Effect of Alpha Tocopherol (Vitamin E) Deficiency on Intestinal Transport of Passively Absorbed Drugs

MAHASEN M. MESHALI * and CHARLES H. NIGHTINGALE ˣ

Abstract □ Alpha tocopherol (vitamin E) deficiency has been shown to cause changes in membrane structure. The present study relates alpha tocopherol deficiency with increased rates of transport and absorption of passively absorbed drugs. The pharmacokinetics of barbital in alpha tocopherol-deficient and control rats was studied. The barbital absorption rate constant in deficient animals increased compared to control values. This finding indicates that alpha tocopherol deficiency affects the intestinal membrane structure. This finding was confirmed by studying the intestinal transport of phenolsulfonphthalein, barbital, and salicylate using the everted gut technique. Phenolsulfonphthalein was transported more rapidly through the alpha tocopherol-deficient gut, but this difference was not significant after 30 min, probably due to membrane decomposition. Barbital, which is more lipid soluble and less dependent on changes in pore volume and size, was transported more rapidly through the deficient gut during the entire experiment. The transport rate of salicylate was not altered by the deficiency state. This result was expected since the drug is normally rapidly transported; therefore, comparatively small changes in permeability such as those induced by alpha tocopherol deficiency would be masked. After the oral administration of phenolsulfonphthalein to intact animals, a significantly higher amount of drug was recovered in the urine of the deficient group.

Keyphrases □ Vitamin E—deficiency, effect on intestinal transport and absorption of barbital, phenolsulfonphthalein, and salicylate, rats □ Barbital—absorption and intestinal transport, alpha tocopherol-deficient rats □ Phenolsulfonphthalein—absorption and intestinal transport, alpha tocopherol-deficient rats □ Salicylate—absorption and intestinal transport, alpha tocopherol-deficient rats □ Absorption, GI—effect of alpha tocopherol deficiency, rats □ Transport rates, GI—effect of alpha tocopherol deficiency, rats □ Alpha tocopherol—effect of deficiency on intestinal transport and absorption of barbital, phenolsulfonphthalein, and salicylate, rats

Alpha tocopherol (vitamin E) is believed to play an important role in the maintenance of normal membrane structure and function. The relationship between alpha tocopherol body levels and membrane structure is not clear. It has been proposed (1) that alpha tocopherol may form a stable "complex" with membrane phospholipids in that the methyl groups at positions 4′ and 8′ of alpha tocopherol fit into pockets created by the *cis* double bonds of the fatty acid. An alpha tocopherol deficiency, therefore, would cause inefficient packing of the polyunsaturated fatty acids and alter membrane structure. An alternative postulation for the mechanism of action of alpha tocopherol in maintaining membrane structure is based upon its antioxidant properties (2–6). Since membrane lipids decompose *via* a free radical peroxidation reaction, a lack of the antioxidant alpha tocopherol would allow random free radical membrane damage to occur.

Regardless of the mechanism involved, alpha tocopherol deficiency has been shown to affect membrane structure. Examples are the increased tendency of red cells to hemolyze (7, 8) and membrane damage to subcellular structure such as mitochondria and ribosomes (6). Changes in the GI membrane structure in humans as well as Peking ducklings deficient in alpha tocopherol were demonstrated (9). Normal membrane structure was observed after treatment with alpha tocopherol. A decrease in the active transport of valine in alpha tocopherol-deficient animals was reported (10).

Since alpha tocopherol deficiency generally occurs in cases of malabsorption or malnutrition, the absorption of nutrients or drugs might be affected by the deficiency state. It is not known whether the general changes observed in membrane structure lead to changes in the transport or absorption rates of drugs. The purpose of this study was to determine the effect of alpha tocopherol deficiency on the passive transport of several drugs through the intestinal membrane.

EXPERIMENTAL

Sprague–Dawley albino male rats[1], 156 ± 10 g, were divided into two groups. The animals were fed either an alpha tocopherol-deficient diet (deficient group) or the deficient diet supplemented with alpha tocopherol (control group). The dietary composition, food consumption, and growth patterns of these animals were described previously (11). The animals were found to be alpha tocopherol deficient 4–6 weeks after being placed on the diets (11), and the experiment was started after that time.

Intestinal Transport Rate Measurements—Rats from each group were fasted 20–24 hr prior to the experiment but were allowed free access to water. The animals were anesthetized with ether, and a midline abdominal incision was made. About 40 cm of the intestine was removed, prepared, and everted as described by Mayersohn and Gibaldi (12). After removal of the intestine, the animals were sacrificed by etherization. The intestine, after discarding the first 10 cm, was divided into two segments and placed into 100 ml of pregassed Kreb's bicarbonate buffer. The pH of the gassed buffer was 7.4 and remained constant throughout the experiment. The buffer contained either sodium salicylate[2] (1 mg/ml), barbital sodium[3] (0.25 mg/ml), or phenolsulfonphthalein[4] (reagent grade) (0.1 mg/ml). One milliliter of the buffer was placed into, and completely filled, the serosal portion of the sac. The entire preparation was maintained at 37° and continuously gassed with oxygen containing 5% (v/v) carbon dioxide. The serosal solution was sampled every 15 min and assayed for drug content.

