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EFFECT OF POLYSORBATE 80 ON THE APPARENT PARTITION COEFFICIENT OF DRUGS AND ON THEIR INTESTINAL ABSORPTION IN THE RAT
II. PHENOBARBITAL

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SUMMARY

The effect of polysorbate 80 on the apparent partition coefficient of phenobarbital between chloroform and a 0.05 M Tris buffer of pH 5.9 were investigated. Three different concentrations of the surfactant were studied; namely 0.001, 0.01 and 1.0% (w/v). The effect of the surfactant in the same medium and at the same concentrations on the absorption of phenobarbital from the rat intestine in situ was also investigated.

The apparent partition coefficient of phenobarbital decreased in the presence of polysorbate 80, reaching a minimum at the CMC, then increased markedly at higher concentration.

Polysorbate 80 at or below the CMC did not produce a significant effect on the per cent phenobarbital absorbed from the rat intestine in 30 min. Higher concentration of polysorbate 80 produced a significant increase in the per cent absorbed. The mechanisms involved in the effect on apparent partition coefficient as well as on the per cent absorbed are discussed.

INTRODUCTION

It has previously been demonstrated (Hikal et al., 1976) that polysorbate 80, in concentrations equal to or higher than the critical micelle concentration (CMC), significantly increased the apparent partition coefficient of salicylic acid. The same study also showed that polysorbate 80 had no significant effect on the absorption of salicylic acid from the rat intestine in situ.

The present work is a continuation of the previous study using a different drug, and a slightly different technique. The purpose of this investigation is to elucidate whether the effect observed with salicylic acid in an alkaline medium (Hikal et al., 1976) could also be observed with a weaker acid, namely phenobarbital, present in an acidic medium. More

studies are presently underway aimed at understanding the mechanisms involved and possible correlation with the physicochemical properties of the drugs.

EXPERIMENTAL

All chemicals were of USP, NF, or Analytical Reagent quality and were used without further purification.

Determination of the apparent partition coefficient

The procedure described previously (Hikal et al., 1976) was followed in this study with the following modifications: the aqueous phase consisted of a 0.05 M Tris buffer adjusted with HCl to pH 5.9 ± 0.02, and containing 0.0, 0.001, 0.01, or 1.0% (w/v) of polysorbate 80 [1]; the volume of the aqueous and of the organic phases was 25 ml; 3 different amounts of phenobarbital [2], namely, 10, 25 and 50 mg were used for each concentration of surfactant; and all solutions were shaken [3] at 250 rpm for 7 days at room temperature. The amount of phenobarbital in the aqueous phase was determined as described below.

Absorption of phenobarbital from the rat intestine in situ

The rats used were male albinos weighing 120–150 g which were fasted for 24 h, water being allowed ad libitum. The rat was anesthetized with ether, and the intestine exposed by a midline abdominal incision. The small intestine was ligated with silk suture at the duodenal and the cecal junctions. About 5 mm from the ligature, the intestine was slit open, a polyethylene cannula [4] was inserted at each end and secured in place with silk suture. The intestine was rinsed out with 100–150 ml of saline warmed up to 37°C. The intestine was cleared by injecting approximately 30 ml of air into it, followed by gentle manual pressure. Ten ml of the drug solution was measured into a syringe fitted with a polyethylene tube [4] which was narrower and about 2 cm longer than the cannula already attached to the duodenal end of the intestine. The polyethylene tube was inserted through the duodenal cannula into the intestine, the cannula was then pulled out of the intestine, and the suture was tightened around the polyethylene tube. Eight ml of drug solution was first introduced; the distal cannula was pulled out, and the intestine was tied securely, then the rest of the drug solution was introduced and timing started. In order to pull out the polyethylene tube without losing any of the solution, the intestine was pressed gently between the thumb and index finger of one hand just below the suture, while the tube was pulled out with the other hand. The suture was tightened around the intestine before pressure was released. The intestine was returned to the abdominal cavity, and the incision was closed with wound clips [5]. The time elapsing between introduction of the drug solution and closure of the incision was less than 2 min. Throughout this

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[1] Atlas Chemical Industries, Wilmington, Dela. U.S.A.
[2] Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.
[3] Eberbach Shaker, Eberbach Corporation, Ann Arbor, Mich. U.S.A.
[4] Intramedic PE 320, Clay Adams, Parsippany, N.J., U.S.A.
[5] Autoclips 9 mm stainless steel, Clay Adams, Parsippany, N.J., U.S.A.

