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Absorption and Distribution of Radioactivity from Suppositories Containing ^{3}H-Benzocaine in Rats

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Abstract □ The effects of the suppository vehicle, drug concentration, and nonionic surfactants on *in vitro* benzocaine dialysis through a cellulose membrane and on rectal absorption in rats of total radioactivity following administration of ^{3}H-benzocaine were investigated. *In vitro* dialysis correlated quite well with *in vivo* absorption, and drug release was greater from water-soluble vehicles than from oleaginous vehicles. Inclusion of a nonionic hydrophilic or lipophilic surfactant in cocoa butter resulted in a statistically significant increase for *in vitro* drug release, while a lipophilic surfactant showed little effect *in vivo* and a hydrophilic surfactant depressed release *in vivo*. Both types of surfactant had small effects on release from polyethylene glycol. *In vitro* release of benzocaine from some commercially available suppositories was compared with experimental preparations. Variation in blood radioactivity following administration of the same concentration of ^{3}H-benzocaine in the same dosage form in male and female rats is reported.

Keyphrases □ Absorption—benzocaine from suppositories, effect of vehicle, drug concentration, and nonionic surfactants, rats □ Distribution—benzocaine from suppositories, effect of vehicle, drug concentration, and nonionic surfactants, rats □ Benzocaine—absorption and distribution from suppositories, rats □ Suppositories—absorption and distribution of benzocaine, rats □ Dosage forms—suppositories, absorption and distribution of benzocaine, rats

It is well recognized that formulation factors can influence the availability of a drug from a dosage form. Surface-active agents included in dosage forms may exert their effects on the active ingredient, the dosage form itself, or the membrane at the absorption site. Surfactants have been reported to increase and to decrease the absorption of drugs (1). Moreover, varying the concentrations of a surfactant can enhance or retard drug absorption, depending on the type of surfactant and whether or not micelle formation occurs (1).

The complex mechanisms of surfactant effects on drug absorption were reviewed previously (2). The *in vitro* release of benzocaine from ointment vehicles was reported (3) and compared (4) to the rate of absorption and resulting total blood level radioactivity following rectal administration of 20% ^{3}H-benzocaine (ethyl *p*-aminobenzoate) from ointment vehicles in rats. This paper reports the effects of suppository vehicles, variations in drug concentration, and the presence of a nonionic hydrophilic or lipophilic surfactant on the *in vitro* dialysis of benzocaine and the absorption of ^{3}H-benzocaine in rats.

EXPERIMENTAL

Dosage Form Preparation—All suppositories were prepared by the fusion method, and commercial products were used as received. The reagents and equipment used were similar to those reported previously (3, 4). Additional materials used in the present experiment were: dialysis membrane, available as a 2.54-cm (1-in.) × 30.5-m (100-ft) roll[1]; cocoa butter[2]; polysorbate 80[3]; and sorbitan monooleate[4].

For *in vivo* studies, ^{3}H-benzocaine was dissolved in the polyethylene glycol vehicle (75% polyethylene glycol 1000 and 25% polyethylene glycol 4000) or suspended in the cocoa butter vehicle. Suppository vehicles containing ^{3}H-benzocaine were poured into plastic, disposable, U-80 insulin syringes, which were refrigerated until completely congealed. The tips of the syringes were cut off, and the excess semisolid was removed.

A suppository volume of 0.5 ml was used for the experiment. The amount of surfactant used was too small to weigh directly, and the aliquot method was used for preparation.

***In Vitro* Dialysis**—Dialysis tubing was cut into 10-cm lengths and soaked for at least 24 hr in distilled water. At the time of the test, the tubing was closed and weighted at one end by tying with a thin strip of the dialysis tubing to a glass stopper. The suppository was introduced into the tubing followed by 2.5 ml of distilled water. The top was tied to form a container, which was as nearly full as possible without loss of water.

