THE IN VITRO RELEASE OF

MEDICATION FROM VARIOUS SUPPOSITORY BASES

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EUGENE H. BARON

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ABSTRACT

Ten selected commercially available fatty suppository bases including cocoa butter and three polyethylene glycol formulations currently in use, were subjected to in vitro testing using the following methods:

(a) Determination of melting and congealing points. The melting point for Class II substances of the United States Pharmacopeia was used for the fatty bases tested and the melting point of cocoa butter was determined by the procedure of the British Pharmacopoeia. A modification of the procedure of the British Pharmacopoeia was used to determine the congealing points of the polyethylene glycol formulations tested.

(b) Dye release. The rate of release of amaranth into an aqueous medium was measured using a colourimetric method of analysis.

(c) Liquefaction Time. The time required for suppositories to liquefy at thirty-seven degrees was measured using a special apparatus.

(d) Release of secobarbital sodium into an aqueous medium. Dialysis of the incorporated active ingredient through a cellophane membrane was measured using a spectrophotometric technique.

The testing procedure adopted established that a combination of polyethylene glycol 1000 nine parts and polyethylene glycol 4000 one part, produced the most satisfactory

water-soluble base tested, and that Estarinum B was superior to all the fat bases tested. Some correlation between dye release and dialysis was shown, and dialysis was found to be the most satisfactory _in vitro_ preliminary screening procedure for measuring the release of water-soluble medication from suppository formulations.

The effect of storage for one year on suppositories prepared from the bases was studied.

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INTRODUCTION

Definition

Suppositories are easily fusible solid preparations of various weights and shapes, usually medicated, in the form of cones, rods or cylinders, adapted for introduction into any orifice of the body except the mouth; and usually melting, softening or dissolving at body temperature. Pharmaceutically, the term connotes a preparation intended for insertion into the rectum, vagina or urethra. Other dosage forms of suppositories used in the ear and nose are termed bougies. (1,2,3)

The word suppository stems from the Latin word "subponere", meaning to place under; however, some historians feel it means substitute, as suppositories were substitutes for enemas. (4) The term pessary or vaginal suppository comes from the Greek word describing a small stone used for playing the game of draughts; while the term bougie comes from the French word meaning wax candle. (5)

The present study will deal only with those dosage forms introduced into the rectum, since suppositories inserted into the vagina and urethra are primarily for local action. The discussion of the development and selection of the suppository base, concentration and distribution of drugs employed and duration of action will be confined to the rectal route.

Because therapeutic efficacy depends upon retention in the rectum of the suppository, the shape and size of this dosage form is of prime consideration. Rectal suppositories are usually cylindrical, tapering to a rounded point at one end and generally made in sizes to meet the needs of the patient - infants, children or adults. The so-called streamlined form has the greatest diameter one-quarter the distance from the tip, the diameter gradually decreases towards the base. Once inserted, rectal contractions cause the suppository to move forward thus minimizing the possibility of expulsion. (2)

The action of medication introduced into the rectum may be classified as follows:

(a) Stimulation of the defecation reflex. The use of glycerin suppositories secures prompt and rapid evacuation due to the hygroscopic effect on the rectal mucosa, which stimulates the defecation reflex.

(b) Local action. The purpose of medication employed is to soothe or heal tissues immediately at the anal opening, in the treatment of haemorrhoids and anal fissures where prolonged and intimate contact of the drug or mixtures of drugs with a small specific surface area is required. This medication is not intended for absorption by the mucous membrane. (6)

(c) Systemic action. Two-thirds of the total number of suppositories on the market today are used for systemic

effects. Preparations used for systemic effects include
sedatives and hypnotics, analgesics, anti-asthmatics,
anti-rheumatics, anti-emetics and tranquillizers. (7)
The drug used must be finely subdivided and evenly dis-
tributed in the base, be readily available and be in
close contact with the rectal mucosa. (6) Absorption
of drugs from the rectum will be discussed under a sep-
arate section.

Properties of the Ideal Base

An ideal suppository base should:

(a) liquefy at body temperature or dissolve in body fluids
within ten minutes.

(b) be stable, forming no polymorphic forms;

(c) be easily handled, and manufactured by any process;

(d) be firm for easy introduction into the rectum;

(e) possess good contraction capability so that it may easily
be removed from a mould and require no lubricant;

(f) possess good emulsion capacity for oils, glycerin or
aqueous vehicles;

(g) be white or almost white, odourless or have an agreeable
odour;

(h) be stable on prolonged periods of storage, requiring no
refrigeration;

(i) not leak from the rectum;

(j) be compatible with a wide range of medicaments;

(k) possess good suspending power, and rapid setting time to
ensure speedy preparation;

(l) be completely non-toxic and non-irritating to the mucous
membrane;

(m) release medication rapidly, enabling prompt absorption
if intended for systemic effects. (8,9,10)

Historical Background

One of the earliest prescriptions for the compounding
of a suppository, translated from an early Assyrian medicinal
clay tablet (ca 2600 B.C.) reads: "Thou shalt reduce lion-
skin, mix with lion-fat; let it dry, mix with cedar oil, make
a suppository; put it to his anus." (11) Vaginal suppositor-
ies were also recommended in the Papyrus Ebers (1550 B.C.),
which was translated as follows: "Fennel, incense, garlic,
sert juice, fresh salt, wasp's dung. Make into a ball and
put in the vagina to cause a woman to deliver." (12) The
medical papyrii of the ancient Egyptians show that they used
suppositories to treat diseases of the rectum, to treat more
remote diseased organs, and to exert general effects. (13)

In ancient India, suppositories were used to treat
painful haemorrhoids and certain rectal diseases. (13) Hippo-
crates used suppositories for the administration of cathartics
in a vehicle of soap and honey. (14) He referred to this

dosage form as "Prosdita" or "Balanoi". (4)

Dioscorides, in the first century A.D., referred to
the use of suppositories for many indications; suppositories
of Hellebore to produce vomiting, and those of poppy seed
and mandrake to produce deep sleep. (13,14)

Two centuries after Dioscorides, Galen concluded that
suppositories were useful only in evacuation of the intestine
and was the first to describe soap suppositories. He used a
comminuted textile material which he saturated with solutions
of medicaments. Galen believed that his suppository formula-
tion would wander into the stomach via the intestine, and for
this reason he attached string to the textile material. (13,14)

Suppositories are mentioned only rarely in the later
Byzantine literature, although Paul of Aegina, the Byzantine
physician, utilized suppositories of Hellebore, "wrapped
round or bound with some wool firmly", as a purge. (15) The
Arabians paid little attention to suppository therapy. The
Latin schools of medicine seemed to have ignored suppository
medication and only the school of Salerno seemed to have de-
voted some attention to this dosage form. (13)

Paracelsus (1493-1541) mentioned suppositories only
briefly and merely recommended them as a mild purgative in
the form of soap suppositories. (16)

The Greco-Roman literature was concerned with the manu-
facture of suppositories. The size of the suppository varied
from that of a pea, to one which filled the entire rectum.

The basic substances that were used included chopped onions, honey, seedless raisins and soap. Comminuted wool, silk and linen were also used. All of these substances were generally used to incorporate medicinal agents. (13)

During the seventeenth and eighteenth centuries, the suppository was a fairly common form of medication. William Salmon recorded in the New London Dispensatory of 1691 formulae for nine suppositories and three pessaries. The base recommended was either "boyled honey" or "the white of an egg and juice of Plantane". Other bases in use during this period were wax, suet, lard and soap. However, it was not until the introduction of cocoa butter that suppositories really gained complete acceptance by the medical profession. (17)

The use of cocoa butter for the preparation of suppositories originated in the early eighteenth century, shortly after Homberg had isolated oil of theobroma. As early as 1766, Baumé (18) described the use of a suppository mould into which the liquefied cocoa butter could be poured. (17)

The use of cocoa butter in America was popularized through an article in the American Journal of Pharmacy in 1852 by Taylor. (19) The innovation was accepted immediately by a large section of the medical profession in America (17) but accepted only gradually by the pharmaceutical profession probably due to the high prices paid for the base, since as Griffenhagen (17) suggests, cocoa beans had to be imported from remote regions. The 1846 edition of Ellis' Medical

Formulary contained only a soap and opium suppository, while
the 1854 edition contained an entire section on the use and
preparation of suppositories. The Fifth Edition of the Phar-
macopeia of the United States listed nine suppositories, all
utilizing cocoa butter. (21)

The use of cocoa butter brought about a change in the
methods of compounding suppositories. Wooden moulds were de-
scribed as early as the seventeenth century, but most suppo-
sitories were hand-rolled until the late nineteenth century.
(13) The first use of metallic suppository moulds was de-
scribed about 1860. (17) A review of the history and evolution
of the suppository mould was presented by Griffenhagen. (17)

Modifications and additions to cocoa butter have been
made to improve such things as its drug-carrying capacity,
stability, hygroscopicity, hardness and temperature resistance.
The disadvantages of cocoa butter such as rancidity, leakage
from the rectum and liquefaction when incorporated with drugs
gave impetus to efforts to modify the properties of cocoa
butter and led to a search for the ideal base.

The twentieth century history of suppositories can be
divided into two separate divisions, those developments prior
to World War II and those during and after the European conflict.
The following developments took place before the war:

In 1913, Van Riel and Van der Wielen (22) added wax to
cocoa butter to improve its viscosity as a suppository base.
Terry (23) suggested the addition of kaolin and two percent

chondrus decoction to cocoa butter in 1918 for the same pur-
pose. In the same year, Behrbalk (24) proposed a cocoa butter
substitute consisting of three parts olive oil with one part
of spermaceti. Two years later, Rhodehamel (25) patented a
process for the coating of cocoa butter with gelatin, glycerin
and acacia mixtures. A United States patent was granted for
the first use of saponification products of higher fatty acids,
in 1924. (26) Eschenbrenner (27) proposed Suppositol as a
suppository base in 1925, which consisted of lard and suet,
but it was quickly discarded due to the fact that it became
rancid. In 1930, hollow forms, into which the medication was
filled into the hollow shell and sealed, were introduced by
Smith. (28) Other authors (29,30) have mentioned the use of
additive substances such as 3% calcium oleate to cocoa butter
and paraffin suppositories and the incorporation of lecithin
and cholesterol into cocoa butter to facilitate water absorp-
tion. In 1934, the use of hydrogenated vegetable oils was
initiated by the introduction of Astra-fat, a hydrogenated
peanut oil. (31) A German patent was issued in the same year
for a suppository excipient composed of cholesterol, lactose
and water heated to 80-90°, then rapidly cooled. This was one
of the first references to the water-soluble or water-miscible
base. (32) An oleaginous base was described in a United
States patent granted to Nitardy et al in 1935. (33) In 1936,
a patent was granted to Bird (34) for a base using glycerol
and glycol ethers of stearic, palmitic and lauric acids called

Monolene. Kremel (35) in 1937, introduced a base composed of
fat dispersed in a colloidal gelatin mass. Patents in Germany
the same year and later in the United States were issued to
Bochmuhl et al for their work in the development of the poly-
ethylene glycols. (36) The most important development of the
following two years, 1937-1939 was made by Caldwell (37) who
suggested the first use of hydrogenated palm kernel oil and
hydrogenated soybean oil as a base resistant to melting for
use in tropical climates.

As a result of the Second World War, restrictions on
trade and the need for ersatz products, a plethora of new pro-
ducts were developed and various substitutes suggested by
various authors in this period, (38-40), but these involved
mainly the addition of emulsifying agents to cocoa butter.
Since the Allied blockade prevented the importation of cocoa
butter, the forced substitution of cocoa butter gave rise to
the possibility that there existed a suppository base that
was close to the ideal. During the post-war period, a vast
number of products were introduced some of which are used to-
day, either in the original form or modified. The most im-
portant developments of this period will be outlined.

The new polyethylene oxide polymer first discovered by
Bochmuhl et al (36) underwent many developments and by 1946,
the use of this new base became widespread in Europe. This
base was called Scurol in France, Postonal in Germany, while
in the North American continent it was termed Carbowax. Many

workers have prepared articles on these bases and have stated
that the W grades of the products are suitable for use as suppo-
sitory bases. (41-52) This base is included in the 17th edi-
tion of the Pharmacopeia of the United States. (53) In 1948,
Schneider (54) described a new base comprised of the neutral
glycerol esters of saturated fatty acids, which was developed
by Chemische Werke Witten. (7) Many other authors (7) have
written reviews and articles on this base now included in the
French, Belgian, Austrian and Italian Codices and incorporated
as adeps solidus in the third supplement to the tenth edition
of the German Pharmacopoeia. (7) These bases, originally term-
ed Imhausenmassen are now called Witepsols. The same year,
Wankmuller (55) described bases known as Supponal O and ON.
Two years later, the same author described Schlüter 200, one
of the first suppository bases to be developed by Edelfettwerke
Werner Schlüter. One year prior to this development, Ward (57)
had obtained a patent for the use of polyoxyethylene sorbitan
esters (Tweens), as a suppository mass and they are currently
in use. (53) Hofman and Hornbogen (58) investigated the syn-
thetic suppository masses Lasupol and Suppobasin, but these
masses are not as popular as other substitutes.

During the following three years, from 1951 to 1953,
hydrogenated colza oil was investigated for use as a suppo-
sitory base (59); the seed oil from Xanthium riparium, which
is the fatty oil obtained from the fruits of Lappa major, was
investigated by Kunert (60); the fat, expressed from the seeds

of _Garcinica indica_ was noted by Affonso (61); and palm kernel
stearin, commercially known as Cebes was introduced. (62)
Tschudi-Steiner (63) investigated Suppositial which consisted
of hydrogenated vegetable fats, hydrocarbons, unsaturated
fatty acids, oxycholesterol and (hexadecyl)-myristyl alcohol.
These bases were available in three types; normal, the export
type for use in tropical regions and for use with fatty oils.
Köhler (64) reported on Stadasuppol which is an oil-in-water
emulsion of a mineral oil mixture (m.p. 37-38°) emulsified
with lecithin and mixtures of higher saturated fatty alcohols,
their sulfates and nonionic surface active agents. Kariyone
(65) patented hydrophilic suppositories, consisting of tri-
laurin mixed with fatty acid esters of polyethylene glycol,
their aliphatic esters and the fatty acid esters of sorbitol
or sorbitan with their corresponding polyethylene glycol
ethers. In 1953, Gross and Becker (1) reviewed all aspects
of suppositories. These authors (66) developed a colourimet-
ric _in vitro_ method for the development of potential release
of medication from a suppository base. They investigated a
total of sixty-seven bases and considered the water-soluble
or water-miscible base to be the universal suppository base.
(67)

Biedebach (68) in 1954 first described a suppository
mass made from the cleavage of natural fats, especially the
seed fats, fractionating and distilling the free fatty acids
obtained, removing the unsaturated and low acids, and esteri-

fying the fraction containing fatty acids, with twelve to
sixteen carbon atoms with an iodine value less than five with
glycerol. These products, developed by Edelfettwerke Werner
Schlüter are now termed Masse Estarinum. This type of product
is mentioned in the British Pharmaceutical Codex (69) as an
alternate base and in other European pharmacopoeias as reported
by the above company. (70) Edelfettwerke Werner Schlüter was
granted further patents in addition to the original. (71-76)

The following developments took place in the next four
years: a formula consisting of sixty parts of monostearyl
acetate and forty parts of methyl stearate was proposed by
Spanish workers in 1954 (77); Japanese workers (78) patented
a formula consisting of Japan wax and octadecyl formate;
Hartman and Larocca (79) investigated the use of hexadienol
and hydrogenated cottonseed oil with Veegum for use in suppo-
sitory bases; Bogs conducted research on Neat's foot oil (80)
and Lasupol EM (81); Chinese workers (82) studied Chinese
vegetable tallow; and Duracao, a greasy synthetic excipient
was described by Del Pozo et al. (83) One of the most import-
ant developments at this time was the introduction of Massupol,
consisting of the "glycerol esters of lauric acid with a very
small amount of glycerol monostearic acid ester", described
first by Soos and Kastel in 1956. (84) The use of this base,
incompatibilities, absorption aspects and disadvantages were
reviewed by Soulsby and Hopkins (8), Pavoir (85), Pennati and
Steiger-Trippi (86) and recently by Schwarz and Bichsel (87).