TABLE 1

APPARENT PARTITION COEFFICIENT OF PHENOBARBITAL IN PRESENCE OF POLYSORBATE 80 (CHLOROFORM/0.05 M TRIS BUFFER AT pH 5.9)

% polysorbate 80 (w/v)	Apparent partition coefficient *
None	3.78 ± 0.14
0.001	3.43 ± 0.19
0.01	3.01 ± 0.22 **
1.00	5.32 ± 0.33 **

* Mean of 3 determinations ± S.D.

** Significantly different from control ($P < 0.01$).

procedure care was taken to maintain the integrity of the intestine and its blood supply. An infra-red lamp was used keep the animal warm.

At the end of 30 min, the wound clips were removed; the intestine segment was also removed and rinsed in normal saline. The intestinal contents were collected in a 50 ml graduated centrifuge tube. The intestine was rinsed with 10 ml of normal saline, and completely emptied by squeezing gently between the thumb and index finger. The combined liquids were treated with 1 ml of a 30% solution of trichloroacetic acid, adjusted to 20 ml with normal saline, and then centrifuged [6] at 4500 rpm for 15 min. Five ml of the supernatant liquid was further purified by filtration through a membrane filter [7].

One ml of the clear filtrate was diluted with 9 ml of an alkaline borate buffer of pH 9.6, and the absorbance was measured at 240 nm [8].

Six rats were used in each phase of the study. The drug solutions were prepared by dissolving 100 mg of sodium phenobarbital [2] in approximately 80 ml of the vehicle, adjusting the pH to 5.9 ± 0.02 and then adjusting the volume to 100 ml. The vehicles used were: (1) 0.05 M Tris buffer of pH 5.9; and (2) 0.05 M Tris buffer of pH 5.9 containing 0.001, 0.01 (equal to the CMC) or 1.0% (w/v) polysorbate 80.

Student's *t*-test was used to determine the significance of the differences observed in the apparent partition coefficients as well as in the per cent absorbed in 30 min.

RESULTS

Values of the apparent partition coefficient of phenobarbital in 0.05 M Tris buffer at pH 5.9 and at various concentrations of polysorbate 80 are listed in Table 1. The apparent partition coefficient seems to decrease in the presence of polysorbate 80 until it reaches a minimum value at the CMC, then it increases markedly at higher concentrations. The decrease in the presence of 0.001% (w/v) polysorbate 80 was found to be statistically not significant ($P > 0.05$, t value for df = 4 was 2.10). However, the decrease in the pres-

[6] IECHN-S Centrifuge, Damon/IEC Division, Needham, Mass. U.S.A.

[7] Millipore, Bedford, Mass., U.S.A.

[8] Perkin-Elmer Coleman 124, double beam spectrophotometer, Hitachi, Tokyo, Japan.

TABLE 2

PER CENT PHENOBARBITAL ABSORBED IN 30 MIN FROM THE RAT INTESTINE IN SITU

Rat weight (g)	Polysorbate 80 (w/v)	Per cent absorbed	Mean ± S.D.
146	None	75.5	75.6 ± 4.88
135		76.1	
134		75.3	
128		77.7	
148		67.1	
139		82.1	
124	0.001	79.5	78.1 ± 2.99
134		77.9	
124		78.9	
129		76.5	
133		73.5	
137		82.4	
125	0.01	74.7	75.3 ± 1.85
141		75.8	
142		76.7	
134		74.3	
133		77.7	
128		72.5	
133	1.0	83.4	84.2 ± 2.80 *
126		83.3	
125		84.8	
140		84.3	
127		80.4	
135		89.0	

* Significantly different from control ($P < 0.05$).

ence of 0.01 per cent w/v and the increase in the presence of 1.0 per cent w/v surfactant both were statistically significant ($P < 0.01$, t-values for df = 4 were 4.18 and 6.08, respectively).

