FACTORS INFLUENCING THE INTESTINAL ABSORPTION OF HYDROCARBON CARCINOGENS

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by

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ABSTRACT

This study was undertaken to determine mechanisms involved in absorption of lipophilic xenobiotics and to define factors which may alter their bioavailabilities. First examined were the *in vitro* partitions of 3 hydrocarbon carcinogens, 7,12-dimethylbenz(a)anthracene (DMBA), 3-methylcholanthrene and benzo(a)pyrene and one polychlorinated biphenyl compound, between an emulsified oil phase and a mixed micellar solution simulating intestinal content. Nearly identical behaviours were exhibited by the 4 hydrocarbons whose solubilization was directly dependent upon the formation of bile salt micelles. Solubilization was enhanced by increasing concentrations of bile salt, reduction of triglyceride concentration and the formation of mixed rather than pure bile salt micelles. Micelles containing long-chain fatty acid and monoglyceride were better able than medium-chain lipid mixed micelles to solubilize the hydrocarbons. The second part of the study examined intraluminal factors which might alter hydrocarbon bioavailability *in vivo*. Conscious, restrained rats were administered ^{3}H-DMBA in various lipid test meals via duodenal cannulae. Subsequent biliary excretion or plasma levels of radiolabel were monitored using common bile duct or tail arterial catheters. In the absence of luminal bile, DMBA was absorbed from triglyceride vehicles to an appreciable extent. Luminal bile replacement did not

influence radiolabel recovery from the medium-chain triglyceride (MCT) but dramatically enhanced it from long-chain triglycerides (LCT) whether mono- or polyunsaturated. With luminal bile present, plasma levels and biliary recovery were significantly greater from both LCTs than from the MCT carrier. In a 6 hour study period, plasma levels of radiolabel were inversely related to the volume of triolein carrier. Rats receiving intraduodenal infusions of ^{3}H-DMBA biliary products subsequently eliminated 83% of the dose in their bile within 24 hours. In rats with lymphatic fistulae, biliary radiolabel excretion was used as an indirect index of portal transport of hydrocarbon. Increasing doses of radiolabel were administered then relative lymphatic and biliary radiolabel recoveries were monitored. Despite a 2000-fold variation in dose (10 µg vs 20 mg) total recoveries as percentage administered were similar. Although some degree of increased lymphatic transport was evident with increasing DMBA burden, at least 75% of absorbed radiolabel was transported in portal blood. This study demonstrates that conditions favouring micellar solubilization of hydrocarbon in the intestinal lumen produce greatest bioavailabilities. Portal transport accounts for the major route of hydrocarbon (or derivative) transport following ingestion with the lymphatic system playing only an auxiliary role. Once in the body, the compound is efficiently excreted in the bile and extensive enterohepatic recycling ensues.

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ABBREVIATIONS:

B(a)P	benzo(a)pyrene
CMC	critical micellar concentration
cpm	counts per minute
DMBA	7,12-dimethylbenz(a)anthracene
dpm	disintegrations per minute
GLC	gas liquid chromatography
h	hour
3MC	3-methylcholanthrene
MCT	medium-chain triglyceride
min	minute
NaTC	sodium taurocholate
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
rpm	revolutions per minute
SEM	standard error of the mean

INTRODUCTION

I. Background information

A. Lipophilic xenobiotics

Many toxic substances in the environment, including certain well-recognised carcinogens, are highly lipophilic and exist in significant amounts in the food chain. Polycyclic aromatic hydrocarbons (PAHs), many of which are carcinogenic, have been detected in foodstuffs throughout the world. Such occurrence can result from methods of preparation such as curing meat and fish by smoke (Gray and Morton, 1981). In the early 1960's Lijinsky and Shubik (1964) detected the presence of many PAHs including the carcinogen benzo(a)pyrene (B(a)P) in charcoal-broiled meat. Even produce grown in or near urban areas is susceptible to contamination by external surface deposition of air-borne pollutant hydrocarbons (Lo and Sandi, 1978; Shabad, 1980). Particulate matter in cigarette smoke containing adsorbed carcinogenic PAHs is swallowed and ultimately reaches the gastrointestinal tract.