Massupol is now mentioned in the British Pharmaceutical Codex (69) as a "hydrogenated vegetable oil" and is used in Australia (88) and in the Netherlands (89) in place of cocoa butter, but has not found wide use on the North American continent. Emulgin (90), a product of glycerol esters with the fatty acids obtained from paraffins was proposed in the same year. The following year, Farr (91) patented a cocoa butter substitute prepared by fractionation of cottonseed oil and palm oil. The same year, Silverman (92) introduced an emollient suppository base consisting of Lantrol (93), the liquid fraction of wool grease, and Wecobee base S, the latter described as a synthetic cocoa butter possessing natural triglyceride structures and being hydrophobic in nature. (94) These bases have found limited use in the United States. (94) In 1960, nonionic surface active agents termed Pluronics were introduced as suppository bases. (95) In 1961, Robertson (96) suggested the use of the fats expressed from certain species of <u>Shorea</u> of the family <u>Dipterocarpaceae</u> as a cocoa butter substitute. Simon and Slaun (97) reviewed a product termed Cao Butta in 1963, and at the same time Giand et al (98) suggested beeswax fractions as suppository bases. The most recent developments include a patent issued to Chemische Werke Witten for a base resistant to melting in tropical climates (99), and the use of higher boiling fractions of ethoxypolysiloxane oil (E.P.S.) in suppositories. (100)

Although hydrogenated products and synthetic substances

have replaced cocoa butter to some extent, the search for the
ideal excipient still continues.

Absorption of Drugs from the Rectum

Absorption of drugs from the colon and rectum is quite
similar. Some of the factors which influence availability
from the rectal-colon area include degree of ionization and
the lipid:water partition coefficient of the undissociated
form of the active ingredient. Weak acids and bases are ab-
sorbed readily, the highly ionized compounds are absorbed
slowly. If the drug is bound to the components of the base
by physical interaction through the surface active agents
present or through preferential solubility of drug or base,
the availability of the drug is lowered. (103)

Gradnick (101) indicated that the rectal mucosa had
a high absorbent capacity comparable to that of the small in-
testine. The greater part of the drug appears to be absorbed
from the mucosa and thence into the portal system and only a
small portion, which by-passes the liver, enters immediately
into the general circulation. According to this author, the
base used is very important. He states that fatty bases give
slow prolonged absorption, and water-soluble bases liberate
medicaments more easily and should be used when a rapid effect
is required, while emulsifying bases are intermediate in effect.
According to Gradnick (101) a substance with a high activity

in small doses should be administered rectally, but the rate of absorption would not be easy to determine.

The lower region of the rectum, with its numerous superficial blood vessels, provides an absorptive surface from which soluble substances can rapidly pass into the venous circulation providing action almost as rapid as that produced by an intravenous injection of the drug. By direct absorption into the venous circulation, drugs suffer no breakdown in the liver. This view is held by many clinicians, but the opposite view, that there is little or no absorption of drugs from the rectum is held by other clinicians. (14)

The statement that suppositories are employed locally where ointments cannot be applied readily has broad implications. Suppositories are intended to ensure uniform diffusion of medicinal components to the internal parts to which they are applied. (2) The statement made by Gradnick (101) regarding the use of fatty bases for drugs which require slow prolonged absorption, and water-soluble bases to liberate medication more readily is not necessarily true. Comparative studies must be conducted for each drug separately and no general statements can be made regarding the release of medication.

The Use of Suppositories

Suppositories are indicated for many and varied reasons. If the drug is not absorbed by the mucous membrane, supposi-

tories can be used advantageously for treatment of local conditions of the rectum, vagina and urethra. A suppository for systemic action is desired and is indicated, where continuous emesis is present, when a patient is unable to swallow other dosage forms, or if the patient is in a comatose condition. Suppositories are well suited for infants and children, where the oral route presents a problem due to the fact that children may not be able to swallow the conventional dosage forms and will refuse medication which is unpalatable. In the case of drugs rendered inactive by digestive fluids of the stomach or intestine, such as insulin or those which interfere with digestive functions, systemic effects can be exerted rectally. (102) The use of morphine suppositories in bedridden patients with terminal cancer has also been reported. (6)

It has been stated that the dosage limits rectal administration, that absorption is irregular, and that the administration of suppositories lacks aesthetic quality. However, the answer to the critics is that the correct dosage and the rate of release of drugs from suppository bases has yet to be determined.

Rectal medication appears to be gaining in importance. Although suppository preparations account for only ten percent of all the dosage forms produced, Chemische Werke Witten reported that this figure represented an increase of almost sixty percent in the last decade. (7) As the pool of knowledge in the sphere of rectal absorption increases, further

developments may be expected.

Manufacturing Procedures

The method of manufacture of suppositories depends on the number of suppositories prepared, the materials used and the equipment available. In general, however, two methods, or modifications of these methods, are used, the cold, and the hot or fusion process. The cold method can be further subdivided into the hand-rolling method and the compression method. (103)

The hand-rolling method involves the moulding of the suppository with the fingers after formation of the plastic mass. The method requires a minimum of equipment, good technique and usually small quantities, less than twelve suppositories are manufactured by this process. (103) This method is rarely used today.

The compression or pressing process requires the formation of a uniform mixture prior to placing the mass into a chamber for moulding. The prepared and grated mass is placed in the chamber of the machine to which the appropriate mould has been attached. A pressure wheel is then turned which extrudes the base from the chamber into the mould. Due to the high pressure developed, the mixture becomes hot in the cylinder and it is advisable to have some means of cooling the cylinder. (103) This method has been largely supplanted by the fusion method in industry, due to the technical problems involved. (104)

The fusion method is used with all suppository bases and all but the most heat-sensitive drugs. It is the method used for glycerinated-gelatin, and polyethylene glycol suppositories because these require a higher temperature for melting than that produced by the compression method. Basically, it consists of melting all constituents and pouring directly into a mould at a suitable temperature. The mixture is stirred to prevent settling of medicaments and poured into prepared moulds just before the setting temperature of the mixture is reached. The moulds are filled three to four millimetres over capacity to allow for contraction on cooling. The moulds may or may not be treated with a lubricant to prevent sticking and may also be chilled. Industrial production is carried out in basically the same manner employing large moulds coated with a special synthetic material to prevent sticking. The moulds are chilled to five degrees centigrade. (104)

The Casting Process can be regarded as an industrial modification of the fusion technique. It is used principally in European countries (7) and has not proven popular in North America. The entire operation is done by machine. The excipient is melted in the stirring and melting portion of the machine and mixed with the active ingredients. It is then transferred to a second vessel and poured into moulds while the machine continues to run. Excess quantities are scraped off and returned to the machine. A cooling coil is fitted directly into the moulds. Before the suppository is expelled,

the moulds are briefly reheated to eliminate cavities in the prepared form. A special device opens the moulds, expels the suppositories, cleans the moulds, releases the suppositories and returns the excess material back to the machine. (7)

Tardos et al (105) reported a difference in absorption of active ingredient between those suppositories made by the compression method and those by fusion. It has been stated that the difference could be due to incompatibility with the components of the machine or due to insufficient mixing before the suppositories were compressed. (103) Tardos (105) attributed the difference to the modification of physical properties of the active compound produced in manufacture.

Testing of Suppositories

The standardization and assay of suppositories with respect to liberation of active ingredients, and therapeutic activity involves two procedures:

(a) in vitro testing of the suppository base.

(b) testing of the suppository with various active ingredients in vitro and in vivo.

"In Vitro" Methods

In vitro testing of bases involves chemical and physical tests: chemical tests for the fatty bases include iodine number, saponification number, and acid value and can

be found in the Pharmacopeia of the United States or the British Pharmacopoeia (106,107); physical tests include consistency of the base, hardness, density, refractive index and melting points, which can be found in the previously mentioned Pharmacopoeias (106,108). The more important modifications of the melting point can be classified into two groups:

(a) those done in a dry environment: Bogs (81) used a micro-melting point apparatus for the determination of the melting behaviour of several suppository bases; and

(b) those done on suppositories in a water bath. Malangeau (109) placed a wire of small diameter in the mould before the suppository solidified. The suppository was held by the wire and immersed in the water while the temperature of the bath was raised uniformly. The melting point was taken as the time the suppository slipped off the wire. A patented melt-testing apparatus was developed by Chemische Werke Witten (7) in order to observe the melting process as a function of time.

An apparatus designed for the mechanical strength testing of bases was first described by Malangeau. (109) He determined the mechanical strength of cocoa butter by the use of weights. Reznek (110) described an apparatus to apply pneumatically a slight pressure to a suppository held at a specified temperature in a water bath, in order to measure the pressure required to deform a suppository. Silverman

(92) determined the softening point of various suppository
bases utilizing the ring-and-ball apparatus of the American
Society for Testing Materials using a slice taken from the
suppository. Chemische Werke Witten (7) has also developed
an apparatus to test the mechanical strength of suppositories.
This apparatus uses sliding weights in order to reduce fric-
tion, and is enclosed in a thermostatically controlled device.
Recently, Azhgikin (111) determined the hardness of cocoa
butter, hydrogenated cottonseed oil and sunflower oil, the
latter two having an added emulsifying agent, by means of the
Kaminskii apparatus which involves determining the load nec-
essary to cut a specific cross-section of fat (solidified under
specified conditions) by a wire.

Suppository testing in vitro using suppositories con-
taining various medicinal ingredients includes microbiologi-
cal testing, colourimetric analysis, liquefaction time and
dialysis techniques.

Microbiological testing involves diffusion into static
media and is designed to measure diffusion of a medicinal, usually
an anti-microbial into some medium, generally agar. This type of
work was initiated by Buchi and Schlumpf (112) utilizing suppo-
sitories containing an antiseptic which developed a zone of in-
hibition on an agar plate inoculated with a microorganism. Ward
(113) measured the antibacterial activity of phenyl mercuric nit-
rate on Staphylococcus aureus in suppositories manufactured from
polyoxyethylene sorbitan mono-oleate and glyceryl laurate.

Silverman (92) used four test organisms; _Proteus Morganii_,
Klebsiella pneumoniae, _Micrococcus_ pyrogenes var. _citreus_
and _Micrococcus_ pyrogenes var. _albus_ to measure the zones of
inhibition produced by medicaments including Penicillin G
potassium tetracycline, nitrofurazone, using cocoa butter and
Lantrol. Silverman used a slice of the suppository base in
question measuring ten millimetres thick and ten millimetres
in diameter. Blissitt et al (114) incorporated Erythromycin
into various suppository bases including cocoa butter, poly-
ethylene glycol and a water-soluble base consisting of poly-
ethylene stearate, wax, water and Aerosol OT, and measured
the zone of inhibition produced in a medium inoculated with
Staphylococcus aureus, employing a thin slice selected at ran-
dom from each suppository. Ghafoor and Huyck (115) incorpor-
ated chloramphenicol into various suppository bases includ-
ing cocoa butter, polyethylene glycol, Aerosol OT, and yellow
wax-polyethylene glycol, and measured the zones of inhibition
produced in a medium consisting of horse serum inoculated with
Staphylococcus aureus.

The microbiological methods employed indicate that a
water-soluble or water-dispersible base may release medication
at a more rapid rate than a base consisting of natural fat. Del
Pozo and Cemeli (116) measured the diffusion of sodium salicy-
late from various suppository masses including glycerinated
gelatin, polyethylene glycol, polyethylene glycol esters, ethyl
and methyl stearate, cocoa butter, Agrasup A and H, which are

two suppository masses developed in Spain, and the Witepsols

into a special medium consisting of agar-ferric chloride.

The diffusion time required to produce a zone of inhibition

of five millimetres was measured. The water-soluble masses

produced the zone in the most rapid time followed closely by

cocoa butter and some of the Witepsols. An important feature

of these tests is that the authors attempted another in vitro

test, which was dialysis. This procedure will be described

later in another section. Recently, Endraszka et al (100)

measured the diffusion rates of two antibiotics, penicillin

and chloramphenicol, into agar media utilizing suppository

bases consisting of cocoa butter, and cocoa butter containing

five percent EPS oil. The purpose of their study was to

examine the feasibility of incorporating EPS oil into suppo-

sitories. Their results show that the inclusion of this oil

was not feasible in suppository manufacture.

Gross and Becker (66) reported that the rapidity of

release of medicament from a base may be measured by incor-

porating a known amount of water-soluble dye into the suppo-

sitory and determining the release of dye in aqueous solutions

at various time intervals. A suppository containing amaranth

was placed in a percolator, stoppered at the orifice and

samples withdrawn at various time intervals while the suppo-

sitory liquefied. The solutions were analyzed spectrophoto-

metrically. The bases tested included cocoa butter and con-

geners, hydrogenated vegetable oils, glycerin and glycerinated-

gelatin, polyethylene glycol, sorbitan fatty acid esters, poly-
oxyethylene sorbitan mono-oleate and polyethylene glycol esters.
They found that the polyethylene glycol esters released the maxi-
mum amount of dye. Hartman and Larocca (79) used a modification
of the Gross and Becker (66) procedure and tested dye release
from hydrogenated vegetable oils in combination with disinte-
grating and emulsifying agents. These authors concluded that
in their opinion emulsifying agents do not greatly affect the
release of medicinals from fatty bases, which is in disagree-
ment with previous authors. (1) Whitworth and Larocca (117)
tested hydrogenated cottonseed oil in combination with sorbitan
fatty acid esters and polyoxyethylene sorbitan mono-oleate and
concluded that dye release was greatest in those bases with
thirty-five to forty percent emulsifying agent and possessing
a melting point less than forty-eight degrees. These authors
(117) attempted to correlate their results with an in vivo study.
They measured the onset of loss of righting reflex in rabbits
using the bases they had developed. They stated that bases
which released no dye were ineffective in vivo, and those which
released forty percent of dye at the end of thirty minutes in
vitro were effective in vivo in less than fifteen minutes. Sil-
verman (92) compared dye release as a function of time with two
bases, however these were evaluated at different temperatures.
Recently, Giand et al (98) used the method developed by Gross
and Becker (66) to test fractions of bleached beeswax as possible
substitutes for cocoa butter.

Some research workers (118) have proposed methods by

which the characteristics of a suppository, particularly the
melting point, can be controlled on the entire suppository.
However, with the present methods, the melting point is meas-
ured in an environment different from that of the rectum.
It gives useful information on fatty bases only, because all
of the water-soluble bases soften at a temperature above body
temperature or dissolve independently of this temperature.
A representative time of melting or softening at body tem-
perature is difficult to achieve with the methods previously
used. Knowledge of this time is essential for suppositories
with drugs intended for systemic action. If the melting time
is too lengthy, the suppository may be expelled before lique-
faction. (118)

Setnikar and Fantelli (118) have stated that the phar-
macopoeias should recommend a maximum liquefaction time for
rectal suppositories just as they fix maximum time periods
for tablet disintegration. According to these authors, the
conditions of the rectum can be simulated in an apparatus
which they have developed. The apparatus consists of a glass
cylinder of specified dimensions, fitted for connection to a
circulating water pump and thermometer and containing a
specified length of cellulose dialyzing tubing. This appara-
tus is intended to simulate the conditions of the rectum,
and has also been used for the determination of the melting
point of suppositories manufactured from fatty bases. These
authors (118) have tested a large number of bases of varying

composition including the Estarinums and the Witepsols, as
well as polyethylene glycol and glycerinated gelatin suppo-
sitories. The authors have also correlated their results
with an in vivo technique involving the incorporation of X-ray
contrast media into one base selected from the fatty bases
and one from the water-soluble masses, and administering
them to humans. The authors (118) recommended that a maxi-
mum time of ten minutes be set for the liquefaction time of
rectal suppositories.

