentration.

Bromperidol levels (C_{min}) determined in the patient plasma samples are summarized in Table II. These data represent the mean bromperidol concentrations determined in samples collected immediately before the daily medication (n = 4–10 d) was administered. The patient plasma samples generally demonstrated the same degree of precision described for the quality control plasma samples with regard to replicate and within-assay variability. These variabilities (*CV*) were 7.1 ± 3.9 (n = 101) and 6.6 ± 4.6 (n = 45), respectively. However, intrasubject variability, the variability observed between the C_{min} values of an individual, ranged from 4.6 to 58.5% with no apparent correlation between the *CV* and the level of drug measured (Table II). In addition, comparison of the mean C_{min} drug levels from subjects receiving approximately the same dose per body weight demonstrated substantial intersubject variability. Specifically, patients A and C (dose, 0.14–0.15 mg/kg) had mean C_{min} levels of 3.73 and 6.67 ng/mL, respectively; patients K and M (dose, 0.55 mg/kg) had mean C_{min} levels of 9.20 and 20.24 ng/mL, respectively; and patients L, N, and O (dose, 0.91–1.00 mg/kg) had mean C_{min} levels of 10.72, 55.13, and 32.23, respectively. Realistically, this intra- and interindividual variability observed in mean C_{min} bromperidol levels cannot be explained solely by assay variability. Rather a combination of metabolism, elimination, and possibly concurrent medication may significantly influence the scatter in these single time point determinations.

In conclusion, this report describes an RIA procedure used to assay the bromperidol content of human plasma samples. Predose plasma samples, obtained from schizophrenic patients chronically receiving bromperidol therapy, were assayed using this RIA method. Substantial intra- and intersubject variabilities in the C_{min} drug levels were demonstrated. The bioavailability and pharmacokinetic information of bromperidol in schizophrenic patients will be reported elsewhere.

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Novel Concept for a Mucosal Adhesive Ointment

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Received December 14, 1982, from the *Institut für Pharmazeutische Technologie der Philipps Universität Marburg/Lahn, Ketzerbach 63, 3550 Marburg*, and the [‡]*Hautabteilung des Luisen-Hospitals, Aachen, West Germany.* Accepted for publication March 8, 1983.

Abstract □ Conventional adhesive ointments cause irritation to the mucous membranes. Therefore, a novel mucosal adhesive ointment based partly on neutralized polymethacrylic acid methyl ester was formulated. The flow curves of the ointment vehicle showed pseudoplastic properties. The rheological behavior as well as the adhesion on the mucosal membrane could be varied by the type and concentration of the polymer used and the base used for neutralization. During clinical studies, the ointment vehicle as well as a tretinoin (vitamin A acid) preparation for the treatment of lichen planus did not cause any local irritation or systemic side effects. Both vehicle and preparation were found to be pleasant for the patients to use. The new system of the mucosal adhesive ointment is not limited to the incorporation of tretinoin as the active agent; combined with other drugs the system could be applied to all types of mucosal membranes.

Keyphrases □ Adhesive ointment, mucosal—tretinoin, irritation-free formulation, clinical assessment □ Lichen ruber planus—treatment with tretinoin, irritation-free formulation, mucosal adhesive ointment □ Tretinoin—mucosal adhesive ointment, irritation-free formulation, clinical testing for lichen ruber planus

Ointments employed for the treatment of the mucous membranes of the mouth must be suited to the special conditions pertaining to that particular site of application. The flow of saliva and the mechanical stress generated by the continuous movements of the buccal cavity prevent any long-term adhesion of the ointment subsequent to its application. The quantity

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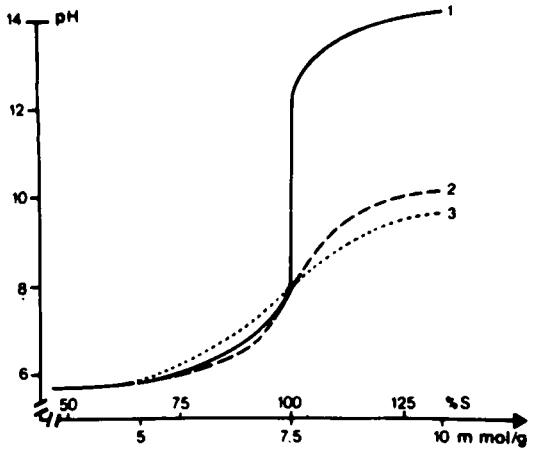

