Factors Involved in the Browning of Spray-Dried Lactose

By K. T. KOSHY, R. N. DUVALL, A. E. TROUP, and J. W. PYLES

The pH profile of the lactose browning reaction was ascertained with various buffers under accelerated stability conditions and compared to the pH browning profile obtained by similar procedures for both dextrose and galactose. The effect of heat, humidity, and tablet lubricants was studied also. A correlation was found between the ultraviolet absorption spectrum and the 5-hydroxymethylfurfural content of samples of spray-dried lactose. The increase in absorption during the browning process afforded a simple procedure for studying this phenomenon. Also, absorption measurement could serve as a purity test for U.S.P. purposes. A procedure involving the use of anion exchange resins was developed for the purification of spray-dried lactose. This method should be adaptable to large scale commercial processes. Lactose which was spray-dried following this purification treatment was less susceptible to browning than the commercially available material.

FLOW AND COMPRESSIBILITY properties of spray-dried lactose (lactose S.D.) have led to an increased use of this material as an excipient in tablet and capsule formulations. However, the discoloration of such formulations is a problem and has been investigated by various workers. Gunsel and Lachman (1), evaluating the tableting properties of lactose, noted that while lactose S.D. was advantageous in several respects, it darkened more rapidly than conventionally processed lactose. The reason for this has not been clearly established. The significant differences between the two grades are that lactose S.D. contains (*a*) about 8% amorphous lactose, (*b*) slightly higher amounts of other sugars, metals, and ash and (*c*) that it has been exposed to heat during the spray-drying process.

Webb (2) investigated the browning of lactose and other milk products and reported that the hydrogen ion concentration is of major importance and that phosphate buffer has a catalytic effect. However, the reaction was studied over a narrow pH range. Patton and Josephson (3) and Ramsey *et al.* (4) proposed that phosphates may be of secondary importance in the browning of milk products. A synergistic effect of phosphate and protein amino groups has been suggested by Pederson *et al.* (5). Recently, Brownley and Lachman (6) have demonstrated a relationship between the discoloration of lactose S.D. and the presence of free 5-hydroxymethylfurfural (HMF).

The large-scale use of lactose in complex pharmaceutical formulations and the lack of adequate information on the stability of lactose under varying conditions of usage in such systems have prompted this study. This paper deals with some of the factors involved in the browning of lactose in the absence of added amines (referred to hereafter as the normal browning of lactose). Browning which takes place in the presence of added amines will be the subject of a subsequent report. A major portion of this study deals with browning related to changes in the ultraviolet absorption spectrum and HMF content of lactose. The effect of pH on the browning of lactose and on the monosaccharide hydrolysis products, galactose and dextrose, was studied. The effects of heat, humidity, commonly used lubricants, and common buffer species were investigated also. Lactose S.D. was purified by treatment with ion exchange resins and respray-dried in an attempt to improve its resistance to browning.

EXPERIMENTAL

Role of HMF in Lactose Browning.—The ultraviolet absorption spectra of 10% solutions of lactose were determined using a Beckman model DB spectrophotometer with 1-cm. cells and compared to the spectrum of HMF (Aldrich Chemical Co., Inc.). The free HMF and/or related compounds in these samples were determined by adaptation of the reaction with 2-thiobarbituric acid (TBA), described by Keeney and Bassette (7), and applied by Brownley and Lachman for the determination of HMF in lactose (6).

Purification of Spray-Dried Lactose with Ion Exchange Resins.—Aqueous solutions containing 15–18% of lactose S.D. were passed through separate 1 × 22-cm. columns containing the following resins: (*a*) weak anion exchange resin, Amberlite IR-45 (Cl^-), (*b*) Amberlite IR-45 (OH^-), (*c*) strong anion exchange resin, Dowex 1-X4 (Cl^-). The purity of the eluate from each column was monitored by scanning the absorption in the 220–340-mμ region. The eluate was then spray-dried with a Nerco-Niro laboratory model spray-dryer (Nichols

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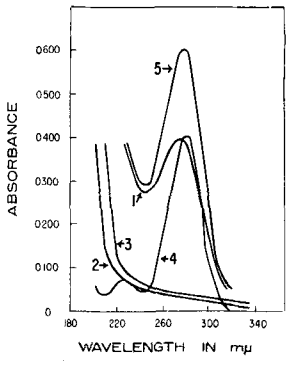

Fig. 1.—Spectra of HMF (4 mcg./ml.) and 10% aqueous solutions of lactose. Key: 1, U.S.P. spray-dried; 2, U.S.P. conventionally processed; 3, analytical reagent grade; 4, HMF; 5, U.S.P. spray-dried with 2 mcg. HMF per milliliter.