Another class of xenobiotic, the polychlorinated biphenyls (PCBs) have been found in the adipose tissue of Canadians (Mes et al. 1982) probably resulting from ingestion. PCBs are deposited in the fatty tissue of fish (Campbell et al. 1977) and are excreted in breast milk

(Wickizer and Brilliant, 1981). Contamination is widespread as a result of huge production volume (85 million pounds per year in the U.S.A. at peak usage) and resistance to both chemical and microbiological degradation (Campbell et al. 1977). Although they have been supplied only for use in closed systems since 1971, their stability renders them persistent environmental pollutants which have accumulated in the food chain, contaminating foods from breakfast cereals to poultry and eggs. Toxicological actions of PCBs include modulation of chemical carcinogenesis as well as porphyria, endocrine, reproductive and skin disorders, and a syndrome characterized by wasting (Safe et al. 1982).

Thus the gastrointestinal tract is often exposed to toxic lipophilic substances and can also serve as a major portal of entry to the organism. Although many studies have examined their metabolism, particularly in the liver, the fate and bioavailability of orally ingested xenobiotic hydrocarbons such as PAHs and PCBs remain largely ignored.

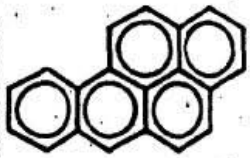
PAHs consist of 3 or more fused benzene rings and as a result are highly hydrophobic. Formed by the pyrolysis of organic substances they are widely dispersed in the environment contaminating soil, air, water and food (for reviews see Blumer, 1976; Baum, 1978). For example, it has been estimated that each year in the United States approximately 1000 tons of B(a)P are discharged into the environment (Dipple, 1983). Although not all PAHs cause cancer, certain of these common hydrocarbons have been used

as standard animal carcinogens and are believed to be potential carcinogens in the human. For example, some populations of the world who frequently consume smoked foods (e.g. China, Japan, Iceland) have a greater incidence of cancer at certain sites, particularly, the gastrointestinal tract (National Research Council, 1982). The most well-known of the powerfully carcinogenic PAHs are B(a)P, 3-methylcholanthrene (3MC), and 7,12-dimethylbenz(a)anthracene (DMBA), whose structures are shown in Fig. 1. Although each of these have been detected in foods (e.g. Lo and Sandi, 1978), they are rarely found alone usually occuring as mixtures with other PAHs, and hence should be considered as an epidemiological group (Berg, 1975).

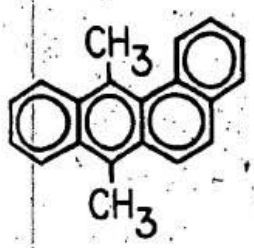
Many non-polar xenobiotics (substances foreign to the body), once in the body, are metabolized to polar or more readily excretable forms. This detoxification mechanism usually involves 2 phases. Phase I produces derivatives with suitable groups such as -OH, $-CO_2H$, -SH and $-NH_2$ which then undergo conjugation (Phase II) to water soluble entities (Williams and Millburn, 1975). The end products of detoxification are usually non-toxic, water soluble substances which are readily excreted in the urine or bile according to molecular weight. Although it is beyond the scope of this thesis to cover this process in detail, the reader is referred to reviews which are cited where appropriate.

Metabolic conversions of PAHs, however, are often associated with the transformation of relatively inert

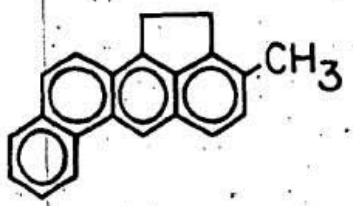
Figure 1: Structures of polycyclic aromatic hydrocarbons used in the study.



