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Title: ABSORPTION OF BENZOCAINE - ^{3}H FROM SEMISOLID DOSAGE FORMS FOLLOWING RECTAL ADMINISTRATION IN RATS

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Dr. James W. Ayres

The effect of concentration, vehicle and surfactant on the absorption of benzocaine - ^{3}H across the rat's rectal mucosa was investigated. Blood radioactivity levels were determined after the application of benzociane - ^{3}H containing suppositories and ointments. A wet oxidation method was used for sample digestion. Since the radioactivity counted is the sum of the total radioactive molecules of any chemical form, thin layer chromotography was used to separate benzocaine from its metabolites. The in vitro hydrolysis of benzocaine in blood and the in vivo hydrolysis of benzocaine was studied. The in vitro method did not show any hydrolysis of benzocaine. However, the in vivo study did show that hydrolysis takes place.

Different concentrations of benzocaine - ^{3}H in the same suppository base gave different blood radioactivity levels. The radioactivity increased with an increase in benzocaine - ^{3}H concentration.

The polyethylene glycol (PEG) suppository base containing benzocaine - ^{3}H gave radioactivity levels several times higher than the same amount of drug in a cocoa butter base. The incorporation of surfactants, Span 80 and Tween 80, into the PEG suppository dosage form did not affect the absorption of benzocaine significantly ($p < .05$). The incorporation of Tween 80 into cocoa butter base significantly decreased the absorption of benzocaine ($p < .05$). However, the incorporation of Span 80 into the cocoa butter suppository vehicle did not show any significant effect. The effect of five different ointment bases on the rectal absorption of benzocaine - ^{3}H were compared. The amount of absorption of benzocaine - ^{3}H was found to be in the following decreasing order: PEG>Neobase®> Aquaphor®:Water (1:1)> Aquaphor®and white petrolatum.

The tissue distribution of benzocaine - ^{3}H was investigated. The radioactivity level of the organs studied increased along with the increase in blood radioactivity levels. The liver and kidney contained the highest levels of radioactivity.

Methemoglobinemia was recently reported after the application of high concentrations of benzocaine containing suppositories. The effect of benzocaine on the degree of methemoglobin formation was investigated by the application of 20% benzocaine -^{3}H in Polyethylene glycol suppository and 20% benzocaine -^{3}H Neobase®. Methemoglobinemia was observed after the application of 20% benzocaine -^{3}H in Polyethylene glycol suppository. However, no methemoglobin could be measured after 20% benzocaine -^{3}H in Neobase® was applied rectally.

Absorption of Benzocaine -^{3}H from Semisolid Dosage Forms Following Rectal Administration in Rats

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSORPTION OF BENZOCAINE -^{3}H FROM SEMISOLID DOSAGE FORMS FOLLOWING RECTAL ADMINISTRATION IN RATS

I INTRODUCTION

Benzocaine is a local anesthetic which is frequently included in non-prescription preparations for the relief of pain and pruritis. The products within which benzocaine is most commonly incorporated are semisolid hemorrhoidal products, burn and sunburn remedies, and some solid preparations, i.e., troches or lozenges. Benzocaine is also found in astringent products, eczema and psoriasis preparations, and poison ivy and poison oak remedies (1-6). The utilization of these benzocaine containing products is rather extensive and uncontrolled. (1, 2) There is a forty-fold difference in benzocaine concentrations, i.e., 0.5 to 20%, in the products sold without a prescription. The popularity of topical benzocaine products is said to be due to its low toxicity. The low toxicity is attributed to the low solubility, thus causing benzocaine to be poorly absorbed cutaneously. Therefore, it is believed to be devoid of systemic toxicity. However, several cases of acute systemic reactions after topical application of benzocaine containing preparations have been reported in the literature. (7-14)

Systemic reactions are usually associated with high blood levels of drug. The degree of local anesthesia and the duration of this effect depends, to a large extent, on the total amount of accumulation of the drug and its binding affinity in nerve tissue.(15) This knowledge suggests that the systemic absorption and elimination of benzocaine is the main characteristic which can be used to consider the effectiveness and safety of the drug.

Statement of the Problem

In spite of the widespread utilization of benzocaine, relatively little is known about the absorption, fate, and distribution of this drug. Benzocaine is frequently applied to the rectal area and therefore, absorption of the drug across rectal mucosa deserves consideration.

Because of benzocaine's poor solubility the amount of systemic absorption, though detectable, is expected to be very low, thus making a specific chemical assay extremely difficult to establish. Radiotracer methods would be sensitive enough to detect this small amount, but certain considerations must be satisfied. For liquid scintillation, the samples have to be completely digested so that a clear and homogenous solution (instead of suspension) will appear after the fluor solution has been added and mixed in order to count with a high efficiency. Quenching of

the counts may be a problem with this method and can be corrected by using the Automatic External Standard ratio to determine the counting efficiency.

Benzocaine is an ester derivative which has been proposed to be hydrolyzed in the circulatory system (7, 8) and research is needed to determine if this is true. It is important to identify the metabolites of benzocaine to know the main substances which cause the methemoglobinemia which has been reported following the application of high concentrations of benzocaine. Also, if benzocaine could be separated from its metabolites, the pharmacokinetic and metabolic pathways could be studied.

Varying the concentration of the active ingredient may have some effect on the release of drug from a semi-solid vehicle and different suppository or ointment vehicles do not show the same effect on the release of different drugs. Benzocaine is commercially available in various concentrations. Therefore, the absorption of benzocaine incorporated in different vehicles at various concentrations needed to be compared to determine an optimum formulation for the release of benzocaine.

Surfactants are often included in semisolid dosage forms to increase the rate of absorption of the active ingredients. However, their effects on the efficiency of drug absorption are variable, i. e., increasing the absorption of the active ingredient from some

formulas while decreasing the absorption of the active ingredient from others. Different concentrations of surfactant sometimes exert opposite effects depending on the type of surfactant and whether or not micelle formation occurs. Therefore, the incorporation of different concentrations of different types of surfactant in benzocaine containing preparations was another aspect to be examined in an attempt to optimize the formulation of a benzocaine containing semisolid.

Tissue distribution of a drug has an important effect on the blood level concentration of the drug. Once the drug enters the blood stream, it will transfer back and forth between blood and tissues. The rates of transfer depend on the affinity of the drug for each particular tissue. The systemic toxicity of benzocaine may be either central nervous stimulation or methemoglobinemia. Therefore, a knowledge of the tissue distribution of benzocaine and its partitioning into blood might be useful in treating cases of benzocaine toxicity. Though methemoglobinemia has been reported as a possible toxicity caused by benzocaine (7-14), no study has been done to confirm the cause and effect relationship.

An *in vitro* study of the release of benzocaine from different types of ointment bases *via* dialysis through a cellulose membrane has been reported. (16) An *in vivo* study was needed to find out

whether or not the results of the in vitro study were predictive of in vivo results.

Purpose of the Study

This study was initiated to determine the amount of systemic absorption of benzocaine -^{3}H from rectally applied suppositories and ointments containing benzocaine -^{3}H. Suppositories and ointments were the two major dosage forms examined because they are commonly used rectally.

The study entailed:

1. The determination of an effective method to digest blood and tissue samples so they could be counted with a liquid scintillation counter with high efficiency.
2. An attempt to separate benzocaine -^{3}H from its metabolites by a thin-layer chromatography method after its assumed hydrolysis in blood. An attempt was made to measure both the in vitro and in vivo metabolism of benzocaine.
3. Examination of the effect of varying the concentration of benzocaine -^{3}H in the dosage form on the rectal absorption from rats of benzocaine -^{3}H.
4. Investigation of the effect of different vehicles on the rectal absorption of benzocaine. Polyethylene glycol

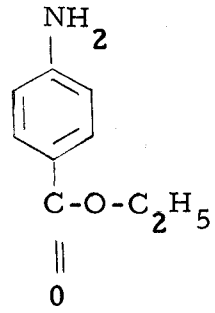
[PEG 1000 (75%) and PEG 4000 (25%)] and cocoa butter vehicles were compared as suppository dosage forms. Five different vehicles were compared as ointments: white petrolatum, Aquaphor®, Aquaphor®: water (1:1), Neobase®, polyethylene glycol (U. S. P.).

5. Investigation of the effect of surfactants on the rectal absorption of benzocaine by including Span 80 and Tween 80 in two different concentrations in polyethylene glycol and cocoa butter suppository vehicles. The concentrations were chosen to correspond with those used in an in vitro study (16). A recent article (17) indicates that both concentrations would be above the critical micelle concentration.
6. Investigating the distribution and relationships of total radioactivity in different tissues and in blood after the rectal absorption of benzocaine - ^{3}H.
7. Measuring the percent of methemoglobinemia after the rectal application of 20% benzocaine - ^{3}H in polyethylene glycol and Neobase vehicles.
8. Comparing earlier in vitro results (16) with the in vivo data obtained.

II REVIEW OF LITERATURE

Benzocaine is a local anesthetic which blocks nerve conduction in a limited area around the site of application when appropriate concentrations are applied (18, 19). Numerous substances are capable of producing the type of blockade of nerve conduction referred to as local anesthesia and the ability to do so is not attributed to any particular molecular configuration (18, 20-23).

Benzocaine



Ethyl para-Aminobenzoate

(benzocaine)

Benzocaine cannot be employed as an aqueous solution because it is too weak a base to form stable salts and the free base is only slightly water soluble (1 g: 2500 ml.). Ointments, suppositories, oily solutions and dusting powders are the main dosage forms used for benzocaine. Benzocaine was found to be ineffective after topical application in concentrations of less than 5%, when converted to the hydrochloride or lactate, or

when incorporated in an acid medium (24). For concentrations greater than 5%, the onset of anesthesia requires less than 30 seconds after topical application. The duration of anesthesia is brief and seldom persists for more than five minutes (25).

Benzocaine is employed for painful wounds and ulcerations of the skin and mucous membranes. It has also been used internally (19) for controlling gastric pain associated with peptic ulcer, cancer and other disease conditions, but the efficacy is questionable and this use is not recommended.

Toxicity

The toxicity of benzocaine, like most anesthetics, may be either systemic or local. Local anesthetic toxicity is not necessarily correlated with *in vivo* effectiveness (26, 27). Occurrence of toxic reactions can be reduced by using the minimal effective concentration and limiting the total quantity used.

Adriani described the general systemic effects of local anesthetics including benzocaine (22). Cutaneous lesions due to allergic responses have been reported following repeated use of benzocaine-containing ointments for sunburn and pruritis (25). Benzocaine is a relatively frequent sensitizer and is a cross-sensitizer with procaine and other local anesthetic drugs (28, 29).

Since benzocaine is poorly soluble, it is expected to be absorbed through mucous membranes in a very small amount. (24, 25) Adriani et al. (30) reported that 20% benzocaine ointment (Americaine ®) has been used in their department for approximately 12, 000 applications per year as a lubricant for intratracheal catheters and for pharyngeal and nasal airways to obtund the pharyngeal and tracheal reflexes. No untoward effects were observed except in one patient who developed methemoglobinemia within 30 minutes after the application of the drug. However, several other cases of methemoglobinemia after application of benzocaine-containing preparations have now been reported (7-14). The possibility of methemoglobinemia formation has become a well-known caution for those who use benzocaine, especially when the drug is applied to infants. Small infants may be especially susceptible to benzocaine induced methemoglobinemia because of a transient deficiency in reduced diphosphopyridine nucleotide (DPNH) dependent methemoglobin reductase (10, 12).

Methemoglobinemia should be suspected whenever cyanosis is present and cardiac or pulmonary failure are not the cause. When approximately 15% of the total body hemoglobin becomes methemoglobin, cyanosis will occur. High levels of methemoglobin in blood result in a characteristic chocolate color which does not change upon agitation in air. Cardiovascular compensatory

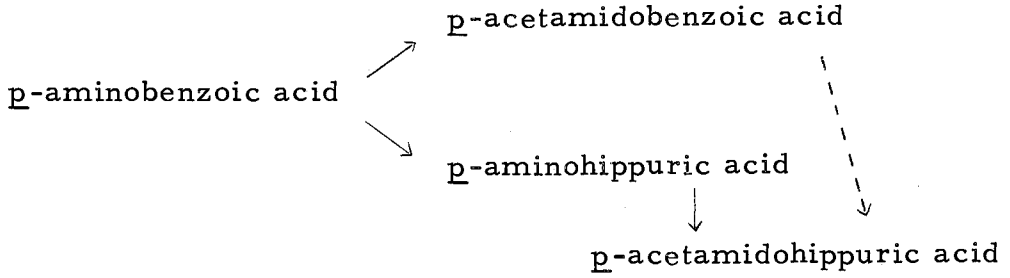
mechanisms are not activated until the concentration of methemoglobin reaches about 40%. Methemoglobin levels of over 60% may cause symptoms of ataxia, prostration and unconsciousness. When the concentration of methemoglobin is about 85%, death will occur (9).

