tended (Table III, solutions 1 and 2) to 36 days *versus* 7 days as recommended (1). The loss in potency after a 36-day storage was <3.2%, and the samples were clear. The expiration date for the frozen samples may be extended to 60 days (solutions 1 and 2, Table III) *versus* 28 days as recommended (1) by the manufacturer. There was a loss of <2 and 7% in potency in 60 days for solutions in 0.9% NaCl and 5% dextrose, respectively. There was no significant change in pH values, and the solutions were clear. Furthermore, the frozen samples can be thawed in <4 min using a microwave oven, without any observable decomposition.

It is interesting to point out that the manufacturer has recommended (1) an expiration of 24 hr under refrigeration for solutions in 5% fructose and 10% dextrose *versus* 7 days for solution in 5% dextrose. In our investigations (Table III, solutions 3–5), there was no difference in the stability of these three solutions. No decomposition was found in any sample for up to 4 days under refrigeration, which was also evident from the absence of any additional peak(s) in the chromatograms. After 14 days of storage there was only slightly more decomposition of drug in solutions containing either 10% dextrose or 5% fructose *versus* 5% dextrose (Table III, solutions 3–5). There were no significant changes in pH values, and all solutions were clear.

The optimum pH of stability (Fig. 2) appears to be ~4.8. The phosphate buffer did not catalyze the reaction (solutions 8 and 9, Table III). Trials to treat the data mathematically using first-order equations were not successful, which may be due to the complexity of the reaction as evidenced by a number of new peaks (Fig. 1C) in the chromatogram. The general information about the degradation of penicillins is available in the literature (2, 3).

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COMMUNICATIONS

Buccal Absorption of Protirelin: An Effective Way to Stimulate Thyrotropin and Prolactin

Keyphrases □ Protirelin—buccal absorption, stimulation of thyrotropin and prolactin, use as a diagnostic tool □ Buccal absorption—evaluation of protirelin, use as a diagnostic tool, measurement of thyrotropin and prolactin stimulation □ Thyrotropin and prolactin stimulation—buccal absorption of protirelin, use as a diagnostic tool

To the Editor:

Most of the biologically active oligopeptides are almost, or completely, inactive if administered perorally. This can be partly attributed to low chemical stability in the course of intestinal passage, and partly to low invasion rates along with rapid plasma degradation. For the same reasons some peptides, like thyrotropin-releasing hormone (TRH) (protirelin), need extremely high peroral doses in order to stimulate biological response. Therefore, intravenous injection is the most common form of peptide administration. However, few studies on nasal (1) and rectal (2) administration have been reported. The purpose of this investigation was to evaluate buccal absorption of protirelin as a model peptide. The major objective was to set the groundwork for future research on buccal peptide delivery and absorption. This study also evaluated buccal protirelin as a diagnostic tool.

Thyroid gland diagnostics by protirelin is mainly a domain of the intravenous test, although some doubts have emerged due to serious side effects (3–5). On the other hand, the peroral protirelin test exhibits only slow response at high doses, and usually needs a 3-hr period to attain maximum stimulation. This is often considered inconvenient for routine clinical diagnostics. Therefore, buccal protirelin could become an appropriate supplement with both intravenous and peroral protirelin, if absorbed properly. This study will show the overall feasibility of buccal protirelin for use in thyroid diagnostics. Buccal absorption will be followed by monitoring thyrotropin and prolactin stimulation.

Ten clinically healthy volunteers, five males and five females, took part in the study. The age range was 23–35 years. For all volunteers an euthyroid state was certified by determinations of triiodothyronine, thyroxine, and thyroxine-binding globulin. The body weights of all volunteers were within normal limits of the ideal body weight according to Broca. All tests were performed beginning at 2 p.m.

For the intravenous test, 200 μg of protirelin[1] was injected in the antecubital vein. Blood samples were taken immediately before and 30 min after injection. For buccal application a polytef disk was prepared with a diameter of ~3.5 cm, corresponding to an area of ~10 cm^2 and a height of 1 cm. The disk had a central circular depression depth of 4 mm, leaving an elevated rim. A previously water-soaked filter paper disk was placed into the depression, and 20 mg of crystalline protirelin[2] was spread onto the filter paper. The protirelin dissolved immediately. Subsequently, the device was put into contact with the buccal mucosa. After 30 min the device was removed, and the mouth was thoroughly washed with tap water. Blood samples were taken at 0, 30, 60, 120, and 180 min *via* a cannula placed into the antecubital vein.