BENZO(a)PYRENE
(B(a)P)



7,12-DIMETHYL-
BENZ(a)ANTHRACENE
(DMBA)



3-METHYLCHOL-
ANTHRENE
(3-MC)

chemicals to highly reactive "ultimate carcinogens" (DiGiovanni and Juchau, 1980; Gelboin, 1980; Sims, 1980; Levin et al. 1982). Many tissues in both human and rat, such as liver, lung and colon, can metabolize PAHs. Certain tissues, however, such as the liver remain refractory in PAH induced carcinogenesis. It is probable that due to their highly reactive nature which prohibits widespread distribution, most ultimate carcinogens are formed in susceptible tissues. Conceivably, however, proximate carcinogens may also be formed in remote sites (e.g. liver) prior to delivery to the target site such as the mammary gland (Sims, 1980).

Besides activating potential carcinogens, the liver is capable of detoxifying these hydrocarbons to hasten their excretion. As proposed in an extensive review on carcinogenesis by Farber (1[illegible]) the ultimate fate of a chemical may depend largely upon the balance between activation and inactivation, with the metabolic patterns of a particular organ or tissue determining its susceptibility to cancer. An important consideration, which is often overlooked, however, is the degree of exposure a tissue liable to tumour induction may receive. As mentioned earlier, the gastrointestinal tract is directly exposed to and also serves as a major portal of entry for xenobiotics such as PAH and PCB but factors governing their uptake remain obscure.

In the diet such highly lipophilic substances are likely to be found in [illegible]sociation with fat. PAHs present in charcoal-broiled meat are located in the lipid portion

(Lijinsky and Shubik, 1964) and the content of PAHs in meat has been shown to correlate with the amount of fat (Gray and Morton, 1981). It is established that the absorption of trace lipid nutrients such a sterols and fat-soluble vitamins is intimately dependent on the normal processes of fat digestion and absorption and that bile salts, which play a facilitatory role in triglyceride absorption are obligatory for absorption of these trace lipids (Hofmann, 1968). It seems likely that lipophilic xenobiotics will be handled in a similar fashion to trace nutrient lipids in the early stages of assimilation by the small intestine.

The object of the present study was to investigate factors which govern the efficiency of lipophilic toxin absorption. Since the basic premise of this work is that foreign lipophilic compounds accompany dietary lipids and are absorbed in parallel with them, it is appropriate to summarize the current understanding of lipid digestion and absorption. Although it is not feasible to present an extensive coverage of this subject here, the reader is referred to several excellent reviews (Borgström, 1974, 1977; Patton, 1981; Shiau, 1981; Thomson and Dietschy, 1981).

B. Lipid digestion and absorption

In the gastrointestinal lumen ingested lipids, nutrient or non-nutrient, exist in an aqueous environment. Since

lipid absorption is passive (Sallee and Dietschy, 1973), sufficient concentrations of lipid in monomolecular solution must exist close to the enterocyte membrane to create the diffusion gradient necessary for uptake. Lipid digestion involves a series of steps which increase the concentration of these hydrophobic compounds in an aqueous phase.

The major dietary lipid species, long-chain triglyceride, is absorbed in the small intestine following hydrolysis by pancreatic lipase. This process is very efficient and it has been estimated that 140 grams of fat per minute can be digested in the normal human small intestine (Patton, 1981). The split products of hydrolysis, fatty acids and monoglyceride (see Fig. 2), are solubilized in mixed bile salt micelles and it is from these micelles that they are thought to be absorbed by the enterocyte.