Metabolism

Metabolic pathways of some local anesthetic drugs have been studied in several animal species but studies in man are lacking. No metabolic studies of benzocaine have been reported. However, it might follow the metabolic pathways of procaine, which is also an ester type local anesthetic, and which has been studied (31, 32). Procaine has shown to be hydrolyzed *in vivo*, to *p*-aminobenzoic acid and diethylaminoethanol. Diethylaminoethanol is further metabolized, but the nature of its metabolic transformation is not known. About 90% of the *p*-aminobenzoic acid produced was excreted in the urine in the form of *p*-aminobenzoic acid and its various conjugates (31). The hydrolysis of procaine was found to occur mainly in the plasma and the hydrolysis was extremely rapid (31). Brodie *et al.* found from an *in vitro* study that about 80 to 100% of procaine was broken down within two minutes when added into fresh human

serum by an enzyme believed to be cholinesterase (32).

Wan et al. (33) have investigated the metabolism of p-aminobenzoic acid in rabbits and reported the metabolic pathways as follows:



p-Acetamidobenzoic acid was found to be the main conjugate excreted. Smaller amounts of p-aminobenzoic acid were excreted as p-aminohippuric acid and p-acetamidohippuric acid. Very negligible amounts were excreted as p-aminobenzoic acid. They also observed that most of the conjugation occurred in the kidney.

Rectal Absorption

All substances absorbed, secreted or excreted by the body are transported across some types of epithelial tissue, each of which in one way or another may act as a barrier (24).

Mucous membranes are generally more permeable than skin to drugs because keratinization is less complete or may be absent. Therefore, a uniform barrier layer is not present in mucous membranes (24, 34).

The mucosa of the rectum is composed of columnar cells and glands. Normally the rectum is empty except for a small amount of mucus. The rectum is devoid of villi, has a relatively small surface area, and does not have a primary absorptive function. However, diffusion of drugs through and between the epithelial cells of the mucosa can occur. The submucosal region receives a rich vascular and lymphatic supply. The venous circulation in the rectal area consists of three branches: the superior, middle and inferior hemorrhoidal veins. The inferior and middle hemorrhoidal veins go from the lower rectum into the inferior vena cava, bypassing the liver where most drugs are modified. The superior hemorrhoidal vein goes from the upper area of the rectum to the hepatic portal circulation via the inferior mesenteric vein. Therefore, drugs absorbed in the lower part of the rectum enter directly into the vena cava and bypass the liver, but a drug placed in the upper part of the rectum should diffuse into blood vessels that lead to the liver. Some drugs applied rectally as the suppository, no matter in what shape, do not remain in the lower portion (37) of the rectum, but are moved upward into the region where the blood vessels lead to the liver. If a suppository disintegrates rapidly, immediate absorption of the active ingredient might take place from the lower portion of the rectum and the drug would bypass the liver. Lymphatic circulation, though exceedingly slow when

compared with the rate of blood flow, is also involved in the absorption of a rectally administered drug (35-37).

In order for a drug to be absorbed through the rectal mucosa into the circulation, at least two steps must occur:

1) release of drug from vehicle into the rectal fluid.

2) diffusion of drug through the rectal mucosa. The movement across the rectal mucosa of most drugs is said to be a passive diffusion process and the process has been found to be first order (37-40).

After absorption into the circulation, the drug concentration in blood is modified by several factors, such as: metabolism, tissue distribution, protein-binding and excretion.

Vehicle Effect on Absorption

The absorption of a drug depends primarily upon the physical-chemical nature of the drug itself. The vehicle in which it is incorporated and whether or not the substance is soluble in the vehicle are of secondary importance (34, 41-43). The vehicle itself generally does not increase percutaneous or mucosal absorption of a lipid insoluble substance and no vehicle can carry a substance which is incapable of penetration itself through the cell membrane.

The incorporated drug and vehicle have to separate from

each other before entering the cells, and both are absorbed at different rates (11). Therefore, the affinity of the vehicle for the drug substance can influence the release of the active substance from the vehicle. Substances with a low solubility in the vehicle are released more readily than substances with a high solubility in the vehicle. The greater the tendency for the drug and vehicle to separate, the greater is the chance of the drug being absorbed. For suspensions, the drug substance needs to be finely distributed throughout the vehicle to produce maximal absorption (43).

In summary, ointment bases usually tend to retard or delay absorption of drugs through the intact epidermis and from mucous surfaces. The penetration of the base itself cannot be used to determine whether or not the absorption of the incorporated drug will occur. It depends on the chemical and physical relationship between the drug and the base. There is no ointment base which has been shown to be an ideal ointment base. Different drugs incorporated in a series of different ointment bases do not show the same relative order of absorption rates (43, 44).

Surface-Active Agents Effect on Absorption

Surface-active agents contain high wetting and detergent properties. They have been combined with medicinal substances for several purposes: to help in the dispersion of finely ground

water-insoluble substances; to stabilize suspensions of solid drugs; to emulsify oils; to solubilize small amounts of lipid-soluble (water-insoluble) substances within micelles and to enhance the absorption of some drugs (40, 45). Surface-active agents have been reported to have variable effects on the efficiency of drug absorption, i.e., sometimes increasing and sometimes reducing the rate of absorption (40). Surfactants may act on the biologic membrane, the drug, or the dosage form (45).

The effects of surface active agents on the rate of absorption of lipid-soluble drugs may result from the withdrawal of the drug from the aqueous phase into the micellar phase (40, 45, 46), thus reducing the concentration of the free drug in the aqueous phase from which diffusion can occur. Micelles are too large to pass through biologic membranes, so drug molecules within these micelles cannot be absorbed. This same effect may also occur with water-soluble drugs which contain a high oil-to-water partition coefficient. Reduction of absorption by surface-active agents may also be caused by anionic surfactants forming insoluble and unabsorbable precipitates with large drug cations or cationic surfactants may interact with large drug anions (38, 40). An incompatibility between anionic and nonionic surface-active agents and bile (40) may also occur to decrease the rate of absorption of the drug. The effect of surfactants to increase the rate of absorption of drugs may

be attributed to decreasing surface tension and mucous peptizing action which results in greater contact between drug and the absorbing membrane.

The activity of surface-active agents can be highly dependent on their concentration. Micelle formation does not occur until the concentration of the surface-active agents increases beyond a certain value known as the critical micelle concentration (CMC). Below the CMC, surface-active agents decrease the surface tension of the solution and enhance wetting or spreading of the solution. This results in a more intimate contact between the drug solution and membrane which means there is a greater chance for drug absorption. Above the CMC, surfactants form micelles. A portion of the drug molecules may be entrapped in the micelles and become unavailable for absorption.

In general, low concentrations of surface-active agents may enhance absorption while a higher concentration may exert absorption-retarding effects. However, prolonged or repeated exposure to high doses of a surface-active agent may destroy the membrane barrier effect by causing disruption of biological membranes (46). In this case, a high concentration of surfactant would increase absorption of the drug.

The influence of the hydrophil-lipophil balance (HLB) of ointment bases on the diffusion of drugs was investigated by

Rhyne et al. (47). From the results of their study they concluded that there should be an optimum HLB at which the diffusion of the medicament from an ointment base is most effective, and the HLB value of an ointment should be taken into consideration when one wishes to adjust that vehicle to increase or decrease the rate of drug release.

The HLB of a surfactant when added to the ointment vehicles largely determined the type of emulsion formed and the degree of emulsification. Emulsification can make the ointment bases more or less miscible with the body fluids depending on the type of emulsion formed, i.e., water-in-oil or oil-in-water (48). The less miscible with body fluids the ointment vehicle is, the less likely that the drug will be released.

Effect of Physiological Changes in Absorption

When the skin or mucous membrane is damaged by trauma, burn, or inflammation, the barrier permeability is changed. This results in a higher than normal rate of drug absorption (34, 44). To prevent drug toxicity, physiological changes should always be kept in mind when a drug is applied to damaged skin or membranes.

Absorption of Topical Local Anesthetics

Local anesthetics are generally thought not to be absorbed

from the unbroken skin (40, 49, 50). They do pass into the blood if the skin is abraded. In studies by Campbell and Adriani (50), aqueous solutions of cocaine and tetracaine as the free base and ointments of tetracaine and benzocaine as the free base were applied to skin burned in varying degrees and no detectable blood levels of the local anesthetics were obtained after application to first or second degree burns. In cases of third degree burns, if the vesicles remained intact and there were no raw surface, detectable drug concentrations were not obtained. Application of the drug to the raw surface after opening the vesicles gave detectable drug levels in the blood. No absorption occurred after application to the eschar of an old burn, but the blood drug levels were measurable after the eschar was removed and the drug was applied to the raw surface. Therefore, the toxicity of local anesthetics should be considered whenever they are applied to a raw surface (20, 49, 50). Dalili and Adriani (51) studied the efficacy of bases and salts of different anesthetics in obtunding cutaneous itching, burning and pain. They found that saturated solutions of all bases tested when dissolved in a mixture of water, 40% alcohol and 10% glycerol, blocked the sensation of itching and burning caused by electrical stimulations. Salts of the same drugs in aqueous solution did not block the sensation. This leads to a conclusion that cutaneous barriers are more easily traversed by the bases of local anesthetic

agents than by their salts (24). However, even for the free anesthetic bases, penetration is limited and not comparable to that occurring through the mucosa surface either in quantity or rate (24). Adriani et al. (24, 51) also tested the efficacy of 30 commercially available local anesthetic preparations. They found that none of them completely obtunded the sensation of itch and burning in intact burned skin when stimulated electrically, with the exception of Americaine® which contains 20% benzocaine. This product showed the blocking effect to last for 10 to 60 seconds after it was removed from the application site.

For absorption from mucous membranes, the salts of local anesthetic agents are as readily absorbed as their bases and can cause nerve blockade. The anesthetic effect on the mucous membranes persists after the drug is wiped off (24). After local anesthetics were applied to a mucous membrane, the drug level in the blood vs. time curves simulated those of rapid intravenous injection (20,49, 25), although the peaks are lower, take longer to develop, and do not rise as abruptly. The rate of drug absorption varies with the mucous surface studied, concentration of drug applied and the total quantity of drug applied (24). Absorption was poor when the free anesthetic base was incorporated into an oily medium, such as petrolatum, and applied to the mucous membrane. The absorption was rapid when the free base was incorporated into water soluble ointments (49).

III EXPERIMENTAL PROCEDURE

Standardization of Benzocaine -^{3}H

The method used for quench correction was the internal standardization method because it is the most accurate (52). The samples were counted with a Packard Tri-carb Liquid Scintillation Spectrometer, model 2405.

Preparation of Benzocaine -^{3}H Solution for Standardization

The following amounts of benzocaine -^{3}H were weighed on an analytical balance and dissolved in dioxane.

Solution A: 1.14 mg. of Benzocaine -^{3}H dissolved in 1 ml. of dioxane.

Solution B: 8.28 mg. of Benzocaine -^{3}H dissolved in 10 ml. of dioxane.

Preparation of Dioxane Scintillation Fluid

1. Naphthalene (56.5 g.), 2, 5-Diphenyloxazole (PPO) (4.125 g.) and 1, 4-bis-2-(4-Methyl-5-phenyloxazolyl) benzene (dimethyl-POPOP) (51.35 mg.) were weighed.
2. The maphthalene was dissolved in about 300 ml. of dioxane, the PPO was dissolved in about 50 ml. of

dioxane and the dimethyl POPOP was dissolved in about 20 ml. of dioxane. These three solutions were well mixed and adjusted to 500 ml. with dioxane.

Procedure for Standardization of Benzocaine - ^{3}H

1. Dioxane scintillation fluid (15 ml.) was pipetted into each of four counting vials labelled, a, b, c, d.
2. 50 μl (0.05 ml.) of solution A, accurately measured with a disposable micropipette, was added into each vial a and b.
3. 50 μl (0.05 ml.) of solution B, accurately measured with a disposable micropipette, was added to each vial c and d.
4. The four samples, a, b, c, d, were counted with Tri-carb Liquid Scintillation Spectrometer, (model 2405) using the ^{3}H quick set, preset time was 4 minutes, preset count was 100,000.
5. 50 μl of the internal standard ^{3}H-Toluene, labeled 1-15-71, 88,400 dpm/50 μl, was added into each vial a, b, c, d.
6. The four samples were recounted with the same preset.

Result and Calculation

The internal standard ^{3}H-toluene was labelled 1-15-71

88,400 dpm/50 μl.

Date that experiment was done = 5-16-73.

Range of time = 2 years and 4 months.

= 2.3 years

Half life of ^{3}H = 12.33 years

$$\lambda = \frac{0.693}{t_{1/2}} = \frac{0.693}{12.33} = 0.0562 \text{ year}^{-1}$$

$$A = A_o e^{-\lambda t}$$

$$= 88,400 \times e^{-0.0562 \times 2.3}$$

$$= 88,400 \times e^{-0.129}$$

$$= 88,400 \times 0.879$$

$$= 77,704 \text{ dpm}$$

The activity of the internal standard at the date that the experiment was done = 77,704 dpm/50μl.

From Table 1 The activity of benzocaine -^{3}H =

0.067 mci/mm (average of samples a, b, c, d).

Benzocaine -^{3}H 165.2 μg. the radioactivity is $0.067 \times 2.22 \times 10^{6}$

Therefore, benzocaine -^{3}H 1 μg, the radioactivity is

Table 1. Data and the Calculation for the Activity of Benzocaine - ^{3}H.

