EFFECT OF OILS ON DRUG ABSORPTION

by

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To my parents

PREFACE

Many drugs exhibit poor and/or erratic absorption from the gastro-intestinal tract. Reports in the literature have suggested that under certain conditions drug absorption may be enhanced by co-administration with a lipid. The physiological and physicochemical mechanisms activated by the presence of the lipid within the gut are poorly understood. A knowledge of these mechanisms may enable a rational approach to drug formulation in lipid vehicles to be adopted, leading to exploitation of a hitherto little used dosage form.

The aim of the present study was to investigate the physiological and physicochemical factors governing oral drug absorption from lipid vehicles and thus to evaluate the potential of lipids as an oral drug delivery system.

The work was performed by collaboration between the Department of Physiology and Pharmacology and the Department of Pharmacy at the University of Nottingham, and Merck, Sharp and Dohme Research Laboratories, Hoddesdon, Hertfordshire, and was funded by the Science Research Council.

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the three phases of a lipid digestion mixture with the potential for drug absorption from each phase.

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(71.5mg/kg) in different vehicles

(30µl volumes) to rats.

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concentrations (dpm/mg) in anaesthetised rats folowing oral administration of ^{3}H-Prednisolone and ^{3}H-Prednisolone-21-Palmitate (20µCi) in different vehicles (30µl volumes).

ABBREVIATIONS

LCT	Long chain triglycerides
MCT	Medium chain triglycerides
MCM	Medium chain monoglycerides
LCFA	Long chain fatty acids
MCFA	Medium chain fatty acids
SCFA	Short chain fatty acids
FABP	Fatty acid binding protein
VLDL	Very low density lipoprotein
CCK	Cholecystokinin
CMC	Critical micellar concentration
DAO	Diamine oxidase
EE	Ethynylestradiol
THC	Δ-9-tetrahydrocannabinol
SL512	1-cyclopropyl-4-phenyl-6-chloro-2-(1H)-quinazoline
MBLA	N-methyl-benzyllinoleamide
PEG	Polyethylene glycol
DDT	2,2,bis(p-chlorophenyl)-1,1,1-trichloroethane
DDE	2,2,bis(p-chlorophenyl)-1,1-dichloroethylene
^{99m}Tc	^{99m}Technetium
^{123}I	^{123}Iodine
ROI	Region of interest
AUC_{x-y}	Area under the plasma concentration versus time profile between x and y hours
T_{max}	Time of the maximum plasma concentration
C_{pmax}	Maximum plasma concentration

SUMMARY

Oil and emulsion vehicles have been shown to alter the oral absorption of many drugs. This may be due to enhanced lymph flow and/or altered gastro-intestinal motility in the presence of the oils.

The oral absorption of a model compound (DDT) in the presence of three chemically different oils, arachis oil, Miglyol 812 and liquid paraffin was investigated in rats, the influence of lymphatic absorption and gastro-intestinal motility being determined. The findings were applied to the formulation of the steroid prednisolone, in an attempt to produce elevated more uniform plasma drug levels by enhancing lymphatic absorption.

The rank order for total DDT absorption from 1ml volumes of different vehicles was arachis oil > Miglyol 812= water containing 6% Tween 80 > liquid paraffin. The concentration of DDT in lymph collected via thoracic duct cannulae in anaesthetised rats was greatest in the presence of arachis oil, there being no difference between Miglyol 812 and liquid paraffin. Using a gamma camera the gastric emptying rate and total intestinal transit of ^{99m}Tc-sulphur colloid, an oil phase marker, was shown to be faster in the presence of 1ml liquid paraffin than the other two oils.

The oral absorption of ^{3}H-prednisolone was independent of the nature of the oily vehicle (30µl volumes) and was not selectively absorbed into the lymph. Esterification to ^{3}H-prednisolone-21-palmitate increased the lipophilicity of the

drug but did not stimulate selective lymphatic absorption and reduced oral absorption following administration in arachis oil. Lymphatic absorption of the ester was not promoted by administration in 1ml arachis oil.

Only the lymphatic absorption of compounds exhibiting selective uptake into the lymph may be enhanced by the presence of a suitable lipid vehicle. Altered gastro-intestinal motility in the presence of lipids may have greater potential for enhancing the absorption of a wider variety of compounds.

CHAPTER 1

INTRODUCTION

Oral administration in lipid vehicles has often been investigated as a potential means of overcoming the variable and incomplete bioavailability associated with poorly water soluble drugs. Although the absorption of a variety of drugs has been shown to be altered in the presence of oils and emulsions, few studies into the underlying mechanisms causing this change have been conducted. The influence of the physiological processes stimulated by lipids and their digestion products, on drug absorption are therefore not well understood. The aim of the present investigation was to determine the affect of these processes on oral drug absorption. From the results obtained it was anticipated that a rational approach to drug formulation in oily vehicles could be adopted.

1.1 Oral Drug Absorption in the Presence of Lipids.

Although there are many reports in the literature of altered oral drug absorption following co-administration of oils, the approach has not been systematic since each study differs with respect to the drug, oil, formulation and species investigated. The formulation details of these studies are summarised in Table 1.1.

The oral absorption of griseofulvin, an antifungal agent, from lipid vehicles has been extensively studied as a

Table 1.1 -Examples of Altered Oral Drug Absorption in the Presence of Lipids

(Formulation (1) is compared with formulation (2). $^{o}/w$ = oil-in-water emulsions)

Drug + Dose	Formulation	Effect of Lipid	Species	Reference
Griseofulvin	1.Co-administration with a high fat meal. 2.Co-administration with a high protein or carbohydrate meal	Plasma levels doubled	Human	Crounse (1961)
Griseofulvin 50mg/kg	1.$^{o}/w$ containing 10mg polsorbate 60 + 10mg digycerides of edible fats per ml 2a)Corn oil suspension containing 10mg polysorbate 60 per ml b)Aqueous suspension containing 10mg polysorbate 60 per ml	2.5 fold increase in bioavailability	Rat	Carrigan & Bates (1973)

Griseofulvin 500mg/kg	1.Corn oil ^{o}/w + 300mg polysorbate 80 per 30g 2a)Aqueous suspension + 300mg b)Commercial tablets	Peak plasma levels increased 3-4 fold Two fold increase in bioavailability	Human	Bates & Sequeira (1975)
Anticonvulsant Piperazine 21 derivative 8mg/kg in 0.1ml/kg	1.Corn oil solution 2a)Corn oil ^{o}/w = 20%^{v}/v oil+0.8% pluronic F-87 b)Suspension in normal saline	Increased ED_{50} Decreasd pharmacological activity and duration	Mice	Sanvordeker & Bloss (1977)
Chlormethiazole 640mg	1.Arachis oil suspension-640mg oil in soft gelatin capsules 2.Chlormethiazole base in soft gelatin capsules	50% increase in peak plasma levels	Human	Fischler et al.(1973)

Hydrophobic antimalarial amine 250mg	1.Soft gelatin capsules-20%^{W}/w drug base in oleic acid solution 2.Hard gelatin capsules-hydrochloride salt	Increased bioavailability	Dogs	Stella et al.(1978)
Flufenamic acid 200mg	1.Soft gelatin capsule-100mg vegetable oil, 40mg hydrogenated vegetable oil, 8mg beeswax and 5mg soya lecithin 2.Hard gelatin capsule-20mg magnesium stearate	Increased plasma levels 34% increase in total drug absorbed	Dogs	Angelucci et al.(1976).
Sulphanilamide acyl derivatives	1.Olive oil suspension 2.Aqueous accacia suspension	Increased therapeutic activity & blood levels	Mice	Feinstone et al.(1940)

Acetyl sulphasoxazole 118mg/0.5ml rat 118mg/2g human	1. $^{O}/_{w}$ - 50% vegetable oils+emulsifiers 2.Commercial aqueous suspension	Plasma levels increased 0-4h.	Rat Human	Svenson et al.(1956)
Sulphisoxazole	As above	No change		
Micronised Phenytoin 20mg/kg	1a)Corn oil suspension b)Corn oil $^{O}/_{w}$ = 0.4g oil/ml 2.Aqueous suspension + 0.5% methyl cellulose All formulations contain 10mg polysorbate polysorbate 80 per ml	Rate of absorption reduced Peak plasma levels 1.5 to 1.9 fold increase	Rat	Chakrabarti & Belpaire (1978)
Ephedrine 20mg	1.Mineral oil solution 1g in 99g 2.Aqueous solution	Decreased % drug recovered in urine over 48h.	Dogs	Lin et al. (1970)

Theophylline =15mg anhydrous theophylline	1.^{o}/w = 50ml peanut oil,4ml polyoxyethylene (4) lauryl ether & 2ml sorbitan trioleate per 100ml 2.Aqueous solution 550mg/100ml	Increased rate of absorption	Rabbits	Diamond (1970)
Nitroglycerin 3.5mg/kg	1.^{o}/w = sesame oil 40%^{v}/v, polysorbate 80 0.4%^{v}/v 2.Aqueous solution	Reduced peak plasma level Unchanged bioavailability	Human	Ogata & Fung (1980)
Lampren	1.Oily suspension 2.Micronised drug	50-85% increase in absorption	Human	Visher (1969)
THC 5mg in 10ml/kg	1.Corn oil suspension 2.10% Tween 80 in saline suspension	Reduced plasma levels	Mice	Mantilla-Plata &Harbison(1975)

THC 35mg	1.0.85ml sesame oil solution 2.$^{O}/_{W}$ = 0.85ml sesame oil+Tween 80	Increased peak plasma levels and rate of absorption	Human	Perez-Reyes et al.(1973)
Androstendione cyclopentyl enol ether 123.6mg/5ml	1.Sesame oil solution in soft gelatin capsules 2.Aqueous suspension of varying particle size +0.15% disodium phosphate	Increased urinary excretion	Human	Bruni et al.(1966)
Quingestrone 100mg	1a)Sesame oil suspension b)Sesame oil solution in 3ml 2.Micronised powder - particle size 10um	Increased urinary excretion	Human	Bruni & Galletti(1970)
Ethynylestradiol -3-cyclopentyl ether 0.5ml	1.Sesame oil solution + 25%$^{W}/_{v}$ glycerol mono-oleate 2a)Sesame oil solution b)Aqueous suspension	Increased lymphatic absorption	Rat	Gianina et al.(1966)

Urogastrone 20mg	1. $^{O}/_{w}$ = trioctanoin or olive oil 2a)Aqueous solution b)0.2% $^{w}/_{v}$ Tween 80 in aqueous solution c) $^{O}/_{w}$= liquid paraffin or diethylphthalate All $^{O}/_{w}$ formulations contain 5% $^{v}/_{v}$ oil+0.2% $^{w}/_{v}$ Tween 80	Increased actvity	Rat	Hori et al.(1977)
Heparin 100mg/kg in 5.0 or 10ml/kg	1. $^{O}/_{w}$ =trioctanoin,corn oil or peanut oil 2a)aqueous solution + sodium taurocholate b) $^{O}/_{w}$=mineral oil All emulsions = 4.5ml oil, 31.5ml water and sodium taurocholate 0.035M	Increased plasma levels	Rat Gerbil	Engel & Fahrenbach (1968)

Indoxole	1a)Lipomul-Oral emulsion b)Soft gelatin capsule containing polysorbate 80 2a)Aqueous suspension +0.1% pluronic F68, 50% sucrose, reservatives and flavouring agent b)Hard gelatin capsules=dioctyl sodium succinate sodium benzoate & magnesium stearate	Increased bioavailability	Human	Wagner et al.(1966)
SL512 0.5mg/kg in 20ul	1.MCT solution 2a)Corn oil suspension b)MBLA solution	Increased plasma levels	Rat	Yamahira et al.(1978)

Dicoumarol 10mg/kg in 5ml/kg	1.Triolein suspension 2.0.5% methyl celluolse aqueous suspension	Increased bioavailability	Rat	Bloedow & Hayton (1976)
Sulphisoxazole acetyl 100mg/kg in 5ml/kg	1a)Triolein suspension b)Trioctanoin suspension 2.0.5% methyl cellulose aqueous suspension	Increased bioavailability	Rat	Bloedow & Hayton (1976)

means of improving the slow, erratic and incomplete gastro-intestinal absorption normally associated with this drug. Crounse (1961) reported a two fold increase in the serum griseofulvin levels after single or multiple dosing to human subjects following a high fat meal compared with fasting conditions or a high protein or carbohydrate meal. It was demonstrated that the elevated serum levels were due to increased drug absorption rather than slowed clearance from the serum. Following a study demonstrating an enhancing effect of corn oil on griseofulvin absorption in rats (Carrigan 1974,Carrigan & Bates 1973) similar experiments were conducted in fasted human subjects (Bates & Sequeira 1975). Each subject was dosed with griseofulvin formulated as a)commercial tablet, b)an aqueous suspension or c)in suspension in a corn oil, oil-in-water emulsion. Urinary analysis of 6-desmethylgriseofulvin, the major metabolite of the antibiotic, showed the emulsion to have produced peak drug levels 3 to 4 times those of the other formulations and a total drug absorption twice that of the other preparations. It was suggested that the underlying mechanism was similar to that demonstrated in the rat (Carrigan 1974,Carrigan & Bates 1973), i.e. that the products of corn oil hydrolysis (linoleic acid and oleic acid) inhibited gastric emptying and proximal intestinal motility, allowing time for more complete drug dissolution and absorption. In human subjects bile secretion stimulated by administration of the oil possibly further enhanced griseofulvin dissolution. Varying the proportion of oil in the emulsion from 0-12g showed maximal absorption of a

250mg dose of the drug to be achieved with 6-12g oil, 6g being comparable to that necessary for initiation of the reflexes reducing gastric motility (Bates et al.1977). Although unemulsified corn oil also enhanced absorption the effect was less marked, 12g oil having the same effect as only 3g emulsified corn oil (Bates & Sequeira 1975). Bloedow & Hayton (1976) found that the bioavailability of griseofulvin in rats was reduced by administration of the drug as a suspension in triolein. It was suggested that differences in the rat strain, lipid volume and drug particle size might account for this unexpected result compared with that obtained with corn oil. Further investigations by Grisafe & Hayton (1978a) using an *in situ* rat intestine model and a perfusate containing monolein (3mM), oleic acid (6mM), triolein (2g/100ml) and/or sodium taurodeoxycholate (8mM), suggested that following dissolution griseofulvin concentrates in the micellar and oil intra-luminal phases from which there is negligible absorption, absorption occuring mainly from the aqueous phase. Thus the ability of a triglyceride to enhance the oral absorption of griseofulvin is apparently due solely to enhanced dissolution and not enhanced absorption of dissolved drug.

The effects of lipid vehicles on drugs other than griseofulvin have been far less extensively investigated.

The therapeutic activity of a series of acyl derivatives of sulphanilamide against streptococcal, pneumococcal and influenza virus infections in mice was enhanced by oral administration of the drugs in olive oil rather than suspensions in accacia (Feinstone et al.1940), and blood level

determinations showed that drug absorption had been increased. A study with another sulphonamide, acetyl sulphisoxazole, showed an oil-in-water emulsion formulation to increase the plasma drug levels during the first four hours after administration in both rats and humans, and to be 3 to 5 times more active against streptococcal and pneumococcal infections in mice than the corresponding aqueous suspension (Svenson et al.1956). Absorption of the parent drug, sulphisoxazole, was unaltered by formulation as an emulsion. It was not established whether acetyl sulphisoxazole absorption was altered by the presence of the oil or the emulsifiers.

Intra-gastric administration of micronised phenytoin to rats, in corn oil suspension and in corn oil emulsion yielded peak plasma levels 1.89 and 1.47 times that obtained with the equivalent aqueous suspension, and significantly higher total drug absorption as determined from the AUC_{0-12h} (Chakrabarti & Belpaire 1978). It was suggested that reduced gastro-intestinal motility in the presence of the corn oil may have contributed to these effects, the time to the peak plasma concentration being slower with the oil formulations. However, the oral absorption of the anticonvulsant, 1-diphenyl-4-[(6-methyl-2-pyridyl) methyleneamino]piperazine, was reduced in the presence of corn oil (Sanvordeker & Bloss 1977). Intra-gastric administration to mice of the drug in corn oil solution resulted in total arrest of anticonvulsant activity and, although administration in a corn oil emulsion restored activity, it was not to the same level attained from an aqueous suspension. The authors suggested that as the drug is very

lipophilic transfer from the corn oil to the intestinal fluid was insignificant, but that following emulsification the available surface area was increased allowing greater drug transfer into the intestinal fluid and hence greater absorption.

Oral administration of drugs in oily vehicles has been achieved by several workers using soft gelatin capsules. A comparison was made in human subjects of chlormethiazole administered in capsules containing chlormethiazole base and in capsules containing the base in arachis oil. The presence of the oil increased the peak plasma level by approximately 50%, although the time to the maximum plasma concentration was unaltered (Fischler et al.1973). This effect was shown to be dependant on the weight ratio of the arachis oil and chlormethiazole administered, chlormethiazole (384mg) plus arachis oil (384mg) yielded plasma levels equal to the base alone whereas chlormethiazole (384mg) plus arachis oil (758mg) produced plasma levels twice that of the base alone. As *in vitro* results showed that the arachis oil did not enhance chlormethiazole dissolution it was suggested that adsorption of arachis oil to the stomach wall resulted in an enlarged contact surface, which facilitated diffusion of the drug across the gastric mucosa. Stella et al.(1978) investigated the effect of oleic acid in a soft gelatin capsule formulation on the bioavailability of a hydrophobic amine antimalarial, dibutylaminomethyl 6,8-dichloro-2-(3,4-diphenyl)-4-quinolinemethanol, normally administered as the hydrochloride in hard gelatin capsules. Female Beagle dogs were each dosed

with either the hard gelatin capsule or three soft gelatin capsules containing the drug in solution in oleic acid. A significantly higher total drug absorption, as determined from the AUC_{0-12h}, was produced by the soft gelatin capsules. It was postulated that the oleic acid formulation allowed the poorly water soluble drug to enter the gastro-intestinal tract in a readily dispersible form facilitating more rapid and complete drug absorption. It is possible that the oil served only to form a solution of the drug and had no physiological effect on drug absorption; further investigations with a control drug solution are therefore needed to determine the effect of the oleic acid. The oral absorption of flufenamic acid, an analgesic and anti-inflammatory agent, was enhanced in dogs by administration of the drug in a soft gelatin capsule containing vegetable oils, beeswax and soya lecithin, rather than in a hard gelatin capsule containing magnesium stearate (Angelucci et al.1976). Higher plasma levels were achieved, except after 30 minutes, and the mean total drug absorption, as determined by the AUC_{0-12h}, was increased by 34%. However, in human subjects higher plasma levels were only attained after 90 minutes, although it was suggested that the sampling regimen had not been optimised and further investigations were therfore required. Ephedrine absorption in dogs was not enhanced by administration in a mineral oil solution contained in a soft gelatin capsule (Lin et al.1974), approximately half of the dose from the soft gelatin capsule being excreted in the urine over the first 48 hours compared with three-quarters from the aqueous solution. Unfortunately absorption from a digestible

oily solution was not investigated. Human absorption studies with Lampren, an antileprotic agent, showed that for a standard dose absorption from coarse crystals was about 20%, from micronised crystals about 50% and from oily suspension about 85%. For clinical purposes the drug was therefore suspended in an oil-wax base and administered in a capsule, resulting in an absorption of about 70% (Vischer 1969). Although the mechanism for absorption is not clear the drug is very lipophilic (log octanol:water partition coefficient of approximately 6.0) and it is possible that it remained with the oil and was absorbed into the lymph.

Higher plasma levels during the first 20 minutes after administration of theophylline to rabbits, were achieved by administering the drug in a peanut oil-in-water emulsion rather than an aqueous solution (Diamond 1970). It was suggested that the fine dispersion of emulsion droplets facilitated rapid drug absorption and that the presence of oil stimulated mesenteric blood flow via hormonal pathways.

Nitroglycerin when formulated in a sesame oil emulsion and intra-gastrically administered to rats was found to yield lower, later peak plasma levels than the equivalent aqueous solution (Ogata & Fung 1980). However, the total drug absorption was the same from the two formulations indicating the potential of the emulsion as a sustained release preparation. The delay in drug absorption was attributed to the time required for drug movement from the oil phase to the water phase prior to absorption, and to gastric emptying effects of the oil.

Solutions of ^{14}C-Δ-9-tetrahydrocannabinol (THC) in corn oil reduced drug absorption in mice compared with absorption from a 1% polysorbate 80 in saline formulation (Mantilla-Plata & Harbison 1975). Further studies showed a decrease in absorption in laboratory animals when oily solutions of THC were compared with emulsions containing Tween 80. However, in human studies the rate of THC absorption and the resulting plasma levels were higher with a sesame oil solution than with a sesame oil emulsion containing Tween 80 (Perez-Reyes et al.1973).

The absorption of a number of steroids has been shown to be altered by administration in oily vehicles. Alibrandi and his co-workers (1960) investigated the biological activity in rats, of testosterone, androstanolone (dihydrotestosterone), 17 α-methyl testosterone, prednisone, prednisolone and a number of short chain fatty acid esters, administered in sesame oil solution and in aqueous suspension. Following repeated daily, intra-gastric dosing, nearly all the test compounds of the androgen series and some of the cortical series exhibited an increase in specific activity when in solution in the sesame oil. A number of possible physiological mechanisms were suggested to explain the results. However it is possible that improved absorption was observed with the sesame oil solution simply because the drug was in solution and a dissolution step was not required as with the suspension. This possibility was further discussed by Bruni et al.(1966). They showed that as long as the particle size of an androstendione aqueous suspension was sufficiently small (4 to 30μm) the absorption,

as determined by urinary analysis, was the same as from a sesame oil solution of the drug. These results are in disagreement with those of Alibrandi et al.(1960), although the experimental protocol was different in the two investigations. The cyclopentyl enol ether of androstendione administered in a sesame oil solution resulted in higher steroidal urinary excretion than administration in an aqueous suspension of particle size 4 to 8μm. Although this result may again be due to the difference in the physical form of the drug, it was suggested that the particle size was sufficiently small for this not to be a problem, and that some other mechanism such as lymphatic absorption was involved (Bruni et al.1966). A similar study was conducted with progesterone 3-cyclopentyl enol ether (Quingesterone), 100mg being administered to one human patient either as micronised powder (particle size <10μm), a suspension in sesame oil or a solution in sesame oil. In the fasted patient the oily formulations gave better absorption than the aqueous suspension, but when the drug was taken after a meal excretion levels from the aqueous suspension approached those observed after ingestion of the oily solution. Thus, there would appear to be little difference in the therapeutic effectiveness of Quingestrone given in oily solution and in solid form taken at meal times (Bruni & Galletti 1970). The absorption of ethynylestradiol was enhanced by administration of the cyclopentyl ether derivative in sesame oil solution, and further enhanced by administration of the cyclopentyl ether derivative in sesame oil containing 25%^{w}/v glyceryl monoleate (Giannina et al.1966).

Preliminary investigations in two adult male subjects showed that higher serum concentrations of indoxole were achieved by administration of the drug in cottonseed oil solution or Lipomul Oral emulsion (cottonseed oil-in-water emulsion) than as a very fine powder in hard gelatin capsules. Further studies (Wagner et al.1966) showed that the bioavailability of indoxole in man decreased in the following order of formulations, emulsions=soft gelatin capsules (containing drug dissolved in polysorbate 80)>aqueous suspension>powder in capsule. The relative potency estimates were 1.00, 1.10, 0.47, 0.16 following a single dose and 1.00, 0.72, 0.27 and 0.16 following the sixth dose of the drug administered on a multiple dose regimen. On the basis of these results and those in the dog which indicated that indoxole is excreted into the bile, it was postulated that joint enterohepatic recycling of the drug and oil resulted in increased absorption from the emulsion. Both Carrigan (1974) and Sequeira (1976) suggested that this in fact was not the underlying mechanism since the serum level-time curves did not exhibit the characteristic multiple peaks and parallel decreases in serum concentration associated with enterohepatic recycling. In addition there was no supporting evidence which would explain how the drug and lipid continued to associate with each other following absorption from the gut. It seems more likely that indoxole absorption is dissolution rate limited and that any solution dosage form would therefore increase the bioavailability of the drug relative to a suspension, as was seen with both the emulsion and polysorbate

80 solution, there being no difference between these two formulations. Similar results were obtained by Kaiser et al.(1967) in the rat.

As the glycoprotein urogastrone inhibits gastric acid secretion its plasma levels may be determined by monitoring gastric acid output. Intra-jejunal administration of urogastrone in aqueous solution, 0.2%w/v Tween 80 solution, liquid paraffin emulsion and diethylphthalate emulsion was shown not to alter gastric acid output in rats, whereas administration in trioctanoin and in olive oil emulsions significantly inhibited gastric acid secretion (Hori et al.1977). This suggests that the digestibility of a lipid vehicle is an important consideration. The inhibitory action of the trioctanoin emulsion on gastric acid output was shown to be dependant on the oil volume, 5%v/v trioctanoin giving the maximum inhibition.

Whilst investigating the physicochemical and physiological properties of lipids which may affect the bioavailability of poorly water soluble drugs, Bloedow and Hayton (1976) showed that in rats the bioavailability of dicoumarol in was increased by administration in suspension in triolein or polysorbate 80, and the bioavailability of sulphisoxazole acetyl was increased by administration in suspension in triolein, polysorbate 80 and trioctanoin. The results of these studies indicated that whilst polar, digestible lipids increased the bioavailability of lipophilic, poorly water soluble drugs without increasing the absorption rate; nonpolar, nondigestible lipids generally did not affect

the bioavailability of these drugs, although they did appear to reduce the absorption rate.

Engel and Fahrenbach (1968) showed that the oral absorption of heparin was insignificant in rats and gerbils following intra-duodenal administration in an aqueous solution, in micellar solutions of monoolein or sodium taurocholate, or in sodium taurocholate stabilised mineral oil emulsion. However, significant absorption was achieved by administration in trioctanoin, corn oil or peanut oil emulsions stabilised with sodium taurocholate. These results would again suggest the importance of oil digestibility for enhanced drug absorption. The mechanism by which absorption of this lipid insoluble drug was enhanced was not determined.

Yamahira and co-workers (1978,1979a,1979b) investigated the effect of oil volume and oil digestibility on the gastro-intestinal absorption of the poorly water soluble antinflammatory agent, 1-cyclopropyl-4-phenyl-6-chloro-2(1H)-quinazolinone (SL512) in the rat. It was suggested that previous animal experiments had utilised large oil volumes that were beyond reasonable comparison with a human dose, distorting the characteristics of the dosage forms, and that an oil volume of 20ul/rat was more comparable to a human clinical dose volume. Serum levels of SL512 1 hour after administration in 20ul volmes of a MCT (medium chain triglyceride) solution, a corn oil suspension, a MCM (medium chain monoglyceride) solution, a MBLA (N-α--methyl-benzyllinoleamide, a poorly digestible lipid) solution and an aqueous suspension, were determined and shown to be in

good agreement with the gastric emptying rate of each preparation, with the exception of the MBLA formulation. It was suggested that as SL512 was readily absorbed from the intestine, the gastric emptying step was rate determining for digestible oils, whereas the rate of MBLA digestion was probably the rate limiting step for drug absorption from this oil. The serum levels of SL512 had the rank order MBLA<corn oil<MCT in intact animals but in rats with ligated bile ducts and in in situ recirculation experiments where no oil digestion occured, SL512 absorption was virtually equal for all three preparations. This again demonstrates the requirement for oil digestion. However, at a dose level of 2ul, the absorption of SL512 was shown to be less dependant on dosage form factors of the lipid formulation such as drug concentration or intestinal digestibility of the lipid vehicle, suggesting that physiological factors might be relatively more important. Results for the MCT formulation at a range of oil volumes indicated that for 10-100µl/rat the decrease in SL512 absorption rate with increasing oil volume was mainly attributable to a decrease in the rate of digestion of the lipid after gastric emptying, but that above 200µl/rat various factors such as depression of the gastric emptying rate or intestinal motility, in addition to a decrease in lipid digestion rate, may be involved in the decreased absorption rate of the drug.

Talbot & Meade (1971) reported that the ingestion of potato chips containing small amounts of methyl polysiloxane, a lipid like agent that enhances crispness, apparently

significantly reduced the absorption of warfarin and phenindione in patients taking these drugs.

Few generalised conclusions can be drawn from these investigations due to the large variation in the experimental conditions and protocol. However it does appear that oil digestibility and oil volume may be important considerations. In addition it is evident that suitable control studies have to be conducted to ensure that changes in drug absorption are due to the presence of the oil and not due to such factors as particle size or the presence of emulsifiers.

This literature review has been limited to simple oil and oil-in-water emulsion systems. Other more complex lipid formulations which have been used to enhance oral drug absorption include:-

a)liposomes (Ryman & Tyrrell 1979)

b)multiple emulsions (Engel et al.1968)

c)water-in-oil emulsions (Nakamoto et al.1979)

Various physiological mechanisms affecting oral drug absorption in the presence of lipids have been proposed:-

a)Altered gastro-intestinal motility changing the residence time of the drug at the site of absorption (Bates & Sequeira 1975).

b)Enhanced mesenteric lymph flow increasing the capacity for lymphatic absorption (DeMarco & Levine 1969).

c)Stimulated bile flow increasing drug dissolution and solubilisation (Bates & Sequeira 1975).

d)Altered intestinal mucosal membrane permeability caused by

reversible combination of fatty acids with the membrane structure (Muranushi et al.1980).

The present study was limited to an investigation of the influence of lymphatic absorption and gastro-intestinal motility on oral drug absorption. To understand these potential mechanisms a knowledge of the process of lipid digestion and the effect of lipids on lymph flow and gastro-intestinal motility is essential.

1.2 Lipid Digestion.

Triglycerides are fatty acid esters of glycerol and form the major portion of dietary lipids. Digestion and absorption of these molecules is dependant on the hydrocarbon chain length and the degree of unsaturation of the fatty acid moiety.

The digestion process is initiated by pharyngeal lipase in man, or by lingual lipase in rats (Hamosh et al.1975) which is mixed with the food as it is swallowed and starts the hydrolysis of the triglyceride molecules within the stomach. The fatty acids liberated stabilise the coarse triglyceride emulsion formed in the stomach from the shear forces generated by gastric motility (Lindhorst et al.1977), and following gastric emptying stimulate the release of the hormones secretin and cholecystokinin (CCK), leading to gall bladder contraction and secretion of pancreatic juice.

Pancreatic juice contains several lipolytic enzymes. "Classical" pancreatic lipase rapidly hydrolyses both tri- and di- glycerides, as it is specific for the primary (1,3) ester

bonds, to produce an equilibrium mixture of tri-, di- and monoglycerides and fatty acids (Borgstrom 1974,Hofman 1966). This enzyme has a greater affinity for long chain and for unsaturated fatty acids (Morely et al.1974). Lipolytic activity depends on the adsorption of the water soluble enzyme to the oil-water interface, enabling binding of the substrate ester bond to the active site. Bile salts at their critical micellar concentration (CMC) have been shown to inhibit lipase activity by removing the enzyme from the oil-water interface, the mechanisms involved are discussed by Borgstrom (1977). However, co-lipase, a polypeptide co-factor for lipase present in pancreatic juice, effects the binding of lipase to the substrate in the presence of bile salts probably by forming a 1:1 complex with the enzyme (Borgstrom & Erlanson 1973). Co-lipase also has a binding site for bile salt micelles so bringing the micelles close to the oil-water interface allowing rapid solubilisation of the lipolytic products. A second lipolytic enzyme present in pancreatic juice is known by a variety of names due to its broad spectrum of action, lipase A, non-specific lipase, sterol-ester hydrolase and carboxylic ester hydrolase. It hydrolyses 2-monoglycerides and generally compliments the action of "classical" lipase so that together they digest most triglycerides present in the diet.

Lipolysis is facilitated by increasing the available oil surface area for enzymic action and by rapid removal of hydrolysis products from the substrate surface (Bennzonana & Desnuelle 1965). Bile salts perform both these functions. Initially bile salts are synthesised in the liver from

cholesterol although the major fraction of bile is derived from bile salts that have been re-absorbed in the ileum by an active transport mechanism, and then transported to the liver in the portal vein where they are reconjugated. In humans, bile is stored in the gall bladder until ejected into the intestinal lumen after hormonal stimulation of the gall bladder following gastric emptying of food. There is no gall bladder in the rat, virtually the entire bile salt pool is present in the intestine (Norman & Sjovall 1958).

Adsorption of bile salts at the oil-water interface reduces the surface tension of the oil droplets thereby increasing the stability of the emulsion. Experiments by Lindhorst et al.(1977) indicated that at the usual intra-luminal pH of approximately 6.5, monoglyceride, fatty acid and bile salt monomers interact to reduce the interfacial tension of oil droplets sufficiently to allow emulsification during the introduction of low intensity shear forces, such as are produced by gastro-intestinal movement. The formation of emulsions of high stability is relatively unimportant as fat digestion normally occurs rapidly.

Within the intestinal lumen bile salts are usually above their CMC and therefore form micelles. The importance of micellar solubilisation of the lipolysis products varies greatly for different fats. The soaps of short chain fatty acids (SCFA) are sufficiently water soluble to give a reasonable rate of absorption even in the absence of bile salt micelles. For less water soluble fatty acids a dynamic equilibrium is established between the fatty acids in the oil

phase, in the micellar phase and in the aqueous phase. Absorption of fatty acids causes a shift in the equilibrium drawing fatty acids and monoglycerides out of the oil phase into the micellar or aqueous phases. For long chain fatty acids (LCFA) the rate of absorption from the micellar phase is faster than from the aqueous phases as they partition in favour of the bile salt micelles, the reverse being true for short and medium chain fatty acids. The absorption surface of the intestinal mucosal cells is considered to be covered by a layer of unstirred water through which the digestion products have to diffuse prior to absorption. As the rate of diffusion is inversely related to the square root of the molecular weight, the micelle diffuses across the unstirred layer at a rate about a tenth of that of the molecule. However, the concentration of the water insoluble lipids in the micellar phase is considered to be 100 times that in the molecular lipid phase. Thus the presence of a micellar phase favours transport of these lipids through the unstirred water layer by a factor of ten. When the micelles have delivered their content of lipid they diffuse back into the bulk phase (Borgstrom 1974,Wilson & Dietschy 1972).

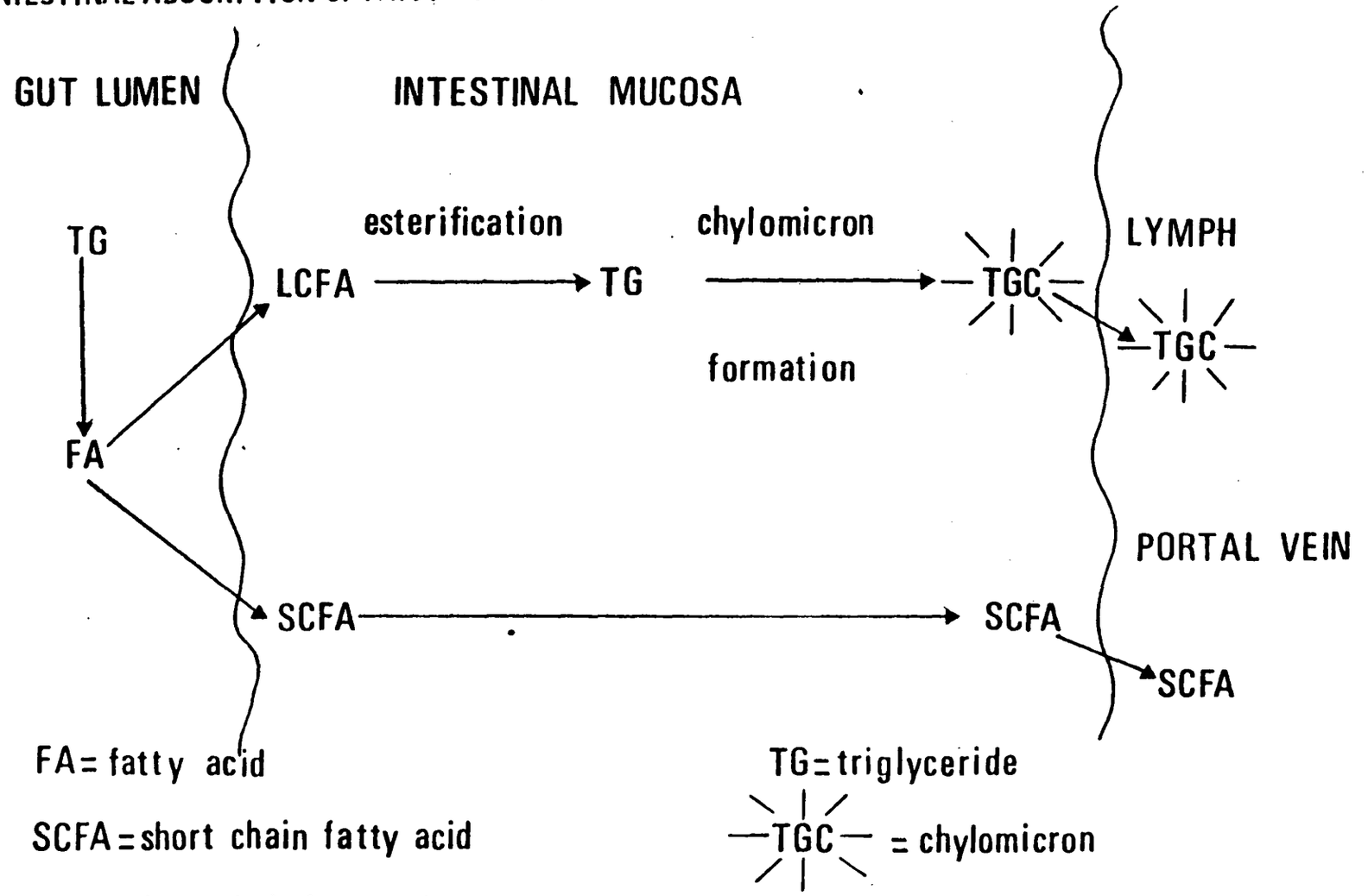
The mechanism by which fatty acids and monoglycerides enter the mucosal cells remains obscure, although the initial uptake is not energy dependant as it occurs in the absence of oxygen, at low temperatures and in the presence of metabolic inhibitors (Mishkin et al.1972,Johnston & Borgstrom 1964). As uptake is a passive process the rate depends on the concentration of free unbound fatty acid external to the cell.

It is possible that there are specific receptor sites for fatty acids in the mucosal membrane but they have not yet been shown to be present. It seems likely that some facilitating mechanism within the cell accounts for the fact that after uptake LCFA rapidly leave the lipoidal membrane to enter the aqueous cytosol of the cell. This movement of fatty acids through the cell has been observed during electron microscopy studies (Ohshima 1977). Ockner and Manning (1974) found that a protein constituent of the mucosal cell, which they called fatty acid binding protein (FABP), was capable of binding to LCFA and that its concentration responded to the fat content of the diet. In addition it was found to have a greater affinity for unsaturated rather than saturated fatty acids.