Recent studies have suggested that the older concept of the lipid microenvironments of the small intestine consisting of an oil phase of unhydrolyzed triglyceride and a micellar phase of bile salts, partial glycerides and fatty acids (Hofmann and Borgström, 1962, 1964) may be an over-simplification. Observing fat digestion by light microscopy, Patton and Carey (1979) were able to demonstrate as many as 4 coexisting phases. Of particular note was a "viscous isotropic phase" containing primarily monoglyceride and protonated fatty acids, although its exact composition was not determined. Of low surface tension, this phase could be

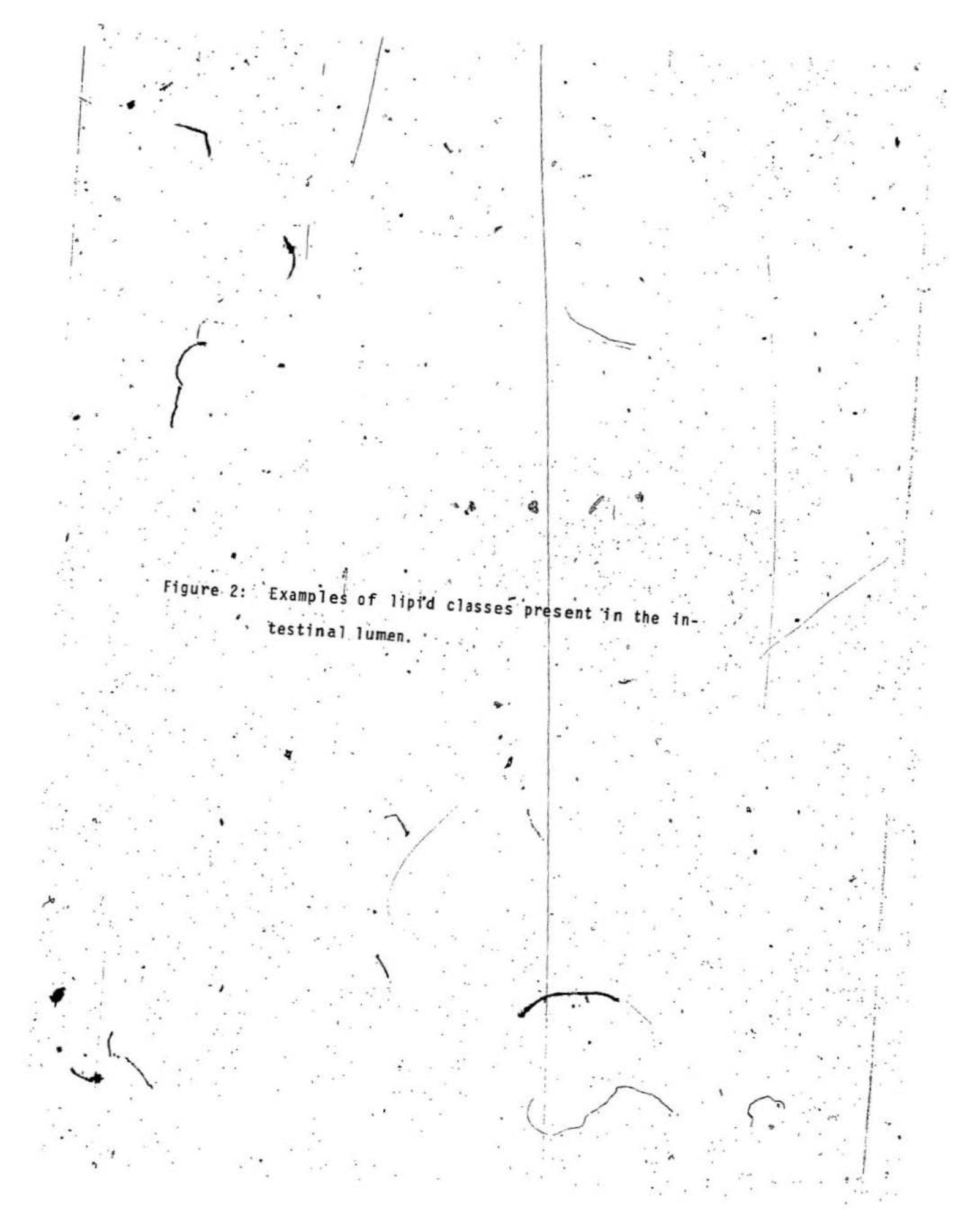
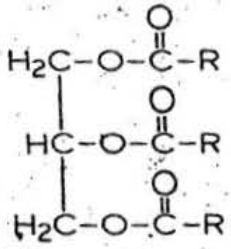
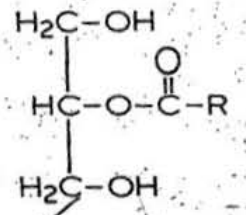


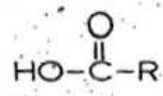
Figure 2: Examples of lipid classes present in the intestinal lumen.