Figure 1—*pH value of polymethyl methacrylate preparations using various bases. Key: (1) NaOH; (2) triethanolamine; (3) diisopropanolamine.*

of saliva produced each day ranges from 0.5 to 1.5 L (1, 2); with suitable stimulation, transient flow rates of up to 10 mL/min have been found (3, 4). On the one hand, therefore, mucosal adhesive ointments should be sufficiently hydrophilic to permit intimate contact with and adhesion to the skin surface; on the other hand, they should include a lipophilic component to prevent the ointment base from being washed away, or at least to delay this process. It is for this reason that such ointments frequently contain a hydrogel constituent suspended in a lipophilic base, which swells and exhibits its adhesive characteristics only after contact with mucous membranes and saliva. Typical ointments that have been developed in this way are a polyacrylate[1]-calcium carbonate–liquid paraffin suspension (5) and a sodium carboxymethylcellulose, pectin, and gelatin combination in a polyethylene–paraffin base[2]. In some cases, highly viscous solutions or hydrogels with no lipophilic admixtures are also used for this purpose, in which case natural and synthetic macromolecular compounds such as tragacanth alginates, pectin, methylcellulose, and polyacrylate[1] are incorporated.

The intended objective, apart from good mucosal adhesion over as long a period as possible, is for the ointment base to be as free from mechanical, physical, and chemical irritant properties as is practical, depending on the indication. Any

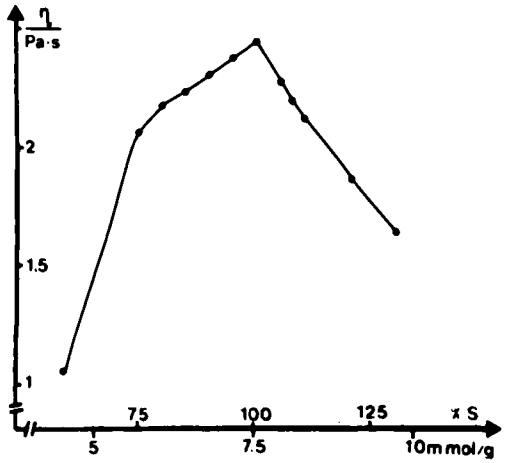

Figure 2—*Viscosity dependence on the stoichiometric saturation of the carboxyl groups (5% polymethyl methacrylate hv neutralized with NaOH; shear rate* $D = 90\ s^{-1}$).

[1] Carbopol; Goodrich, Cleveland Ohio.
[2] Orabase; Squibb Pharmaceuticals, Mooton, England.

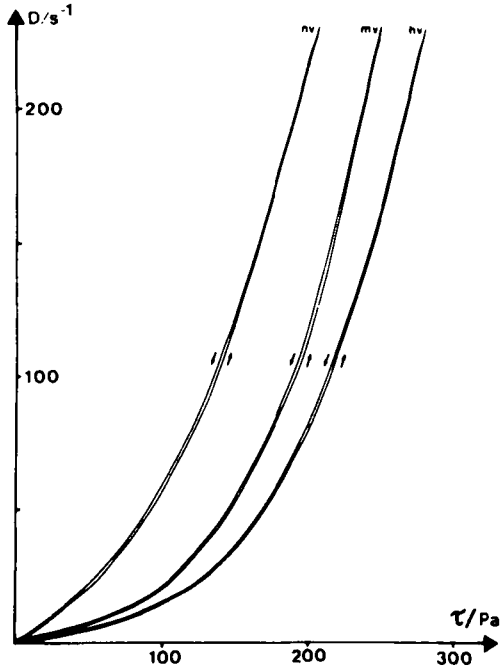

Figure 3—*Flow curves of polymethyl methacrylate-Na (100% S) using 5% polymethyl methacrylate nv, mv, and hv.*

irritation of the oral mucosa should be strictly avoided, as it is extremely sensitive. This whole problem has to be faced, for example, in developing a mucosal adhesive ointment for the treatment of lichen planus mucosae oris with tretinoin (vitamin A acid). Tretinoin, described by Günther (6) in 1972 in the treatment of lichen ruber planus, has been employed with great success by many clinicians. However a number of patients have complained of reddening of the mucosa, pain, burning sensations, and maceration (7–10); the ointment bases were considered to have caused irritation to the sensitized oral mucosa. Therefore, there has been a need to formulate a nonirritating mucosal adhesive ointment containing tretinoin.