The amount of drug transported by each segment was calculated and statistically compared within each group. Since differences in drug transport between the segments of each group were not observed, the data were combined and transport rates, expressed as clearance, were compared between groups. Clearance was calculated by dividing the combined amount of drug transported every 15 min by the mucosal concentration.

Salicylate was determined colorimetrically by the method of Trinder (13). Barbital was assayed spectrophotometrically by a modification of the method described by Brodie *et al.* (14) for pentobarbital. Two milliliters of serosal solution (1 ml of sample and 1 ml of washings) was mixed in a 50-ml glass-stoppered tube with 1

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Allied Chemical Corp., New York, N.Y.
[3] Merck and Co., Rahway, N.J.
[4] Fisher Chemical Co., Pittsburgh, Pa.

ml of 0.1 *N* HCl, 1 g of sodium chloride, and 12 ml of chloroform. The mixture was shaken mechanically at high speed for 1 hr and then centrifuged at 3000 rpm for 10 min. Ten milliliters of the chloroform layer was accurately measured and filtered through filter paper[5] into a glass-stoppered centrifuge tube containing 4 ml of pH 11 phosphate buffer. The filter paper was washed with 1 ml of chloroform, and the washing was added to the original filtrate.

The mixture was shaken mechanically for 20 min and then centrifuged. The absorption of the aqueous phase was determined at 240 nm against a similarly prepared blank. Phenolsulfonphthalein was assayed by mixing 2 ml of serosal solution (1 ml of sample and 1 ml of washings) with 1 ml of sodium hydroxide and 2 ml of Kreb's bicarbonate buffer. The mixture was centrifuged at 3500 rpm for 10 min, and the supernate was read spectrophotometrically at 560 nm against a similarly prepared reagent blank.

Urinary Excretion of Phenolsulfonphthalein after Oral Administration—Five rats from each group were fasted for 20 hr prior to drug dosing but allowed free access to water. The rats were lightly anesthetized with ether, and 1.5 ml of Kreb's bicarbonate buffer containing 1 mg/ml of phenolsulfonphthalein was administered directly into the stomach by oral intubation. Each animal was then placed into individual metabolic cages for urine collection and allowed free access to water. The cumulative amounts of urine excreted during the first 9 hr after dosing and then during the subsequent 15 hr were collected quantitatively.

The volume of each sample was measured, and phenolsulfonphthalein was assayed as described previously except that the supernate was filtered through a 0.22-μm cellulose filter. The solution was read spectrophotometrically against a similarly prepared 24-hr urine blank for each rat.

Pharmacokinetic Study of Barbital in Rats—*Oral Dosing*—Eight rats from each group, deficient and supplemented, weighing 350–400 g, were fasted for 20–24 hr prior to drug dosing but were allowed free access to water. Barbital sodium was dissolved in pH 6.8 phosphate buffer to produce a final concentration (pH 8.9) of 100 mg of drug/ml of buffer. The solution was administered to the animals by oral intubation at a constant dosage level of 200 mg/kg.

Blood samples were collected from the tail artery 5 min after administration of the drug and then at 15-min intervals for the 1st hr. Samples were also taken every hour for the next 3 hr and then every 12 hr for a total of 3 days. Each blood sample was centrifuged, and a 0.2-ml aliquot of the plasma was accurately transferred into a 50-ml glass-stoppered extraction tube. The plasma sample was frozen until assayed for drug content.

Intracardiac Dosing—The experiment was essentially the same as that described for the oral dosing, except that barbital sodium was injected intracardially. Blood samples were collected from the tail artery into heparinized tubes at 5-min intervals for 20 min, every 15 min for the subsequent 30 min, and then every 12 hr for a total of 3 days.

Determination of Barbital in Plasma—Plasma samples (0.2 ml) were mixed with 1 ml of pH 5.5 phosphate buffer, 1 g of sodium chloride, and 18 ml of chloroform. The process was then completed following essentially the same procedure used for determining barbital in the serosal solution of the everted gut, except for measuring and filtering 15 ml of the chloroform extract instead of 10 ml.

To determine the percent recovery of barbital from plasma, five samples of plasma, 0.2 ml each, were collected from rats not receiving the barbiturate for at least 2 weeks. Different concentrations of barbital, ranging from 2 to 12 μg/ml, were added to the plasma samples. This assay procedure was carried through using a plasma blank. Recovery of the drug was 98 ± 2.0%.

RESULTS AND DISCUSSION

Barbiturates are usually classified as short or long acting, according to the duration of their effects. It is assumed that the short-acting barbiturates are readily destroyed in the liver while the long-acting compounds are either eliminated through the kidneys or partly destroyed and partly eliminated (15). Barbital, the longest-acting barbiturate, is subjected to very slight, if any, metabolic breakdown. The drug is quantitatively excreted unmetabolized in dogs (16), 93% in mice (17), 95% or more in rats (18), 98% in guinea pigs (19), and 75–95% in humans after oral administration (20).

