Noninvasive Assessments of the Percutaneous Absorption of Methyl Nicotinate in Humans

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Abstract □ Percutaneous penetration of the vasodilator methyl nicotinate (methyl 3-pyridinecarboxylate) has been monitored *in vivo* in humans with the noninvasive techniques of laser Doppler velocimetry and photopulse plethysmography. These optical methods use different technologies to generate a voltage output which is related to perfusion of the cutaneous microcirculation. The procedures are therefore sensitive to the pharmacologic stimulus and duration of local vasodilation. Following topical application of methyl nicotinate, excellent correlation was found between the response of both methods and the visual observation of erythema. Lower drug concentrations delayed the onset and magnitude of the response and shortened the time period for which elevated microperfusion was observed. These techniques appear to provide a useful noninvasive assessment of the time course of drug behavior in the region of skin to which topical application is made.

Keyphrases □ Methyl nicotinate—noninvasive assessments of percutaneous absorption, laser Doppler velocimetry, photopulse plethysmography, pharmacokinetics □ Absorption, percutaneous—of methyl nicotinate, noninvasive assessments by laser Doppler velocimetry and photopulse plethysmography, pharmacokinetics □ Pharmacokinetics—of percutaneous absorption of methyl nicotinate in humans, noninvasive assessments by laser Doppler velocimetry and photopulse plethysmography

Presently, there exists limited understanding of the kinetics associated with drug absorption into the skin, retention or metabolism of compounds within the tissue, and subsequent elimination into the systemic circulation. Such knowledge, however, is valuable both for devising rational dermatological chemotherapy and for topical dosage form design and optimization. In this paper two noninvasive procedures for monitoring blood perfusion through the cutaneous microcirculation are discussed, and methodology is suggested that may improve pharmacokinetic and pharmacologic measurement of percutaneous absorption.

The techniques employed are laser Doppler velocimetry (LDV) and photopulse plethysmography (PPG). The methodologies employ different optical principles to generate an output related to either velocity (LDV) or amount (PPG) of cutaneous blood vessel perfusion. In 1975, it was shown that LDV could indicate changes in microvascular perfusion (1). Validation and development of the method for clinical evaluation of local blood flow in the skin surface (2–4) was followed by its application to assess perfusion changes in response to injection trauma (5) and its employment as an indicator of skin blood flow (as compared with heater power) during transcutaneous oxygen monitoring (6). A recent review (7) has summarized the current technological and theoretical status of the procedure and has discussed possible clinical applications.

The PPG technique has been used for many years to assess skin blood flow (8), alone and in conjunction with other procedures, to assess various physiological functions. Although there are examples in the literature which have indicated the ability of PPG to demonstrate drug-induced changes in skin blood flow (9–12), there has been no attempt to apply the method specifically to follow the passage of drug into the body following topical administration.

To characterize and test the two techniques, the percutaneous absorption of methyl nicotinate in humans has been considered. This nicotinic acid ester crosses the skin rapidly and elicits a distinctive erythema (13), the time of onset of which is reportedly a function of drug concentration (14). After its appearance, erythema intensity first increases to a maximum, and the area of redness expands, before gradually fading away and becoming no longer visually detectable (15, 16).

EXPERIMENTAL

LDV experiments were performed with a recently developed capillary perfusion monitor[1]. The method uses the Doppler principle. Light at 632.8 nm from a 5-mW helium–neon laser is transmitted to the skin through a quartz optical fiber. The light is backscattered from stationary skin components and by erythrocytes moving in the dermal capillaries[2], which are encountered as the radiation penetrates to a depth of 1–1.5 mm. A second optical fiber collects the reflected light and the electronic configuration of the instrument then separates out the frequency-shifted (*i.e.*, Doppler) component and converts it to a single flow parameter, which is registered as a voltage output. That this quantity is related to peripheral cutaneous perfusion, as measured by other means, has been shown (4). The optical fibers supplying and detecting the radiation are supported in a small, essentially cylindrical probe (1.9-cm diameter, 0.5-cm height) which is held securely to the skin surface by double-sided adhesive tape.