TRIGLYCERIDE



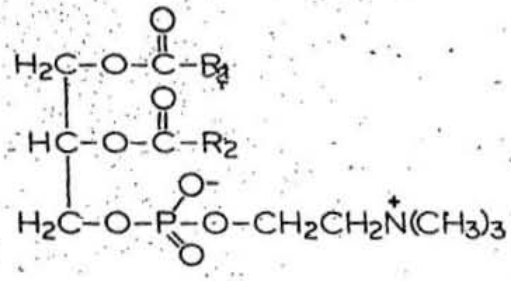
MONOGLYCERIDE



FATTY ACID

OLEIC SERIES (18:1) $R = -(CH_2)_7C{=}C(CH_2)_7CH_3$

PHOSPHATIDYL CHOLINE (LECITHIN)



dispersed by bile salts with subsequent formation of a mixed micellar phase containing the products of triglyceride hydrolysis. There is also evidence that bile salt micelles coexist with larger particles of identical composition termed liposomes (Stafford et al. 1981).

A thick unstirred water layer variously estimated to be 100 to 500 microns in thickness covers the absorptive surface of the small intestinal mucosa (Westergaard and Dietschy, 1974). This unstirred water layer is considered to be a barrier for diffusion of lipid substances which becomes increasingly rate limiting with expanding fatty acid and monoglyceride chain length (Sallee and Dietschy, 1973). It is feasible that diffusion through this layer becomes completely rate limiting for long chain fatty and non-polar solutes such as cholesterol which must permeate this barrier before they can be absorbed (Wilson et al. 1971; Hofmann, 1976). The diffusive flux of long-chain lipolytic products through the unstirred layer is increased by a factor of 100 to 200 by solubilization in bile salt micelles (Hofmann, 1976). Patton (1981) has suggested the concept of a continuous hydrocarbon domain through which the split products of hydrolysis flow. This "hydrocarbon continuum" allows non-polar molecules to move from a non-dispersible oil phase to a phase dispersible in an aqueous medium and yet remain constantly in association with a hydrophobic microenvironment.

It is thought that lipid absorption occurs from a monomer phase in equilibrium with a micellar phase (Wilson and Dietschy, 1972; Westergaard and Dietschy, 1976). It is postulated that micelles dissociate near the enterocyte membrane, an action probably favoured by an acid microclimate (Shiau, 1981), and fatty acid and monoglyceride are subsequently absorbed. Bile salts remain in the lumen continuously bringing triglyceride digestion products to the enterocyte border. Some passive absorption of bile acids occurs in the upper small intestine, but the majority are absorbed by an active process in the terminal ileum (Dietschy, 1968). Under normal conditions fat absorption is a remarkably efficient process (Thomson and Dietschy, 1981) where removal of even 40% of the small bowel in the rat has very little effect on absorption (Bennett, 1964).