	Data		Calculation					
Sample	cpm of sample + Spike (p)	cpm of sample without Spike (Q)	cpm of spike (R=p-Q)	Efficiency = $\frac{R \times 100}{\text{Activity of spike}}$	dpm of sample (50μl) $\frac{Q \times 100}{\text{Efficiency}}$	dpm of whole solution (A=1 ml.= 1.14 mg) (B=10 ml.= 8.28 mg)	dpm of 1 mM of Benzocaine - ^{3}H (165.2 mg)	Activity of Benzocaine - ^{3}H in mci/mM
a	57,985	22,812	35,173	$\frac{35,173 \times 100}{77,704}$ = 45.3%	50,358	1,007,160	145,937,484	$\frac{145,937,484}{2.22 \times 10^{9}}$ = 0.0657
b	57,622	22,595	35,027	45.1%	50,100	1,002,000	145,189,800	0.0654
c	52,745	17,548	35,197	45.3%	38,738	7,747,600	154,952,000	0.0698
d	50,913	16,533	34,380	44.2%	37,405	7,481,000	149,620,000	0.0674

$$\frac{0.067 \times 2.22 \times 10^{6}}{165.2} \text{ dpm} = 900 \text{ dpm.}$$

Preparation of Dosage Forms

Suppositories and ointments are the two dosage forms which were prepared.

Suppositories. All suppositories were prepared by the fusion method. The bases were melted first on the water bath, then benzocaine -^{3}H was added and mixed with the bases with the help of a stirring rod. With polyethylene glycol, benzocaine -^{3}H was dissolved in the polyethylene glycol so it was uniformly distributed through the vehicle. Suppository vehicles containing benzocaine -^{3}H were poured into plastic disposable U-80 insulin syringes which were placed in the refrigerator until the contents became completely congealed. The tips of the syringes were cut off and the excess suppositories were removed to leave a suppository volume of 0.5 ml. in each syringe which was the amount used for the experiment.

The cocoa butter suppositories were prepared by heating the vehicle on the water bath until it was about half melted, then removing it from the water bath and stirring with a glass rod until it was all melted. The benzocaine -^{3}H was added and the mixtured stirred continuously until the base was congealed enough

to yield a uniform dispersion of the drug, which was then poured into a syringe. These were then handled the same as the polyethylene glycol suppositories. This procedure was necessary because benzocaine -^{3}H does not dissolve in cocoa butter and tended to precipitate when the base was completely melted and still clear.

Since the amount of surfactant used is too small to weigh directly, it has to be premixed with the base in a larger amount and an aliquot method used.

9.75 g. of base was mixed with 0.25 g. of surfactant, to make 10.00 g. of base + surfactant.

In the formulas which contain 1% of surfactant. aliquot 2.0 g. of base + surfactant above(which is composed of 1.95 g. of base and 0.05 g. of surfactant), add 2.85 g. of base (without surfactant) and 0.15 g. of benzocaine -^{3}H.

In the formulas which contain 0.05% of surfactant, aliquot 0.1 g. of base + surfactant above (which is composed of 0.0975 g. of base and 0.0025 g. of surfactant), added 4.75 g. of base (without surfactant) and 0.05 g. of benzocaine -^{3}H.

Ointments. Benzocaine -^{3}H was first reduced to a fine state of subdivision by using a mortar and pestle. Then it was incorporated in the vehicle with the help of a spatula and an ointment slab by first levigating with a small portion of the vehicle until the mixture appeared smooth, then incorporated into the remainder of the

vehicle and mixed well to make sure that the drug was uniformly distributed in the vehicle. Ointment (0.5 ml containing benzocaine -^{3}H was then loaded into a plastic disposable U-80 syringe.

With the Aquaphor®:water vehicle, the water was added gradually to Aquaphor® and each time the water was added, it was mixed until the base appeared homogeneous. When all of the water needed in the formula was mixed, benzocaine-^{3}H was incorporated.

Generally, the suppository and ointment formulas were prepared about one week before the experiment and were kept in the refrigerator until used.

Pharmacological Procedures

Female Sprague-Dawley rats raised at the Oregon State University, School of Pharmacy were used. Their weights varied between 100-280 g. Statistical analysis indicated that weight variation of the rats accounted for less than 10% of the difference observed among formulation effects. The rats were anesthetized with sodium pentobarbital by intraperitoneal injection of 55 mg/kg. After the rat was anesthetized, the hair in the abdominal area and the ventral

portion of the neck was removed with an electric clipper. The trachea was surgically exposed and cannulated with polyethylene tubing (P. E. 200) in order to facilitate respiration during the entire experiment. The inferior vena cava was then exposed by a mid-line abdominal incision. Part of the fat that surrounded the vessel was removed and the inferior vena cava was cannulated with poly-ethylene tubing (P. E. 60). The abdominal incision was closed with autoclips after the cannulation was finished.

The suppository or ointment was inserted rectally from the syringe in which it was prepared. A short, blunt, glass rod was inserted rectally as a plug. A 0. 1 ml. sample of blood was with-drawn via the inferior vena cava cannulation before the drug was inserted and at 5, 10, 20, 40, 60, 90, 120, 180, 240, and 300 minutes after the suppository or ointment was inserted. The same volume of heparin-normal saline solution (7. 5 units of heparin/ml of normal saline) was injected immediately after each blood sample was obtained. All the blood samples were placed directly into the counting vials. When necessary 2. 75 mg/kg of sodium pentobarbital solution was injected to maintain the anesthesia. For the tissue distribution studies the liver, kidney, brain, heart, lung, spleen, part of the abdominal muscle and some fat were surgically removed from the animals after they were sacrificed. At the conclusion of each experiment the animals were sacrificed by administering a

lethal dose of sodium pentobarbital.

Blood and Tissue Analysis

The blood and tissue samples were digested to a clear solution before the scintillation solution was added and counted for activity.

Two methods of digestion were compared. One was a wet oxidation method used by Mahin and Lofbert (53), and the other was accomplished by using Unisol® + complement (54).

Unisol® + Complement

Unisol® + complement (Isolab's) is a two component system of tissue solubilization and radioassay. Unisol is a quaternary ammonium hydroxide compound provided in concentrated aqueous solution. It was claimed to be able to dissolve all animal tissue including hair and fingernails (54). The complement is a toluene-based solution containing fluors and a sufficient quantity of a balancing acidic solubilizer to accommodate over 10% (v/v) of Unisol-solubilized tissue as a clear and efficient liquid scintillation counting cocktail. The procedure was as follows:

1. Weighed about 100 mg. of wet weight tissue into the counting vial (if the sample was blood, the whole 0.1 ml. could be digested). Add 1 ml. of Unisol to completely wet or cover the

tissue specimen.

2. Tightly cap the vial and store at room temperature for one to two days until no undigested fibrous material remains. Opalescence due to lipids can be ignored.

3. After the digestion was completed, 0.5 ml. water-free methanol was added, the mixture agitated and 10 ml. of Unisol-complement was added. The mixture was capped and shaken and the cocktail was clear and ready to be counted.

Wet Oxidation Method

The procedure was as follows:

1. Approximately 100 mg. of tissue (or 0.1 ml. of blood) was placed in each counting vial.
2. 0.2 ml. of 70% perchloric acid was added to each sample vial and the contents agitated until the acid was well-mixed with the sample.
3. 0.4 ml. of 30% hydrogen peroxide was added and the contents were mixed again.
4. The vial was tightly capped to minimize evaporation of fluid during digestion, then was placed in a water bath at about 70°C for 60 minutes or until the contents of the vial became clear and colorless. Some fine or flocculent precipitate remained with some types of

tissue, but it was soluble in the final solution and caused no problem with respect to counting efficiency.

5. After the samples were cooled, 8 ml. of 2-methoxyethanol and 10 ml. of toluene scintillation solution were added. The vial was capped and shaken and a clear solution was formed.

Toluene Scintillation Solution

Omnifluor	4 mg.
Toluene to make	1 liter

Note: Omnifluor is composed of 98% PPO and 2% p-bis-0-Methylstyroyl) benzene (bis-MSB).

All samples were counted in a Packard Tri-carb liquid scintillation spectrometer, model 3375, using the 3H quick set and the preset time was 10 minutes. The counting efficiency was determined by the use of external standardization (i. e., the A. E. S. ratio).

Separation of Benzocaine - ^{3}H from its Metabolites by Thin Layer Chromatography

Thin layer chromatography was used to separate benzocaine from its metabolites and two solvent systems were compared. One was the system used by Khemani et al. (55) for the separation of aminobenzoic and salicylic acid derivatives. This was a five component solvent system and the solvent composition was

petroleum either (b.p. 30-60°):chloroform:methanol:glacial acetic acid:benzene (70:10:5:5:10). The other was the system used by Wan et al. (33) for separation of p-aminobenzoic acid and its metabolites. The solvent composition was benzene:p-dioxane: acetic acid (90:75:8).

The chromato-plates used in the experiment were UNIPLATE, precoated silica gel G-F thin layer chromatography plates (thickness - 250 microns).

In vitro study of hydrolysis of benzocaine in the blood: 2 ml. of blood was freshly withdrawn from the inferior vena cava of the rat and placed in a small beaker containing 1 drop of heparin solution (1000 units/ml.). Two drops of benzocaine -^{3}H solution (50 mg/ml. in aocohol) were added. At 1, 5, 30 and 60 minutes, 0.1 ml. of blood was taken from the beaker and immediately put into a tube containing one drop of 50% sodium arsenite solution to inhibit the activity of the enzyme in the blood (31) and one drop of 10% saponin solution to lyse the red blood cells, and mised well. The sample was then spotted as a row of small spots on a 10 x 20 cm. precoated thin layer plate, and developed with the three components solvent system. The chromato-plates were examined under U. V. light to locate the benzocaine and its metabolites. The same procedure was repeated with a saturated solution of benzocaine -^{3}H in water and 50 mg. benzocaine -^{3}H/ml. in polyethylene glycol 400 but the sample was

taken at 60 minutes only. The blood was maintained at 37°C in a water bath.

In vivo study: The rat was canulated the same way as before and 20% benzocaine -^{3}H in PEG ointment base was inserted into the rectum. At 30, 60, 120, 180, 240 and 300 minutes, 0.1 ml. of blood was withdrawn through the canula and put immediately into a small tube containing one drop of 50% sodium arsenite solution and one drop of 10% saponin solution and mixed well. The sample was then spotted, developed and after the solvents had evaporated the plate was examined under the U. V. light in the same way as the in vitro study. Finally, the entire chromatographic band from origin to solvent front was divided into one centimeter successive zones and the adsorbent of each zone was removed with a razor blade and put into counting vials. Eight ml. of methoxyethanol and 10 ml. of Omnifluor toluene scintillation fluid (same as before) were added and mixed. After the silica gel had completely set in the bottom of the vials, each vial was counted for 10 minutes in a Tri-carb Liquid Scintillation Spectrometer, model 3375, using external standardization to determine the counting efficiency.

An attempt was made to solve the problem of possible loss of radioactivity by adsorption on the support by igniting the silica gel samples in a Packard Oxidizer (56). The principle of this approach is to combust samples of (^{3}H) or (^{14}C) so that all the radioactivity is in the form of water or soluble carbonate. Scintillation fluor

was automatically added by the instrument and the samples were counted as previously indicated.

Determination of Methemoglobinemia

The method was similar to that used by Evans et al. (57, 37) which was modified from the methemoglobin procedure of Evelyn and Malloy (58). The method used is based on a spectrophotometric measurement of the decrease in the percent transmittance at 630 nm with the increase in the amount of methemoglobin. The instrument used was the Bausch and Lomb "Spectronic 20" with a red filter and lamp to make it suitable for measurement when the wavelength of interest is in the red region (above 610 nm).

Procedure

Since the results from the previous section (blood analysis in different vehicles) indicated that 20% benzocaine -^{3}H in PEG vehicle gave the highest absorption of drug and 20% benzocaine -^{3}H in Neobase® was the second, these two formulas were investigated for their possibility of causing methemoglobinemia.

The test procedure was the same as before, except that only three rats were used for each formula. Blood samples (0.1 ml.) were withdrawn at time 0, 15, 30, 45, 60, 75, 90, 105, 120, 150,

180, 210, 240, 270, and 300 minutes.

The freshly drawn blood was put into a spectrophotometer cuvette containing 7.0 ml. of M/60 phosphate buffer. One drop of 10% saponin solution was added and the components were mixed by inverting the capped cuvette several times in order to lyse the red blood cells. The percent transmittance was then read at 630 nm ($\%T_1$) against a buffer blank containing a drop of saponin solution. One drop of 10% potassium ferricyanide solution was added to convert all the hemoglobin (Hb) to methemoglobin (MHb), mixed well, and about three minutes later the percent transmittance was read again at 630 nm ($\%T_2$) against the saponin blank. (Ferri-cyanide reagent does not absorb at 630 nm, so it is not necessary in the blank).

To determine percent MHb a calibration chart was made for each rat on semilog graph paper. The horizontal axis was scaled from 0 to 100 percent MHb, and the log scale assigned percentage T values. Assuming no MHb in the blood sample at time 0, the percentage T_1, at time 0 was plotted as the reference point on the 0% MHb axis. These two reference points were connected with a straight line. Percent T of each sample was extended horizontally to the diagonal. A vertical to the MHb scale from this horizontal intersection determines the percentage MHb.