One of the required characteristics of a suppository
base is the rapid and complete release of incorporated active
principle. Eckert and Mühlemann (119) discussed the influence
of the properties of the base on release of medication. They
concluded that if the drug dissolves in the base, release and
absorption are much slower and more continuous than in the
case when the drug is in suspension. Release of a water-
soluble drug depends on melting state, melting interval, and
the time when the melt is clear. Release of a drug soluble
in a fat base is affected little by the melting state and
much less drug is released per unit of time. Water-soluble
bases release medication less quickly than fatty bases inde-
pendent of time. The fat base must have a clear melting point
less than or equal to thirty-six point five degrees, a small
melting interval and a small hydroxy number in order to re-
lease the maximum amount of medication. If a quick effect is
desired, a fatty base should be used in which the drug is

insoluble and in suspension. For a delayed effect, the drug must be dissolved. (119)

Various workers have shown dialysis to be a rapid, convenient and valid method for the determination of the rate of release of drug from suppository bases. (120-127) Peterson and Guida (120) measured the dialysis of theophylline incorporated into suppositories through filter paper into normal saline. They surveyed fatty bases, including cocoa butter and water-soluble bases, mainly the polyethylene glycol type containing emulsifying agents. These authors reported that water-soluble bases gave the most rapid release of theophylline, and that prolonged storage affects theophylline release from a cocoa butter base. These authors (120) also compared the release of theophylline from a suppository when compounded using different forms of the drug. This test demonstrated that slight changes in the physico-chemical properties of the drug-base relationship influenced the rate of release of the drug. Del Pozo (121) attempted to determine the effect of emulsifying agents on the rate of release of potassium iodide in suppositories prepared by suspension and emulsification using cocoa butter as the base. Dialysis of potassium iodide was measured through a tube prepared from collodion in a special apparatus by withdrawing samples at intervals. He concluded that the emulsifying agent itself has little effect on the release of potassium iodide from suppositories but that emulsion-type suppositories release

medication at a faster rate than suspension-type suppositor-
ies. Del Pozo and Cemeli (116) incorporated sodium salicylate
and acetylsalicylic acid into various suppository bases includ-
ing some of the Witepsols, glycerinated-gelatin, polyethylene
glycol esters, Agrasup A and H, and polyethylene glycol, and
measured dialysis of the drugs across a cellophane membrane.
Dialysis of sodium salicylate and acetylsalicylic acid was
the greatest with Witepsol H-15. The authors (116) found
little or no correlation between dialysis and the diffusion
into agar media which was described previously. In another
study by the same authors (122) it was demonstrated that the
Witepsol masses released more sodium salicylate than cocoa
butter. However, in vivo testing involving the determination
of the blood level of the drug in rabbits gave practically
the reverse results. Cemeli and Bardet (123) measured the
dialysis rate of disodium hydrogen phosphate with radioiso-
topically labelled P_{32} through a cellophane membrane utiliz-
ing cocoa butter, Witepsol H-15, polyethylene glycol 1540
and 4000, and various other polyethylene glycol combinations
as bases. Maximum dialysis of the drug was obtained with
Witepsol H-15 followed by the water-soluble masses. These
authors (123) also attempted correlation with an in vivo
test and administered the same drug to guinea pigs. The
fatty masses showed instantaneous diffusion of P_{32} into the
bloodstream, while the water-soluble masses gave counts only
after four minutes. Krowczynski (124) stated that release

of medication depended upon the melting point of the vehicle,
absence of adhesive substances and the presence of surfactants.
This same author also stated that the method of manufacture of
suppositories has no effect on release, contrary to the report
by Tardos. (105) In the case of drugs soluble in the vehicle,
the liberation of medicament was markedly slower, which is in
agreement with the work of Eckert and Mühlemann. (119) Plaxco
(125) compared the relative rate of dialysis of aminophylline
through nylon and cellophane membranes and concluded that di-
alysis through cellophane was the more uniform, although slower.
This author compared the rate of release of aminophylline by
dialysis from cocoa butter, a hydrogenated vegetable oil base,
a polyethylene glycol base, a polyoxyethylene sorbitan mono-
oleate base, an anhydrous liquid base and a petroleum-paraffin
base. Dialysis of aminophylline was most rapid with the poly-
ethylene glycol base, closely followed by cocoa butter. Plaxco
(125) stated that the temperature of the dialyzing solutions
must be kept constant and above the melting temperature of
those suppositories that liquefy. This author also indicated
that a definite relationship exists between the rate of release
of medication from suppository bases and rate of dialysis.
Kakemi et al (126) measured the diffusion of sulfonamide from
fatty bases such as cocoa butter and some of the Witepsols and
the water-soluble masses and surfactants such as polyethylene
glycol 4000, polyoxyethylene acids such as Myrj 51, polyoxy-
ethylene sorbitan mono-oleate (Tween 65), and sorbitan monostear-
ate (Span 60) across a cellophane membrane using much the same

method as previous authors. (120-125) However, instead of removing an aliquot portion from the solution, the withdrawal was subsequently replaced with fresh medium. These authors (126) attempted an _in vivo_ study by measuring the blood level of sulfonamide in rabbits after insertion of the drug incorporated into the above mentioned bases. They concluded that the hydrophile-lipophile balance system (HLB) which is the ratio between the lipophilic (nonpolar) and hydrophilic (polar) portion of a molecule of a surface active agent is related to absorption rate and that this is controlled by mixing surface-active agents of different HLB. Absorption rate of the sulfonamide was accelerated by the use of a surfactant in a fatty base. Release rate of the drug was the greatest factor affecting the absorption rate in the _in vitro_ study. Neuwald and Kunze (127) employed cellophane dialyzing tubing to measure dialysis using acetylsalicylic acid, sodium salicylate, and calcium salicylate in cocoa butter and the Witepsols. They placed the bag containing the suppository and a portion of distilled water in beakers at definite time intervals and analyzed the contents. It was found that water solubility has a limited influence on release from fatty bases, and that the composition of bases was of secondary importance. Analysis of samples showed quite pronounced salicylate levels with the different drugs _in vitro_. These workers (127) administered the same drugs rectally to humans and found that all test substances gave good blood levels, irrespective of their water

solubility. Release of the test substance in vitro depended largely on the water solubility of the medication whereas water solubility had a limited influence on release in vivo. Endraszka et al (100) used the dialysis method utilizing penicillin and sodium chloride to determine the effect of EPS oil in suppositories. These authors found that the suppositories did not dialyze after one month's storage and concluded that this substance was unsuitable for use in suppositories.

"In Vivo" Methods

In vivo methods can be classified as follows:
(a) those involving single end-point studies.
(b) those involving multi-point absorption studies.

Single end-point studies involve a single pharmacological response and include loss of righting reflex in animals, single blood level, urine level, and mydriatic response.

Hassler and Sperandio (9) incorporated amobarbital sodium, secobarbital sodium, and pentobarbital sodium in varying doses into polyethylene glycol formulations using cocoa butter as a control base for each test. Absorption was more rapid in those suppositories manufactured with cocoa butter, but the duration of effect of the water-soluble bases was much more prolonged. Whitworth and Larocca (117) incorporated pentobarbital sodium into several bases and administered

the drug to female albino rabbits, utilizing ten animals for each test. In addition to this in vivo work, these workers compared loss of righting reflex using the bases they have developed with a commercial product having a cocoa butter base. The commercial brand of the suppository formulation had the largest standard deviation. Whitworth (128) stated that although much had been done to attempt in vitro testing, little had been done to standardize in vivo work. This author incorporated pentobarbital sodium into suppositories consisting of surface active agents in combination with hydrogenated vegetable oils. Rabbits were used as test animals. An attempt was made to correlate the result of this test with that of administering sulfathiazole sodium in suppository form to the same test animals. The bases were graded equal in both tests with one exception. Correlation between the two in vivo methods appeared to be significant, as both drugs used were water-soluble and the same test animal was used.

Cacchillo and Hassler (102) used acetylsalicylic acid incorporated in masses of glycerinated-gelatin, polyethylene glycol mixture and cocoa butter. The suppositories with a cocoa butter base were commercial examples; the others were prepared for the test. The acetylsalicylic acid in suppository form was administered to human volunteers and a sample of blood removed and analyzed after two hours. Absorption was most rapid from the polyethylene glycol base followed by cocoa butter and glycerinated-gelatin. The glycerinated-

gelatin produced a high incidence of burning and two of the

test subjects expelled the suppository. Because of these

undesirable side effects and the fact that this base is in-

compatible with several substances, it is not a good base

for general use in suppositories intended to exert a systemic

action.

An early method of testing the physiological absorp-

tion of medicaments was discussed by Rapp. (129) He tested

various suppository masses and described a test utilizing

fifty milligrams of methylene blue absorbed from the dosage

form and recognized by discolouration of the urine in human

subjects. Following this test, Schroff (30) incorporated

sodium salicylate, sodium iodide, lecithin and cholesterol

into cocoa butter. Sodium iodide was detected in thirteen to

twenty minutes in the urine and sodium salicylate in twenty-

five to thirty-six minutes when administered to human subjects.

The author stated that the manner of incorporation of ingred-

ients was of prime importance. Buchi and Oesch (130) admini-

stered suppositories to human volunteers using cocoa butter

as the base and incorporating sodium salicylate and acetyl-

salicylic acid by various methods and employing various emul-

sifying agents. The first appearance of the drug in the urine

was employed as the test criterion. The urine was collected

periodically, tested and analyzed for salicylate. They stated

that emulsions released water-soluble medication faster and

gave a faster release of medication than a suspension of the

drug. Block and Dekker (131) administered methylene blue in-
corporated in cocoa butter, a polyethylene glycol base and
Massuppol to human volunteers and measured the elapsed time
between application of the suppository and appearance of the
drug in the urine. The second part of this experiment involved
the cumulative excretion of quinine from the same test bases.
Absorption of the test substances from polyethylene glycol was
better than the other two bases and cocoa butter was probably
more superior to Massuppol.

Dilatation of pupils by released drugs in test animals and
human volunteers has been used as a criterion of availability
of medication. Tardos et al (105) incorporated atropine sul-
fate into several suppository bases including cocoa butter,
polyoxyethylene 40 monostearate and two water-soluble compounds
and administered them to rats. These authors (105 measured the
dilatation of pupils produced every five minutes for thirty min-
utes. The experiments were repeated incorporating emulsifying
agents into the cocoa butter. The authors found that synthetic
suppository masses released medication only after dissolution in
body fluids, and that emulsifying agents accelerate absorption.
Cocoa butter produced the most rapid absorption of atropine sul-
fate and polyoxyethylene 40 monostearate was the most efficient
synthetic mass tested. Neuwald et al (132) repeated this type
of experiment utilizing rabbits and incorporating the atropine
sulfate into cocoa butter and adeps solidus. The examples of adeps
solidus used were Witepsol and Stadimol. These authors found
that absorption of atropine was more rapid from adeps solidus

than cocoa butter.

Delay and Thuillier (133) observed the effects of curarization of rabbits. The use of suppositories containing D-tubocurarine permitted slower curarization, more persistent effect and slower recuperation of muscular contractility. Heite et al (134) determined the level of bismuth in the blood of rats after parenteral and rectal administration of the drug. These authors reported that although the bismuth was not absorbed to an appreciable extent rectally, it was not significantly different from that produced by parenteral administration. However, their main purpose was to determine the efficacy of commercially available preparations, and was not intended for any comparison. Lechat and Boissier (135) observed the time taken to produce a bitter taste in the mouth of human volunteers after rectal administration of suppositories containing sodium dehydrocholate.

Multi-point absorption studies involve the measurement of a pharmacological response at varying time intervals such as peripheral rise in temperature after rectal administration by suppository, radioactive procedures or the level of a drug in the blood or urine.

Many experiments have been carried out to measure the level of administered drug in the blood of test subjects. Whitworth (128) and Kakemi (126) measured the level of sulfonamides in the blood following administration of this drug by suppositories to rabbits. Aoki and Fukuchi (136) administered

diphenhydramine and its hydrochloride, p-toluene sulfonate and
2-,4-,5-trichlorobenzenesulfonate to rabbits in vehicles of cocoa
butter, polyethylene glycol ester and polyethylene glycol 1500
and 2000 and periodically determined the concentration of drug
released in the blood. The hydrophilic bases showed better re-
lease of medication from suppositories than the hydrophobic bases.
The partition-coefficients of the drugs between oil and water
affected the rate of absorption. The authors (136) concluded
that water solubility of medication was the prime requirement
for absorption rate. Cutting and Sultan (137) administered
suppositories containing sulfapyridine, sulfanilamide and sul-
fathiazole to dogs and humans, and measured the blood level of
sulfonamide. The authors found that sulfanilamide was released
from the suppository bases and absorbed into the blood to a
variable but considerable extent, but the blood levels obtain-
ed with sulfapyridine and sulfathiazole could barely be de-
tected. Waxler and Schack (138) administered aminophylline
to human volunteers by various dosage forms and took blood
samples at periodic intervals to measure the level of the
aminophylline in the blood. They found that the rectal route
of administration of this drug gave the greatest scattering
of values and pronounced individual differences. Del Pozo
and Cemeli (122) measured the level of salicylate in the blood
of rabbits after rectal administration of this drug incorpor-
ated into suppository bases. Trandafilov et al (139) measu-
red the blood level of penicillin after rectal administration

of potassium benzylpenicillin in a base consisting of cocoa
butter, hydrogenated vegetable oil and refined beeswax. The
authors reported that the required rectal dosage of this drug
was one and one-half times the oral dosage. Similarly, Backe-
Hansen (140) compared the blood levels produced in renal
ligitated rabbits after the administration of sodium benzyl-
penicillin in bases of cocoa butter, Witepsols, polyethylene
glycol 1000 and glycerinated-gelatin and measured the serum
concentrations of penicillin using an agar cup technique with
Sarcina lutea. The highest serum concentrations were found
in the fatty bases. The author further stated that penicillin
stability in the fat bases was good, and poor in water-soluble
bases.

Pennati and Steiger-Trippi (86) incorporated sulfasomi-
dine, sulfasomidine sodium and sulfachlorpyridazine into bases
of cocoa butter, glycerinated-gelatin, polyethylene glycol and
Massuppol, and administered these drugs rectally to rabbits.
After regular time intervals, a blood sample was removed and
analyzed for sulfonamide. They stated that in order to achieve
high blood levels, the sulfonamides must be as water-soluble
as possible. They reported also that the highest blood con-
centration-time produce and the highest blood level was
achieved with sulfasomidine sodium in Massuppol, followed by
glycerinated-gelatin, cocoa butter and the polyethylene glycols.
By comparison, Schwarz and Bichsel (87) in a similarly con-
ducted experiment using human volunteers, reported best results

were obtained with a polyethylene glycol base.   Oral admini-
stration was twice as effective as rectal administration with
these sulfonamides; the sodium salt was absorbed almost twice
as much from suppositories as the free acid.   Delfs and Kuhne
(141) concluded that the sodium salts of the sulfonamides are
absorbed in sufficient quantities from fatty bases after their
experiments involving the serum and urine levels produced by
rectal administration by suppository of sulfonamides.   Hobel
and Tabelian (142) measured the absorption of N-acetyl-p-
aminophenol from gelatin suppositories and those of adeps
neutralis.   They reported that there was twice as much absorp-
tion from gelatin suppositories as there was from the natural
fat suppositories.   Those suppositories containing twenty-
seven to twenty-eight percent glycerin were considered to be
the best.   Neuwald et al (132) measured serum blood levels
attained using the following compounds and their sodium salts:
tolbutamide, thiopental, and hexabarbital in rabbits, using
bases of cocoa butter and adeps solidus.   In all cases absorp-
tion was more rapid from adeps solidus.   Examples chosen were
Witepsol H-15 and Stadimol.

The periodic examination of the urine of the test sub-
jects at stated time intervals has also been measured as a
criterion of release of medication from suppositories.   Hofman
and Hornbogen (58) studied the patented German masses Lasupol,
Suppobasin, Suppositol, the K-masses (synthetic paraffin bases)
and the polyethylene glycols.   The authors incorporated sodium

salicylate into the test masses and administered them to human
volunteers. The urine was collected and analyzed for salicyl-
ate every four hours. All synthetic masses showed a greater
release of salicylate than cocoa butter. Suppobasin released
the maximum amount. The fatty bases tested released more sali-
cylate than the water-soluble bases.

Radioactive procedures involve tracing the path of a
radioactive substance through a biological system. One of
the first reports of the use of isotopes was by Peterson and
co-workers. (143). These workers incorporated radioactive
sodium iodide labelled with iodine 131 into cocoa butter and
four water-soluble or water-miscible bases, and administered
the suppositories rectally to rats. After the desired time
interval, the rat was sacrificed and the amount of iodide ex-
tracted from the blood, thyroid, other organs and urine was
measured for radioactivity. Absorption of sodium iodide was
greatest from the water-soluble masses, in particular glycer-
inated-gelatin, whereas cocoa butter yielded the poorest re-
sults. Canals et al (144) administered calcium gluconate
labelled with radio-calcium rectally by suppository to rats
and concluded that the calcium salt in suppository form is
absorbed to a greater extent rectally than orally. In con-
trast to this report, Cremer and co-workers (145) stated that
the rectal absorption of a calcium salt administered by suppo-
sitory was low. Their experiments involved the administration
of suppositories incorporated with calcium acetate utilizing

radio-calcium to rats.

Charonnat et al (146) incorporated methyl nicotinate into glycinerated-gelatin, cocoa butter and polyethylene glycol suppository bases and introduced the suppository into the rectum of guinea pigs. The rubrefaction produced by the drug in the ear of the animal was measured by means of a thermocouple every two to three minutes. The bases were classified as follows: glycinerated-gelatin, cocoa butter and polyethylene glycol.

Summary and Purpose of Present Study

Many authors (92,104,112-116) have indicated that the water-soluble or water-miscible bases tend to release medication at a more rapid rate than the fat bases. However, Eckert and Muhlemann (119) have stated that water-soluble bases release medication less quickly than the fat bases, providing that the fat base in question has a melting point less than or equal to 36.5°, a small melting interval and a low hydroxy number. Fat bases meeting the above requirements were selected for this work and include the Witepsols, Massuppol, Estarinums and the Wecobees.