Experiments with PPG utilized a photoplethysmograph[3] in conjunction with two photopulse probes[4] and a dual-channel recorder[5]. As with LDV, information is collected by a small (2 × 1 × 0.7-cm) probe attached with tape to the skin surface. In this case, however, the optical source is a diode (LED) emitting IR radiation at 800–940 nm. A phototransistor positioned in the probe beside the LED detects the reflected part of the incident source, and the photoplethysmograph then converts this information for display as a fluctuating voltage on the chart recorder. The frequency of the input radiation covers wavelengths strongly absorbed by hemoglobin. It follows that changes in blood volume in the region of skin under the probe cause the PPG output to change because the percentage of incident radiation absorbed is altered. The penetration depth of the light from the PPG probe is stated to be similar to that of the LDV apparatus, 1–2 mm. Whereas the recording displayed by the LDV unit can be damped so that output oscillations due to the heartbeat can be eliminated, this is not the case with the PPG instrument. The results from the latter appear as a series of pulses on which, at high sensitivities, the dicrotic notch may be observed (Fig. 1).

Percutaneous penetration experiments were carried out on two healthy male volunteers. Methyl nicotinate[6] (I) solutions of 150, 15, and 1.5 m*M* were prepared in distilled water and used directly. The application procedure was as follows: A 1-cm diameter standard test patch[7] was saturated

[1] Medpacific LD5000, Medpacific Corp., Seattle, Wash.
[2] Medpacific, technical information.
[3] Medasonics PPG-13, Medasonics, Mountain View, Calif.
[4] Medasonics PH77, Medasonics, Mountain View, Calif.
[5] Medasonics R12A, Medasonics, Mountain View, Calif.
[6] Sigma Chemical Co., St. Louis, Mo.
[7] Al-test, Imeco-ab, Södertälje, Sweden.

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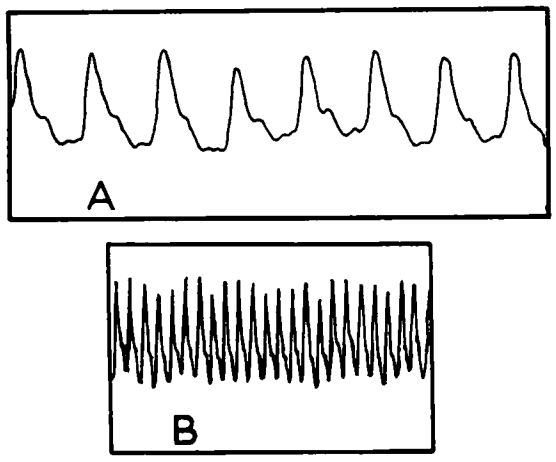

Figure 1—*Typical pulsatile output from the PPG apparatus (probe attached to the forefinger). The dicrotic notch is visible at both (A) fast (25 mm/sec) and (B) slow (5 mm/sec) chart recorder speeds.*

with the appropriate solution of I and applied with tweezers to the midpoint of the flexor aspect of the subject's forearm. The patch was in contact with the skin for 15 sec before being removed; any excess solution left on the skin was wiped away with a tissue.

The LDV or PPG probe was then placed directly over the drug application site (the center of the probe being coincident with the position occupied by the center of the patch) and recordings were made from this point. For LDV measurements, continuous readings were taken throughout the duration of an experiment (~1 hr). For PPG, data collection was continual for the first 10–15 min and thereafter, 30-sec recordings were made every 3–5 min for the remainder of the run. The PPG procedure is much more sensitive to subject movement than LDV and it was found that periodic measurement as described provided both volunteer comfort and measurement clarity.