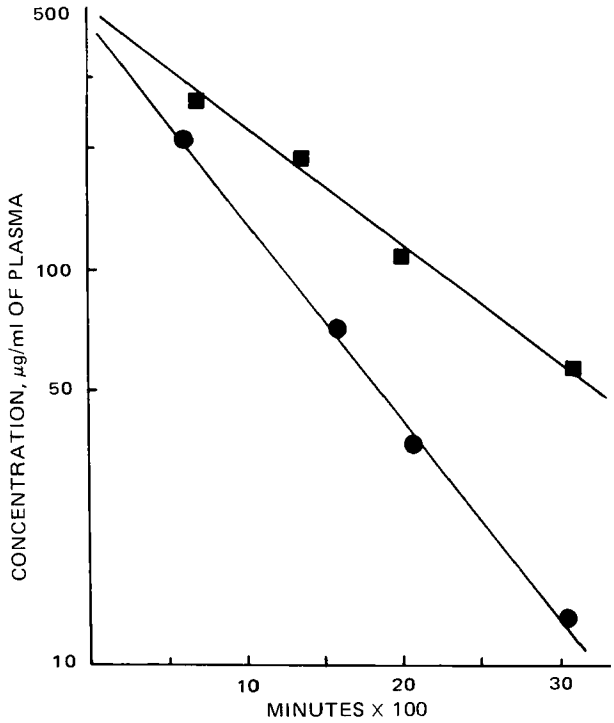

Figure 1—*Plasma concentration of barbital as a function of time following intracardiac administration of 200 mg/kg to rats. Key:* ●, *control; and* ■, *alpha tocopherol deficient.*

Barbital is also excreted very slowly; *i.e.*, 7–10 days is required for the complete excretion of a single dose by some mammals (21). This slow elimination rate, coupled with a zero value protein-binding capacity (22), implies that the drug is filtered through the kidney and is largely reabsorbed. Giotti and Maynert (21), by comparing barbital renal clearance to creatinine clearance, showed that barbital was excreted by glomerular filtration in dogs. By comparing barbital concentration in both the plasma and urine, these investigators proved that the drug was reabsorbed in the renal tubules as the free acid by a process of back-diffusion.

Being excreted almost unmetabolized, barbital was found to be the most suitable barbiturate for studying the effect of alpha tocopherol deficiency on drug absorption, distribution, and elimination, since it was reported that alpha tocopherol interferes with barbiturate metabolism (23).

A plot of the logarithm of plasma concentration *versus* time after intracardiac dosing of barbital to both the deficient and control groups (200 mg/kg) is presented in Fig. 1. The drug was distributed rapidly, and an equilibrium between blood and body tissues was achieved within 10 min after administration. The plasma level of barbital then declined slowly in a monoexponential fashion over 3–4 days. These data suggested that the pharmacokinetics of barbital in the rat can be adequately described by a one-compartment open model (Scheme I):

$$D_B \xrightarrow{K_E} D_C$$

Scheme I

where D_B is the amount of barbital in the body at any time t, D_C is the amount of barbital eliminated from the body at any time t, and K_E is the apparent first-order overall elimination rate constant. Since barbital is eliminated only by excretion in the urine, K_E is the first-order excretion rate constant of barbital through the kidney.

By definition, $D_B = (V_d)(c)$, where V_d is the apparent volume of distribution of barbital, and c is the plasma concentration of the drug at any time t.

The apparent elimination rate constant in each rat was calculated from the slope of the least-squares fit of log plasma concentration *versus* time plots. The apparent volume of distribution of bar-

[5] Whatman No. 1.

Table I—Calculated Pharmacokinetic Parameters for Barbital after Intracardiac Administration[a]

Parameter	Control Group	Alpha Tocopherol-Deficient Group	Significance Level of Difference between Groups[b]
K_E, min^{-1}	0.0010 (0.0001)	0.0006 (0.0001)	S ($p < 0.05$)
V_d, ml	214.10 (34.8)	214.40 (39.0)	NS ($p > 0.05$)
Clearance, ml/min	0.227 (0.060)	0.127 (0.031)	S ($p < 0.05$)

[a] Standard deviation in parentheses. [b] The Student t test. S = significant, and NS = not significant.

bital was calculated by dividing the intracardiac dose (D) by c_0, the plasma concentration extrapolated to time zero:

$$V_d = D/c_0 \qquad \text{(Eq. 1)}$$

Total body clearance was obtained from the relationship:

$$\text{clearance} = V_d K_E \qquad \text{(Eq. 2)}$$

The values of K_E, V_d, and clearance for each group of rats were analyzed statistically (Table I). Although the mean volume of distribution was not statistically different between the two groups, there existed a significant difference in the overall elimination rate constant. In the alpha tocopherol-deficient animals, barbital elimination was at a slower rate than with the control animals. Since the kidney is the main route of elimination for barbital, any change in the elimination rate constant could be attributed to changes in the kidney function as a consequence of alpha tocopherol deficiency. Alpha tocopherol deficiency caused degenerative changes in the kidney tubules of rats (24–28). Such a decrease in the elimination rate constant can then be explained by a decrease in glomerular filtration and/or an increase in the drug's tubular reabsorption. The renal tubular epithelium, like the GI epithelium, is lipoid in nature; it is possible that the barrier membrane responsible for glomerular filtration and tubular reabsorption might be influenced by alpha tocopherol deficiency.