After centrifugation the plasma was separated from the blood and stored at −20° until analysis of thyrotropin and prolactin.

Measurements of thyrotropin[3], prolactin[4], thyroxine-binding globulin[5], triiodothyronine[6], and thyroxine[6] were performed in duplicate using commercially available radioimmunoassays. Standard errors of the radioimmunoassays were from 6 to 8% within kits and from 3 to 9% between kits. A second, but slightly modified, test was per-

[1] Antepan, Henning, D-Berlin.
[2] Hoechst, D-Frankfurt.
[3] TSHK-PR, CIS-CEA-Sorin, I-Saluggia.
[4] PROLK-PR, CIS-CEA-Sorin, I-Saluggia.
[5] RIA-gnost TBG-kit, Behringwerke, D-Marburg.
[6] ARIA II, Becton and Dickinson, Paramus N.J.

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Table I—Baseline and Stimulation Levels of Thyrotropin after Buccal Application of Protirelin

Subject[a]	Intravenous Test[b] (min)		Buccal Application[c] (min)					
	0	30	0	15	30	60	120	180
A	1.8	8.9	1.6	—	1.8	4.9	4.1	3.0
A[d]	—	—	1.4	2.1	2.9	5.7	5.4	3.4
B	1.8	7.9	1.7	—	3.3	4.2	4.8	6.8
B[d]	—	—	1.5	1.7	3.0	3.8	2.5	2.7
C	1.8	9.5	1.6	—	4.4	5.5	9.7	12.8
D	1.0	4.0	1.4	—	2.4	3.2	2.4	2.6
E	1.4	4.9	1.2	—	2.5	3.4	2.9	4.9
F	1.9	8.7	1.6	—	4.3	6.2	5.6	4.9
G	4.3	25.1	5.0	—	10.8	17.5	28.0	29.4
H	1.0	4.7	1.1	—	2.8	10.9	5.7	7.2
I	1.7	5.9	0.9	—	5.6	5.0	6.2	7.9
J	3.4	20.3	2.7	—	3.0	6.1	5.0	4.4

[a] A–E = male; F–J = female. [b] Intravenous dose = 0.2 mg of protirelin; plasma levels in μU/ml. [c] Buccal dose = 20 mg of protirelin; plasma levels in μU/ml. [d] Extra test under removal of excess saliva.

Table II—Baseline and Stimulation Levels of Prolactin after Buccal Application of Protirelin

Subject[a]	Intravenous Test[b] (min)		Buccal Application[c] (min)					
	0	30	0	15	30	60	120	180
A	7.6	27.0	7.0	—	13.0	24.4	11.9	10.5
A[d]	—	—	7.1	8.1	14.3	25.3	16.9	10.3
B	10.1	36.0	9.3	—	31.2	22.7	16.5	13.6
B[d]	—	—	6.4	9.5	14.3	13.4	10.1	8.4
C	<3.0	7.6	<3.0	—	<3.0	<3.0	3.6	<3.0
D	3.0	24.5	5.2	—	11.7	14.9	9.8	7.4
E	5.7	23.5	7.3	—	15.8	13.1	7.2	13.1
F	18.2	44.0	5.9	—	26.0	24.6	14.0	12.3
G	11.6	44.0	14.8	—	35.7	31.4	27.6	31.8
H	7.4	17.6	8.4	—	12.3	26.4	10.2	8.3
I	7.8	30.8	6.2	—	31.3	18.1	21.4	12.8
J	38[e]	90[e]	10.5	—	19.7	33.3	16.5	11.0

[a] A–E = male; F–J = female. [b] Intravenous dose = 0.2 mg of protirelin; plasma levels in ng/ml. [c] Buccal dose = 20 mg of protirelin; plasma levels in ng/ml. [d] Extra test under removal of excess saliva. [e] Increased prolactin levels due to metoclopramide medication previous to intravenous test; data excluded from further evaluation; no metoclopramide medication previous to buccal application.

formed with two of the subjects. In addition to the aforementioned procedure, excess saliva was constantly withdrawn by aspiration to prevent undesirable GI absorption of swallowed protirelin, which might stimulate thyrotropin and prolactin after intestinal absorption. Because plasma thyrotropin and prolactin levels of the subject could not be considered normally distributed, the Wilcoxin-Mann-Whitney *U*-test was used for statistical evaluation (6).