The literature contains many conflicting reports concerning release of medication in vitro from suppository bases, and few authors have attempted correlation between various in vitro methods. Microbiological methods employ diffusion of a medicament, usually an antimicrobial into an agar medium and

the zone of inhibition produced is measured. Del Pozo and
Cemeli (116) have shown that no correlation existed between
microbiological methods of testing and other in vitro tests
attempted. Furthermore, no in vivo correlation has been re-
ported utilizing microbiological methods and any standard in
vivo procedure.

Since dialysis involves dynamic diffusion, and correla-
tion with in vivo test procedures has been reported (92,116)
dialysis was chosen as a method for in vitro testing of diffu-
sion of medicament from bases rather than the microbiological
methods previously described in the introduction. Plaxco (125)
has stated that a definite relationship exists between dialy-
sis and rate of release of medication from suppository bases.

The dye release test is a comparative study under con-
trolled conditions with the variable factors limited. An
attempt to correlate dye release with an in vivo study has
been reported. (117) Furthermore, correlation in vitro with
other methods also seems possible.

The liquefaction time test provides a method for re-
cording a representative time of melting or softening of
suppositories. To be useful medicinally, the base should
liquefy within ten minutes in this test procedure. (118)
Setnikar and Fantelli (118) have reported that suppositories
tested in this manner and found to liquefy within ten minutes
also were effective in vivo.

There are a great many factors affecting release of
medication in vitro. The melting point of a vehicle, absence

or presence of surfactants, the water-solubility of a drug, whether dissolved or in suspension, and the absence or presence of surfactants are only a few. Many _in vitro_ methods have been proposed, as a means of comparing the release of medication from suppository bases. From these methods the melting point, dye release, liquefaction time and dialysis tests were chosen for this study to:

(a) determine the most effective polyethylene glycol formulation _in vitro_ of those chosen for study.

(b) determine the most effective fatty base _in vitro_ of those selected.

(c) investigate the possibility of correlation between the most efficient _in vitro_ methods.

(d) develop a suitable _in vitro_ preliminary screening procedure for measuring the release of water-soluble medication from suppository bases.

EXPERIMENTAL

Determination of Melting and Congealing Points of Bases Used

U.S.P. melting point determinations for Class II substances were carried out on the following bases: (147)

1. <u>Cao Butta</u>. Samples were supplied by the Rugar Chemical Company, New York, N.Y. Simon and Slaun (97) have given some chemical and physical data pertaining to this base and have stated that the base contains lecithin, making the base hydrophobic in nature.

2. <u>Cocoa Butter</u>. Cocoa butter, lot number AC-2556 was purchased from Anachemia Chemicals Limited, Toronto, Ontario.

3. <u>Estarinum Bases</u>. Estarinum A, lot number A-820, and Estarinum B, lot number A-855 were supplied by Edelfettwerke Werner Schlüter, Hamburg-Eidelstedt, Germany. These bases are described as "mixtures of mono, di, and triglycerides of the saturated fatty acids $C_{17}H_{23}COOH$ to $C_{17}H_{35}COOH$". (70)

4. <u>Massuppol</u>. Supplies of this base were donated by Afd. Crok and Laan, Wormerveer, Holland. This base is described as consisting of "the glycerol esters of lauric acid, plus a very small amount of glycerol monostearic acid ester". (89)

5. <u>Wecobee Bases</u>. Wecobees R and W were supplied by the Drew Chemical Corporation, New York, N.Y. These bases "possess natural triglyceride structures and are therefore oil soluble products, very hydrophobic in nature". (94)

6. <u>Witepsol Bases</u>. Witepsols H-19, lot number H-19007; H-15 lot number H-15181; and W-45 lot number W-45025, were supplied by Chemische Werke Witten, Witten Ruhr, Germany. These bases consist of "triglycerides of saturated vegetable fatty acids of normal nutrient type with varied portions of the corresponding partial glycerides". (7)

A modification of the U.S.P. XVII procedure for materials of Class II was used in the determination of the melting temperature of the above bases, with the exception of cocoa butter, which was done by the method of the B.P. 1963. (108)

For the U.S.P. determinations, a 2000 ml. graduated beaker was used as the glass container, and was filled with water to the 1100 ml. mark. A thermometer, American Society for Testing Materials designation 91C, graduated from 20.0 to 50.0 in $1/10^{\circ}$ was used in the determinations. The controlled source of heat was a hot plate, connected in series to a rheostat. The thermometer was clamped so that the tip of the bulb remained about 20 mm. from the bottom of the water bath. The base tested was melted at as low a temperature as possible, using water not more than 1° warmer than the highest temperature in the reported melting range. The base was drawn into a capillary tube (which met the specifications of the U.S.P. XVII), open at both ends to a depth of 10 mm. The charged tube was cooled in a refrigerator set at 5° for 24 hours prior to attaching it to the thermometer by means of an elastic band. Adjustment was made in the water bath so

that the upper edge of the material was 10 mm. below the water
level. The bath was then heated with constant stirring using
a metal rod, so that the temperature rose approximately 3° per
minute. As soon as the temperature came within 5° of the
lower limit of the reported melting range, the rise in tem-
perature of the bath was regulated to 0.5-1.0° per minute.
The temperature at which the material was observed to rise
in the capillary tube was then taken as the melting tempera-
ture. This procedure was repeated four times, each time the
water in the bath was changed and reheated. If the results
were within 0.2° of each other, the average was taken. If
the results were within 1.0° of each other, a further two
determinations were made and the average of six taken. If
the results were greater than 1° apart, a further sample was
melted as before, drawn into a capillary tube and cooled in
contact with ice for two hours. The determination of the
melting point was then repeated, and an average of six de-
terminations taken to be the melting temperature. Results
are shown in Table I.

The melting point of cocoa butter was determined using
a modification of the B.P. 1963 method. The sample was melted
at not more than 10° above the point of complete fusion, shaken
and drawn into a capillary tube, so that a column 10 mm. in
height was produced. The tube and its contents were cooled
rapidly to 15°, and maintained in a refrigerator at a tempera-
ture not less than 15° and not greater than 17° for not less

than 48 hours. The apparatus used in the determination of
the melting point of cocoa butter was identical to that of
the tests previously described. The thermometer was suspen-
ded in the beaker containing water at 15° so that the lower
end of the column of the substance was 30 mm. below the sur-
face of the water. The water was heated so that the tempera-
ture rose approximately 2° per minute, with constant stirring.
The temperature at which the partially melted cocoa butter
began to rise in the capillary tube was taken as the melting
point. The procedure was repeated four times and the results
averaged. Results are shown in Table I.

Due to the difficulty encountered in the adaptation
of a suitable colourless fluid to serve as a bath in order
to determine the melting temperature of polyethylene glycol
bases, the technique of determining the congealing tempera-
ture was used. This temperature is frequently referred to
in commerce as the melting temperature. The polyethylene
glycol bases used in this study included PEG 1000, lot num-
ber M-35439; PEG 6000, lot number M-81317; PEG 1540, lot
number CCC-1837D; and PEG 400, lot number CCC-1814E. These
were purchased from Union Carbide (Canada) Limited. Poly-
ethylene glycol 4000, lot number 23125 was purchased from
British Drug Houses (Canada) Limited.

Combinations of the polyethylene glycols in the follow-
ing proportions were tested for their congealing temperature:

Polyethylene Glycol Base A

        Polyethylene Glycol 1000   9 Gm.

        Polyethylene Glycol 4000   1 Gm.

Polyethylene Glycol Base B

        Polyethylene Glycol 1540   7 Gm.

        Polyethylene Glycol 6000   3 Gm.

Polyethylene Glycol Base C

        Polyethylene Glycol 400   3 Gm.

        Polyethylene Glycol 1540   3 Gm.

        Polyethylene Glycol 4000   4 Gm.

The apparatus used was similar to that used in the B.P. 1963 to determine the freezing point, with the following modifications: a stout-walled test tube 25 x 150 mm. was placed inside a larger stout-walled test tube 40 x 200 mm., which acted as an air-jacket, and was weighted with lead shot. The inner tube was closed by a two-holed rubber stopper fitted with a wire stirrer and a suitable thermometer, arranged in such a manner that the bottom of the bulb was about 10 mm. above the bottom of the inner tube. The wire stirrer was bent at its lower end into a horizontal loop around the thermometer. The inner tube, with its jacket, was placed in a 2000 ml. graduated beaker and clamped into place so that the

level of the water in the beaker was not below the sample of the substance in the inner tube.

In each case, 25 Gm. of material were melted and placed in the inner tube. The suppositories were melted over a water bath, using water heated to not more than 20° above the expected congealing point, which was previously determined by cooling the sample rapidly. The tube was allowed to cool until the contents were about 5° higher than the expected congealing point. The beaker was then filled with water at a temperature 5° lower than the expected congealing point, the inner tube inserted into the jacket and the sample stirred continuously during the remainder of the test by moving the wire loop up and down throughout the entire depth of the sample at a regular rate of approximately twenty cycles per minute. The readings of the thermometer in the inner tube were recorded every 30 seconds. The temperature fell gradually at first, then rose slightly before becoming constant, then fell gradually. The highest temperature recorded after the slight rise was taken to be the congealing temperature of the base tested. The temperature was recorded every 30 seconds until 3 minutes had elapsed after the highest temperature had been reached. This test was repeated twice and the average result taken. These results are shown in Table I.

TABLE I

EXPERIMENTAL AND REPORTED MELTING AND

CONGEALING POINTS OF BASES TESTED

Base	Experimental	Reported
Polyethylene glycol A	$40.8 \pm 0.1^\circ C.$	
Polyethylene glycol B	$52.0 \pm 0.1^\circ C.$	
Polyethylene glycol C	$45.4 \pm 0.1^\circ C.$	
Cao Butta	$38.8 \pm 0.1^\circ C.$	$33-36^\circ C.$ (97)
Cocoa Butter	$32.2 \pm 0.1^\circ C.$	$31-35^\circ C.$ (148)
Estarinum A	$37.4 \pm 0.1^\circ C.$	$33-35^\circ C.$ (70)
Estarinum B	$34.5 \pm 0.1^\circ C.$	$33.5-35.5^\circ C.$ (70)
Massuppol	$33.0 \pm 0.1^\circ C.$	$35-37^\circ C.$ (89)
Wecobee R	$34.4 \pm 0.1^\circ C.$	$33.5-35.5^\circ C.$ (94)
Wecobee W	$33.1 \pm 0.1^\circ C.$	$31.1-33.3^\circ C.$ (94)
Witepsol H-15	$35.2 \pm 0.1^\circ C.$	$33.5-35.5^\circ C.$ (7)
Witepsol H-19	$35.1 \pm 0.1^\circ C.$	$33.5-35.5^\circ C.$ (7)
Witepsol W-45	$34.4 \pm 0.1^\circ C.$	$33.5-35.5^\circ C.$ (7)

Effect of Storage on Suppositories

Preparation of Suppositories

All suppositories were prepared using the fusion process. A 2 Gm. conical, nickel plated brass mould, containing twelve holes was used to prepare the suppositories. Suppositories manufactured using cocoa butter required a lubricant to prevent the suppositories from sticking to the mould. The formula of the lubricant used was as follows: (149)

Castor Oil U.S.P.	1 ml.
Castile Soap N.F.	2 Gm.
Alcohol	18 ml.
Water	2 ml.

The fatty-type bases were melted in a clean, dry, evaporating dish by placing them over hot water at a temperature no higher than 1° over the melting temperature previously determined. The water-soluble bases were melted on a hot plate, set at low heat. The fatty bases were first shredded before use and refrigerated until required. When the melted base showed a tendency to solidify, it was poured into the prepared mould. It was essential that the mould be clean and dry in the preparation of all suppositories except cocoa butter, which required lubrication. Chilling of the mould prior to pouring was unnecessary, since the mould was

fairly new and the inner surfaces were very smooth. Chilling

of the suppositories produced was found necessary in a few

cases for periods between 10 to 20 minutes at 10°. After the

suppositories had been allowed to set, either in a refrigera-

tor or at room temperature, the tops were trimmed uniformly.

A total of thirty-six blank suppositories were prepared in

this manner from each base listed in Table II and stored in

100 ml. amber glass jars with bakelite caps in a refrigerator

set at 10° until required.

Storage

After 2 weeks, twelve blank suppositories were selected

at random and removed from the refrigerator. Six suppositories

were placed in a clear glass 100 ml. jar and six in an amber

jar. These suppositories were stored in a room with subdued

light with a temperature range from 15° to 35°. The bases

were observed at the following intervals: 1 and 2 weeks,

1, 2, 4, 6, 10 months and 1 year. The bases were examined for

appearance and odour. Results after storage for one year are

shown in Table II.

TABLE II

EFFECT OF STORAGE FOR ONE YEAR
ON BLANK SUPPOSITORIES

Base	Description	Grading
Polyethylene glycol A	Deformed, strong odour	Poor
Polyethylene glycol B	Cracked	Fair
Polyethylene glycol C	Deformed	Poor
Cao Butta	Lustre gone	Very good
Cocoa Butter	Shrivelling	Fair
Estarinum A	Turned slightly yellow	Very good
Estarinum B	Very slight odour	Very good
Massuppol	Turned slightly yellow	Very good
Wecobee R	Lost original lustre	Very good
Wecobee W	Lost original lustre	Very good
Witepsol H-15	Very slight loss of lustre	Excellent
Witepsol H-19	Slight buttery odour	Very good
Witepsol W-45	Strong coconut odour	Very good

Colourimetric Methods of Analysis

Amaranth C.F. (F.D.&C Red No. 2) lot number 23744 purchased from British Drug Houses (Canada) Limited was used as the dye. The dye was passed through a two hundred mesh screen prior to incorporation into the test bases.

The bases used were identical to those used in the previous sections. They were the polyethylene glycol formulations A, B, C, Cao Butta, cocoa butter, Estarinum A and B, Massuppol, Wecobee R and W, and Witepsols H-15, H-19 and W-45.

So that results could be expressed in mg./ml., a graph of known concentrations of dye versus optical density was prepared. An amaranth calibration curve was plotted using a Beckman DU spectrophotometer at a maximum wavelength of 519 mu. Since a plot of optical density versus concentration (Figure 1) resulted in a straight line, the dye obeys the Beer-Lambert Law.

All suppositories were prepared by the fusion process. Although all the suppositories did not require lubrication, the same mould lubricant was used throughout the test to ensure uniformity. In order to ensure uniform lubrication of the mould, a cotton pledget was used to apply the lubricant and the mould was allowed to drain prior to pouring the molten mass.

1 Gm. of the screened dye was weighed in an evaporating dish on a torsion balance, having a sensibility reciprocal of

FIGURE 1
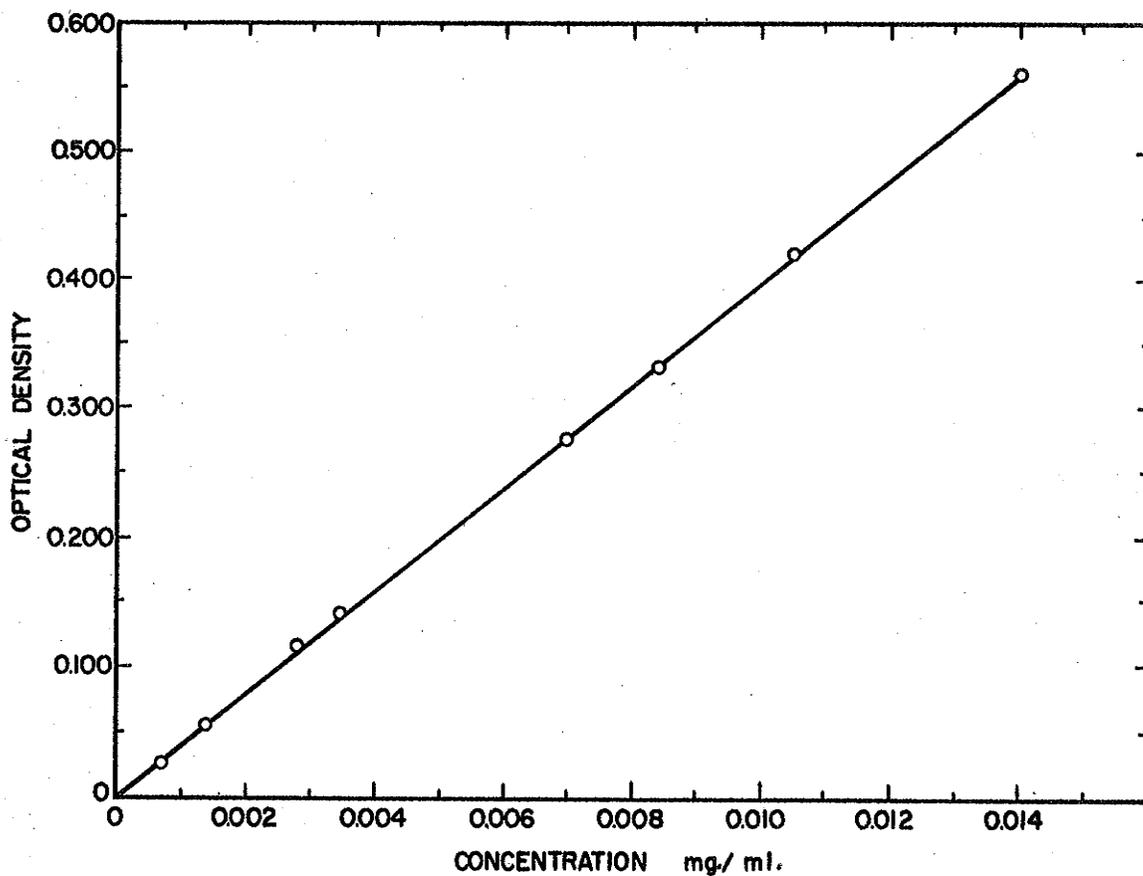
CALIBRATION CURVE OF AMARANTH AT 519 mu

A Beer plot of optical density against the con-
centration of amaranth in water at 519 mu and 25°.
Absorption Coefficient$_{519}$=40.0 (mg./ml.)$^{-1}$cm.$^{-1}$.