IV. RESULTS AND DISCUSSION

Various concentrations of benzocaine -^{3}H in suppository and ointment dosage forms 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 minutes and the total radioactivity present determined. The means of the radioactivity detected are shown in Figures 1-7. The ranges are included in some figures but not shown in others due to crowding. A point by point comparison of the means obtained at each sample time was made using a students t-test (95% confident level). The results are shown in Tables 2-7, and 9.

Two methods of sample digestion were compared and the results indicated that a wet oxidation method worked better than a Unisol® method with blood samples because color quenching was much higher with the Unisol® method. With tissues, the Unisol® method gave less chemical quenching in most tissues. Although the wet oxidation method gave a somewhat lower counting efficiency than the Unisol® method for tissue samples, the efficiency was still high enough to give satisfactory results.

It was convenient to use only one method for all samples. Since the main samples studied in the experiment were blood samples, wet oxidation was chosen as the best method and was used for treating all samples (blood and tissues) throughout the experiment.

When the radioactivity of a sample is counted, the total radioactivity is the sum of the total number of radioactive molecules of any chemical form in the sample. Generally, each metabolite formed from the drug has its own rate of formation, rate of elimination and volume of distribution. Therefore, their pharmacokinetic models are different from each other and also different from the original drug. Therefore, a thin layer radiochromatographic method of separating benzocaine from its metabolites was investigated in an attempt to study the pharmacokinetic model of benzocaine -^{3}H.

Two different solvent systems (see experimental, page 30) were compared for their ability to separate benzocaine -^{3}H from its proposed metabolite, para-aminobenzoic acid (PABA). The Rf value of para-aminobenzoic acid (PABA) for the five component solvent system was 0.10 and of benzocaine -^{3}H was 0.39. With the three component solvent system the Rf value of PABA was 0.70 and of benzocaine -^{3}H was 0.85. The three component solvent system was chosen for further use in the experiment because the Rf values of both substances were higher, thus leaving more space to detect polar metabolites. The metabolites formed were expected to be the conjugation forms and the Rf values were expected to be lower than the original substance. Another reason for the preference of three component solvent system was that when

chromatographing whole blood, the five component system tended to move part of the red blood cells along the plate, thus causing streaking of the plate and some uncertainty in the Rf values.

Various amounts of benzocaine -^{3}H in different solvents (see experimental, page 31) were introduced into heparinized blood. At specified time intervals the blood enzymes were inactivated, the cells lysed, and the samples chromatographed. The chromatoplates were scraped off and counted in a liquid scintillation spectrometer but the distribution of the total radioactivity was not well delineated. Some zones on the plate, below that of benzocaine's zone, did show slightly higher counts than background but were not determined to be significant. Scintillation counting showed much higher counts than background only in the zones for benzocaine as detected with U. V. light. Therefore, no in vitro hydrolysis of benzocaine was found with the method used in this study. The reasons could be: (1) benzocaine was not hydrolysed in the blood, (2) the amount of benzocaine added was so large that the enzyme was inactivated, (3) the enzyme was inactive under the in vitro conditions, and (4) the method used here was not sensitive enough to detect the amount that was hydrolysed.

The in vivo study did show evidence of benzocaine -^{3}H hydrolysis. The 30 minute blood sample did not show any counts above background in the zones where benzocaine should be located.

The counts were high in the zones where the Rf values were approximately 0.7, which is the Rf value of PABA. For the 60, 120, 180, 240 and 300 minute samples, the Rf value of the zones where the highest counts were located was approximately 0.60 to 0.66. Another radioactive zone with an Rf value of 0.40 to 0.50 was present. The Rf values obtained in this study can only be approximations because the exact positions of the spots could not be determined using scintillation. Oxidation of the silica gel increased the counts but did not provide any further information concerning the hydrolysis of benzocaine.

From the results of this study, it is probable that benzocaine is hydrolysed in vivo to PABA which is then conjugated (33). However, it is not possible to determine if benzocaine hydrolysis took place in the blood until further work is completed.

The absorption of benzocaine from different concentrations of benzocaine -^{3}H in polyethylene glycol (PEG) suppositories (1%, 3%, 5%, 10%, 20%) and cocoa butter suppositories (3%, 10%, 20%) was examined (Figures 1 and 2, Tables 2 and 3). For rectal absorption the absolute amount of drug absorbed is directly proportional to the initial saturation concentration present and not to any excess beyond this amount. The amount of drug absorbed will increase with the increasing of drugs in the rectal fluids until the amount of drug in the rectal fluids is above a particular amount,

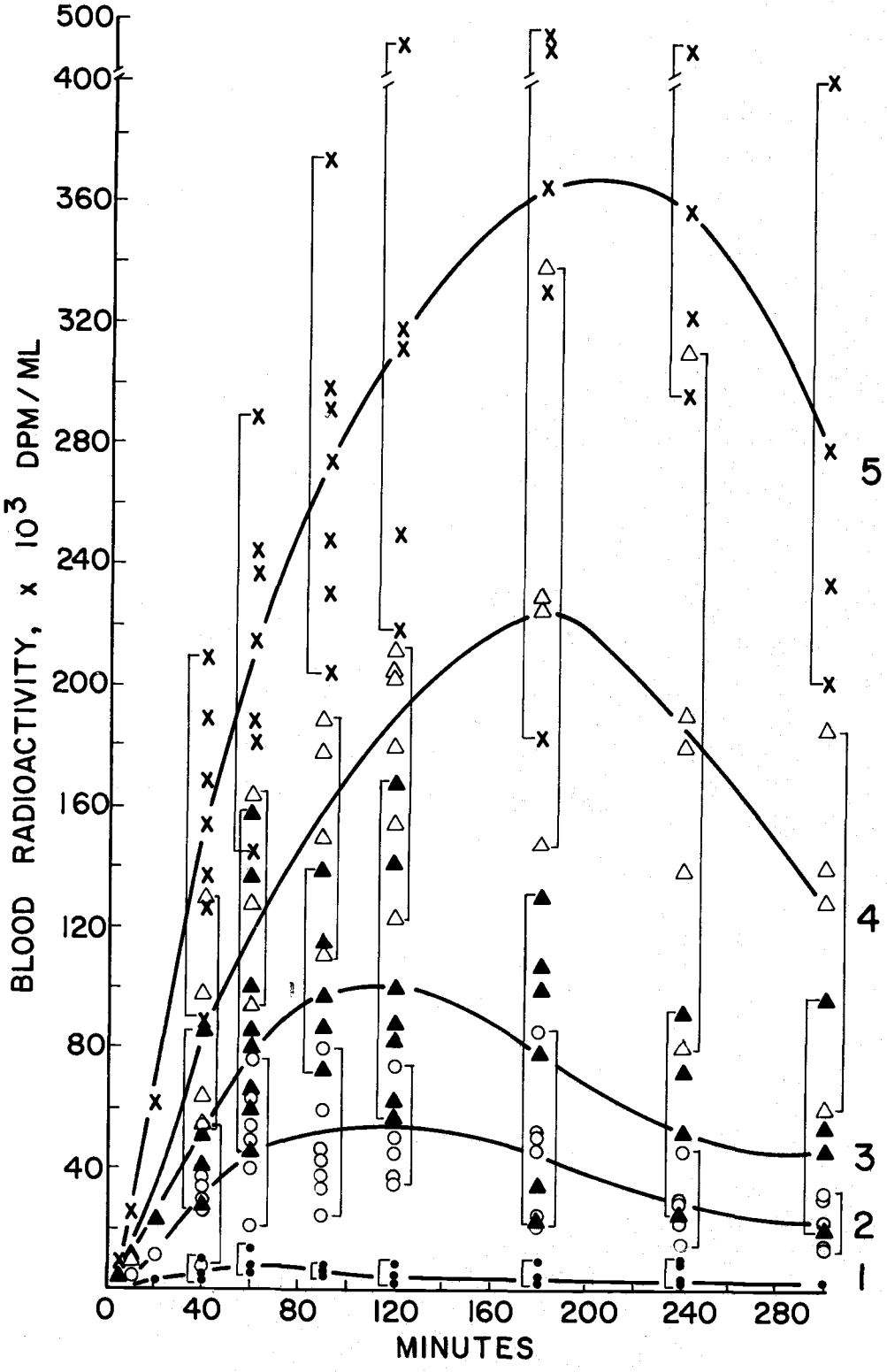


Figure 1. Blood radioactivity after the application of benzocaine -^{3}H in polyethylene glycol suppository base. 1=1%, 2=3%, 3=5%, 4=10%, and 5=20% benzoicaine -^{3}H.

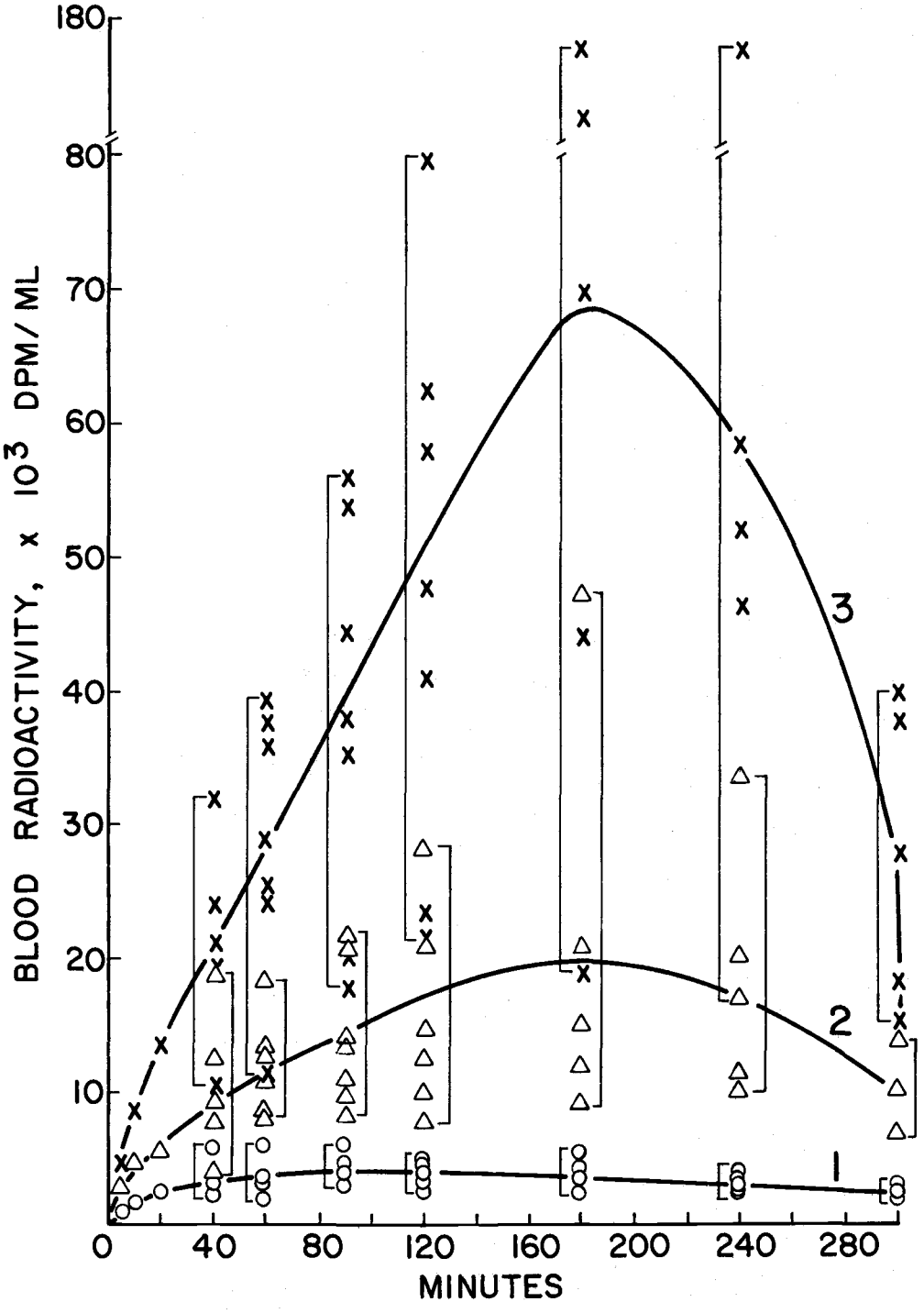


Figure 2. Blood radioactivity after the application of benzocaine -^{3}H in cocoa butter suppository base. 1=3%, 2=10%, and 3=20% benzocaine -^{3}H.

Table 2. T-test 95% Confidence (0.05 Level) of Different Curves (1=1%, 2=3%, 3=5%, 4=10%. 5=20%) in Figure 1.

Curve A. v.s.B.	Time (min.)									
	5	10	20	40	60	90	120	180	240	300
1 v.s. 2	+	+	+	+	+	+	+	+	+	+
1 v.s. 3	+	+	+	+	+	+	+	+	+	+
1 v.s. 4	+	+	+	+	+	+	+	+	+	+
1 v.s. 5	+	+	+	+	+	+	+	+	+	+
2 v.s. 3	-	+	-	-	-	+	+	-	-	-
2 v.s. 4	-	-	+	+	+	+	+	+	+	+
2 v.s. 5	+	+	+	+	+	+	+	+	+	+
3 v.s. 4	-	-*	-	+	+	+	+	+	+	-
3 v.s. 5	+	+	+	+	+	+	+	+	+	+
4 v.s. 5	+	+	+	+	+	+	+	-	+	-

(+ = > critical t-value, - = < critical t-value, * = A > B.)