Short and medium chain fatty acids (less than C_{10}) are sufficiently water soluble to move through the mucosal cell into the hepatic portal vein by diffusion alone. Fatty acids having a longer chain lengths are re-esterified within the mucosal cell, incorporated into chylomicra and transported into the lymph (see figure 1.1). The distribution of fatty acids between these two absorption pathways is discussed in section 1.5.1. Two pathways for the re-esterification of LCFA are recognised (Isselbacher 1967), the synthetic enzyme system responsible being located in the smooth endoplasmic reticulum (Ohshima 1977,Sabesin & Frase 1977). Intra-cellular triglyceride formation is promoted by an increased rate of fatty acid delivary to the mucosa, mucosal penetration being the rate-limiting step in fatty acid absorption.

The mechanism by which re-synthesised triglycerides are

Figure 1.1
INTESTINAL ABSORPTION OF FATTY ACIDS.



formed into chylomicra is not clearly understood. Chylomicra consist of an oil droplet of liquid triglyceride containing small amounts of dissolved, esterified cholesterol, and a stabilising layer of more polar lipid (phospholipid) together with protein lying at the interface between the oil and aqueous medium (Johnston 1968). It is possible that the oil droplets are formed in a proteinaceous medium within the membrane bound cisternae (smooth endoplasmic reticulum) of the cell so that a layer of lipophilic protein forms around each drop (Cardell et al.1967). The proteins are probably formed in the ribosomes of the rough endoplasmic reticulum passing through communications between the cisternae of the rough and smooth endoplasmic reticulum to associate with re-synthesised triglycerides forming the chylomicra. Inhibition of protein synthesis using puromycin (Yousef et al.1976,Friedman & Cardell 1972,Kayden & Medick 1969,Redgrave 1969,Redgrave & Zilversmitt 1969) and acetoxycycloheximide (Glickman et al.1972) has shown that lipoprotein synthesis has a marked influence on the absorption and transport of long chain triglycerides. However, the reliabiliy of this technique has been questioned (Barrowman 1978).

During fat absorption chylomicra can be seen to accumulate in Golgi vacuoles in the cytoplasm close to the lateral borders of the epithelial cells and in enlarged intracellular spaces between neighbouring cells at the level of their nuclei (Cardell et al. 1967). Polay and Karlin (1959) proposed that chylomicra leave the cell by reverse pinocytosis, and E.M. studies (Ohshima 1977,Sabeisin & Frase 1977) have

shown Golgi vacuoles containing lipid droplets fused with the lateral cell membrane following absorption of LCFA. Recent studies by Glickman et al.(1976) using colchicine have indicated that microtubules may be involved in the extrusion process. From the intercellular spaces chylomicra appear to pass through the basement membrane of the epithelial cells into the lamina propria of the villus and thence gain access to the lymphatic vessels. Chylomicra enter the terminal lymphatics by passing between the epithelial cells (Casley-Smith 1962, Sabesin 1976), or by passing through the cells in vesicles (Casley-Smith 1962,Dobbins 1971,Dobbins & Rollins 1970).

Triglycerides may also enter mesenteric lymph in the form of very low density lipoproteins (VLDL). The mechanism by which resynthesised triglycerides are assigned to one of these carrier proteins appears to be specific and dependant on the degree of unsaturation of the constituent fatty acids (Ockner et al.1969,Leat & Harrison 1974). Caselli et al.(1979) administered corn oil, peanut oil, rapeseed oil and canbra oil to rats and found that the higher the proportion of unsaturated fatty acid ingested the more numerous and the greater the size of the chylomicra, whereas absorption of saturated fatty acids gave rise to numerous VLDL like particles and small chylomicra. The size of chylomicra also varies with the fat load in the intestine, the mean size of fat particles in the lymph increases during absorption and their phospholipid content decreases (Borgstrom & Laurell 1953,Fraser et al.1968,Bouquillon et al.1974). Glickman et al.(1972) showed that the size of chylomicra is dependant on the rate of mucosal

protein synthesis, inhibition of protein synthesis caused a marked, sustained increase in chylomicra size following intra-duodenal lipid infusion in rats, in an attempt to "package" lipid more efficiently. Shepherd and Simmonds (1959) suggested that there is a limit to the rate at which fat can be transfered to lymph.

Upon release of chylomicra and VLDL particles into the vascular system the triglyceride is hydrolysed by lipoprotein lipase bound to the luminal surface of capillaries. The fatty acids released bind to albumin and remain in circulation or are taken up by the liver. The other fractions of chylomicra are also taken up by the liver (Scow et al.1980).

Several workers (Knoebel 1972,Sabesin et al.1975,Noguchi et al.1977) have shown that the proximal region of the intestine is the principal site for fat absorption, although absorption in the distal region may occur in adverse conditions. The rate of fatty acid re-esterification is greater in the proximal region (Kotler et al.1980) and Wu et al.(1980) concluded that the distal intestine is defective in utilising phospholipid for chylomicra synthesis. Under conditions of high lipid infusion absorption of triglyceride may occur at a low rate in the distal intestine.

1.3 Effect of Fat on Gastro-Intestinal Transit.

The gastro-intestinal smooth muscle cells are characterised by spontaneous activity and autorhythmicity. External factors such as mechanical or chemical stimulation of

the mucosal receptors, extrinsic or intrinsic nerve impulses and hormones modulate this spontaneous actvity to give rise to the different movements of the gut. Inhibition of gastro-intestinal transit by fats has been observed by many workers, one of the earliest being Roberts (1931) who showed gastric motility in humans to be inhibited by a variety of natural triglycerides. The precise mechanism by which fats exert this effect is unclear.

Distention of the stomach stimulates gastric emptying but as soon as fresh food enters the duodenum it initiates the responses that then inhibit further emptying (Wiepkema et al.1972). Aberdeen et al.(1960) found that in rats, 15-20% of a test meal containing emulsified coconut oil, PEG and saline was immediately recoverable from the small intestine but that no further material left the stomach during the first hour. Once gastric emptying re-started it proceeded at a steady rate which was slower in the presence of 1.0ml oil than with either 0.5ml or 0.25ml oil present.

Exit of chyme from the stomach is associated with antral peristaltic waves moving all the way to the pyloric sphincter. Upon approaching the pylorus the contraction produces a pressure gradient of 2-4mmHg in humans, which is sufficient to initate passage of chyme through the pylorus into the duodenum. The pylorus then begins to contract increasing the resistance to the outlet and causing a sharp rise in pressure in the antrum. Evacuation is stopped when the closure of the pylorus is complete. Peristaltic action in the first portion of the duodenum is closely associated with that of the antral stomach

(Jacobson & Shanbour 1974,Cohen et al.1979) and the evacuated contents are rapidly propelled into the second portion of the duodenum while the pylorus remains closed preventing reflux. The role of the pyloric sphincter in the regulation of gastric emptying is not clear, there being evidence to suggest that it may have a role in the emptying of solids only. This is discussed in a review by Cooke (1975).

Usually gastric emptying is slowed by a depression or abolition of the propulsive antral pumping activity, affected by neural and humoral mechanisms (Thomas 1957). Hunt and Knox (1968a) have defined three types of receptors in the duodenum which respond to acid, osmotic pressure and lipids present in the intestinal contents by indirectly inhibiting gastric emptying. Neural inhibition is transmitted by the vagus, stimulation of the receptors influences the vagal nucleus which in turn co-ordinates gastric motility (Thomas & Baldwin 1968). This is discussed at greater length by Cohen et al.(1979). In the presence of fat humoral inhibition is mediated via the enterogastrone hormones CCK and secretin, released from the mucosa of the upper intestine in response to fatty acids in the lumen. It is not known whether hormone release is by direct action of the fatty acids on the endocrine cells, or at another site via a neural-hormonal reflex. Secretin inhibits both gastric and intestinal motiliy as well as gastric emptying (Chey et al.1970), whereas CCK inhibits gastric emptying but stimulates gastric and intestinal motility at physiological concentrations (Debas et al.1975). Yamagishi and Debas (1978) investigated the effectiveness of the C-terminal octapeptide of

CCK in inhibiting the emptying of 300ml saline in dogs, and concluded that CCK inhibits gastric emptying by relaxation of the proximal stomach and contraction of the pyloric sphincter. Again this is discussed at greater length by Cohen et al.(1979). The pyloric shincter has also been shown to contract in response to acid, fat, amino acids and glucose preventing emptying (Fischer & Cohen 1973).

Biliary and pancreatic secretions are essential for the inhibiton of gastric motility by fat, as fatty acids are required for receptor stimulation having a much lower threshold for inhibition than triglycerides (Quingley & Meschan 1941,Menguy 1960,Long & Weiss 1974). The chemical structure of the fatty acid is important in determining the extent of the inhibition of gastric emptying. Hunt and Knox (1968b) investigated the effect of straight chain fatty acids on gastric emptying and found that from acetic (C_2) to octanoic acid (C_8) there was little difference in the effect on gastric emptying, from C_{10} to C_{14} fatty acids there was a significant increase in the ability to delay gastric emptying, but C_{16} (palmitic) and C_{18} (stearic) acids were not as effective as C_{14} (myristic) acid. Unsaturated fatty acids delay gastric emptying more than saturated fatty acids of the same chain length (Cooperman & Cook 1976).

Upon arrival in the descending duodenum the chyme is subjected to the action of a complicated series of contractions, relaxations and tonus changes, resulting from segmentation (stationary ring contractions) and peristalsis (moving ring contractions) of the intestinal muscles that

affects mixing and then propulsion. The occurence and force of contraction at a given point depends on the inter-relationship among several factors; intrinsic and extrinsic nerve reflexes, activity of the autonomic nervous system and various locally released chemical agents (serotonin, histamine, prostaglandins, etc.). Simmonds (1957) observed that pressure patterns associated with propulsive motility are decreased in the proximal jejunum by fat feeding whilst non-propulsive motility is increased.

The amount of food transported per unit time through the intestine and the speed of overall progression along the gut are greatly influenced by the rate of gastric emptying (Derbloom et al.1966,Pirk 1967). Thus inhibition of gastric emptying produced by contact of food with the intestinal mucosa, contributes to slower progression through the gut. Investigations into the influence of fats on intestinal transit have suggested that gastric emptying and not intestinal motility is the determining factor in gastro-intestinal transit (Aberdeen et al.1960,Derbloom et al.1966,Yamahira et al.1978).

Thus fats slow gastric emptying through a complex response, mediated by neural and humoral mechanisms, to the stimulation of duodenal receptors by fatty acids in the intestinal lumen. Propulsive intestinal motility is also slowed by fatty acids although intestinal transit is largely dependant on the rate of gastric emptying.

1.4 Lymphagogue Effect of Fats.

A number of authors have discounted lymph as a major route of drug transport due to its low flow compared to blood; blood flow being 500-700 times that of lymph under normal conditions (Bollman et al.1948,Reininger & Sapirstein 1957). Lowrimore (1977) suggested that any drug not exclusively absorbed by the lymph would therefore be taken up to a greater extent by the portal system. It follows that the percentage of drug absorbed via the lymphatic route may be increased by stimulation of lymph flow.

Water and fats are the only dietary materials that have been shown to stimulate lymph flow (Simmonds 1954,Gallo-Torres & Muller 1969a) although there is a distinct difference in the underlying mechanisms causing this change. Borgstrom and Laurell (1953) studied lymph and lymph proteins during fat and saline absorption in rats. Both materials caused an increase in lymph flow but with saline there was a corresponding decrease in lymph protein concentration, whereas this was increased by fat. In addition, optimal water flow was reached two hours earlier than optimal lipid flow following fat administration. It was suggested that the increase in lymph flow following saline administration was due merely to increased fluid absorption from the intestinal lumen, whereas increased lymph flow and protein levels following fat feeding was due to increased blood filtration following a rise in blood flow through the mesenteric vessels. It is possible to visualise plasma protein escape from the vascular system by binding Evans

Blue dye to plasma albumin. Using this technique Wollin and Jacques (1973) showed that following intra-gastric administration of olive oil (2mg/kg) or glucose solution (0.5g/ml) to rats, far higher concentrations of extravascular dye were found in the fat-fed rats. These observations were in agreement with the findings of Simmonds (1957) who suggested that the lymph flow changes following fat feeding are mediated by some humoral effect on the mucosal blood flow or motility or both.

Studies by Fara et al.(1969,1972) in the cat showed that intra-duodenal administration of fat caused an increase in mesenteric blood flow, inhibited gastric and duodenal motility and increased gallbladder pressure and pancreatic secretion. In addition intravenous administration of hormones which are released during fat digestion (CCK or secretin) was found to have a significant vasodilator effect on the superior mesenteric vascular bed. CCK was also found to increase gut motility but this required a cholinergic link. Further investigations using anaesthetised cats showed that pentagastrin and CCK induced motor contraction of the small intestine and a transient increase in regional blood flow, whilst secretin had no effect on motility but caused a sustained increase in blood flow (Fasth et al.1973). Secretin appears to increase submucosal blood flow while CCK enhances mucosal blood flow (Fara & Madden 1975). Following this work in cats, Turner and Barrowman (1977) showed that intravenous administration of CCK and its synthetic C-terminal octapeptide caused a transient augmentation of intestinal lymph flow in

rats which was suggested to be a result of the effect of these agents on mesenteric blood flow.

Biber et al.(1973) proposed that vasodilatation in the gut depends partly on the release of intestinal hormones and partly on local mechanisms evoked by mechanical and possibly chemical stimulation. They showed that intra-arterial administration of 5-hydroxytryptamine produces a similar vascular response in the small intestine to that of CCK and secretin. In addition, studies by Wollin and Jacques (1974,1976) implicated histamine in the mechanisms increasing intestinal capillary permeability. They were able to prevent the increase in lymph flow and plasma protein escape in the rat following intra-gastric administration of olive oil, by blockage of H_1 and H_2 receptors or by stimulation of diamine oxidase (DAO) release by prior injection of heparin. They proposed that histamine was released locally after olive oil feeding and that this increased the permeability of the intestinal capillaries to plasma proteins. DAO levels in lymph were found to be elevated during fat feeding again suggesting histamine release; DAO is released to limit the biological response to histamine when it is liberated in large quantities (Buffoni 1966). Charbon et al.(1980) showed that histamine and 2-methyl-histamine also increase blood flow in the vascular beds of the superior mesenteric artery, left gastric artery and common hepatic artery of dogs.

It has been suggested that intestinal blood flow may be increased by increased gut motility following CCK release (Frank and Kern 1968,Lee 1965). However, Simmonds (1957) had

shown by in vivo experiments with anaesthetised rats, that gut motility is not essential for the propulsion of lymph, and that the increase in the formation and propulsion of lymph during fat absorption was not dependant on a high level of intestinal motility. Depression of intestinal motility in rats by intra-duodenal infusion of atropine had no effect on the ability of the intestine to absorb and transport fat (Bennett & Simmonds 1962). Glucagon has a vasodilator action on splanchnic circulation but inhibits intestinal motility. Barrowman et al.(1978) found that glucagon caused a rapid but transient increase in lymph flow again suggesting that intestinal motility is unimportant in the lymphagogue effect of fat.

It can be seen therefore that the increase in lymph flow following fat feeding is due to a complex mechanism of humoral and local effects causing vasodilatation and increased capillary permeability, giving rise to increased plasma filtration. The precise manner in which these mechanisms interact is as yet not fully understood.

1.5 Intestinal Lymphatic Absorption.

Although few studies have been performed a number of isolated reports in the literature suggest that under certain conditions some foreign compounds can exhibit significant lymphatic absorption. LCFA and some dietary compounds are absorbed primarily into the intestinal lymph. A knowledge of the factors affecting the absorption of these compounds will help in determining the criteria for lymphatic absorption and

the potential for enhanced lymphatic drug absorption in the presence of fats.

1.5.1 Lypmhatic Absorption of Fatty Acids.

A number of studies (Powell 1932, Hughes & Wimmer 1935, Bloom et al.1950, Reiser & Bryson 1951, Bloom et al.1951, Chaikoff et al.1951, Kiyasu et al.1952, Bergstrom et al.1954, Bloomstrand 1954) have shown that following intestinal absorption fatty acid molecules of greater than C_{14} chain length are transported primarily as fatty acid esters in the chylomicra of the mesenteric lymph, whereas medium chain fatty acids (MCFA) with a chain length of C_8-C_{12}, are carried primarily as free fatty acids in the portal vein.

A small proportion of LCFA will normally partition into the portal vein during absorption and be transported by this route (Bloom et al.1950, Blomstrand 1954). This partitioning into the portal vein appears to be dependant on the chemical structure and on the quantity of fatty acid administered. McDonald et al.(1977,1980) showed that for linoleic acid the percentage absorbed into the lymph of rats was directly related to the intra-duodenal infusion rate of the oil. From a similar study with a variety of fatty acids it was concluded that at physiological concentrations of fatty acids in the intestinal lumen the portal pathway accounts for more LCFA transport than previosly realised, especially for unsaturated fatty acids. However, this system is saturable at a level that varies for each fatty acid and then the remainder has to be transported in the lymph. Failure in the digestive mechanisms for LCFA at any stage will increase the partitioning into the portal vein.

Several workers have shown that a deficiency of bile salts within the gut lumen reduces re-esterification of fatty acids in the mucosal cells leading to greater transport of LCFA through the portal vein (Gallagher et al.1965, Dawson & Isselbacher 1960, Saunders & Dawson 1963, Borgstrom 1953). Kayden & Medick (1969) found that inhibition of protein synthesis by puromycin reduced chylomicra formation, again resulting in an increase in LCFA absorption into the portal vein.

Absorption of MCFA into the lymph does not occur as readily as absorption of LCFA into the portal vein. Borgstrom (1955) suggested that the route by which fatty acids are transported following absorption is determined by the extent of their re-esterification within the mucosal cell. It has been shown that within these cells the re-esterifying enzymes (see section 1.2) are specific for LCFA (Brindley & Hubscher 1966) and that there are also short and medium chain glyceride hydrolases present (Borgstrom 1974). Formation of short or medium chain triglycerides within the mucosal cell does not readily occur therefore, with the result that only a small proportion of MCFA is transported in the lymph, as illustrated by Hyun et al.(1967). They found that following administration of 150mg ^{14}C-octanoic acid (C_8) or 292mg ^{14}C-oleic acid (C_{18}) in 3mls emulsion to rats with lymph and portal vein cannulae, 85% of the absorbed oleic acid was transported by the lymphatic system but as much as 15% was transported directly by the portal vein. 50% of the oleic acid detected in the portal blood was as free, unesterified fatty acid. However after

administration of octanoic acid 95% of the absorbed fatty acid was transported by the portal system and the small amount present in the lymph was predominantly as free fatty acid. It is possible that this small amount of ^{14}C-activity was transferred from the blood to the lymph during general circulation.

It would appear that MCFA absorption is restricted almost totally to the portal route whereas LCFA absorption is primarily via the mesenteric lymph but a small proportion is also absorbed into the portal vein. During abnormal conditions the portal system may form the primary route of absorption for LCFA.

The degree of unsaturation of the hydrocarbon chain also affects fatty acid absorption. Unsaturated fatty acid have been shown to be more rapidly absorbed into the mucosal cells, more readily bound to fatty acid binding protein and more easily esterified than saturated acids of equal chain length (Ockner et al.1972,Gangl et al.1980,Simmonds et al.1968,Chow & Hollander 1979).

Thus the rate and route of absorption of fatty acids is dependant on:-

a)the lipophilicity of the molecule as determined by the hydrocarbon chain length and the degree of unsaturation,

b)the fatty acid load within the intestinal lumen,

c)the affinity of the fatty acid for the enzymes and carrier proteins involved in the digestion process.

1.5.2 Lymphatic Absorption of Dietary Compounds.

Cholesterol is the major sterol found in animal tissue

being an important structural component of cell membranes and a precursor for the formation of bile salts and steroidal hormones. It is almost totally absorbed via the lymphatic system both in rats (Sylven & Borgstrom 1968) and humans (Hellman 1960). Sieber et al.(1974) showed in rats that all of the absorbed activity from a labelled 0.72μmol/kg dose of cholesterol was recovered in the lymph, almost all in the form of cholesterol esters in the chylomicron fraction. As a result of this property cholesterol has been used in some absorption studies to assess the degree to which a test compound is transported by the lymph.

The fat soluble vitamins, A,D,E and K, all exhibit significant, if not total absorption via the lymphatic route. Absorption of vitamin A can occur via the portal route in rats (Murray & Grince 1961,Yeung & Veen-Baigent 1972) especially in abnormal conditions such as diverted bile flow (Gagnon & Dawson 1968, Hollander 1980) but normally it partitions between the lymph and the blood in favour of the lymph. Schachter et al.(1963,1964) demonstrated in the rat that vitamin D is absorbed maximally in the jejunum and transferred chiefly to the lymph as the free biologically active sterol. The tocopherols (vitamins E) are absorbed from the gut unchanged and the intestinal lymph acts as the major route of absorption (Peake et al.1972,Johnson & Pover 1962,MacMahon et al.1971). Using ^{14}C-labelled vitamin K preparations Jacques et al.(1954) found that approximately 50% of a dose of vitamin K_1 and all of a dose of vitamin K_3 was absorbed by rats. Vitamin K_1 appeared to be transported mainly via the intestinal lymphatics and

vitamin K_3 by the portal venous route. These results were later confirmed by Mezick et al.(1968).

The presence of bile salts has been found to be essential for the lymphatic absorption of cholesterol (Chaikoff et al.1952) and the fat soluble vitamins D (Schachater et al.1963,1964) and E (Gallo-Torres 1970). Due to the low water solubility of these materials solubilisation by bile salt micelles is required to enable movement of the molecule through the aqueous enviroment of the gut lumen to the mucosal membrane. It has also been suggested that bile salts may be essential for the hydrolysis of cholesterol and vitamin esters that is necessary prior to absorption, and may aid penetration of the molecules into the mucosal cell itself (Gallo-Torres 1970). Vitamin A is sufficiently water soluble to be absorbed directly into the portal vein in the absence of bile (Gagnon & Dawson 1968).

Co-administration with long chain triglcerides (LCT) has been shown to enhance the lymphatic absorption of cholesterol and vitamins D and E by a variety of mechanisms. Incorporation of fatty acids and monoglycerides into the bile salt micelle increases the solubility of the cholesterol in the hydrophobic micellar centre giving rise to increased absorption (Hofman & Small 1967,Treadwell & Vahouny 1968). In addition, LCT stimulate chylomicra synthesis thereby increasing the capacity of the lymph to transport cholesterol (see section 1.2). Thompson et al.(1969) ascribed the enhanced absorption of cholesterol and vitamin D following co-administration with LCT to increased transport of the compounds out of the mucosal cell

into the lymphatics. The enhanced absorption of vitamin E in the presence of LCT was attributed to stimulation of bile flow giving rise to increased solubilisation of the poorly water soluble vitamin (Gallo-Torres et al.1971,MacMahon & Thompson 1970). All these proposed mechanisms are feasible and it is likely that it is a combination of these effects that results in increased lymphatic absorption. The effectiveness of this triglyceride stimulation is related to the chain length of the constituent fatty acids, only LCT enhance absorption as only LCFA are absorbed via the lymphatic pathway. In addition, if cholesterol is administered in a hydrocarbon oil, absorption is decreased almost to zero (Sylven & Borgstrom 1969,Mattson et al.1976) because cholesterol partitions in favour of the oil which is not absorbed and the cholesterol is therefore retained within the intestinal lumen.

Thus the presence of bile salts is essential for the lymphatic transport of cholesterol and vitamins D and E due to their low solubility in the aqueous enviroment of the gut lumen. Co-administration with LCT enhances the absorption of these compounds by increasing their solubility in the bile salt micelles, by stimulating chylomicra synthesis and/or increasing the transport out of the mucosal cells into the lymph.

1.5.3 Lymphatic Absorption of Foreign Compounds.

Robbins (1929) found that in the presence of dietary fat (cream) there was a marked increase in the absorption of carbon tetrachloride in the dog. It was suggested that this may be due to absorption of the drug with the fat thereby avoiding first-pass metabolism. Analysis of lymph samples showed that

carbon tetrachloride was only present following co-administration of cream.

The lymphatic absorption of the steroid ethynylestradiol (EE) in rats was found to be enhanced by the addition of a lipophilic grouping and by administration in an oily vehicle (Gianna et al.1966). Only 0.4-0.6% EE was recovered in the lymph when given in aqueous or oily vehicles, whereas 1.4% of a dose of ethynylestradiol cyclopentylether was recovered in the lymph following administration in aqueous suspension, 7.5% following administration in sesame oil solution and 15.7% following administration in sesame oil containing 25%^{w}/v glyceryl mono-oleate. Similarly Coert et al.(1975) showed that the androgenic activity of testosterone in rats over 7 days was greatly improved by esterification to the apolar testosterone undecanoate. This effect was further augmented by dissolving the ester in arachis oil. It was demonstrated that the absorption of testosterone undecanoate occured via the lymphatic route. These findings were confirmed in human patients (Horst et al.1976). This suggests that both the lipophilicity of the compound and the nature of the vehicle are important in determininig the extent of lymphatic absorption.

In 1969 DeMarco and Levine reported an attempt at a more systematic investigation into the role of the lymphatics in intestinal absorption and distribution of drugs. They considered the absorption in rats of intra-duodenal doses of para-aminosalicylic acid (PAS) (40mg in 2ml water) and tetracycline hydrochloride (20mg in 1ml water) and concluded that under normal conditions the lymphatic route contributes

little to the overall absorption of the two drugs. It was suggested that as there was no selective uptake into the lymph the major route of absorption would be the blood due to its far higher flow rate. Pre-dosing with tripalmitin to increase the lymph flow was found to increase the level of both drugs in the lymph (Levine 1970), and ligation of the superior mesenteric vein to decrease the blood flow also increased PAS lymph levels. Thus for compounds not selectively absorbed into the lymph co-administration of lipid may increase the lymphatic absorption by stimulation of lymph flow.

Lowrimore (1977) performed a series of investigations in order to elucidate the relationship between the physicochemical properties of drugs and their absorption and distribution by the lymphatic system. Lymph levels following intra-gastric injections of aqueous, radiolabelled solutions of the acidic drugs phenobarbital and thiopental, the basic drugs d-amphetamine and imipramine, and the neutral compounds ethanol and prednisolone, were determined in anaesthetised, female mongrel dogs. Only prednisolone appeared in significantly greater concentrations in the lymph than in the portal blood during the absorptive phase. It was suggested that the ability of a compound to be absorbed by the lymph rather than the portal venous system is significantly, but not exclusively governed by the lipid solubility of the compound. A correlation was observed between the relatively selective lymphatic absorption of compounds and their ability to become incorporated into the lipid phase of the lymph.

Similar criteria for lymphatic absorption were suggested

by Sieber et al.(1974). They studied the lymphatic transport of a large series of radiolabelled foreign and natural compounds intra-duodenally administered in ethanol/water solution to rats with thoracic duct fistulae. In most cases the activity recovered in the lymph over 24 hours was only a small percentage of the total amount absorbed. The portal route appeared to be more important in the intestinal absorption of benzene, benzoic acid, aniline, p-aminosalicylic acid, salicylic acid, phenanthrene, oestradiol, testosterone, digoxin, hexanoic acid, hexylamine, hexanol, antipyrine, isoniazid and caffeine. Only a few compounds appeared to be selectively absorbed via the intestinal lymphatics, namely octadecanol, cholesterol and p,pDDT, all of which are highly lipid soluble. It was shown that for compounds poorly absorbed into the lymph most of the radioactvity was carried in the infranatant phase of the lymph, whereas for those compounds more readily absorbed the radioactivty was present in the chylomicra. This suggests that the lipid fraction of the lymph is important in carrying compounds well absorbed into the lymphatics and it could be that the extent of absorption is a function of lipid solubility. However, a further study by Sieber (1976) on the lymphatic absorption of DDT and some structurally related compounds which varied markedly in lipophilicity, suggested that there was not a strict parallel between lipophilicity and lymphatic absorption. Kamp and Neurmann (1975) concluded that while lipid solublity appeared to be a major pre-requisite, specific structural requirements contributed to determining the extent to which compounds are

lymphatically absorbed.

In a series of investigations with rats, into the absorption of 17-methyl-^{14}C-oestradiol (Blockage et al.1953), 17α-methyl-^{14}C-testosterone (Hyde et al.1954) and cortisone-4-^{14}C-acetate (Blockage et al.1955), it was concluded that the lymphatic absorption of these steroids was insignificant with respect to the total absorption. Only 0.2 to 0.4% of a 0.2mg dose of 17α-methyl-^{14}C-testosterone was recovered in the lymph over 24 hours regardless of whether it was administered in a 50% ethanol solution or in corn oil. Similarly with a 0.2mg dose of cortisone-4-^{14}C-acetate, none was recovered in the lymph although 46% of the dose was absorbed. It was suggested that in the former case the small amount of steroid detected in the thoracic duct lymph could have been derived from the plasma. Following oral administration of labelled hydrocortisone and testosterone in human patients Hellman et al.(1956) also concluded that these compounds were not transported to a significant degree in the thoracic duct lymph. These results are in general agreement with those reported by Sieber et al.(1974) in which it was suggested that these steroids were not sufficiently lipid soluble to be selectively absorbed into the lymph.

The same conclusion can be applied to the cardiac glcosides. Forth et al.(1969) studied the intestinal absorption of aqueous solutions of tritium labelled digoxin, digitoxin and oubain in the intestinal loops of anaesthetised cats. Drug concentrations always remained below those in the portal blood; the amount of glycoside passing into the lymph during one hour

being only 0.006 to 0.02% of the administered dose, with oubain displaying the highest lymph levels followed by digitoxin and digoxin. Administration of digtoxin as an olive oil emulsion failed to increase the lymphatic absorption and it was concluded that these glycosides appear in the lymph indirectly by way of the blood. These findings for digoxin and digitoxin were confirmed by Oliver et al.(1971) and Beerman & Hellstrom (1971). In addition only trivial amounts of proscillaridin A, a cardiac glycoside derived from squill, were found in human thoracic duct lymph after oral dosing of the drug as a tablet to two patients (Anderson 1977).

A major criterion for lymphatic absorption would therefore appear to be high lipid solubility such that the material is carried in the chylomicra of lymph. Increasing lipophilicity by structural modification such as esterification, and stimulation of lymph flow by co-administration with oils, may enhance lymphatic absorption.

1.6 Objectives and Proposals.

In summary it has been suggested that drug absorption in the presence of lipids may be affected by 4 main physiological factors a)gastro-intestinal motility, b)lymph flow, c)bile flow and d)membrane permeability, associated with fat digestion.

Inhibition of gastric emptying and intestinal motility stimulated by fatty acids via neural-humoral pathways (see section 1.3), prolongs the time of the drug at the site of dissolution and absorption which may result in enhanced

absorption.

Stimulation of mesenteric lymph flow by fatty acids increases the potential for drug absorption via this route (see sections 1.4 & 1.5.). The lymphatic absorption of drugs offers a number of advantages. First, it may be possible to divert drug absorption from the portal system, reducing first-pass metabolism. Second, the absorption of lipophilic agents showing irregular absorption might be enhanced. Finally, if the absorption of a drug into the mesenteric lymph can be promoted it may be possible to deliver high concentrations of cytotoxic drugs into the lymphatic circulation.

Bile flow is stimulated by fat hydrolysis. In the presence of an increased bile salt concentration both drug dissolution and absorption may be enhanced. Solubilisation of drug in bile salt micelles will aid the dissolution process and incorporation of drug into mixed bile salt micelles may enhance absorption (see below).

Several workers have attributed enhanced drug absorption in the presence of fatty acids to changes in the mucosal membrane permeability (Hori et al.1977,Grisafe & Hayton 1978b,Inui et al.1976,Muranushi et al.1980).Grisafe & Hayton (1978b) suggested that short and medium chain fatty acids present in the intestinal epithelial cell membranes may induce a phase transition in the lipid bilayers from organised gel to a randomised liquid crystalline phase, and the associated change in membrane fluidity results in a change in membrane permeability. A similar hypothesis was proposed by Muranushi et al.(1980) who suggested that mixed bile salt micelles enhance

the intestinal absorption of poorly absorbed drugs by facilitating the incorporation of the lipid component of the mixed micelle into the mucosal membrane. This then interacts with the polar region of the membrane phospholipids and increases the fluidity and the permeability of the membrane. Such a change in permeability is reversible. No conclusive evidence has yet been reported to substantiate these theories.

The aim of the present study was to determine the potential for enhancing oral drug absorption by stimulating lymphatic absorption in the presence of a suitable oily vehicle. The effect of different oily vehicles on the oral absorption of a model compound (DDT) was investigated, the influence of lymphatic absorption and gastro-intestinal transit being determined. The results from this study were applied to the formulation of the steroid prednisolone, in an attempt to produce elevated more uniform plasma levels of the drug by enhancing the lymphatic absorption.

CHAPTER 2.

ORAL ABSORPTION OF DDT.

2.1 Introduction.

The selection of a model compound to determine the possiblity of enhancing lymphatic absorption by oral administration in an oily vehicle is determined by several factors. First that co-administration of the compound with oils enhances its oral absorption, second that it exhibits a degree of lymphatic absorption, and third that it is readily assayed in biological fluids.

DDT (2,2,-bis(p-chlorophenyl)1,1,1-trichloroethane) was first synthesised by Zeilder in 1874 but attracted little interest until the early 1940's when its insecticidal properties were discovered. Toxicity studies at this time indicated that DDT orally administered as an oily solution was more toxic than the corresponding aqueous suspension. Konst and Plummer (1946) found that in rats the tremor and the mortality ratio induced by DDT was ten times higher following administration as a corn oil solution than as an aqueous suspension at concentrations of 2%, 5% and 20%^{w}/v. In a similar study Woodward et al.(1944) showed the mortality ratio of rats following administration of DDT in increasing doses (140-300mg/kg) to be higher with a corn oil solution than with an aqueous suspension. However in both sets of experiments it is possible that the difference between the aqueous and oily vehicles was due only to the difference in the physical form in

which the DDT was administered, i.e. a suspension compared with a solution. Sieber et al.(1974) orally administered to rats DDT (0.64mg/kg) in solution in 0.5ml 50% ethanol and in 1ml olive oil and found that 82% of the dose was absorbed from ethanol compared with 79% from olive oil. However, only 20% of the absorbed dose was recovered in the lymph following dosing with ethanol whereas 33% of the absorbed dose was recovered in the lymph following dosing in olive oil. This suggests that the presence of an oil may alter the route, but not the extent of DDT absorption.

Several groups of workers have shown that following oral administration to rats, DDT is largely absorbed via the lymphatic pathway, incorporated in the triglyceride core of the chylomicra (Pocock & Vost 1974,Sieber et al.1974,Rothe et al.1957). There is, however, some discrepancy between these investigators as to the proportion of DDT absorbed via this route. Pocock and Vost (1974) recovered 62% of a 100nM dose of DDT dissolved in sunflower seed oil, from the lymph within 12 hours, whereas Sieber et al.(1974) recovered only 33% of the absorbed dose (=26% original dose) in the lymph in 24 hours following oral administration at a dose level of 0.64mg/kg in 1.0ml olive oil. Rothe et al.(1957) administered DDT (10mg/kg) to rats in 0.2ml emulsion containing 15% peanut oil and recovered 47±5% of the dose, 60% of this being recovered from the lymph. As different doses of DDT in different vehicles were used in these experiments it is not possible to compare the results directly, although they do suggest that following oral administration a proportion of DDT is absorbed into the lymph.

Hayes (1959) in a review article suggested that the nature of the oily vehicle was important in determining the extent of DDT absorption, he stated that "the formulation in which DDT is applied is more important than the purity of the compound in determining the toxicity and often depends on the amount and character of the oil in the formulation". It is possible that the chemical composition of the oil is important in determining the lymphatic absorption of DDT.

Early investigators had to rely on relatively insensitive analytical methods to determine DDT levels in biological fluids. One widely used technique is based on the titration of the chlorine in the DDT molecule with silver nitrate (Smith & Stohlman 1944). Other techniques include spectrophotometry following a complex chemical reaction (Ofner & Calvery 1945,Judah 1949) and a biological assay based on the toxic response of the housefly to DDT contaminated material (Laug 1946). More recently the development of GLC and the electron capture detector has provided an accurate analytical method for determining trace levels (nanogram quantities) of DDT in biological fluids (Dale et al.1970).

DDT would therefore seem to be a suitable model compound for the present investigation. It is highly lipophilic having a water solubility of the order of 1-5ppb and an octanol:water partition coefficient of 1.6×10^6 (Chiou et al.1977). It shows some dependance on formulation as to the extent of oral toxicity and is in part absorbed via the lymphatic route. In addition a sensitve analytical technique is available for the determination of DDT in biological fluids and tissues.

Three oils that are used in pharmaceutical preparations but which have chemically different structures were selected as vehicles for oral administration of DDT to rats:-

a)Arachis oil B.P.(peanut oil), a natural oil consisting of triglycerides the fatty acid constituents of which are chiefly oleic acid (C_{18} containing one double bond) and linoleic acid (C_{18} containing two double bonds) with smaller amounts of palmitic, arachidic, lignoceric and stearic acids.

b)Miglyol 812, a synthetic oil produced by hydrolysis, fractionation and re-esterification of coconut oil, consisting of a mixture of triglycerides of saturated fatty acids of medium chain length (C_8 - C_{12}).

c)Liquid paraffin B.P., a mineral oil consisting of a mixture of liquid hydrocarbons.

The oral absorption of DDT from solution in each oil was investigated in rats, to determine whether DDT absorption could be altered by the presence of an oily vehicle and whether the nature of the oil used was an important consideration.

1.2 Materials and Apparatus.

2.2.1 Materials.

a)p,pDDT and p,pDDE - Aldrich Chemicals Co.Ltd.,Gillingham, Dorset.

b)Miglyol 812 - Dynamit Nobel, Slough, Berks.

c)Liquid paraffin B.P.- Shell. Ltd., London.

d)Arachis oil B.P. - Evans Ltd., Liverpool.

e)Tween 80 - Sigma (London) Chemicals Co.Ltd., Poole, Dorset.

f)Amethocaine hydrochloride 1% B.P. Minims, Metoclopramide and Propantheline bromide - Pharmacy Dept., University Hospital, Nottingham.

g)n-Hexane SLR and Formic acid AR 97% - Fisons, Loughborough, Leices.

h)Heptachlorepoxide - Vesicol Chemicals Corporation, Chicago, USA.

2.2.2 Apparatus.

a)Silverson Blender - Silverson Machines Ltd., Waterside, Chesham, Bucks.

b)QPR hand Homogeniser - Omerod Engineers Ltd., Rochdale, Lancs.

c)Coulter counter model TA - Coulter Electronics Ltd., Harpenden, Herts.

d)Heparinised haematocrit capillary tubes - Gelman Hawsksley Ltd., Brackmills, Northhants.

e)Haematocrit centrifuge - MSE Scientific Instruments, Crawley, Sussex.

f)Dreyer's tubes - R.B.Radleys & Co.Ltd., Sawbridgeworth, Herts.

g)Dosing needles - formed from a curved syringe needle (Size 19g X 2") with a smoothed rounded piece of silver solder at the end.

h)Gas chromatograph - Perkin Elmer Series Model F17 fitted with an electron capture detector ^{63}Ni foil source. Operating conditions - injector temperature 275^0, oven temperature 225^0, nitrogen carrier gas inlet pressure $170kN/m^2$ and flow rate 78ml/min, pulse setting 6, range 10.

i)Rheometer - Deer Variable Stress Rheometer, London.