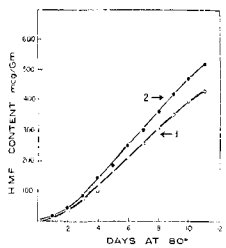

Fig. 2.—HMF content by the ultraviolet absorption and TBA methods of a 10% aqueous solution of U.S.P. conventionally processed lactose stored at 80°. Key: 1, HMF by TBA method; 2, HMF by the ultraviolet absorption method.

Engineering and Research Corp.). The respray-dried material was stored under accelerated conditions and its stability compared with that of the raw material by visual observation of resultant browning and by the intensity of the absorption maximum at 275–285 mμ.

pH Profiles of the Dextrose, Galactose, and Lactose Browning Reactions.—Ten per cent solutions of lactose analytical reagent, lactose U.S.P. (conventionally processed), and lactose U.S.P. spray-dried were prepared in buffers of pH 1 through 10. These solutions were stored at 50° and changes in ultraviolet absorption spectra noted at suitable intervals. The relative hydrolysis of lactose in these solutions was determined with reagent strips.[1] The buffer systems were: pH 1, hydrochloric acid–potassium chloride; pH 2–8, citric acid–disodium hydrogen phosphate; pH 8–10, boric acid–sodium hydroxide. The pH profiles of dextrose and galactose also were obtained to ascertain further the significance of hydrolysis as a factor in the browning process.

Catalysis of Buffer Species.—Ten per cent solutions of lactose S.D. were prepared in buffers at pH 4 and 8 with different buffer concentrations at each pH. These solutions were stored at 50° for 1 week, and any change in the absorbance at 276 mμ was noted. The buffer systems were: pH 4, tartaric acid–sodium hydroxide; pH 4, hydrochloric acid–potassium citrate; pH 4, acetic acid–sodium acetate; pH 8, citric acid–disodium hydrogen phosphate.

Effect of Heat and Moisture on Browning.—Samples of lactose S.D. were stored at 50° dry heat, 50° and 70% relative humidity, and room temperature and 70% relative humidity. Ten per cent solutions of each were prepared after 2 weeks of storage and their ultraviolet absorption spectra compared to that of the original.

An 18% aqueous solution of lactose S.D. (containing 5% moisture as loss on drying) was respray-dried with inlet and outlet temperatures of 150–155° and 70–80°, respectively, resulting in a product containing 1.4% moisture. This was stored in sealed containers at elevated temperatures and evaluated visually and by measurement of the absorption at 276 mμ.

Effect of Tablet Lubricants on Browning.—One-gram tablets containing lactose S.D. and 2% of commonly used lubricants were prepared and stored at 50° dry heat in both capped and uncapped bottles and at 50° and 70% relative humidity. At suitable intervals, the tablets were observed visually and the ultraviolet spectra of filtered aqueous solutions determined and compared to the initial spectra of comparable solutions.

DISCUSSION

Role of HMF in Lactose Browning.—Figure 1 shows the typical ultraviolet absorption spectra of aqueous solutions of various grades of lactose, a HMF standard, and the effect of adding HMF to a solution of lactose S.D. All samples of lactose S.D. scanned had an absorption maximum in the region of 275–285 mμ. Solutions of reagent grade and U.S.P. conventionally processed lactose under accelerated conditions developed a peak in this region. For all grades of lactose, the intensity of the peak increased as the solution discolored. This phenomenon was noted recently by Brownley and Lachman (8), who suggested that it might be due to changes in molecular structure. The almost superimposable nature of the absorption curves of lactose S.D. and of HMF and the curve obtained after adding HMF to lactose S.D. solutions, along with other evidence for the presence of this compound in lactose (6), indicated that the absorption peak might be due to HMF and/or closely related compounds. The presence of HMF in concentrates of ethyl ether extracts of solid raw materials was