Table 3. T-test 95% Confidence (0.05 Level) of Different Curves (1=3%, 2=10%, 3=20%) in Figure 2.

Curve A. v.s. B.	Time (min.)									
	5	10	20	40	60	90	120	180	240	300
1 v.s. 2	+	+	+	+	+	+	+	-	+	+
1 v.s. 3	+	+	+	+	+	+	+	+	-	+
2 v.s. 3	+	+	+	+	+	+	+	-	-	+

which varies with the drug, and then the rate of absorption will not change with further increases in drug (35). However, in suppositories, concentration does play a role in determining the rate of release of drug from suppository bases (35).

Increasing the concentration of benzocaine -^{3}H in both polyethylene glycol and cocoa butter suppository bases resulted in a higher total radioactivity in the blood (Figures 1 and 2 and Tables 2 and 3). Since the volume of suppositories applied was equal with every concentration, the total dose was also increased with increasing concentrations. Both concentrations and total doses should be taken into account as possible causes for the difference in the blood level curves using the same suppository base (59).

The increase in blood radioactivity in the first five hours (Figure 1) was higher than the proportional increase of benzocaine -^{3}H concentration in the formulas for 1% and 3% benzocaine -^{3}H in PEG suppository, but for 3%, 5%, 10% and 20% PEG suppositories, the increases were nearly proportional to the increase in drug concentrations. The areas under the curve in the first five hours for 1%, 3%, 5%, 10% and 20% benzocaine -^{3}H in PEG vehicle were approximately 2:14:22:51:93, respectively. With the cocoa butter (Figure 2), the increases in absorption were higher than proportional increases of benzocaine -^{3}H in the formulas. The areas under the curve in the first five hours for 3%, 10%, 20% benzocaine

-^{3}H in cocoa butter were approximately 1, 5, and 15, respectively. This result correlated well with in vitro results (16) which showed the increase in the amount of benzocaine dialyzed through a cellulose membrane when the concentration of benzocaine in the vehicle was increased. The decrease in drug release with increased drug concentration which occurred with polyethylene glycol vehicle in the in vitro study did not occur with the in vivo study.

The initial rate of absorption (Figures 1 and 2) was higher with the higher concentration and this might be explained by the higher release rate from the vehicle. The peak of the higher concentration curve was also higher which might be caused by the increase in the total dose and also the increase in absorption rate. At the same time, the higher the peak, the slower was the peak time, and this result could come from a complicated series of events due to the interrelationship of the vehicle disintegration, drug dissolution, drug release rate, drug absorption rate, total dose and elimination rate.

Benzocaine -^{3}H in polyethylene glycol suppository vehicle gave counts in the blood many times higher than the same concentration of benzocaine -^{3}H in cocoa butter (Figures 1 and 2 and Table 4). Polyethylene glycol is a water-soluble suppository vehicle, which dissolves in the rectum. Benzocaine dissolved in the polyethylene glycol vehicle, and therefore, was in solution and

Table 4. T-test 99% Confidence (0.05 Level), Compared the Total Radioactivity in the Blood after the Application of Benzocaine -^{3}H in Polyethylene Glycol and Cocoa Butter Suppository Bases.

[1 = 3% benzocaine -^{3}H in Polyethylene glycol
3 = 10% benzocaine -^{3}H in Polyethylene glycol
5 = 20% benzocaine -^{3}H in Polyethylene glycol
2 = 3% benzocaine -^{3}H in cocoa butter
4 = 10% benzocaine -^{3}H in cocoa butter
6 = 20% benzocaine -^{3}H in cocoa butter]

Curve	Time (min.)									
A vs. B	5	10	20	40	60	90	120	180	240	300
1 v.s. 2	-*	-*	+*	+*	+*	+*	+*	+*	+*	+*
3 v.s. 4	-*	-*	+*	+*	+*	+*	+*	+*	+*	+*
5 v.s. 6	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*

(+ = > critical t-value, - = < critical t-value, * = A>B)

ready to partition into the rectal mucosa. At the same time, cocoa butter does not dissolve but melts in the rectum. Before liquefication, dissolution of drug in rectal fluids is limited to the drug located at the surface of the suppository. Benzocaine was insoluble in cocoa butter and was incorporated in this vehicle as a suspension and therefore, even after liquefication, it was very slowly dissolved in the rectal fluid. Because benzocaine has to be in solution to be absorbed, a very small amount was available for absorption from the cocoa butter suppository.

The amounts of rectal absorption in rats of radioactivity from 20% benzocaine -^{3}H in five different ointment bases (i.e., White Petrolatum, Aquaphor®, Aquaphor®:water (1:1), Neobase® and polyethylene glycol) were compared and the results are shown in Figure 3 and Table 5. For the drug to be absorbed from the rectum it must be released from the vehicle and distributed through the surrounding fluids to the sites of absorption where it is absorbed through the rectal mucosa. The rectum contains fluids in which water-soluble vehicles can dissolve. However, the quantity of this fluid is not sufficient to effect rapid dissolution of a suppository (37). The release of drug from the suppository vehicle depends on the relative affinities of drug for the vehicle and the rectal fluid. Low affinity of the drug for the vehicle or ready solubility of drug in the aqueous rectal fluid

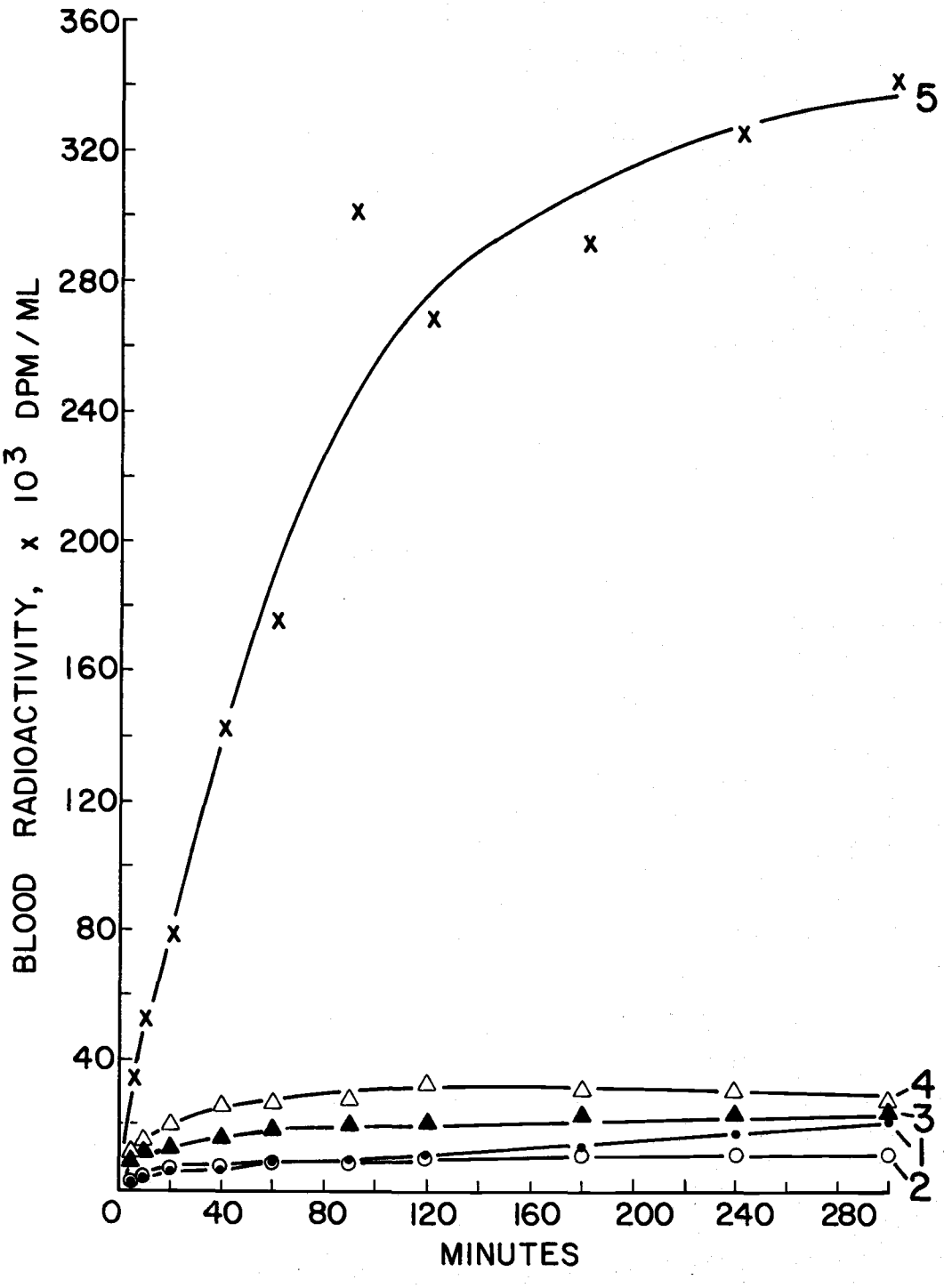


Figure 3. Blood radioactivity after the application of benzocaine -^{3}H in different ointment bases. 1=20% benzocaine -^{3}H in white petrolatum, 2=20% benzoicaine -^{3}H in Aquaphor® 3=20% benzocaine -^{3}H in Aquaphor®:water (1:1), 4=20% benzocaine -^{3}H in Neobase® , 5=20% benzocaine -^{3}H in Polyethylene glycol.

Table 5. T-test 95% Confidence (0.05 Level) of Different Curves (1=20% Benzocaine-^{3}H in White Pet., 2=20% Benzocaine -^{3}H in Aquaphor, 3 = 20% Benzocaine -^{3}H in Aqua:Water, 4= 20% Benzocaine -^{3}H in Neobase, 5= 20% Benzocaine -^{3}H in PEG) in Figure 3.

Curve A vs. B	Time (min.)									
	5	10	20	40	60	90	120	180	240	300
1 v.s. 2	-*	-	-	-	-	-*	-*	-*	-*	-*
1 v.s. 3	+	+	+	+	+	+	+	+	-	-
1 v.s. 4	+	+	+	+	+	+	+	+	+	-
1 v.s. 5	+	+	+	+	+	+	+	+	+	+
2 v.s. 3	+	+	+	+	+	+	+	+	+	+
2 v.s. 4	+	+	+	+	+	+	+	+	+	+
2 v.s. 5	+	+	+	+	+	+	+	+	+	+
3 v.s. 4	-	-	+	+	+	-	+	-	-	-
3 v.s. 5	+	+	+	+	+	+	+	+	+	+
4 v.s. 5	+	+	+	+	+	+	+	+	+	+

(+ = > critical t-value, - = < critical t-value, * = A>B.)

favors release of drug. Conversely, high affinity of drug for the vehicle of poor drug solubility in aqueous rectal fluid decreases the amount of released drug (37). The rate of drug release from the vehicle is highly dependent on the lipid/water distribution coefficient. If the drug has a lipid-water coefficient favoring lipid solubility, it is released slowly from the suppository vehicle. In fat-base suppositories, water-soluble, i.e., oil-insoluble medicaments, will be released more rapidly than a water-insoluble medicament (35). For water-soluble suppository vehicles, a water-soluble drug will be released and absorbed more rapidly than an oil-soluble drug (35). One of the primary rate-limiting steps in drug absorption from suppositories is the partitioning of the dissolved drug from the vehicle rather than the dissolution of the drug in the body fluids (35). Although it is desirable that the partition coefficient of the drug favor solubility in the rectal fluids rather than in the vehicle, a drug that is very insoluble or suspended in the vehicle will be absorbed slowly. This is because drugs must be in solution to be absorbed. The larger the particle size of a suspension, the slower the rate of dissolution and absorption. Drugs which are suspended in polyethylene glycol or oleagenous bases will give prolonged absorption because the drug is slowly eluted into the rectal fluids. Once the drug is released from the suppository vehicle and reaches the sites of absorption, the lipid-soluble undissociated drug is the most readily absorbed form.

White petrolatum U. S. P. is an example of a lipophilic vehicle. It is greasy, anhydrous and unable to absorb or mix with water. Aquaphor® (brand of "Eucerite") is a cholesterolized absorbent ointment base. It is anhydrous but is advertised to be miscible with water or aqueous solutions several times its own weight. Aquaphor®: Water (1:1) is a creamy water-in-oil emulsion type ointment base. It is hydrous and water absorbable. Neobase® (Burroughs Wellcome) is a greaseless, water miscible ointment base which is an oil-in water emulsion. Polyethylene glycol is a water soluble type ointment base.

After 20% benzocaine - ^{3}H in these ointment bases were applied into the rectum of the rats, the total radioactivity found in the blood was in the following decreasing order: Polyethylene glycol > Neobase® > Aquaphor®:water > Aquaphor® and white petrolatum. Aquaphor® and white petrolatum did not give significant differences in the amount absorbed (Figure 3, Table 5). The results of this study show that benzocaine was absorbed to a higher amount when it was incorporated in the more hydrophilic ointment base. The more soluble in water the vehicle is, the higher was the absorption. The reason might be explained by the same theory as for the suppository dosage forms above, i. e., with a high vehicle solubility in the rectal fluid, there was a corresponding increase in the amount of benzocaine which came in contact with the rectal fluid, more

benzocaine dissolved and thus resulted in an increased probability for the drug to be absorbed through the rectal mucosa.