With the PPG technique, for which there were two probes available, the second probe was positioned on the identical site on the other arm to which no drug was applied. This probe acted as a control to indicate whether any changes in the monitored outputs were induced by the apparatus alone in contact with the skin. On no occasion was there any change in output from the control site during the course of a normal experiment (of ~1-hr duration). Finally, in a separate set of experiments, the same drug application procedure was followed and the onset of erythema determined visually with no probe on the skin. Erythema onset was defined as the appearance of redness at the skin position to which the patch had been applied (14).

RESULTS AND DISCUSSION

The results are presented in Table I. Figure 2 shows a typical initial response to the drug observed with LDV, and in Fig. 3 the pattern of microperfusion changes detected by PPG during an experiment is summarized. Good correlation between erythema onset and instrument response was found for both techniques, and the expected concentration dependence of drug effect (*i.e.*, increased concentration, faster onset, and longer duration) was confirmed. Furthermore, there is potential to begin to quantify in an objective fashion both the initial response following

Table I—Summary of LDV and PPG Experimental Results [a]

Methyl Nicotinate Concentration, m*M*	Onset of LDV Response, sec	Onset of PPG Response, sec	Observed Onset of Erythema, sec	Decay Time of Response[b], min
Subject 1				
150	120	125	125	40–60
15	160	180	165	20–35
1.5	250	270	260	15–25
Subject 2				
150	135	150	180	40–60
15	300	325	370	30–50
1.5	615	635	645	20–35

[a] All data represent the average of duplicate or triplicate experiments (±10%). [b] Approximate time ranges covering the postapplication period between the start of the response decay and the return to baseline LDV or PPG output.

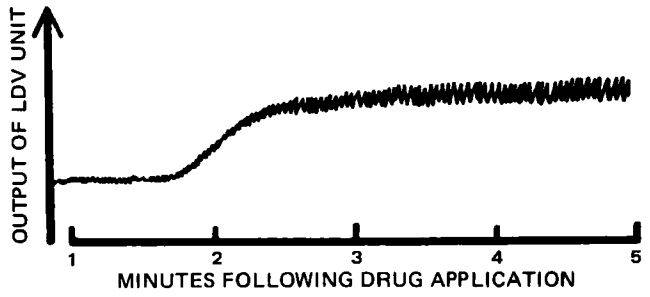

Figure 2—*Early time portion of a characteristic LDV response curve (Subject 1; applied concentration of methyl nicotinate = 150 m*M*).*

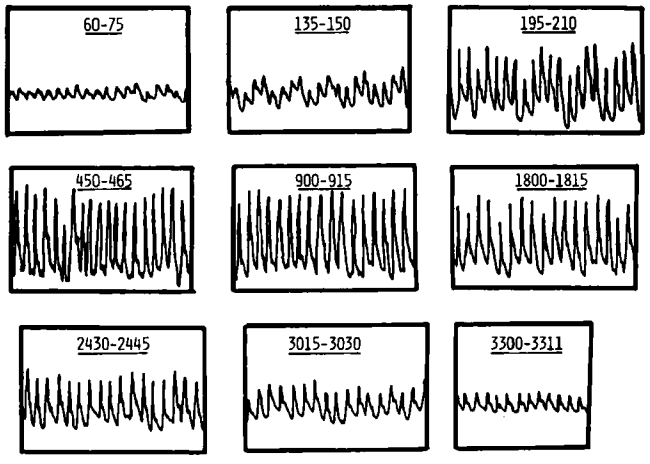

Figure 3—*PPG recording at various times (in seconds) after a 15-sec application of 150 m*M *methyl nicotinate to Subject 1.*

absorption (*e.g.*, Fig. 2) and the subsequent increase and eventual decay (Fig. 3) of the microcirculation stimulus. Certainly for the nicotinic acid esters, it is now possible to follow the latter part of the local time course of the drug.

