2. The pH profiles of the browning of lactose, dextrose, and galactose were ascertained. Relative order was lactose > galactose > dextrose. The possible presence of monosaccharides does not appear to be a major factor.

3. Browning of lactose was catalyzed by phosphate, tartrate, citrate, and acetate ions, whereas borate ions gave remarkable protection.

4. Moisture, in addition to HMF and/or related compounds, was shown to be a major contributory factor.

5. Water repellent lubricants, such as magnesium and calcium stearate, showed good protection against browning of lactose tablets.

6. Treatment with anion exchange resins prior to spray-drying or spray-drying to a lower moisture content resulted in a product less susceptible to browning.

REFERENCES

(1) Gunsel, W. C., and Lachman, L., THIS JOURNAL, **52**, 178(1963).
(2) Webb, B. H., *J. Dairy Sci.*, **18**, 81(1935).
(3) Patton, S., and Josephson, D. V., *ibid.*, **32**, 222 (1949); through *Chem. Abstr.*, **45**, 276*e*(1951).
(4) Ramsey, R. J., Tracy, P. H., and Ruehe, H. A., *J. Dairy Sci.*, **16**, 17(1933); through *Chem. Abstr.*, **27**, 1953 (1933).
(5) Pederson, C. S., Beattie, H. G., and Stotz, E. H., *N. Y. State Agr. Expt. Sta. Bull.*, **728**, 3(1947); through *Chem. Abstr.*, **42**, 3869*c*(1948).
(6) Brownley, C. A., Jr., and Lachman, L., THIS JOURNAL, **53**, 452(1964).
(7) Keeney, M., and Bassette, R., *J. Dairy Sci.*, **42**, 945(1959).
(8) Brownley, C. A., Jr., and Lachman, L., THIS JOURNAL, **52**, 86(1963).
(9) Berl, W. G., and Feazel, C. E., *J. Agr. Food Chem.*, **2**, 37(1954).
(10) de Jong, G. J., and Jansen, J. C., *Starke*, **9**, 147 (1957); through *Chem. Abstr.*, **52**, 5864*i*(1958).
(11) Kitriene, D., *Mokslas ir Tech.*, (No. 3) 33(1960); through *Chem. Abstr.*, **55**, 12896*i*(1961).
(12) Noller, C. R., "Chemistry of Organic Compounds," 2nd ed., W. B. Saunders Co., Philadelphia, Pa., 1957, p.376.

Factors Influencing Percutaneous Absorption II

Absorption of Methyl Ethyl Ketone

By DALE E. WURSTER and ROBERT MUNIES

A method is described for following the percutaneous absorption process with a vapor-phase chromatography technique. Data showing the absorption of methyl ethyl ketone (MEK) from a forearm surface of 91.5 cm.^{2} under both normal and hydrous conditions of the stratum corneum are presented. Hydration of the stratum corneum enhanced the percutaneous absorption rate of methyl ethyl ketone, but this penetrant subsequently partially dehydrated the stratum corneum. The resulting steady-state concentration of MEK in expired air, however, was still higher than that obtained with normal skin.

THE INFLUENCE of hydration of the stratum corneum on the percutaneous absorption of various salicylate esters in humans was studied previously (1). This study showed that the actual absorption rate expressed in terms of moles cm.$^{-2}$ hr.$^{-1}$ could be increased several fold by the hydration of this tissue. The magnitude of this increase was shown to be related to the oil/water distribution coefficient and the water solubility of these chemically similar salicylate esters. In general, the absorption of the more water-soluble compounds was enhanced more greatly by the hydration effect, but the precise relationship between the physical constants of the chemical and percutaneous absorption remains obscure. However, the single but strong influence of skin hydration on percutaneous absorption now has been shown to occur with several chemical agents including ethyl nicotinate (2), naphazoline (3, 5), steroids (4), aniline (6, 7), benzidine, dichlorobenzidine, dianisidine, and *o*-toluidine (8).

This paper is thus a part of a larger study in which an attempt will be made to elucidate relationships between the percutaneous absorption rate, the functional groups, and physical constants of the absorbed chemical.

