Investigation of Some Factors Influencing Percutaneous Absorption III

Absorption of Methyl Ethyl Ketone

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The percutaneous absorption of methyl ethyl ketone (MEK) was followed for a period of 8 hr. under normal, dehydrated, and hydrated skin conditions. Data are presented showing a delay in absorption when the skin is pretreated with a strong desiccant; however, the final steady-state concentration of MEK in expired air was of the same magnitude as that obtained under normal skin conditions. Hydration, as noted previously, continued to enhance absorption. Apparent first-order expired air elimination rates following percutaneous absorption were determined and compared to those obtained after oral ingestion of MEK.

ALTERING environmental skin conditions has been shown to be an effective method of influencing the percutaneous absorption of different chemical agents (1–8). In a preliminary phase (9) of the current investigation, using methyl ethyl ketone (MEK) as the test penetrant, it was observed that a marked increase in absorption occurred under hydrous skin conditions. Shelmire (10) also stated that hydration of the stratum corneum is one of the most important factors in the penetration by a medicament. Fritsch and Stoughton (11), using excised skin, showed the effects of both temperature and humidity on the penetration of acetylsalicylic acid containing ^{14}C. These observations and others have led to the use of occlusive plastic films for the purpose of increasing the state of hydration of the stratum corneum in both therapeutic and investigational procedures (12–15).

In designing the current investigation the assumption was made that percutaneous absorption occurs *via* a diffusional process, with the rate-limiting step being the transfer of the penetrant across the skin. This approach was based in part on previous work (1) supporting this concept. The test penetrant, MEK, was selected for this study because it is a liquid with a simple chemical structure, possesses a high vapor pressure so that it may be easily detected in the expired air by vapor phase chromatography, and is nontoxic in the amounts absorbed, as was indicated previously (9). By maintaining an excess of the penetrant on a constant skin surface area, a steady state was achieved, with the rate of absorption and the rate of elimination of the penetrant being equal. Under these conditions absorption was studied using hydrous, anhydrous, and normal skin. Due to the differences existing between the skin of animals and that of humans, the current study was again limited to humans.

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EXPERIMENTAL

Absorption Cell, Sample Collection, and Analysis.—The absorption cell and the manner in which it was affixed to the forearm of the test subject have been previously described (9). Similarly, the collection and analysis of expired air samples were accomplished according to the method used in the preliminary investigation, except for the following minor modifications. Instead of discarding the first 1.5 L. of expired air, the entire volume of air expired by the test subject was collected. This procedure was immediately repeated so that the components from approximately 9 L. of expired air were collected for analysis at each time period. Modifications in the gas chromatography method of analysis pertained only to the column employed. Better separation of the volatile components of the expired air was obtained using a column prepared according to the technique described by Ikeda *et al.* (16). With the exception of the first 6 in. nearest the detector, the column was packed with 15% diethylene glycol adipate on 70–80 mesh Anakrom ABS which served as the supporting material. The first 6 in. consisted of 1% boric acid along with the already coated packing material. This was done by mixing powdered boric acid with the coated packing material in petroleum ether in a rotating vacuum evaporator. The column was conditioned at 200° for 8–12 hr. Upon heating, the boric acid loses water to form metaborate, pyroboric acid, or the anhydride (16). Both ethanol and methanol were then retained on the column, and excellent chromatographic separation of the methyl ethyl ketone was obtained. Samples could only be injected once every 30 min., and this was a slight disadvantage since samples had to be kept frozen until just prior to analysis. Although the test chemical was eluted in 3.5 min., it took about 30

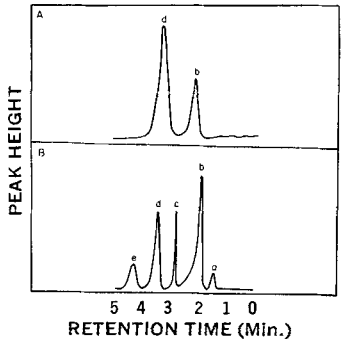

Fig. 1.—Chromatograms of 5.0-μl. sample of the condensate from expired air. Key: A, column composed of diethylene glycol adipate and boric acid; B, *N,N,N¹,N¹*-tetramethyl azelamide, heavy alcohol column. Peak a, acetaldehyde; b, acetone; c, methanol; d, MEK; e, ethanol.