The absence of any difference in the volume of distribution between the deficient and the supplemented animals indicated that barbital was distributed throughout the body to the same extent in both groups. However, a difference in the rate of penetration to various tissues might exist.

Since the value of K_E was different between the groups while the V_d was unchanged, the clearance was significantly different between the groups (Table I). The plasma of alpha tocopherol-deficient animals was cleared of drug at a slower rate than that of the control animals. This result underlines the role of alpha tocopherol deficiency in drug clearance through the kidney, a parameter that could influence the duration of the pharmacological effect.

To determine the effect of alpha tocopherol deficiency on the absorption of barbital, a 200-mg/kg dose was administered orally.

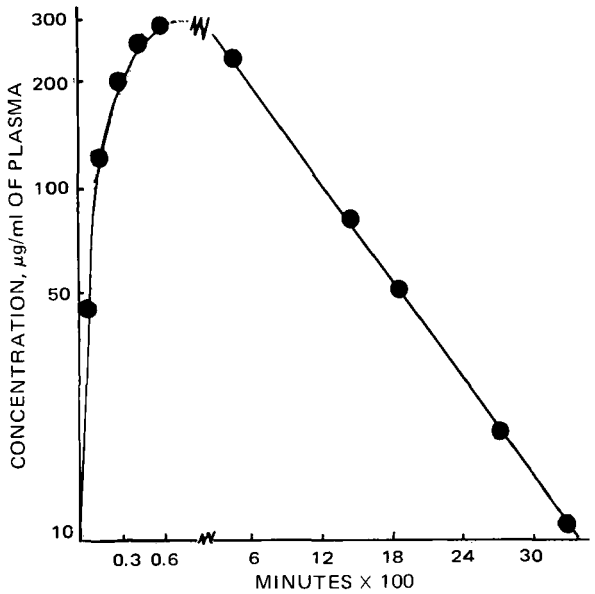

Figure 2—*Plasma concentration of barbital as a function of time following oral administration of 200 mg/kg to control rats.*

The overall elimination rate constant and the volume of distribution were determined and compared to those calculated after intracardiac administration. Two other important parameters that reflect the efficiency of the GI absorption of barbital were also considered. One parameter, the plasma drug concentration peak height (C_{max}), reflects the intensity as well as the duration of pharmacological effect. The other parameter, the time of occurrence of the peak height concentration (T_{max}), reflects the onset of occurrence of the drug's pharmacological effect.

After oral administration, the plasma barbital concentration–time data were plotted semilogarithmically (Fig. 2). The drug entered the plasma rapidly, reaching a maximum level and then exhibiting a slow exponential decline. The shape of the curve indicated that the pharmacokinetics of barbital could be described, as in the case of the intracardiac dosing, by a one-compartment open model (Scheme II):

$$D_A \xrightarrow{K_a} D_B \xrightarrow{K_E} D_C$$

Scheme II

where D_A is the amount of barbital in the GI fluid at any time t, and K_a is the first-order absorption rate constant of the drug from the GI fluids. All other parameters were as previously described. The plasma barbital concentration, c, at time t is determined by integrating the equation resulting from Scheme II, which gives:

$$c = \left(\frac{FD}{V_d}\right)\left(\frac{K_a}{K_a - K_E}\right)\{\exp[-K_E(t)] - \exp[-K_a(t)]\} \qquad \text{(Eq. 3)}$$

where F is the fraction of the oral dose (D) absorbed, and all other parameters are as previously defined. The absorption rate constant, K_a, was graphically determined for each individual rat by the method of feathering. The elimination rate constant, K_E, was calculated from the slope of the terminal elimination phase. The data were then fitted with the aid of a digital computer, nonlinear regression analysis program (NONLIN). Excellent computer fits to the experimental data were obtained using Eq. 3 for a one-compartment open model (Fig. 2).

The time of occurrence of the peak height and peak concentration for both groups is found using Eqs. 4 and 5, respectively:

$$T_{max} = \frac{\ln(K_a/K_E)}{K_a - K_E} \qquad \text{(Eq. 4)}$$

$$C_{max} = \frac{FD\, e^{-K_E T_{max}}}{V_d} \qquad \text{(Eq. 5)}$$

Table II represents the mean value of the computer-generated nonlinear least-squares regression estimates of K_a, K_E, and V_d/F for each group of animals. The calculated mean values of T_{max} and C_{max} and the statistical analysis of all parameters obtained for both groups of animals are included in the table. An examination of the mean absorption rate constants revealed a significant difference between the alpha tocopherol-deficient and control animals. The deficient group absorbed barbital at a rate twice that of the control animals. In addition, the deficient animals eliminated the drug at a slower rate.