3 mg. Ten blank suppositories were selected at random from those prepared for storage tests. As before, the fat-base suppositories were melted over hot water and the polyethylene glycol bases on a hot plate. 1 Gm. of the melt was weighed out and discarded to compensate for the added amaranth disregarding the displacement factor. The melted base was then added a little at a time to the dye, mixing thoroughly by means of a bent-glass stirring rod after each addition. No water, solvents, or emulsifying agents were used to incorporate the dye into the base. As soon as the base uniformly incorporated with the dye, showed a tendency to solidify, it was poured into the previously lubricated mould. On the average, this procedure yielded seven suppositories, each containing 100 mg. of amaranth. The prepared suppositories were stored in amber 100 ml. containers with bakelite caps and placed in a refrigerator set at 10° for 2 weeks to allow for possible isomerization of such bases as cocoa butter.

Colourimetric Testing Technique

Before testing, the suppositories were removed from the refrigerator and allowed to come to room temperature. A suppository was then selected at random from the suppositories previously prepared. The suppository containing the dye was placed in a 1000ml. glass Oldberg-type percolator stoppered at the orifice with a plug cut from a rubber stopper and inserted into place using stopcock grease. The percolator was

mounted on a retort stand and placed in a water bath. 500 ml.
of distilled water from the same source and pH were then added
to the percolator . The temperature of the water bath was ad-
justed so that the water in the percolator was maintained at
a constant temperature of $37.0\pm0.1^{\circ}$. The percolator was
equipped with a small blade stirrer attached in series to a
rheostat, and operated at the same low speed throughout the
test. A thermometer, graduated from $20.0-50.0^{\circ}$ in $1/10^{\circ}$ was
fitted to the apparatus to ensure accurate temperature read-
ings. A Bronwill constant temperature heater was used to
maintain the temperature. A diagram of the apparatus is
shown in Figure 2. The apparatus provided convenient with-
drawal of samples and maintained uniform concentration of
liquid. As the suppository liquefied or dissolved, 5 ml. of
liquid were removed by means of a pipette at 1, 2, 4, 6, 8
and 10 minute intervals and drained uniformly into 100 ml.
volumetric flasks. The flasks were then brought to volume with
distilled water. After the test interval was completed, the
apparatus was removed, cleaned and set in place again. The
test was then repeated with a blank suppository. Each solu-
tion was then placed in a 1 cm. quartz cuvette, which had
been previously rinsed several times with the dilution. The
procedure was repeated with the corresponding blank. Read-
ings were then taken on the Beckman DU spectrophotometer set
at a wavelength of 519 mu.

FIGURE 2.
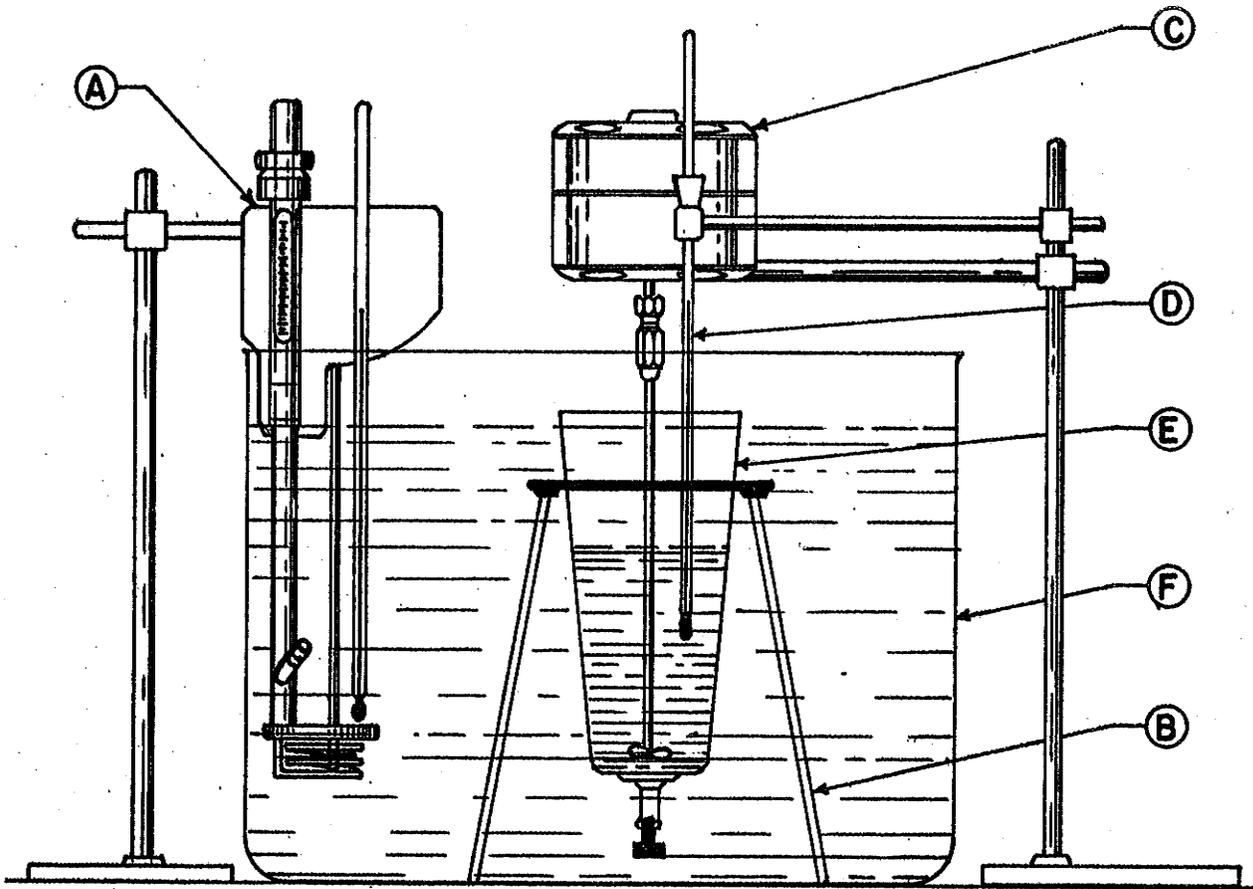
COLOURIMETRIC APPARATUS

A. BRONWILL CONSTANT TEMPERATURE HEATER
B. RETORT STAND
C. SMALL BLADE STIRRER
D. THERMOMETER
E. PERCOLATOR
F. WATER BATH

The slope of the graph of concentration versus optical density was calculated. From the calculation of the slope and the readings obtained using the Beckman DU spectrophotometer, the number of milligrams present in each dilution were calculated. From this figure and the knowledge of the number of milligrams present at the start of the test, the percentage of dye release was calculated as shown in Table III.

Assay of Suppositories

An assay procedure was developed to establish the percentage error involved in incorporating the dye into the suppositories. One example was selected from each class, the polyethylene glycols, the synthetic fat bases, and cocoa butter.

Suppositories manufactured with polyethylene glycol base B and amaranth were selected as an example of those suppositories manufactured from the water-soluble masses. Two control suppositories each containing 100 mg. of amaranth were prepared as follows. Two evaporating dishes were accurately weighed on an analytical balance, and 100.0 mg. of dye added to each dish. A blank suppository was added to the accurately weighed dye in each evaporating dish. The suppositories were melted on a hot plate and allowed to solidify. Hot distilled water was used to remove the solidified masses and the solutions were transferred to 1000 ml. volumetric

TABLE III

PERCENTAGE DYE (AMARANTH) RELEASE FROM
BASES AT VARYING TIME INTERVALS

Base	Percent Dye Release					
	Minutes					
	1	2	4	6	8	10
Polyethylene glycol A	–	4.2	8.3	28.0	40.7	51.5
Polyethylene glycol B	1.8	4.2	11.5	18.7	28.3	36.2
Polyethylene Glycol C	2.0	5.0	10.6	15.1	19.3	23.9
Cao Butta	--	–	–	1.9	2.9	4.1
Cocoa Butter	4.3	8.4	15.2	29.5	40.7	54.8
Estarinum A	1.8	4.2	5.9	6.8	8.4	8.8
Estarinum B	6.3	15.6	20.7	32.0	42.5	52.0
Massuppol	2.0	6.9	13.3	15.8	29.2	30.5
Wecobee R	2.0	5.0	10.3	15.3	18.8	21.1
Wecobee W	11.0	19.4	38.0	49.8	59.6	68.2
Witepsol H-15	4.3	14.1	19.7	27.3	39.5	48.5
Witepsol H-19	2.3	12.9	42.1	47.3	62.3	77.5
Witepsol W-45	2.3	6.7	13.5	19.5	26.8	35.3

flasks, using analytical techniques. The evaporating dishes were rinsed well with distilled water and the rinsings transferred to the volumetric flask. The solutions were allowed to come to room temperature and then made up to volume in the flasks. A blank suppository was then added to another 1000 ml. volumetric cylinder, dissolved and brought to volume. 10 ml. were removed and allowed to drain uniformly into 100 ml. volumetric flasks. The flasks were brought to volume and the dilution read on the Beckman DU spectrophotometer, taking care to rinse each cuvette with dilution. The blank served to correct for any absorption due to the base alone. In both cases, it was found that 100% of the dye was recovered. Two test suppositories were selected from those previously prepared containing amaranth and added to 1000 ml. volumetric flasks and allowed to dissolve before making up to volume. As before, an aliquot portion was removed, diluted and analyzed. Results are shown in Table IV.

TABLE IV

ASSAY OF AMARANTH IN POLYETHYLENE

GLYCOL SUPPOSITORIES

Polyethylene Glycol Suppository	Theoretical mg. dye	Recovered mg. dye	% Error
Control 1	100.0	100.0	-
Control 2	100.0	100.0	-
Test 1	100.0	102.3	2.3%
Test 2	100.0	102.5	2.5%
Average Error in Manufacturing			2.4%

Suppositories manufactured with dye and Witepsol H-15 were selected as an example of those manufactured from a synthetic fat, and suppositories manufactured with cocoa butter, as an example of natural fat base suppositories. The procedure used to assay both was identical. Control suppositories each containing 100 mg. of amaranth were prepared as follows: 100.0 mg. of amaranth were accurately weighed into two evaporating dishes and one blank suppository added to each dish. These were melted using hot water and allowed to cool. 75 mg. of chloroform U.S.P. were added to the evaporating dishes, mixed with the contents and then transferred to 325 ml. separatory funnels. The dishes were then rinsed with distilled water and the rinsings added to the separatory funnels. The funnels were shaken vigorously and the contents allowed to separate into layers. The chloroform layers were drawn off and the water layers added to 1000 ml. flasks. The chloroform was replaced in the separatory funnels and the extraction procedure repeated using 100 ml. portions of distilled water until no colour remained in the water layer. This procedure required five 100 ml. portions of water and one further 100 ml. portion of water was added to ensure complete extraction. The volumetric flasks were brought to volume with rinsings from the funnels after the chloroform layer had been removed. A portion of these solutions was filtered through two successive layers of Whatman #1 filter paper. 10 ml. were diluted to volume in a 100 ml. volumetric

flask.   Extraction was then carried out on two test supposi-
tories and also with a blank suppository containing no amar-
anth.   Readings were then taken on the Beckman DU spectro-
photometer with allowance made for the blank.   Results are
shown in Table V.

TABLE V

ASSAY OF AMARANTH IN FAT SUPPOSITORIES

| Suppository	Theoretical mg. dye	Recovered mg. dye	% Error
Witepsol H-15			
Control 1	100.0	100.0	-
Control 2	100.0	100.0	-
Test 1	100.0	102.4	2.4%
Test 2	100.0	103.0	3.0%
Average Error in Manufacturing			2.7%
Cocoa Butter			
Control 1	100.0	100.0	-
Control 2	100.0	100.0	-
Test 1	100.0	103.5	3.5%
Test 2	100.0	104.3	4.3%
Average Error in Manufacturing			3.9%

Liquefaction Time

The suppository masses used in this test were identical to those used in the colourimetric test. No active ingredient was incorporated into the test masses.

2 Gm. suppositories were prepared by the fusion technique. The lubricant, previously described, was necessary only in the case of cocoa butter suppositories to prevent sticking. A total of twenty-four suppositories from each base were prepared and stored at 10° for 2 weeks prior to use in 100 ml. amber glass containers with bakelite caps.

Testing Technique

Prior to testing, the suppositories were removed from the refrigerator and allowed to come to room temperature. An apparatus as shown in Figure 3 was designed along the same lines as described by Setnikar and Fantelli (118). The apparatus consists of a glass cylinder, external diameter 50 mm., narrowing down to 22 mm. at either end for a length of 30 cm. The cylinder is fitted with two connections through which water circulates from a Bronwill constant temperature water heater, thermoregulated in such a manner that the water maintains a temperature of $37.0\pm0.1^\circ$. A 34-35 cm. length of cellophane tube, size inflated diameter 1-1/8" (Fisher catalogue 8-667) was moistened, opened and placed in

FIGURE 3.
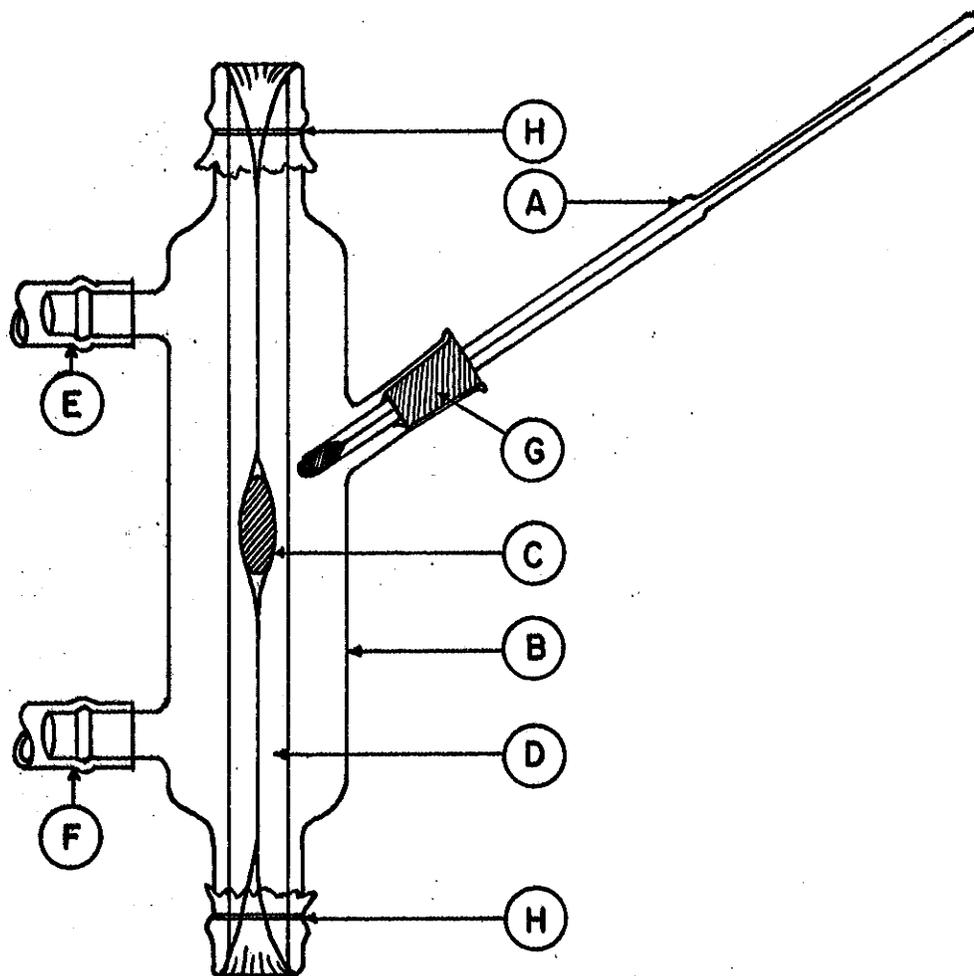
LIQUEFACTION TIME APPARATUS

A. THERMOMETER

B. CYLINDER

C. SUPPOSITORY UNDER TEST

D. CELLULOSE DIALYZER
   TUBING

E. CONNECTION TO BRONWILL

F. CONNECTION FROM BRONWILL

G. CORK

H. ELASTIC BANDS TO SECURE
   TUBING

the glass cylinder. The tube was drawn out of either end of
the cylinder and secured with elastic bands. The Bronwill
heater was clamped into a water bath and connected to a rheo-
stat. A thermometer, graduated from 20.0 to 50.0 in $1/10^{\circ}$
was fitted into the apparatus by means of a cork. The tubes
were then attached to the apparatus, the heater turned on
and water was circulated through the apparatus. The appar-
atus was raised or lowered, keeping it vertical, until the
level was reached at which the lower half of the cellophane
tube was collapsed and the upper half gaping. When the level
was reached at which the tube started to close in, the hydro-
static pressure of the water in the apparatus was about zero.
Six suppositories were then selected at random from the
twenty-four prepared. When the water temperature reached
a stable reading of 37.0°, a suppository was dropped in, the
apparatus lowered about 30 cm., and measurement of the lique-
faction time was begun. The apparatus was raised and lowered
periodically to facilitate dispersion of the material already
liquefied. When the measurement was completed, the apparatus
was raised, drained, and a new cellophane tube inserted. The
measurement was then repeated five times and the average re-
sult taken to be the liquefaction time. Results are shown in
Table VI and are compared where possible with those obtained
by other workers. (118)