Although reduced, appreciable absorption of long-chain fatty acid can occur in the absence of bile (Gallagher et al, 1965; Morgan and Borgstrom, 1969) but the absorption of cholesterol and fat-soluble vitamins is virtually abolished (Hofmann, 1968). The exact means by which bile salts enhance trace lipid absorption is not clear. Feldman and Cheng (1975) postulated that cholesterol absorption from a micellar solution requires a specific interaction of bile salt and fatty acid or monoglyceride with the absorbing membrane. It is also probable that since these trace lipids are rather non-polar they may simply depend on bile salt micellar solubilization to achieve sufficiently high

monomeric concentrations in aqueous solution near the enterocyte membrane to promote uptake. MacMahon and Thompson (1970) showed that while a polar lipid, oleic acid, was absorbed nearly as well from an emulsion as from a bile salt micellar solution, α-tocopherol uptake from the emulsion into intestinal mucosa was lower than that from a micellar solution. This indicates the importance of micellar solubilization for non-polar lipids. It therefore appears that appreciable absorption of trace nutrient lipids is dependent upon the creation of a continuous lipid domain within the lumen. Through this continuum a trace lipid substance could be expected to pass with relative ease, from initial dissolution in dietary fat to eventual transfer to the enterocyte in mixed lipid-bile salt micelles.

Following passive absorption of long-chain monoglyceride and fatty acid they are resynthesized to triglyceride within the enterocyte. Triglyceride droplets are given a polar coat of protein, phospholipid and cholesterol thereby forming chylomicra. These are the major products of the enterocyte during fat absorption which exit from the lateral plasma membrane to travel through the mucosal interstitium and enter the lymphatic system. The oil core of the chylomicra is a solvent for a number of non-polar compounds including lipovitamins (Barrowman, 1983), cholesterol and its esters (Sylvén and Borgström, 1968), and a number of lipophilic xenobiotics including DDT (Pocock and Vost, 1974).

Short- and medium-chain triglycerides (which have fatty acid chain lengths of less than 12 carbon atoms) are handled differently than long-chain lipid and their digestion may not provide the continuous lipid medium seen during long-chain triglyceride digestion and absorption. Medium chain fatty acids and monoglycerides are water-soluble and therefore, do not depend upon solubilization in bile salt micelles to reach the enterocyte. Also, although they are readily split by lipase, hydrolysis is not obligatory prior to absorption. Trioctanoin, a medium chain triglyceride of molecular weight 471 can be absorbed intact and hydrolysed in the enterocyte (Bennett Clark and Holt, 1968). Within the enterocyte, medium chain fatty acids are not reesterified but leave the cell bound to proteins such as albumin and are transported in portal venous blood. During rapid medium chain triglyceride digestion and absorption, without the formation of an effective micellar phase, non-polar solutes may not have a route to the enterocyte. As chylomicra are not elaborated, exit of trace solutes from the cell may also be compromised.

C. Hydrocarbon micellar solubilization and absorption

A number of model hydrophobic compounds have been studied with a view to their micellar solublization *in vitro*. In a study in 1967 Borgström examined the partition of various lipids between emulsified oil and micellar phases

of glyceride:fatty acid:bile salt dispersions. Non-glyceride lipids in such a system partitioned between oil and micellar phases; the distribution seemingly governed by their polarity. Thus cholesteryl oleate partitioned much more in favour of the oil phase than cholesterol. Fat-soluble vitamins such as β-carotene and retinol (El-Gorab and Underwood, 1973) and α-tocopherol (Takahashi and Underwood, 1974) are also dissolved in bile salt micelles. Non-polar solutes are more readily solubilized when polar lipids are present and mixed micelles rather than pure bile salt micelles are formed (Carey and Small, 1970). It is generally believed, as discussed above, that the mixed micellar phase is the preferred medium for the absorption of non-polar lipids. The ability of a bile salt micelle to solublize a particular solute may determine the efficiency of the intestinal mucosa to absorb the solute.

Micellar solubilization is not limited to compounds of biologic necessity and bile salt micelles are able to solubilize non-nutrient substances. Savary and Constantin (1967) found the hydrocarbon hexadecane to undergo micellar solubilization, which like other solutes (e.g. retinol) was greater in mixed than in pure micelles. These workers also discovered an increased appearance of hexadecane in thoracic duct lymph <u>in vivo</u> when hexadecane was administered with oleic acid or triolein rather than when given alone. Another straight chain hydrocarbon octadecane, was

investigated as a micellar solute by Borgstrom (1974) who obtained similar *in vitro* results. *In vivo* he demonstrated octadecane absorption to be directly related to the amount of carrier triolein fed to rats. Early studies by Ekwall and associates (1948, 1951) established the aqueous solubilization of several PAH carcinogens when present with high concentrations of bile salt (in excess of 100 mM). Norman (1960) demonstrated 3MC solubilization in solutions of conjugated and unconjugated bile salts beginning at 12 mM, although no further studies of this phenomenon appear to have been made.