The sample was then placed in a 600-ml beaker containing 500 ml of distilled water maintained at 37.5°. It floated upward, being held near the center of the container by the glass stopper weight. At the appropriate time periods, 5-ml samples were pipetted from the beaker and 5 ml of distilled water (37.5°) was returned to the beaker. Care was taken to draw each sample from as close to the same place in the beaker as possible and to avoid stirring.

Analytical Method—The analysis of the benzocaine released during the *in vitro* test was carried out by the method of Matsumoto *et al.* (5). Aliquot portions of a sample solution were pipetted into a test tube followed by 2 *N* HCl (2 ml) and 0.2% $NaNO_2$ (0.4 ml), and the mixture was shaken for 5 min. Then 0.5% $NH_4SO_3NH_2$ (0.4 ml) was added, and the mixture was shaken for 3 min. *N*-(2-Diethylaminoethyl)-1-naphthylamine hydrochloride (1.0 ml of 0.5%) was then added with shaking.

After 30 min of intermittent shaking, the percent transmittance was measured at 550 nm and the concentration of benzocaine was determined from a standard curve.

***In Vivo* Studies**—Female Sprague–Dawley rats were used for all experiments except the male *versus* female study. Animal weights varied between 100 and 280 g. Surgical preparation, cannulation,

[1] Seamless regenerated cellulose dialysis tubing, Catalog No. 25225-226, VWR Scientific Supplies, Portland, Ore.
[2] Hershey Food Corp., Hershey, Pa.
[3] Tween 80, J. T. Baker, Phillipsburg, N.J.
[4] Span 80, J. T. Baker, Phillipsburg, N.J.

Table I—Composition and Benzocaine Concentration of Various Types of Suppository Bases

Suppository	Vehicle	Surfactant	Average Weight for Dialysis Study[a], g	Benzocaine, %	Total Benzocaine for Dialysis, mg	Total Benzocaine Dialyzed in 5 hr, mg	Percent Dialyzed in 5 hr
A	Polyethylene glycol[c]	None	—	20	—	—	—
B	Polyethylene glycol	None	2.35	10	235.0	80.5	34.3
C	Polyethylene glycol	None	—	5	—	—	—
D	Polyethylene glycol	None	2.41	3	72.3	57.0	78.8
E	Polyethylene glycol	Sorbitan monooleate, 1%	2.42	3	72.6	60.5	83.3
F	Polyethylene glycol	Sorbitan monooleate, 0.5%	2.42	3	72.6	45.8	63.0
G	Polyethylene glycol	Polysorbate 80, 1%	2.41	3	72.3	57.8	79.9
H	Polyethylene glycol	Polysorbate 80, 0.05%	2.33	3	69.9	55.5	79.4
I	Polyethylene glycol	None	—	1	—	—	—
J	Cocoa butter	None	—	20	—	—	—
K	Cocoa butter[d]	None	1.63	10	163.0	22.9	14.0
L	Cocoa butter	None	1.74	3	53.2	10.0	18.8
M	Cocoa butter	Sorbitan monooleate, 1%	1.77	3	53.1	11.9	22.4
N	Cocoa butter	Sorbitan monooleate, 0.05%	1.76	3	52.8	12.4	23.5
O	Cocoa butter	Polysorbate 80, 1%	1.67	3	50.1	13.9	27.6
P	Cocoa butter	Polysorbate 80, 0.05%	1.60	3	48.0	11.7	24.4
Q	Oleaginous[e,f]	Unknown	2.14	11	235.0	17.5	7.5
R	Cocoa butter[e,g]	Unknown	2.65	4.9	130.0	15.5	12.0
S	Cocoa butter[e,h]	Unknown	2.40	5.4	130.0	13.2	10.1

[a] Average weight of 10 suppositories. [b] Average weight of six or more suppositories. [c] Polyethylene glycol vehicle consisted of polyethylene glycol 1000 (75%) [d] Hershey's. [e] Commercially available product. [f] Also contains hexachlorophene, ephedrine sulfate, and bismuth subgallate. [g] Also contains oxyquinoline sulfa peru. [h] Also contains ephedrine sulfate, oxyquinoline sulfate, zinc oxide, bismuth subgallate, and balsam peru.