2.3 Methods.

2.3.1 Assay Procedures.

a)Standard solutions - a stock solution of the internal standard (heptachloepoxide) in n-hexane (70ng/ml) was prepared. Approximately 100mg DDT was accurately weighed into a 10ml flask and made up to volume with the internal standard solution. By serial dilution a range of standard DDT solutions from 50-400ng/ml were prepared which were run at intervals during the analysis.

b)Extraction procedure - plasma samples were mixed for one minute on the vortex mixer with an equal volume 97% formic acid saturated with n-hexane. Two volumes of internal standard stock solution were added to each sample which were mixed for a further five minutes. The aqueous and organic phases were then separated by centrifugation at 3,000 rpm for five minutes. Samples of the hexane layer were either injected directly onto the column or diluted as necessary with stock internal standard solution prior to injection.

c)Efficiency of the extraction procedure - rat plasma samples (1ml) were spiked with a concentrated ethanolic solution of DDT to give final concentrations of 50, 100, 200, 300 and 400 ng/ml. The plasma was shaken overnight and six 50µl aliquots from each plasma sample were analysed to determine the percentage extraction of D.D.T.. Aliquots (3X50µl) of a plasma

sample taken by cardiac puncture from a rat dosed 24 hours previously with DDT (100mg/kg), were analysed on three different days at weekly intervals and the coefficient of variation determined.

d)Cleaning of glassware - due to the sensitivity of the assay, glassware had to be thoroughly washed to remove all traces of D.D.T.. Glassware was cleaned with acetone and water, allowed to stand overnight in aqueous Decon and then re-washed in acetone and then n-hexane using an ultra-sonic bath.

2.3.2 Preparation of Dosage Forms.

a)Oily solutions - in all experiments in which at least 1ml oil was administered the same dose of DDT (100mg/kg) was used. Oily solutions containing this dose in 2ml and in 1ml of each oil were prepared. To determine the effect of 30µl oil on DDT absorption oily solutions containing 7.5mg/kg DDT in 30µl were prepared.

b)Emulsions - oil-in-water emulsions were prepared from each oil using the Silverson blender and the QPR hand homogenisor and the same basic formula:-

Oil (containing 20mg/ml DDT)	50%^{v}/v
Tween 80	6%^{v}/v
Water	to 100%

Each rat was dosed with 2mls emulsion. Prior to administration the droplet size distribution and viscosity of the emulsion was determined using the Coulter Counter and rheometer, and the total surface area of the emulsion

calculated. Preparations containing (oil + DDT and 6% Tween 80) in a volume of 2mls were made to determine the effect of the surfactant on DDT absorption.

c)Control - a fine suspension of DDT (20mg/ml) in water was prepared by dissolving DDT in 6% Tween 80 and making up to volume with warm water. The particle size distribution of the suspension was determined using the Coulter Counter. The dose micellised in the aqueous phase was calculated by allowing the suspension to settle and then analysing an aliquot of the clear supernatant.

2.3.3 Plasma Level Determinations.

Groups (n=4 per group) of male Wistar rats, weight range 180-200g, received a single formulation cotaining D.D.T.

Immediately after oral administration of the dose each rat was restrained to allow blood to be taken from the tail tip. 1% amethocaine hydrochloride (0.05ml) was injected subcutaneously into the lower half of the tail five minutes prior to excision of the last millimetre of the tail. To encourage bleeding the tail was gently stroked along its length towards the tip. Duplicate blood samples were then collected into 50ul heparinised capillary tubes. Each capillary tube was approximately three-quarters filled and the end sealed in a flame before centrifuging. The haematocrit value of each blood sample was noted in the attempt to monitor any haemodynamic changes due to repeated sampling. The capillary tubes were broken at the plasma/red blood cell boundary and the plasma collected into Dreyer's tubes. Repeated sampling was carried out in the same manner; bleeding was re-started by wiping the

tail tip with a damp cloth. Between sampling the rats were returned to the cage containing the other rats of the group.

This general procedure was used to determine the plasma concentration of DDT in rats dosed with the following formulations:-

1. 2ml oil
2. 1ml oil
3. 2ml emulsion
4. 2ml (oil + 6% Tween 80)
5. 30ul oil
6. 1ml (water + 6% Tween 80)
7. 1ml arachis oil or Miglyol 812 orally administered two hours after intra-peritoneal injection of an aqueous solution metoclopramide (10mg/kg)
8. 1ml liquid paraffin or Miglyol 812 orally administered two hours after intra-peritoneal injection of an aqueous solution of propantheline bromide (5mg/kg).

2.4 Results.

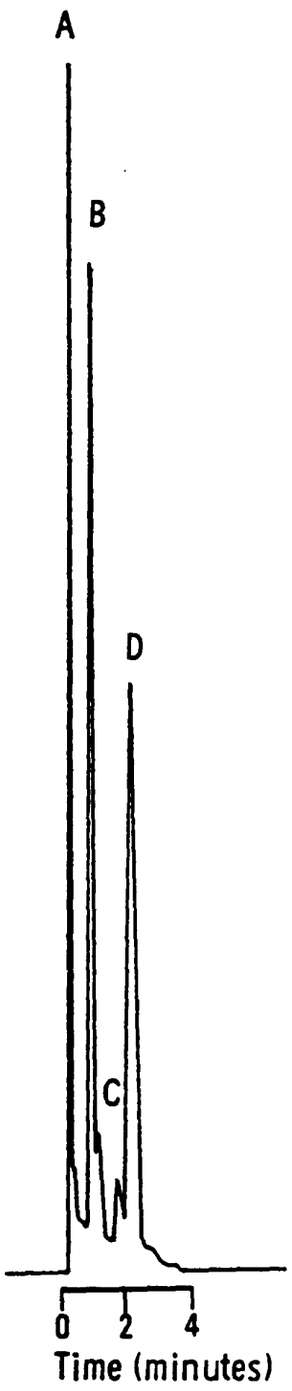
2.4.1 Assay Procedure.

The linear sensitivity range of the electron capture detector for DDT was 50-400ng/ml (see figures 2.1 & 2.2). As the response of the detector tended to vary standard solutions were run at various time intervals during the day.

The mean efficiency of the plasma extraction procedure for the range 100-400ng/ml was 90.6±2.4% (see table 2.1) with a daily coefficient of variation of 6.1%.

FIGURE 2.1

GAS CHROMATOGRAM FOLLOWING INJECTION OF EXTRACTED PLASMA SAMPLE.



A=Solvent Peak

B=Internal Standard
(Heptachlorepoxide
70 ng/ml)

C=p, p DDE

D=p, p DDT

FIGURE 2.2 REGRESSION LINE FOR STANDARD SOLUTIONS OF D.D.T. (100 - 400 ng/ml) AND FOR EXTRACTED PLASMA SAMPLES SPIKED WITH D D T.

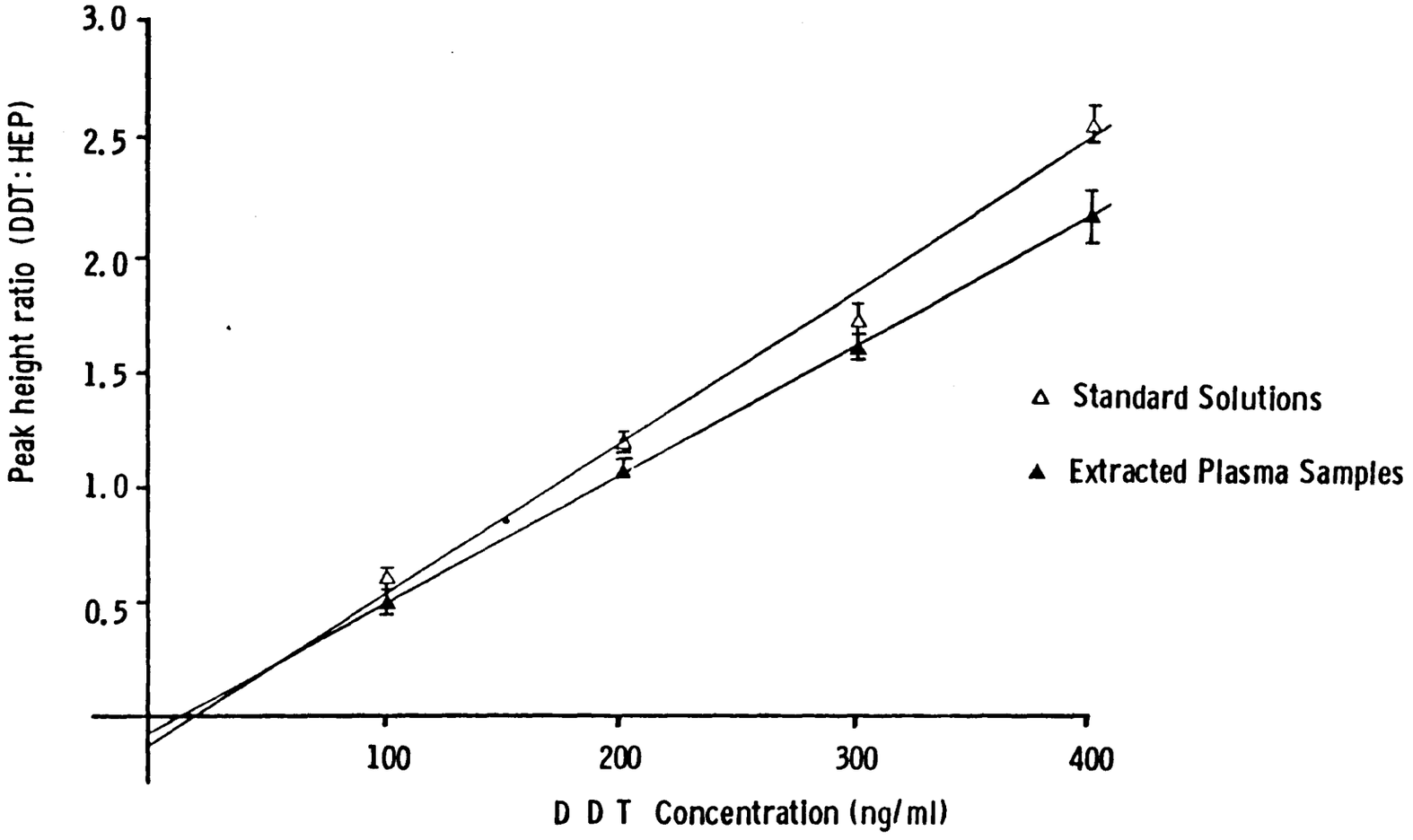


Table 2.1 -Efficiency of the Plasma Extraction Procedure for DDT Over the Concentration Range 400 to 100 ng/ml.(n=6 per sample group).

	D.D.T. concentration ng/ml	Peak height ratio (mean±S.D.)	Percentage extraction %
std.soln. plasma sample	400	2.58 ± 0.09 2.21 ± 0.09	89.0
std.soln. plasma sample	300	1.74 ± 0.08 1.62 ± 0.05	88.3
std.soln. plasma sample	200	1.18 ± 0.04 1.09 ± 0.05	91.9
std.soln. plasma sample	100	0.59 ± 0.05 0.50 ± 0.05	93.4

2.4.2 Absorption Studies.

For each dosage form the plasma DDT concentration versus time profile was constructed (see figures 2.3-2.10). From the data the maximum plasma concentration (Cp_{max}) and the time of the Cp_{max} (T_{max}) were determined, and the total drug absorption as determined from the area under the curve between 0 and 24 hours (AUC_{0-24h}), was calculated using the trapezoidal method (see table 2.2). Comparison of these parameters for the different formulations was made using the Student's"t"test or the Mann Whitney"U"test.

The droplet size distribution, total oil surface area and viscosity of the emulsions were not significantly different ($p>0.05$) (see table 2.3), therefore the differences in DDT absorption from the emulsions could not be attributed to these physical parameters.

The aqueous suspension of DDT had a particle size distribution of 5.4 ± 2.7um (geometric mean diameter $\pm$ geometric standard deviation) and only 0.32mg/ml DDT was micellised by the Tween 80 in the aqueous phase.

During the animal experimentation it was observed that certain groups of rats became hyperexcitable. Initially they over-reacted to external stimuli and then they began to shake and when excited would exhibit characteristic "shadow boxing". The severity of the symptoms was directly related to the plasma concentration of DDT.

FIGURE 2.3

THE EFFECT OF DIFFERENT VEHICLES (1 ml volumes) ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/ kg) IN RATS (Mean + S.E.M., n= 4 per group).

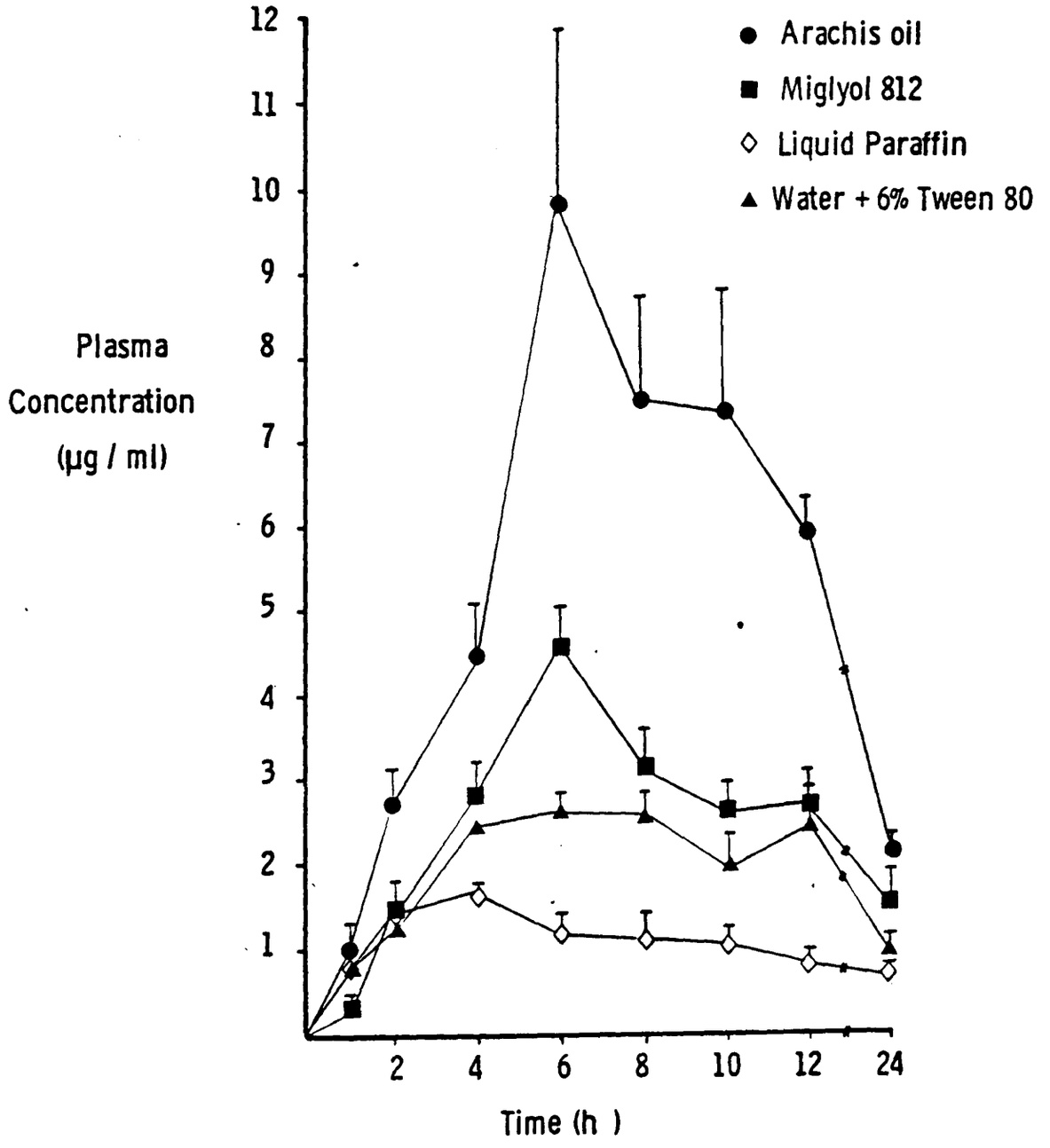


FIGURE 2.4

THE EFFECT OF DIFFERENT ARACHIS OIL FORMULATIONS ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/kg) IN RATS (Mean + S.E.M., n=4 per group).

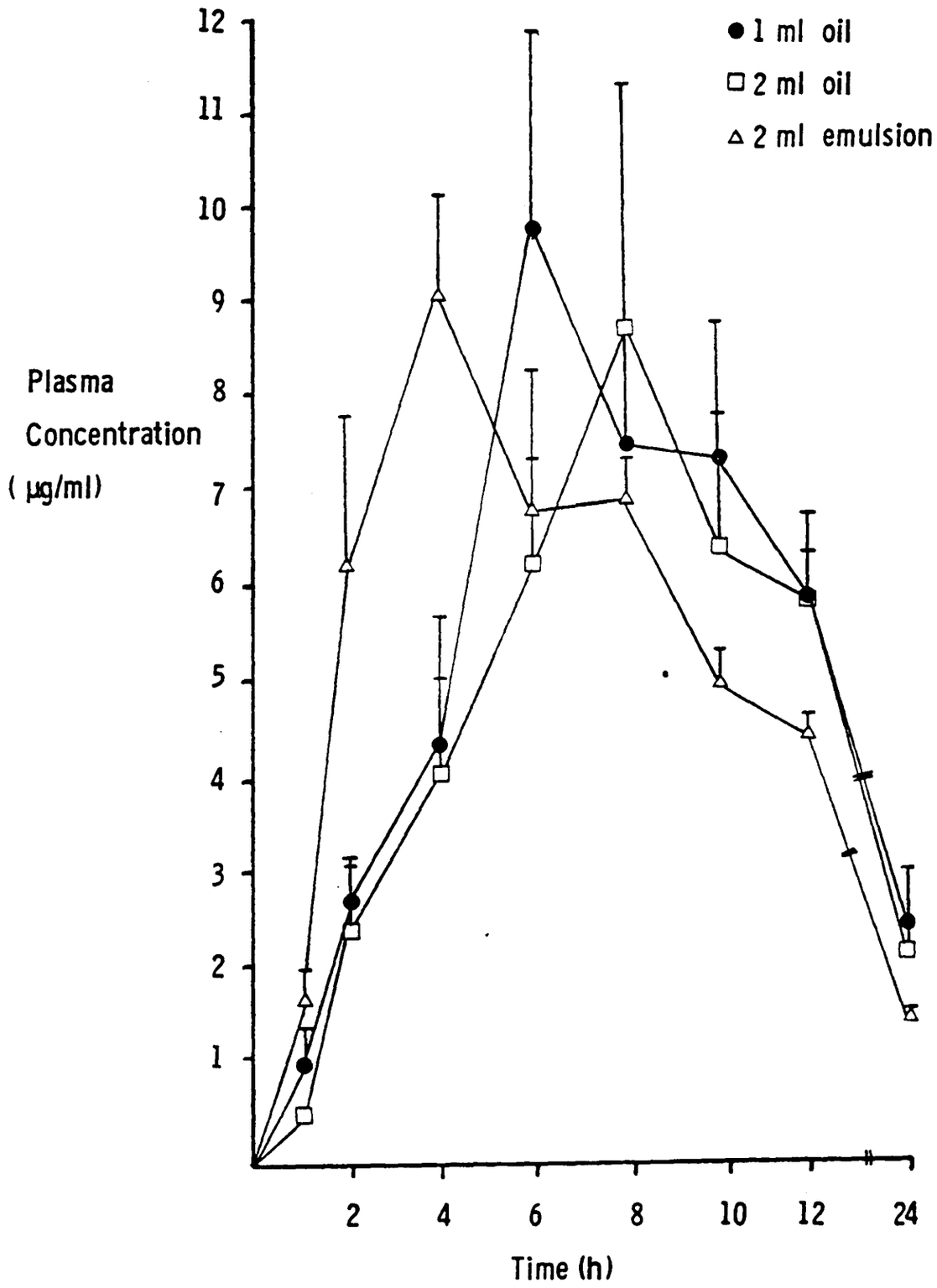


FIGURE 2.5

THE EFFECT OF DIFFERENT MIGLYOL 812 FORMULATIONS ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/kg) IN RATS (Mean + S.E.M., n= 4 per group)

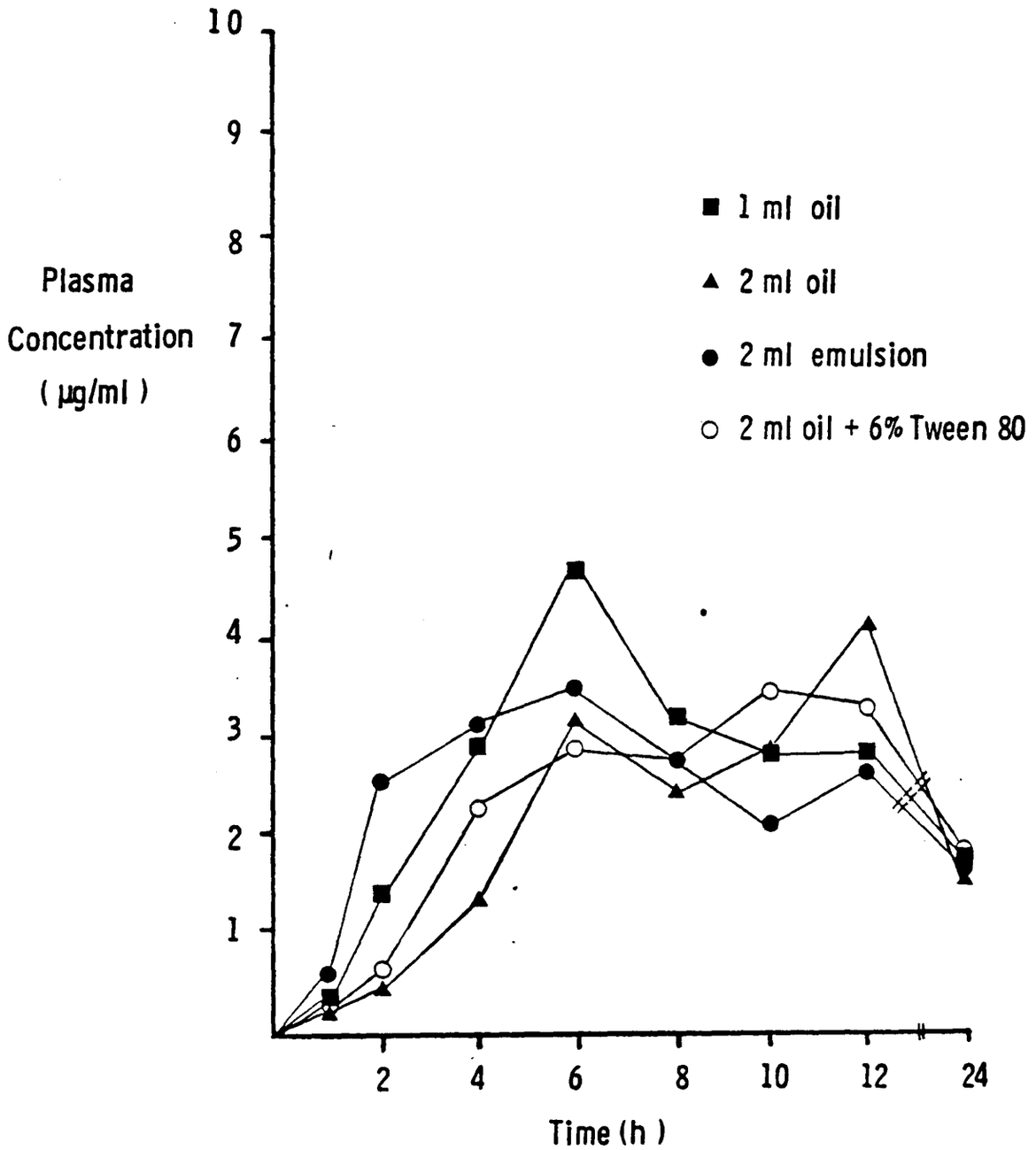


FIGURE 2.6

THE EFFECT OF DIFFERENT LIQUID PARAFFIN FORMULATIONS ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/kg) IN RATS (Mean + S.E.M., n= 4 per group)

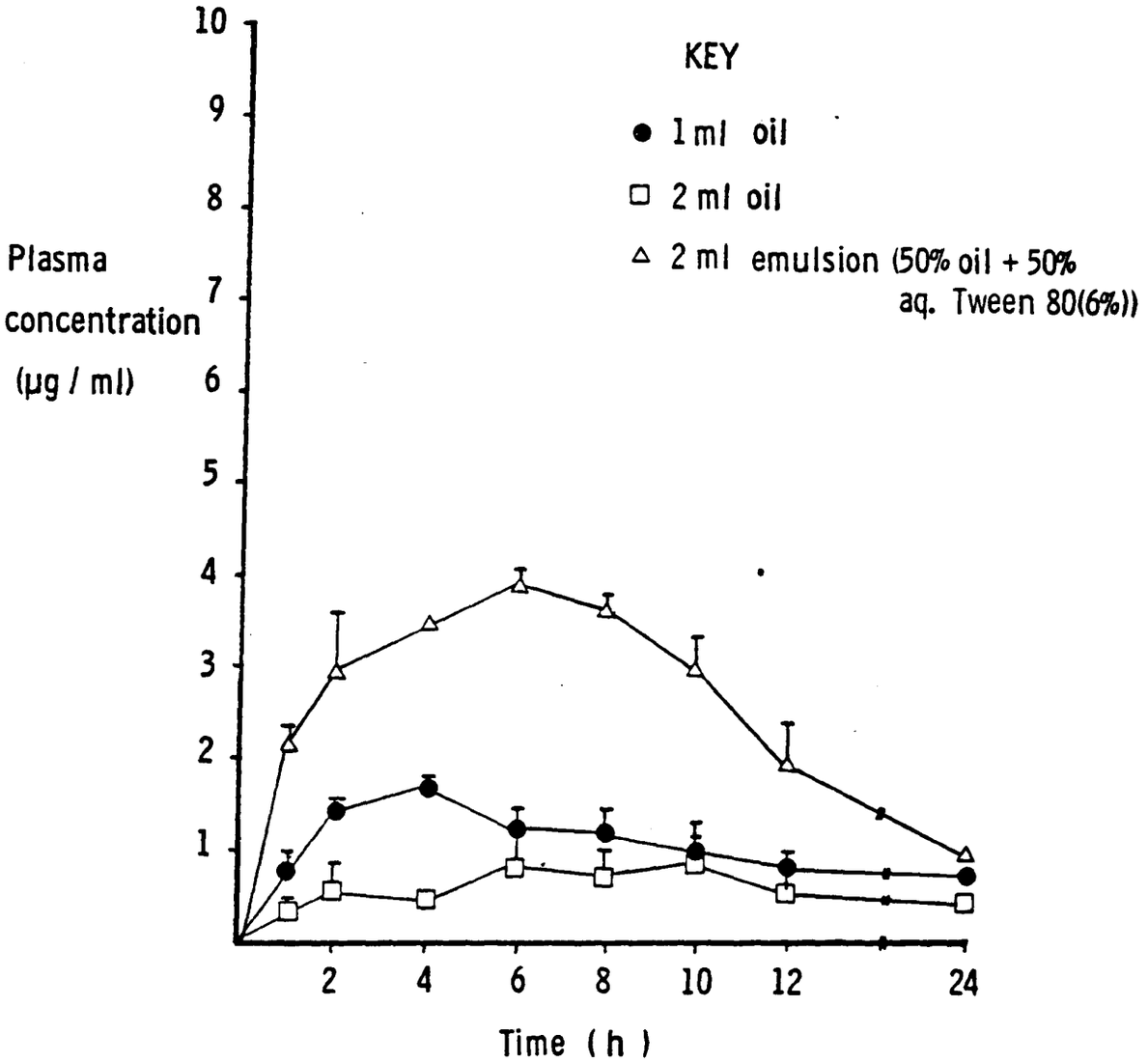


FIGURE 2.7

THE EFFECT OF DIFFERENT OILS (30 µl volumes) ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (7.5 mg/ kg) IN RATS (Mean + S.E.M., n= 4 per group).

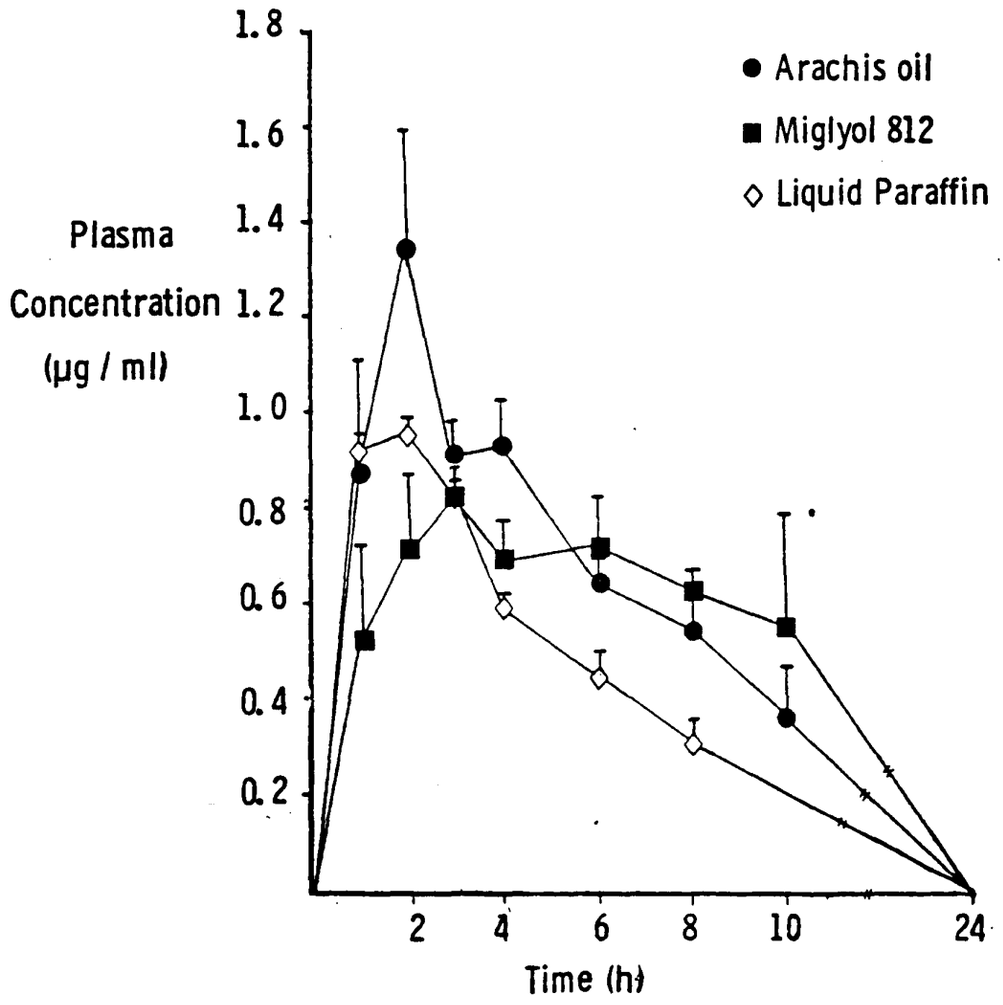


Figure 2.8

THE EFFECT OF METOCLOPRAMIDE (10mg/kg) ON THE ABSORPTION IN RATS OF ORALLY ADMINISTERED DDT (100mg/kg) IN ARACHIS OIL (1ml)

(Mean + S.E.M. n=4per group).

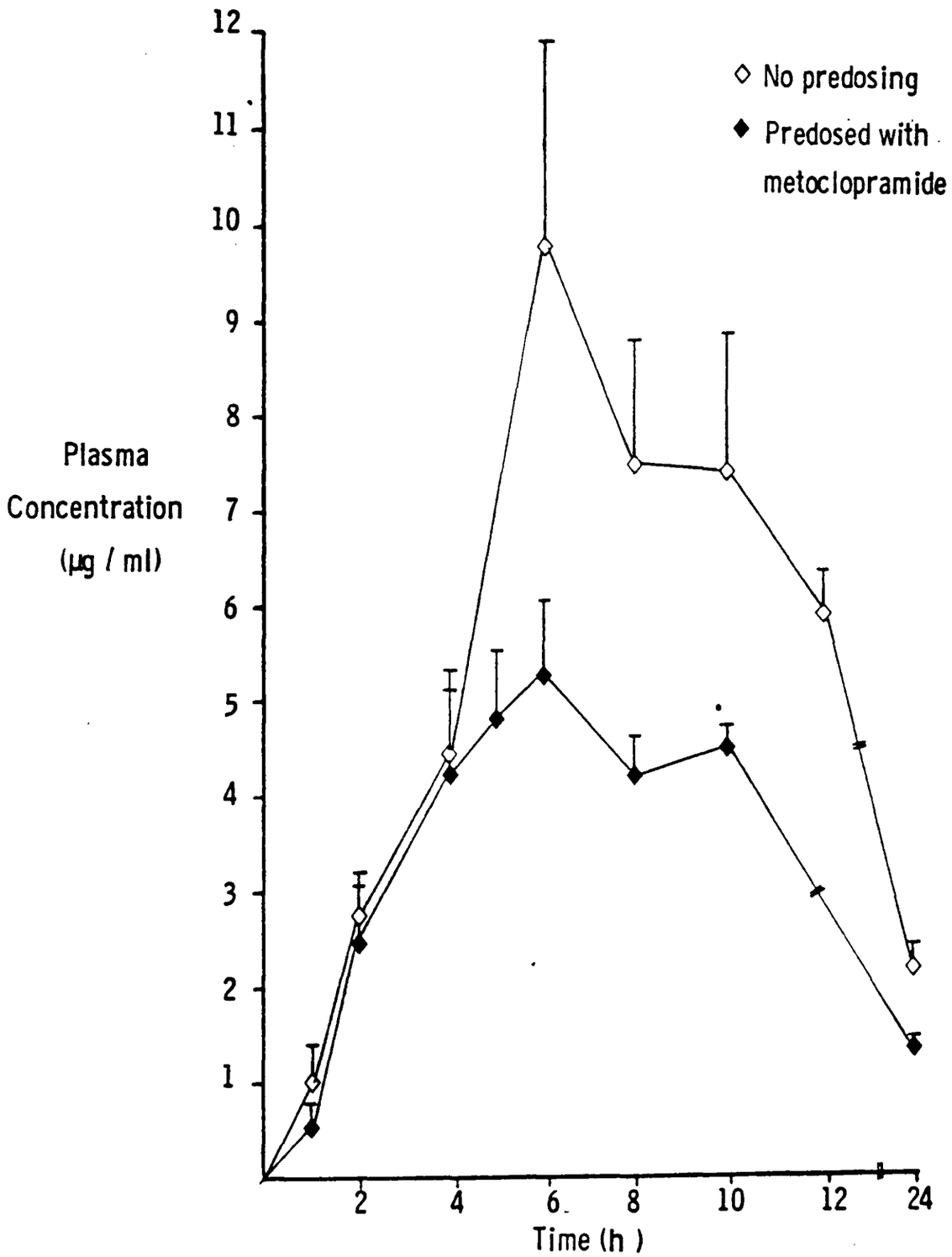


Figure 2.9

THE EFFECT OF PROPANTHELINE (5mg/kg) AND METOCLOPRAMIDE (10mg/kg) ON THE ABSORPTION IN RATS OF ORALLY ADMINISTERED DDT (100mg/kg) IN MIGLYOL 812 (1ml) (Mean + S.E.M., n=4 per group).

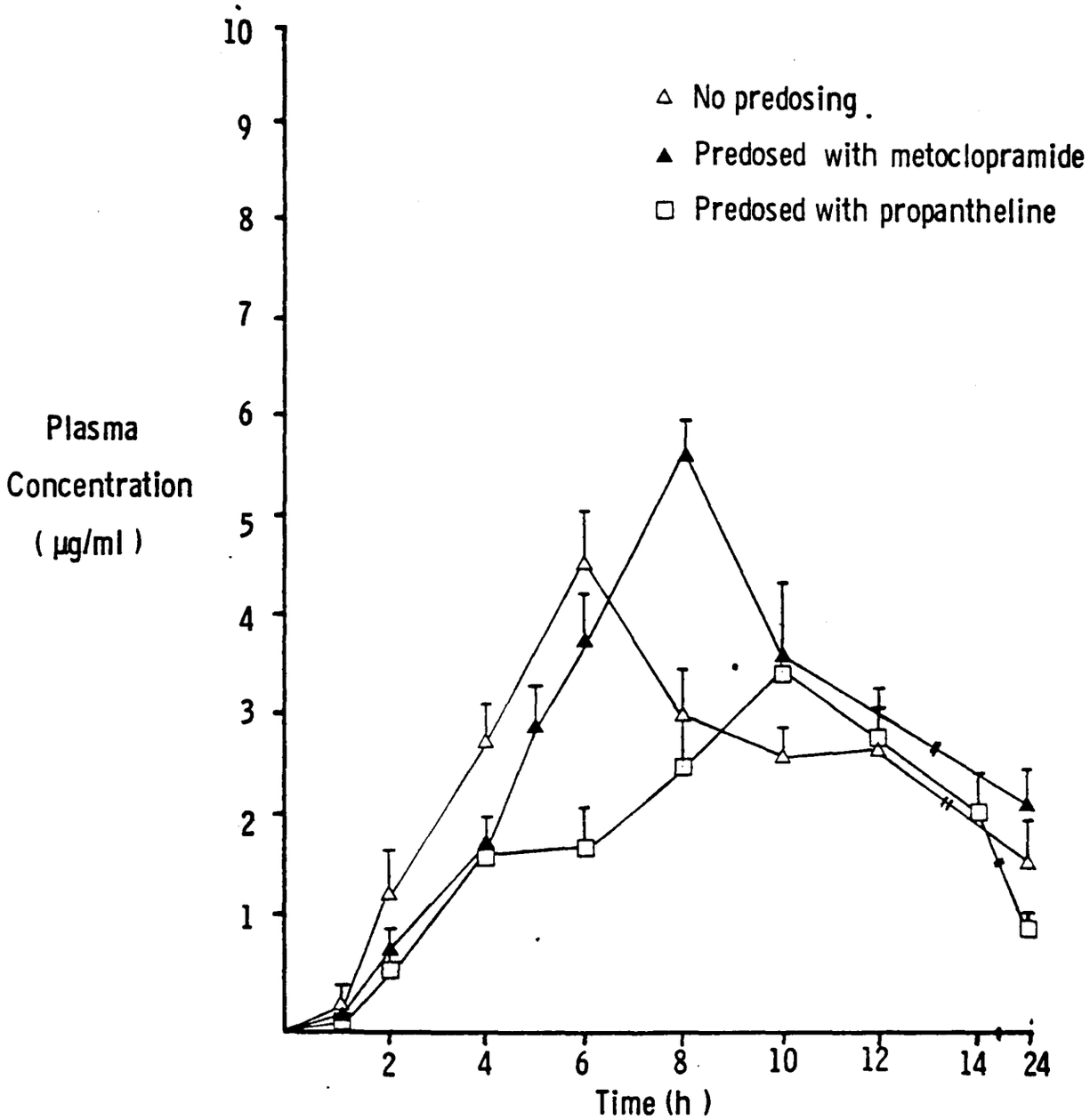


Figure 2.10

THE EFFECT OF PROPANTHELINE (5mg/kg) ON THE ABSORPTION IN RATS OF ORALLY ADMINISTERED DDT (100mg/kg) IN LIQUID PARAFFIN (1ml) (Mean + S.E.M., n=4per group).