No statistical difference was observed between the groups with respect to V_d/F. A comparison of the elimination rate constant, K_E, obtained after intracardiac (Table I) and oral dosing revealed no difference in this parameter. The value of V_d/F obtained from the computer-generated nonlinear least-squares regression after oral administration was not statistically different from the value of V_d calculated from the least-squares fit of log plasma concentration *versus* time after intracardiac dosing. This finding can be true only if F is equal to one, since the volume of distribution of a drug is constant and independent of the route of administration. To prove that F in Eq. 2 is unity, the area under the plasma concen-

Table II—Calculated Pharmacokinetic Parameters for Barbital after Oral Administration[a]

Parameter	Control Group	Alpha Tocopherol-Deficient Group	Significance Level of Difference between Groups[b]
K_a, min^{-1}	0.017 (0.004)	0.037 (0.014)	S ($p < 0.05$)
K_E, min^{-1}	0.0011 (0.0001)	0.0008 (0.00015)	S ($p < 0.05$)
V_d/F, ml	223.5 (26.0)	213.8 (28.5)	NS ($p > 0.05$)
T_{max}, min	179.96 (42.46)	116.0 (42.42)	S ($p < 0.05$)
C_{max}, μg/ml	287.64 (52.36)	331.22 (51.54)	S ($p < 0.05$)

[a] Standard deviation in parentheses. [b] The Student t test. S = significant, and NS = not significant.

tration–time curve for a representative number of orally dosed amounts was calculated using Simpson's rule. The fraction of the dose absorbed, F, was calculated using:

$$\text{area under the curve} = \frac{FD}{V_d K_E} \qquad \text{(Eq. 6)}$$

The fraction of the dose absorbed, F, was equal to unity, indicating that while the rate of absorption was faster in the deficient compared to the control group, the extent of absorption was the same. This result also indicated that barbital was not subjected to any metabolic process in the GI tract or in its first or subsequent passes through the liver.

Table II shows that T_{max} for the deficient group was significantly shorter than that of the control group, obviously due to the higher rate of absorption in the deficient animals. It was found that C_{max} was significantly higher in the deficient group. This finding implies that the pharmacological effect of barbital would be of a higher intensity and longer duration in the deficient than in the supplemented animals.

When unmetabolized drugs, like barbital and phenolsulfonphthalein, are administered orally, their plasma levels are a function of three factors: (*a*) the rate of absorption from the GI tract, (*b*) the rate of diffusion from the blood to other tissues, and (*c*) the rate of excretion from the blood out of the body, mainly in the urine. The present study showed that alpha tocopherol deficiency caused an increase in the absorption rate of barbital (Table II). A reasonable explanation is that a change in the structural integrity and stability of the GI epithelium resulted as a consequence of alpha tocopherol deficiency. This assumption is supported by the barbital everted gut transport study, where increased barbital permeability was observed in the alpha tocopherol-deficient animals.

To confirm the effect of alpha tocopherol deficiency on drug absorption and transport, phenolsulfonphthalein was used as a marker compound. Phenolsulfonphthalein was chosen because it is absorbed mainly from the small intestine by a simple diffusion process. It is a highly charged molecule and, as such, is poorly absorbed. Therefore, the rate of phenolsulfonphthalein absorption would be sensitive to changes in membrane structure. In addition, it is easily assayed in biological fluids and has been used to test the effect of several compounds, such as edetic acid (29, 30) and bile salt (31, 32), on GI permeability in rats. Since it is eliminated essentially unmetabolized in the urine, the amount excreted depends on the amount of phenolsulfonphthalein absorbed. A measure of the rate of phenolsulfonphthalein excreted in the urine would then reflect its rate of absorption.

The amount of phenolsulfonphthalein excreted in the urine after oral dosing to both groups, control and deficient, is presented in Table III. In the first 9 hr after dosing, the amount of phenolsulfonphthalein excreted by the deficient group was more than double that excreted by the control group. However, there was no significant difference in the amount of drug excreted by both groups in the subsequent 15-hr period. The total amount excreted in 24 hr after dosing was 62% more in the deficient group than in the control group.

Table III—Urinary Excretion of Phenolsulfonphthalein in Alpha Tocopherol-Deficient and Control Groups after Oral Administration[a]

Hours	Deficient Group	Control Group	Significance Level of Difference between Groups[b]
0–9	48.18 ± 9.77	24.76 ± 7.49	S ($p < 0.005$)
9–24	9.19 ± 1.78	10.53 ± 2.34	NS ($p > 0.05$)
Total in 24	57.38 ± 8.38	35.29 ± 7.81	S ($p < 0.05$)

[a] Average of five rats in micrograms ± *SD*. [b] The Student t test. S = significant, and NS = not significant.

Passive absorption occurs mostly in the small intestine, where the surface area is large, and gradually decreases in the more distal portion of the intestine, where the surface area decreases and the viscosity of the intestinal contents increases. Therefore, phenolsulfonphthalein absorption is expected to occur mainly in the small intestine during the first 9 hr after administration. The larger amount of phenolsulfonphthalein excreted in the urine of the deficient group in that period reflected the greater extent of absorption of this compound. In the subsequent 15-hr period, some phenolsulfonphthalein was absorbed while the unabsorbed drug passed through the GI tract. This additional amount absorbed was small compared to that absorbed during the first 9 hr.