TABLE VI

EXPERIMENTAL AND REPORTED LIQUEFACTION
TIMES AT 37.0° OF BASES TESTED

Base	Experimental	Reported (118)
Polyethylene glycol A	22.58 min.	-
Polyethylene glycol B	42.58 min.	-
Polyethylene glycol C	25.89 min.	-
Cao Butta	Softening 10-20 min. No liquefaction during 1 hour.	-
Cocoa Butter	4.88 min.	6.09 min.
Estarinum A	10.35 min.	8.58 min.
Estarinum B	16.41 min.	30.00 min.
Massuppol	10.35 min.	-
Wecobee R	8.01 min.	-
Wecobee W	6.46 min.	-
Witepsol H-15	10.32 min.	9.00 min.
Witepsol H-19	9.42 min.	7.42 min.
Witepsol W-45	9.37 min.	10.25 min.

Dialysis

Preliminary Experiments

Selection of Drug

Quinalbarbitone sodium B.P. 1963 (secobarbital sodium) lot number 298-A-30 was purchased from May and Baker (Canada) Limited and used as one of the test substances. The barbiturate was passed through a two hundred mesh screen prior to use.

Methyl iso-nicotinate (m.p. 14.5-15.5°) lot number 7860MX1135 was purchased from Matheson, Coleman and Bell, Inc., and used as a test substance.

These drugs were selected with a view towards future in vivo testing and adaptation to a spectrophotometric method of analysis.

Preliminary experimentation was carried out and calibration curves of concentration versus optical density of secobarbital in N/10 sodium hydroxide (pH 12.0$\pm$0.2) and methyl iso-nicotinate in water were prepared using the Beckman DU spectrophotometer. Maximum wavelengths were found to be 244 mu for the secobarbital sodium and 275 mu in the case of methyl iso-nicotinate. Since plots of optical density versus concentration (Figures 4 and 5) resulted in straight lines, both drugs obey the Beer-Lambert Law.

FIGURE 4
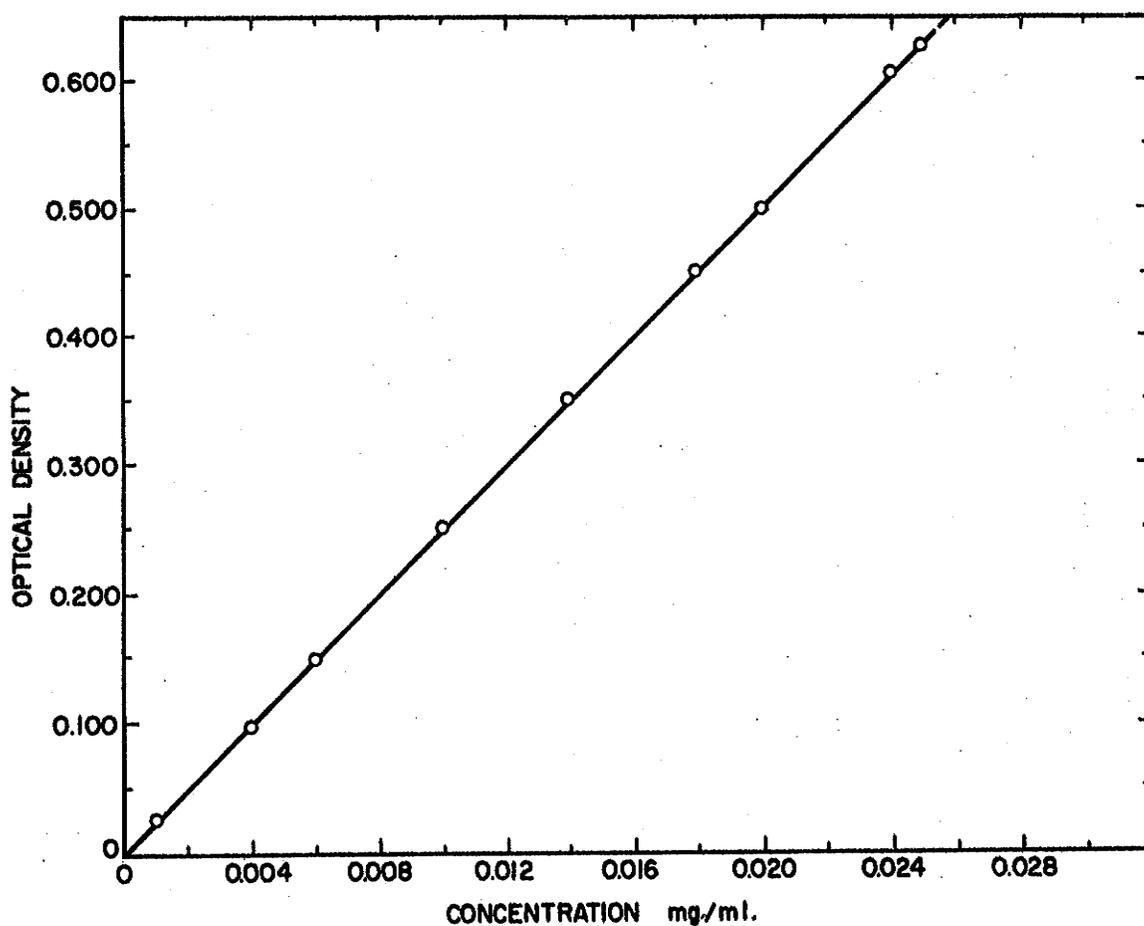
CALIBRATION CURVE OF SECOBARBITAL SODIUM AT 519 mu

A Beer plot of optical density against the concentration of secobarbital sodium in 0.1N sodium hydroxide at 244 mu and 25°. Absorption Coefficient$_{244}$ = 25.0 (mg./ml.)$^{-1}$cm.$^{-1}$.

FIGURE 5
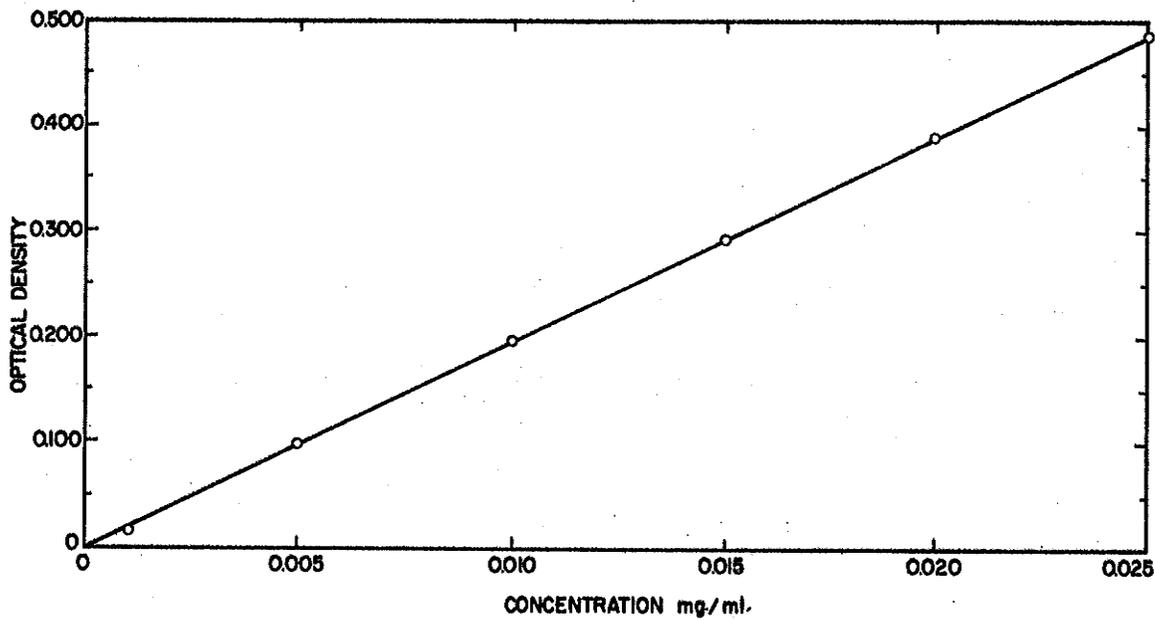
CALIBRATION CURVE OF METHYL ISO-NICOTINATE AT 244 mu

A Beer plot of optical density against the concentra-
tion of methyl iso-nicotinate in water at 275 mu at
25°.   Absorption Coefficient$_{275}$=19.5 (mg./ml.)$^{-1}$cm.$^{-1}$.

Selection of Membrane

Experiments were carried out using two types of dialyzing membranes:

(a) cellophane dialyzing tubing (size inflated diameter 1.81", Central Scientific Company of Canada, Catalogue number 70160-2), and

(b) Naturalamb dialyzing bags, supplied by the Young's Rubber Company of Canada Limited. These bags, which are prepared from sheep cecum, were selected as an example of a natural membrane.

The cellophane dialyzing bags were prepared in the following manner: a strip of dialyzing tubing approximately 9 inches long was double-tied at the bottom with dental floss and then cut. The prepared bag was tested for leaks and soaked for not less than 24 hours prior to use. The Naturalamb bags were used as received after thorough washing in lukewarm water to remove the moistening agent and soaked prior to use for the same period.

Dialysis Using Solutions of Drugs

Secobarbital sodium was chosen for further dialysis studies. On the day of the test, the membranes were drained, 8 Gm. of glass beads added for weight and placed in wide-mouth, 275 ml. glass bottles to which 200 ml. of water

had been added. The bottles were than placed in a water bath
and brought to a temperature of 37.0°. 25 ml. of an aqueous
solution of secobarbital sodium at 37.0° containing 1.028
mg./ml. in the case of the cellophane dialyzing bags and
1.036 mg./ml. for the Naturalamb bags were transferred into
the bags by means of a pipette. The tops of the bags were
pulled through a hole cut in the number eight rubber stopper
and the excess trimmed. This procedure left the final length
of the bags approximately six inches with four inches exposed
to the water in the bottle. A plastic covered magnetic stirrer
was then placed in the bottom of each bottle. Each bag was
adjusted in its bottle so that the level of the water outside
the bag was even with the level of the water inside the bag.
Care was taken to ensure that the surface area exposed to the
water in the bottle was the same for each bag. A cork was
then fitted into the top of each bottle. A diagram of the
apparatus is shown in Figure 6. The bottles were set up in
a water bath and the temperature maintained at 37.0±0.1° by
a Bronwill constant temperature water circulator. 5 ml.
samples from outside the bags and 0.5 ml. samples from inside
the bags were withdrawn by means of a pipette at 15 minute
intervals up to 1 hour and drained uniformly into 25 ml. volu-
metric flasks containing 2.5 ml. of N/1 sodium hydroxide.
The volume of water removed by pipette was replaced each time
from a burette, using water at 37.0°. The bags were left in
the apparatus for a total of 24 hours. The flasks were brought

FIGURE 6.
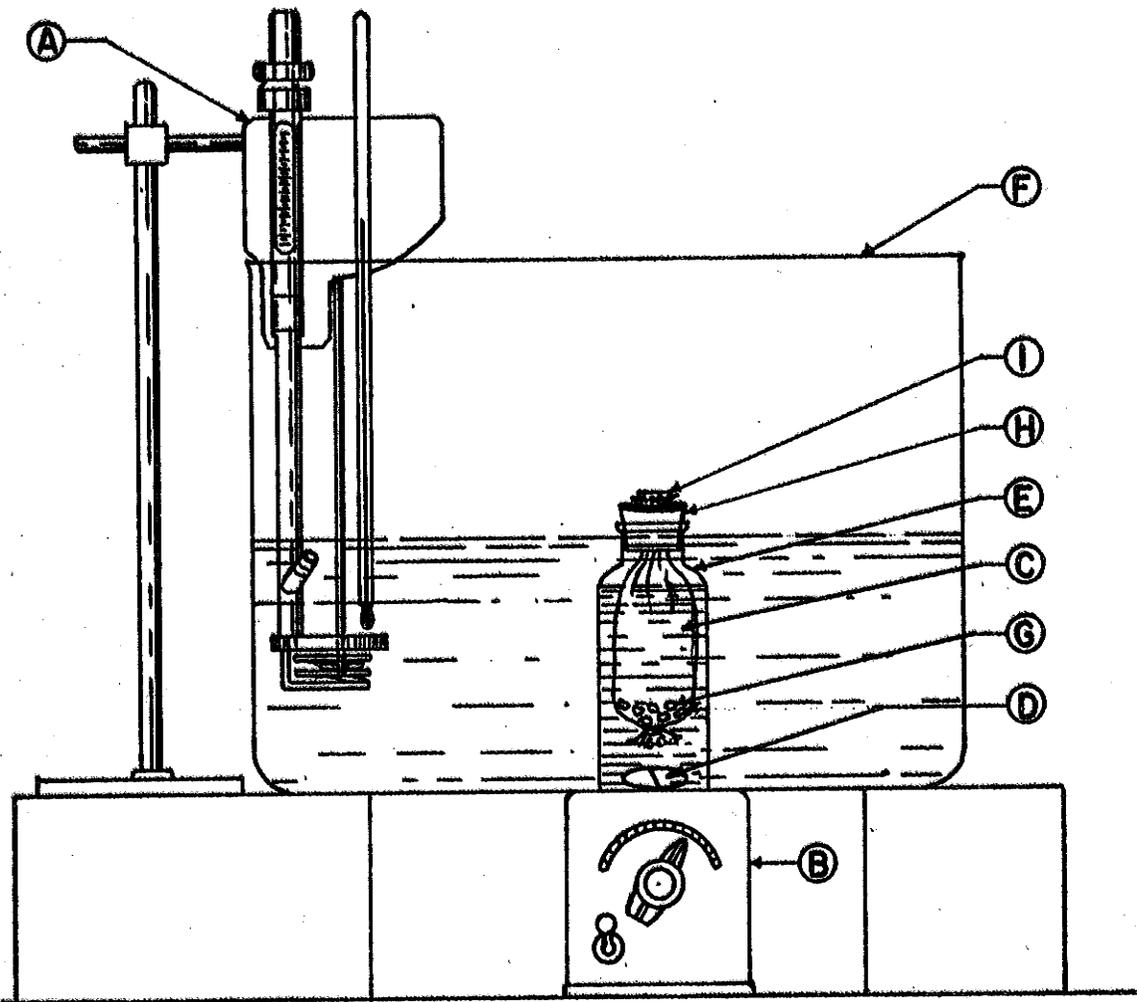
DIALYSIS APPARATUS

A. BRONWILL HEATER
B. MAGNETIC STIRRER
C. CELLOPHANE TUBING
D. MAGNETIC IMPELLER
E. BOTTLE

F. WATER BATH
G. GLASS BEADS
H. #8 RUBBER STOPPER
I. CORK

to volume using distilled water from the same source and pH throughout. Each solution prepared was placed in a quartz cuvette, 1 cm. in thickness, previously rinsed several times with the dilution. N/10 sodium hydroxide was used on the blank. Readings were then taken on a Beckman DU spectrophotometer set at 244 mu and the concentrations of drug inside and outside the bags calculated. The results are shown in Table VII.