The results obtained from this in vivo study corresponded well with the in vitro study by Ayres et al. (16) which dealt with the release of benzocaine from these same five ointment bases via dialysis through a cellulose membrane to an aqueous sink. The amounts of benzocaine released (in vitro) and the amounts of benzocaine absorbed (in vivo) from these vehicles were in the same order while white petrolatum and Aquaphor® gave very close results with no significant difference between them.

Surfactants (Tween 80 and Span 80) were included in the 3% benzocaine -^{3}H in polyethylene glycol and cocoa butter suppositories to determine if they would affect the amount of benzocaine absorption. Incorporation of Span 80 and Tween 80 with 3% benzocaine -^{3}H in polyethylene glycol suppository base did not show any significant increase or decrease in the amount of total radioactivity in the blood (Figure 4, Table 6). The total counts appeared to increase when 1% Span 80, 1% Tween 80, or 0.05% Tween 80 was incorporated but the increase was not statistically significant at the 95% significance level. With 3% benzocaine -^{3}H in cocoa butter base, Span 80 in both concentrations studied (1% and 0.05%) showed no significant influence on the amount absorbed (Figure 5, Table 7), but Tween 80 in both concentrations studied decreased the total

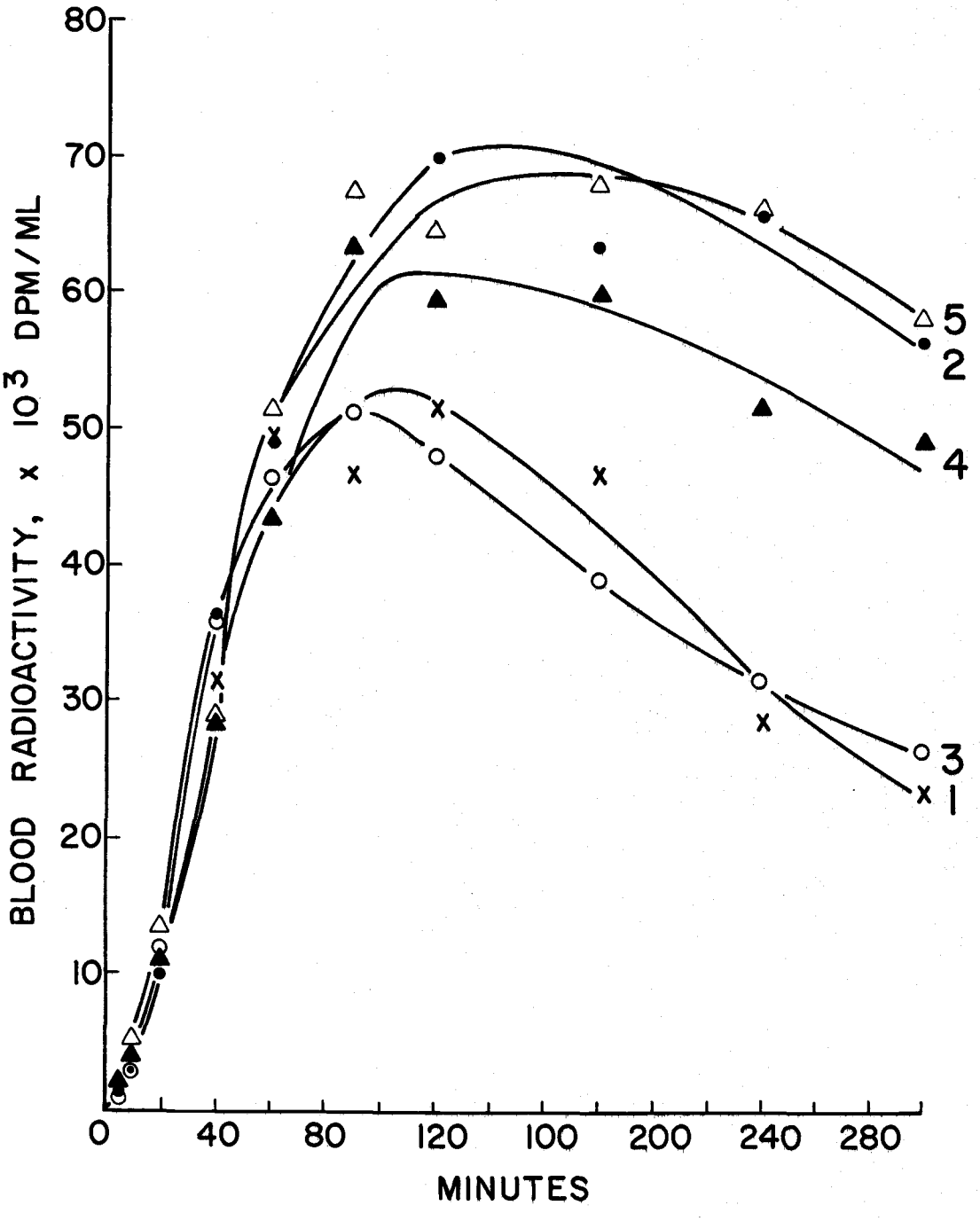


Figure 4. Blood radioactivity after the application of 3% benzocaine -^{3}H and surfactants in PEG suppository base. 1=3% benzocaine -^{3}H, 2=3% benzocaine -^{3}H + 1% Span 80, 3=3% benzocaine -^{3}H + 0.05% Span 80, 4=3% benzocaine -^{3}H + 1% Tween 80, 5=3% benzocaine -^{3}H + 0.05% Tween 80.

Table 6. T-test 95% Confidence (0.05 Level) of Different Curves (1=3% Benzocaine -^{3}H, 2=3% Benzocaine -^{3}H + 1% Span 80, 3=3% Benzocaine -^{3}H + 0.05% Span 80, 4= 3% Benzocaine -^{3}H + 1% Tween 80, 5 = 3% Benzocaine -^{3}H + 0.05% Tween 80) in Figure 4.

Curve	Time (min.)									
A vs. B	5	10	20	40	60	90	120	180	240	300
1 v.s. 2	-*	-*	-*	-	-*	-	-	-	+	+
1 v.s. 3	-*	-*	-	-	-*	-	-*	-*	-	-
1 v.s. 4	-	-*	-	-*	-*	-	-	-	+	+
1 v.s. 5	-	-	-	-*	-	-	-	-	-	-
2 v.s. 3	-*	-	-	-*	-*	-*	-*	-*	+*	+*
2 v.s. 4	-	-	-	-*	-*	-	-*	-*	-*	-*
2 v.s. 5	-	-	-	-*	-	-	-*	-	-	-
3 v.s. 4	-	-	-*	-*	-*	-	-	+	+	+
3 v.s. 5	-	-	-	-*	-	+	-	-	-	-
4 v.s. 5	-	-	-	-	-	-	-	-	-	-

(+ = > critical t-value, - = < critical t-value, * = A>B.)

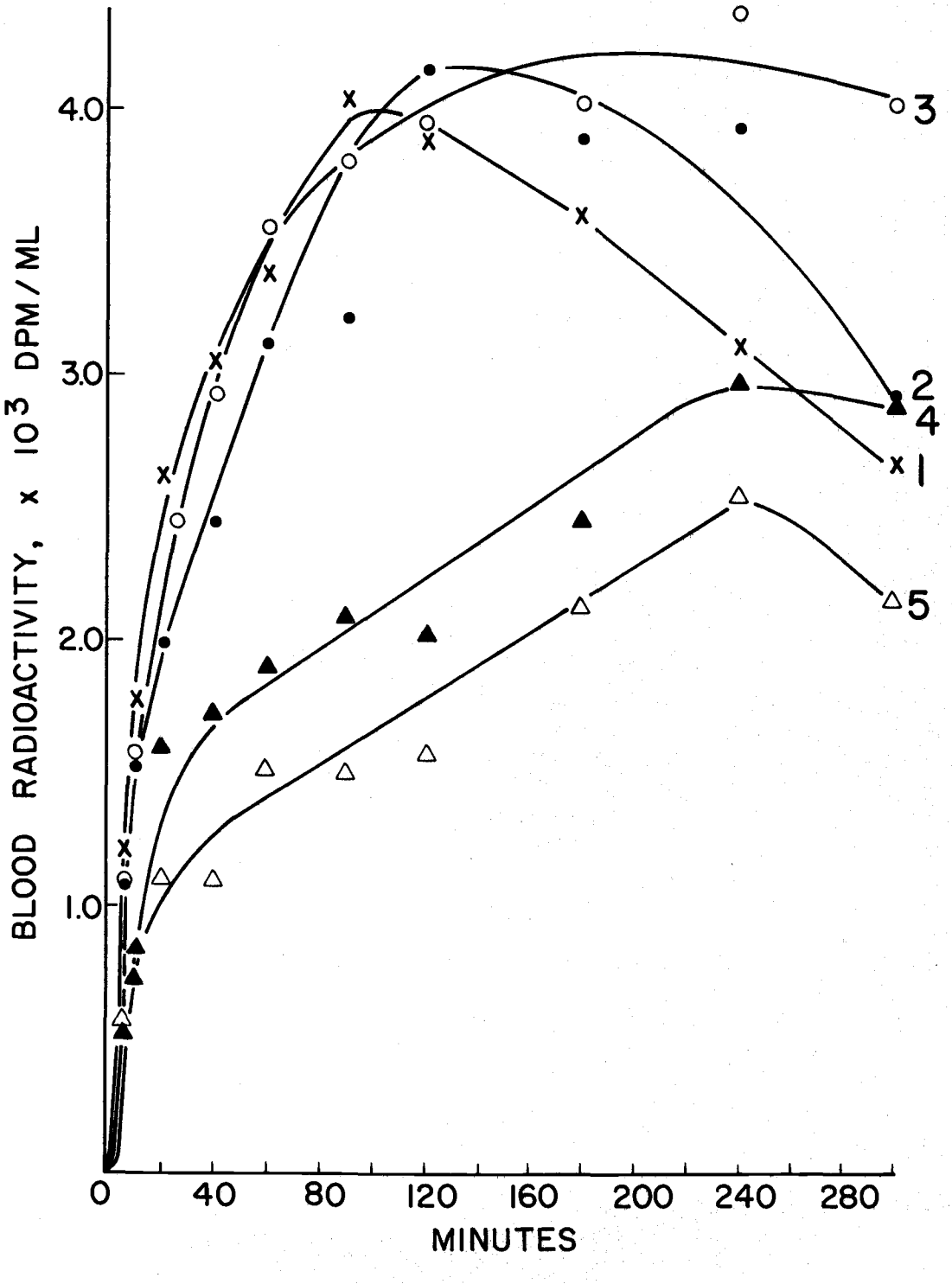


Figure 5. Blood radioactivity after the application of 3% benzocaine -^{3}H and surfactants in cocoa butter suppository base. 1=3% benzocaine -^{3}H, 2=3% benzocaine -^{3}H + 1% Span 80, 3-3% benzocaine -^{3}H + 0.05% Span 80, 4=3% benzocaine -^{3}H + 1% Tween 80, and 5=3% benzocaine -^{3}H + 0.05% Tween 80.

Table 7. T-test 95% Confidence (0.05 Level) of Different Curves (1=3% Benzocaine -^{3}H, 2=3% Benzocaine -^{3}H + 1% Span 80, 3=3% Benzocaine -^{3}H + 0.05% Span 80, 4-3% Benzocaine -^{3}H + 1% Tween 80, 5 = 3% Benzocaine -^{3}H + 0.05% Tween 80) in Figure 5.

Curve A vs. B	Time (min.)									
	5	10	20	40	60	90	120	180	240	300
1 v.s. 2	-*	-*	-*	-*	-*	-*	-	-	-	-
1 v.s. 3	-*	-*	-*	-*	-	-*	-	-	-	-
1 v.s. 4	+*	+*	-*	-*	+*	+*	+*	-*	-*	-
1 v.s. 5	=*	=*	+*	+*	+*	+*	+*	+*	-*	-*
2 v.s. 3	-	-	-	-	-	-	-*	-	-	-
2 v.s. 4	+*	+*	-*	-*	-*	-*	+*	-*	-*	-*
2 v.s. 5	-	+*	+*	+*	+*	-*	+*	-*	-*	-*
3 v.s. 4	+*	+*	-*	+*	+*	+*	-*	-*	-*	-*
3 v.s. 5	-*	+*	+*	+*	+*	+*	+*	-*	-*	-*
4 v.s. 5	-	-*	-*	-*	-*	-*	-*	-*	-*	-*

(+ = > critical t-value, - = < critical t-value, * = A>B.)

counts in the blood significantly with most of the times under investigation. This might be caused by the interaction of benzocaine with the surfactant micelles. The amount of free benzocaine could be decreased, therefore less amount was released to partition into the rectal fluid, and thus reduced the chance of being absorbed.

