Effect of Some Nonionic Surfactants on the Rate of Absorption of Aminophylline from Suppositories in Rabbits

By J. M. PLAXCO, JR., and F. FOREMAN*

The rate of absorption of aminophylline from theobroma oil base suppositories containing 5 percent of various nonionic surfactants was determined. The results were erratic and unpredictable, with no correlation with *in vitro* results reported in a previous communication.

SUPPOSITORIES HAVE received much attention in recent years as a means of administering medication. In the U. S. more attention has been directed toward *in vitro* investigations of drug release. European literature (1–9) contains many reports of *in vivo* work using different drugs and various animals. A previous paper from this laboratory (10) reported the *in vitro* effects of some nonionic surfactants on the rate of release and dialysis of aminophylline and of ephedrine and ephedrine hydrochloride from theobroma oil suppositories.

The purpose of the current investigation was to determine the effects, *in vivo*, of these same surfactant-containing aminophylline suppositories. Because of the reports of Waxler and Schack (11) and Glass and his coworkers (12), it was felt that the concentration of aminophylline (theophylline) in the plasma was a reliable indicator of the rate of absorption of the drug. Waxler and Schack (11) showed that aminophylline (theophylline) is restricted to the plasma, does not penetrate the red cell membrane, and is bound only slightly to the proteins of the blood and to tissue proteins.

EXPERIMENTAL

Materials—The suppositories were those prepared and investigated for *in vitro* release (10). Each suppository contained 5% surfactant and 10 mg. of aminophylline in theobroma oil.

Procedure—Young male white rabbits weighing initially approximately 2 Kg. were used. At the conclusion of the investigation they weighed approximately 3–4 Kg. each. At least 1 week lapsed between tests on each rabbit to allow the rabbits to recuperate and to eliminate completely all traces of drug from the system. As many tests were run on each suppository as time permitted, usually 5 or 6 different rabbits per suppository, though in a few instances only 3 were used. Twenty rabbits were used, mainly in succession, which resulted in different animals testing one suppository. In a few instances in which repeat tests were run, the same animal was used. The variability was less using the same animal than when using different animals but unfortunately insufficient data were available for analysis.

The rabbits were fasted overnight to rid the rectum of fecal matter, and each experiment was started at approximately the same time each morning (9:00 a.m.) for uniformity and to allow ample time to use another rabbit if the suppository was expelled. After weighing the rabbit, a suppository was inserted into the rectum and pushed about 2 cm. with a glass rod lubricated with white petrolatum. No satisfactory method of closing the anus to prevent expulsion of the suppository was found, and occasionally it was expelled, necessitating the use of another rabbit. After insertion of the suppository the animal was returned to its cage for about one-half hr.

The rabbit was then placed in a rabbit holder with its head exposed. At the stated times, approximately 4 ml. of blood was collected from the marginal or medial ear veins in a heparinized tube and 3 ml. of this was pipeted into a separator containing 30 ml. of chloroform-isopropyl alcohol (20:1) mixture. The aminophylline (theophylline) concentration was determined by the method of Schack and Waxler (13) with modifications as noted.

Chloroform NF and isopropyl alcohol NF were freshly distilled weekly and stored in glass-stoppered bottles of 500 ml. capacity. This precaution of fresh solvents had previously been noted by Glass *et al.* (12) and others.

The 3 ml. of blood was extracted for 3 min. with two 30-ml. portions of chloroform–isopropyl alcohol mixture and the portions mixed. Following filtration, 40 ml. of the solution was extracted with 6.0 ml. of 0.1 *N* NaOH and centrifuged. The absorbance at 277 mμ was determined on a Beckman model DB spectrophotometer and compared with a standard curve prepared by adding a known amount of aminophylline to bovine blood and extracting, using the same technique as described. This method gave more uniform and reproducible results than using a standard curve prepared from the absorbance of varying concentrations of aminophylline in 0.1 *N* NaOH and correcting for the absorbance of the blood. Schack and Waxler (13) found "the mean blank value of human blood (300 analyses) to be an optical density of 0.07 ± 0.02 per cc. 0.1 *N* NaOH. Blood and tissues of other species follow this closely." Our experiments confirmed these values for rabbit, swine, and cattle blood. It was also found that following the insertion of blank surfactant-containing suppositories (no aminophylline) the same values were obtained.