METHOD

A liquid chemical compound of simple structure was desired to eliminate the complicating factors involved in the release of the drug from the physical system. Additional requirements for the penetrant indicated that it should be nonirritating, have low systemic toxicity, and be excreted unchanged mainly *via* one pathway. Because of the difficulty in analyzing excretions and body fluids for the absorbed compound, a substance which possessed a high vapor pressure and which could be detected easily in the expired air by vapor phase chromatography was desired also. Methyl ethyl ketone (MEK) appeared to meet most of the above requirements.

To simplify working with the complex biological system further, the study was designed so that

Received August 26, 1964, from the School of Pharmacy, University of Wisconsin, Madison.
Accepted for publication December 3, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.
Abstracted in part from a dissertation submitted by Robert Munies to the Graduate School, University of Wisconsin, Madison, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported in part by the University of Wisconsin Research Committee, Vick Divisions Research, Mount Vernon, N. Y., and the Malstrom Chemical Corp., Newark, N. J.

advantage could be taken of known absorption and elimination kinetics. Initially, the absorption rate exceeds the elimination rate, and the concentration in the blood and other fluids increases. Ultimately, the elimination rate, usually first order, increases to the point where the absorption and elimination rates are the same; a steady state then exists, provided, of course, that the absorption rate remains constant. By maintaining an excess of the test chemical on a constant skin surface area, the above conditions were met and zero-order kinetics established for this percutaneous absorption system.

EXPERIMENTAL

Absorption Cell.—A somewhat flexible absorption cell was constructed from 0.063-cm. stainless steel. A more flexible plastic cell, like that described in the previous investigation (1), could not be used due to the excellent solvent properties of MEK. The cell was 1.5 cm. in depth, 6.5 cm. wide at one end, 15.25 cm. long, and tapered to a width of 5.5 cm. at the other end so that it could be worn comfortably on the forearm of the human test subjects. The cell was surrounded by 1.5 cm. flanges, which were bonded firmly to the skin surface with tape (Okonite) and surgical appliance adhesive (Davol). When fixed in place, the cell defined a skin surface of 91.5 cm.^{2}. The good fit of the cell and the strong adhesive prevented this area from changing during the experiment. Other features of the absorption cell included an absorbent cotton pad inside the cell to hold the chemical in contact with the skin and orifices in the top through which chemicals could be injected.

Since absorption was followed by determining the concentration of the test chemical in the expired air, it was necessary that none of the MEK be inhaled. Elaborate precautions were taken to insure that the absorption cell provided the only portal of entry. The test subject wore a gas mask, and the arm to which the absorption cell was attached was maintained in a fume hood. Previously, it had been established that the mask was effective in eliminating the ketone from the inspired air. Separate experiments were conducted also to show that another test subject exposed to the same atmosphere as the individual wearing the absorption cell did not acquire significant amounts of the ketone. No container of the test chemical was ever opened in the same room in which the absorption experiment was conducted. Thus, in a remote room a large syringe was filled with MEK. One-hundred milliliters of the ketone then was injected through orifices in the top of the absorption cell and the orifices immediately sealed.

Sample Collection and Analysis.—The collection and analysis of samples was similar to that described previously by Eriksen (9, 10). At the appropriate time interval, the test subject took a maximum air inspiration, then held his breath for 30 sec. Then 1.5 L. was expired into a spirometer. This expired air was discarded. Continuing to hold his breath, the subject then expired an additional 3.0 L. at a slow and near steady rate into a collecting vessel. This vessel was maintained in liquid nitrogen; thus, substances such as water, alcohols, ketones, etc., present in the expired air were immediately condensed to the solid state. The above procedure was repeated immediately so that the components from

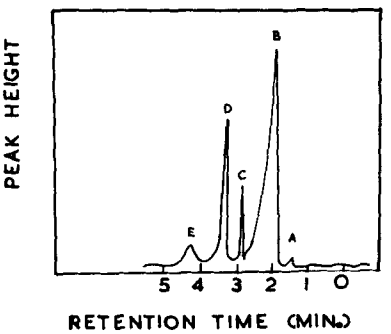

Fig. 1.—Chromatogram of a 5-μl. sample of expired air condensate. Key: A, acetaldehyde; B, acetone; C, methanol; D, methyl ethyl ketone; E, ethanol.