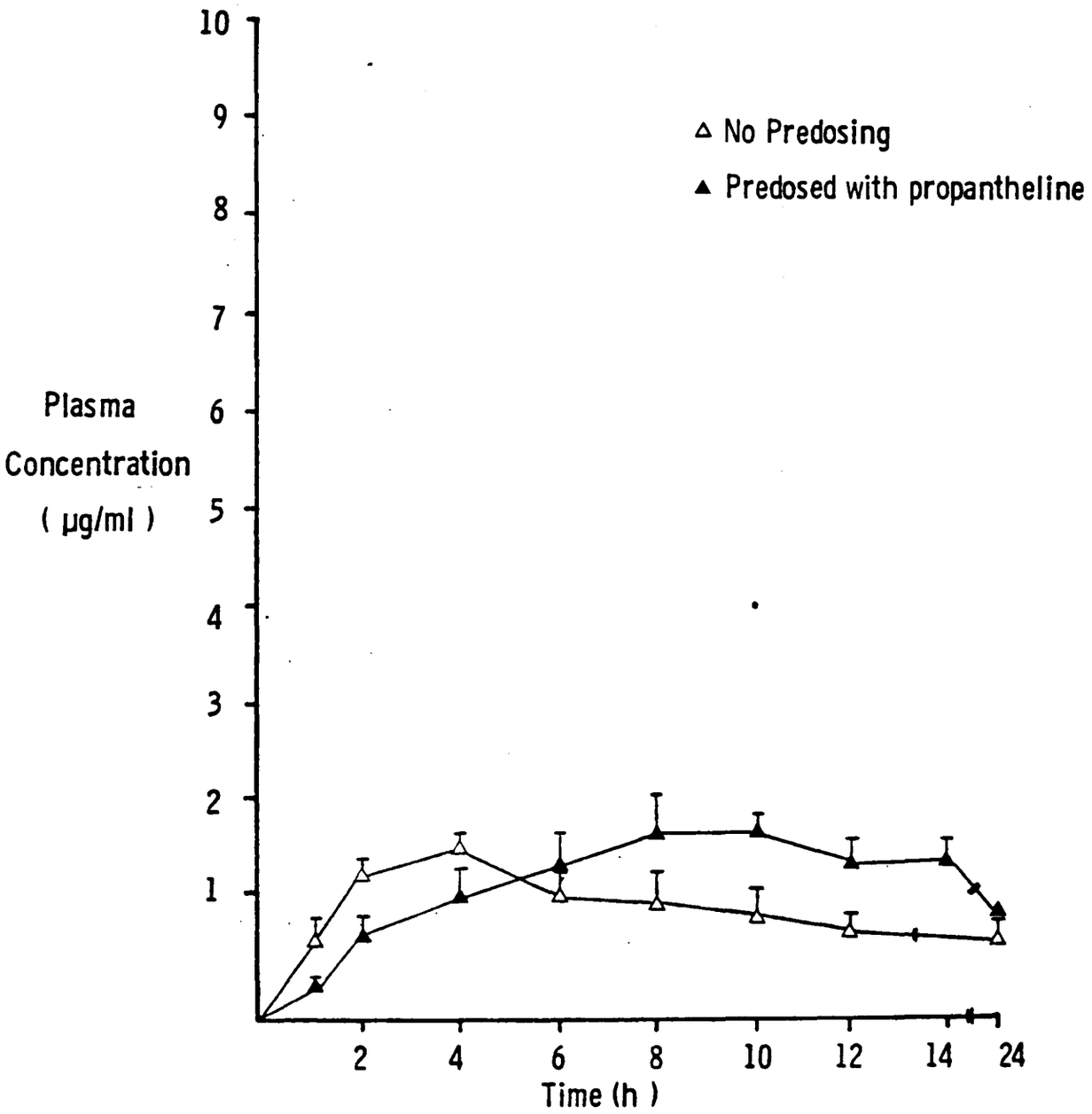


Table 2.2 - Absorption Data Following Oral Administration of DDT in Different Vehicles to Rats

(Mean ± S.D., n=4 per group).

	Arachis Oil			Miglyol 812			Liquid Paraffin		
	C_{pmax} ug/ml	T_{max} h.	AUC_{0-24h} mg ml/h.	C_{pmax} ug/ml	T_{max} h.	AUC_{0-24h} mg ml/h.	C_{pmax} ug/ml	T_{max} h.	AUC_{0-24h} mg ml/h.
1ml oil	11.08 ±1.95	7.0 ±2.0	118.3 ±6.6	4.67 ±0.74	6.0 ±0.0	57.91 ±10.22	1.72 ±0.05	4.0 ±0.0	23.90 ±4.23
2ml oil	10.24 ±3.62	10.0 ±2.3	112.17 ±14.74	4.21 ±1.88	11.5 ±1.0	54.52 ±23.61	1.39 ±0.59	6.5 ±3.4	13.25 ±7.36
2ml emulsion	9.18 ±2.07	4.0 ±0.0	105.81 ±12.54	3.61 ±0.59	5.5 ±5.5	54.52 ±3.42	4.25 ±0.42	5.0 ±2.0	54.27 ±8.20
2ml oil (containing 6% Tween 80)	9.99 ±2.52	7.0 ±1.41	112.21 ±26.11	3.67 ±0.99	9.0 ±3.46	55.97 ±19.02	1.87 ±0.38	8.0 ±1.63	27.11 ±5.25
Predosing with Propantheline	/	/	/	3.56 ±0.73	10.0 ±0.0	46.08 ±15.44	1.99 ±0.52	9.0 ±1.16	31.80 ±6.15
Predosing with Metoclopramide	5.52 ±1.58	5.75 ±0.5	76.76 ±12.23	/	/	/	/	/	/

Control C_{pmax} = 2.88±0.37ug/ml T_{max} = 10.0±2.0h. AUC_{0-24h} = 44.59±5.32mg ml/h.

Table 2.3 -Physical Properties of the Emulsion Formulations Containing DDT.(n=3)

Oil	Droplet geometric diameter (mean±S.D.)	Surface Area (m^2/ml)	Viscosity (poise)
Arachis oil	7.1 ± 2.5	0.537	0.00440
Miglyol 812	6.6 ± 1.5	0.525	0.00436
Liquid paraffin	7.0 ± 2.6	0.608	0.00410

2.5 Discussion.

The results of these studies showed clearly that the oral absorption of DDT is dependant on the nature and the formulation of the vehicle in which it is administered.

The plasma DDT concentration versus time profiles for DDT administered in 1ml volumes of arachis oil, Miglyol 812, liquid paraffin and water containing 6% Tween 80, exhibit significant differences (see figure 2.3). Whilst arachis oil promoted absorption ($p<0.05$ for Cp_{max} and AUC_{0-24h} values) liquid paraffin gave rise to a shorter absorption phase resulting in lower total drug absorption and earlier peak plasma concentrations than for the other vehicles ($p<0.05$ for T_{max} and AUC_{0-24h} values). Comparison of DDT absorption from Miglyol 812 and water showed that although a higher peak plasma level occured with Miglyol 812 ($p<0.05$) there was no difference ($p>0.05$) in total DDT absorption over 24 hours as determined from the AUC_{0-24h}. However, one hour after dosing the plasma concentration of DDT following administration in Miglyol 812 was less than after administration in any other vehicle ($p<0.05$). This difference no longer existed after 2 hours ($p>0.05$) suggesting that there is an initial time lag prior to DDT absorption from Miglyol 812.

Ideally, an aqueous solution of D.D.T. would have formed the control formulation for this set of experiments. However, as DDT is highly insoluble in water and also in 50% aqueous ethanol, which is often used in such circumstances, an aqueous suspension of DDT was used. A fine suspension suitable for

administration was formed by dissolving the DDT in Tween 80 (6%) prior to the addition of water. Although it is possible that the surfactant itself may have altered the absorption of DDT studies using oil containing 6% Tween 80 in a volume of 2mls suggested that this did not occur (see below).

Increasing the volume of the vehicle administered to 2ml changed the plasma DDT profiles for the individual oils but did not alter the differences between the oils (see Figures 2.4,2.5 & 2.6). Whereas 2ml arachis oil and Miglyol 812 only slowed the rate of absorption ($p<0.05$ for T_{max} values), with 2ml liquid paraffin the total absorption and the peak plasma concentration of DDT were also reduced ($p<0.05$ for AUC_{0-24h}, Cp_{max} and T_{max} values) compared to the 1ml volumes of oil. Thus doubling the oil volume increased the time to peak plasma concentration and, in the case of liquid paraffin, the total drug absorption and peak plasma concentration.

Administration of DDT in oil-in-water emulsions altered the absorption in different ways with each oil. With arachis oil emulsification increased the rate ($p<0.05$ for T_{max} values) but not the extent of absorption compared with 1ml and 2ml volumes of the oil (see Figure 2.4). Although with Miglyol 812 emulsification increased the initial rate of DDT absorption, the 2 hour plasma concentration being higher ($p<0.05$), the T_{max} value was unchanged (see Figure 2.5). Administering the oil as a preformed emulsion by-passes the emulsification step in the digestion process (see introduction) enabling absorption to commence more rapidly.

DDT absorption from liquid paraffin was greatly enhanced

by emulsification, the total drug absorption and peak plasma levels being higher ($p<0.05$) than for 1ml or 2ml volumes of oil (see Figure 2.6). Liquid paraffin is not readily absorbed but when it is presented to the mucosal membrane as a preformed emulsion of mean droplet diameter <0.5um it is absorbed as easily as olive oil (Frazer et al.1944,McWeeny 1957). As the mean particle size of the liquid paraffin emulsion in which DDT was administered was larger than 0.5um it is unlikely that the liquid paraffin was absorbed as easily as these authors suggest, but it is probable that absorption of the smaller emulsion droplets readily occured.

There were no differences ($p>0.05$) between the plasma DDT concentration time profiles for 2ml oil and 2ml oil containing 6% Tween 80, suggesting that Tween 80 was not exerting any influence on DDT absoption from the emulsions (see Table 2.2).

DDT was administered in 30µl volumes of oil (approximately equivalent to a 10ml dose for a 70kg man on a body weight basis) to determine whether the differences in absorption from the different oils were still observed at low oil volumes (see figure 2.7). The total absorption of DDT over 24 hours was still less ($p<0.05$) from liquid paraffin than from arachis oil or Miglyol 812 but there was no longer a difference ($p>0.05$) between the latter two oils. The peak plasma level was still higher ($p<0.05$) following administration in arachis oil than with the other two oils, but the time to peak plasma concentration was now longer with Miglyol 812 than with arachis oil or liquid paraffin. The profiles of the plasma DDT

concentration with 30µl volumes of liquid paraffin and arachis oil are similar in that there is a rapid rise in the plasma DDT levels followed by a steady fall, although absorption from arachis oil was greater, as evidenced by the higher plasma DDT concentration at any given time. With Miglyol 812 the profile was flatter the peak plasma levels being lower and achieved after a longer time period but sustained for longer. Reducing the oil volume therefore had a significant effect on DDT absorption in the presence of all three oils but maintained characteristic differences in absorption between them.

The influence of metoclopramide and propantheline bromide on the absorption of DDT from 1ml volumes of oil was investigated to determine whether gastro-intestinal motility was an influencing factor in the present studies. Metoclopramide increases gastro-intestinal motility by increasing the size of antral contractions (Johnson 1971), whereas propantheline bromide slows gastro-intestinal motility by its anticholinergic properties (Hurwitz et al.1977,Brodie 1966). Jamali and Axelson (1977) investigated the effect of intra-peritoneal doses of metoclopramide (10mg/kg) and propantheline (5mg/kg) on the gastro-intestinal absorption of griseofulvin in rats when given as an aqueous suspension in 0.5% polysorbate 80 or as a solution in polyethylene glycol 600. Metoclopramide preadministration was shown to increase the gastric emptying rate of the formulations whereas propantheline retarded it. The effect on griseofulvin absorption induced by the two drugs was dependant on the dosage form of the griseofulvin administered. Predosing with metoclopramide

increased the bioavailability of the PEG formulation but decreased the bioavailability of the griseofulvin suspension; propantheline had the opposite effect to metoclopramide in both instances. This showed that griseofulvin absorption was dependant on both the formulation and on gastro-intestinal motility. The same experimental protocol that was used for this griseofulvin study was adopted in the present investigation.

Pretreatment with metoclopramide reduced total DDT absorption and the peak plasma concentrations from 1ml arachis oil ($p<0.05$) although during the first four hours there was no difference ($p>0.05$) in the plasma levels (see Figure 2.8). This suggests that the effect of the drug on gastric emptying was insignificant compared to that of the oil but then, under influence of the drug the transit of the oil through the upper gastro-intestinal tract was increased reducing the time available for DDT absorption. However with Miglyol 812 pretreatment with metoclopramide appeared to increase DDT absorption and the lag-time prior to absorption (see Figure 2.9).

Total absorption of DDT from liquid paraffin following pretreatment with propantheline bromide was increased ($p<0.1$) as was the time to the peak plasma level ($p<0.05$) although there was no change in the actual plasma levels of D.D.T.(see Figure 2.10). This suggests that slowing the gastro-intestinal transit of liquid paraffin allowed absorption of DDT to occur over a longer time period. Figure 2.9 shows the effect of pre-treatment with propantheline on the absorption of DDT from Miglyol 812.

Whatever the underlying mechanisms these studies showed that changes in gastro-intestinal motility can alter DDT absorption from these oils. It is therefore possible that the effect of the individual oils on gastro-intestinal motility contributes to the differences in DDT absorption from the three oils.

In most studies a large variation in the plasma levels of DDT within the same group of animals was observed, and this may be attributed to slow, incomplete absorption of DDT Judah (1949) found that 72-80% of a 125mg intra-gastric dose of DDT in nut oil could be recovered from the rat gut three hours after administration. Smith & Stohlman (1945) dosed rabbits with 300mg/kg DDT in olive oil and recovered 5-50% of the dose in the faeces and in addition, they found that the relative elimination of DDT in the faeces as compared to the urine was greater with larger doses. Using male Sprague-Dawley rats orally dosed with 10mg DDT in 0.5ml peanut oil, Bishara et al.(1972) found that 40% of the dose was eliminated in the faeces in 24 hours yet only 0.9% of the same dose was excreted in rats dosed intra-peritoneally. It was concluded that a large percentage of the orally administered dose was not absorbed. DDT absorption from the gut would seem to be slow, and a relatively large fraction of an oral dose is excreted in the faeces as a result of not being absorbed or possibly biliary excretion (see below). It may be anticipated that such factors would contribute to large intra-group variation in DDT absorption such as observed in the present study.

The hyperexcitability of the rats observed during some

of the experiments is characteristic of DDT poisoning (Cameron 1945,Domenjoz 1944). Experiments by Dale et al.(1963) with rats suggested that the severity of the symptoms of poisoning after a single oral dose of DDT are directly proportional to the concentration of the compound in the brain, which in turn is in equilibrium with the plasma DDT levels (Radmoski 1970). Thus, the observed symptoms are directly related to the plasma concentration of DDT as determined in the present experiment. The pharmacological action of DDT on the brain does not seem to be clearly understood, although it is thought to be due to a reduction in brain acetylcholine (Martin & Nigar 1979,Judah 1949,Colhourn 1959,St.Omer & Ecobichon 1971).

Pharmacokinetic analysis of the plasma profiles of DDT was not attempted as it was difficult to determine the most suitable compartmental model to apply. Semilogarithmic plots of the plasma DDT concentration versus time for arachis oil suggested that a one compartment model was applicable, whereas the plots for liquid paraffin suggested that a two compartment model was more suitable. As DDT accumulates in the adipose tissue (Gillette 1973) a two compartment open model is possibly the more suitable. However, the plasma profiles for DDT administration in Miglyol 812 and to a lesser extent in arachis oil, exhibited two peak plasma levels as is characteristic of biliary recycling. High concentrations of DDT in the bile have been found in rats killed after acute oral dosing with DDT (Smith 1944,1945). More recently Jensen et al.(1957) have demonstrated small quantities of DDT present in the bile and shown that biliary excretion is responsible for almost all of

the DDT metabolites in faeces. The pharmacokinetic picture for DDT following oral administration to rats is therefore complex and there was insufficient data from the present study to attempt any such analysis.

An increase in the height of peak C (see Figure 2.1) was observed in the chromatograms for sequential plasma samples following dosing of D D T. Further investigation showed that peak C had similar chromatographic properties to p,pD D E , 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene, which is known to be a major metabolite of p,pD D T.

The present studies have shown that the vehicle in which DDT is orally administered is critical in determining the oral toxicity of the compound. In addition it was shown that altering gastro-intestinal motility, using the drugs metoclopramide and propantheline bromide, alters the plasma levels of D D T. The lymphatic absorption of DDT in the presence of arachis oil, Miglyol 812 and liquid paraffin was investigated to determine whether this factor was contributing to the observed differences in oral DDT absorption.

CHAPTER 3.

LYMPHATIC ABSORPTION OF DDT.

3.1 Introduction.

From literature reports it was anticipated that the partitioning of DDT between the portal and lymphatic absorption pathways could be affected by the presence of lipids of different chemical structure (see sections 1.5 and 2.1). The lymphatic absorption of DDT in the presence of Miglyol 812, arachis oil and liquid paraffin was therefore investigated to determine whether this was a factor in the differences observed in the plasma DDT profiles following administration in the three oils.

3.1.1 Investigative Techniques.

Investigations into the role of the lymphatic system in the transport of absorbed material from the intestine are dependant on cannulation of the lymph vessels. It is preferable to cannulate the main intestinal lymph vessel, tying off the accessory trunk which lies close to it, but as this is a difficult surgical procedure the thoracic duct is often cannulated immediately above the cisternae chyli. In small animals such as the rat, it may be difficult to be certain whether or not the liver lymph is included in this drainage and therefore whether metabolic derivatives of the test substance are being sampled. Mezick et al.(1968) whilst investigating the lymphatic transport of ^{14}C-menadione (vitamin K) in the dog, determined the contribution of hepatic lymph to the thoracic

duct lymph. They found that in almost every case all the radioactivity in the thoracic duct lymph represented transport from the intestine, total recovery in the thoracic lymph over 90 minutes being unchanged when the hepatic lymphatics were ligated. The hepatic lymph transport was only important during the initial period of intestinal absorption and transport; the initial lymphatic transport being decreased in animals with closed hepatic lymphatics. Noguchi et al.(1977) suggested that the concentration of compounds absorbed via the intestinal lymphatics is always lower in the thoracic lymph than in mesenteric lymph due to lymph drainage from other vessels into the cisternae chyli.

In recent years the availability of fine flexible plastic tubing has made possible the preparation of chronic lymphatic fistulae in conscious animals. Prior to this fine glass cannulae were used which only allowed acute studies in anaesthetised animals. Virtually all the studies that have been conducted to determine the contribution of the intestinal lymph to the absorption of compounds from the gastro-intestinal tract have used the chronic cannnulation procedure described by Bollman et al.(1948), for either the thoracic lymph duct or small intestinal lymph vessel. Slight modifications to this procedure have been descibed by Warshaw (1972) and Gallo-Torres & Muller (1969b). There are several major problems associated with this method;

a)Securing the cannula.

b)Clot formation within the cannula.

c)Metabolic disturbances due to removal of body fluid.

d)Opening of lymphatico-venous anastomoses.

It is difficult to secure the cannula in position in the thoracic duct so that there is no leakage of lymph and its position is not disturbed by animal movement. Initially the cannula was tied in position by means of a ligature around the duct but this is a difficult surgical procedure and more recently biological glues such as cyanoacrylic cements, have been used to glue the cannula in place.

Due to the low lymph flow rate there is a tendancy for clots to form within the cannula either hindering or stopping further flow. This can sometimes be avoided by heparinisation of the cannula prior to use and by the addition of heparin to the infusion fluid.

Metabolic disturbances may be caused by drainage of lymph over long periods of time, resulting in loss of body fluid and plasma proteins and phospholipids. Body fluid may be replenished by intra-venous infusion of a suitable buffer solution.

It is likely that the communications which exist between intestinal lymph vessels and the portal veins are not normally functionary but exist as potential routes which open when subjected to increased lymphatic pressure such as may occur when a cannula is partially obstructed, or when lymph flow is particularly high. Therefore, when investigations are being made to define the relative importance of portal venous and lymphatic routes in the transport of absorbed material from the intestine, the presence of these lymphatico-venous anastomoses has to be considered.

In an attempt to overcome the latter two problems Giradet and Beninghoff (1977) developed two alternative procedures to allow continuous circulation of lymph within the body whilst removing small samples as required, these are the thoracic duct to duct shunt and the t-tube-side thoracic duct fistula, for use in rat or man.

In the present study the lymph levels of DDT were determined in rats following intra-gastric administration of DDT (100mg/kg) in 1ml arachis oil, Miglyol 812 or liquid paraffin. Owing to the technical problems associated with recovery operations with thoracic duct cannulated rats, lymph was collected from anaesthetised animals. Corresponding lymph flow and plasma DDT levels were determined.

3.2 Marterials and Apparatus.

a)Sugical implements - forceps, coarse scissors, fine scissors, iris scissors (J.Weiss & Son Ltd., 11, Wigmore St., London) Spencer-Wells forceps, glass disectors (home-made by drawing out, bending and fire polishing 1.5mm glass rod), surgical needle (1/2 circular triangular pointed no.15, Aseptic, Leeds), trochar (able to accomodate PP50 tubing), sterile gauze, cotton buds, iris retractor (Weiss, modified to take an extra rounded flange 13mm wide by 17mm depth on one side, dimensions 70mm for each arm, 22mm across the base, 36mm between the tips when relaxed).

b)Cannulas - i)thoracic duct- Portex poythene tubing (PP50) about 50cm bent into a 12-14mm diameter semicircle at one end and with that end bevelled at about 45^0. Portex Ltd., Hythe, Kent.

ii)jugular vein- Portex flex nylon 00 tubing.

c)Slow infusion pump - Scientic Research Instruments Ltd., Croydon, Surrey.

d)Dulbecco's A+B solution - Oxoid Ltd.,Basingstoke, Hants.

e)Heparinised capillary tubes - Gelman Hawksley Ltd., Brackmills, Northants.

f)Dreyer's tubes - R.B.Radleys&Co.Ltd., Sawbridgeworth, Herts.

g)Miglyol 812- Dynamit Nobel, Slough, Berks.

h)Arachis oil B.P. - Evans Ltd., Liverpool.

i)Liquid paraffin B.P.- Shell Ltd., London.

k)p,p D.D.T.-Aldrich Chemicals Co.Ltd., Gillingham, Dorset.

3.3 Methods.

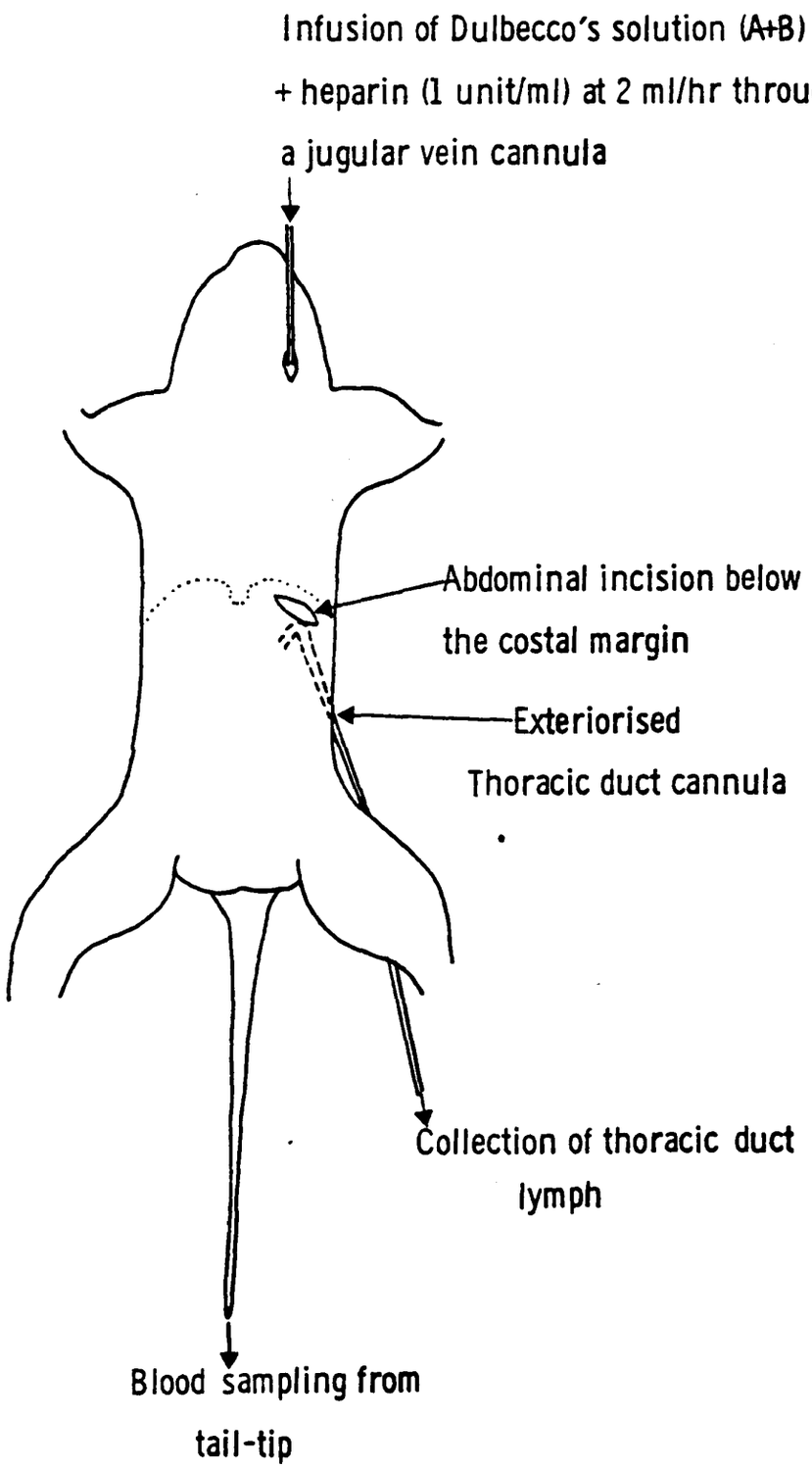
3.3.1 Thoracic Duct Cannulation.

(The experimental procedure is represented diagramatically in figure 3.1).

Each rat (200g male Wistar) was orally dosed with 1ml or 30µl oil containing 20mg or 1.5mg DDT respectively. Rats dosed with 1ml oil volumes were then left for 45 minutes before proceeding. This allowed time for the oil to be swallowed and to begin emptying from the stomach. Each rat was anaesthetised with pentobarbital sodium (Sagatal 90mg/ml) approximately 0.3ml

FIGURE 3.1

CANNULATION OF THE RAT THORACIC DUCT (Acute Preparation).



intra-peritoneally, and a maintainance dose of 15mg/kg was administered intra-peritoneally at approximately half hourly intervals to maintain the animal in a state of deep anaesthesia throughout the experiment. The left jugular vein was cannulated at the level of the anterior and posterior facial branches and Dulbecco's solution (A+B) containing heparin (1 unit/ml) was infused at a rate of 2ml/hr to restore the body fluid loss due to drainage of the thoracic duct lymph. The abdominal thoracic duct was cannulated immediately above the cisternae chyli using the method described by Ford & Hunt (1967). As the animal remained anaesthetised the incision was not closed merely covered with saline moistened gauze. Lymph was collected continuously from the cannula through heparinised capillary tubes into Dreyer's tubes. Samples of lymph were taken for analysis at half-hourly intervals between 2 and 6 hours after dosing and the lymph flow rate was determined by weighing the lymph collected. Tail tip blood samples were taken at 2, 2.5, 3, 3.5, 4, 5 and 6 hours after dosing. Lymph and plasma levels of DDT were determined by G.L.C. analysis. The normal lymph flow in anaesthetised animals under these conditions was determined using the above procedure with undosed animals.

3.3.2 Plasma Levels in Anaesthetised Rats.

Rats were orally dosed with 1ml oil containing DDT (20mg) and then left for 45 minutes before being anaesthetised as in the previous section.. An abdominal incision was made and the organs overlying the thoracic duct were packed to one side as in the cannulation procedure. The state of anaesthesia was maintained for six hours after dosing. Tail tip blood samples

were taken at 2, 2.5, 3, 4, 5 and 6 hours after dosing and the plasma levels of DDT determined by G.L.C. analysis.

3.4 Results.

Statistical comparison of parameters was made using the Student's "t" test or the Mann Whitney "U" test.

Insufficient DDT was absorbed from the 30µl oil volumes to be accurately detected in either the lymph or plasma samples. A comparison of the different formulations could not therefore be made.

Anaesthesia and "sham" operation procedure significantly ($p<0.05$) reduced plasma DDT levels in rats dosed with 1ml arachis oil and Miglyol 812 at the three data points available for comparison with unanaesthetised rats i.e. 2, 4 and 6 hours after dosing (see figures 3.2 & 3.3). However in those rats dosed with D.D.T.in liquid paraffin, the reduction was only significant ($p<0.05$) 4 hours after dosing (see figure 3.2 & 3.3).

The plasma DDT levels in anaesthetised rats dosed with Miglyol 812 rose steadily from 0.43 ± 0.02ug/ml at 2 hours to 1.76 ± 0.08ug/ml at the end of the experimental period. This reflected the steady increase in lymph flow from 0.36 ± 0.04 ml/hr to 0.67 ± 0.07 ml/hr, that was observed over the same time period in the thoracic duct cannulated rats, a characteristic which was not observed following dosing with arachis oil or liquid paraffin. In the former case, the lymph flow remained

Figure 3.2

THE EFFECT OF DIFFERENT VEHICLES (1ml volumes) ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100mg/kg) IN UNANAESTHETISED RATS (Mean + S.E.M. n= 4 per group).

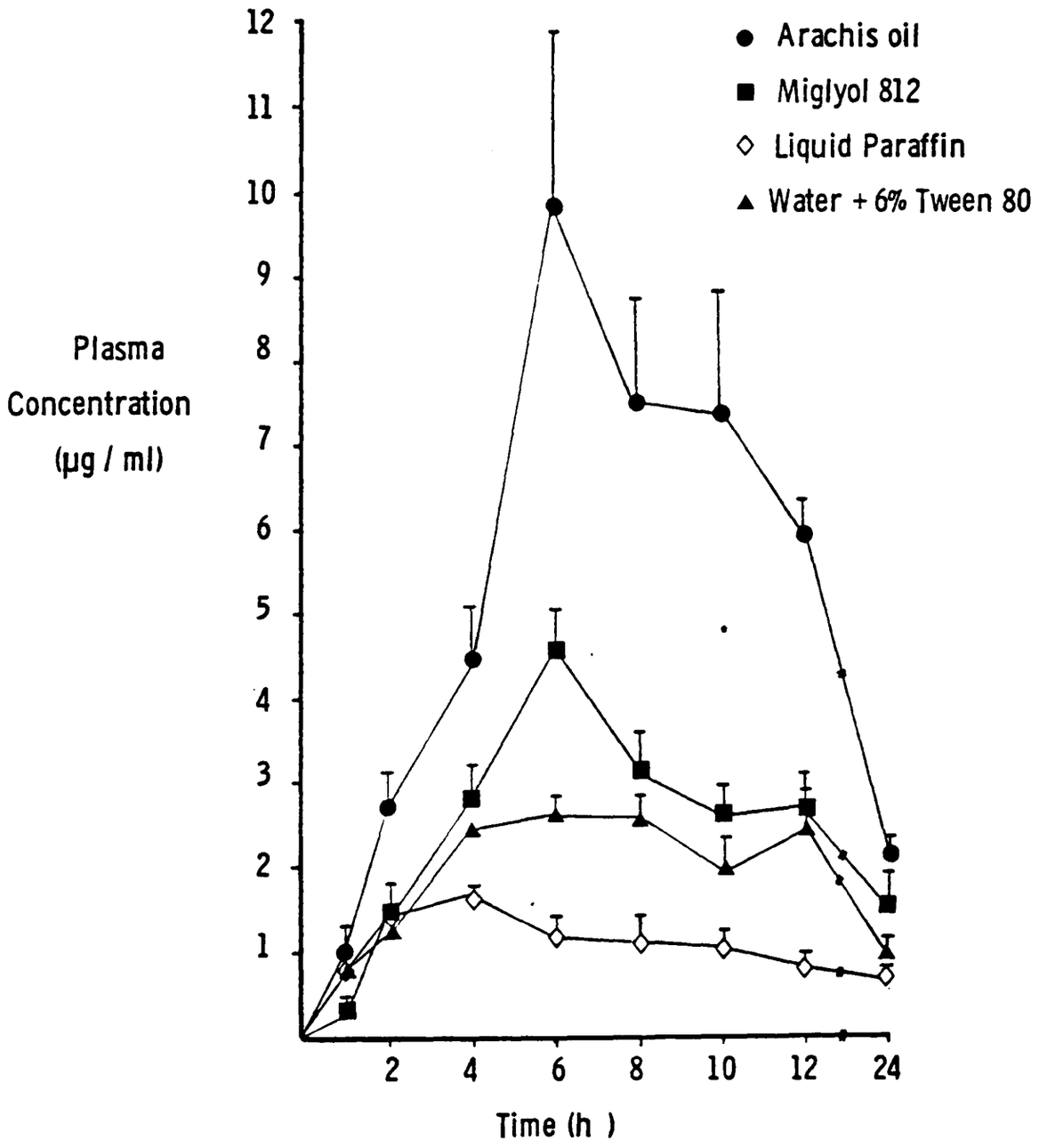
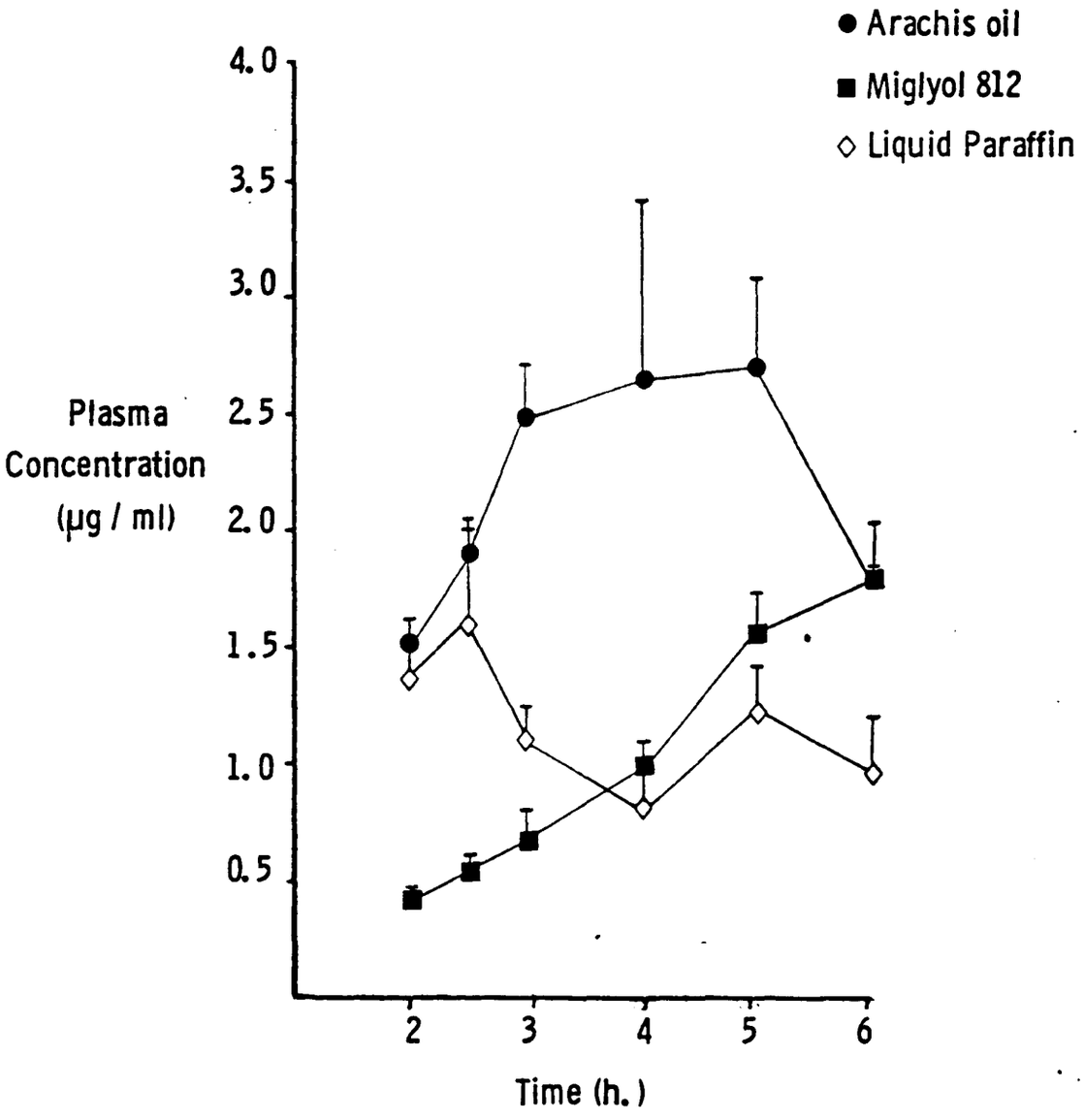


FIGURE 3.3

THE EFFECT OF DIFFERENT OILS (1 ml volumes) ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/kg) IN ANAESTHETISED RATS (Mean + S.E.M., n= 4 per group)



relatively constant at approximately 1.0ml/hr until it began to fall 4 hours after dosing, and in the latter case although the lymph flow was slightly less and more erratic, it was not different (p 0.05) to that found with arachis oil. The lymph flow in the control group ranged from 0.58±0.06ml/hr initially to 0.65±0.067ml/hr 4 hours later (see figure 3.4).