Polymethyl methacrylate[3] would seem to be a logical candidate for these tests, as it is a polymer with lipophilic methyl groups and hydrophilic carboxyl and ester groups. Both of these components were mentioned earlier as being essential for a mucosal adhesive ointment. The solubility of this polymer in water and its adhesiveness and viscidity can be varied, within certain limits, by using different degrees of neutralization (11). However, as polymethyl methacrylate has not previously been used as a mucosal adhesive ointment, the drug-free ointment base was first clinically investigated in volunteers. For dermatological reasons, no preservations, surfactants, stabilizers, or taste adjusters were added either to the ointment base or to the final tretinoin preparation, because of their potential irritant and allergenic effects.

EXPERIMENTAL

Production of the Polymethyl Methacrylate Base—Dilute polymethyl methacrylate salt solutions (<10%) were produced from concentrated master batches (≥10%) and, following the neutralization reaction (~1 h), made up to the required end concentration with water. For the master batch, polymethyl methacrylate was steeped for 10 min in 3–4 times its volume of cold water (20°C), and the amount of base required for neutralization was added cold with the remaining water (20°C) with continuous stirring. A 7.5-mmol volume of base/g of polymethyl methacrylate is needed for complete neutralization

[3] Eudispert hv; Röhm Pharma, Darmstadt, West Germany.

Table 1—Tretinoin Grain Size Distribution as a Function of Storage Temperature and Time

Storage Temp.	Particle Size, μm	Storage Time		
		Nil	3 months	6 months
8°C	10	97%	98%	97%
	10-20	2.8%	1.7%	2.7%
	20	0.2%	0.2%	0.3%
25°C	10	98%	97%	98%
	10-20	1.8%	2.7%	1.7%
	20	0.2%	0.3%	0.3%

(Fig. 1). It was possible to accelerate the reaction by heating the mixture to ~ 60°C *in vacuo*. A detailed procedure has been described previously (11). The final stage in preparing the ointment base involved its sterilization for 20 min at 121°C in a steam autoclave.

Production of the Adhesive Ointment Base—The following quantities were used to prepare a 1-kg batch: 50 g of polymethyl methacrylate in 200 g of water and 12 g of NaOH in 238 g of water. The sterilized polymethyl methacrylate ointment base was produced from the above ingredients, into which the following components were incorporated under aseptic conditions: 1 g of tretinoin[4], 200 g of glycerol (85%)[5], and 299 g of water. The tretinoin was micronized with the aid of a high-efficiency mill[6] and a three-roller mill[7]. The ointment was placed into 20-g tubes for the clinical tests.

Flow Characteristics—The flow characteristics of the aqueous polymethyl methacrylate solutions were investigated with a rotational viscometer[8] at 25°C. The number of rpm was changed by 25 s^{-1}. The pH value was measured with a digital pH meter[9].

Tretinoin Determination—The tretinoin content in the finished preparation was determined with a UV-visual spectrophotometer at 351 nm ($E^{1\%}_{1cm}$ = 1500). For this purpose, 1 g of ointment was dissolved in 50 mL of water, brought to 100 mL with isopropyl alcohol, and measured against a blank made from drug-free ointment base, water, and isopropyl alcohol. This complies essentially with the requirement described in the USP XX (12). The particle size of the active agent in the ointment was determined microscopically immediately after manufacture and after 3 and 6 months of storage at room temperature and at 8°C. The drug content was also checked after storage.