An additional feature of the data is the demonstration that, within minutes, sufficient drug is able to penetrate and produce the threshold concentration necessary to elicit an observable pharmacological effect. Because of the design of these experiments, steady-state transport across the stratum corneum cannot be established, and conventional *in vivo–in vitro* penetration assessment parameters (*e.g.*, steady-state permeability coefficient and lag time) are not appropriate for describing the data. It is interesting, however, to note that classically measured lag times are often on the order of hours and always in significant excess of 2–10 min. It seems clear that the lag period between application and physiological response for methyl nicotinate must be quite different from the lag time necessary to establish a quasi-steady-state concentration profile across the barrier.

At present, it appears reasonable to suggest that the data reported here strongly hint that local cutaneous pharmacokinetics may prove accessible to study *in vivo* and that the noninvasive procedures described warrant further attention.

REFERENCES

(1) M. D. Stern, *Nature (London)*, **254**, 56 (1975).

(2) G. A. Holloway, Jr. and D. W. Watkins, *J. Invest. Dermatol.*, **69**, 306 (1977).

(3) D. W. Watkins and G. A. Holloway, Jr., *IEEE Trans. Biomed. Eng.*, **BME-25**, 28 (1978).

(4) M. D. Stern, D. L. Lappe, P. D. Bowen, J. E. Chimoskey, G. A. Holloway, Jr., H. R. Keiser, and R. L. Bowman, *Am. J. Physiol.*, **232**, H441 (1977).

(5) G. A. Holloway, Jr., *J. Invest. Dermatol.*, **74**, 1 (1980).

(6) L. Enkema, G. A. Holloway, Jr., D. W. Paraino, D. Harry, G. L. Zick, and M. A. Kenny, *Clin. Chem.*, **27**, 391 (1981).

(7) R. F. Bonner, T. R. Chen, P. D. Bowen, and R. L. Bowman, in "Scattering Techniques Applied to Supramolecular and Non-Equilibrium Systems," (NATO ASI Series B, Vol. 73), S. H. Chen, B. Chu, and R. Nossal, Eds., Plenum, New York, N.Y., 1981, pp. 685–702.

(8) A. V. J. Challoner, in "Non-Invasive Physiological Measurements, Vol. I," P. Rolfe, Ed., Academic, New York, N.Y., 1979, pp. 125–151.
(9) A. B. Hertzmann and W. C. Randall, *J. Appl. Physiol.*, **1**, 234 (1948).
(10) E. G. Cummings, *J. Invest. Dermatol.*, **53**, 64 (1969).
(11) P. Thune, *Acta Derm. Venerol.*, **51**, 261 (1971).
(12) C. Ramsay, *Br. J. Dermatol.*, **81**, 37 (1969).
(13) R. B. Stoughton, W. E. Clendenning, and D. Kruse, *J. Invest. Dermatol.*, **35**, 337 (1960).
(14) W. J. Albery and J. Hadgraft, *J. Pharm. Pharmacol.*, **31**, 140 (1979).
(15) R. B. Fountain, B. S. Baker, J. W. Hadgraft, and I. Sarkany, *Br. J. Dermatol.*, **81**, 202 (1969).
(16) R. H. Guy and H. I. Maibach, *Arch. Dermatol. Res.*, **273**, 91 (1982).

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Stabilizing Effect of Fructose on Aqueous Solutions of Hydrocortisone

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Received July 1, 1982 from the *Department of Allied Health and Industrial Sciences, College of Pharmacy and Allied Health Professions and the School of Chemistry, St. John's University, Jamaica, NY 11439.* Accepted for publication August 26, 1982.