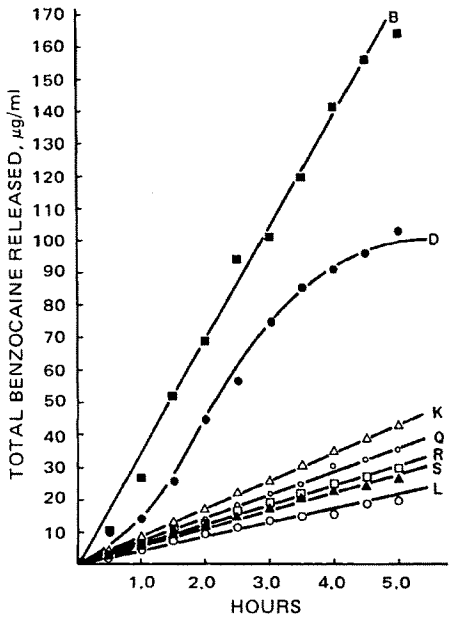

Figure 1—*Effect of suppository vehicle composition on release of drug from preparations containing benzocaine. Key: see Table I.*

blood sample collection, and blood analysis methodology were followed as previously reported (4).

RESULTS AND DISCUSSION

Table I shows the composition of suppositories selected for the investigation of variations in dialysis and rate of release for absorption of benzocaine. Figure 1 illustrates the variation in drug release of some experimental suppository formulations and the commercial products investigated. Since the active ingredient content of many commercial products is reported as a percentage, rather than an amount, Products A–P were prepared on a percentage weight per weight basis. The specific gravity of the polyethylene glycol vehicle used was about 1.4

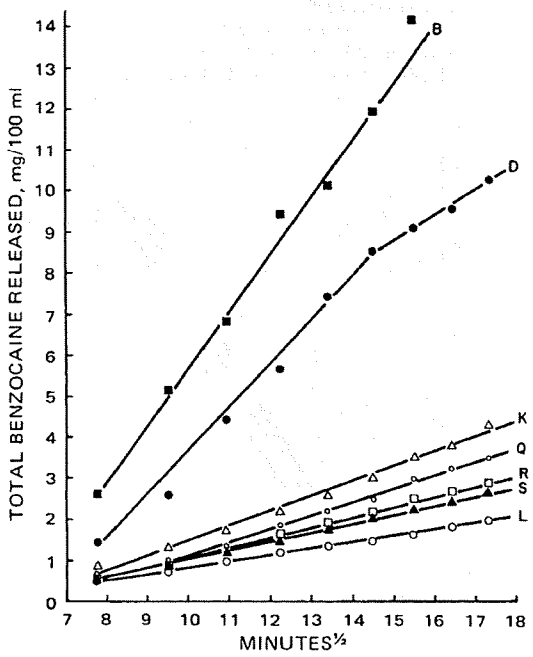

Figure 2—*Relationship between total mass of drug dialyzed and time$^{1/2}$. Key: see Table I.*

Table II—Summary of Calculated Statistical Parameters

Product	Intercept (b_0)	*SE* of Intercept	Regression Coefficient (b_1)	*SE* of Regression Coefficient
B	−88.9583	4.2313	113.5101	2.4429
D	−62.6965	7.3624	76.0611	4.2507
E	−90.9342	8.1531	96.8973	4.5225
F	−69.2085	5.8930	74.6488	3.2689
G	−76.7483	5.5375	87.5097	3.0717
H	−81.1662	4.4574	86.9697	2.4725
K	−21.1737	1.3366	27.8889	0.7717
L	−6.8433	0.5277	11.6569	0.3047
M	−8.5915	0.3772	14.3776	0.2178
N	−10.7434	0.5166	15.6600	0.2982
O	−10.6500	1.3357	17.8333	0.7712
P	−8.6347	0.6929	14.5876	0.4001
Q	−18.4070	1.0351	23.6619	0.5976
R	−11.9034	0.5641	16.9296	0.3257
S	−13.0224	0.8578	18.6843	0.4952

times that of the cocoa butter, and this difference was reflected in the variation in the total amount of benzocaine present in suppositories containing the same percent of benzocaine but different vehicles.