In each experiment the plasma level of DDT following thoracic duct cannulation was less than 1µg/ml (see figure 3.5). These levels are virtually negligble in comparison to the corresponding concentrations of DDT in the lymph. This was also observed in those rats dosed with 30µl oil volumes in which sufficient DDT was absorbed to be detected.

To show the comparative effects of the different oils on DDT uptake into the lymph it was necessary to allow for the differences in lymph flow in each rat caused by the different oils. Thus, the concentration of DDT (µg/hr) at the thoracic duct end of the cannula was calculated from the flow rate, the volume of the cannula and the DDT concentration in the lymph, and plotted against time. By interpolation from this graph the concentration of DDT (µg/hr) at the same time intervals after dosing with 1ml oil volumes for each rat was determined, and the mean and standard deviation for each group calculated (see figure 3.6).

Oral administration of DDT dissolved in 1ml arachis oil gave higher ($p<0.05$) peak concentrations of DDT in the lymph than in either Miglyol 812 or liquid paraffin. There was no difference ($p>0.05$) between the DDT concentrations in the lymph following administration in these two oils. However the time to

FIGURE 3.4

THORACIC DUCT LYMPH FLOW IN ANAESTHETISED RATS FOLLOWING ORAL ADMINISTRATION OF DIFFERENT OILS (1 ml volumes) CONTAINING DDT (100 mg/kg)

(Mean + S.E.M., n= 4 per group)

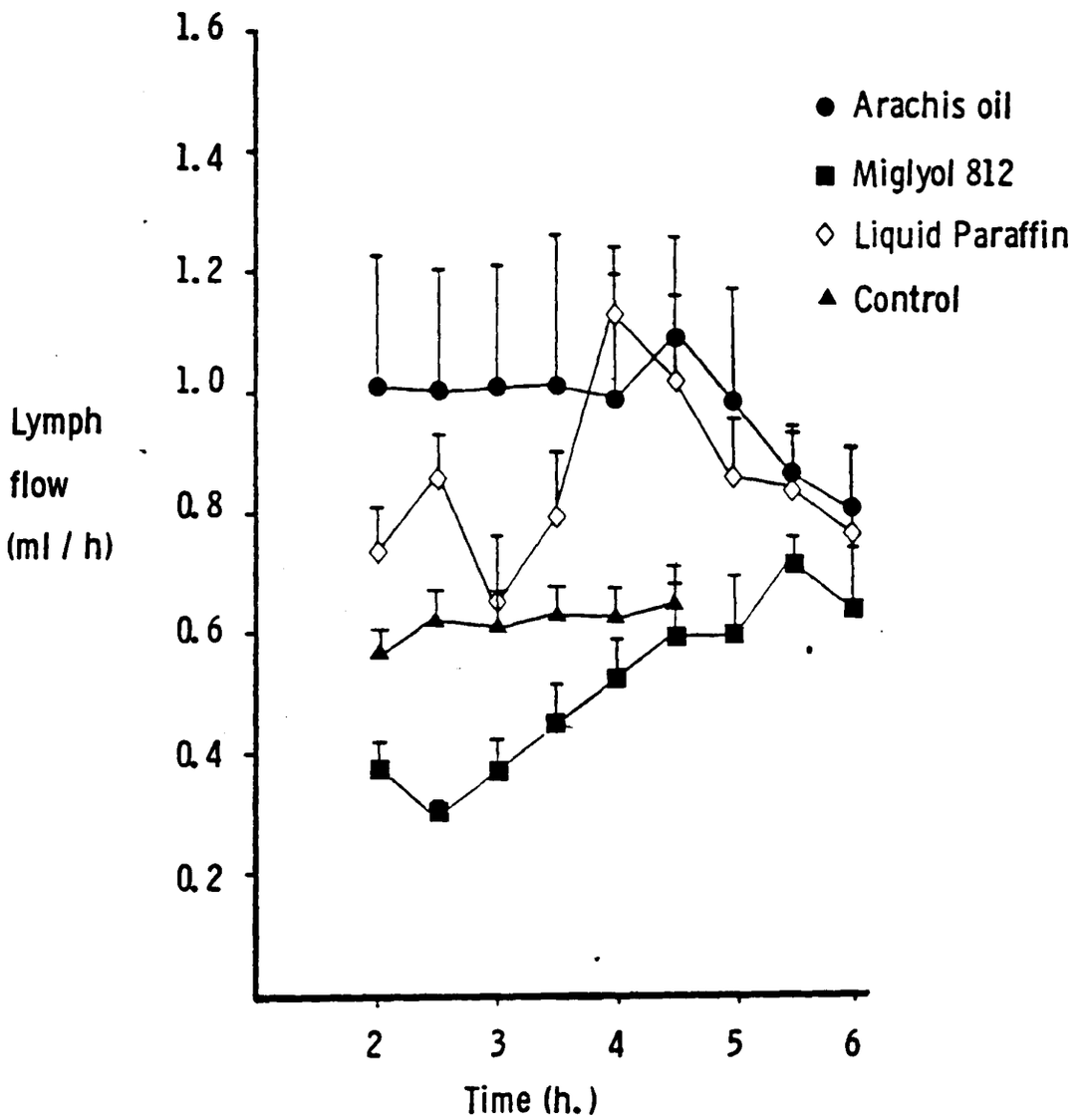


FIGURE 3.5

THE EFFECT OF DIFFERENT OILS (1 ml volumes) ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/kg) INTO LYMPH AND INTO PLASMA IN ANAESTHETISED RATS (Mean + S.E.M., n= 4 per group)

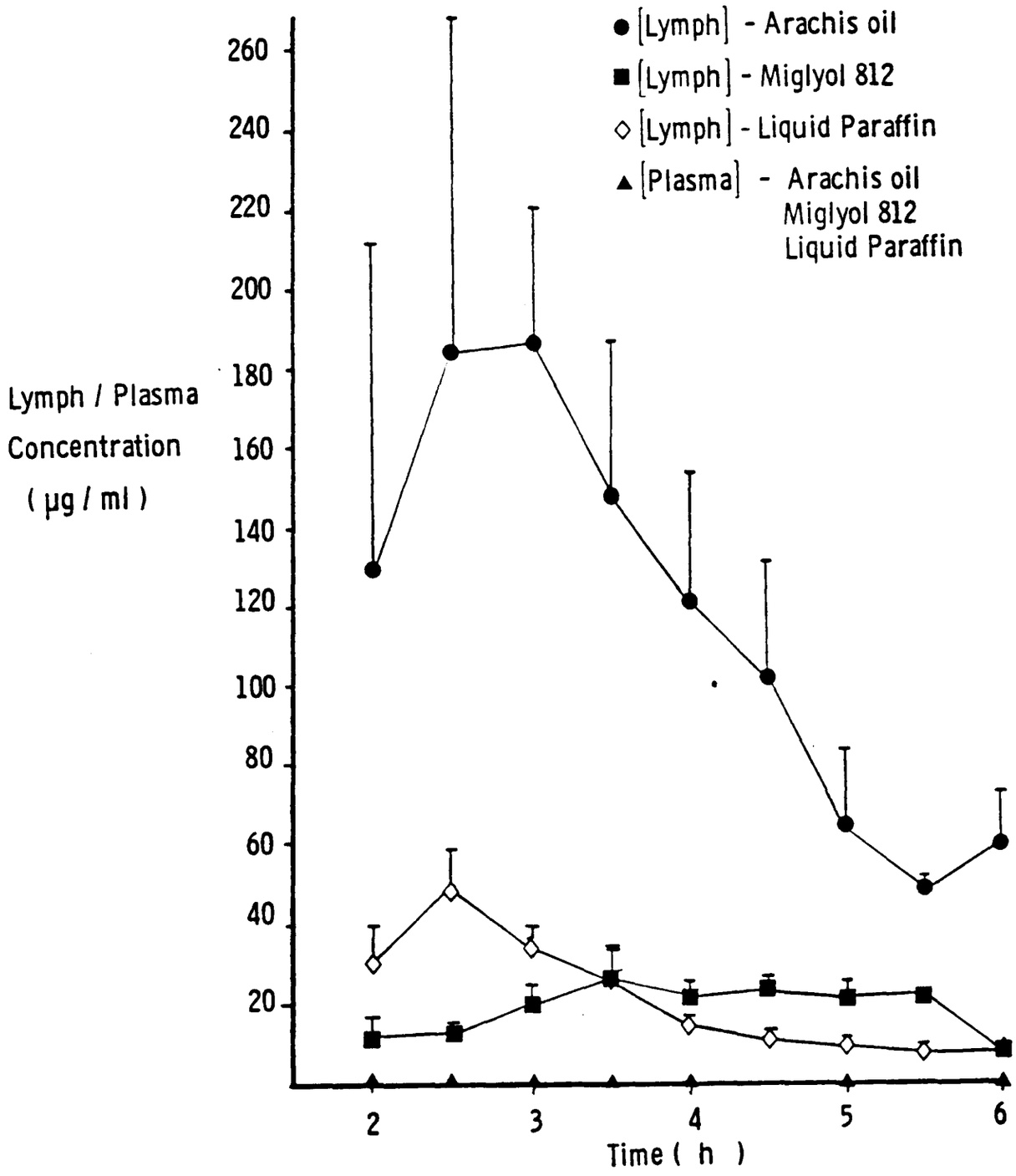
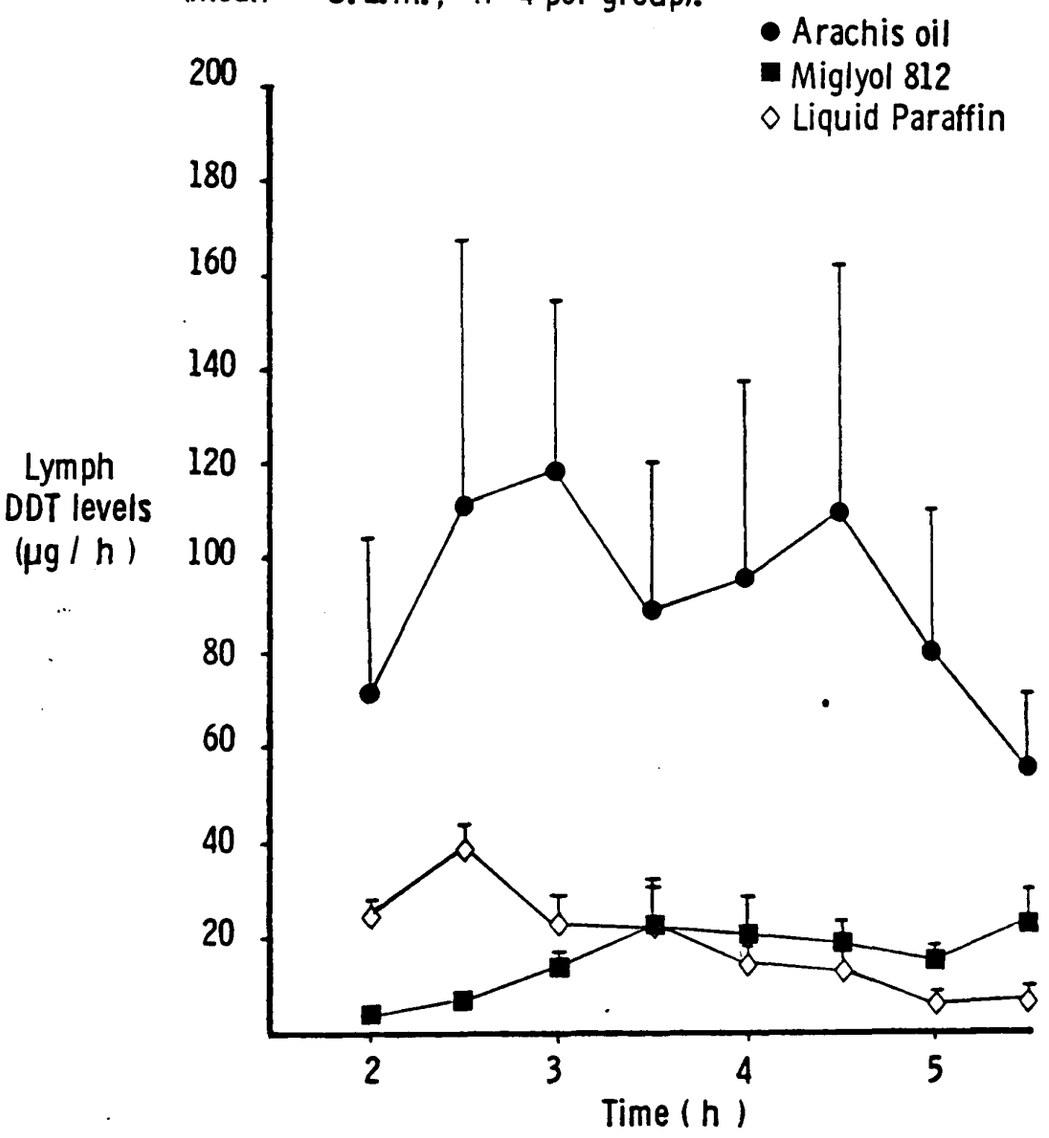


FIGURE 3.6

TOTAL THORACIC DUCT LYMPH OUTPUT OF DDT IN ANAESTHETISED RATS FOLLOWING ORAL ADMINISTRATION OF DDT (100 mg/ kg) IN DIFFERENT OILS (1 ml volumes) (Mean + S.E.M., n= 4 per group).



reach the peak concentration was faster ($p<0.05$) following administration in liquid paraffin than in either Miglyol 812 or arachis oil (see figure 3.6).

3.5 Discussion.

The data in this set of experiments were obtained using anaesthetised animals in order to minimise the technical procedures required. It is necessary to consider the effect the anaesthesia may have had on the physiological mechanisms being investigated.

As pentobarbitone anaesthesia is known to reduce blood flow in the splanchnic bed and reduce gut motility (Lee 1965,Green 1979), it was anticipated that drug absorption in the anaesthetised animals would be different to that in the conscious animals. This was shown to be true in those rats dosed with DDT in 1ml arachis oil or Miglyol 812, the plasma levels being higher ($p<0.05$) in the unanaesthetised animals (see figures 3.1 & 3.2). However, in rats dosed with DDT in liquid paraffin the plasma levels were only higher ($p<0.05$) in the conscious animals 4 hours after dosing (see figures 3.1 & 3.2). It is likely that slower intestinal transit of liquid paraffin in anaesthetised rats allowed more time for DDT absorption, thereby compensating for reduced absorption resulting from other physiological changes.

General anaesthesia has also been shown to alter lymph flow and composition, with each anaesthetic having its own

particular effect (Lowrimore 1977). Polderman et al.(1943) investigated the effect of ether and pentobarbital sodium on lymph flow in mongrel dogs by cannulation of the cervical lymphatics. Barbiturate anaesthesia was found to reduce flow by 50% from that under local anaesthesia, with an associated rise in lymph protein concentration, whereas ether anaesthesia had the reverse effect. It was suggested that the barbiturate caused a shift of fluid from the tissue to the blood stream and that the reverse occured with ether. These findings were confirmed by Hungerford and Reinhardt (1950) whilst investigating the effects of sodium pentobarbital and ether on the flow rate and cell content of rat thoracic duct lymph. Lee (1965) investigated the effect of a number of drugs on intestinal motility and lymph flow using in vitro rat intestine preparations, and found that sodium pentobarbital (15mg/100ml) abolished motility and produced a sustained reduction in lymph flow (49%) with no observable changes in lymph contractility.

Evidence from this literature suggests that low lymph flow rates could be expected in the present study since sodium pentobarbitone (Sagatal) was the anaesthetic agent used. Turner and Barrowman (1977) found that in conscious rats intra-duodenally dosed with 0.5ml LCT (olive oil) and 0.5ml 0.9% saline the lymph flow increased from a resting rate of approximately 1.3ml/hr to a peak flow rate of 5.1ml/hr after 40-80 minutes. Similarly in rats intra-duodenally dosed with 0.5ml MCT and 0.5ml 0.9% saline, the lymph flow rose to a peak of 3.5±0.3ml/hr over the same time period. Experimental peak lymph flows of 1.2±0.29ml/hr after 4.5 hr and 0.73±0.07ml/hr

after 5.5 hr were recorded following dosing with 1ml arachis oil and Miglyol 812 respectively (see figue 3.4). The delay in the time of peak lymph flow rate compared to the literature data will be due in part to intra-gastric and not intra-duodenal administration of the oil, and in part to the anaesthesia reducing gut motility. The experimental lymph flow rates were, as anticipated, lower than in the conscious animal but were in good ageement with the values obtained by Nakamoto et al.(1979) whilst investigating lymphatic absorption of 1-(2-tetrahydrofuryl)-5-fluorouracil from the rat intestinal loop. These rats were anaesthetised with sodium pentobarbitone, and 2ml saline was given intra-peritoneally each hour. Lymph flows of 0.6 to 0.8ml/hr were recorded following administration of an aqueous solution and sesame o/w and w/o emulsions.

As anaesthesia and the surgical procedures have such a marked effect on absorption and lymph flow it is not possible to compare directly the results from this study with those obtained in unanaesthetised animals.

In anaesthetised rats peak plasma levels were higher ($p<0.05$) following administration in 1ml arachis oil than the other two oils but there was no difference between the peak levels following administration in Miglyol 812 and liquid paraffin (see figure 3.3). However, the liquid paraffin formulation gave earlier ($p<0.05$) peak plasma levels due to the faster gut transit of this oil in comparison to the other two oils (section 4.5). Thus, under conditions of anaesthesia the difference in the extent of DDT absorption from Miglyol 812 and liquid paraffin is abolished but is maintained with respect to

arachis oil.

Comparison of the lymph flows in the presence of the different oils (see figure 3.4) shows that arachis oil and liquid paraffin stimulated lymph flow whereas Miglyol 812 caused inhibition. Both LCT and MCT have been shown to exert a lymphagogue effect. Simmonds (1955) observed that although tributyrin did not appear in the lymph it produced an increase in lymph flow accompanied by a fall in protein concentration. More recently Barrowman and Turner (1977,1978) showed that intra-duodenally administered MCT caused a lymphagogue effect 75% that of LCT over approximately the same time period, and the total protein moved was also 75% that of LCT. On the basis of these reports the lymph flow in the prescence of Miglyol 812 should only have been slightly less than that with arachis oil, whereas the experimental lymph flow profile (see figure 3.4) suggests that initially lymph flow is being severly inhibited and that this effect then gradually decreases. The only indication of such an inhibitory mechanism in the plasma level data in unanaesthetised rats following administration of DDT in Miglyol 812, is at the hour one data point when the plasma DDT concentration was less ($p<0.05$) than in the presence of the other oils (see figure 3.2). It is suggested that by some unknown mechanism Miglyol 812 initially inhibits lymph flow but that the effect is only short-term in conscious animals. In anaesthetised animals due to the depression of blood flow in the intestinal region and the reduction in gut motility, recovery is not as rapid resulting in the gradual rise in lymph flow and in plasma DDT levels observed during the experimental

period.

The experimental data showed that liquid paraffin exerted a lymphagogue effect almost equal to that of arachis oil. There is no literature data available with which to compare these results. As liquid paraffin is not comprised of fatty acids and is not digested the mechanism for this effect cannot be the same as for triglyceride materials. Unemulsified liquid paraffin is absorbed in very small quantities from the gastro-intestinal tract of rats (Albro & Fishbein 1970) and is then oxidised in the mucosal cells to long chain fatty acids (McWeeny 1957) which are presumably incorporated into chylomicra in the normal way. In conditions of excess absorption liquid paraffin may be transported unchanged in the lymph and oxidised in the liver. In either situation liquid paraffin or its metabolites are transported in mesenteric lymph and this may be the means by which liquid paraffin enhances lymph flow.

Following dosing approximately 1.5 hours elapsed before the thoracic duct lymph flow was interupted. DDT absorbed into the lymph during this period would pass into the general circulation. To compensate for this time delay the DDT concentration in the first plasma sample was subtracted from that in the subsequent samples. On this basis the maximum plasma concentration of DDT in rats dosed with DDT in arachis oil=0.29±0.47ug/ml, Miglyol 812 = 0.18±0.09ug/ml, and liquid paraffin = 0.03±0.19ug/ml (mean ± S.D.). It is possible that lymph containing DDT was by-passing the cannula and entering the plasma by way of veno-lymphatic anastamoses as suggested by

other workers (Rothe et al.1957,Pocock & Vost 1974), or a small fraction of the dose was absorbed directly into the portal vein. In either situation it can be concluded that in the presence of all three oils the major, if not the only route for DDT absorption was via the lymphatic system. This is in agreement with the findings of Sieber et al.(1974), Pocock and Vost (1974) and Rothe et al.(1957).

The peak lymph levels of DDT following administration in arachis oil were higher ($p<0.05$) than the other two oils. There was no difference between the levels following administration in liquid paraffin and Miglyol 812 (see figure 3.5). This suggests that there is some facet of arachis oil digestion and absorption that enhances the lymphatic uptake of DDT and which is absent with the other oils (see section 6).

The results of these experiments show that DDT is largely absorbed via the lymphatic route and that the presence of different oils does not alter the route of absorption but effects the extent to which DDT absorption into the lymph occurs. Thus, the enhanced oral absorption of DDT in the presence of arachis oil is due at least in part to increased lymphatic absorption of DDT. However this study does not explain the difference in the oral absorption of DDT in the presence of Miglyol 812 and liquid paraffin. It was shown that the plasma levels of DDT may be altered by changing gastro-intestinal motility using the drugs metoclopramide and propantheline bromide (see section 2.5). It is possible that the differences in DDT absorption from Miglyol 812 and liquid paraffin is due in part to the different affect of these oils

on gastro-intestinal motility. This was investigated using a gamma camera and oils spiked with a gamma emitting nuclide, as discussed in the following chapter.

CHAPTER 4.

GASTRO-INTESTINAL MOTILITY STUDIES IN THE RAT.

4.1 Introduction.

Gastric emptying and intestinal motility may have a profound effect on drug activity following oral administration by altering the residence time of the drug at the site of absorption, or at the site of metabolism and deactivation (Prescott 1974,Varga et al.1975,Franklin 1977). Pharmacological modification of gastro-intestinal motility using therapeutic agents such as metoclopramide and propantheline, has shown the absorption of a number of drugs to be influenced by the rate of gastric emptying and intestinal transit e.g.digoxin (Manninen 1973), paracetamol (Nimmo et al.1973), L-dopa (Algeri et al.1976), phenylbutazone (Consolo et al.1970), and griseofulvin (Jamali & Axelson 1977). It follows from this that drug absorption may be altered by administration with a fatty meal which inhibits gastro-intestinal motility. For example, the absorption of griseofulvin is enhanced by oral administration in the presence of a high fat meal (Crounse 1961) or when given in a micronised form in corn-oil-in-water emulsion (Carrigan & Bates 1973,Bates & Sequeira 1975). Further investigations suggested that in the presence of fat the gastric emptying and intestinal transit of griseofulvin were inhibited, allowing more time for dissolution and absorption (Jamali & Axelson 1977).

4.1.1 Determination of Gastric Emptying and Intestinal Transit.

The majority of studies of gastric emptying and intestinal transit in rats, have employed the same basic technique. The rats are dosed with a non-absorbable marker and are then left for the requisite time period before being killed and the distribution of the marker along the gastro-intestinal tract determined.

The non-absorbable markers that have been used in these studies are of two types:-

a) Non-radioactive - glass beads, small seeds, or inert indicators such as carbon, chromic oxide or barium sulphate. Various problems are associated with such markers; beads and seeds can become separated from food, dye indicators may not be accurately located, and barium sulphate may retard gastric emptying (Nimmo 1976).

b) Radioactive - two types of monitoring techniques have been employed with radionuclide markers. Either the gut is cut into segments and the activity in each segment counted, or the entire gastro-intestinal tract is removed in one continuous section, stretched out on a perspex plate and scanned with a detector connected to a scaler or ratemeter, to produce a profile of the activity distribution through the gut. (Examples of these studies are listed in table 4.1).

The main disadvantage of these methods is that gastric emptying cannot be continuously followed in the same animal and that a large number of animals have to be sacrificed in order to obtain statistically significant results.

In the study of gastric emptying in humans one of the

Table 4.1- Markers Used in Gastric Emptying and Intestinal Transit Studies in the Rat.

1.Non-Radioactive Markers.

Author	Marker	Study
Macht (1930)	Charcoal	Propulsive motility
Schurch et al.(1950)	Chromic oxide	Gastro-intestinal transit
Goodman et al.(1952)	Dye T1824	Gastro-intestinal transit
Reynell & Sprey (1956)	Phenol red	Gastric emptying Gastro-intestinal transit
Aberdeen et al.(1960)	PEG	Gastric emptying
Bennett & Simmond (1962)	PEG	Gastric emptying
Brodie (1966)	Amberlite pellets	Gastric emptying

2.Radioactive Markers - Gut Segmentation monitoring

Author	Marker	Study
Cramer & Copp (1959)	Radiostrontium $^{89}SrCl_2$	Intestinal transit in fed and fasted rats
Marcus & Lengemann (1962)	Radionuclide ^{91}Y	Intestinal transit
Nygaard (1967)	$^{25}Na_2CrO^4$ PEG Phenol red	Intestinal motility
Purdon & Bass (1973)	$^{133}BaSO_4$	Gastro-intestinal transit
Varga (1976)	^{85}Sr-labelled microspheres	Gastro-intestinal motility

3.Radioactive - Monitoring of the Intact Gut.

Author	Marker	Study
Derbloom et al.(1966)	$^{51}NaCrO_4$ +	Simultaneous,
	^{125}I-polyvinyl-	independant
Poulakos & Kent (1973)	pyrrolidone	determination of
	(PVP)	gastric emptying &
		intestinal transit
Patterson et al.(1976)	^{125}I-PVP	Intestinal transit
Hinder et al.(1976)	^{125}I-RIHSA	Gastric emptying
Gustavsson et al.	^{125}I-PVP	Calcium absorption+
(1979)	^{47}Ca	gut motility

most recent innovations involves the use of technetium-99m (^{99m}Tc) labelled markers for *in vivo* imaging using a gamma camera with an associated computer (Chaudhuri 1974, Van Dam 1974, Chaudhuri et al.1976, Meyer et al.1976, Ostick et al.1976, Grimes & Goddard 1977). Although several millicuries of radionuclide are generally administered during the imaging studies, the effective radiation dose is low due to the lack of particulate emission (α or β) and the short half life (6 hours) of the radionuclide. In addition, the low energy gamma rays (140keV) emitted by ^{99m}Tc are at the optimum energy level for detection by the camera.

A full review of gamma camera techniques for determining gastric emptying is outside the scope of the present work and has recently been carried out by Hardy & Wilson (1981).

As the methodology for gastric emptying studies using the gamma camera is well established and has been successfully applied to rabbits (Curt 1977) it was decided to apply the same technique to rats, and if possible, determine the gastric emptying rate and intestinal transit of arachis oil, Miglyol 812 and liquid paraffin.

4.2 Materials and Apparatus.

4.2.1 Materials.

a)Arachis oil B.P.- Evans Ltd.,Liverpool.

b)Miglyol 812 - Dynamit Nobel, Slough, Berks.

c)Liquid paraffin B.P.- Shell Ltd., London.

d)Oleic acid - B.D.H. Chemicals Ltd., Poole, Dorset.

e)^{123}I-oleic acid, ^{123}I-arachis oil - prepared using the method descibed by Lubran and Pearson (1958), in the Radiopharmacy Department, General Hospital, Nottigham.

f)^{99m}Tc-Sulphur colloid - prepared in a ^{99}Mo-^{99m}Tc generator, from Amersham International Ltd., Bucks. Particle size distribution of colloid - 72%=400-600nm, 18%<100nm, 7%>600nm.

g)Barium sulphate suspension - Baritop 100 suspension, Concept Pharmaceuticals Ltd., Rickmansworth, Bucks.

4.2.2 Apparatus.

a)Gamma camera - i)Searle U.K. Ltd.

ii)International General Electric of New York Ltd.

These consist of three parts:-

i)camera head and stand consisting of a collimator, a detector crystal (sodium iodide, thallium activated) and a bank of 37 interconnected photomultiplier tubes.

ii)operating console

iii)display system

When the nuclidie ^{99m}Tc is placed on the collimator surface the gamma radiation emmitted by the isotope is detected. Gamma radiation passing perpendicularly to the detector face passes into the sodium iodide crystal exciting electrons to a higher energy state. The electrons drop back to their original energy level and in doing so emit photons which are detected by the photomultiplier tube and converted into electrical signals. These signals are electronically combined to produce a spot on an oscilloscope screen corresponding in

position with the site of interaction of the gamma ray with the crystal. By photographing the events on the oscilloscope an image representing the distribution of radioactivity can be obtained. Data is recorded and analysed by computer (Varian Data Machines Ltd. and Link Systems Ltd.), the images being displayed on a television monitor. Hard copy from the computer of the graphs and images is produced using an electrostatic printer (Statos, Varian Data Machine Ltd.). Information can be stored on magnetic tape for subsequent analysis.

b)Perspex restraining cages - consisting of a perspex tube approximately 5cm×19cm, with a perforated perspex plate at one end and a removable pespex restraint at the other.

4.3 Methods.

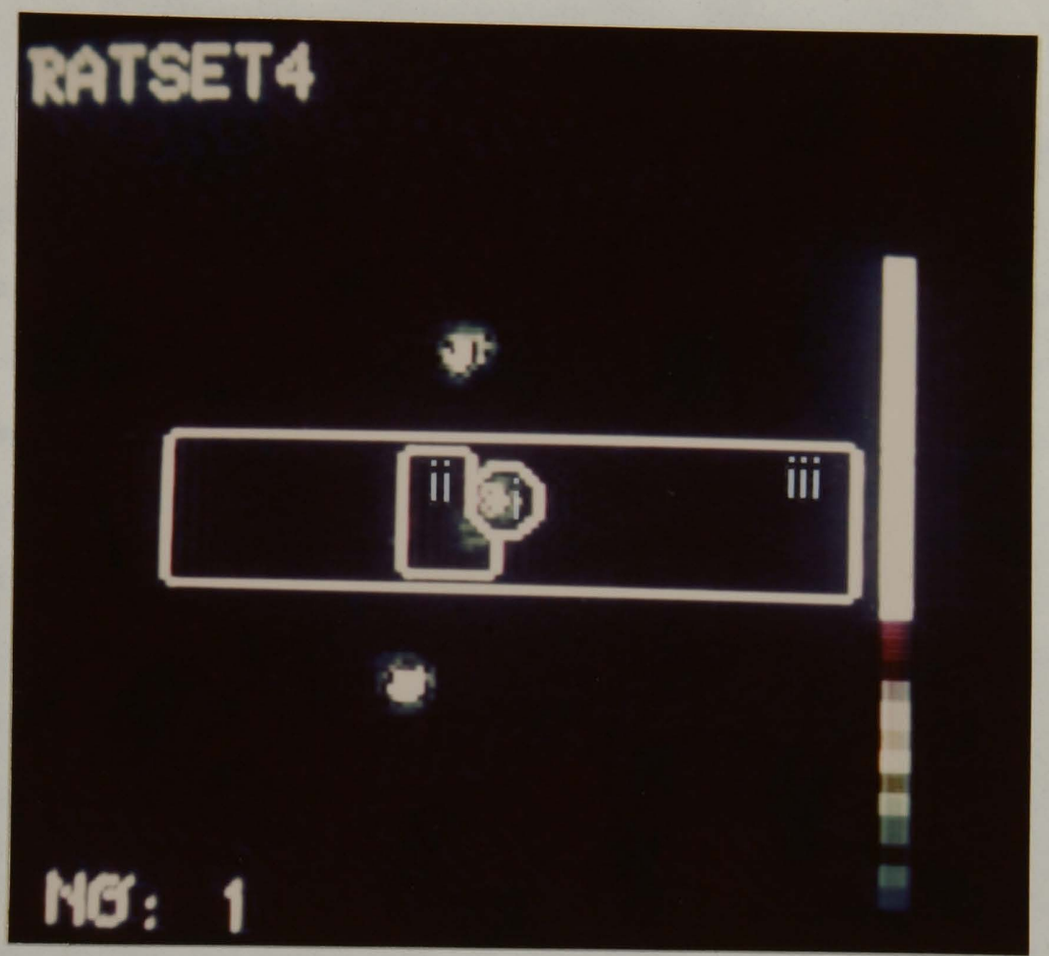
4.3.1 X-ray investigation.

Three male, Wistar rats were orally dosed with 1ml barium sulphate suspension. Dorsal, ventral and lateral radiographs of the rats in cages were taken at 1, 2 and 3 hours after dosing.

4.3.2 Radionuclide Imaging Study.

Each rat was orally dosed with oil plus radionuclide marker and immediately placed in a perspex restraining cage at the camera face. It was possible to image three rats simultaneously by use of a small rack so that the rats were positioned one above the other. For the large oil volume study 90 one minute images were taken, whereas for the small oil

Figure 4.1 - Regions of Interest Constructed For Analysis of Radionuclide Computer Images.



i) stomach region

ii) intestinal region

iii) whole body region

volume study 72 75 second images were taken. In each study the first frame was started when the first rat was placed at the camera face.

4.3.3 Analysis of Radionuclide Imaging Study.

For each rat a series of images taken at 5 minute intervals were analysed such that for each formulation the images analysed were at the same time period after dosing for each animal. On each image regions of interest (ROI) of a standard size were constructed around 1)the stomach (30 cells) 2)the intestine (142 cells) and 3)the whole body (363 cells) (see figure 4.1) and the counts within the regions computed. The counts for the stomach region and the intestinal region were divided by the whole body counts to allow for any variation in the activity of each dose. Assuming that the total dose is in the stomach on the first image, the ratio stomach:whole body counts at t=0 will be 1 and the ratio intestinal:whole body counts will be zero. Graphs were constructed of the decrease in the stomach counts ratio with time and the increase in the intestinal counts ratio with time.

4.3.4 Preparation of Dosage Forms.

Preliminary investigations were conducted with several oil markers to determine the most suitable. In each case the oil was either mixed or emulsified with the marker prior to administration. Formulations investigated:-

a) ^{99m}Tc-sulphur colloid in water.

0.3ml (≈500uCi)	sulphur colloid
1.2ml	oil (arachis oil, Miglyol 812

or liquid paraffin)

b) ^{123}I-oleic acid

0.2ml (≈60uCi)	^{123}I-oleic acid
1.3ml	oil (arachis oil, Miglyol 812 or liquid paraffin)

c) ^{123}I-arachis oil

0.3ml (≈190uCi)	^{123}I-arachis oil
1.2ml	arachis oil

^{99m}Tc-sulphur colloid was found to be the most suitable marker (see discussion) and was used in all further investigations.

Formulation for large oil volume study:-

0.5ml (≈500uCi)	^{99m}Tc-sulphur colloid
1.0ml	oil (arachis oil, Miglyol 812 or liquid paraffin)

Formulation for the small oil volume study:-

15ul (≈150 uCi)	^{99m}Tc-sulphur colloid
30ul	oil (arachis oil, Miglyol 812 or liquid paraffin)

4.3.5 Total Gut Transit Time Study.

Using the same animals and continuing directly from the

radionuclide imaging studies with ^{99m}Tc-sulphur colloid and the large oil volumes and small oil volumes, each rat was imaged at hourly intervals for 100secs (large oil volume study) or 75secs (small oil volume study) and the faeces collected and counted. A standard dose was counted simultaneously so that radioactive decay could be compensated for by expressing the whole body counts in terms of a ratio with the standard. The study was continued until the mean standardised whole body count for each formulation had fallen by 60% from its highest value.

4.4 Results.

Statistical comparison of parameters was made using the Student's "t" test or the Mann Whitney "U" test.

4.4.1 X-ray investigation.

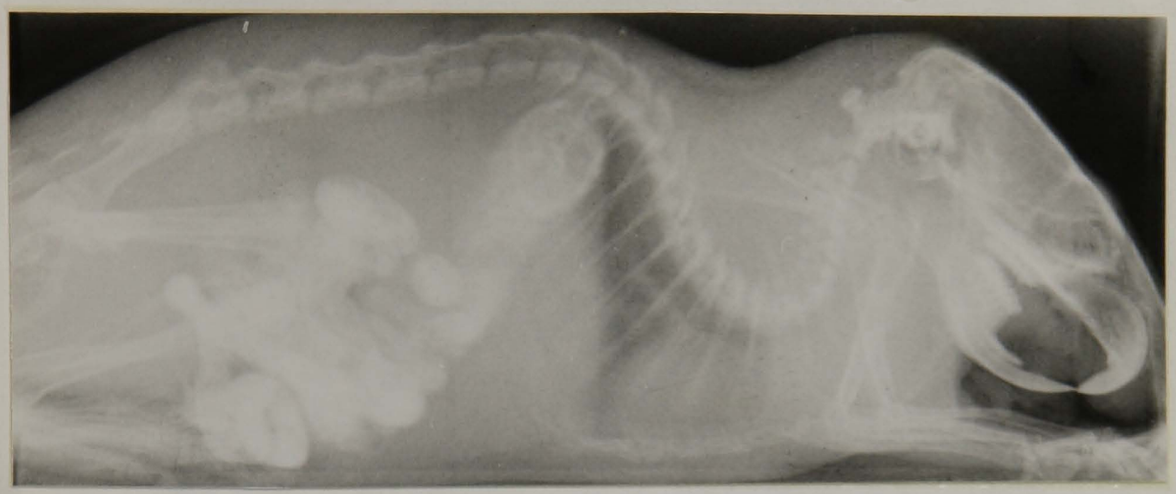
The radiographs of the rats showed that the right lateral view gave the best single plane discrimination of the stomach and the intestinal regions (see Figure 4.2). This view was subsequently used in all the gamma camera studies.

4.4.2 Radionuclide Imaging Study.

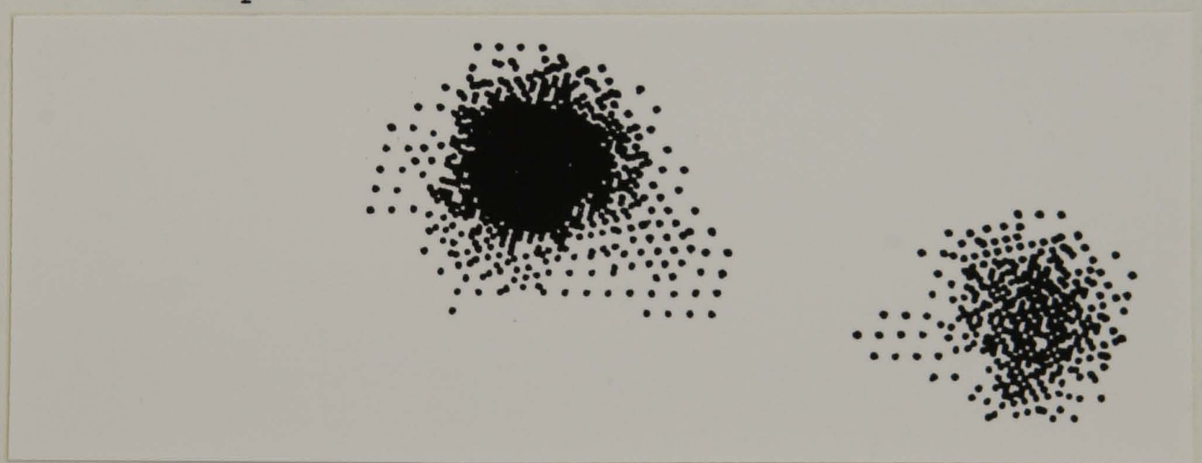
The high activity present in the stomach region masked any loss of activity into the intestinal region so the graphs showing the change in the stomach counts ratio with time did not clearly illustrate gastric emptying (see figure 4.3). However, the rise in intestinal activity as gastric emptying occured was shown clearly by plotting the intestinal counts ratio against time (see figure 4.4). Gastric emptying was

Figure 4.2 - Lateral Images of a Rat One Hour After a Barium Meal and Following Administration ^{99m}Tc-Sulphur Colloid.

a)Lateral radiogram of a rat one hour after a barium meal.



b)Lateral scintogram of a rat immediately after dosing with ^{99m}Tc-sulphur colloid in water (0.5ml).



c)Composite of a) and b).