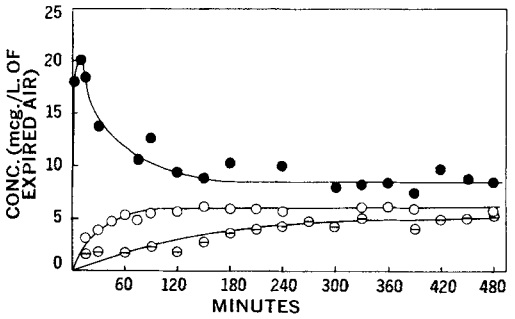

Fig. 2.—Individual expired air elimination data showing the influence of hydration and dehydration on the percutaneous absorption of MEK. Key: ●, hydrated system; ○, normal system; ⊖, dehydrated system.

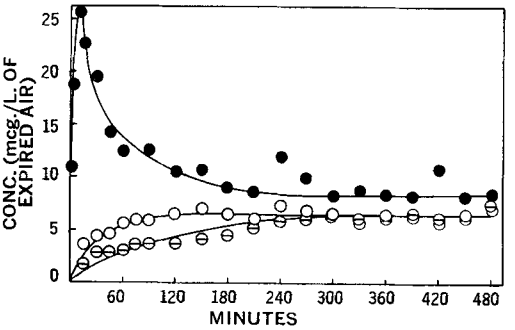

Fig. 3.—Average expired air elimination data showing the influence of hydration and dehydration on the percutaneous absorption of MEK. Key: ●, hydrated system; ○, normal system, ⊖, dehydrated system.

min. for the recorder to return to the baseline as two unidentified substances were eluted at about 9 min. and exhibited considerable tailing. These were not the primary alcohols, methanol and ethanol, as they would only begin to emerge after the column had been in use for 3–4 months. At this time a new column was prepared, and separation was duplicated with only a slight variation of retention times under the same operating parameters. Analysis with this column was performed with the oven temperature and injection port at 85 and 128°, respectively. The flow rates of the nitrogen and hydrogen were both at 20 ml./min. The better separation obtained with this column is shown in the chromatogram in Fig. 1 (A). The chromatogram in Fig. 1 (B) was obtained in the previous study (9).

Normal and Hydrated Stratum Corneum Studies.—The preparation of the absorption cell and site for percutaneous absorption under normal and hydrous skin conditions again was similar to that described in the earlier investigation (9); however, the length of time the test subject was exposed to the penetrant was extended from 3 to 8 hr.

Dehydrated Stratum Corneum Study.—The compartment in the absorption cell which normally contained the cotton pad saturated with the penetrant was filled with magnesium perchlorate. The compartment was then covered with filter paper in order to prevent any possible irritation to the skin due to direct contact by the desiccating agent. This cell was then worn on the forearm of the test subjects for a period of 5 hr. At this time the cell was removed and the usual absorption cell was affixed to the same site as rapidly as possible and the ketone injected. The cell again remained on the arm for 8 hr.

Determination of Distribution Coefficient of MEK.—Tightly closed screw-cap vials sealed with paraffin containing 10 ml. of water, 10 ml. of olive oil, and 5 μl. of MEK in one case and 10 ml. of water and 5 μl. of MEK in the other were shaken in a water bath at 30 ± 1° for 24 hr. After equilibrium was attained, the aqueous phase of each vial was analyzed *via* vapor phase chromatography and the olive oil/water distribution coefficient calculated.

Comparative Elimination Rates Between Orally and Percutaneously Absorbed MEK.—Test subjects ingested a known amount of MEK (usually about 375 mg.) contained in a gelatin capsule. Samples were collected and analyzed in the manner previously described. Immediately after the release of the ketone from the capsule (approximately 5 min.), eructation was experienced by the test subjects. Since some of the test chemical thus reached the mouth directly, expired air samples were not collected until 0.5 hr. after the ingestion of the capsule.

To study elimination after absorption through normal untreated skin, expired air samples were collected after removal of the absorption cell from the forearm. Following removal, the forearm was thoroughly washed with diethyl ether to remove any remaining ketone from the skin. To avoid direct inhalation of the chemical the same precautions (the arm was maintained in the fume hood and test subject wore a gas mask), as previously described, were taken during this procedure (9).