The total mass of drug transferred from semisolids under the conditions of the *in vitro* experiment was nearly linear with respect to the square root of time for the times investigated (Fig. 2). The linear portions of such benzocaine release curves were used in generating a linear least-squares regression line, and comparisons among the estimated parameters (Table II) were made (Table III) using the usual null hypothesis. The data points for Product D were approximated with two linear portions (Fig. 2), and the line for the early time period was arbitrarily chosen for statistical comparisons (Tables II and III).

In vitro testing of suppositories involves many considerations and some compromises in simulating conditions operating during rectal absorption. The conditions (6) that should be emulated are: (*a*) an average temperature of 36.9°; (*b*) water not present in the liquid state but present in the semisolid feces, which are 77–82% water; (*c*) rectal mucosa acting as a semipermeable membrane, allowing passage of water both away from and into the blood, depending on the osmotic gradient; (*d*) practically no peristaltic movement; (*e*) pressure on rectal contents varying from 0 to 50 cm of water, according to posture; and (*f*) possible presence of feces.

In normal people, fecal material is present in the rectum just prior to defecation only (7). Most of the time, this organ is free of solid matter which could physically interfere with absorption. Therefore, it is not necessary to introduce a material for *in vitro* testing that would simulate the presence of feces. It is necessary, however, to expose the dosage form to some fluid so that the drug has an opportunity to dissolve. While this exposure may seem to violate Condition *b*, a positive correlation between *in vitro* testing and *in vivo* results would indicate that such exposure to fluids is acceptable for testing purposes.

Testing at body temperature is critical, especially for products that melt in the rectum. Conditions *a* and *c* are readily satisfied by using a temperature-controlled water bath and placing the suppository inside a commercially available, semipermeable, dialysis membrane tubing. Condition *d* can be met by placing the dosage form in an unstirred medium. Although this procedure may allow a buildup of drug around the dosage form, which can slow drug release, such a static dialysis method may have a closer relationship to the absorption of drugs through a biological membrane than dialysis when the bulk phase is stirred.

The data reported here were obtained using a simple dialysis procedure, without stirring of the bulk receptor phase, which exposed the suppositories tested to a single, uniform pressure and approximated to some degree Conditions *a*, *c*, and *d*.

Examination of the results for the commercial products (Fig. 1) reveals less release from the 11% preparation than the 10% preparation and somewhat greater release from the 4.9% preparation than the 5.4% preparation, although the latter difference is not significant. Formulation factors other than concentration that could be playing a role include the presence of other ingredients that might interact with the benzocaine as well as different vehicle effects. The experimental cocoa butter formulations were completely melted within 10 min and the polyethylene glycol vehicles were dissolved within 1 hr. There was, however, no visible change in any commercial product during the 5-hr dialysis period. Each commercial suppository retained its shape, al-

Table III—Individual Comparisons Made[a]

Products	S	R	Q	P	O	N	M	L	K	H	G	F	E	D	B
B	—	—	*	—	—	—	—	—	*	*	*	*	*	*	—
D	—	—	—	—	—	—	—	*	*	ns	ns	ns	*	—	
E	—	—	—	—	—	—	*	—	—	—	ns	*	—		
F	—	—	—	—	—	*	*	—	—	~*	—	—			
G	—	—	—	—	*	—	—	—	—	ns	—				
H	—	—	—	*	—	—	—	—	—	—					
K	—	—	*	*	*	*	*	*	—						
L	*	*	—	*	*	*	*	—							
M	*	*	*	—	*	*	—								
N	*	ns	*	~*	—	—									
O	ns	ns	*	*	—										
P	*	*	*	—											
Q	*	*	—												
R	ns	—													
S	—														

[a] Comparisons among rates of benzocaine release from the semisolid as measured by the regression coefficient of the total benzocaine released with respect to the square root of time were made. All of these comparisons are not statistically independent. Therefore, when a comparison is noted as significant at the 95% confidence level, it is meant that the rates of release of benzocaine for the two products under comparison are likely to be different but at a confidence level slightly less than 95%. The result is that some of the differences in release rate noted as ~* might not prove to be significantly different under more rigorous testing; ns = not significant, ~* = barely significant, * = significant, and — = not tested for significance.

though they all became somewhat more pliable at the end of the experiment than at the beginning.