The results of the study in terms of the baseline levels and the levels after stimulation by intravenous and buccal application of protirelin are shown in Table I for thyrotropin, and Table II for prolactin. After intravenous application of protirelin, the thyrotropin concentrations increased from a baseline range of 1.0–4.3 μU/ml to a 30-min stimulation range of 4.0–25.1 μU/ml. After buccal application there was a rise from the baseline range of 0.9–5 μU/ml to a 30-min range of 1.8–10.8 μU/ml and to a 60-min range of 3.2–17.5 μU/ml. The subsequent part of the plasma profiles did not show a general tendency among subjects: in some subjects there was a further increase of thyrotropin, whereas in others there was a drop-off. (A graph of the profile is shown in Fig. 1.) The increase from the initial values to the 30-min and the 60-min values is significant, as evidenced by the *U*-test ($p = 0.01$). With respect to prolactin, the intravenous test gave rise to levels in the range of 7.6–44.0 ng/ml after 30 min, with the baseline between 3.0–18.2 ng/ml. In the case of buccal application, the baseline range was 5.2–14.8 ng/ml, whereas upon stimulation prolactin increased to 11.7–35.7 ng/ml after 30 min, and to 13.1–31.4 ng/ml after 60 min. One subject was omitted from further evaluation because several plasma levels were below the limit of analytical sensitivity at 3.0 ng/ml of prolactin. In the subsequent part of the prolactin plasma profiles there was a decrease in levels in all but one subject (see Fig. 1). Like thyrotropin, the increase of prolactin above its baseline level after buccal protirelin application is statistically significant ($p = 0.01$) for the 30-min range, as well as for the 60-min range.

An additional set of data on two subjects showed that an increase of thyrotropin and prolactin levels also could be achieved when the buccal application of protirelin is accompanied by a constant withdrawal of excess saliva from the oral cavity. The values are presented in Table I; for graphical depiction see Fig. 2. It is also interesting to note that following the intravenous test all subjects reported side effects such as nausea, urge to urinate, and facial flushing, but no side effects were observed after buccal protirelin administration.

The conclusion drawn from the presented data is that protirelin is readily absorbed *via* the buccal mucosa, as indicated by a significant stimulation of both thyrotropin and prolactin. The response pattern observed seems to indicate that females exhibit a greater stimulation by protirelin than males do and that peak levels appear more rapidly with prolactin than with thyrotropin. This parallels the typical intravenous stimulation characteristics (3). Compared with the peroral application of protirelin, buccal absorption leads to earlier attainment of maximum thyrotropin and prolactin levels, at half the dose usually taken for the peroral test. Apparently, there is no difference in the maximum increments achieved by both routes of administration, as indicated by comparison with literature data on peroral protirelin stimulation (7).