Clinical Testing—Clinical testing was carried out in three sections: study

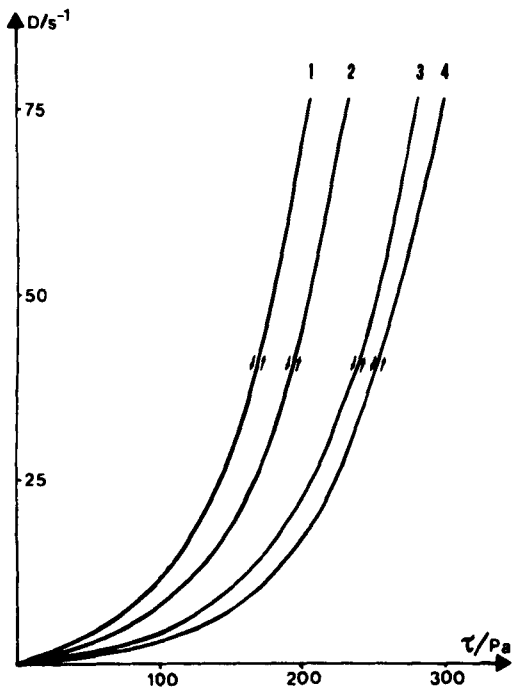

Figure 4—*Flow curves of 5% polymethyl methacrylate hv (100% S) neutralized with $Na_2B_4O_7$ (1), NaOH (2), triethanolamine (3), and diisopropanolamine (4).*

[4] Fa. Hoffmann-La Roche, Basle, Switzerland.
[5] Merck, Darmstadt, West Germany.
[6] Ultra-Turrax TP 18/10: Janke und Kunkel, Stauffen, West Germany.
[7] Kupper, Troisdorf, West Germany.
[8] Model RV 12 with MV-System; Haake, Karlsruhe, West Germany.
[9] Model 532; Metrohm, Herisau, Switzerland.

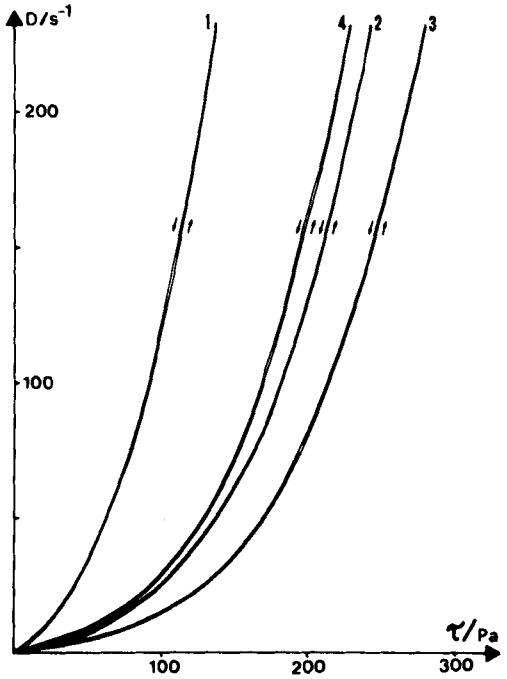

Figure 5—*Flow curves of 5% polymethyl methacrylate hv neutralized with NaOH. Key: (1) 50% S; (2) 75% S; (3) 100% S; (4) 125% S.*

of the ointment base on (A) skin and (B) mucous membranes and (C) application of the tretinoin adhesive ointment.

Section A: Patch Testing of the Polymethyl Methacrylate Ointment Base—A nonallergenic test patch[10] to which the ointment base under investigation had been applied was affixed to the flexor surface of the right forearm of each of 16 subjects, aged 17 to 43 years (10 females and 6 males), with no skin or mucosal disorders. The patch was removed after 24 h. The skin reaction was read after 24, 48, and 72 h and once again after 1 week, in accordance with the procedure of Bandmann and Dohn (13).

Section B: Application of the Polymethyl Methacrylate Ointment Base to the Oral Mucosa—The same ointment base was then tested on the oral mucosa of the same 16 subjects. The test was carried out over a period of 14 d in each case. The subjects were each provided with a 20-g tube of ointment base and were instructed to apply the ointment four times daily to the same area of the oral mucosa. They were reexamined and questioned after 1 and 2 weeks. Fifteen of the subjects completed the study; one dropped out prematurely due to a recurrence of perlèche, which was apparently associated with poorly fitting dentures.