The dialysis of drug from saturated benzocaine solutions was studied, and the ratio of total benzocaine to free benzocaine increased proportionately as the concentration of a nonionic hydrophilic surfactant[3] was increased from 0 to 7%. Addition of the surfactant to a solution containing a fixed amount of benzocaine increased the dialysis rate compared to a solution without surfactant (5, 8). Therefore, 0.05 and 1.0% of sorbitan monooleate or polysorbate 80 were incorporated into both the polyethylene glycol and the cocoa butter vehicle containing 3% benzocaine.

Figure 3 shows the effect on the cocoa butter suppositories and the increase in the amount and rate of benzocaine released. The greatest increase in release was due to the presence of 1% polysorbate 80. Since the membrane was not controlling the rate of diffusion (as evidenced by increasing diffusion with an increased concentration), the surfactant must have been increasing the rate of dissolution of the drug. This finding is consistent with work showing that an increase in benzocaine dialysis from surfactant-containing solutions is due to the increased solubilization of the drug because of surfactant–drug interactions, followed by a rapid release of free drug as dialysis takes place (5, 8).

Figure 4 shows the results of dialysis from polyethylene glycol suppositories containing surfactants. Between 63 and 80% of the active ingredient was released (Table I).

It is not possible to use the actual amount of drug released from the different products (Table I) as a measure of the effect of the vehicle on dialysis of drug, since the amount present varies with the product considered. The percent of drug released, however, can be used for this purpose. The total released from the polyethylene glycol vehicle containing 10% benzocaine was almost 2.5 times the total released from the corresponding cocoa butter preparation when the percent benzocaine dialyzed was considered. The ratio was 2.9 to 4.2 when comparing the corresponding preparations containing 3% benzocaine. When considering the release from the 3 *versus* 10% preparations of a single vehicle type, it can be seen that the 10% formulation released a larger amount of drug but a smaller percent of drug during the dialysis period (Table I).

Various concentrations of ^{3}H-benzocaine in suppository dosage forms with and without surfactant (Table I) were selected for *in vivo* testing and were inserted into the rectum of female Sprague–Dawley rats. Blood samples (0.1 ml) were taken from the inferior vena cava at 5, 10, 20, 30, 40, 60, 90, 120, 180, 240, and 300 min, and the total

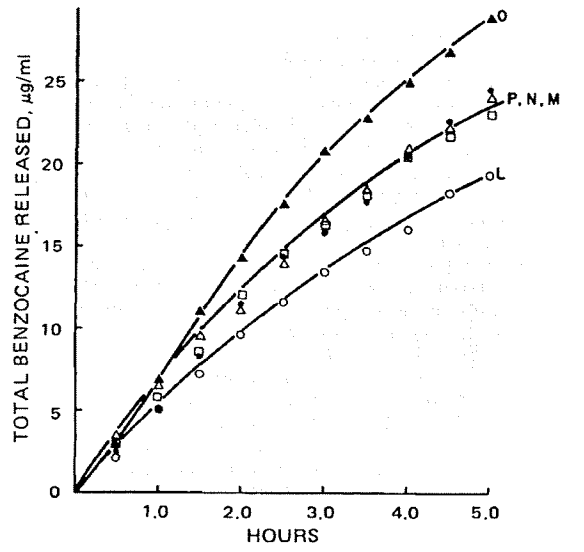

Figure 3—*Effect of surfactants on drug release from cocoa butter suppositories. Key: see Table I.*