6.0 L. of expired air were collected for analysis at each time period. The temperature of the collection vessel was allowed to increase until the contents liquefied. A 5.0-μl. aliquot then was taken for analysis. The instrument employed in the vapor-phase chromatography procedure was an Aerograph Hy Fi, model 600C, with a hydrogen generator and flame ionization detector. A 5.0-ft. stainless steel column of 1/8-in. i.d. was packed with an 8% mixture of heavy alcohols (30% arachidyl, 25% behenyl, 30% stearyl, 15% cetyl) and 2% *N,N,N',N'*-tetramethylazelamine on 70–80 mesh Anakrom ABS as a support. The column was heated to 60°, and the flow rates of the hydrogen and the nitrogen carrier gas were 20 and 14 ml. min.$^{-1}$, respectively. The injection site was 120°.

Hydrated Stratum Corneum Study.—The preparation of the absorption cell and site for percutaneous absorption under hydrous skin conditions were similar to the procedure previously described, with the following exception. Two hours prior to the absorption experiment, a large absorbent cotton pad was saturated with water, placed over the absorption site, and the entire forearm wrapped with an occlusive plastic film (Saran Wrap). At the end of the stated time period, the above materials were removed and the absorption cell fitted in place. Not more than 5 min. elapsed between the removal of the hydrating materials and the injection of the MEK into the percutaneous absorption cell.

RESULTS AND DISCUSSION

Exposure of Normal Skin to the Test Penetrant.—Following the exposure of the skin surface, the test penetrant could be detected in the expired air in a remarkably short period of time. In some cases, the MEK was detected after only a 3-min. time lapse following the introduction of the penetrant in the absorption cell. Figure 1 is a chromatogram of the expired air containing the MEK. In plotting the concentration of the ketone in the expired air as a function of time, plateau values were observed to occur rather rapidly, an indication that the desired steady state for the system had been achieved. Of course, this implies that an equilibrium has been obtained involving the absorption, distribution, elimination, etc., processes with respect to the MEK. Table I gives the steady-state concentration of the penetrant in the expired air and the steady-state intercept. As indicated, there is apparently

considerable amount of biological variation between the human test subjects. However, duplicate determinations for the same individual are somewhat more uniform than might be expected in an experiment of this nature. The steady-state intercept was, of course, obtained by extrapolating the straight line portion of the accumulation plot (Fig. 2) to zero concentration. Also, the slope of this line is the elimination rate under the described conditions. Of particular interest is the fact that the largest value obtained for the steady-state intercept was only 45 min., which again shows that this state was achieved quite rapidly.

Exposure of Hydrated Stratum Corneum to the Test Penetrant.—Under hydrated stratum corneum conditions, the MEK was detected in easily measurable amounts (9.3 mcg. $L.^{-1}$ expired air) within 30 sec. after the penetrant was introduced into the percutaneous absorption cell. However, this value is somewhat misleading since 2 min. were required to inject the penetrant into the cell. Nevertheless, it is obvious that the transport of the penetrant across the skin is extremely fast.

The subjects used to study the effect of hydration of the stratum corneum on the percutaneous absorption of MEK were selected carefully and represent both the high and low extremes of the steady-state concentrations (Table I) and one individual having an intermediate value.