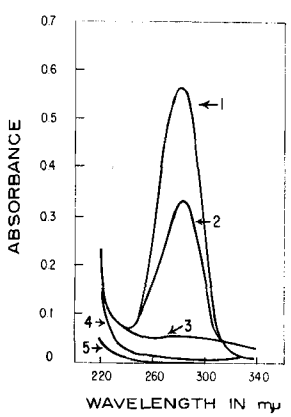

Fig. 3.—Spectra of aqueous solutions of HMF before and after treatment with anion exchange resins; 20 ml. containing 4.4 mcg./ml. passed through 2 Gm. of resin (20–50 mesh) in 1-cm. O.D. column. Key: 1, before treatment; 2, Dowex 1-X4 (Cl^-); 3, Amberlite IR-45 (OH^-); 4, Dowex 1-X4 (OH^-); 5, Amberlite IR-45 (Cl^-).

[1] Clinistix, Ames Co., Inc., Elkhart, Ind.

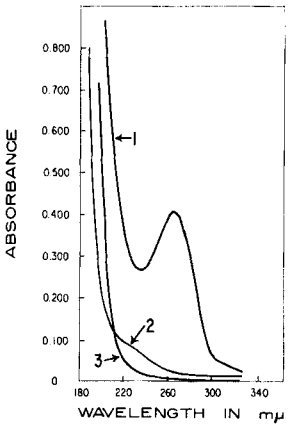

Fig. 4.—Spectra of aqueous solutions of spray-dried lactose before and after treatment with anion exchange resins; 20 ml. of a 10% solution passed through 1 Gm. of resin (20–50 mesh) in 1-cm. O.D. column. Key: 1, before treatment; 2, Dowex 1-X4 (Cl^-) and Amberlite IR-45 (Cl^-); 3, Amberlite IR-45 (OH^-) and Dowex 1-X4 (OH^-).

confirmed by thin-layer chromatography. The concentrates were spotted on silica gel plates, 0.25-mm. thick, and developed with *n*-butyl acetate. HMF was detected by spraying with an aqueous solution made of equal volumes of 0.05 *M* TBA and 40% trichloroacetic acid.

Additional proof for the relationship between the absorbance at 276 mμ and the free HMF content was obtained from the data on a 10% unbuffered aqueous solution of lactose U.S.P. conventionally processed and stored at 80°. The free HMF concentration of this solution was determined daily by the TBA method and from the absorbance at 276 mμ. The data are presented in Fig. 2. Initially, there was no absorbance and no free HMF by the TBA method, but the HMF content increased steadily with time, with a corresponding increase in the absorbance at 276 mμ. The ultraviolet absorption method gave consistently higher values than the TBA method. The reason for this is not known but could be due to the degradation of lactose beyond the HMF stage, resulting in the formation of interfering substances. Since the absorption at 276 mμ increased with browning, it appeared that the intensity of absorption in the region of 275–285 mμ could be used to study this process.

It was felt that if the impurities in lactose S.D. which were strongly suspected to be HMF and/or related compounds could be removed, the resulting material would be less susceptible to browning. Berl and Feazil (9) used a strong anion exchange resin to remove the chromogens from alkaline solutions of a few simple sugars. De Jong and Jansen (10) removed HMF from a starch hydrolyzate with an anion exchange resin partly charged with sulfur dioxide. Kitriene (11) has reported that ion exchange resins could eliminate sulfite treatment in the refining of syrup. Weak and strong anion exchange resins in both their chloride and hydroxyl forms were found to remove HMF from solution, as illustrated in Fig. 3. The strong anion exchange resin in the chloride form was only partially effective, but it was found later that larger quantities of the resin removed HMF completely. Figure 4 shows how effectively the weak and strong anion exchange resins, in both their chloride and hydroxyl forms, removed the chromogens from lactose S.D. Four-hundred milliliters of a 10% solution was passed through a 1 × 22-cm. resin column, and the last portion of eluate still had no absorption in the ultraviolet region.