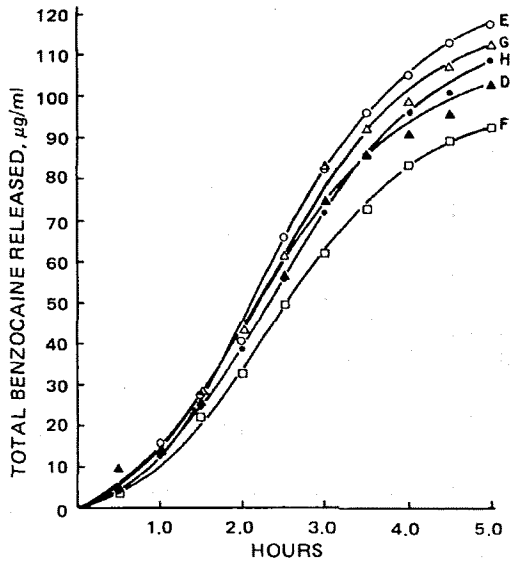

Figure 4—*Effect of surfactants on drug release from polyethylene glycol suppositories. Key: see Table I.*

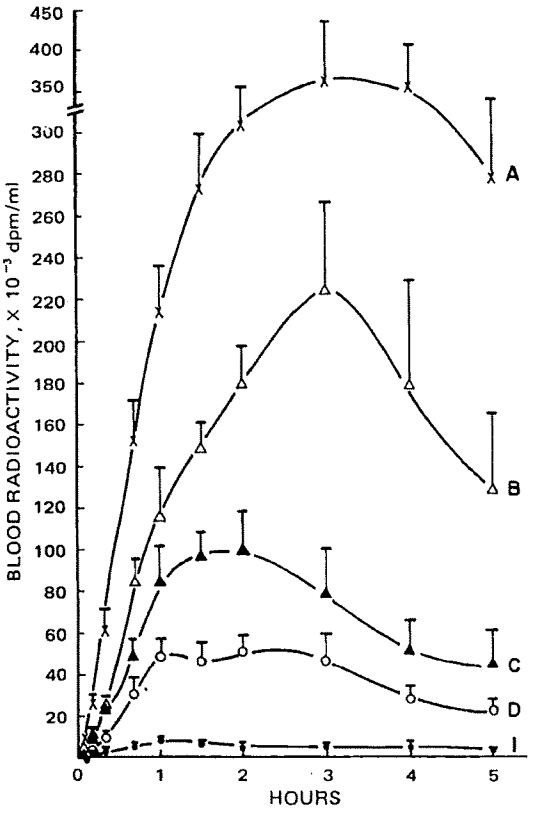

Figure 5—*Blood radioactivity after the application of ^{3}H-benzocaine in a polyethylene glycol suppository vehicle. Key: see Table I. A t-value larger than the critical t-value was obtained for all point comparisons except curve I versus B at 5 and 10 min; curve C versus B at 5, 10, 20, and 300 min; and curve B versus A at 180 min. (One side of the standard error of the mean is shown.)*

radioactivity present was determined. The means of the radioactivity detected are shown in Figs. 5–9.

Statistical analysis using unequal variance techniques indicated that weight variation among animals accounted for less than 5% of the variation following different dosage formulations. The standard error of the mean is included in some figures but not in others due to crowding. A point-by-point comparison of the means obtained at each sample time for the nonlinear curves was made using the Student *t* test (95% confidence level), and the results are summarized in the figure legends.

Due to the relatively large dispersion of experimental values, the ability to distinguish between mean values, which appear quite distinct, is compromised. An example of this situation can be seen when comparing the results from Formulation A *versus* Formulation B in Fig. 5 for the 180-min sample. The mean values for A and B were significantly different for each sample time (95% confidence level) except at 180 min due to the relatively large variances at that time. Increasing the number of animals in the study may have resulted in a significant difference in this case.

The blood level radioactivities from different concentrations of ^{3}H-benzocaine in polyethylene glycol suppositories (1, 3, 5, 10, and 20%) and cocoa butter suppositories (3, 10, and 20%) are shown in Figs. 5 and 6. Increasing the concentration of ^{3}H-benzocaine in both polyethylene glycol and cocoa butter suppository bases resulted in a higher total radioactivity in the blood. Since the volume of suppositories was equal with every concentration of drug administered, the total dose was increased by increasing the drug concentration. Both concentration and variation in total dose may be causes for the difference in the shape of the blood level curves using the same suppository vehicle.