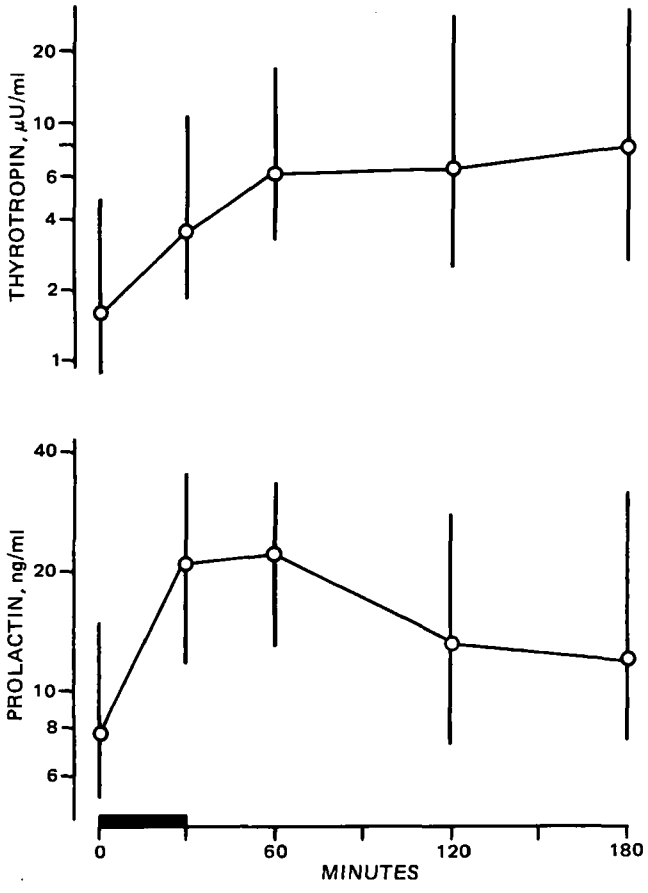

Figure 1—*Plasma thyrotropin and prolactin profiles after buccal application of 20 mg of protirelin. Circles are geometric means of 10 subjects (thyrotropin) and nine subjects (prolactin), respectively. Vertical bars indicate observed range. Horizontal bar indicates time of application.*

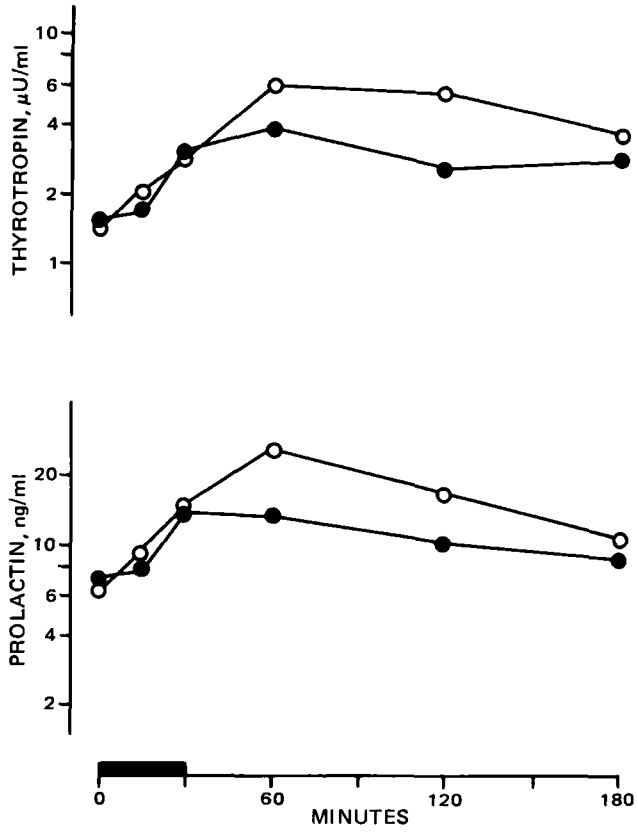

Figure 2—*Plasma thyrotropin and prolactin profiles after buccal application of protirelin under constant withdrawal of excess saliva. Filled and open circles indicate two subjects. Horizontal bar indicates time of application.*

These results also reveal that buccal protirelin administration is a clear alternative to both the peroral and the intravenous tests. As compared with the intravenous test, the obvious advantage of buccal protirelin is the fact that none of the aforementioned side effects were observed. This appears to be due to the lower incremental increase of plasma protirelin and, therefore, to the more moderate stimulation kinetics after buccal administration. In addition, buccal protirelin with a maximum stimulation 30 to 60 min after application, provokes a faster response than the peroral test, which usually requires a period of 2–3 hr to reach its peak (3, 7). We therefore suggest this test be evaluated for diagnostic use. Suitable dosage forms are presently under investigation.

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R. Anders
H. P. Merkle ˣ

Institute of Pharmaceutical Technology and Biopharmaceutics

W. Schurr
R. Ziegler

Department of Internal Medicine (Endocrinology)
University of Heidelberg
D-6900 Heidelberg
Federal Republic Germany

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Practical Solution to the Michaelis–Menten Equation

Keyphrases □ Michaelis–Menten equation—mathematical solution

To the Editor:

The mathematical solution of the Michaelis–Menten equation apparently only exists in implicit form. The explicit form required in practical usage is obtained by numerical means either using numerical integration or by solving the implicit form using a root-solving algorithm. To avoid this numerical complexity methods have been proposed which are based on a numerical solution in-

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