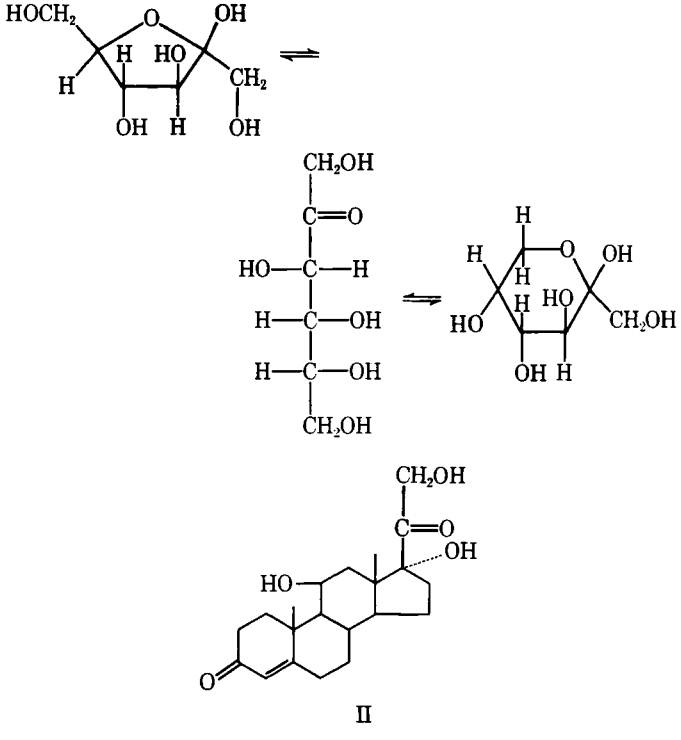
Abstract □ Accelerated stability studies (37°, 47°, and 57°) were conducted on buffered aqueous solutions (pH 7.4, 8.4, and 9.4) of hydrocortisone in the presence of various molar ratios of D-fructose. First-order degradation was observed. Significant improvement in hydrocortisone stability was seen in those solutions containing a 25 *M* excess of D-fructose. Hydrocortisone solutions containing dextrose, lactose, sucrose, sorbitol, propylene glycol, or glycerin in the same molar ratio were not stabilized.

Keyphrases □ Hydrocortisone—stability in aqueous buffers, stabilization by D-fructose □ Stability studies—hydrocortisone, stability in aqueous buffers, stabilization by D-fructose

A ketol side chain is present at the 17-position in the following therapeutically important glucocorticoids: cortisone, hydrocortisone, dexamethasone, prednisone, prednisolone, and 6-α-methylprednisolone. Removal of the ketol side chain results in a significant loss of therapeutic activity.

The reactivity of the ketol side chain of the adrenocorticosteroids is well documented. Base-catalyzed rearrangements and eliminations have been shown to occur under both aerobic and anaerobic conditions (1–5). Guttman and Meister (6) showed that at least three parallel first-order reactions were involved in the base-catalyzed degradation of prednisolone. Neutral and acidic degradation products were obtained. Caspi *et al.* (7) studied oxidative cleavage of the ketol side chain of cortisone with lead tetraacetate. Lewbart and Mattox (8) reported that trace amounts of copper in actinic glassware caused destruction of cortisone and related steroids.

Mauger *et al.* (9) followed the degradation of 21-hydrocortisone hemisuccinate at 70° in aqueous solutions buffered at pH 6.9, 7.2, and 7.6. The data obtained indicated that the overall kinetic pathway at each pH value could be interpreted as consecutive first-order reactions. The blue tetrazolium assay confirmed that the production of a species devoid of the ketol side chain at the 17-position occurred after the steroid alcohol was formed. The ketol group imparts reducing properties to the glucocorticoid molecules similar to those of fructose (Scheme I), which contains a similar ketol group. It was speculated that the presence of excess fructose in aqueous solutions of hydrocortisone (II) might retard the base-catalyzed degradation of the latter. The significance of the ketol group would be checked by using other polyols lacking this group.



II

EXPERIMENTAL

Materials—The following were obtained from commercial sources: hydrocortisone, USP (lot 15C-6126)[1], blue tetrazolium[1], tetramethylammonium hydroxide[1], D-fructose[2], dextrose USP[3], glacial acetic acid

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Pfaltz and Bauer, Inc., Flushing, N.Y.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.

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