RESULTS AND DISCUSSION

Exposure of Normal Stratum Corneum to MEK.—Following the exposure of the skin surface to MEK, the test penetrant could be detected in the expired air in a remarkably short period of time. Normally, expired air samples were not collected until 15 min. of time elapsed following the introduction of the penetrant into the absorption cell; however, some

TABLE I.—CONCENTRATION OF MEK (mcg./L.) IN THE EXPIRED AIR OF HUMAN TEST SUBJECTS FOLLOWING APPLICATION OF THE PENETRANT UNDER VARIOUS SKIN CONDITIONS

Time, min.	Normal		Anhydrous		Hydrous	
	Av.	Range	Av.	Range	Av.	Range
0.5–1	...	...	...	...	11	...
3	...	...	...	...	18.8	14.7–23.8
8–10	...	...	...	...	25.7	19.8–39.7
15	3.6	2.5–4.4	1.7	1.0–4.0	22.7	18.4–34.5
30	4.3	3.2–5.9	2.9	1.8–4.3	19.6	13.8–35.3
45	4.4	3.2–4.9	3.0	2.2–4.0	14.2	9.6–23.3
60	5.6	4.1–7.4	3.1	1.9–4.6	12.4	7.0–22.1
75	6.0	4.6–8.0	3.6	2.5–4.5	18.4	10.6–26.2
90	6.0	4.6–9.6	3.8	2.5–4.9	12.4	6.6–22.2
120	6.6	5.1–8.4	3.7	2.0–5.1	10.5	6.2–17.1
150	7.0	6.2–8.4	4.3	2.8–5.3	10.7	6.2–16.5
180	6.5	4.9–8.3	4.6	3.5–6.7	9.0	8.3–10.3
210	6.1	4.4–8.5	5.2	4.1–8.1	8.7	7.4–11.4
240	7.3	6.0–8.8	5.9	4.4–7.4	12.1	10.1–14.1
270	6.9	5.6–7.9	6.1	4.9–7.4	10.1	9.9–10.3
300	6.5	4.8–8.3	6.4	4.4–8.0	8.4	6.6–12.3
330	5.7	5.5–6.0	6.2	5.2–8.0	8.7	7.5–12.3
360	6.5	4.4–9.0	6.3	4.6–8.4	8.5	5.8–12.8
390	6.3	3.6–8.8	6.5	4.1–9.5	8.4	7.2– 9.7
420	5.8	4.6–6.9	6.2	5.0–8.6	10.7	9.7–12.3
450	6.5	5.0–8.0	6.3	5.1–8.3	8.3	7.2–10.0
480	7.0	5.7–8.4	7.5	6.1–8.8	8.6	6.8–12.1

initial tests showed the MEK to be present in expired air in only 2.5–3 min.

In plotting the concentration of the ketone in the expired air as a function of time, plateau values were observed to occur within 2–3 hr. (Figs. 2 and 3). When the plateau is reached and maintained, it is an indication that the desired steady state for the system has been achieved. This, of course, implies that an equilibrium has been obtained involving the absorption, distribution, elimination, etc., processes with respect to the MEK. The flat portion of the curve thus indicates a zero-order type system with respect to either elimination or absorption: $dMEK/dt = k$, that is, the rate of excretion is a constant, k, as determined by the rate of diffusion through the skin, the skin being completely covered by an excess of the ketone and the exposed surface remaining constant.

As would be expected, some biological variation does occur between the human test subjects, but the average plateau values were more consistent than might be expected in this type of experiment, particularly in the normal skin condition. Figure 2 shows the data obtained in single experiments in the same test subject under the three different skin conditions. Figure 3 shows the average data from five experiments with the same test subject under the different conditions. Table I shows the data collected on another test subject and is characteristic of the data obtained with the other individuals employed in this study.

Dehydrated Stratum Corneum Study.—The effect of pretreating the skin with a strong desiccant, followed by application of the penetrant, is also graphically illustrated in Figs. 2 and 3. From the data obtained it appears that there is no significant difference in the excretion rate between normal and initially dehydrated skin when the steady state has been attained. Examination of the complete curve, however, shows a very definite change in the approach to the steady state. The anhydrous experiments did not attain plateaus until 4–5 hr. after application of the test chemical, whereas in the normal skin experiments the steady state was reached much earlier. The effect was quite apparent in all the test subjects. This observation is in accord with the concept that a dry condition offers a more effective barrier to percutaneous absorption. If the desiccant were present during the entire course of the experiment, one would expect a decreased steady-state excretion rate in addition to a delay in reaching this equilibrium.