A concentration of 10 mg. of aminophylline per suppository was satisfactory for the *in vitro* work but the plasma levels for this concentration were at the lower end of the curve for the *in vivo* work. It is believed that more uniform results would have been obtained with more than 10 mg. of aminophylline per suppository. However, the object of the investigation was to compare *in vitro* and *in vivo* release from the same batches of suppositories.

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TABLE I—AMINOPHYLLINE BLOOD LEVELS FROM THEOBROMA OIL SUPPOSITORIES CONTAINING 5% NONIONIC SURFACTANTS

Surfactant Chemical Type or Composition	HLB	Mg. Dialyzed, 1 hr.	Blood Levels, mcg./100 ml. Blood 1 hr.	3 hr.	4 hr.	5 hr.
Theobroma oil only	0	1.0	400	250		200
Sorbitan fatty acid esters[a]						
Sorbitan monostearate	4.7	1.2	250	300		200
Sorbitan monopalmitate	6.7	1.7	240	160		80
Sorbitan monolaurate	8.6	2.1	220	230		100
Polyoxyethylene sorbitan fatty acid ester-ethers[b]						
POES monostearate	9.6	2.9	230	160		80
POES tristearate	10.5	2.7	260	150	190	...
POES monolaurate	13.3	4.1	200	220		80
Polyoxyethylene fatty acid esters[c]						
POE (8) stearate	11.1	6.0	200	150		80
POE (40) stearate	16.9	1.5	400	90		50
POE (50) stearate	17.9	1.7	200	120		negligible
Polyoxyethylene fatty ethers[d]						
POE (4) lauryl ether	9.7	9.8	300	300		80
POE (23) lauryl ether	16.9	6.6	340	240	250	...
POE (2) cetyl ether	5.3	1.3	200	240		200
POE (10) cetyl ether	12.9	7.8	400	100		50
POE (20) cetyl ether	15.7	2.6	150	300		300
POE (10) stearyl ether	12.4	7.4	350	...		200
POE (20) stearyl ether	15.3	2.1	400	450		50
POE (20) oleyl ether	15.3	5.6	50	50		negligible

[a] Trademarked as Spans 60, 40, and 20, respectively. [b] Trademarked as Tweens 61, 65, and 21, respectively. [c] Trademarked as Myrjs 45, 52, and 53, respectively. [d] Trademarked as Brijs 30, 35, 52, 56, 58, 76, 78, and 98, respectively, all trademarked by the Atlas Chemical Industries, Inc., Wilmington, Del.

RESULTS AND DISCUSSION

Table I shows the concentration of aminophylline in the blood plasma. A study of this table reveals no pattern of release. The addition of the surfactants did not result in an increased rate of absorption as compared to theobroma oil. In most instances there was even a decrease in plasma concentration. HLB values, which had a pronounced effect in our and other *in vitro* work, had no discernible effect in the *in vivo* work. No relationship was found between chemical type or structure and effect. There was also no correlation between *in vitro* and *in vivo* results.

Figures 1 and 2 show the scatter pattern of the individual aminophylline plasma concentrations from selected suppositories. Individual differences were great but were more uniform than data obtained using human subjects (11, 12). This was to be expected as conditions were more closely controlled with rabbits than would be possible with humans.

Generally, it has been found that the addition of surfactants to oleaginous bases (suppository or ointment) increased the *in vitro* rate of release (10–15).

Reports of *in vivo* work have been contradictory. Riegelman and Crowell (16) found that the rate of absorption of sodium iodide was accelerated by the presence of surfactants but the rate of absorption of the sodium salt of 2,4,6-triiodophenol was retarded. Kakemi *et al.* (17) showed that the rectal absorption of sulfonamides in the rat was reduced by the presence of nonionic surfactants due to the entrapment of part of the drug within the micelles of the surfactants.