It is clear from Fig. 5 that an increase in the ^{3}H-benzocaine in polyethylene glycol increased the area under the curve up to 5 hr. Although this finding indicates an increase in the amount of drug being absorbed from the rectum, since the drug had to be absorbed

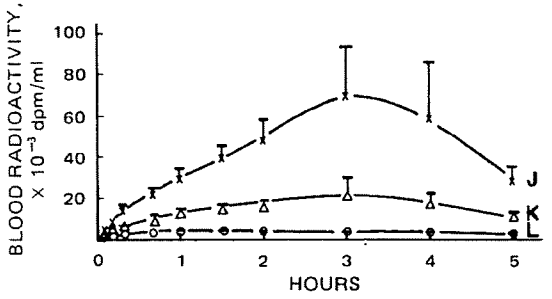

Figure 6—*Blood radioactivity after the application of ^{3}H-benzocaine in a cocoa butter suppository vehicle. Key: see Table I. A t-value larger than the critical t-value was obtained for all point comparisons except curve L versus K at 180 min, curve L versus J at 240 min, and curve K versus J at 180 and 240 min. (One side of the standard error of the mean is shown.)*

for the radioactivity to appear, under the conditions of this experiment the total radioactivity represents several metabolites rather than intact drug (4). Therefore, no pharmacokinetic analysis using the blood level radioactivity *versus* time curves was done in the present experiment.

A previous *in vitro* study (3) found that an increased concentration of benzocaine in polyethylene glycol ointment caused a decrease in release through a dialysis membrane. That decrease was explained on the basis of a decreased solubility and precipitation of the benzocaine in a polyethylene glycol–water solution, which formed under the *in vitro* conditions. This effect was not observed during the current dialysis experiments and is apparently not occurring *in vivo*, as evidenced by increasing absorption from an increased concentration of drug in this water-soluble vehicle.

The absorption from a cocoa butter suspension of drug is much less in rate and amount when compared to equal concentrations of drug in polyethylene glycol (Fig. 6). The latter vehicle is water soluble and can dissolve in the rectum. Benzocaine was dissolved in the polyethylene glycol vehicle and, therefore, was available to partition into the rectal fluids and the rectal mucosa during liquefaction (dissolution) of the polyethylene glycol. Cocoa butter does not dissolve but melts in the rectum.

Before liquefaction, dissolution of drug in rectal fluids is limited to the drug located at the surface of the suppository. Diffusion through the semisolid suppository is probably of little importance, since melting occurs readily at body temperature. After liquefaction, the

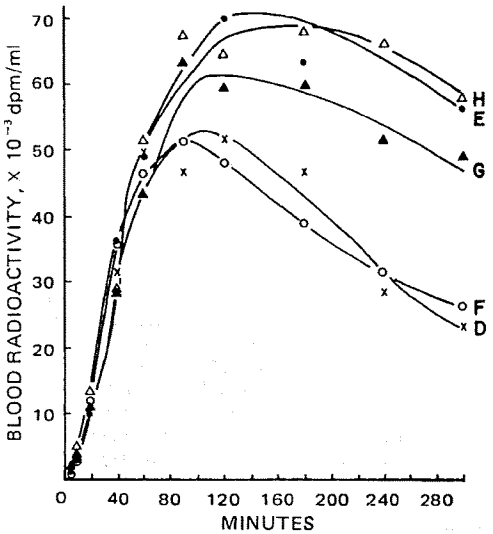

Figure 7—*Blood radioactivity after the application of 3% ^{3}H-benzocaine and surfactants in a polyethylene glycol vehicle. Key: see Table I. A t-value larger than the critical t-value was only obtained for point comparisons on curve D versus E after 240 min, curve D versus G after 240 min, curve E versus F after 240 min, and curve F versus H at 90 min.*

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