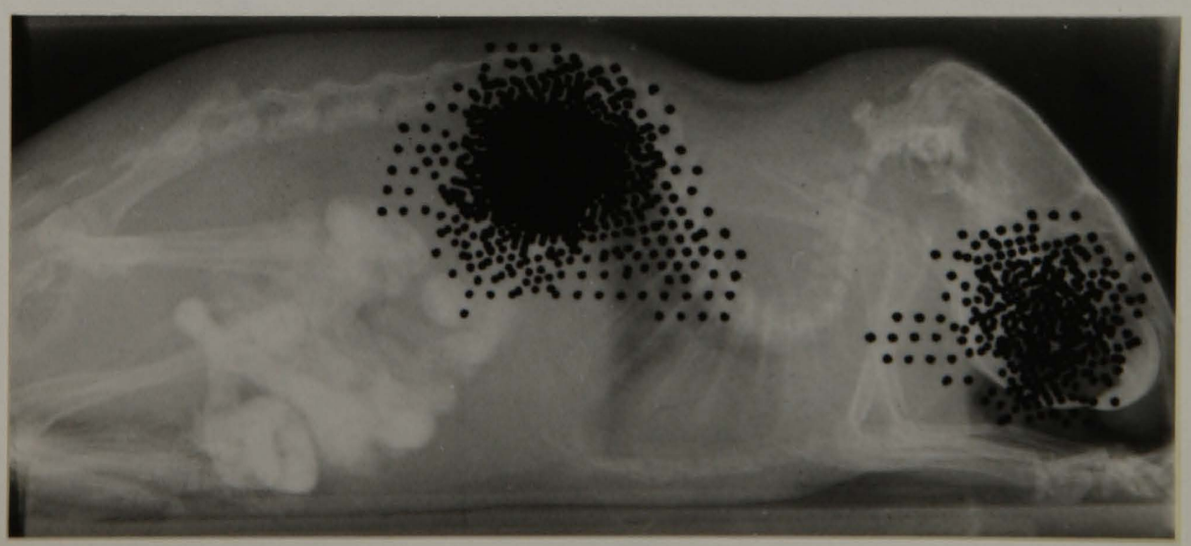


Figure 4.3

EFFECT OF DIFFERENT VEHICLES (1ml volumes) ON THE LOSS OF (^{99m}Tc)-SULPHUR COLLOID FROM THE RAT STOMACH

(Mean values, n=6 per group).

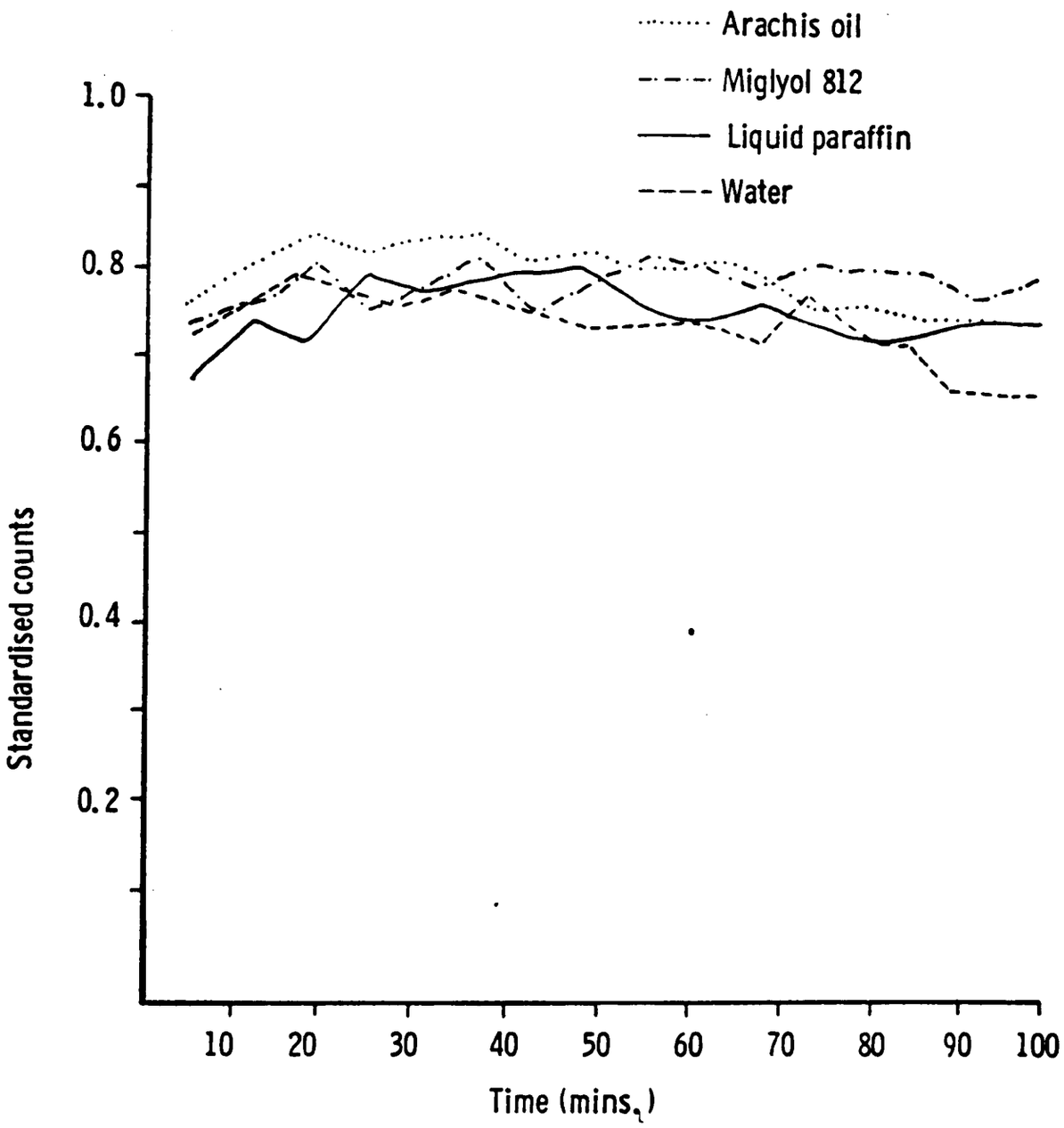


Figure 4.4

EFFECT OF DIFFERENT VEHICLES (1 ml volumes) ON THE APPEARANCE OF (^{99m}Tc)- SULPHUR COLLOID IN THE RAT INTESTINE (Mean values, n=6 per group).

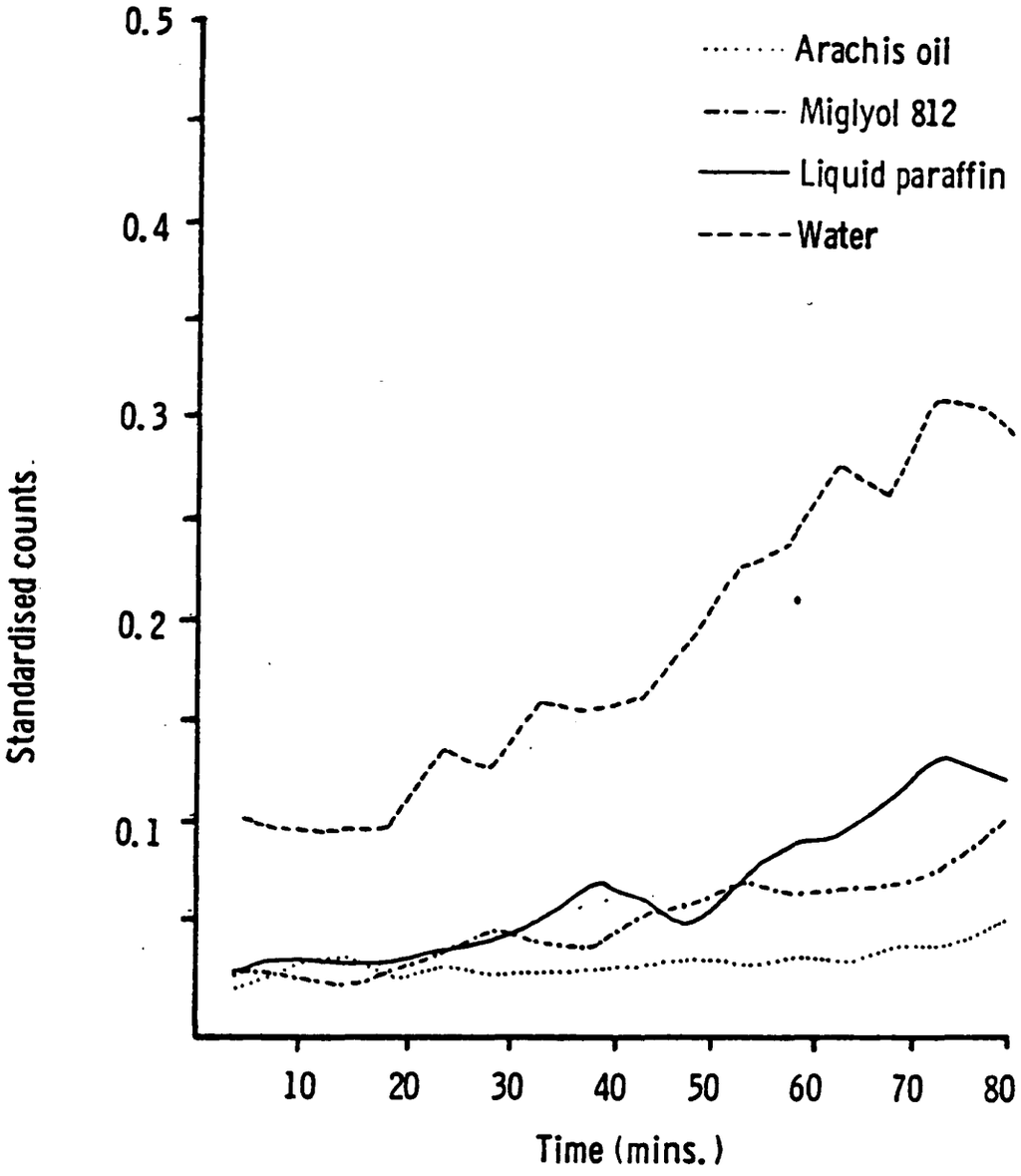


Figure 4.5

THE APPEARANCE OF (^{123}I)-ARACHIS OIL IN THE RAT INTESTINE FOLLOWING ORAL ADMINISTRATION IN ARACHIS OIL (1.5ml) (Mean value, n=3).

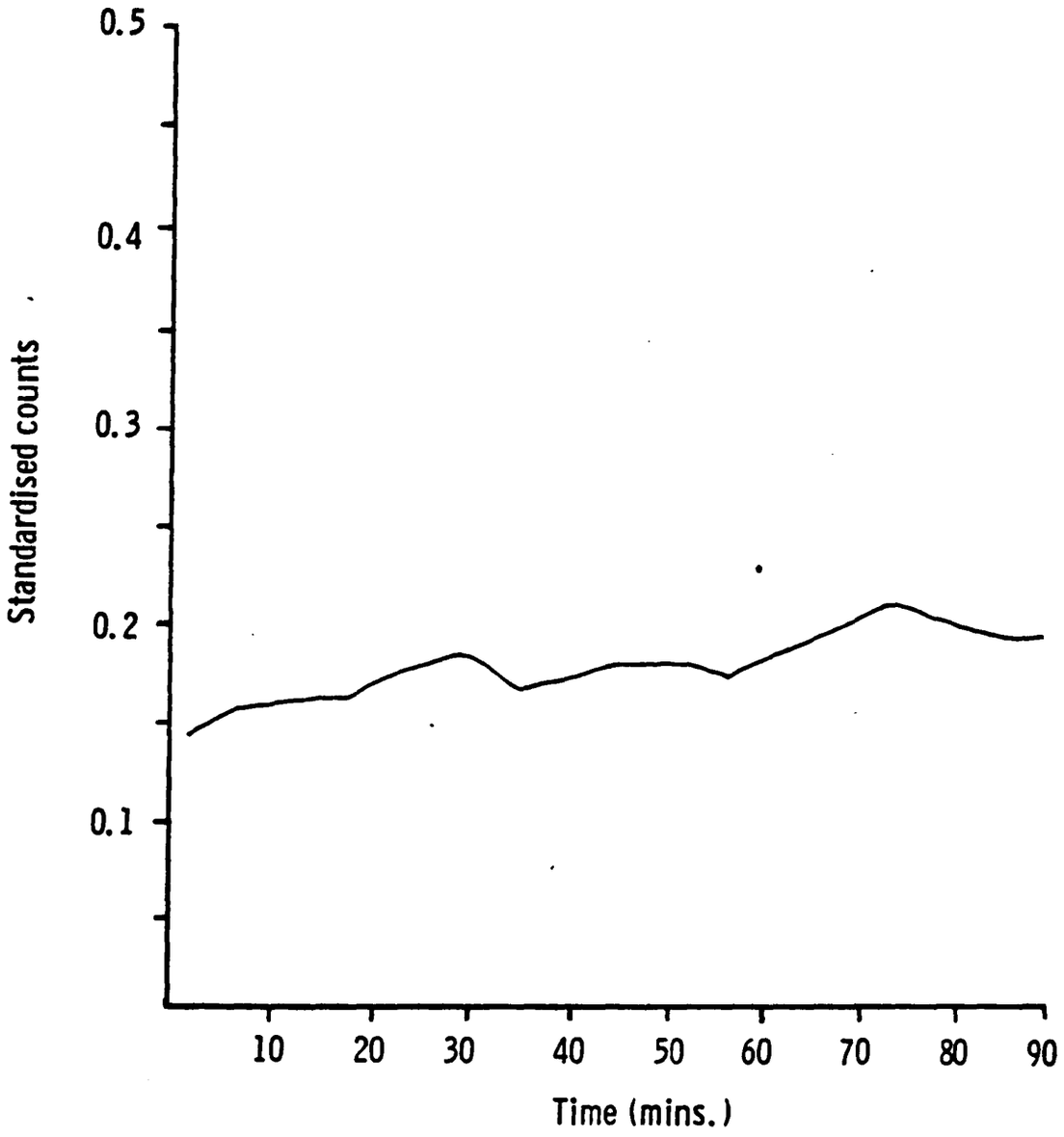


Figure 4.6

EFFECT OF DIFFERENT VEHICLES (1.3 ml volumes) ON THE APPEARANCE OF (^{123}I)- OLEIC ACID IN THE RAT INTESTINE (Mean values, n=3 per group).

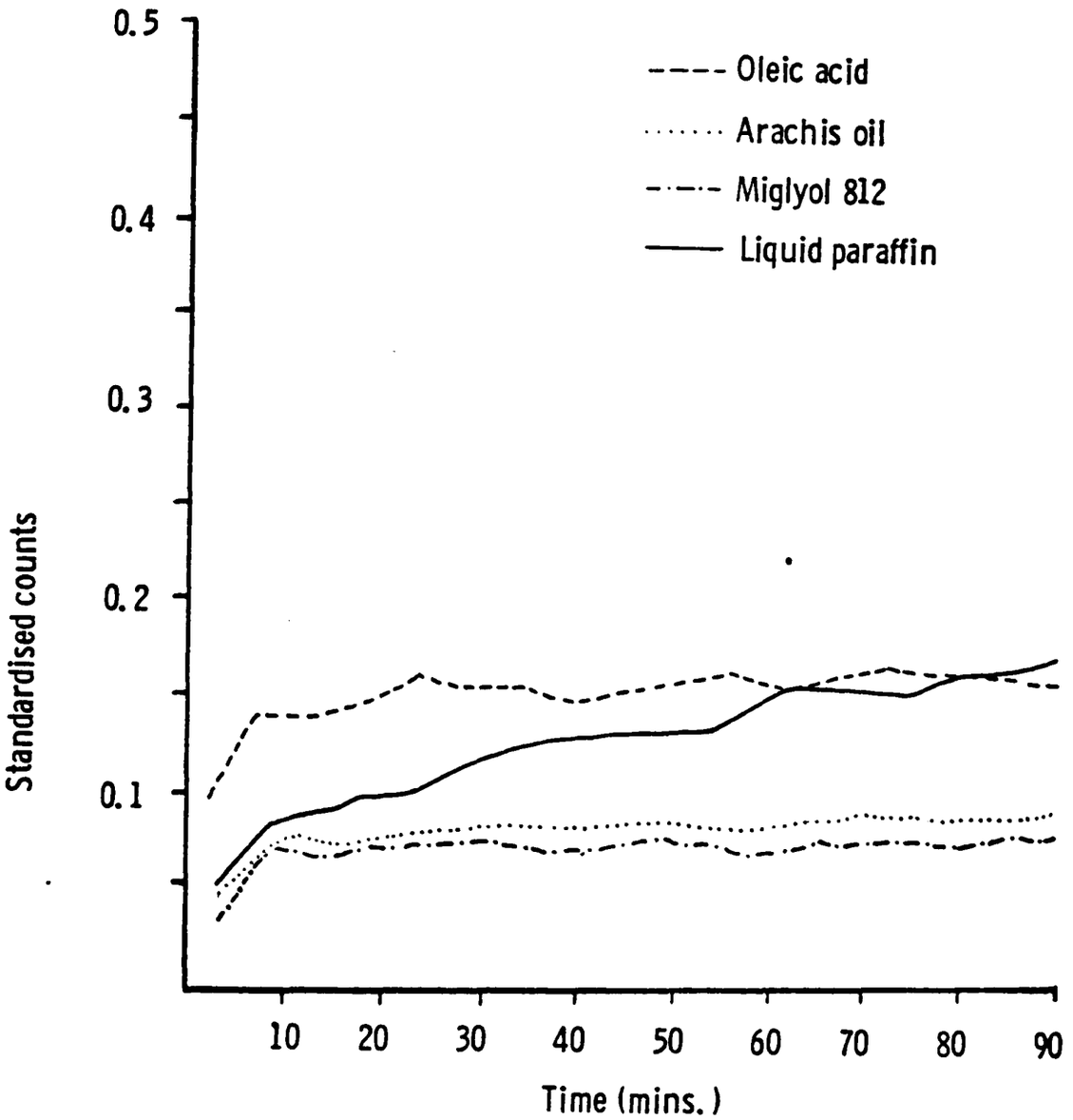
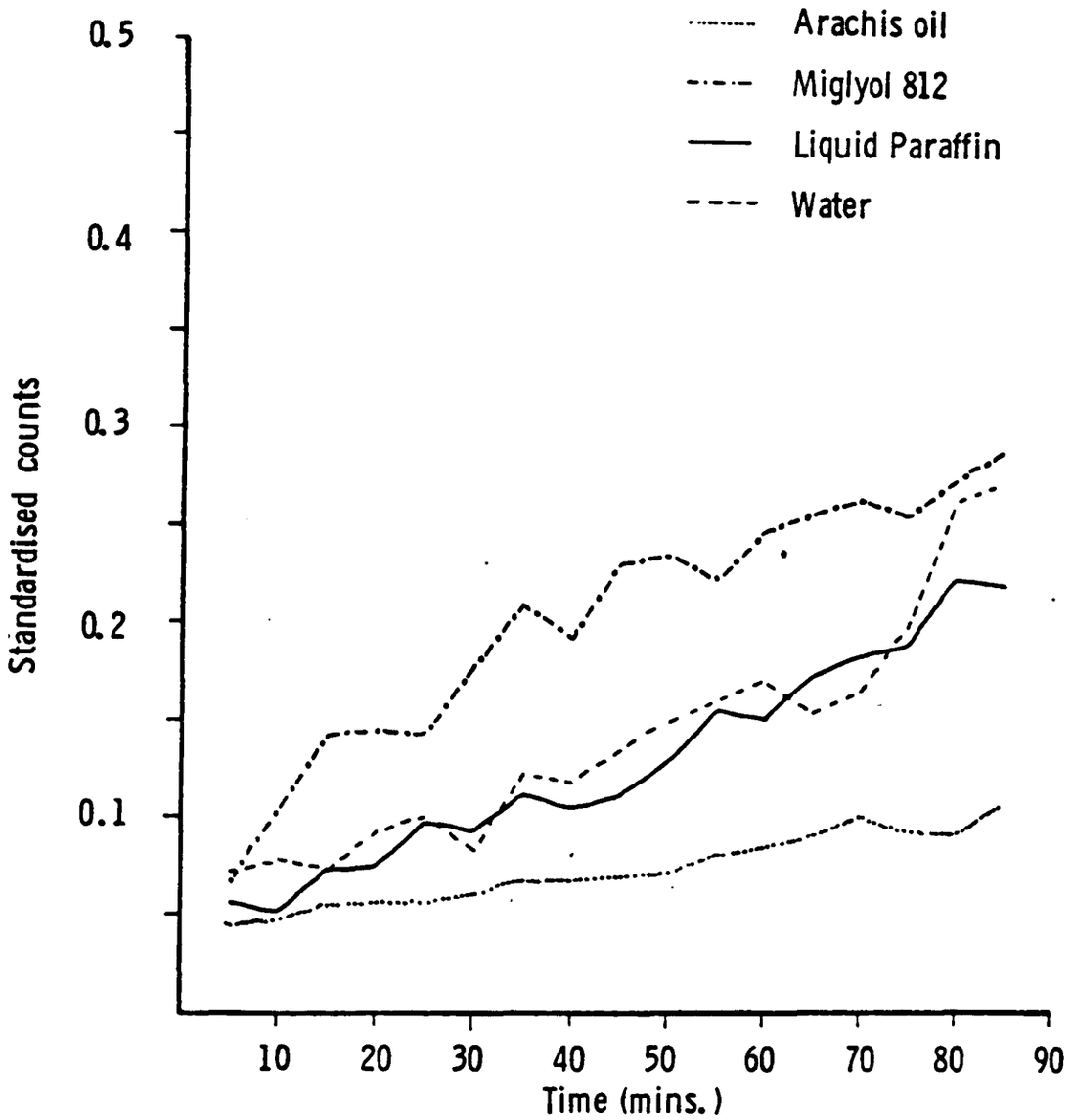


Figure 4.7

EFFECT OF DIFFERENT VEHICLES (30µl volumes)ON THE APPEARANCE OF (^{99m}Tc)-SULPHUR COLLOID IN THE RAT INTESTINE (Mean values, n=6 per group).



therefore detemined in terms of the activity entering the intestinal region.

Determination of gastric emptying in groups of 6 rats using ^{99m}Tc-sulphur colloid (0.5ml) dispersed in each oil (1.0ml) showed there to be no difference ($p>0.05$) between the oils as determined from the difference in the intestinal counts ratio at t=5min and at t=80min, although the mean values had the rank order liquid paraffin > Miglyol 812 > arachis oil. All three oils significantly inhibited (p 0.05) the gastric emptying of ^{99m}Tc-sulphur colloid compared to water (see figure 4.4).

With the smaller volumes of oil (30µl) the difference in the intestinal activity at t=5min and at t=85min was lower ($p<0.05$) following administration in arachis oil compared with water, liquid paraffin and Miglyol 812 (see figure 4.7), but there was no difference ($p>0.05$) in the gastric emptying of ^{99m}Tc-suplhur colloid in the presence of water, liquid paraffin and Miglyol 812.

4.4.3 Total Gut Transit Study.

As all the rats did not receive an identical dose of radioactivity the whole body:standard dose ratios were expressed as a percentage of the highest ratio value for each rat (see figures 4.8 & 4.9). The time taken for the mean standardised whole body count to fall to 60% of its highest value was taken as an index of total gut transit time (see table 4.2). As the faecal samples were not homogenised and made up to equivalent volumes the counting efficiency was not the same for each sample and in addition a standard count was not

Figure 4.8

EFFECT OF DIFFERENT VEHICLES (1 ml volumes) ON THE EXCRETION OF (^{99m}Tc)- SULPHUR COLLOID FROM THE RAT (Mean values, n= 6 per group).

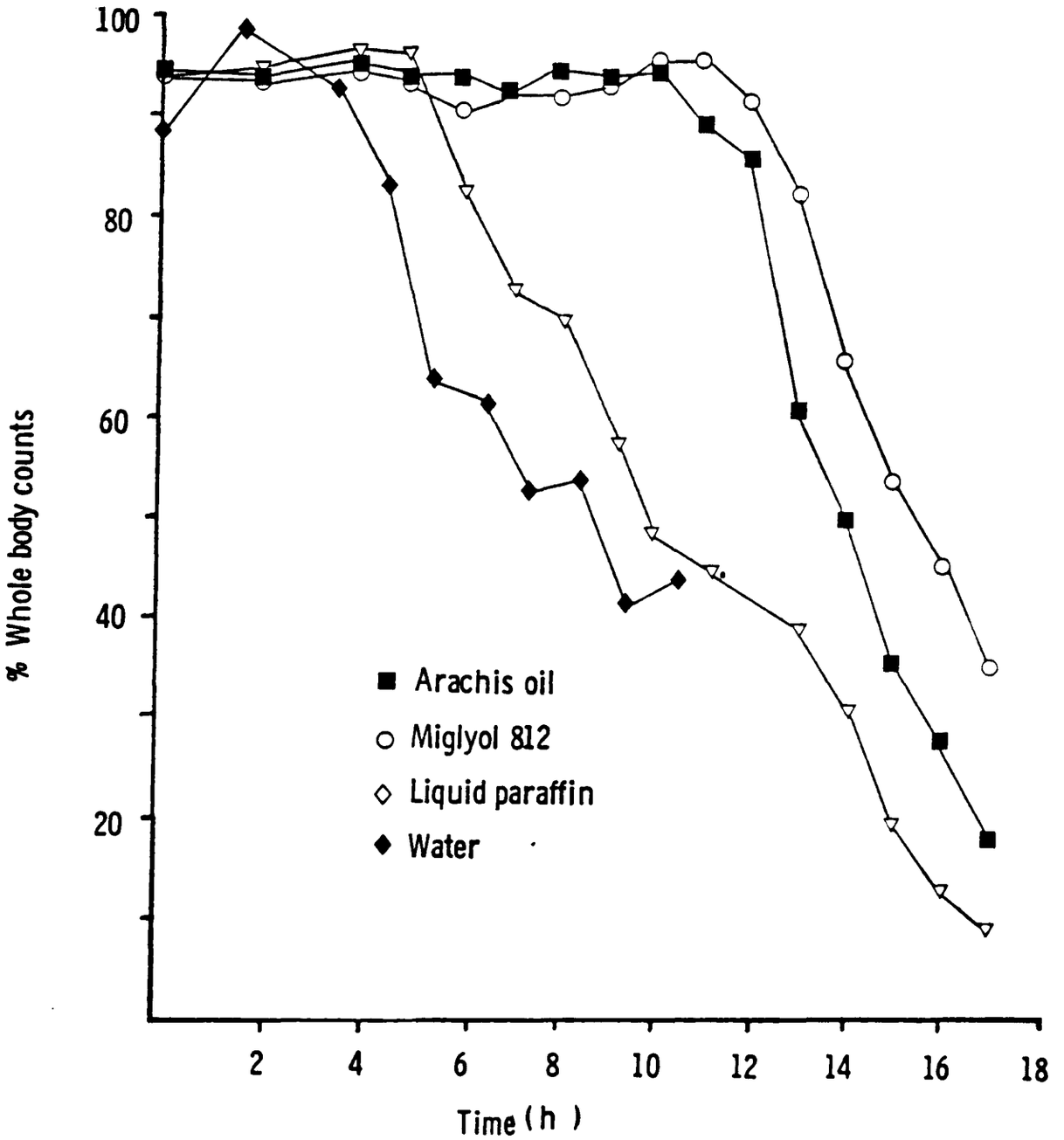


Figure 4.9

EFFECT OF DIFFERENT VEHICLES (30 µl volumes) ON THE EXCRETION OF (^{99m}Tc)- SULPHUR COLLOID FROM THE RAT (Mean values, n=6 per group).

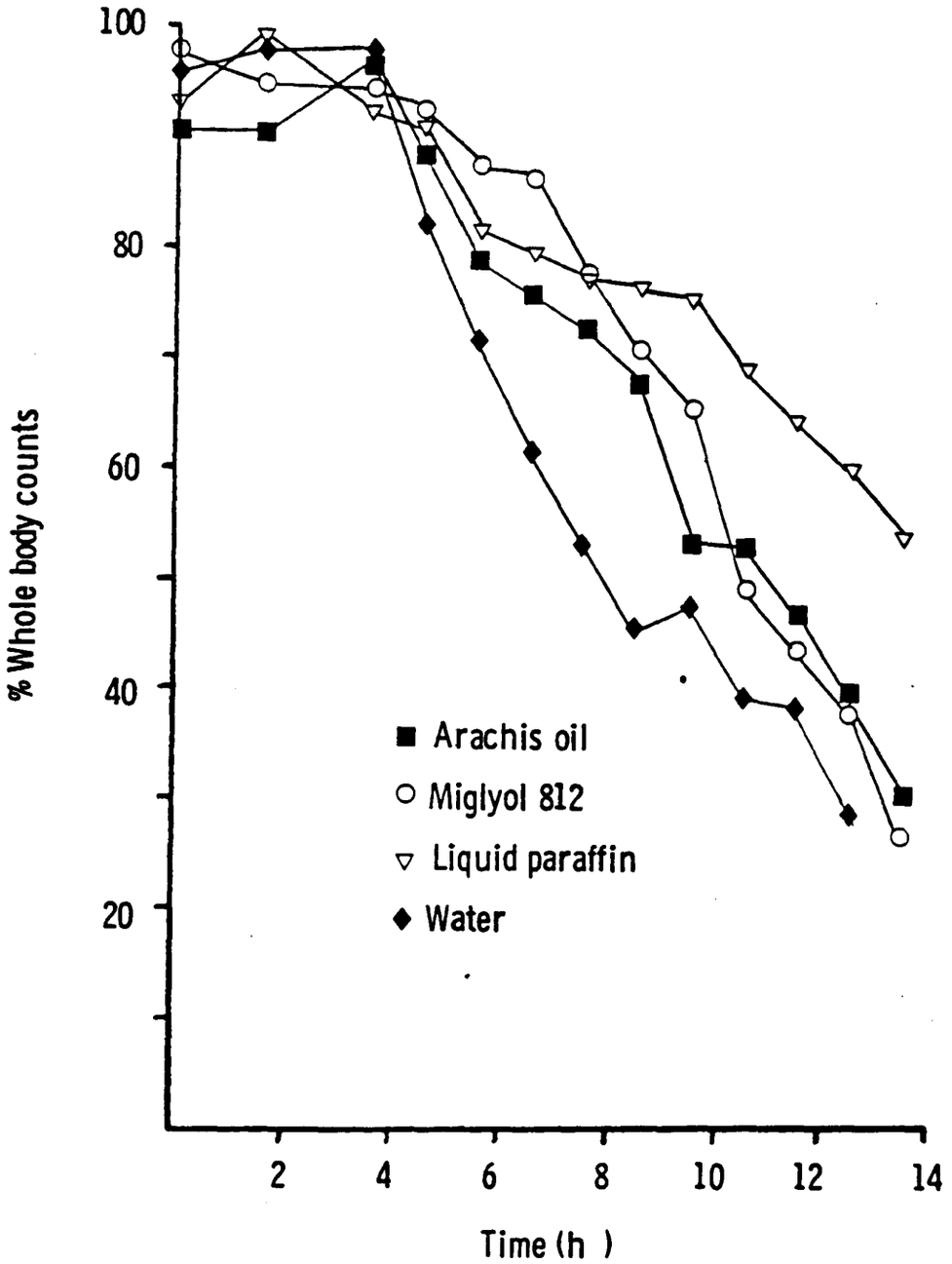


Table 4.2 -Time Taken for Standardised Whole Body Counts to Fall to 60% of the Original Value Following Oral Administration of ^{99m}Tc-Sulphur Colloid in Different Vehicles to Rats.(Mean ± S.D., n=6 per group).

Formulation	$t_{60\%}$ (h.)	
	1ml	30µl
Arachis oil	13.33 ± 1.1	9.23 ± 1.4
Miglyol 812	14.83 ± 2.1	9.26 ± 1.6
Liquid paraffin	9.22 ± 3.4	12.42 ± 1.8
Water	6.05 ± 2.1	7.01 ± 1.6

made. The standardised whole body counts were therefore used to determine the total gut transit time of the ^{99m}Tc-sulphur colloid.

In the rats dosed with 1ml oil, the total gut transit time of ^{99m}Tc-sulphur colloid as determined from the $t_{60\%}$ values was faster ($p<0.05$) in the prsence of liquid paraffin and water than in the presence of arachis oil and Miglyol 812 (see table 4.2 & figure 4.8). With the small volumes of oil (30µl) the total gut transit time of ^{99m}Tc-sulphur colloid was slower ($p<0.05$) in the presence of liquid paraffin than the other formulations (see table 4.2 & figure 4.9).

4.5.Discussion.

Preliminary studies were performed to determine first whether gastric emptying in rats could be monitored using the gamma camera technique and second, which of the radionuclides available would be the most suitable oil phase marker.

Radiographs of restrained rats orally dosed with barium sulphate suspension showed that the lateral view of the animal was the most suitable for the present studies as it gave the best single plane discrimination of the areas of interest, namely the stomach and the intestines. By combining a lateral radiograph and a scintigram, taken immediately after dosing a rat with ^{99m}Tc-sulphur colloid, it was seen that initially all the activity was in the stomach and the mouth (see figure 4.2). With time, activity moved out of the stomach region into the area corresponding to the intestines, and by computer analysis

it was possible to follow the the decrease in stomach counts and the increase in intestinal counts as this occured, and thus monitor gastric emptying. As clearer results were obtained from the intestinal counts, these were used to determine gastric emptying.

The requirments for an ideal radionuclide marker for the oil phase were that:-

a)it was not absorbed, as activity passing into the general circulation would cause errors in the analysis.

b)it was attached to or at least miscible with, the oil so that gastric emptying of the oil and not the marker alone was monitored.

c)it exerted no independant effect on gastric emptying which would alter the results.

As no radionuclide marker with all these properties was available a suitable compromise had to be found.

Attaching a marker to the molecules of the oil would ensure that it was the gastric emptying of the oil that was being monitored and that the marker was not altering the gastric emptying rate. Unfortunately arachis oil was the only oil to which a radionulcide could be added due to the presence of unsaturated bonds. ^{123}I-arachis oil was prepared, orally administerd to rats and the results were used for comparison with the other markers (see figure 4.5).

^{123}I-oleic acid was prepared as this is miscible with each oil. The studies with ^{123}I-oleic acid + arachis oil gave the same gastric emptying profile as was obtained with ^{123}I-arachis oil (see figure 4.6) suggesting that the presence

of oleic acid was not altering gastric emptying. As the energy of the ^{123}I-iodide emmissions is not optimal for detection by the collimator available a relatively large scatter of activity was detected, resulting in a high background count. This made it difficult to establish the regions of interest on the scintigrams correctly, giving rise to inaccurate counts for each region.

^{99m}Tc-sulphur colloid has been widely used in human gastric emptying studies (Hardy & Wilson 1981) being non absorbable, and as it was readily available it was examined as a potential oil phase marker. The gastric emptying profile for ^{99m}Tc-sulphur colloid in the presence of arachis oil was very similar to that for ^{123}I-arachis oil and ^{123}I-oleic acid + arachis oil. However, the emptying of the colloid in the presence of Miglyol 812 was greater than for ^{123}I-oleic acid + Miglyol 812, suggesting that the oleic acid had inhibited the emptying of Miglyol 812. Oleic acid is a long chain unsaturated fatty acid and may therfore be expected to inhibit gastric emptying. This would not have been seen with the arachis oil as there was virtually no emptying of either marker, ^{123}I-arachis oil or ^{123}I-oleic acid, within the experimental period. There was, however, little difference in the gastric emptying of ^{99m}Tc-sulphur colloid and of ^{123}I-oleic acid in the presence of liquid paraffin.

^{99m}Tc-sulphur colloid would seem to be a more suitable marker than ^{123}-oleic acid being less likely to exert any pharmacological action, being non absorbable and emitting gamma rays at an optimum energy level for detection by the camera. It

is, however, immiscible with oil and is a solid, although the particles are very small, and it was considered possible that it was the gastric emptying of the colloid and not that of the oil that was being monitored. However if the gastric emptying of the colloid had been unaffected by the presence of the oils the same result would have been anticipated for each formulation. As this did not occur it was considered that the gastric emptying of the colloid was being altered by the affects of the oils on gut motility. The effect of each oil on gastric emptying could therefore be determined from the changes in the gastric emptying of ^{99m}Tc-sulphur colloid administered with the oil.

On the basis of these investigations ^{99m}Tc-sulphur colloid was selected for use in all further gastric emptying studies.

Having shown that it is possible to monitor gastric emptying in rats using the gamma camera and ^{99m}Tc-sulphur colloid as a marker the effect of 1ml and 30µl volumes of arachis oil, Miglyol 812, liquid paraffin and water on gastric emptying in the rat were investigated.

With 1ml volumes, ^{99m}Tc-sulphur colloid emptied faster ($p<0.05$) in the presence of water than the oils, and there was no significant difference ($p>0.05$) in the intestinal counts ratios for the oils after 80minutes, although the mean values had a rank order of liquid paraffin > Miglyol 812 > arachis oil. Within each group of rats there was a wide variation in the rate of gastric emptying due in part to biological variation and in part to analytical difficulties (see later),

such that, although possible differences in gastric emptying were observed, the values were not different ($p>0.05$). The preliminary study using ^{123}I-oleic acid gave the same ranking of the mean values for the intestinal counts ratio after 80 minutes as this experiment, in that liquid paraffin exhibited faster emptying than the other oils. It is therefore suggested that 1ml volumes of liquid paraffin emptied from the stomach faster than the 1ml volumes of arachis oil, but that due to large intra-group variation and analytical errors a statistically significant ($p<0.05$) effect was not observed.

With the smaller volumes of oil (30µl), arachis oil inhibited ($p<0.05$) the gastric emptying of ^{99m}Tc-sulphur colloid compared with water, Miglyol 812 and liquid paraffin, there being no difference ($p>0.05$) between the latter group.