Various attempts have been made to correlate *in vitro* and *in vivo* data regarding drug release from

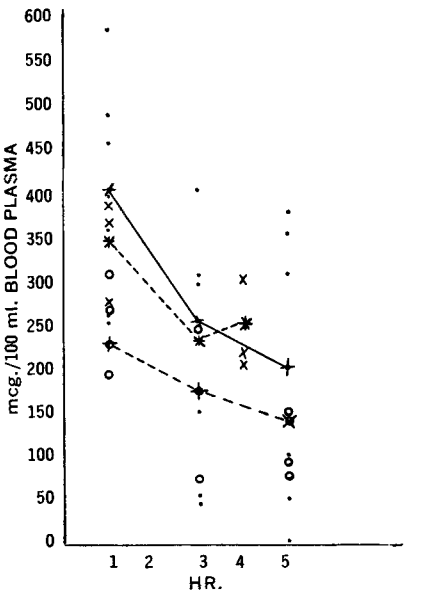

*Fig. 1—Aminophylline plasma concentrations. Key: •, theobroma oil; ⊠—, theobroma oil-av.; O, POES monostearate; ⊠- - -, POES monostearate-av.; ×, POE (23) lauryl ether; *- - -, POE (23) lauryl ether-av.*

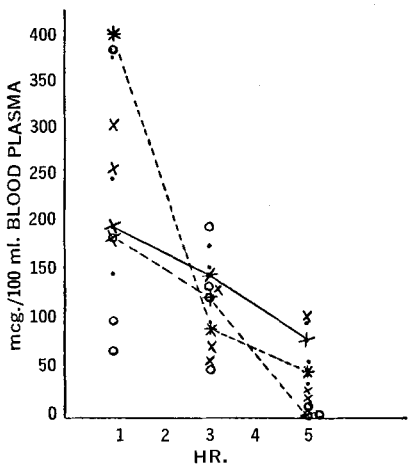

*Fig. 2—Aminophylline plasma concentrations. Key: •, POE (8) stearate; ✕—, POE (8) stearate-av.; O, POE (50) stearate; ✕- - -, POE (50) stearate-av.; ×, POE (40) stearate.; *- - -, POE (40) stearate.*

suppositories. Since *in vitro* conditions cannot take into account the conditions prevailing in the rectum, especially regarding fluid content, the viscosity and chemical composition of the mucus, and the pressure of the walls on the suppository, they have been unsuccessful. Recently Setnikar and Fantelli (18) described an apparatus which more closely reproduces the rectal environment. Using this apparatus, the *in vitro* disintegration time compared favorably with the disintegration time of radiopaque suppositories in man. This was an improvement over other techniques but still omits the effect of many factors.

Kuhne (4) reported results of studies relating *in vitro* to *in vivo* experiments. He prepared suppositories of two pairs of drugs, phenobarbital and its sodium salt, and atropine and atropine sulfate, in several bases. The suppositories were administered to rabbits for the *in vivo* experiments, and dialyzed through a membrane for the *in vitro* experiments. The water-insoluble drugs, phenobarbital and atropine, did not pass from the suppositories through the membrane in the *in vitro* work, but all drugs passed readily through the rectal mucosa into the blood in the *in vivo* experiments. He concluded that *in vitro* results cannot serve as a basis for the prediction of *in vivo* results.

Neuwald and Kunze (5) compared *in vitro* results with blood levels in man of three salicylates: acetylsalicylic acid, calcium acetylsalicylate, and sodium salicylate. The plasma concentrations of all three salicylates were about the same after 2 hr., but there was very wide variation in the amounts of drug diffused *in vitro*. Their *in vivo* results do not agree with animal experiments of others and contradict their own *in vitro* results. They drew the conclusion that the evaluation of suppository medication for man cannot be based on animal experiments, much less on *in vitro* diffusion experiments.

Our data support the unpredictability of absorption of drugs from suppositories. Several explanations may be advanced as to why the addition of the nonionic surfactants retarded the absorption of aminophylline by the rabbits.

It has been shown (19) that, at least for salicylates, drugs are initially absorbed as rapidly as they are released from the suppository—*i.e.*, the rate of release and diffusion to the rectal mucosa is the rate-limiting step. Our *in vitro* study (10) revealed that the addition of nonionic surfactants increased the rate of release and diffusion of aminophylline. Hence, it is evident that the interference with absorption is not due to lessened release.

The cellophene membrane used in the *in vitro* study cannot be compared with the living rectal mucosa. While most drugs are absorbed from the rectum by a simple diffusion process, this diffusion through a lipoidal membrane is different from a cellophene membrane. As reported by Riegelman and Crowell (16) and Kakemi *et al.* (17) it is probable that part of the drug is trapped within the micelles of the surfactant. The nature of the membranes and the size openings were probably such that the surfactants were able to pass through the cellophane membrane much more readily than through the rectal membrane.