Section C: Application of the Tretinoin Adhesive Ointment to the Oral Mucosa—A total of 18 patients (7 females and 11 males) with lichen ruber planus, confirmed histologically in each case, were treated over a nearly 2-year period. The changes in the mucous membranes had first appeared on the average 20.8 months earlier and had been treated, unsuccessfully, with adhesive ointments and lozenges containing such active ingredients as cortisone, psychopharmacological drugs, external agents with a local anesthetic action, and antimycotics. The condition affecting 17 of the patients was a striate or reticular form of lichen ruber planus, while one patient had lichen planus annularis of the mucosa, which manifested the same kind of changes found on the skin. No erosive or verrucose lesions were observed. While two patients were found to have eruptions on the tongue in addition to buccal changes, only the upper surface of the tongue was affected in two others.

RESULTS AND DISCUSSION

One of the most important criteria in assessing the consistency of ointments is the flow behavior. Among other factors the rheological behavior of aqueous polymethyl methacrylate preparations was influenced by the degree of the neutralization. Strong bases such as sodium hydroxide produced a sharp rise in the pH at the end point corresponding to 7.5 mmol of base/g of the polymer. Weaker bases produced a flatter curve (Fig. 1). The neutralization characteristics of polymethyl methacrylate were similar to those of a weak acid. Maximal viscosity was achieved on the stoichiometric neutralization (100% S) of the carboxyl groups (Fig. 2).

[10] Fin Chambers on Scanpor; Hermal Chemie, Hamburg, West Germany.

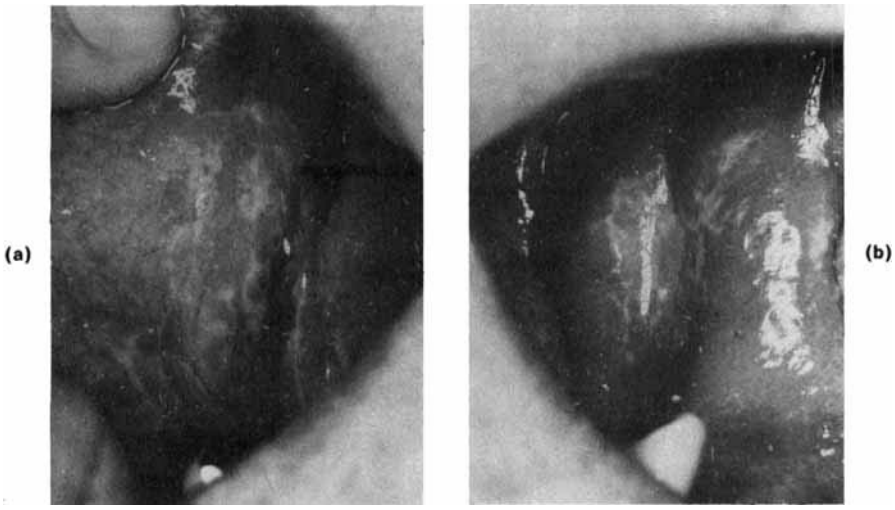

Figure 6—*Buccal mucosa of a patient with lichen planus mucosae oris, right (a) and left (b) sides.*

Admittedly, rheological measurements of polymethyl methacrylate had already been carried out by various researchers (14–16), but these were mainly discontinuous measurements which had lent themselves to widely differing interpretations. To obtain some unequivocal evidence, therefore, it would seem advisable to repeat an investigation of the flow characteristics of the polymethyl methacrylate preparations with a modern rotational viscometer. For this purpose the three standardly marketed types of polymethyl methacrylate (nv, low; mv, medium; hv, high viscidity) were investigated as 5% solutions neutralized with sodium hydroxide. Viscous texture flow curves typical of macromolecular disperse systems were obtained.

The flow characteristics were pseudoplastic; no pronounced thixotropic effect was observed. The highest viscosity was found with polymethyl methacrylate hv (Fig. 3), in line with the molecular weight which, according to the type, varied between 5×10^5 and 2×10^6 (17). In addition to the molecular weight, the solvation status of the polymer coil is also a significant factor in the viscosity of the solution.