The results obtained for the small volumes of oil may be anticipated from the literature data. Miglyol 812 contains C_8-C_{12} fatty acids which are unlikely to inhibit gastric emptying to the same extent as the long chain unsaturated fatty acids of arachis oil (Hunt & Knox 1968b,Cooperman & Cook 1976). Liquid paraffin is unlikely to inhibit gastric emptying as it is non-digestible so there are no intra-luminal digestion products to stimulate the inhibitory responses. Thus, only arachis oil is likely to delay gastric emptying with respect to water. For the larger volumes of oil it is difficult to draw any conclusions as to the underlying mechanisms as the experiment was not conducted over a long enough time period to determine any significant ($p<0.05$) differences in the gastric emptying of the oils. The lower ($p<0.05$) gastric emptying rate

of the oils in comparison to water may be due in part, to a viscosity effect. Following mixing with chyme the low volumes (30µl) of oil would not have significantly altered the viscosity of the stomach contents, but the large volumes (1ml) of oil probably markedly increased the viscosity of the stomach chyme thereby retarding gastric emptying (Yamahira et al.1978,Levy & Jusko 1965).

Comparison of the gastric emptying profiles for 1ml and 30ul volumes of oil suggest that with the larger volumes of oil there was an initial delay before gastric emptying started; for Miglyol 812, liquid paraffin and water this was approximately 20 minutes whereas for arachis oil it was approximately 30 minutes. In addition, comparison of figures 4.4 and 4.7 suggests that for the larger volumes of oil the rate of gastric emptying was slower. Oil volume would therefore appear to have a marked effect on gastric emptying.

As previously stated a number of problems were encountered with the computer analysis of the radionuclide imaging studies which gave rise to error in the counts recorded:-

a)The analysis was done in two dimensions and it was not possible to allow for activity which moves through the intestinal region lying in the same horizontal plane as the stomach and all the activity was attributed to the stomach.

b)Longtitudinal movement of the rats during the study could be compensated for by moving the ROI correspondingly, but rotational movement could not be accounted for and gave rise to errors as the view presented to the camera changed. In addition

any movement of the rat during an image could not be allowed for and caused errors in the counts from that frame.

c)As the ^{99m}Tc-sulphur colloid passed along the intestinal tract its distance from the camera face changed causing errors as the nearer activity is to the camera face the higher the counts that are reccorded.

d)Standardisation to allow for the slight variation in the dose received by each rat was based on the assumption that in the first complete image all the activity would be in the stomach. If the rat did not swallow the whole dose the stomach:whole body count ratio was not equal to 1. In a later frame if the rat swallowed the activity remaining in the mouth or oesophagus the stomach count ratio suddenly increased. The intestinal counts ratio did not always start at zero due to background activity in the ROI.

e)Errors caused by incomplete resolution by the camera of the anatomical structure being examined.

As ^{99m}Tc-sulphur colloid is not absorbed it was possible to determine the total gut transit of the colloid by whole body gamma scintigraphy and faecal analysis and relate this to gastro-intestinal motility in the presence of each oil. The time taken for the standardised whole body count ratio to fall to 60% of its highest value was used as an index for total gut transit time. Variations in the whole body counts were seen throughout the experiment due to movement of activity towards and away from the camera face and it was considered that a 60% fall in whole body activity would only occur when the colloid

was excreted in the faeces.

For the large volumes of oil the colloid was excreted faster ($p<0.05$) in the presence of water and liquid paraffin than arachis oil or Miglyol 812 (see table 4.2). As gastric emptying is an important factor in intestinal transit these results correlate with the faster gastric emptying of the colloid in the presence of water, and the suggestion that liquid paraffin causes faster emptying of the colloid from the stomach than arachis oil. With such large volumes of oil it may be anticipated that the laxative properties of liquid paraffin would markedly increase intestinal transit, resulting in a low total gut transit time.

With the smaller volumes of oil and water there was not the same correlation between gastric emptying and total gut transit time. ^{99m}Tc-sulphur colloid was excreted faster ($p<0.05$) in the presence of arachis oil and Miglyol 812 than liquid paraffin but slower ($p<0.05$) than in the presence of water, whereas with the gastric emptying study arachis oil significantly inhibited ($p<0.05$) emptying compared to the other formulations. This suggests that the intestinal transit of the colloid in the presence of arachis oil is faster than with the other two oils reducing the time available for its absorption and, conversely, in the presence of liquid paraffin intestinal transit of the colloid is slowed allowing more time for absorption. Unemulsified liquid prarffin is absorbed in small quantities from the gastro-intestinal tract of rats (Albro & Fishbein 1970) and metabolised to long chain fatty acids within the mucosal cells (McWeeny 1957) which are then transported in

the chylomicra of the lymph (section 1.2). It is possible that although liquid paraffin itself does not initially inhibit gastro-intestinal motility the LCFA produced within the mucosal cells following absorption do stimulate an inhibitory response. With 1ml volumes this effect would have been negligible with respect to the laxative action of the relatively large proportion of liquid paraffin remaining within the intestinal lumen. However, with the smaller oil volume it is possible that gut motility was inhibited by this mechanism, especially as the liquid paraffin moved further along the gastro-intestinal tract when less remained in the intestinal lumen. Also liquid paraffin absorption may have continued along the length of the small intestinal tract causing a continued suppression of motility. The other two oils are completely absorbed within the upper regions of the intestine and so would only inhibit motility for a relatively short time. In this way it is possible that following rapid gastric emptying, the intestinal transit of liquid paraffin was slowed causing the observed delay in total gut transit of ^{99m}Tc-sulphur colloid in the presence of this oil.

Transit of material through the colon is an important consideration in total gut transit, being the region through which food takes the longest time to pass (Cumming & Wiggins 1976). In the present studies arachis oil, Miglyol 812 and water would have been absorbed within the small intestine so that in each experiment the same material, ^{99m}Tc-sulphur colloid, was entering the colon. Thus the colonic transit was not dependant on the vehicle in which the colloid was

originally administered and the differences in total gut transit could be related to the influence of the vehicle on the small intestinal transit of the colloid. The same arguament can probably be applied to small volumes of liquid paraffin as it is likely that most, if not all, is absorbed within the small intestine. With the larger volumes of this oil the laxative effect would have caused rapid transit of the colloid throughout the entire intestinal tract.

The present study has shown that the gastric emptying rate and intestinal transit of 1ml volumes of arachis oil and Miglyol 813 are similar ($p>0.05$), whereas the gastric emptying rate and intestinal transit of 1ml liquid paraffin is faster ($p<0.05$) than that of arachis oil and Miglyol 812. The differnce in the oral absorption of D D T from 1ml volumes of Miglyol 812 and of liquid paraffin may therefore be partially explained in terms of differences in the gastro-intestinal residence time of D D T in the presence of the two oils (see section 6).

CHAPTER 5.

ORAL ABSORPTION OF PREDNISOLONE.

5.1 Introduction.

The preceeding studies have shown that it is possible to enhance the lymphatic absorption of a model compound, DDT, by co-administration with arachis oil. On the basis of these findings the study was extended to examine the lymphatic uptake of the glucocorticoid, prednisolone. Although prednisolone is extensively used clinically plasma levels and total drug absorption (calculated as the area under the plasma level-time profile) exhibit wide inter-subject variation following oral administration. This has been attributed to poor water solubility and to first-pass metabolism (Pickup 1979,Thiesen 1976). It is possible that elevated, more uniform plasma levels may be achieved by enhancing the lymphatic absorption of the steroid.

A number of steroids, namely oestradiol and testosterone (Sieber et al.1974), 17-methyl-^{14}C-oestradiol (Blockage et al.1953), 17-α-methyl-^{14}C-testosterone (Hyde et al.1954), cortisone-4-^{14}C-acetate (Blockage et al 1955) and testostereone and hydrocortisone (Hellman et al.1956), have been shown not to exhibit selective lymphatic absorption, being preferentially absorbed into the portal vein. It was suggested that these compounds are not sufficiently lipophilic to be absorbed into the lymph (see section 1.5.3). However, a study by Lowimore (1977) showed that in dogs, prednisolone concentrated in the

lymph preferentially to the portal blood during the first 20 minutes after intra-gastric administration of an aqueous radiolabelled drug solution; the lymph concentrations were 2.9 and 2.2. times those of the portal plasma 5 and 15 minutes after dosing. It was also shown that prednisolone and its metabolites preferentially distributed into the lipid rather than the aqueous phase of the lymph.

It was concluded from the literature review (see section 1.5) that the absorption of a drug into the lymphatic system may be increased by the introduction of a lipophilic grouping into the molecule and by stimulation of lymph flow by co-administration of oil. The lymphatic absorption of ethynyloestradiol in rats was enhanced by synthesis of the cyclopentyl ether ester and by administration in sesame oil (Gianina et al.1966). Similarly, Coert et al.(1975) showed that the biological activity of testosterone in rats, was enhanced by esterification of the steroid to testosterone undecanoate and by administration in arachis oil. Absorption of the ester was demonstrated to be via the lymphatic route. In a number of studies Bruni and his co-workers (1966,1970) showed in man that the oral absorption of several Δ-3-ketosteroids could be increased by administration of the enol ether derivative and by administration in oily solution (see section 1.1). Although no investigations were conducted to determine the underlying mechanisms enhanced lymphatic absorption was suggested.

It may therefore be possible to enhance the partitioning of prednisolone into the lymph by co-administration of the drug with oils and by the introduction of a lipophilic grouping into

the molecule. In the present study the plasma and lymph radioactivity following oral administration of ^{3}H-prednisolone and ^{3}H-prednisolone-21-palmitate (198nM/kg body weight) in 30µl arachis oil, Miglyol 812, liquid paraffin and water were determined in rats.

5.2 Materials and Apparatus.

5.2.1 Materials.

a)Prednisolone B.P. - M.S.D., Cramlington, Tyne & Wear.

b)Pyridine - B.D.H., Poole, Dorset.

c)Octanol - B.D.H., Poole, Dorset.

d)Palmitoyl chloride - Aldrich Chemical Co., Gillingham, Dorset.

e)Chloroform - Cambrian Chemicals, Croydon, Surrey.

f)Methanol - East Anglia Chemicals, Hadleigh, Sulfolk.

g)Ether - May & Baker Ltd., Dagenham, Essex.

h)Acetronitrile - Fisons, Loughborough.

i)Fisoflor - Fisons, Loughborough.

j)Brij 97 (polyoxyethylene oleyl ether) - Honeywell Atlas Ltd., Carshalton, Surrey.

k)Miglyol 812 - Dynamit Nobel, Slough, Bucks.

l)Arachis Oil - Evans Ltd., Speke, Liverpool.

m)Liquid Paraffin - Shell, London.

5.2.2 Apparatus.

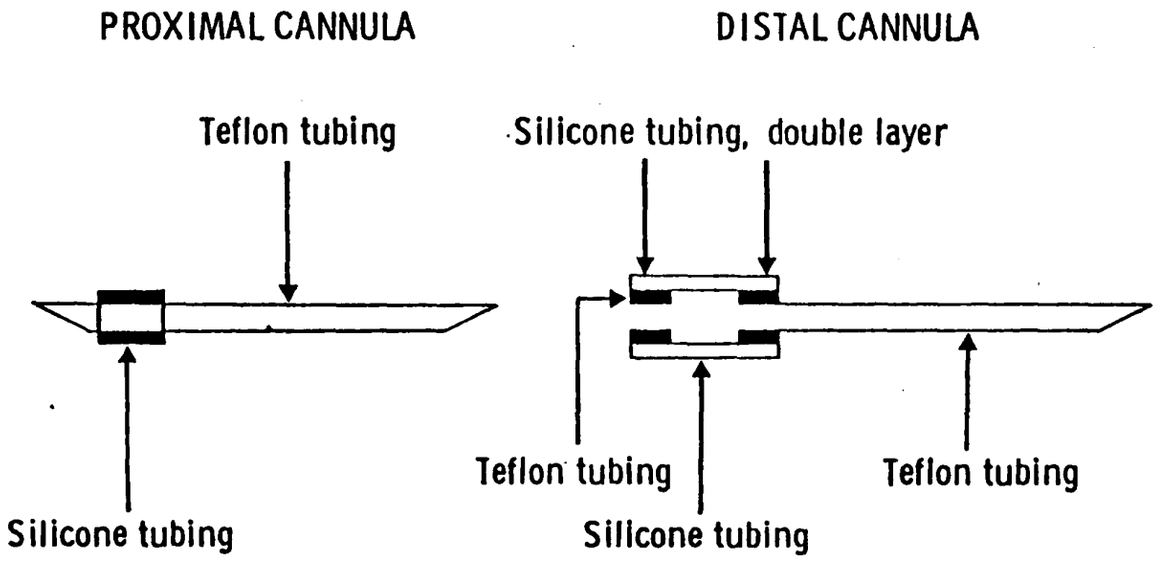
a)TLC plates - Kieselgel 60 F_{254}, Merck, Germany.

i)analytical - 5cm×10cm×0.25mm

ii)preparative - 20cm×20cm×5mm

Figure 5.1

DETAILS OF CANNULAE.



b)Rotary evaporator - Buchi, Switzerland.

c)Peristaltic pump - Watson-Marlow Ltd., Bucks.

d)Cannulae - (see figure 5.1) Teflon tubing - Portex Ltd., Hythe, Kent.

Silicone tubing - Esco Rubber, Teddington, Middlessex.

e)Homogeniser - Ultra-Turrax, Janke & Kunkel KG, Staufen, Breisgau.

f)Agla micrometer syringe - Wellcome Reagents Ltd., Beckenham, Middlessex.

i)HPLC - Varian Model 5000 Liquid Chromatograph, Model 5020/5030 Column Heater, Hewlett Packard Model 3390A Integrator, UV 50 Detector at 245nm, Zorbax ODS Column. Operating conditions - a)prednisolone - Temp.=40^0. Flow rate=1.0ml/min. Mobile phase= 45%acetronitrile and 55% water. R_t = 4.2 minutes.

b)prednisolone-21-palmitate - Temp.=40^0. Flow rate 1.5ml/min. Mobile phase=96% acetronitrile and 4% water. R_t= 10.8 minutes.

5.3 Methods.

5.3.1 Synthesis of Prednisolone-21-Palmitate.

Prednisolone (4g,11.1mM) was dissolved in dry pyridine (25mls) in an ice bath, under nitrogen. Palmitoyl chloride (6.1g,22.2mM) dissolved in ether (15mls), was added through a dropping funnel over a 30 minute peroid whilst the reaction mixture was stirred continually. After a further 30 minutes the

ice-bath was removed. TLC analysis of the reaction mixture on silica gel F_{254} using chloroform:methanol (8:1^{v}/v) as the solvent system, showed that after two hours there was no unreacted prednisolone. The reaction mixture was poured into 150mls iced water and the precipitate produced was extracted twice with chloroform. The chloroform phase was collected and washed with excess hydrochloric acid (1.0M) to remove the pyridine, and with sodium bicarbonate solution to remove excess hydrochloric acid and palmitic acid. The organic phase was then dried over sodium sulphate. Rotary evaporation in vacuo, of the filtered chloroform yielded a "glass" from which the product was crystallised by the addition of ether (approximately 10mls) and scratching of the flask surface. The crystals were collected and recrystallised from aqueous ethanol using charcoal to decolourise the solution. The purified crystals were dried to constant weight and the character and purity of the product was determined using NMR, IR, HPLC, TLC and micro-analysis.

The preceeding method was modified when synthesising ^{3}H-prednisolone-palmitate from ^{3}H-prednisolone due to the small quantities of starting material used. ^{3}H-prednisolone (13.6µg,2mCi,37.7nM) and prednisolone (1.42mg,3.94µM) were dissolved in dry pyridine (0.8ml) and palmitoyl chloride (approximately 0.002ml) was added. The reaction flask was left shaking for 48 hours when TLC analysis indicated that the reaction of prednisolone was complete. The pyridine was removed by rotary evaporation at 35^{0} in vacuo and then left for 12 hours under vacuum in a vacuum oven. The residue was dissolved

in methanol (approximately 1ml) to remove unreacted palmitoyl chloride and then evaporated to dryness by rotary evaporation in vacuo before being redissolved in chloroform. This solution was spotted onto a preparative TLC plate which was developed in chloroform:methanol (8:1^{v}/v) solvent system. The silica at the R_f corresponding to prednisolone palmitate (0.7) was collected and washed with 3x20ml aliquots of chloroform. These washings were collected and rotary evaporated in vacuo at 35^0, to dryness and then the residue was redissolved in a known volume of chloroform. The purity of the product was determined by TLC and the yield was calculated from the activity in known aliquots of the solution.

5.3.2 Solubility Determinations.

Prednisolone or prednisolone-21-palmitate (100mg) was added to each oil (6ml) contained in 10ml glass screw capped bottles and the bottles were vigoroursly shaken at 20^0 for 4 days. The oil was centrifuged at 2,000rpm for 15 minutes to sediment the undissolved drug and an aliquot was taken for analysis. Drug concentrations in Miglyol 812 and arachis oil were determined by HPLC following dilution of each oil sample with ethanol (1 in 10^{w}/v for Miglyol 812 and 1 in 50^{w}/v for arachis oil). Diluted ethanolic solutions of liquid paraffin (1 in 10^{w}/v) were assayed by UV spectroscopy at 245nm.

5.3.3 Partition Coefficient Determinations.

The octanol:water partition coefficient for prednisolone and for prednisolon-21-palmitate and the ether:water partition coefficient for prednisolone were determined. 5ml aliquots of drug solutions (1mg/ml) in water saturated octanol were shaken

with 25 ml octanol saturated water in stoppered glass centrifuge tubes for 10 hours at 20^0. The tubes were centrifuged at 2,000rpm for approximately 20 minutes to separate the phases. The aqueous phase was filtered through a 0.47um Millipore filter and a sample of the filtrate taken for analysis. Aliquots of the octanol phase were diluted with ethanol (1 in 10^V/v) prior to analysis. 10ml samples of prednisolone in water saturated ether (1.5mg/ml) were shaken with 20ml ether saturated water in stoppered glass centrifuge tubes at 20^0 for 6 hours. The mixture was allowed to settle and the phases separated. 1ml aliquots of each ether layer were evaporated to dryness under nitrogen and then re-constituted in acetonitrile (1ml) prior to analysis. Samples of the water phase was taken directly for analysis. All the samples were assayed by HPLC.

5.3.4 Preparation of Dosage Forms.

Each rat was orally dosed with 39.6nM prednisolone or prednisolone-21-palmitate containing 20µCi ^{3}H-prednisolone or ^{3}H-prednisolone-21-palmitate dissolved or suspended in 30ul vehicle. Solutions containing the tritiated and the cold material were evaporated to dryness under nitrogen, the required volume of vehicle added and a solution or suspension of the drug produced by sonication. Formulations containing prednisolone in arachis oil, Miglyol 812, liquid paraffin, aqueous ethanol (20% ethanol in water) and water, and prednisolone-21-palmitate in arachis oil were prepared in this way. A solution containing 20µCi ^{3}H-prednisolone-21-palmitate plus 0.25mg cold drug (=0.0042mM prednisolone-21-palmitate) in

1ml arachis oil was also prepared.

Solutions of prednisolone and prednisolone-21-palmitate for the *in situ* gut loop study were prepared using the non-ionic surfactant Brij 97. 10mg drug was dissolved in 1g Brij 97 by warming and made up to 100mls with 0.9% saline.

5.3.5 Oral absorption Study.

Groups (n=6 per group) of male, Wistar rats in the weight range 195-200g were starved for 18 hours with free access to water. 30ul volumes of the formulations were orally administered to the rats using an Agla micrometer syringe unit fitted with a dosing needle. Following oral dosing tail tip blood samples were taken, (see section 2.3.3), at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 24.0 hours after dosing. After 4 hours the rats were given free access to food. Weighed plasma samples were added to 10mls Fisoflor containing 50µl water to solubilise the plasma, and the activity determined by liquid scintillation counting.

The oral absorption of ^{3}H-prednisolone from 30µl Miglyol 812 , arachis oil, liquid paraffin, aqueous ethanol and water, and of ^{3}H-prednisolone-21-palmitate from 30µl arachis oil, was determined.

5.3.6 Lymphatic Absorption Study.

Starved male, Wistar rats as in section 5.3.5 were used for each formulation (n=4 per group). Following oral dosing the rats were anaesthetised, and jugular vein and thoracic duct cannulations performed as previously described (see section 3.3.1). Rats dosed with 30µl volumes were anaesthetised immediately whereas rats dosed with 1ml volumes were left for 5

minutes to allow the dose to be swallowed. Tail tip blood samples and lymph samples were collected at half-hourly intervals between 0.5 and 4.0 hours after dosing and the lymph flow was determined from the weight of the lymph collected. The lymphatic absorption of ^{3}H-prednisolone from 30ul arachis oil, Miglyol 812 and liquid paraffin, and of ^{3}H-prednisolone-21-palmitate from 1ml and 30ul volumes arachis oil were determined.

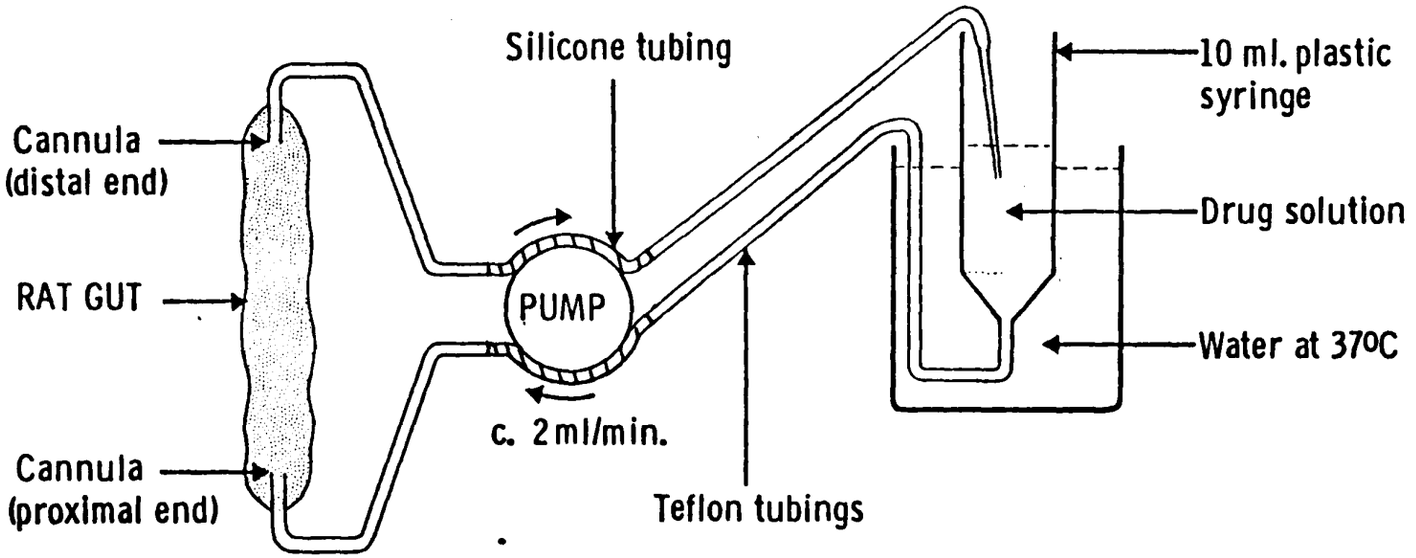
During the studies with prednisolone-21-palmitate, 100ul aliquots of lymph were collected at intervals between the samples taken for scintillation counting, and extracted with chloroform. The phases were separated by centrifugation at 3,000rpm for 10minutes, and the chloroform layer collected and evaporated to dryness under nitrogen. Each sample was reconstituted with 50µl chloroform and 10µl was spotted onto a TLC plate. Standard solutions of prednisolone and prednisolone-21-palmitate were also spotted onto each plate. The plates were developed in chloroform:methanol (8:1^{v}/v) solvent system, and uniform areas of silica around each spot were removed and the activity determined by scintillation counting.

5.3.7 In situ Gut Loop Study.

Starved male, Wistar rats as in section 5.3.5 were used (n=3 per group). Each rat was anaesthetised and the abdomen opened by a mid-line incision along the linea-alba. The stomach was exposed and a small plastic cannula inserted through a slit in the wall of the pyloric region, and passed into the small intestine. It was secured in place with a ligature placed

Figure 5.2

THE RECIRCULATION PERFUSION SYSTEM



behind the bulge in the cannula. A slit was made in the small intestinal wall just proximal to the ileo-caecal junction. The gut segment was cleaned by infusing with warm saline and the fluid removed by displacement with air. A second cannula was then introduced into the distal end of the gut segment and secured in place with a ligature, taking care not to damage the mesenteric blood vessels. The proximal cannula was fitted to the perfusion circuit, the pump started and the loop filled with drug solution. The loop was perfused through a recirculating system at a rate of 2ml/min using 25mls drug solution (see figure 5.2). 0.5ml samples were taken from the resevoir at time zero (the time at which the drug solution appeared at the distal end of the gut loop) and thereafter at 5 minute intervals until 35 minutes. Throughout the experiment the rats were kept on a heated table at 45^0 and anaesthesia maintained by administration of phenobarbital. At the end of the experiment the gut loop was excised, blotted dry and weighed. The absorption of prednisolone and prednisolone-21-palmitate from 0.9% saline containing 1% Brij 97 was determined in this way. Control experiments were performed to determine the adsorption of both drugs to the tubing of the re-circulating system.

5.3.8 In vitro Hydrolysis of Prednisolone-21-Palmitate.

Starved male Wistar rats as described in section 5.3.5, were orally dosed with 0.5ml arachis oil containing Sudan Black and left for 30 minutes before being anaesthetised. The abdomen was opened and the section of the small intestine (approximately 33cm) containing Sudan Black was excised. The

luminal contents were added to 10ml 0.1M phosphate buffer pH7.4 and mixed using a homogeniser. The gut wall was cut into sections and homogenised with 10ml 0.1M phosphate buffer pH7.4. 3ml aliquots of homogenised gut wall or luminal contents were added to flasks containing 5 mg prednisolone-21-palmitate suspended in 4.5ml 0.1M phosphate buffer+0.5ml arachis oil or 5.0ml 0.1M phosphate buffer respectively. The flasks were shaken at 35^0 for 1 hour and then the reaction mixture was extracted with 10ml chloroform. Samples of the chloroform layer were analysed by TLC using the solvent system of chloroform:methanol (8:1^v/v). Control experiments without either drug or rat tissue were performed.

5.4 Results.

5.4.1 Synthesis of Prednisolone-21-Palmitate.

The identity of the reaction product as prednisolone-21-palmitate was confirmed using NMR, IR, and micro-analysis. The product had a melting point of $138-140^0$. HPLC and TLC showed the presence of a low concentration impurity with slightly greater R_t and R_f values than prednisolone-21-palmitate, which was not removed by further recrystalisation of the product. It is likely that this impurity was prednisolone-21-stearate formed due to contamination of the palmitoyl chloride with stearoyl chloride. A final yield of 66% was obtained from the large scale preaparation and a 29.3% yield of ^{3}H-prednisolone-21-palmitate,

this product being 88.9% pure as determined by TLC.

5.4.2 Solubility Determinations.

The solubility of prednisolone and prednisolone-21-palmitate in arachis oil, Miglyol 812, and liquid paraffin is shown on table 5.1. The concentration of both drugs in liquid paraffin was below the detection limit of the UV spectrophotometer.

5.4.3 Partition Coefficient Determinations.

The ether:water partition coefficient for prednisolone was 1.06 and the octanol:water partition coefficient was 40.22 (mean of two determinations). The octanol water partition coefficient for prednisolone-21-palmitate was $> 3.05 \times 10^3$, the exact value could not be determined as the drug concentration in the water phase was below the limit of the detector.

5.4.4 Dosage Forms.

The solubility data suggested that prednisolone would not form a solution in Miglyol 812 or arachis oil at a concentration of 14.3ug/30µl. However microscopic examination of both preparations made in exactly the same manner as the original formulations but containing no radioactive material, failed to detect any drug particles. Prednisolone also formed a solution in aqueous ethanol but formed a suspension in liquid paraffin and water. Prednisolone-21-palmitate formed a fine suspension in the 30µl arachis oil formulation and a solution in the 1ml arachis oil formulation. Microscopic examination of the prednisolone and prednisolone-21-palmitate suspensions showed the particles to be conglomerates of needle shaped crystals. The individual crystals were of the order of 2-100µm

Table 5.1 -Solubility (mM/gram) of Prednisolone and Prednisolone-21-Palmitate in Arachis Oil, Miglyol 812 and Liquid Paraffin at 20^0.(Mean of 2 determinations).

	Arachis Oil	Miglyol 812	Liquid Paraffin
Prednisolone	5.16×10^{-4}	1.83×10^{-4}	$< 1.57 \times 10^{-4}$
Prednisolone-21-Palmitate	31.73×10^{-4}	12.67×10^{-4}	$< 0.95 \times 10^{-4}$

Prednisolone solubility in water at 25^0 = 6.74 mM/gram (Hayton & Grisafe 1975).

in size but this varied considerably and the true particle size distribution could not be determined.

5.4.5 Oral Absorption Study.

The plasma activity versus time profiles for the various formulations are shown in figure 5.3 and 5.4. The plasma activity was expressed in terms of the fraction of the total activity administered to allow for the difficulties in administering the exact dose of a suspension. From the data the peak plasma activity (C_{pmax}) annd the time of peak plasma activity (T_{max}) were determined and the area under the curve between 0 and 24 hours (AUC_{0-24h}) calculated using the trapezoidal method (see table 5.2). A comparison of these parameters for the different formulations was made using the unpaired Student's "t" test.

5.4.6 Lymphatic Absorption Study.

To show the comparative effects of the different vehicles on the uptake of activity into the lymph it was necessary to allow for the differences in lymph flow in each rat. The lymph activity at the end of the cannula in the thoracic duct, at the same time interval after dosing for each rat, was interpolated from the experimental data and the lymph:plasma activity ratio calculated (see table 5.3).

The activity in each spot from the TLC plates was determined in terms of dpm/100µl and the ratio of the prednisolone-21-palmitate to prednisolone activity for each sample calculated. For the 30µl arachis oil formulation this ratio remained relatively constant being 0.62±0.10, 0.587±0.09, and 0.680±0.16 (mean ± standard deviation) between 1-2, 2-3,

Figure 5.3

EFFECT OF DIFFERENT VEHICLES (30µl volumes) ON THE PLASMA CONCENTRATION OF TRITIUM FOLLOWING ORAL ADMINISTRATION OF ^{3}H-PREDNISOLONE (20µCi) TO RATS (Mean + S.E.M., n=6 per group).

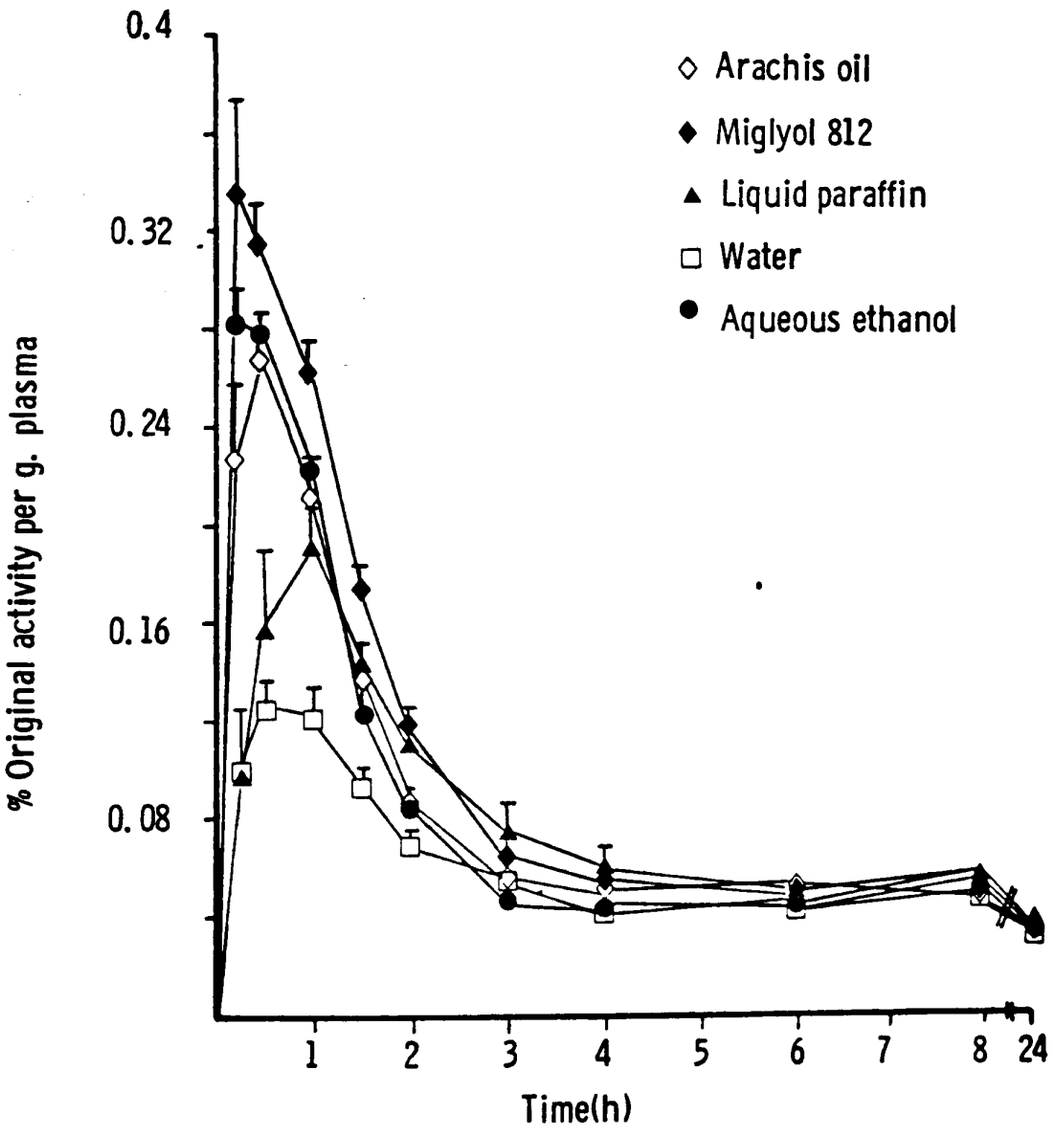


Figure 5.4

PLASMA CONCENTRATION OF TRITIUM FOLLOWING ORAL ADMINISTRATION OF ^{3}H-PREDNISOLONE OR ^{3}H-PREDNISOLONE-21-PALMITATE (20µCi) IN ARACHIS OIL (30µl) TO RATS. (Mean + S.E.M., n=6 per group).

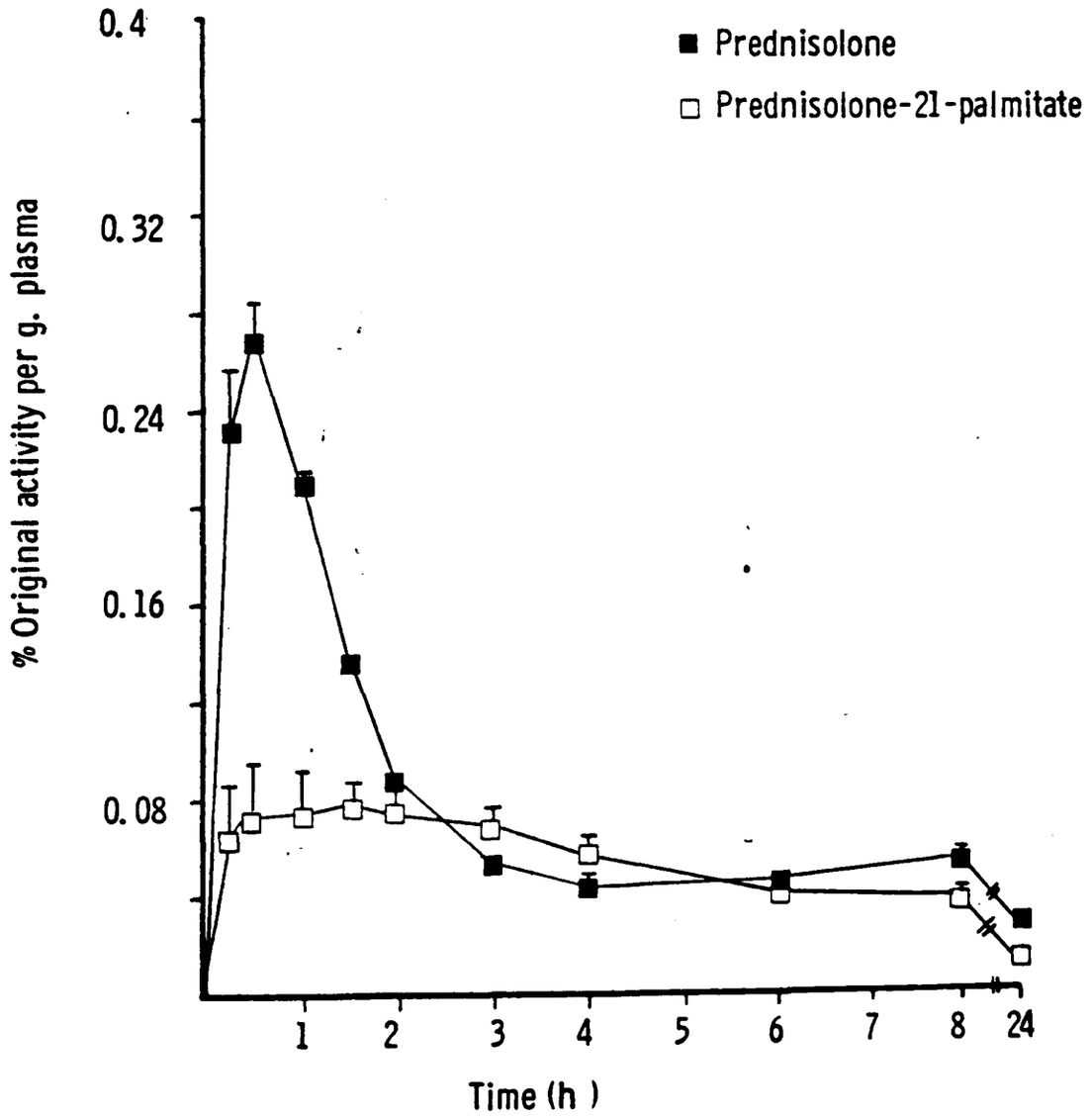


Table 5.2 -Absorption Data Following Oral Administration of Prednisolone (71.5µg/kg) in Different Vehicles (30µl volumes) to Rats(mean±S.D., n=6 per group).

Vehicle	AUC_{0-24h}	C_{pmax}	T_{max} (h.)
Arachis oil	13.34±0.87	2.96±0.47	0.38±0.15
Miglyol 812	16.05±1.03	3.57±0.78	0.31±0.13
Liquid paraffin	12.87±1.78	2.09±0.58	1.0±0.55
Aqueous ethanol (20%^{v}/v)	13.16±1.31	2.92±0.15	0.30±0.11
Water	10.64±1.13	1.47±0.24	0.71±0.33

AUC_{0-24} = area under the curve of fraction of dose administered against time between 0 and 24 hours, determined by the trapezoidal method.