The free HMF content of 10% solutions of different lots of lactose S.D., lactose U.S.P. conventionally processed, and lactose reagent grade was determined by the TBA method before and after ion exchange treatment. The results presented in Table I confirm the removal of free HMF.

pH Profile of Lactose Browning Reaction.—The effect of pH on the browning of 10% buffered aqueous solutions of lactose is shown in Fig. 5. This pH profile was obtained by measuring the increase in ultraviolet absorbance of solutions stored at 50° for 1 week. In all lactose samples there was a marked increase in absorbance at pH 7 and 8 in phosphate–citrate buffer. The curve for lactose S.D. was displaced upward because it had a de-

TABLE I.—FREE HMF CONTENT OF LACTOSE (mcg./Gm.) BY THE TBA METHOD BEFORE AND AFTER ION EXCHANGE TREATMENT[a]

Sample[b]	Before	After[c]
A	5.1	1.0
B	5.5	0.5
C	6.8	1.0
D	5.9	0.5
E	7.8	1.0
F	7.6	1.3
G	4.8	0.2
H	7.8	0.2
I	0.3	0.0
J	1.3	0.0
K	1.2	0.0

[a] Amberlite IR-45 (OH^-). [b] Samples *A* through *H* were spray dried; sample *I* was analytical reagent grade; samples *J* and *K* were U.S.P. conventionally processed. [c] Solutions after ion exchange treatment had negligible ultraviolet absorption in the range of 0–1 mcg./Gm. of HMF.

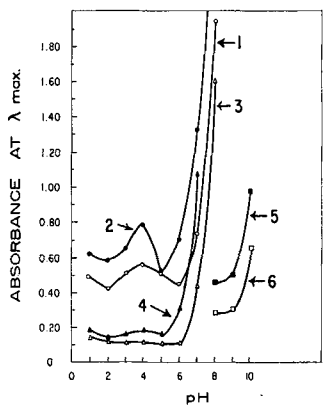

Fig. 5.—pH profile of lactose browning; 10% solutions stored at 50°. Key: 1, U.S.P. spray-dried, 1 week; 2, U.S.P. spray-dried, 2 weeks; 3, analytical reagent grade, 1 week; 4, analytical reagent grade, 2 weeks; 5, U.S.P. spray-dried in borate buffer, 1 week; 6, analytical reagent grade in borate buffer, 1 week. Buffer systems: pH 1, hydrochloric acid–potassium chloride; pH 2–8, citric acid–disodium hydrogen phosphate; pH 8–10, boric acid–sodium hydroxide.

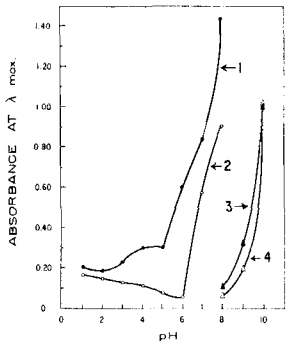

Fig. 6.—pH profile of dextrose and galactose browning; 10% solutions stored at 50° for 1 week. Key: 1, galactose, C.P.; 2, dextrose, analytical reagent; 3, galactose, C.P., in borate buffer; 4, dextrose, analytical reagent, in borate buffer. (Buffer systems the same as in Fig. 5.)

finite peak initially, whereas other grades of lactose did not have such a maximum. The inflection in the curve for lactose S.D. at pH 3–4 was thought at first to be due to experimental error. However, the experiment was repeated, and similar results were obtained. Two-week data were similar, except that the curve was displaced further upward. A slight inflection also was noticeable at this check period at pH 3–4 for both reagent grade and conventionally processed lactose.

Visual observations of the solutions revealed that after 1 week, the solutions at pH 1 were faint yellow, pH 2 through 6 colorless, pH 7 faint yellow, and pH 8 deep yellow. While solutions at pH 2 through 6 were colorless, they exhibited an absorption peak at 275–285 mμ. On prolonged storage, these solutions discolored. It was interesting that, while solutions at pH 8 in phosphate buffer were deep yellow and showed a great increase in absorbance, corresponding solutions in borate buffer were only faintly yellow. Even at pH 9, lactose solutions in borate buffer were discolored only slightly. This protection could be due to complex formation between borate and lactose since many polyhydroxy compounds are known to form complexes with alkali borates or boric acid (12).