It is clear from the data in Table III that the amount of phenolsulfonphthalein excreted in the urine during the subsequent 15-hr period was not significantly different between both groups. One possible explanation for this observation is that other factors, such as diffusion through the viscous medium present in the large intestine, might interfere with the absorption process. Examination of the intestinal contents of rats fasted for 24 hr revealed appreciable amounts of semisolid and solid material in the more distal segments of the intestine. Since the rate of passive absorption is a function of the diffusion coefficient which, in turn, is inversely proportional to viscosity, one would expect that unabsorbed drug incorporated in this semisolid viscous mass would diffuse to the absorption site at a slower rate compared to that found in the proximal portion of the intestine.

In addition, the surface area for absorption found in the distal segments of the intestine is much smaller than that of the proximal segments. Due to the smaller absorptive surface, factors affecting membrane structure would not be expected to have as dramatic an effect on absorption through the distal as compared to the proximal segments of the intestine. These facts allow one to speculate that the phenolsulfonphthalein absorption differences between the control and deficient animals during the period when

Table IV—Phenolsulfonphthalein Transport as a Function of Time through the Everted Gut[a]

Minutes	Amount Transported[b], μg	Clearance, ml/15 min
	Alpha Tocopherol-Deficient Group	
15	3.09 (0.45)[c]	0.03
30	6.57 (0.65)	0.07
45	10.84 (1.74)	0.11
60	16.51 (2.59)	0.17
75	19.37 (3.08)	0.19
90	21.96 (2.65)	0.22
	Control Group	
15	2.26 (0.26)	0.02
30	5.38 (0.60)	0.05
45	11.25 (1.77)	0.11
60	14.56 (1.15)	0.15
75	17.83 (2.03)	0.18
90	20.21 (3.38)	0.20

[a] Average of six rats in each group; mucosal concentration of phenolsulfonphthalein was 0.1 mg/ml of Kreb's bicarbonate buffer. [b] Values are significantly different only at 15 and 30 min ($p < 0.05$). [c] Standard deviation in parentheses.

Table V—Barbital Transport as a Function of Time through the Everted Gut[a]

Minutes	Amount Transported[b], μg	Clearance, ml/15 min
	Alpha Tocopherol-Deficient Group	
15	55.24 (5.18)[c]	0.22
30	59.89 (6.27)	0.24
45	58.88 (7.39)	0.24
60	66.37 (8.83)	0.27
75	64.88 (10.47)	0.26
90	67.28 (5.72)	0.27
		Mean = 0.25 (0.02)
	Control Group	
15	46.35 (10.02)	0.19
30	53.37 (4.79)	0.21
45	53.54 (7.16)	0.21
60	57.95 (8.39)	0.23
75	55.88 (8.50)	0.22
90	58.24 (7.48)	0.23
		Mean = 0.22 (0.02)

[a] Average of five rats in each group; mucosal concentration of barbital sodium was 250 μg/ml of Kreb's bicarbonate buffer. [b] Significant difference between both groups at all 15-min intervals ($p < 0.05$) except the 45-min period. [c] Standard deviation in parentheses.

unabsorbed drug should be in the more distal portions of the intestine (9–24 hr) are probably masked by a decreased rate of diffusion to the absorptive site.

Table III shows that the cumulative amount of phenolsulfonphthalein excreted in the urine in the 24-hr period was higher in the deficient group than in the supplemented animals. Feldman *et al.* (31) reported that phenolsulfonphthalein absorption, as evidenced by urinary excretion studies, occurs within 24 hr and that the urine is completely free of the compound after 24 hr. In this study, the cumulative amount of phenolsulfonphthalein excreted in the urine by the control animals was within the range reported by these investigators and the extent of absorption was significantly greater in the deficient than in the control animals.

Although the results suggest that the observed increase in passive GI absorption of phenolsulfonphthalein in the alpha tocopherol-deficient animals is due to changes in membrane permeability, other factors may partially account for the results. These factors include altered stomach emptying, GI secretion, bile or blood flow, and kidney function. It was, therefore, decided to measure the intestinal transport of this compound in the absence of these variables, *i.e.*, *via* the *in vitro* everted gut preparation. The results (Table IV) show that phenolsulfonphthalein was transported significantly faster through the everted gut of the deficient animals as compared to the controls during the first 30 min of the experiment. Thereafter, differences in transport could not be detected.

Table VI—Salicylate Transport as a Function of Time through the Everted Gut[a]

Minutes	Amount Transported[b], mg	Clearance, ml/15 min
	Alpha Tocopherol-Deficient Group	
15	0.234 (0.03)[c]	0.23
30	0.270 (0.01)	0.27
45	0.290 (0.01)	0.29
60	0.296 (0.02)	0.30
75	0.315 (0.02)	0.32
90	0.340 (0.04)	0.34
		Mean = 0.29 (0.03)
	Control Group	
15	0.235 (0.02)[c]	0.24
30	0.285 (0.02)	0.29
45	0.278 (0.02)	0.28
60	0.307 (0.01)	0.31
75	0.314 (0.02)	0.31
90	0.344 (0.03)	0.34
		Mean = 0.29 (0.03)

[a] Average of five rats in each group; mucosal concentration of salicylate was 1 mg/ml of Kreb's bicarbonate buffer. [b] No significant difference between groups. [c] Standard deviation in parentheses.