Experimentation using both membranes revealed that more uniform results could be obtained using cellophane dialyzing tubing as the dialysis bag and further tests on suppotories were carried out using cellophane only.

TABLE VII

SECOBARBITAL SODIUM DIALYSIS THROUGH DIALYSIS BAGS

Cellophane Bag

Time hours	Concentration Outside mg./ml.	Total mg.	Concentration Inside mg./ml.	Total mg.	Total Secobarb.Sod.
0.25	0.018	3.60	0.884	22.10	25.70 mg.
0.50	0.024	4.85	0.818	20.45	25.30 mg.
0.75	0.035	7.00	0.710	17.76	24.76 mg.
1.00	0.042	8.38	0.652	16.30	24.68 mg.
10.00	0.100	20.09	0.174	4.36	24.45 mg.
24.00	0.107	21.40	0.121	3.03	24.43 mg.

Naturalamb Bag

Time hours	Concentration Outside mg./ml.	Total mg.	Concentration Inside mg./ml.	Total mg.	Total Secobarb.Sod.
0.25	0.041	8.24	0.664	16.60	24.84 mg.
0.50	0.063	12.69	0.446	11.64	24.33 mg.
0.75	0.083	16.52	0.308	7.69	24.21 mg.
1.00	0.093	18.53	0.232	5.80	24.33 mg.
8.00	0.095	18.92	0.104	2.60	21.52 mg.
24.00	0.113	22.16	0.124	3.09	25.25 mg.

Dialysis Using Suppositories

The suppository masses used in this test were identical to those used in the colourimetric and liquefaction tests, namely, the polyethylene glycol bases, Cao Butta, cocoa butter, the Estarinums, Massuppol, the Wecobees and the Witepsols.

All suppositories were prepared using the fusion process. Cocoa butter was the only base requiring the previously described mould lubricant. A total of forty-eight blank suppositories were prepared for each base in the manner previously described.

For each of the bases studied, 250.0 mg. of secobarbital sodium were accurately weighed into an evaporating dish using an analytical balance. Ten blank suppositories were selected at random and melted using a water bath in the case of the fatty bases and a hot plate for the polyethylene glycol bases. An aliquot portion of the base was not weighed out and discarded due to the small displacement coefficient of the barbiturate. The melted base was then carefully added a little at a time to the secobarbital sodium until all had been transferred, mixing thoroughly after each addition with a curved glass stirring rod. As soon as the base, uniformly incorporated with the drug, showed a tendency to solidify, it was poured into the mould. On the average, this procedure yielded seven complete suppositories each containing 25 mg. of secobarbital sodium. The prepared suppositories and the

remaining blanks were then stored in amber containers with bakelite caps in a refrigerator set at 10^0 for 2 weeks to allow for possible isomerization of such bases as cocoa butter.

Testing Technique

Before testing, the suppositories were removed from the refrigerator and allowed to stand at room temperature for 3 days. The morning of the test, the bag was drained, 8 Gm. of glass beads added, and the bag placed in the 275 ml. wide-mouth bottle containing 200 ml. of distilled water. 25. ml. of water were transferred to the bag by means of a pipette. The bag was trimmed and adjusted as previously described, the bottle placed in a water bath and maintained at 37.0 ± 0.1^0. The magnetic stirrer was turned on and adjusted so that it operated at the same speed throughout the tests. As soon as both solutions reached the required temperature, the apparatus was removed from the water bath, opened, a suppository containing the barbiturate added, the apparatus reassembled and replaced in the water bath. Time measurement was initiated at this point. As the suppository liquefied or dissolved, 5 ml. samples were removed from outside the bag at 15 minute intervals up to one hour and drained uniformly into 25 ml. volumetric flasks containing 2.5 ml. of N/1 sodium hydroxide. The fluid thus removed was replaced with water at 37^0 as before. No samples were withdrawn from inside the bag. After

the test interval had been completed, the apparatus was re-
moved, and a new bottle complete with cellophane tubing set
in place. The test was then repeated with a blank supposi-
tory, and with another suppository selected from the remain-
der of those containing secobarbital sodium. If the results
did not agree within 5%, the suppositories were prepared once
again and the procedure repeated. Each solution prepared in
the above manner was brought to volume and placed in quartz
cuvettes, as previously described. Readings were taken on
a Beckman DU spectrophotometer set at a wavelength of 244 mu.

The number of milligrams present was calculated from
the slope of the line and the optical density readings obtain-
ed with the unknown solutions. Allowance for the drug re-
moved for dilutions was made using the following correction
factor obtained from Nelson: (150)

$$C_n = C_n{}^0 + \frac{V_w(C_n-1)}{V}$$

Where

C_n = corrected concentration of nth withdrawal

$C_n{}^0$ = concentration actually determined

C_{n-1} = corrected concentration of sample withdrawn
immediately before the nth

V_w = volume in withdrawal

V = volume of dissolution medium.

After allowances were made for the amount of drug re-
moved for dilutions the percent dialysis was calculated.
Results of the tests with suppositories are shown in Table VIII.

TABLE VIII

DIALYSIS OF SECOBARBITAL SODIUM FROM SUPPOSITORIES

| Base | % Dialysis from Bases of Secobarbital Sodium Time (minutes) | | | |
	15 min.	30 min.	45 min.	60 min.
Polyethylene glycol A	4.0	9.4	15.6	20.7
Polyethylene glycol B	4.3	10.2	15.4	19.6
Polyethylene glycol C	2.7	6.8	12.2	16.3
Cao Butta	–	–	–	5.0
Cocoa Butter	7.7	14.6	21.8	27.0
Estarinum A	3.0	10.6	17.2	22.0
Estarinum B	15.2	34.0	38.3	39.7
Massuppol	9.6	18.2	23.3	26.5
Wecobee W	7.7	17.0	21.7	25.2
Wecobee R	2.7	4.2	5.6	6.2
Witepsol H-15	16.6	21.9	24.2	25.6
Witepsol H-19	6.2	16.0	21.8	25.4
Witepsol W-45	1.6	5.6	7.0	8.2
Water only	14.0	18.9	27.2	32.6

Assay of Suppositories

An assay procedure was developed to establish the percentage error involved in incorporating the secobarbital sodium into the suppositories. One example was selected from each class - the polyethylene glycol, the synthetic fat bases and cocoa butter.

Suppositories manufactured with secobarbital sodium and polyethylene glycol base A were selected as an example of these suppositories manufactured from the water-soluble bases. Control suppositories containing accurately weighed quantities of secobarbital sodium were prepared as follows. An accurately weighed quantity of secobarbital sodium was added to each of two evaporating dishes (25.0 and 24.7 mg. respectively), and a blank suppository was added to each dish containing the barbiturate. The suppositores were melted on a hot plate and allowed to solidify. Hot distilled water was used to remove the solidified masses and the solutions were transferred to 1000 ml. volumetric flasks containing 100 ml. of N/1 sodium hydroxide. The evaporating dishes were rinsed well with distilled water and the rinsings transferred to the flask. The solutions were allowed to come to room temperature and then made up to volume. A blank suppository was then added to another 1000 ml. volumetric flask containing the same amount of N/1 sodium hydroxide, dissolved and brought to volume. A portion of solution was then placed in quartz cuvettes, taking

care to rinse out the cuvettes with solution, and read on the
Beckman DU spectrophotometer set at 244 mu. The blank served
to correct for any absorption due to the base alone. In both
cases it was found that 100% of the drug was recovered. Two
test suppositories were then selected and added to 1000 ml.
volumetric flasks containing 100 ml. of N/1 sodium hydroxide.
The suppositories were allowed to dissolve before making the
solutions to volume. As before, the solutions were placed in
quartz cuvettes and analyzed. Results are shown on Table IX.

TABLE IX

ASSAY OF SECOBARBITAL SODIUM IN
POLYETHYLENE GLYCOL SUPPOSITORIES

Polyethylene glycol Suppository	Theoretical Secobarbital Sodium	mg. of Secobarbital Sodium Recovered	% Error
Control 1	25.0	25.0	-
Control 2	24.7	24.7	-
Test 1	25.0	26.0	4.0
Test 2	25.0	25.6	2.4
Average Error in Manufacture			3.1

Suppositories manufactured with secobarbital sodium and Witepsol W-45 were selected as an example of those manufactured from a synthetic fat and suppositories manufactured with cocoa butter as an example of natural fat base suppositories. The following analytical procedure was used for both bases. 26.2 mg. and 25.7 mg. of secobarbital sodium were accurately weighed into each of two evaporating dishes and a blank cocoa butter suppository added to each dish. Similarly, 24.9 and 26.0 mg. of secobarbital sodium were weighed into evaporating dishes and blank Witepsol W-45 suppositories added. These were melted using hot water and allowed to set. 75 ml. of chloroform U.S.P. were added to the evaporating dishes and then transferred to 325 ml. separatory funnels. The dishes were then rinsed with distilled water and the rinsings added to the separatory funnels. The two layers were shaken vigorously and allowed to settle. The chloroform layers were drawn off and the water layers added to 1000 ml. flasks containing 100 ml. of N/1 sodium hydroxide. The chloroform was replaced in the separatory funnels and the extraction procedure repeated thirteen times each with 50 ml. portions of distilled water. The volumetric flasks were then brought to volume with rinsings from the funnels after the chloroform layer had been removed. A portion of these solutions was filtered through two successive layers of Whatman #1 filter paper. Extractions were then repeated using two suppositories and with a blank. It was established that secobarbital sodium was insoluble in chloroform.

Readings were taken on a Beckman DU spectrophotometer with

allowance made for the blank. Results are shown in Table X.

TABLE X

ASSAY OF SECOBARBITAL SODIUM

IN FAT SUPPOSITORIES

Cocoa Butter Suppository	Theoretical Secobarbital Sodium mg.	mg. Secobarbital Sodium Recovered	% Error
Control 1	26.2	25.8	1.5
Control 2	25.7	24.5	4.7
Average Error in Assay Procedure			3.1
Test 1	25.0	24.9	0.4
Test 2	25.0	24.2	3.2
Average Error in Manufacturing			1.8

Witepsol W-45 Suppository	Theoretical Secobarbital Sodium mg.	mg. Secobarbital Sodium Recovered	% Error
Control 1	24.9	20.8	16.5
Control 2	26.0	22.7	12.7
Average Error in Assay Procedure			14.6
Test 1	25.0	25.6	2.4
Test 2	25.0	26.1	4.4
Average Error in Manufacturing			3.2

DISCUSSION

Determination of Melting and Congealing Points of Bases Used

The melting point of a suppository base which is not soluble in body fluids should be no greater than normal body temperature. This requirement would therefore apply to all of the suppository bases tested with the exception of the water-soluble polyethylene glycol formulations.

Table I shows that the congealing points of the polyethylene glycol formulations used are all above 37°. It might be expected, however, that polyethylene glycol formulation A would yield the most satisfactory results in release of medication due to its lower congealing point.

Those fatty bases that yielded the most reproducible results and fell within the limits specified by the manufacturer included Estarinum B, Wecobee W, and the Witepsols. Cocoa butter fell within the official limits mentioned in the B.P. 1963. (148) Cao Butta and Estarinum A gave quite erratic results and their melting points fell above those reported by the manufacturer. Melting points were irregular with Wecobee R, although the average of six determinations fell within the manufacturer's range. In view of these erratic melting points, it was felt that difficulties might be encountered in further _in vitro_ tests involving Cao Butta, Estarinum A and Wecobee R.

Fatty bases with melting points higher than body tem-

perature as in the case of Cao Butta and Estarinum A might be expected to give poor release of medicament _in vitro_.

Effect of Storage on Suppositories

Several criteria were used in the selection of the fatty suppository bases chosen for _in vitro_ testing. These were:

(a) reasonable melting temperature - around body temperature.

(b) reasonable liquefaction time, some reported by Setnikar and Fantelli. (118)

(c) favourable reports in the literature concerning toxicity, stability and compatibility.

Many observations were made during the preparation of suppositories. The use of a lubricant can be questioned in the preparation of all the suppositories except cocoa butter. Lubrication of the mould produced inferior suppositories, in some cases causing the suppositories to shrivel. It is also essential that the mould be perfectly clean and dry prior to lubrication or staining of the mould will result. If the mould is fairly new, clean and dry, chilling of the mould prior to pouring was found to be unnecessary. Chilling of the suppositories after pouring was necessary only in a few cases, but prolonged chilling caused some suppositories to crack and split.

Polyethylene glycol base A made the most superior

suppositories of the three formulations tested, while base B
yielded the poorest suppositories. In spite of all efforts,
this formulation produced suppositories that cracked and
split. Polyethylene glycol base C can be considered midway
between the two, with respect to ease of manufacture. Slight
chilling after pouring (10 minutes at 10^0) was helpful in the
manufacture of suppositories with bases A and C, but caused
base B to split. The fatty bases produced excellent suppo-
sitories and the following observations were noted. Supposi-
tories manufactured from Cao Butta were excellent, however
slight chilling (10 minutes at 10^0) after pouring was found
to be most helpful. Cocoa butter suppositories required
chilling (30 minutes at 10^0) after pouring. Estarinums A and
B produced excellent suppositories but required chilling (no
more than 20 minutes at 10^0) after pouring. Prolonged chill-
ing caused the suppositories to crack. Massuppol suppositories
do not require chilling, and, in fact, chilling caused the
suppositories to crack. The Wecobees required slight chill-
ing (10 minutes at 10^0) after pouring, and produced excellent
suppositories. Suppositories manufactured with the Witepsols
bases produced excellent suppositories. Chilling after pour-
ing was not necessary in the manufacture of suppositories from
Witepsol H-15. Very slight chilling (5 minutes at 10^0) was
beneficial in the case of suppositories manufactured with
Witepsol H-19, and moderate chilling (10-15 minutes at 10^0)
with Witepsol W-45. Special care must be taken in the pre-

paration of Witepsol W-45 suppositories. Very slight and
moderate stirring was necessary, as prolonged stirring or
beating caused bubbles to form in the molten state which pro-
duced suppositories which were somewhat porous in nature.

Witepsol H-15 was considered to be the best fatty base
tested from the standpoint of ease of preparation and appear-
ance of the finished suppository.

The storage tests were designed to test the shelf-life
of the tested bases under normal conditions of storage. The
suppositories were examined at periodic intervals for appear-
ance and odour. A long shelf-life is one of the criteria for
an acceptable suppository base.

Suppositories manufactured from polyethylene glycol
bases A and C lost their original form and the former also
developed a strong sardine-like odour. Polyethylene glycol
base B cracked during storage.

Cao Butta suppositories lost their original lustre and
became dull, but they retained their original shape, as did
the Wecobee bases and the Witepsols. Suppositories manufac-
tured with cocoa butter became shrivelled soon after obser-
vations commenced and became progressively worse. Both
Massuppol suppositories and Estarinum A suppositories turned
slightly yellow, while those made with Estarinum B developed
a very slight odour. Witepsol H-19 suppositories developed
a slight buttery odour, while Witepsol W-45 suppositories
developed a strong coconut odour.

Of all the bases tested, Witepsol H-15 gave the best storage results with respect to the appearance of the finished suppositories.

Colourimetric Methods of Analysis

Previous authors (66,79,92,98,117) have reported maximum absorption at a wavelength of 525 mu for amaranth, while this experimental work established the wavelength at 519 mu. This discrepancy can perhaps be attributed to the different standards and sources for the dye. The reported figure of 525 mu was for Amaranth U.S.P., while in this work Amaranth C.F. as described in The Food and Drugs Act and Regulations of Canada (153) was employed.

The time intervals chosen were selected for several reasons. It has been found by previous authors (66,79,92,98, 117) that after an interval of 10 minutes, dye release is almost constant. It was also stated earlier that one of the criteria of a suppository base is that it should liquefy or dissolve in body fluids within 10 minutes. Further, the base must be liquefied or dissolved to eliminate the defecation reflex.

The dye release test is a comparative study under controlled conditions with the variable factors limited. Since cocoa butter has been used in commercial products for many years, it was taken as the standard basis for comparison.

The tested bases were judged either inferior or superior to cocoa butter for the release of dye in this test.