In Fig. 3, the data obtained in comparing the absorption from a normal skin surface to that of a hydrated surface in the same individual are shown. This individual had one of the slowest percutaneous absorption rates for this penetrant. In the upper curve the concentration under hydrous conditions after only 40 sec. is 13.5 mcg. $L.^{-1}$ of expired air which is greater than the plateau value of the normal skin. Also of interest is that the concentration rises to a maximum value of 37 mcg. $L.^{-1}$, then declines and ultimately reaches a plateau level of 7.6 mcg. $L.^{-1}$ of expired air. The attainment of the high level and subsequent decline to a lower steady-state level can be explained logically on the basis of the barrier change. It is probable, therefore, that the excess MEK in the absorption cell causes a partial dehydration of the stratum corneum, which then causes a decrease in the absorption rate. The higher steady-state level in the hydrous experiments implies that the stratum corneum, in the time interval shown here, did not return to a state of such low water concentration as that in the normal skin of this test subject.

All test subjects in the hydrous experiments noted a mild burning sensation as soon as the penetrant was injected into the cell. This was not experienced in the normal skin experiments. This effect was probably due to the higher concentration of MEK in the tissues than that obtained under the less hydrous condition. Thus, all the test subjects in the hydrous experiments tended to show a similar pattern. Even the ratio of the maximum concentration to the steady-state concentration in each case is approximately 5. (See Table II.)

TABLE I.—STEADY-STATE CONCENTRATION AND INTERCEPTS FOR THE PERCUTANEOUS ABSORPTION OF MEK ON NORMAL SKIN

Test Subject	Steady-State Concn. of MEK, mcg./L. of Expired Air	Steady-State Intercept, min.
1	3.20	21
2	2.97	9
3	2.54	21
3	3.19	35
3	5.31	33
4	3.40	9
5	2.70	42
5	4.87	42
5	4.67	33
6	6.15	30
7	3.84	12
8	4.26	21
8	4.67	45
9	2.70	18
10	8.30	9
10	13.00	23
11	8.90	23
12	5.54	15
12	3.31	30

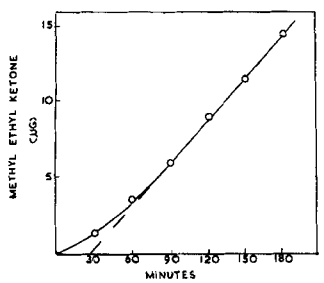

Fig. 2.—Cumulative plot of exhaled methyl ketone (normal skin).

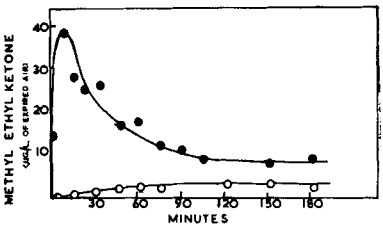

Fig. 3.—Expired air data showing the influence of moisture on the percutaneous absorption of methyl ethyl ketone. Key: O, normal skin; ●, hydrated skin.

TABLE II.—PEAK AND PLATEAU CONCENTRATIONS FOR THE PERCUTANEOUS ABSORPTION OF MEK UNDER PREVIOUSLY HYDRATED CONDITIONS

Test Subject	Max. Concn. of MEK, μl./L. of Expired Air	Plateau Concn. of MEK, μl./L. of Expired Air
9	37.0	7.6
10	81.0	14.8
12	52.5	10.0

REFERENCES

(1) Wurster, D. E., and Kramer, S. F., THIS JOURNAL, **50**, 288(1961).
(2) Cronin, E., and Stoughton, R. B., *Nature*, **195**, 1103 (1962).
(3) Cronin, E., and Stoughton, R. B., *Brit. J. Dermatol.*, **74**, 265(1962).
(4) McKenzie, A. W., and Stoughton, R. B., *Arch. Dermatol.*, **86**, 608(1962).
(5) Vickers, C. F. H., and Fritsch, W. C., *ibid.*, **87**, 633 (1963).
(6) Piotrowski, J., *J. Hyg. Epidemiol. Microbiol. Immunol. Prague*, **1**, 23(1957).
(7) Dutkiewicz, T., *Med. Pracy.*, **8**, 25(1957).
(8) Meigs, J. W., *et al.*, *Arch. Ind. Hyg. Occupational Med.*, **9**, 122(1954).
(9) Eriksen, S. P., *New Scientist*, **21**, 608(1964).
(10) Eriksen, S. P., and Kulkarni, A. B., *Science*, **141**, 639 (1963).