The type and concentration of the base (degree of neutralization) can influence the viscosity of the polymer solution. This influence exerted by the base was investigated with sodium tetraborate, sodium hydroxide, triethanolamine, and diisopropanolamine. Relatively small cations, such as sodium ions, afforded relatively low viscosities; sterically expansive cations such as those of the amines, afforded higher viscosities (Fig. 4).

Different concentrations of the base influenced the viscosity of the polymer solution equally (Fig. 5). Fully neutralized carboxyl groups, corresponding to a 100% S (100% saturation) degree of neutralization, resulted in a high viscosity. A probable explanation of this behavior may be the degree of solvation of the polymer. Polymethyl methacrylate as a weak acid is badly dissociated and insoluble in aqueous systems (11). After neutralization of the polymer it can be assumed that nearly all acid groups are dissociated and the polymer is completely solvated, resulting in a viscous solution. Partly neutralized preparations as well as those with an excess of base were less viscous; in the first case, not all the carboxyl groups were completely solvated, and in the second, free cations interfered with complete solvation.

The polymethyl methacrylate preparations became increasingly tacky as the degree of neutralization was reduced, which is a desirable feature for good mucosal adhesion. Since saliva is weakly acidic—the values quoted in the literature vary between pH 5.8 and 7.8 (18–20), with average normal saliva at pH 6.4—only partly neutralized polymethyl methacrylate (80% S) was used for the mucosal adhesive ointment. The resulting ointment was free of irritation due to isohydria and had good mucosal adhesion, due to partial neutralization. The active substance, tretinoin, is stable in the weakly acidic milieu afforded by the ointment base.

Solubilizers and organic solvents such as those in tretinoin preparations for external application (21) should not be used to incorporate the active substances for mucosal ointments. Consequently, the micronized drug could only be suspended in the ointment base. Hence, there is a possibility of sedimentation due to the base lacking any thixotropic effect; in addition, crystal growth is possible and had to be tested for. To that end, the fineness and degree of dispersion of the active substance were determined as a function of storage conditions. The majority of the particles were <10 μm. Larger particles up to 60 μm were observed sporadically. Table I shows that there is no appreciable recrystallization as judged by the grain size distribution as a function of storage time.

If due allowance is made for the variations that occur with the analytical method, it will be seen that the drug content was practically constant over the observation period (Table II). Neither sedimentation nor any loss of active substance occurred during storage. These qualities go far beyond the requirements of the USP XX with regard to tretinoin ointments, which allow contents to vary between 90 and 130% (12). For the clinical tests, the ointment was supplied to the patients in 20-g tubes. This was at most a 2-week supply.

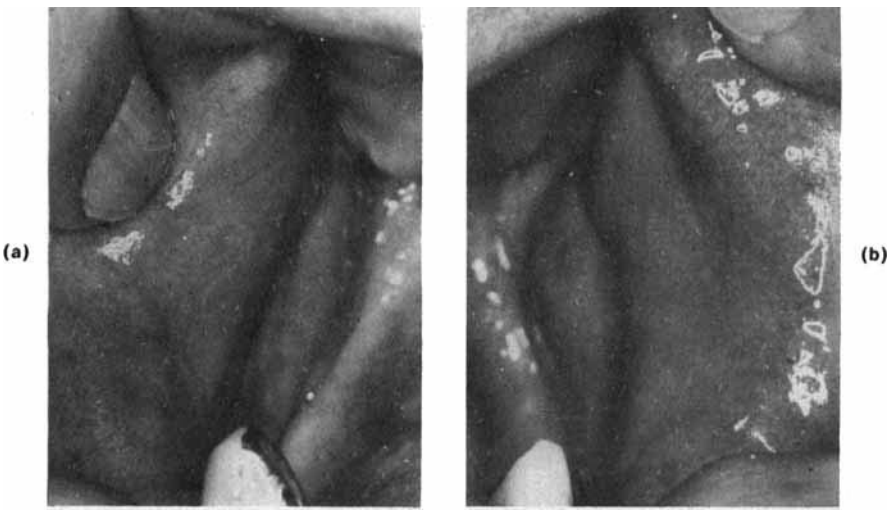

Figure 7—*Buccal mucosa of the same patient after treatment with the tretinoin adhesive ointment, right (a) and left (b) sides.*