Table VII—Comparative Data for Clearance of Salicylate, Barbital, and Phenolsulfonphthalein in Alpha Tocopherol-Deficient and Control Animals

Drug	pKa	Partition[a] Coefficient	Group	Clearance 0–30 min
Salicylate	3.0	2.9	Deficient	0.50
			Control	0.52
Barbital	7.2	0.7	Deficient	0.46[b]
			Control	0.40
Phenol-sulfonphthalein	2 7.9	0.0001	Deficient	0.10[b]
			Control	0.08

[a] Partition coefficient of the undissociated compound between chloroform–water (36, 38). [b] Significantly different from control group; the Student *t* test ($p < 0.05$).

One criterion for adequate characterization of the transfer of compounds across the everted gut is that the viability of the epithelium must be known throughout the entire experiment. In a histological evaluation of the everted gut technique for studying drug transport, the intestinal sacs of rats were morphologically intact after eversion but progressively lost their structural integrity (32). The effect of such structure changes on the transport of drugs through the everted gut was evaluated (33); the clearance of poorly lipid-soluble compounds such as methyl orange showed a continual increase in clearance over a 2-hr period. This finding is supported in the present study.

Phenolsulfonphthalein is a lipid-insoluble compound and is transported very slowly, mainly through pores or aqueous channels present in the GI membrane (34–36). Therefore, progressive decomposition changes in the everted gut preparation would reflect a progressive increase in the normally low transport rate of phenolsulfonphthalein. Biochemical changes in membrane structure due to alpha tocopherol deficiency might also produce significant changes in the transport. The differences in phenolsulfonphthalein transport observed during the first 30 min between the deficient and control groups apparently can be attributed to differences in membrane integrity due to the vitamin deficiency. After 30 min, the everted gut degenerates such that differences in membrane permeability, induced by the deficiency, are masked.

Drugs that have a somewhat greater lipid solubility were transported across the everted gut at a constant rate for at least 2 hr (33). Since these compounds are transported *via* a partitioning process involving the lipid components of the membrane, changes in aqueous pore volumes or diameters should not appreciably affect the overall absorption processes as compared to substances such as phenolsulfonphthalein. Barbital, a drug classified (37) as moderately transported, passed through the everted gut at a constant rate for the entire 90-min experimental period (Table V). During each time period (except the 45-min period), the transport of barbital through the everted gut of the alpha tocopherol-deficient animals was greater than that of the control group, indicating a definite effect of alpha tocopherol deficiency on GI membrane permeability.

Rapidly transported drugs such as salicylate (33, 37) would not be expected to be sensitive to changes in membrane structure due to alpha tocopherol deficiency. Slight changes in permeability of the everted gut preparation would cause comparatively slight increases in the normally high transport rate of salicylate. The data in Table VI show that salicylate transport, like barbital, did not change during the experiment.

A comparison of the *in vitro* transport characteristics of the compounds studied showed the following rank order: salicylate > barbital > phenolsulfonphthalein. Table VII represents the comparative data for clearance of the three compounds during the first 30 min of the experiment, as well as their pKa and partition coefficients. The clearance data for these substances seem to correlate with their partition coefficients.

The *in vitro* data also suggest that the changes in membrane structure induced by the deficiency state are different from that observed after *in vitro* membrane decomposition. The transport of rapidly absorbed drugs appears to be the least affected by alpha tocopherol deficiency. This finding seems reasonable intuitively

since it is difficult to see increases in the rate of transport of substances for which the GI membrane ordinarily offers little resistance.

Based upon the increased rate of barbital absorption *in vivo*, the increased rate of barbital transport *in vitro*, the increased *in vitro* transport observed for phenolsulfonphthalein through the everted gut of alpha tocopherol-deficient animals, and the enhanced excretion of phenolsulfonphthalein after oral administration in the same animals, it appears that morphological changes in membrane structure previously observed with alpha tocopherol-deficient animals and humans result in increased membrane permeability, at least to the drugs used in this study. The significance of these changes in terms of pharmacological effect is currently under study.

REFERENCES

(1) J. A. Lucy, *Ann. N.Y. Acad. Sci.*, **203**, 4(1972).

(2) A. L. Tappel, *Vitam. Horm.*, **20**, 493(1962).

(3) L. A. Witling, *Progr. Chem. Fats Lipids*, **9**, 519(1970).

(4) M. K. Horwitt, *Fed. Proc.*, **24**, 68(1965).

(5) A. L. Tappel, in "The Fat Soluble Vitamins," H. F. DeLuca and J. W. Suttie, Eds., University of Wisconsin Press, Madison, Wis., 1969, pp. 369–373.

(6) A. L. Tappel, *Ann. N.Y. Acad. Sci.*, **203**, 12(1972).

(7) R. E. Heikkila, J. A. Mezick, and D. G. Cornwell, *Physiol. Chem. Phys.*, **3**, 93(1971).