Table 2 lists the per cent phenobarbital absorbed in 30 min in the presence of various concentrations of polysorbate 80. The presence of 0.001 or 0.01% (w/v) surfactant did not produce any significant change in the per cent phenobarbital absorbed ($P > 0.05$, t-values for df = 10 were 0.98 and 0.13, respectively). A 1.0% (w/v) surfactant produced a slight but significant increase in the per cent phenobarbital absorbed in 30 min ($P < 0.05$, t-value for df = 10 was 3.42).

DISCUSSION AND CONCLUSION

Polysorbate 80 at concentrations equal to or higher than the CMC has previously been shown to produce a significant increase in the apparent partition coefficient of salicylic acid (Hikal et al., 1976). A possible micellar complexation with the salicylate anion was

postulated. The present data (Table 1) show a significant increase in the apparent partition coefficient of phenobarbital in the presence of 1.0% (w/v) surfactant. It is possible, therefore, that a micellar complex is formed between polysorbate 80 and phenobarbital which is more soluble in the lipid phase than the uncomplexed drug. Having a pK_a of 7.41 (Martin et al., 1969), phenobarbital would be better than 96% undissociated at pH 5.9, and any complexation would, therefore, involve the undissociated acid. Non-ionic surfactants have previously been shown to form micellar complexes with undissociated salicylic acid (Levy and Reuning, 1964) and secobarbital (Levy and Anello, 1968).

The decrease in the apparent partition coefficient of phenobarbital in the presence of surfactant concentrations equal to or lower than the CMC might be an indication of a different type of complex, namely, one between the drug and the surfactant monomer. Unlike the micellar complex, the drug–surfactant monomer complex seems to keep the drug in the aqueous phase, thus reducing its apparent partition coefficient. The possibility of a non-micellar complex formation between polysorbate 80 and another barbiturate, secobarbital, has previously been raised (Levy et al., 1966). The present data also indicate that complexation between phenobarbital and surfactant monomers increases with increasing surfactant concentration, reaching a maximum at the CMC. It is also likely that the net effect observed above the CMC is the resultant of two opposing effects, namely, monomer complexation keeping the drug in the aqueous phase and micellar complexation pushing it into the lipid phase.

Intestinal absorption data (Table 2) show that polysorbate 80 at 0.001 and 0.01% (w/v) did not produce any significant change in the per cent phenobarbital absorbed in 30 min. Similar concentrations of polysorbate 80 have previously failed to produce any significant change in the per cent salicylic acid absorbed in the rat intestine (Hikal et al., 1976). Complex formation between drugs and surfactants, therefore, does not necessarily have to influence drug absorption even though the apparent partition coefficient may be influenced.

The effect of 1.0% (w/v) of surfactant on the per cent phenobarbital absorbed (Table 2) is worth noting. Despite the fact that the increase observed was rather small, slightly over 10%, yet it was statistically significant ($P < 0.05$). Polysorbate 80 was found to have no effect on the permeability of the rat intestine to salicylate anions (Hikal et al., 1976) or salicylamide (Yamada and Yamamoto, 1965), but it increased the permeability of the goldfish membrane to secobarbital (Levy and Anello, 1968) and to 4-aminoantipyrine (Anello and Levy, 1969). In the meantime, the permeability of the goldfish membrane to ethanol was not influenced by polysorbate 80 (Levy et al., 1966). It is possible that phenobarbital, secobarbital and 4-aminoantipyrine are absorbed mainly through the lipoid portion of the membrane, while salicylate anions, salicylamide, and ethanol can be absorbed through pores in the membrane. The differing effects of polysorbate 80 on the absorption of these drugs can then be explained on the basis that the surfactant affects drug absorption across the lipoid barrier portion of the biological membrane but does not affect diffusion of drugs through membrane pores.

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