Tissue distribution was studied after administration of 1%, 3%, and 5% benzocaine -^{3}H in polyethylene glycol suppository base. The samples were obtained 300 minutes after the suppository was inserted. The DPM/100 mg. and DPM/organ of each tissue studied increased with increasing concentrations of benzocaine -^{3}H in the base, but the amount increased was less than the proportional increase in blood concentration (Table 8). At 300 minutes, the average blood concentration of DPM from the 3% product increased about seven fold from that of the 1% product but the concentrations of most tissues increased about five fold. The blood concentration increased about two fold from the 3% product to the 5% product while the concentrations of most tissues increased approximately one and one half fold. Liver and kidney were the two organs containing the highest counts. This may be due to the detoxification and elimination function of these organs. The concentrations in other organs studied (heart, lung, spleen, muscle, fat, brain) were not much different, with lung a bit higher, and brain contained the

Table 8. Tissue Distribution at 300 Minutes After the Application of Suppository. (Blood level at 300 Min. of 1%=3,211 DPM/ml; 3%=23,406 DPM/ml; 5%=45,894 DPM/ml.)

Dosage Form	Organ	Rat 1 DPM 100 mg	Rat 1 DPM Organ	Rat 2 DPM 100 mg	Rat 2 DPM Organ	Rat 3 DPM 100 mg	Rat 3 DPM Organ	Rat 4 DPM 100 mg	Rat 4 DPM Organ	Rat 5 DPM 100 mg	Rat 5 DPM Organ	Average DPM 100 mg	Average DPM Organ
1%	Liver	1,671	131,828	1,886	124,052	--	--	1,337	86,828	1,859	158,635	1,692	125,335
Benzo-	Kidney	887	15,853	6,384	90,323	--	--	3,380	43,016	1,531	30,514	3,046	44,926
Caine	Brain	127	1,828	198	2,595	--	--	140	2,075	161	2,629	157	2,282
--^{3}H	Heart	207	1,622	266	1,450	--	--	296	2,039	181	1,710	238	1,705
in PEG	Lung	207	2,361	332	3,559	--	--	368	3,267	201	2,946	277	3,033
Sup-	Spleen	146	865	275	1,085	--	--	255	785	134	646	203	845
posi-	Muscle	214	--	282	--	--	--	596	--	190	--	320	--
tory	Fat	91	--	447	--	--	--	262	--	109	--	228	--
3%	Liver	13,167	694,006	10,525	637,012	9,914	636,050	8,276	545,554	9,479	533,325	10,273	609,189
Benzo-	Kidney	8,695	110,599	10,898	157,031	--	--	9,849	155,659	5,001	73,349	8,611	124,160
Caine	Brain	901	12,324	670	10,761	781	11,811	537	6,556	650	10,513	707	10,393
--^{3}H	Heart	1,307	9,448	1,721	9,977	1,781	10,637	824	6,118	1,105	5,764	1,348	8,389
in PEG	Lung	1,793	14,147	1,760	13,860	2,050	17,505	966	8,132	1,443	13,553	1,602	13,440
Sup-	Spleen	1,565	8,021	1,326	6,803	1,462	5,769	790	3,533	1,008	4,042	1,229	5,634
posi-	Muscle	1,655	--	1,588	--	1,247	--	770	--	962	--	1,246	--
tory	Fat	1,196	--	1,235	--	1,407	--	932	--	913	--	1,136	--
5%	Liver	18,560	1,116,112	16,859	958,625	12,199	737,418	9,547	769,476	8,847	726,047	13,201	871,536
Benzo-	Kidney	36,513	650,480	32,579	447,214	7,449	93,109	5,376	87,704	5,532	93,402	17,491	274,382
Caine	Brain	1,381	19,598	1,792	27,077	1,166	15,953	750	10,420	1,009	15,691	1,219	17,748
--^{3}H	Heart	3,002	20,440	3,561	23,964	1,473	8,193	1,004	7,243	1,321	10,076	2,075	13,983
in PEG	Lung	3,653	35,241	3,963	31,108	1,809	15,593	1,177	10,392	1,405	12,749	2,401	21,017
Sup-	Spleen	2,953	13,444	3,065	11,839	1,341	4,975	960	4,893	1,154	5,888	1,895	8,208
posi-	Muscle	4,004	--	4,801	--	1,520	--	887	--	1,065	--	2,455	--
tory	Fat	3,257	--	3,446	--	1,126	--	1,291	--	521	--	1,928	--

least amount. Among all the tissues, the concentrations in kidney varied most among different rats.

The amount of methemoglobin formation, which is believed to be a hazardous toxic effect resulting from absorbed benzocaine from some benzocaine-containing preparations (7-14), was measured after the application of 20% benzocaine -^{3}H in polyethylene glycol suppository base. The blood was observed to have an obvious chocolate brown color. The percentage of methemoglobinemia (MH increased to its maximum at 60 minutes and then started to decrease while the total counts per minute in the blood were still increasing (Figure 6). There should be at least one substance, which could be benzocaine itself and/or one or more of its metabolites, which caused the methemoglobinemia. It is quite possible that benzocaine itself is the main cause. After 60 minutes, part of the benzocaine might be hydrolysed to its metabolites, thus, the amount of methemoglobin was decreasing but the total counts were still increasing because they included both benzocaine and metabolites. However, further study is needed before valid conclusions can be drawn about the exact cause of methemoglobinemia. It was quite clear from this study that preparations which contain high percentages of benzocaine may cause methemoglobinemia in rats when applied rectally. No sign of methemoglobinemia was found with the method used here when 20% benzocaine -^{3}H in Neobase® was

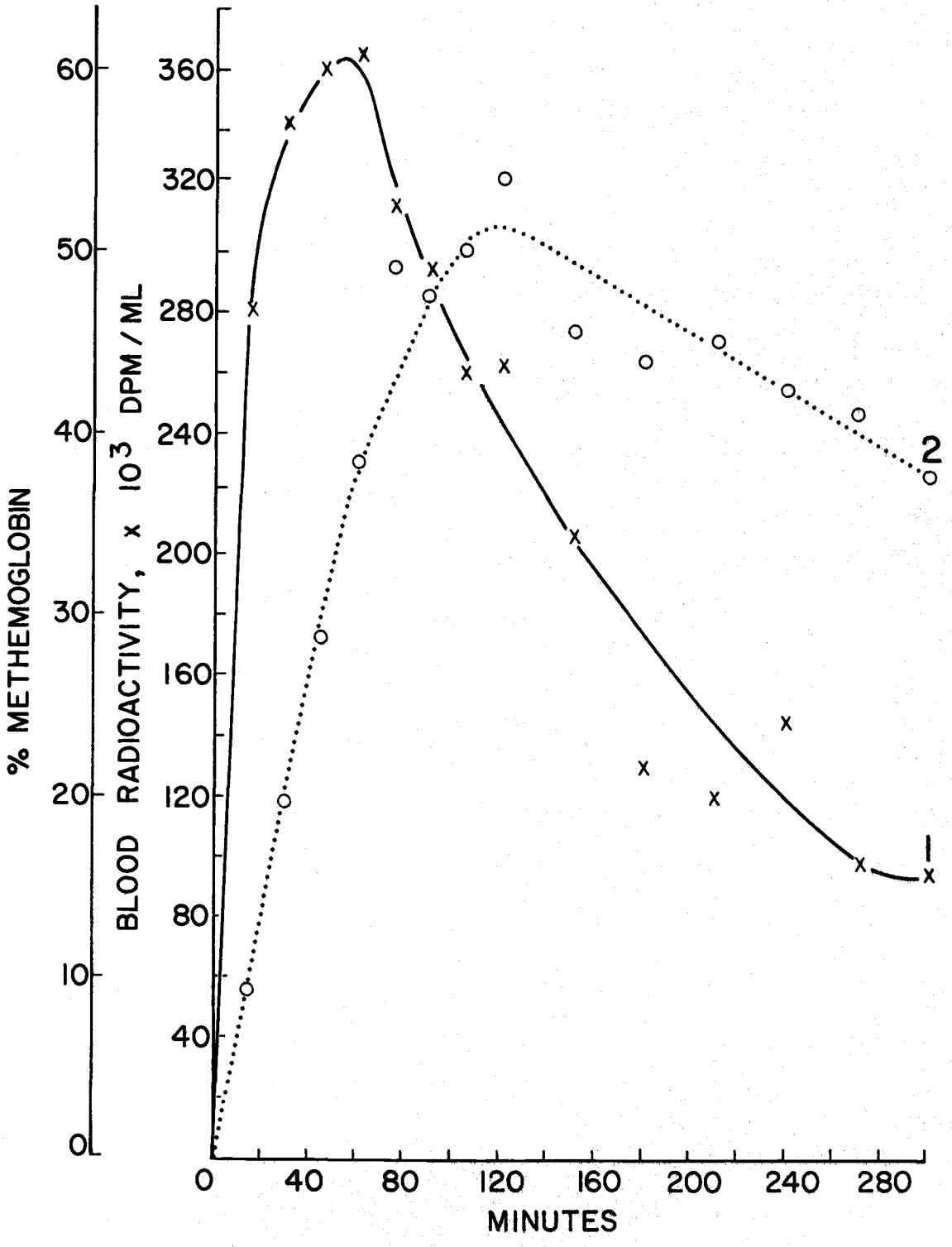


Figure 6. 1= Percent methemoglobin vs. time curve (average from 3 rats), 2= Blood radioactivity vs. time curve (average from 3 rats), after the application of 20% benzocaine -^{3}H in PEG suppository base.

applied, though the blood did appear to have a brown color. This change in color might be caused by some other factors besides methemoglobinemia or methemoglobinemia might occur but the method used here may not be sensitive enough to detect small amounts. This method was chosen because it was simple, rapid, and gave enough information for this initial study.

Effect of Sex

In the earlier part of the study, some experiments were run to measure the blood level concentration of radioactivity vs. time using male-Sprague-Dawely rats. It was surprising to note that female rats showed a total radioactivity in the blood about twice that of male rats (Figure 7, Table 9). The reason for this has not yet been determined.

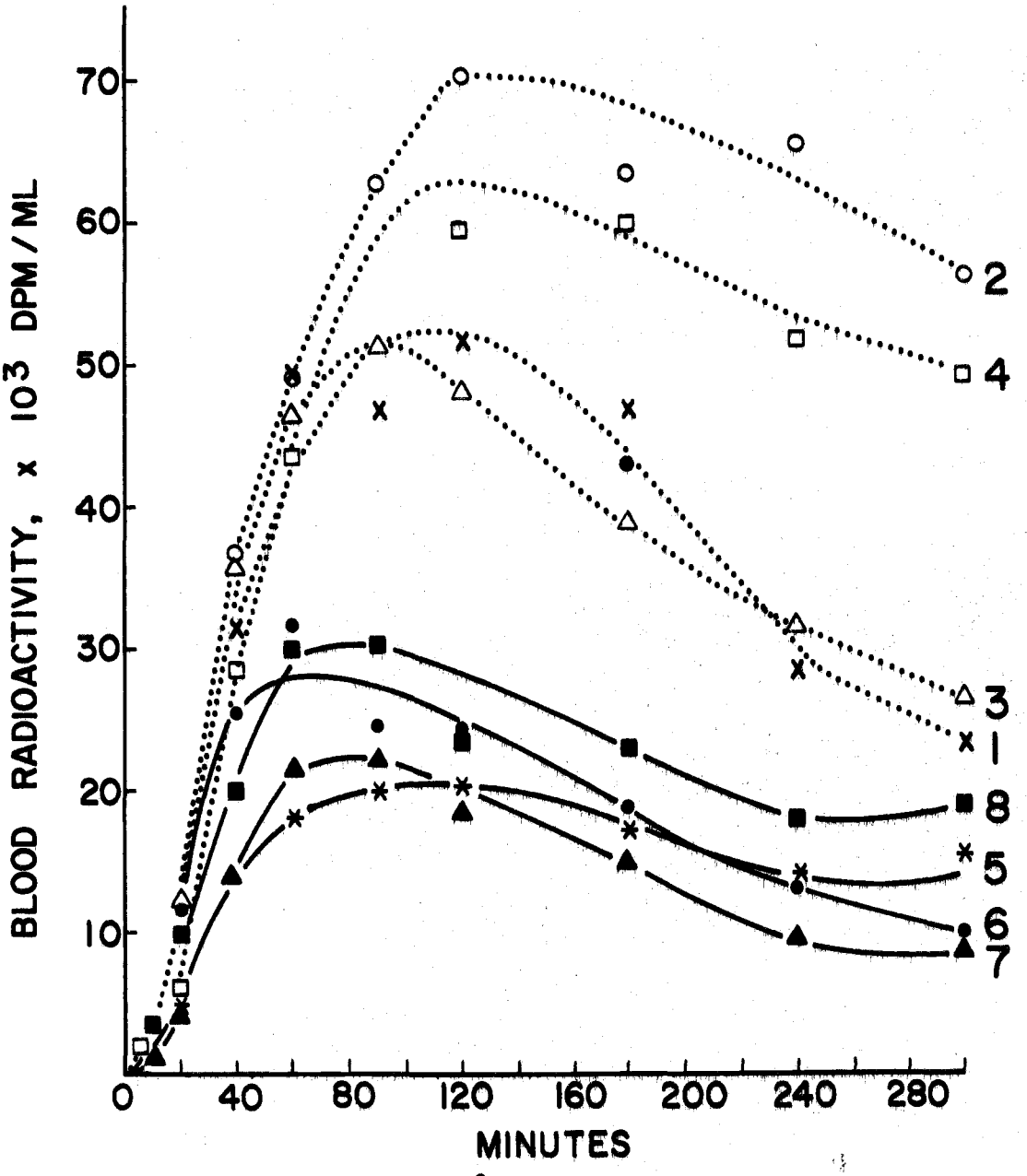


Figure 7. 1=3% benzocaine -^{3}H in Polyethylene glycol (female), 2=3% benzocaine -^{3}H + 1% Span 80 in Polyethylene glycol (female), 3=3% benzocaine -^{3}H = 0.05% Span 80 in Polyethylene glycol (female), 4=3% benzocaine -^{3}H = 1% Tween 80 in Polyethylene glycol (female), 5=3% benzocaine -^{3}H in Polyethylene glycol (male), 6=3% benzocaine -^{3}H = 1% Span 80 in Polyethylene glycol (male), 7=3% benzocaine -^{3}H to 0.05% Span 80 in Polyethylene glycol (male), 8=3% benzocaine -^{3}H + 1% Tween 80 in Polyethylene glycol (male).