(8) S. Gross and D. K. Melhorn, *Ann. N.Y. Acad. Sci.*, **203**, 141(1972).

(9) I. Molenaar, F. A. Hommes, W. G. Braams, and H. A. Polman, *Proc. Nat. Acad. Sci. USA*, **61**, 982(1968).

(10) R. H. Imami, S. Reiser, and P. A. Christiansen, *J. Nutr.*, **100**, 101(1970).

(11) M. M. Meshali and C. H. Nightingale, *J. Pharm. Sci.*, **63**, 1084(1974).

(12) M. Mayersohn and M. Gibaldi, *ibid.*, **60**, 225(1971).

(13) P. Trinder, *Biochem. J.*, **57**, 301(1954).

(14) B. B. Brodie, J. J. Burns, L. C. Mark, P. A. Lief, E. Bernstein, and E. M. Papper, *J. Pharmacol. Exp. Ther.*, **108**, 26(1953).

(15) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1970, pp. 98–120.

(16) E. W. Maynert and H. B. Van Dyke, *J. Pharmacol. Exp. Ther.*, **98**, 184(1950).

(17) A. Dorfman and L. R. Goldbaum, *ibid.*, **90**, 330(1947).

(18) A. G. Ebert, G. K. W. Yim, and T. S. Miya, *Biochem. Pharmacol.*, **13**, 1267(1964).

(19) J. J. Burns, C. Evans, and N. Trousof, *J. Biol. Chem.*, **227**, 785(1957).

(20) P. Lous, *Acta Pharmacol. Toxicol.*, **10**, 147(1954).

(21) A. Giotti and E. W. Maynert, *J. Pharmacol. Exp. Ther.*, **101**, 296(1951).

(22) A. Goldstein, L. Aronow, and S. M. Kalman, "Principles of Drug Action," Harper and Row, New York, N.Y., 1969, pp. 106–205.

(23) N. J. Giarman, G. N. Bowers, P. G. Quie, and L. J. Hampton, *Arch. Int. Pharmacodyn. Ther.*, **97**, 473(1954).

(24) A. J. P. Martin and T. Moore, *Chem. Ind.*, **57**, 973(1938).

(25) A. J. P. Martin and T. Moore, *J. Hyg.*, **39**, 643(1939).

(26) T. Moore, *Chem. Ind.*, **58**, 651(1939).

(27) V. M. Emmel, *J. Nutr.*, **61**, 51(1957).

(28) V. M. Emmel and P. L. LaCelle, *ibid.*, **75**, 335(1961).

(29) C. S. Tidball, *Amer. J. Physiol.*, **206**, 243(1964).

(30) M. M. Cassidy and C. S. Tidball, *J. Cell Biol.*, **32**, 685(1967).

(31) S. Feldman, M. Salvino, and M. Gibaldi, *J. Pharm. Sci.*, **59**, 705(1970).

(32) R. R. Levine, W. F. McNary, P. J. Kornguth, and R. LeBlanc, *Eur. J. Pharmacol.*, **9**, 211(1970).

(33) M. Gibaldi and B. Grundhofer, *J. Pharm. Sci.*, **61**, 116(1972).

(34) H. Kunze and W. Vogt, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, **256**, 139(1967).

(35) H. Kunze, *ibid.*, **259**, 260(1968).

(36) C. S. Tidball, *Amer. J. Physiol.*, **206**, 239(1964).

(37) C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **125**, 275(1959).

(38) L. S. Schanker, P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *ibid.*, **120**, 528(1957).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 5, 1974, from the *School of Pharmacy, University of Connecticut, Storrs, CT 06268*

Accepted for publication May 7, 1975.

Supported by Grant 107 from the University of Connecticut Research Foundation and by a grant from Lederle Laboratories, Pearl River, N.Y.

* Present address: University of Assiut, Assiut, Egypt.

ˣ To whom inquiries should be directed.

Automated *In Vitro* Dissolution Rate Analysis of Potassium in Plastic Matrix Slow Release Tablets

ALF ENGDAHL *, BO KARLBERG ˣ, and SIDSEL THELANDER

Abstract □ A fully automated system for dissolution rate analysis of potassium in slow release tablets is described. Aliquots are removed after 1, 2, and 4 hr from six samples, and potassium is analyzed in a flame photometer at 768 nm. A complete study of six samples takes 5.5 hr. The system may be run overnight. During the time intervals between the removal of aliquots, the system can be used for the determination of the total assay of tablets.

Keyphrases □ Potassium chloride—slow release tablets, dissolution rate analysis by automated system □ Dissolution rate analysis—potassium chloride slow release tablets, automated system □ Automated analysis—dissolution rate of potassium chloride slow release tablets

Potassium chloride tablets are often administered in the form of sustained-release preparations (1, 2). The dissolution rates of 12 different potassium chloride tablets were measured using a potassium selective glass electrode (2). The selectivity for potassium ions over other ions is poor; sodium and hydrogen ions in particular interfere strongly (3). Fortunately, sodium ions are not present; in the *in vitro* methods