Other possible explanations are that the surfactants irritated or in some other manner changed the permeability of the rectal membrane. This is unlikely as Nissim (20) has shown that some nonionic polyoxy surfactants (polyoxyethylene sorbitan esters) exhibited no untoward action on the mucosa.

Another possible explanation is that the surfactants emulsified or in some other manner modified the mucus lining the rectum, causing it to trap or interfere with the absorption of the aminophylline.

SUMMARY AND CONCLUSIONS

(*a*) The rate of absorption of aminophylline from theobroma oil and from theobroma oil containing 5% nonionic surfactants was determined.

(*b*) The addition of the surfactants generally resulted in decreased absorption of the drug.

(*c*) There was little, if any, difference noted in results related to HLB, chemical structure, or chemical type of the surfactant.

(*d*) There was no correlation between *in vitro* and *in vivo* data of aminophylline release and absorption. The evaluation of suppository medication, in rabbits and probably for man, cannot be based on *in vitro* dialysis experiments.

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Keyphrases

Aminophylline suppositories—*in vivo* absorption rate
Nonionic surfactant effect—*in vivo* absorption rate
Blood levels—aminophylline from suppositories
UV spectrophotometry—analysis

Datura Tissue Cultures: Production of Minor Alkaloids from Chlorophyllous and Nonchlorophyllous Strains

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The highest medium concentration of manganese used (14 p.p m.) appeared to stimulate the production of cuscohygrine, choline, and pseudotropine from *Datura stramonium* L., strain 5450, suspension cultures. Amino acid precursors, or chlorophyllous tissue had no significant effect upon the production of these alkaloids. No hyoscyamine or scopolamine was detected. Significant amounts of *Datura* suspension tissues were grown in multiliter fermentors.

RECENTLY, ALKALOIDS have been reported produced by *Catharanthus* (1), *Conium* (2), *Datura* (3), *Ipomoea* (4), *Nicotiana* (5), and *Rauwolfia* (6) tissue cultures. In the past a number of investigators have studied alkaloid production by plant tissue or organ cultures, and their results are comprehensively discussed in review articles (7–9). Plant tissue cultures have been used to biochemically alter alkaloids (10) and cardenolides (11).

The tropane alkaloids are reportedly produced by *Datura* (3, 12, 13) and *Hyoscyamus* (14) callus tissue cultures, and to be potentiated by the addition of certain amino acids to the medium (13). The tropane alkaloids may also be degraded and metabolized by microorganisms (15). Manganese ion supplements stimulated alkaloid production and arginase activity in both sand and field cultures of *Datura stramonium* plants (16).

The principal objective of this study was to determine the nature of the alkaloids present in *Datura* suspension cultures and if they might be increased or modified by altering the manganese ion concentration in the medium, by adding tropane alkaloid precursors to the medium, or by inducing chloroplast formation.

EXPERIMENTAL

Tissue Cultures—The *Datura* tissue culture principally studied was 36 month-old seed callus of *Datura stramonium* L., strain 5450. This culture was established in June 1963 (13) from plants believed to be high in alkaloid content and subcultured to the liquid medium in June 1966. The long-term experiment shown in Table I represents seven continuous subcultures of this strain which were grown as previously described (13, 17).

Forty-one month-old seed callus of *D. stramonium* (13); 36 month-old seed callus of *D. quercifolia* L., strain 52146 and *D. innoxia* Mill. (13); and 4 month-old seed callus of *D. stramonium* (seed origin: Drug Plant Greenhouse, Univ. of Nebraska) were grown as suspension cultures for approximately 3 months, and subsequently the suspension cultures were analyzed for their alkaloid content. A continuous light period was provided (150 ftc. from 40-w. cool-white, fluorescent tubes; Amplex Corp.) to establish and maintain chlorophyllous suspension cultures (*D. stramonium* L., strain 5450).

Three 7.5-L. fermentors (model FS-300, New Brunswick Sci. Co., New Brunswick, N. J.) containing 3.4 L. of medium with 14 p.p.m. of manganese were each inoculated with 12 day-old suspension

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