One of the reasons for the difference in stability between spray-dried and conventionally processed lactose might be the presence of larger quantities of the hydrolytic products, dextrose and galactose, in the former (8). Determination of dextrose in lactose solutions after 1 week at 50° indicated more than 0.5% at pH 1 through 3 and less than 0.25% at pH 4 through 8. Inasmuch as the maximum discoloration and increase in absorbance were observed in solutions of pH 7 and 8 (phosphate–citrate buffer), it is evident that the pH and the buffer species are more significant than the concentration of dextrose and galactose.

The yellow- to brownish-colored material formed in this browning process was extremely water soluble and could not be extracted from aqueous solutions with chloroform, ethyl ether, or *n*-butanol. This general solubility distinguishes the normal browning of lactose from that occurring in the presence of primary amines, evidence of which will be presented in a following communication.

The pH profiles for dextrose and galactose (Fig. 6) were similar to that of lactose, except that galactose discolored over a wider pH range, 5 and above, whereas the other two sugars discolored at pH 7 and above. However, as can be seen from the curves, the relative intensity of the browning of the three sugars was lactose > galactose > dextrose. This also apparently eliminates hydrolysis or the presence of monosaccharides as an important factor in the normal browning of lactose since the extent of browning in a 10% lactose solution is greater than that which would be obtained from the corresponding amounts of monosaccharide hydrolysis products. As in the case of lactose, borate buffer had a protective effect on both monosaccharide browning reactions.

Catalysis of Buffer Species.—Webb (2) demonstrated the catalytic effect of phosphate ions in lactose browning. Figure 7 demonstrates the general base catalytic effect of phosphate, acetate, citrate, and tartrate ions as determined by the increase in absorbance of solutions of lactose S.D. stored at 50° for 1 week. Data in solutions of lower concentration of buffer at pH 8 can be considered only semiquantitative since there was a slight decrease of pH in these solutions. At pH 4, no such change was noticed. It is significant that acetate,

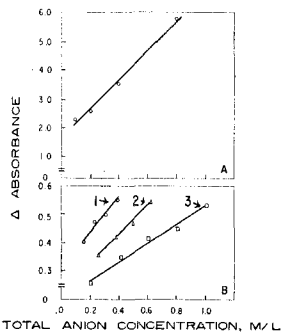

Fig. 7.—Catalytic effect of buffer species on the browning of 10% solutions of spray-dried lactose stored at 50° for 1 week. Key: A, pH 8, citric acid–disodium hydrogen phosphate. B, pH 4; 1, hydrochloric acid–potassium citrate; 2, tartaric acid–sodium hydroxide; 3, acetic acid–sodium acetate.

TABLE II.—EFFECT OF LUBRICANTS ON BROWNING OF SPRAY-DRIED LACTOSE TABLETS[a]

Sample	50° and 70% RH for 2 Mo. A_{276}	Relative Degree of Browning[c]	50° for 1 Mo. A_{276} Capped Bottles[d]	50° for 1 Mo. A_{276} Uncapped Bottles
Lactose control	1.27	+++	1.04	0.55
Talc A[b]	0.85	++	0.75	0.50
Talc B[b]	0.81	++	0.79	0.49
Mg stearate	0.54	0	0.78	0.51
Ca stearate	0.67	0	0.67	0.56
Starch	0.71	+	0.70	0.63
Na lauryl sulfate	1.25	++++	1.17	0.51

[a] Absorbance measurements were made on filtered 10% aqueous solutions. [b] Aqueous slurries of talcs A and B had a pH of 6.1 and 8.4, respectively. [c] From 0 to ++++ indicates relative increase in browning. [d] All samples had browned noticeably.