Polyethylene glycol formulation A appeared to be the best water-soluble base for in vitro dye release compared with the other two polyethylene glycol formulations. This base was almost identical to cocoa butter in dye release at the end of the tested interval, the others being inferior. Since this base has the lowest congealing temperature of the three, a correlation between congealing temperature and dye release may exist. However, since this relationship does not hold for the other two formulations, it would seem that other factors such as relative solubility of the components of the base are equally as important as the congealing temperature.

Cao Butta suppositories containing amaranth released very little dye. Release was so slow that readings at the 1, 2 and 4 minute intervals could not be detected with any accuracy. The melting point of this base was the highest of all the fatty bases used. If the melting point of a fatty base was higher than 37.0°, it did not release dye in vitro to any great extent. This is further shown in the case of the Estarinum A suppositories. Suppositories manufactured with Estarinum B have greater dye release than cocoa butter at all intervals except 10 minutes. Massuppol, Wecobee R and Witepsol W-45 were inferior to cocoa butter with respect to the release of the water-soluble dye, whereas Witepsol H-19 and Wecobee W suppositories were superior. The results with

Witepsol H-15 were very close to those obtained with cocoa
butter. Of the bases tested, it would seem that those suppo-
sitories manufactured with Witepsol H-19 are the most superior
in view of _in vitro_ dye release. No correlation can be made
regarding melting and good dye release.

Satisfactory suppositories which appeared uniform in
colour were prepared using the bases listed in Table II. A
method of analysis was developed to determine whether or not
uniform and accurate incorporation of the dye had been achieved
and it was found that this manufacturing error was less than
5% (Tables IV and V). This indicated that the methods em-
ployed in the manufacture of the suppositories for this work
yielded suppositories that fell well within the limits of the
U.S.P. XVII which allows 10 percent deviation from stated
amounts in all included suppositories but one, where the maxi-
mum error is 7 percent. (154-157) Difficulty was encountered
in finding a solvent best suited for the extraction procedure
and after several trials, chloroform was found to be the most
satisfactory.

Liquefaction Time

This test was designed to record a representative time
of melting or softening of suppositories at body temperature.
It differs from the melting point in that the length of time
required for uniform 2 Gm. masses (suppositories) to liquefy

at 37° is measured. Many of the conditions of the human rectum can be reproduced in the apparatus designed by Setnikar and Fantelli. (118)

In the study of the polyethylene glycol suppositories the most rapid liquefaction time was obtained with base A and the slowest with base B. These times can be directly correlated to the congealing temperature. The lower the congealing temperature of the polyethylene glycol formulations tested, the more rapid the liquefaction time. No correlation can be made regarding congealing temperature and dye release or liquefaction time and dye release, although it would appear from a consideration of the data for base A that the lower the congealing temperature the greater the dye release and the more rapid the liquefaction time. However, results on bases B and C do not follow this pattern.

Cao Butta, as might be expected from the melting point data, did not liquefy at all during the test procedure. However, correlations regarding melting points and liquefaction time are doubtful, as previous authors (118) have pointed out that a suppository will liquefy in the test procedure regardless of melting temperature.

Cocoa butter had the most rapid liquefaction time of all the fatty bases tested. This may be directly related to the rising temperature melting point, but it is doubtful whether correlation can be made regarding in vitro release of dye. It would appear the lower the melting point, the more

rapid the liquefaction time. The reported liquefaction time
of cocoa butter (118) is 6.09 minutes compared with an experi-
mental time of 4.88 minutes (Table VIII). The difference can
be attributed to either slight modification in design of the
apparatus, experimental technique or differences in the source
of cocoa butter.

The liquefaction time of Estarinum A was considerably
shorter than that of Estarinum B, whereas the melting point
of the A grade was higher.

The Wecobees and the Witepsols do show some correlation.
The lower the melting point with the Wecobees, the more rapid
the liquefaction time and the greater the dye release. How-
ever, the percentage dye release at all time intervals for
Wecobee R is much less than for Wecobee W and indeed, much
less than would be expected from a consideration of the differ-
ences between their relative melting points and liquefaction
times. The lower the melting point with the Witepsols, the
more rapid the liquefaction time.

From a consideration of all the bases tested, little
correlation can be established between liquefaction time and
dye release, although it can be stated that if a fatty base
does not liquefy within one hour in the test procedure, dye
release will be very poor. Cocoa butter has the shortest
liquefaction time (4.88 minutes) but it is surpassed in dye
release by Witepsol H-19 and Wecobee W with liquefaction times
of 9.42 minutes and 6.46 minutes respectively. Estarinum B

is quite close to cocoa butter in dye release, but the lique-
faction times are relatively far apart.

Differences between the results reported and those
established in the experimental work can be accounted for by
examining the modifications to the apparatus, experimental
technique and variations in the samples of the bases received.

Dialysis

Preliminary Experiments

The two drugs, secobarbital sodium and methyl iso-
nicotinate, were chosen with a view toward future in vivo
testing. These drugs have been shown in this study to be
adaptable to spectrophotometric methods of analysis (Figures
4 and 5) and in vivo tests have been reported in the litera-
ture for them. (1,146)

Secobarbital sodium was chosen as the test drug for
dialysis studies in suppositories instead of the methyl iso-
nicotinate because the barbiturate is used widely in medicine
as a short acting barbiturate, and is of current medicinal
interest. Preliminary data are presented for the methyl
iso-nicotinate but no suppositories of this drug were pre-
pared for this study.

Extensive experimentation was carried out using the
two dialyzing membranes, cellophane tubing and the Naturalamb

dialysis bags. In individual dialysis equilibrium tests, near-
ly all of the secobarbital sodium could be accounted for using
the cellophane bags, whereas this was not the case with the
Naturalamb dialyzing bags. Further, dialysis was regular and
uniform using cellophane, however with Naturalamb, it was erra-
tic. This might possibly be due to adsorption of the drug on
the Naturalamb membranes or because of the varying thickness of
the membranes. Although the cellophane membranes required a
longer period of equilibrium to be established than the Natura-
lamb, and dialysis was less rapid, uniform results could be
obtained using the cellophane membranes. Although the results
shown in Table VII indicate that the drug is not in equilibrium,
individual tests carried out show that equilibrium was esta-
blished at 24 hours and 8 hours respectively for the cellophane
and Naturalamb membranes, with little loss in drug in the case
of the cellophane membrane. The progressive loss of drug in
Table VII for the cellophane bag may be attributed to experi-
mental technique and in making the dilutions, while the loss
of drug in the case of the Naturalamb may be due, in addition
to the above factor, to the adsorption of the drug on the
membrane. Further evaluation of the Naturalamb membrane
was considered to be beyond the scope of this study, but
since the rate of dialysis is substantially increased using
this membrane, further study may be warranted. The cellophane
membrane was found useful for routine analysis and results
indicate that the surface area exposed for each test was

relatively constant.

Dialysis Using Solutions of Drugs

A water bath was found to be preferable to an incubator for controlling the temperature in these tests.

Dialysis was considered to be a better technique for the measurement of diffusion than the microbiological methods previously described. (92,100,112-116) Microbiological methods employ diffusion into a static medium, whereas dialysis employs diffusion into a dynamic medium. Since the human body is a dynamic medium, there seemed to be a better chance of correlation between in vivo studies and dialysis methods. Literature reports (92,116) indicate that although attempts were made to correlate the microbiological methods with other in vitro work, no attempt was made to correlate in vivo. Further, correlation between dialysis and in vivo methods had been attempted with limited success. (122,123,126,127)

Table VII shows the results of dialysis with aqueous solutions of secobarbital sodium using both membranes. The concentration of drug outside the membrane gradually increases with time until equilibrium, while the concentration inside diminishes. The table also shows the comparative speed of dialysis using the two membranes.

Dialysis Using Suppositories

Dialysis of secobarbital sodium from the polyethylene glycol formulation A was the greatest followed closely by base B, while dialysis of the drug from base C was the slowest (Table VIII). Dialysis of secobarbital sodium from the polyethylene glycol bases was inferior to that of cocoa butter.

Dialysis results with Cao Butta were very poor. As can be seen in Table VIII, it was not possible to obtain accurate determinations of the drug at the 15-30 and 45 minute intervals.

Estarinum A showed a surprising result in the dialysis test. The base released more secobarbital sodium than the polyethylene glycols after 15 minutes and more than Witepsol W-45, Wecobee R and Cao Butta at all time intervals.

Estarinum B released the greatest amount of secobarbital sodium of all suppositories tested. Results in Table VIII indicate that dialysis of secobarbital sodium was greater in the case of this base than it was for water alone. A thorough investigation of this phenomenon was not carried out, but it might be due to the presence of long chain fatty acids or emulsifying agents incorporated in the base.

Massuppol released more secobarbital sodium than cocoa butter. In view of the literature reports (8, 84-87), this was to be expected, but not in view of previous work carried

in this study.

The Wecobees released the secobarbital sodium as might be expected from a consideration of dye release, melting points and liquefaction time of these bases. Dialysis of secobarbital sodium from Wecobee W was almost equal to that of cocoa butter, while the R grade was definitely inferior.

The Witepsols did not release the drug according to what might be expected from previous in vitro tests. Witepsol H-15 and Witepsol H-19 released the drug at approximately the same rate as cocoa butter, while Witepsol W-45 gave a slower release.

Assay of Suppositories

Previous authors (100,116,120-127) have carried out dialysis experiments using different drugs, but no mention is made of an independent assay procedure, to establish uniform incorporation of drug in the suppositories. Results in this experimental work indicate that the procedures adopted for preparing the suppositories resulted in an error no greater than 5%. Table IX shows the results of the assay of polyethylene glycol suppositories containing secobarbital sodium. In each control case, 100% of the drug was recovered, and no difficulties were encountered with the assay procedure. However, difficulties were experienced with the fatty bases (Table X). A large error was encountered with the extraction of the drug from the synthetic fat suppositories. This

procedure was repeated with other examples, namely Witepsol
H-15, H-19 and Estarinum B. In each case there was a large
error in the assay procedure. The reason for this large error
is not known at this time, but it may be due to association
of the compound with the base in the chloroform layer.

Correlation Between In Vitro Test Procedures

Table XI summarizes the results of the in vitro tests.
The polyethylene glycol formulations were inferior to cocoa
butter in all respects, melting point, storage, dye release,
liquefaction time and dialysis and dye release techniques.
In both the dialysis and dye release studies, polyethylene
glycol base A released more material than base B, and B more
than C. No correlations exist between the other in vitro
tests.

Cao Butta showed consistently poor results in all in
vitro tests. If a fatty base does not liquefy in the test
procedure, it probably will not release medication to any
extent in vitro. Correlation does not exist between melting
point and liquefaction time with this base. It is doubtful
whether this base would release medication in vivo.

No correlation between the in vitro tests can be made
with Estarinum A. Results of dialysis with this base were
the opposite to what might be expected from a consideration
of dye release. However, Estarinum B had excellent dialysis

TABLE XI

SUMMARY OF in vitro TEST PROCEDURES

Base	Melting or Congealing Point °C.	Percentage Amaranth Release After Ten Minutes	Lique-faction Time (min.)	Dialysis Secobarb. Sodium at End of One Hour
Polyethylene glycol A	40.8	51.5	22.58	20.7
Polyethylene glycol B	52.0	36.2	42.58	19.6
Polyethylene glycol C	45.4	23.9	25.89	16.3
Cao Butta	38.8	4.1	(a)	5.0
Cocoa Butter	32.2	54.8	4.88	27.0
Estarinum A	37.4	8.8	10.35	22.0
Estarinum B	34.5	52.0	16.41	39.7
Massuppol	33.0	30.5	10.35	26.5
Wecobee R	34.4	21.1	8.01	25.2
Wecobee W	33.1	68.2	6.46	6.2
Witepsol H-15	35.2	48.5	10.32	25.6
Witepsol H-19	35.1	77.5	9.42	25.4
Witepsol W-45	34.4	35.3	9.37	8.2

(a) No liquefaction during one hour testing.

and good dye release and this supports the theory that a direct correlation may exist between these two methods. This base proved to be the most favourable base in the light of _in vitro_ testing.

Correlation between the _in vitro_ tests was not possible using Massuppol.

In all tests, Wecobee W was superior to the corresponding R grade. A direct correlation existed between dialysis and dye release using these bases. Furthermore, the lower the melting point of the Wecobee bases, the more rapid the liquefaction time and the greater the amount of medication released by dialysis and dye release.

No correlation between _in vitro_ tests existed for the Witepsol bases, although Witepsol W-45 appeared to be least satisfactory with respect to dialysis and dye release.

In the majority of cases, direct correlation _in vitro_ existed for the dialysis and dye release tests. These bases were: the polyethylene glycols, cocoa butter, Cao Butta, Estarinum B and the Wecobees. No correlation can be shown between the other _in vitro_ tests.

SUMMARY AND CONCLUSIONS

The melting point of a suppository which is not soluble
in body fluids should be no greater than normal body tempera-
ture. Fatty bases with a melting point greater than body tem-
perature gave poor release of medication _in vitro_. Polyethyl-
ene glycol base A had the lowest congealing point of the three
formulations tested. Estarinum B, Wecobee W, and the Witepsols
had melting points which fell within the manufacturers' speci-
fications while the melting point of cocoa butter fell within
the official limits.

Polyethylene glycol base A yielded the most superior
suppositories of those formulations tested, while base B pro-
duced the most inferior suppositories. All of the fatty bases
tested produced satisfactory suppositories.

Of the polyethylene glycol formulations tested, base A
proved to be the best base with respect to _in vitro_ release of
dye, the results being almost identical to cocoa butter. How-
ever, no correlation can be made with this test and congealing
temperature. Witepsol H-19 was the most superior base tested
with respect to release of dye, followed closely by Wecobee W.
Estarinum B and Witepsol H-15 were close to cocoa butter in
this respect, but all the other fatty bases tested were infer-
ior. No correlation exists between melting point and dye re-
lease for the fat bases.

A method of analysis was developed to determine if uniform

and accurate incorporation of dye had been achieved in manu-
facturing. It was found that the error due to manufacturing
was less than five percent, which more than meets the require-
ments specified by the U.S.P. XVII.

Cocoa butter had the most rapid liquefaction time of
the bases tested, whereas Cao Butta did not liquefy at all
during the test procedure. This fact, coupled with other data,
seems to indicate that if a fatty base does not liquefy within
one hour, release of medication will be poor. No further cor-
relation was shown between dye release and liquefaction time
for the fat bases.

With respect to the polyethylene glycol bases tested,
base A had the most rapid liquefaction time and base B the
slowest. The lower the congealing temperature of the poly-
ethylene glycol base, the more rapid the liquefaction time.
There appears to be no correlation between congealing temper-
ature and dye release or liquefaction time and dye release
from the polyethylene glycol bases.

Differences between experimental and reported lique-
faction times may be attributed to either slight modifications
of apparatus and procedure or variations in the samples used.

Both secobarbital sodium and methyl iso-nicotinate are
suitable for spectrophotometric analysis. Cellophane tubing
was found to be superior to Naturalamb dialysis bags for rout-
ine analysis. Although dialysis was much more rapid with the
Naturalamb bags, it was also erratic, possibly due to adsorption

of the drug on the membrane.

Studies on the polyethylene glycol formulations revealed that dialysis of secobarbital sodium from polyethylene glycol base A was the greatest followed closely by base B. All the polyethylene glycol bases were inferior to cocoa butter with respect to dialysis of the drug. Estarinum B proved to be the best of all bases tested in the dialysis work, while Cao Butta was the poorest.

Melting points, liquefaction time, and release of water-soluble drug as measured by dialysis and dye release were erratic with Cao Butta, Estarinum A and Wecobee R.

All suppositories underwent apparent physical deterioration on storage for one year at room temperature except those prepared with Witepsol H-15, Cao Butta, Estarinum A, Estarinum B, Massuppol, the Wecobees, Witepsol H-19 and W-45 either lost their original lustre, turned slightly yellow, or developed odours. Cocoa butter suppositories shrivelled, and those prepared with polyethylene glycol melted or cracked.

Polyethylene glycol base A proved to be the most effective formulation in vitro of the polyethylene glycol bases tested.

Estarinum B appears to be the most effective fatty base tested, and in fact is superior to those polyethylene glycol bases tested.

In the majority of cases there was a direct correlation in vitro between the dialysis and dye release tests.

Dialysis appears to be the most suitable _in vitro_ preliminary screening procedure for measuring the release of water-soluble medication from suppository bases.

It is suggested that the following topics be the subject of future work:

(a) Effect of surfactants on dye release and rate of dialysis, for example the investigation of the observation that Estarinum B released more secobarbital sodium when measured by the dialysis technique than the aqueous solution of the drug.

(b) Dialysis equilibrium studies on the bases tested to determine if any binding exists between the base and the drug used.

(c) Effect of pH on dialysis.

(d) An attempt to correlate the _in vitro_ studies conducted with _in vivo_ test procedures.

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