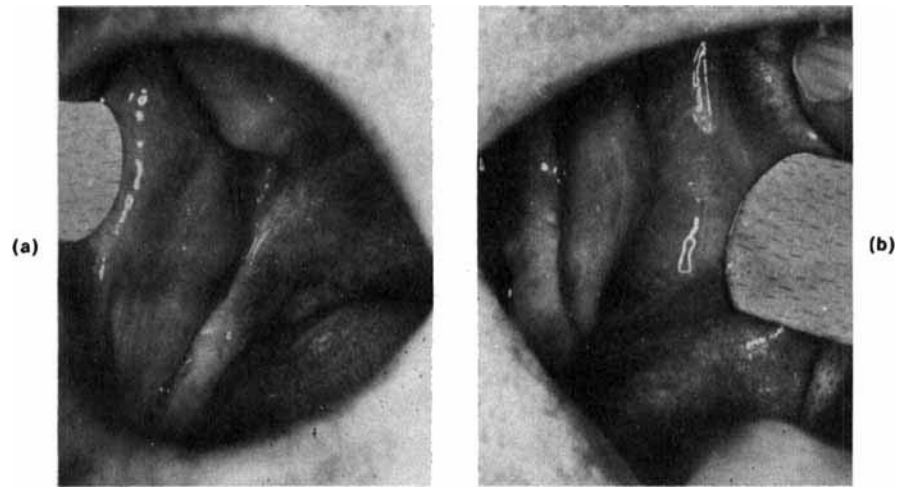

Figure 8—*Buccal mucosa of the same patient 20 months after completing a 3-week course of treatment with the tretinoin adhesive ointment, right (a) and left (b) sides.*

The use of this package size reduced to a low level the risk of any secondary contamination of the ointment.

No positive skin reaction was found in any of the subjects when the patch test was evaluated. No observable objective changes in either the treated or untreated areas were found when the oral mucosa of the subjects remaining in the trial were examined macroscopically with illumination of the buccal cavity. Subjectively, a feeling of "numbness" or of "stickiness" at the site of application lasting some 10–15 min after application of the ointment was reported.

With twice-daily treatment with tretinoin mucosal adhesive ointment, the macroscopic lesions disappeared after an average of 3.12 weeks in 15 of 18 patients (Figs. 6 and 7). This observation was also in accordance with the histological findings in two patients who agreed to an exploratory excision of tissue after completing therapy. To examine the long-term effect, the patients returned for a checkup 11.4 months (on average) after discontinuing therapy; this revealed that none had suffered a relapse (Fig. 8). The two patients who had lesions on the tongue only were among the three subjects in whom therapy was unsuccessful. The tongue lesions of one patient who had buccal changes as well also failed to respond to treatment. However, other authors have also reported that tongue changes in lichen ruber planus exhibit similar resistance to therapy (22). No undesirable side effects of the oral mucosa occurred in any of our cases. The ointment caused no unpleasant sensations and had good adhesive qualities.

As a novel concept, aqueous polymethyl methacrylate preparations can be employed as extremely well-tolerated ointment bases for use in mucous membranes. These ointment bases are characterized by an excellent adhesion. A description of a model for the determination of the adhesive time of mucosal adhesive ointments *in vitro* will be published elsewhere. The preparation of the polymethyl methacrylate ointment base as well as the incorporation of the active agent is easily carried out. The system can be utilized for other drugs, other symptoms, and all mucous membranes, with possible application to oral and vaginal mycoses, herpes labialis, pyoderma, and aphthosis.

Table II—Percentage of Tretinoin in Polymethyl Methacrylate Mucosal Adhesive Ointment as a Function of Storage Temperature and Time

Storage Temp.	Storage Time		
	Nil	3 months	6 months
8°C	0.102%	0.101%	0.100%
25°C	0.101%	0.098%	0.099%

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