Upon removal of the absorption cell from the test subject after an 8-hr. period, the effect of the MEK was quite pronounced. Though no irritation or inflammation was ever noted, it was obvious that the penetrant had dehydrated the skin and removed lipids from the skin surface. This effect cannot be attributed to the desiccant as this was observed in the normal skin studies where there was no prior alteration of the skin environment.

Exposure of Hydrated Stratum Corneum to MEK.—Under hydrated stratum corneum conditions the MEK was detected in easily measurable amounts within 30 sec. after the penetrant was introduced into the percutaneous absorption cell. In a majority of the experiments, the concentrations at this short time period were greater than final plateau values obtained during the normal and anhydrous experiments.

Of considerable interest in this phase of the study is the shape of the curve obtained. As can be seen in Figs. 2 and 3, the concentration rises to maximum in several minutes and then declines and ultimately reaches a plateau. It should be pointed out that it was not possible to determine the exact time at which the maximum occurred since the collection of the expired air sample itself takes approximately 2 min. However, in 80% of all the hydrous experiments the peak was reached in 10 min., and within 15 min. the maximum had either been reached or passed in 100% of the experiments.

Upon removal of the absorption cell, the skin appeared to be in the same condition as previously described for the normal and dehydrated studies. From these observations it is possible to explain the

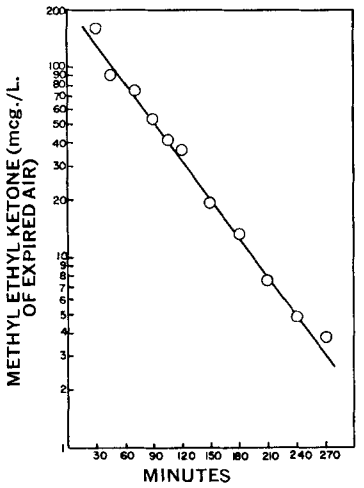

Fig. 4.—Elimination of MEK in expired air following oral administration.

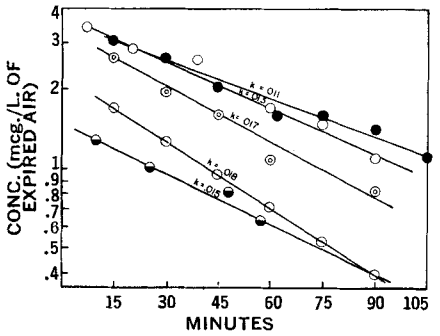

Fig. 5.—Excretion of MEK in expired air following percutaneous absorption.

decline of ketone concentration from a high value to the lower plateau level. It appears that the excess MEK in the absorption cell causes a partial dehydration of the stratum corneum which then causes a decrease in the absorption. By removing the lipids and other substances, the penetrant probably also alters the water-holding capacity of keratin. The chemical substances associated with the skin keratin are believed to be partially responsible for its water-holding capacity (17). The above changes in the stratum corneum alter the partitioning of the penetrant and decrease the diffusion rate.

Partition Coefficients.—In many investigations (17–19) the partition coefficient has been shown to influence percutaneous absorption greatly. Griesemer (20) stated that "a substance in which the partition coefficient between polar and nonpolar solvents is nearly 1.0 will have the highest penetration rate." The average partition coefficient for MEK in an olive oil–water system (P.C. = C_{oil}/C_{H_2O}) was determined to be 0.93 by the described gas chromatography method of analysis.

Comparative Elimination Rates After Oral and Percutaneous Absorption.—As would be expected, theoretically, a previous investigation (1) showed that the urinary elimination rate of salicylate esters when absorbed through the skin was nearly identical with that obtained after the intravenous administration of sodium salicylate. Therefore, limited studies were performed comparing elimination rates in expired air after MEK was absorbed both orally and percutaneously.

Following the oral ingestion of MEK by human test subjects, the elimination was found to follow first-order kinetics, with the k values ranging from 0.016 to 0.018 min.$^{-1}$. A plot of the elimination of MEK in the expired air following oral administration is shown in Fig. 4. Figure 5 shows several first-order excretion plots obtained with human test subjects following the percutaneous absorption of the test chemical. In these tests the elimination constants ranged from 0.011 to 0.018 min.$^{-1}$.

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