C_{pmax} = maximum plasma activity expressed as the fraction of the dose administered.

T_{max} = time of maximum plasma activity.

For prednisolone-21-palmitate (118.7µg/kg) in arachis oil (30µl), AUC=8.43±0.78, C_{pmax}=1.06±0.30, T_{max}=1.60±0.96.

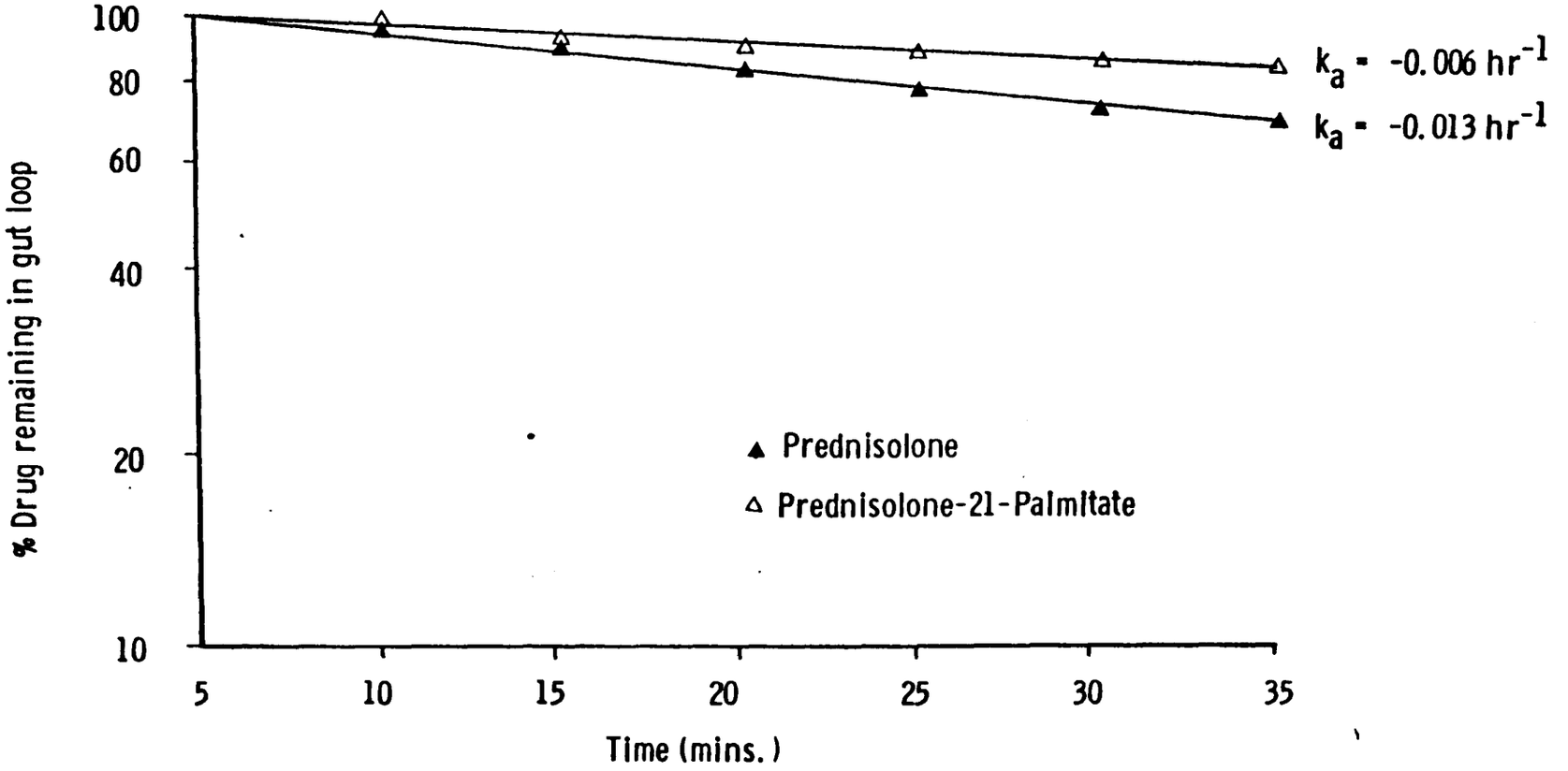
Table 5.3 - Lymph:Plasma Ratios of Tritium Concentrations (dpm/mg) in Anaesthetised Rats Following Oral Administration of ^{3}H-Prednisolone and ^{3}H-Prednisolone-21-Palmitate (20µCi) in Different Vehicles

(Mean ± S.D., n=4 per group).

Time / Formulation	0.5h.	1.0h.	1.5h.	2.0h.	2.5h.	3.0h	3.5h	4.0h
Prednisolone+ arachis oil 30µl	0.62 ±0.03	0.79 ±0.21	0.73 ±0.09	0.77 ±0.16	0.85 ±0.15	0.70 ±0.14	1.05 /	0.73 /
Prednisolone+ Miglyol 812 30µl	0.81 ±0.18	0.77 ±0.01	0.74 ±0.11	0.75 ±0.12	0.77 ±0.15	0.78 ±0.12	0.86 ±0.25	0.59 ±0.56
Prednisolone+ Liquid paraffin 30µl	0.79. ±0.53	0.67 ±0.05	0.76 ±0.15	0.80 ±0.14	0.85 ±0.12	0.77 ±0.07	0.74 ±0.18	0.66 ±0.06
Prednisolne-21-palmitate +arachis oil 30µl	0.58 ±0.33	0.62 ±0.24	0.76 ±0.15	0.80 ±0.25	0.83 ±0.21	0.85 ±0.12	0.72 ±0.60	1.12 ±0.42
Prednisolone-21-palmitate +arachis oil 1ml	0.88 ±0.11	0.99 ±0.19	0.93 ±0.08	1.03 ±0.11	1.18 ±0.17	1.06 ±0.15	0.81 ±0.37	/ /

Figure 5.5

ABSORPTION OF PREDNISOLONE AND PREDNISOLONE-21-PALMITATE IN 1% BRIJ 97 SOLUTION FROM A SMALL INTESTINAL GUT LOOP IN THE RAT (mean values, n=3 per group, k_a = absorption rate constant).



and 3-4 hours after dosing respectively. Due to difficulty in maintaining a steady state of anaesthesia in the animals dosed with 1ml volumes of arachis oil insufficient lymph was collected for analysis.

5.4.7 In Situ Gut Loop Study.

The drug concentration in the test solution at different time intervals was expressed as a percentage of the drug concentration at t=5minutes. This allowed for dilution effects due to saline remaining in the gut loop. From the log-linear time plot of percentage drug remaining versus time, the absorption rate constant for each drug was determined (see figure 5.5).

5.4.8 In Vitro Hydrolysis of Prednisolone-21-Palmitate.

No prednisolone was detected in the extracts incubated with homogenised gut wall. Very low concentrations of prednisolone were detected in the extracts incubated with homogenised luminal contents.

5.5 Discussion.

The oral absorption of prednisolone was not enhanced by administration in oily vehicles and was significantly reduced following esterification to prednisolone-21-palmitate. In addition, neither drug was selectively absorbed via the lymphatic pathway.

The plasma activity versus time profiles constructed represent the concentration of ^{3}H-label in the plasma and not the concentration of drug. No attempt was made to follow drug

metabolism and therefore the distribution of the ^{3}H-label within the plasma. A study by Pickup et al.(1977) showed that in human subjects following intra-venous administration of ^{3}H-prednisolone the total counts in the plasma up to about 1.5 hours after dosing were almost entirely due to prednisolone and its major metabolite prednisone. Later, further metabolites such as glucuronides were produced. Thus the total counts do not reflect the kinetics of prednisolone alone. In the present study the absorption phase is all that is of interest, and as the presence of the ^{3}H-label in the plasma represents absorption of the drug molecule this can be used to determine the effect of different oily vehicles on drug absorption.

The levels of plasma activity following administration of ^{3}H-prednisolone varied considerably with the vehicle in which it was administered (see figure 5.3). This did not result from differences in the lymphatic absorption of the drug. The lymph:plasma activity ratio remained less than one in each experiment (see table 5.3) suggesting that there was no selective lymphatic uptake and that the activity present in the lymph was derived from the plasma. Prednisolone was in solution in the three vehicles (Miglyol 812, arachis oil and aqueous ethanol) which yielded the highest peak plasma levels and in suspension in the other two formulations (liquid paraffin and water). The difference in plasma activity levels may therefore be due to the physical form in which the drug was administered. As dissolution of suspension particles has to occur prior to absorption this may also have contributed to the delay in the peak activity levels that were observed with the liquid

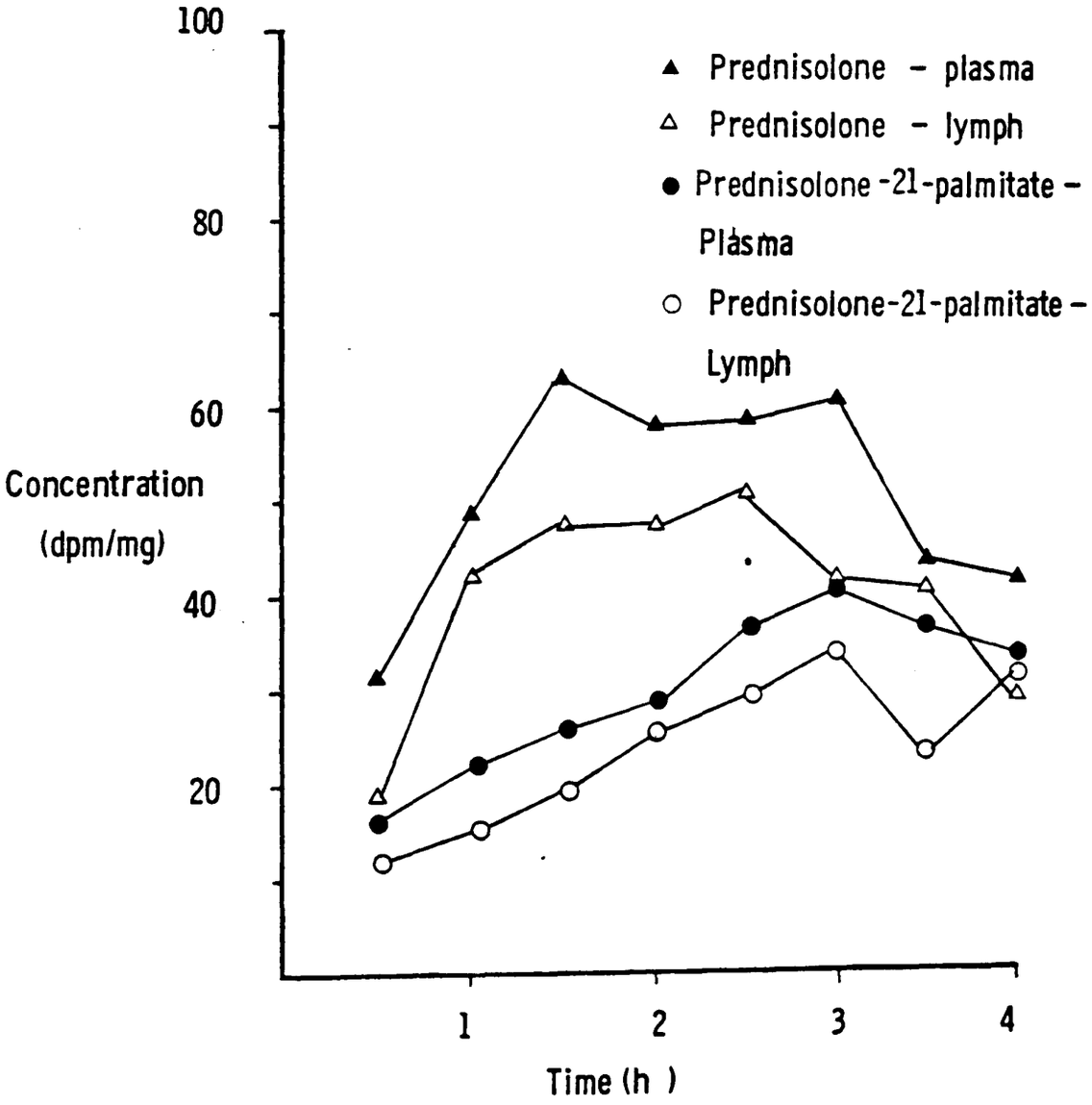
paraffin and water formulations compared with the arachis oil, Miglyol 812 and aqueous ethanol formulations ($p<0.05$ for T_{max} values).

Solubility determinations suggested that prednisolone was insufficiently soluble in arachis oil and Miglyol 812 to form a solution at the required dose level of 14.3µg/30µl (= 1.30×10^{-3}/gram) (see table 5.1). The formation of prednisolone solutions in these oils with the experimental formulations may have resulted from differences in the physical form of the prednisolone used. The solubility determinations were made using prednisolone powder whilst the formulations were produced from prednisolone crystallised from toluene/ethanol solution.

The low oil solubility and octanol:water partition coefficient determined for prednisolone suggested that the drug was not sufficiently lipophilic to exhibit selective lymphatic absorption. By esterification of prednisolone to prednisolone palmitate the solubility of the drug in both arachis oil and Miglyol 812 and the lipoophilicity of the molecule as determined from the octanol:water partition coefficient, was significantly increased. The experimental value for the log ether:water partition coefficient for prednisolone (0.027) was of the same order as that determined by Flynn (1971), 0.053. On the basis of the steroidal ether:water partition coefficients determined by Flynn (1971), McGowan et al.(1979) proposed a theoretical model for the estimation of this parameter for other steroidal molecules. Using this model the log ether:water partition coefficient for prednisolone-21-palmitate was estimated to be of the order of 9.00. As DDT has a log

Figure 5.6

LYMPH AND PLASMA CONCENTRATION OF TRITIUM IN ANAESTHETISED RATS FOLLOWING ORAL ADMINISTRATION OF ^{3}H-PREDNISOLONE AND ^{3}H-PREDNISOLONE-21-PALMITATE (20µCi) IN ARACHIS OIL (30µl) (mean values, n=4 per group).

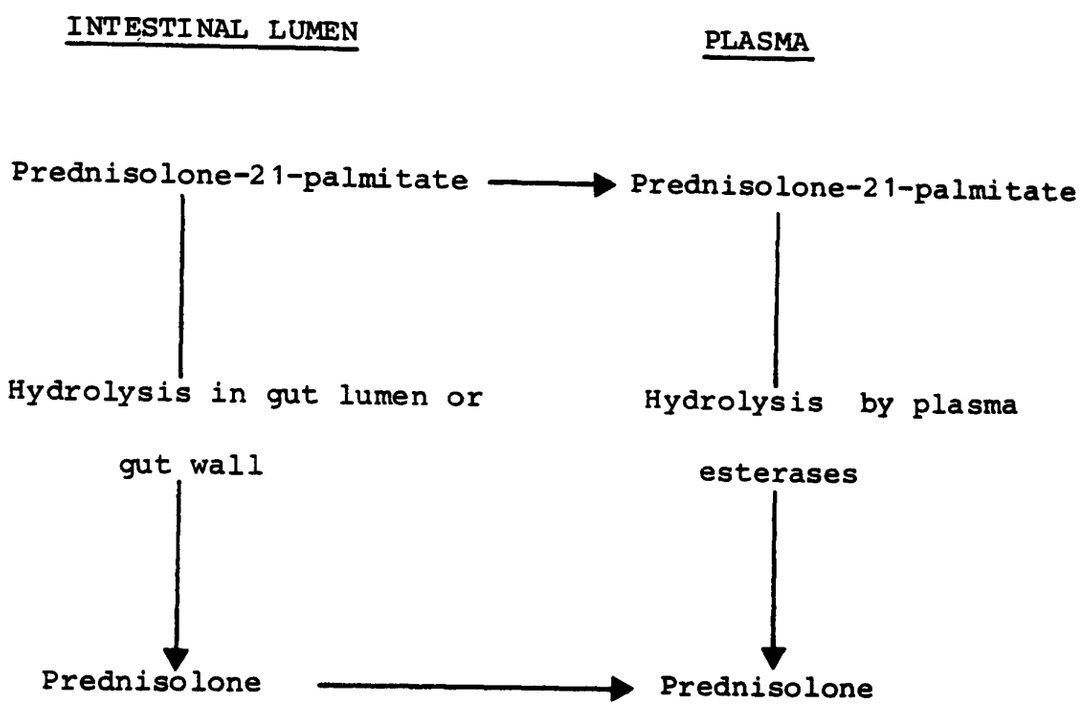


octanol:water partition coefficient of only 6.19 (O'Brien 1975) it was anticipated that prednisolone-21-palmitate would be sufficiently lipophilic to be lymphatically absorbed. However this estimation does not take into account factors arising from the addition of a long chain alkyl group to the original molecule, such as micelle formation, which could introduce errors into the calculation.

The levels of plasma activity following administration of ^{3}H-prednisolone-21-palmitate in 30µl of arachis oil were significantly less than that after ^{3}H-prednisolone administration in the same vehicle (see figure 5.4). There was no selective uptake of prednisolone-21-palmitate into the lymph, the lymph:plasma activity ratio remaining below unity throughout the experimental period (see figure 5.6 & table 5.3).

There are several possible explainations for these results. The difference in the absorption may be due to the difference in the physical form of the drugs, administered, prednisolone was in solution whereas prednisolone-21-palmitate was in suspension. The greater size of the prednisolone-21-palmitate molecule may have reduced the rate of drug absorption into the plasma. It is possible that hydrolysis of the ester had to occur prior to absorption and that this was the rate-limiting step. Hydrolysis of straight chain fatty acid esters of several natural and foreign compounds has been shown to be essential prior to absorption e.g. cholesterol esters (Shiratori et al.1965,Vahouny & Treadwell 1958,1964) Vitamin A (Lawrence et al.1966,Yeung & Veen-Baigent 1972,Mahadevan et

al.1966) Vitamin E (Gallo-Torres et al.1971,Gallo-Torres 1970,1973). Chloramphenicol palmitate (Glazko et al.1958).The possible mechanisms for prednisolone-21-palmitate absorption from the gastro-intestinal tract can be represented by the following scheme:-



The detection of both prednisolone and prednisolone-21-palmitate by TLC analysis of lymph samples demonstrates that at least a proportion of prednisolone-21-palmitate is absorbed in the unhydrolysed form. It is possible that the drug is absorbed only as the ester and is then rapidly hydrolysed by the plasma esterases, prednisolone being detected in the lymph due to redistribution from the plasma. Data derived from the gut loop experiments shows that the rate of absorption of prednisolone is signficantly faster than that of prednisolone-21-palmitate although the effect of the Brij 97 is unknown. This suggests

that if hydrolysis of prednisolone-21-palmitate does occur within the gut lumen the greater portion of the drug will be absorbed as prednisolone. Unfortunately, the data from the in vitro hydrolysis studies were inconclusive. Janssen et al.(1962) demonstrated the in vitro hydrolysis of prednisolone-21-monoacetate by homogenised rat intestine, which suggests that the prednisolone molecule is liable to hydrolysis at the 21 position.

From the data available it is proposed that the greater portion of prednisolone-21-palmitate is hydrolysed within the gut lumen and is absorbed as prednisolone into the plasma. However, a small portion of the ester is absorbed into the plasma unchanged but at a slower rate than the prednisolone due the larger size of the molecule. The administration of prednisolone-21-palmitate as a suspension may have reduced the rate of enzymatic hydrolysis, as has been demonstrated in studies with chloramphenicol palmitate (Glazko et al.1958) and testosterone undecanoate (Coert et al.1975), thereby limiting the absorption of prednisolone and reducing the overall levels of plasma activity. Further investigation is obviously required to confirm these proposals.

The lymphatic absorption of DDT was promoted using 1ml volumes of arachis oil. The volume of arachis oil and the dose of prednisolone-21-palmitate were therefore increased to 1ml and 25mg respectively to determine whether lymphatic absorption could be promoted by these elevated levels of drug and vehicle. Although the mean lymph:plasma ratio rose above 1 between 2 and 3 hours after dosing this was not observed in all the

experimental animals. Increasing the oil volume and the dose of the drug did not therefore promote lymphatic absorption.

The present study has shown that the absorption of prednisolone cannot be enhanced by oral administration in oily vehicles nor is there any selective uptake into the lymph as suggested by Lowrimore (1977). Esterification of prednisolone to form the more lipophilic moiety prednisolone-21-palmitate did not enhance lymphatic absorption.

CHAPTER 6.

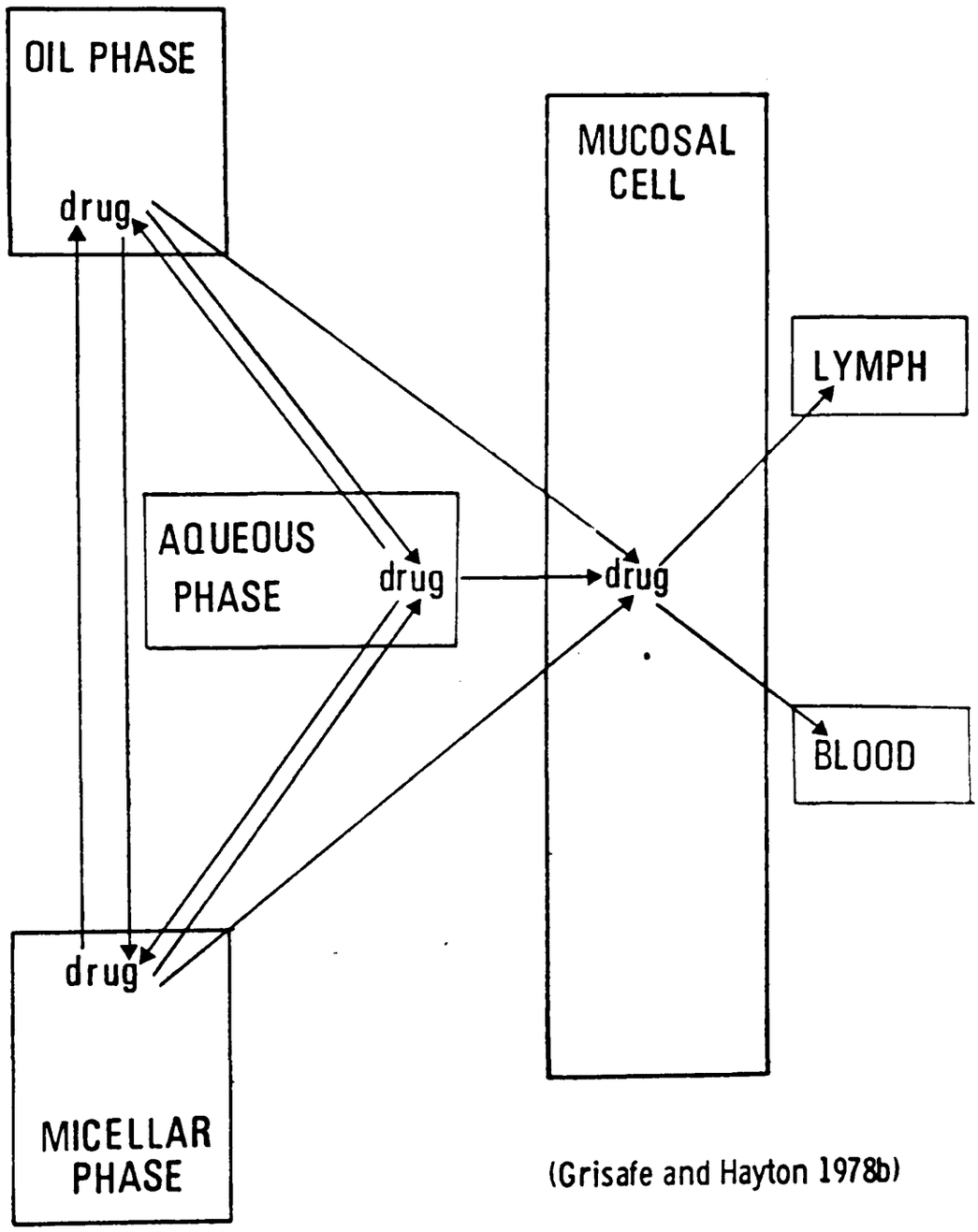
FINAL DISCUSSION AND CONCLUSIONS.

In the present investigation the oral absorption of DDT in rats was shown to be altered by the presence of lipids. The plasma DDT concentration versus time profiles following administration of DDT in solution in 1ml volumes of different vehicles varied considerably (see figure 2.3), with the rank order for total DDT absorption over 0-24 hours being arachis oil > Miglyol 812 = water containing 6% Tween 80 > liquid paraffin. Investigation of the lymphatic absorption of DDT in anaesthetised rats, showed the DDT concentration in thoracic duct lymph to be highest following administration in arachis oil, there being no difference between Miglyol 812 and liquid paraffin. Studies on the effect of these oils on gastro-intestinal motility showed that the marker ^{99m}Tc-sulphur colloid, emptied from the stomach and passed through the intestinal tract more rapidly in the presence of 1ml volumes of liquid paraffin than in the presence of equivalent volumes of either Miglyol 812 or arachis oil. Thus increased DDT absorption in the presence of arachis oil is due in part to enhanced lymphatic absorption, whereas reduced DDT absorption in the presence of liquid paraffin is due in part to the shorter gastro-intestinal residence time of this oil.

The variation in these physiological mechanisms may be attributed to differences in the chemical composition of the oils. As stated in the introduction arachis oil is comprised of long chain saturated and unsaturated fatty acids, Miglyol 812

Figure 6.1

DISTRIBUTION OF A LIPOPHILIC DRUG AMONG THE THREE PHASES OF A LIPID DIGESTION MIXTURE WITH THE POTENTIAL FOR DRUG ABSORPTION FROM EACH PHASE.



(Grisafe and Hayton 1978b)

of saturated medium chain fatty acids whereas liquid paraffin is a non digestible mineral oil. Such differences in structure may also affect the physicochemical processes occuring within the gut during absorption. A lipid digestion mixture within the small intestinal lumen is a 3-phase system comprised of an oil phase, an aqueous phase and a micellar phase. Drug absorption can occur from one or more of these phases (see figue 6.1), although reports in the literature have suggested that drug is absorbed from either the aqueous or the micellar phase; absorption from the oil phase being negligible (Armstrong & James 1980). Absorption of DDT probably occurs only from the micellar phase as it is a highly lipophilic, water insoluble structure, with an octanol:water partition coefficient of approximately one million. The presence of different lipids within the digestion mixture can alter the partitioning of DDT between the three phases and hence absorption.

Following administration of Miglyol 812 the intestinal digestion mixture consists of Miglyol 812, simple bile salt micelles and an aqueous phase. As the oil is digested and absorbed, DDT molecules partition into the micellar phase from which absorption can occur. The capacity of the micellar phase to solubilise DDT is limited by the concentration of the bile salt micelles and the solubility of the DDT molecule within the micelle. If this capacity is exceeded DDT precipitates out in the aqueous phase from which it is not absorbed and is excreted in the faeces. As Miglyol 812 is unlikely to stimulate bile flow due to the nature of the constituent fatty acids, the absorption of DDT may be limited by the bile salt concentration

within the gut. Water containing 6% Tween 80 is unlikely to stimulate bile flow so DDT absorption from this vehicle may also depend on the bile salt concentration within the rat gut. This would explain the similarity in the plasma DDT concentration time profiles for the two vehicles (see figure 2.3).

The digestion products of Miglyol 812 are sufficiently water soluble to be absorbed into the portal vein. Fatty acids that are absorbed into the lymph stimulate the formation of VLDL rather than chylomicra as they are saturated in structure. DDT transported in the lymph has been shown to be present in the triglyceride core of chylomicra (Sieber et al.1974). Thus neither Miglyol 812 nor its digestion products are likely to aid DDT absorption into the lymph.

Digestion of arachis oil liberates long chain saturated and unsaturated fatty acids which are incorporated into bile salt micelles forming mixed bile salt micelles. This increases the capacity of the micelles to solubilise lipophilic molecules such as cholesterol and DDT (see section 1.5). In addition, bile flow may be stimulated by the LCFA. Thus the capacity of the bile salt micelles to solubilise DDT is increased in the presence of arachis oil more than in the presence of Miglyol 812. It has been suggested that mixed bile salt micelles containing long chain unsaturated fatty acids increase mucosal membrane permeability (Muranishi et al.1980), which may further aid DDT absorption. The presence of LCFA within the intestinal lumen may enhance the lymphatic absorption of DDT due to:-

a)stimulation of lymph flow, although MCFA stimulate lymph flow

the effect is only about 75% that of LCFA (Barrowman & Turner 1977,1978),

b)stimulation of chylomicra synthesis by unsaturated fatty acids,

c)association of DDT with LCFA as they pass through the mucosal cells into the lymph vessels.

Thus arachis oil, via a series of physiological responses to its digestion products, may promote the lymphatic absorption of DDT by increasing the capacity of bile salts to solubilise D D T , the mucosal membrane permeability and the capacity of the lymph to transport D D T.

Liquid paraffin is non-digestible and a large proportion of the oil is retained within the small intestinal lumen. However, as a small quantity is normally absorbed (Albro & Fishein 1970), DDT may be absorbed directly with the oil phase or following partitioning into the micellar phase. As the volume of oil absorbed is small and partitioning into bile salt micelles is limited, not being enhanced by the formation of mixed bile salt micelles, a relatively large proportion of DDT is probably retained in the liquid paraffin and excreted in the faeces. Similar findings have been reported for cholesterol and DDT in the presence of sucrose polyester, a non-absorbable lipid (Mattson et al.1976,Volpenhein et al.1980). A marked increase in DDT absorption was observed following emulsification of liquid paraffin. As absorption of liquid paraffin has been shown to be enhanced by emulsification (Frazer et al.1944) this result shows the dependance of DDT absorption on that of the oil. The rapid transit of large

volumes of liquid paraffin through the gastro-intestinal tract probably further reduced DDT absorption. In anaesthetised rats gastro-intestinal motility is reduced and DDT absorption from liquid paraffin was found to be equal to that from Miglyol 812. This suggests that the difference in oral absorption of DDT from liquid paraffin and from Miglyol 812 is due to the difference in the gastro-intestinal transit of the two oils. The increase in DDT absorption from arachis oil as compared with liquid paraffin is probably due to a combination of the effects of slower gastro-intestinal transit and more favourable conditions for lymphatic absorption.

The chemical composition of a lipid vehicle is therefore critical, as it determines the complex series of physiological and physicochemical responses occuring within the gastro-intestinal tract during lipid digestion, which in turn influences drug absorption within the gut. In addition, lipid absorption would appear to be essential for significant absorption of a lipophlic drug.

The present investigation also showed that the volume of lipid administered is an important parameter in drug absorption. Differences were observed in the plasma DDT concentration time profiles when the same dose of DDT was administered in 1ml and 2ml volumes of arachis oil, liquid paraffin and Miglyol 812 (see figure 2.4-2.6). Reducing the oil volume administered from 1ml to 30µl had a marked effect on the differences between the plasma profiles for the individual oils (see figure 2.3-2.7).

With the exception of Hori et al.(1977) and Yamahira et

al.(1978&1979) the importance of oil volume was not considered with respect to either the experimental design or the discussion of the results, in any of the investigations sited in the introduction. In many animal studies the oil volume was large, beyond comparison with a human clinical dose, thereby distorting the characteristics of the dosage form. In a series of studies in the rat, Yamahira et al.(1978&1979) clearly demonstrated that changing the oil volume can have a marked effect on drug absorption. It was considered that from 10 to 100µl MCT/rat the observed decrease in the absorption of SL512, a poorly water soluble anti-inflammatory agent, was mainly attributable to a decrease in the rate of digestion of the lipid after gastric emptying. Above 200µl/rat various factors such as depression of gastric emptying rate and intestinal motility, and a decrease in the digestion rate of the lipid, were considered to contribute to the decrease in drug absorption. Thus changing the oil volume may alter drug absorption by affecting the various physiological mechanisms involved in fat digestion such as, gastro-intestinal motility, the rate and extent of oil digestion, bile flow, lymph flow and chylomicra synthesis. Reducing the oil volume administered from 1ml to 30µl was shown to have a marked effect on the gastric emptying and total gut transit of ^{99m}Tc-sulphur colloid (see chapter 4). Delayed gastric emptying and intestinal transit, caused by increasing the oil volume from 1-2ml, may account for the observed delay in the peak plasma concentration of DDT following administration in arachis oil and in Miglyol 812 (see figure 2.4 & 2.6). However, as liquid paraffin has no

inhibitory effect on gastro-intestinal motility, increasing the oil volume served only to increase the laxative action of the oil.

Yamahira et al.(1978) considered that the dose volume for lipid formulations for human clinical use should be restricted to 0.5ml/capsule or as an upper limit 5ml/human. On the basis of this they decided that 20µl/200g rat was a suitable dose volume. In the present investigation an oil volume of 30µl/200g, 10ml/70kg body weight was used. However, such a scaling down of the dose volume on a body weight basis is of limited use as it does not take into consideration other important physiological factors such as, differences in the basic rate of metabolism and the absence of a gall bladder in the rat. It is therefore apparent that the effect of oil volume on drug absorption should be carefully considered especially in the design of experimental protocol.

The plasma DDT concentration versus time profiles for DDT administered in 30µl volumes of arachis oil, liquid paraffin and Miglyol 812 did not exhibit the marked differences that were observed between the 1ml volumes. However, there were still distinct differences in DDT absorption from the oils which can not be explained in terms of gastric emptying rates and intestinal transit of the oils. Investigation of the lymphatic absorption of DDT from this oil volume was inconclusive and further studies are required.

The oral absorption of prednisolone from 30µl volumes of arachis oil, liquid paraffin, Miglyol 812, aqueous ethanol and water was found to be independant of the nature of the vehicle

but dependant on the physical form of the drug. Higher levels of plasma activity were achieved when the drug was administered in solution rather than in suspension. It is probable that the drug is sufficiently water soluble to be absorbed directly from the aqueous phase of the digestion mixture into the portal vein. Therefore drug absorption would only be affected by the presence of oils if the prednisolone partitioned more favourably into one oil than another.

The lymphatic absorption of prednisolone was not stimulated by the presence of arachis oil, Miglyol 812 or liquid paraffin. Attempts to promote selective lymphatic absorption of prednisolone by synthesis of prednisolone-21-palmitate, a more lipophilic structure, were also unsuccessful, even when the ester was administered in 1ml arachis oil. Further, the oral absorption of tritium was reduced by administration with ^{3}H-prednisolone-21-palmitate compared with ^{3}H-prednisolone. It was suggested that this was because:-

a)prednisolone-21-palmitate was administered as a suspension and prednisolone as a solution,

b)there is a difference in the intestinal absorption rate of the two compounds, the absorption rate constant for prednisolone-21-palmitate being less than that for prednisolone,

c)ester hydrolysis must occur prior to absorption; this has been demonstrated for a number of esters of natural compounds absorbed via the lymph, as discussed in section 5.5.

For a drug to be absorbed into the lymph it has to be

preferentially taken up against a far higher blood flow than lymph flow, even in conditions of fat stimulated lymph flow. One of the major criteria for selective lymphatic absorption is lipophilicity, as discussed in section 1.5. It is likely that lymphatic absorption may only be enhanced by co-administration with oil if the molecule being considered exhibits selective lymphatic absorption from a non oily environment. In the presence of a suitable lipid the partitioning of such a molecule into the lymph may be enhanced by lipid stimulation of suitable conditions for lymphatic absorption, that is increased lymph and bile flow, mixed bile salt micelle formation, chylomicra synthesis and possibly increased membrane permeability. DDT absorption was shown to be primarily via the lymphatic route and in the presence of arachis oil, absorption was enhanced. However, prednisolone was not selectively absorbed into the lymph from an aqueous vehicle and lymphatic absorption was not stimulated by administration in arachis oil.

Modification of drug molecules, for example by esterification, may promote selective lymphatic absorption by increasing lipophilicity. Kimbel (1961) suggested that steroids esters not hydrolysed by intestinal esterases are transported by the lymph to a greater extent than the corresponding free compounds. However, Schedl (1965) concluded that except for certain configurations these esters are rapidly split by the intestinal mucosa. Carbonyl ester hydrolase found in the pancreatic juice has been shown to be capable of hydrolysing the ester bond of a variety of LCFA, including monoglycerides and cholesterol (Barrowman & Borgstrom 1968). <u>In vitro</u> studies

with homogenised rat gut showed that the esters of steroid hydroxyl groups at 3α, 3β, 6β, sec 17β and 21 positions are hydrolysed whereas steroids acylated at 11α and tert 17α-and-β-OH groups tend to be stable (Janssen et al.1962). It would appear that the lymphatic absorption of drug molecules may only be stimulated if the modified structure is stable within the gastro-intestinal tract. Meli (1963) suggested that the biological activity of testosterone propionate in rats was the same as testosterone because the ester grouping was hydrolysed prior to absorption. When ^{3}H-testosterone-17-undecanoate was administered in arachis oil to rats, a large proportion was metabolised in the intestinal wall and absorbed into the portal vein, but the unmetabolised fraction and two metabolites containing the undecanoate grouping, were exclusively absorbed via the lymphatic system (Coert et al.1975). The lymphatic absorption of ethynyloestradiol was found to be enhanced by administration as ethynylestradiol cyclopentyl ether (Gianina et al.1966). Similarly the oral absorption, as determined by urinary excretion, of androstendione and progesterone was enhanced by administration of the 3-cyclopentyl enol ether form of each steroid, it being suggested that this was due to enhanced lymphatic absorption (Bruni et al.1966,1970). Thus the addition of a stable lipophilic grouping to the steroid structure increased the oral/lymphatic absorption. However, the biological activity of these modified steroids was not investigated.

The potential for promoting lymphatic absorption by increasing the lipophilicity of the drug molecule would appear

to be limited by the following requirements:-

a)the modified drug molecule must be stable within the environment of the gastro-intestinal tract,

b)following absorption the active drug must be rapidly released and the pharmacological properties of the drug unimpaired,

c)the modified drug molecule must not be too large to be absorbed across the intestinal mucosal membrane.

The potential of the lymphatic pathway for drug absorption would seem to be limited, being applicable only to highly lipophilic drugs or to pro-drugs which are suitably modified to increase lipohilicity and are stable within the gastro-intestinal lumen. Promotion of lymphatic absorption is not due solely to enhanced lymph flow as previously suggested (DeMarco & Levine 1969), as liquid paraffin and arachis oil had similar lymphagogue effects. Further investigation is required to determine whether the increase in lymphatic absorption of DDT in the presence of arachis oil was due to stimulation of:-

a)bile flow,

b)mixed bile salt formation and/or membrane permeability,

c)chylomicra synthesis.

Enhancement of drug absorption may be achieved by retardation of gastro-intestinal motility following administration of oils, and stimulation of this physiological response may have far wider application than lymphatic absorption.

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