TABLE III.—COMPARISON OF THE BROWNING OF UNTREATED AND RESIN TREATED U.S.P. SPRAY-DRIED LACTOSE

Sample	Initial Moisture % LOD	Storage Temp., °C.	Relative Degree of Browning[a] — Storage Time, Days 0	4	7	14	28
A, raw material	5.0	80	0	++	++	++	+++
B, respray-dried	1.4	80	0	0	0	++	++
C, through Dowex 1-X4 (Cl^-) and respray-dried	2.4	80	0	0	+	++	++
D, through IR-45(OH^-) and respray-dried	1.2	80	0	0	0	0	++
A, raw material	5.0	70	0	+	++	...	...
E, sample *C* with added moisture	5.0	70	0	0	0	...	...
F, sample *D* with added moisture	5.0	70	0	0	0	...	...
A, raw material	5.0	50	0	...	...	0	++
B, respray-dried	1.4	50	0	...	...	0	++
C, through Dowex 1-X4 (Cl^-) and respray-dried	2.4	50	0	...	...	0	0
D, through IR-45(OH^-) and respray-dried	1.2	50	0	...	...	0	0

[a] From 0 to +++ indicates relative increases in browning.

citrate, and tartrate ions which are commonly encountered in pharmaceuticals as anions of amine salts, buffer constituents, and as excipients all have a deleterious effect on lactose stability.

Effect of Heat and Humidity on Lactose Browning.—Lactose S.D. powder stored at 50° and 70% relative humidity was discolored markedly, while that at 50° and dry heat was discolored only slightly. The sample at 20° and 70% relative humidity was not discolored. The absorbance values followed the same pattern—namely, highest for the most discolored and no significant change in the sample with no discoloration. These results indicate that a combination of heat and moisture accelerates browning more than either heat or humidity. Thus, if the moisture content of lactose S.D. could be reduced, the tendency for browning could be retarded considerably.

Effect of Tablet Lubricants on Browning.—The effect of lubricants on the browning of lactose tablets is shown in Table II. Results indicate that, except for sodium lauryl sulfate, all other lubricants had some protective effect. One might expect that the alkalinity of the stearate salts would have a deleterious effect since the pH profile showed a general base catalysis. However, the moisture repellent nature of these salts appears to be more important since both magnesium and calcium stearate gave good protection. Sodium lauryl sulfate, which is water soluble, gave no protection against browning, again emphasizing the importance of moisture in this process. This point also was brought out vividly by storing tablets in both capped and uncapped bottles. All tablets in the capped bottles browned noticeably; the corresponding tablets in uncapped bottles, which would not retain the moisture, were similar to controls. The ultraviolet absorption values of filtered aqueous solutions of these tablets agreed with visual observations.

Purification of Spray-Dried Lactose.—Two factors of major significance in the browning process were the presence of HMF and/or related compounds and moisture. It was shown that HMF could be removed effectively by anion exchange resins. Accordingly, 15–20% solutions of lactose S.D. were treated separately with weak and strong anion exchange resins and then respray-dried, as described previously. The stabilities of these samples were compared to those of the raw material (which contained 5% moisture) and raw material respray-dried to a lower moisture level but without ion exchange treatment.

The results presented in Table III clearly demonstrate the effect of moisture and anion exchange resin treatment on the browning of lactose. Lactose S.D. raw material containing 5% moisture discolored after 4 days at 80°. The same material respray-dried to a lower moisture level of 1.4% had not discolored after 7 days. Treatment with Amberlite IR-45 (OH^-) improved the stability considerably; the material was not discolored even after 14 days. The same effect was noticed at 70°. The three samples at this temperature contained the same amount of moisture. While the untreated raw material discolored after 4 days, there was no change in the resin treated samples after 7 days. Data for the samples stored at 50° demonstrated that, even at lower moisture levels, resin treatment further improved the stability. Samples *B*, *C*, and *D* contained 1.4, 2.4, and 1.2% moisture, respectively. While sample *B* discolored after 28 days, samples *C* and *D*, treated with anion exchange resins, were not discolored.

SUMMARY

1. Some of the factors involved in the browning of lactose in the absence of added amines were studied. Spray-dried lactose was found to have an ultraviolet absorption maximum, whereas U.S.P. conventionally processed and analytical reagent grade lactose did not. A relationship between the intensity of this maximum and the HMF content was shown. This absorbance was related to browning and affords a simple procedure for studying this phenomenon and could serve as a purity test for U.S.P. purposes.

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.

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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

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