Table 9. T-test 95% Confidence (0.05 Level), Compared the Total Radioactivity in the Blood after the Application of Benzocaine -^{3}H in Suppository Dosage Form into the Rectum of the Male and Female Rats.

[1 = 3% benzocaine -^{3}H in Polyethylene glycol (female)
2 = 3% benzocaine -^{3}H+1% Span 80 in Polyethylene glycol (female)
3 = 3% benzocaine -^{3}H+0.05% Span 80 in Polyethylene glycol (female)
4 = 3% benzocaine -^{3}H+1% Tween 80 in Polyethylene glycol (female)
5 = 3% benzocaine -^{3}H in Polyethylene glycol (male)
6 = 3% benzocaine -^{3}H+1% Span 80 in Polyethylene glycol (male)
7 = 3% benzocaine -^{3}H to 0.05% Span 80 in Polyethylene glycol (male)
8 = 3% benzocaine -^{3}H+1% Tween 80 in Polyethylene glycol (male)]

Curve	Time (min.)									
A vs. B	5	10	20	40	60	90	120	180	240	300
1 v.s. 5	+*	+*	+*	+*	+*	+*	+*	+*	-*	-*
2 v.s. 6	-*	-	-	-*	-*	+*	+*	+*	+*	+*
3 v.s. 7	+*	-*	+*	+*	+*	+*	+*	+*	+*	+*
4 v.s. 8	-*	-*	-*	-*	-*	+*	+*	+*	+*	+*

V CONCLUSIONS

From the results of this investigation, the following statements can be made:

(1) The rate and amount of benzocaine absorbed through the rectal mucosa were dependent on the concentration and the total dose of the drug in the vehicle. The total radioactivity in the blood increased with increasing concentration of benzocaine -^{3}H in the same vehicle.

(2) The amount of benzocaine absorbed was higher when it was incorporated in the more hydrophilic vehicle. The more soluble in water the vehicle was, the higher was the absorption with the five ointment bases under investigation, the absorption of benzocaine -^{3}H was in the following decreasing order: Polyethylene glycol > Neobase® > Aquaphor®:Water < Aquaphor® > white petrolatum. Aquaphor® and white petrolatum did not show much difference in effect but the difference among others was quite significant.

(3) Surfactants (Span 80 and Tween 80) did show some effect on the amount of benzocaine absorbed, but not a significant effect. Including Span 80 or Tween 80 in the concentrations used in the polyethylene glycol suppository base did not increase or decrease the absorption of benzocaine at the 95% confidence level, though

the means (from six rats) did show some increase with 1% Span 80, 1% Tween 80 and 0.05% Tween 80. As for cocoa butter base, the incorporation of Span 80 did not show any significant effect, but including Tween 80 in the formula decreased the absorption of benzocaine quite significantly.

(4) The radioactivity in each tissue studied was found to be related to the total radioactivity in the blood at that time. The total counts in each tissue were increased with the increase of total counts in the blood. Liver and kidney were two organs containing the highest counts, brain contained the least. Kidney gave the most inconsistency of counts among different rats.

(5) Preparations which contain high percentages of benzocaine may cause methemoglobinemia when applied rectally. Methemoglobinemia did occur in rats when 20% benzocaine -^{3}H in polyethylene glycol suppository base was applied but no methemoglobin was detected when 20% benzocaine -^{3}H in Neobase® was applied. Further work is needed to find the critical concentration of benzocaine in the blood that can cause methemoglobinemia. Since the plot between the total counts and percent methemoglobin did not show a significant relationship, more work is needed to find which substance is the real cause of methemoglobinemia and this could be benzocaine itself and/or one or more of its metabolites.

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APPENDIX

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REAGENTS

1. Benzocaine - ^{3}H (G. L.); Cal Atomic, Sp. Act. 0.1 mci/mM
2. Dioxane (Scintillar); Mallinckrodt Chemical Works
3. Naphthalene (purified); Mallinckrodt Chemical Works
4. PPO; 2, 5-Diphenyloxazole (Scintillation Grade); Packard Instrument Company
5. Dimethyl POPOP; 1, 4 bis [2-(4-Methyl-5-Phenyloxazolyl) - Benzene (Scintillation Grade); Packard Instrument Company
6. Standard ^{3}H-Toluene, labelled 1-15-71, 88,400 DPM/50 μl
7. Polyethylene Glycol (PEG) 1000; J. T. Baker Chemical Company
8. Polyethylene Glycol (PEG) 4000 (U.S.P.); J. T. Baker Chemical Company
9. Cocoa Butter (Pure Cocoa Butter); Hershey Foods Corporation
10. Polyoxyethylene Sorbitan Monooleate (Tween 80); J. T. Baker Chemical Company
11. Sorbitan Monooleate (Span 80)
12. White Petrolatum, Matheson Coleman & Bell
13. Aquaphor (Eucerite), Duke Lab. Inc.
14. Neobase; Burroughs Wellcome and Company
15. Polyethylene Glycol Ointment U.S.P.
16. Sodium Pentobarbital
17. Sodium Heparin Injection U.S.P. (1000 U.S.P. Units per ml.); Lilly

18. Unisol + Complement; IsoLab Incorporated

19. Water-free Methanol

20. 70% Perchloric Acid; Mallinckrodt Chemical Works

21. 30% H_2O_2 (Analytical Reagent); Mallinckrodt Chemical Works

22. Methoxyethanol (Ethylene Glycol Monomethyl Ether) (Analytical Reagent); Mallinckrodt Chemical Works

23. Toluene (Scintillar); Mallinckrodt Chemical Works

24. Omnifluor; New England Nuclear, Pilot Chemicals Division

25. Anhydrous Disodium Monohydrogen Phosphate; Mallinckrodt Chemical Works

26. Anhydrous Monopotassium Dihydrogen Phosphate ("Baker Analyzed" Reagent); J. T. Baker Chemical Company

27. Saponin Merck (Purified); Merck & Company

28. Potassium Ferricyanide (Crustal), Baker's Analyzed Chemicals

29. Sodium Arsenite; Mallinckrodt Chemical Works

30. p-Aminobenzoic Acid, Merck Company

31. Benzene

32. Dioxane (Stabilized, Analytical Reagent); Mallinckrodt Chemical Works

33. Glacial Acetic Acid (DUPONT); Van Water & Rogers

34. Petroleum Ether (b.p. 30-60°C)

35. Chloroform (Analytical Reagent); Mallinckrodt Chemical Works

36. Methanol (Methanol Aceton Free Analytical Reagent); Mallinckrodt Chemical Works

37. Benzocaine; Merck & Company

EQUIPMENT

1. Packard Liquid Scintillation Spectrometer
2. Spectronic 20 (Bausch and Lomb) and Test Tubes
3. Counting Vials; Packard Instrument Company
4. Disposable Micropipette
5. Plastic Disposable U-80 Insulin Syringes (1 cc.); Becton Dickinson and Company
6. Polyethylene Tubing (P.E. 200); Clay Adams
7. Polyethylene Tubing (P.E. 60); Clay Adams
8. Autoclips; Clay Adams
9. Glass Syringe (Glaspak, 1 cc., Tuberculin), Becton Dickinson and Company
10. Hypodermic Needle (Yale, Sterile Disposable 22 g.); Becton Dickinson and Company
11. Water Bath
12. Short Blunt Glass Rods
13. Uniplate [Precoated Thin Layer Chromatography Plates (Precoated with Silica Gel G.F. 250 Microns)]; Analtech, Inc.
14. Chromatogram Tank
15. U. V. Lamp

Suppository Formulas

1% Benzocaine -^{3}H in PEG

Benzocaine -^{3}H	0.10 g.
PEG 1000 (75%)	7.42 g.
PEG 4000 (25%)	2.48 g
to make	10.00 g.

3% Benzocaine -^{3}H in PEG

Benzocaine -^{3}H	0.15 g.
PEG 1000	3.64 g.
PEG 4000	1.21 g.
to make	5.00 g.

5% Benzocaine -^{3}H in PEG

Benzocaine -^{3}H	0.25 g.
PEG 1000	3.56 g.
PEG 4000	1.19 g.
to make	5.00 g.

10% Benzocaine -^{3}H

Benzocaine -^{3}H	0.50 g.
PEG 1000	3.37 g.
PEG 4000	1.13 g.
to make	5.00 g.

20% Benzocaine -^{3}H in PEG

Benzocaine -^{3}H	1.00 g.
PEG 1000	3.00 g.
PEG 4000	1.00 g.
to make	5.00 g.

3% Benzocaine -^{3}H in Cocoa Butter

Benzocaine -^{3}H	0.15 g.
Cocoa Butter	4.85 g.
to make	5.00 g.

10% Benzocaine -^{3}H in Cocoa Butter

Benzocaine -^{3}H	0.50 g.
Cocoa Butter	4.50 g.
to make	5.00 g.

20% Benzocaine -^{3}H in Cocoa Butter

Benzocaine -^{3}H	1.00 g.
Cocoa Butter	4.00 g.
to make	5.00 g.

3% Benzocaine -^{3}H + Polyoxyethylene Sorbitan Monooleate 1% in PEG

Benzocaine -^{3}H	0.15 g.
Polyoxyethylene sorbitan monooleate	0.15 g.
PEG 1000	3.60 g.
PEG 4000	1.20 g.
to make	5.00 g.

3% Benzocaine -^{3}H + Polyoxyethylene Sorbitan Monooleate 0.05% in PEG

Benzocaine -^{3}H	0.15 g.
Polyoxyethylene sorbitan monooleate	0.0025 g
PEG 1000	3.635625 g.)
PEG 4000	1.211875 g.) 4.8475 g.
to make	5.000000 g.

3% Benzocaine -^{3}H + Sorbitan Monooleate 1% in PEG

Benzocaine -^{3}H	0.15 g.
Sorbitan monooleate	0.05 g.
PEG 1000	3.60 g.
PEG 4000	1.20 g.
to make	5.00 g.

3% Benzocaine -^{3}H + Sorbitan Monooleate 0.05% in PEG

Benzocaine -^{3}H	0.15 g.
Sorbitan Monooleate	0.0025 g.
PEG 1000	3.635625 g.
PEG 4000	1.211875 g.
to make	5.000000 g.

3% Benzocaine -^{3}H + Polyoxyethylene Sorbitan Monooleate 1% Cocoa Butter

Benzocaine -^{3}H	0.15 g.
Polyoxyethylene sorbitan monooleate	0.05 g.
Cocoa Butter	4.80 g.
to make	5.00 g.

3% Benzocaine -^{3}H + Polyoxyethylene Sorbitan Monooleate 0.05% in Cocoa Butter

Benzocaine -^{3}H	0.15 g.
Polyoxyethylene sorbitan monooleate	0.0025 g.
Cocoa Butter	4.8475 g.
to make	5.0000 g.

3% Benzocaine -^{3}H + Sorbitan Monooleate 1% in Cocoa Butter

Benzocaine -^{3}H	0.15 g.
Sorbitan monooleate	0.05 g.
Cocoa Butter	4.80 g.
to make	5.00 g.

3% Benzocaine -^{3}H + Sorbitan Monooleate 0.05% in Cocoa Butter

Benzocaine -^{3}H	0.15 g.
Sorbitan monooleate	0.0025 g.
Cocoa Butter	4.8475 g.
to make	5.0000 g.

Ointment Formulas

20% Benzocaine -^{3}H in White Petrolatum

Benzocaine -^{3}H	1.0 g.
White Petrolatum	4.0 g.
to make	5.0 g.

20% Benzocaine ^{3}H in Aquaphor

Benzocaine -^{3}H	1.0 g.
Aquaphor	4.0 g.
to make	5.0 g.

20% Benzocaine -^{3}H in Aquaphor:H_2O (1:1)

Benzocaine -^{3}H	1.0 g.
Aquaphor	2.0 g.
Water	2.0 g.
to make	5.0 g.

20% Benzocaine -^{3}H in Neobase

Benzocaine -^{3}H	1.0 g.
Neobase	4.0 g.
to make	5.0 g.

20% Benzocaine -^{3}H in PEG Ointment Base U.S.P.

Benzocaine -^{3}H	1.0 g.
PEG Ointment base	4.0 g
to make	5.0 g.