In addition to studies on micellar solubilization of foreign hydrocarbons some work has been done relating to their intestinal absorption *in vivo*. Heptadecane is largely absorbed when present as a dietary contaminant (Tulliez and Bories, 1978). Other straight chain hydrocarbons are known to be converted to their fatty acid counterparts in the enterocyte (McCarthy, 1964). Micellar solubilization appears necessary for passively absorbed lipids and might be a prerequisite for absorption of hydrocarbons, including PAHs, which are probably also absorbed passively. Rees et al. (1971) demonstrated B(a)P accumulations in everted sacs of small intestine increased exponentially with increases in concentration of B(a)P in the incubation medium. Iodoacetate or anaerobic conditions were unable to significantly influence accumulation.

Fragments of evidence indicate that concomitant fat feeding greatly enhances PAH absorption. The absorption from lipid vehicles of PAH and organochlorine compounds has been repeatedly affirmed (Daniel et al. 1967; Rees et al. 1970; Jahss and Moon, 1970; Kamp and Neumann, 1975; Wilson et al. 1982) and shall be treated at greater length in the Discussion section of this thesis. An excellent example of the improved bioavailability of one PAH when administered in fat is provided by Dao (1969). He observed that during the absorption of 3MC in mice, the compound fed in an aqueous suspension was absorbed to less than 6% of the extent achieved when fed in sesame oil. Following absorption these compounds have been detected in thoracic duct lymph. Kamp and Neumann (1975) recovered 9% of an administered dose of 3MC in lymph but only 0.3 to 2.7% of administered aromatic dimethylamines, suggesting that increased lipid solubility favours lymphatic versus portal venous transport.

II. SUMMARY

From the foregoing data it is evident that small amounts of hydrophobic substances present in the intestinal lumen are likely to be contained in lipophilic microenvironments: moving through the "hydrocarbon continuum" from initial dissolution in dietary fat, through a viscous isotropic phase and finally into the hydrophobic cores of mixed bile salt micelles. As passively absorbed

lipid soluble substances, their assimilation in the body may depend on their particular solute properties and the ease with which they undergo micellar solubilization prior to uptake by the intestinal mucosa. The process of long-chain triglyceride absorption offers a hydrocarbon continuum but not so medium-chain triglyceride digestion and absorption. These latter fats have no absolute requirement for micellar solubilization as medium-chain triglyceride and its hydrolysis products are water-soluble, and hence they may be less efficient at creating mixed micelles to solubilize the hydrocarbons.

That these xenobiotics are transported in the lymph to a significant extent after administration in a long-chain lipid vehicle denotes a possible dependence on concomitant fat absorption. Transport in chylomicra implies that the intestinal enterocyte is the only cell through which these compounds pass until they reach the general circulation and the chylomicra are finally disassembled. The ability of the enterocyte to metabolize PAHs may govern their transport; polar derivatives destined for the liver in portal blood and non-polar compounds (perhaps as parent hydrocarbon) favouring lymphatic transport and direct entry to the systemic circulation.

III. Objectives

The present study was undertaken to assess dietary and physiological influences on the intestinal intraluminal

behaviour and absorption of hydrophobic xenobiotics. Experiments with PAH's comprised the bulk of this work and were chosen on account of their toxic potential and their ubiquitous distribution in the food chain. A preliminary *in vitro* study on a PCB compound, another wide-spread lipophilic toxin was performed to lay the ground-work for future experiments.

Of primary interest was the influence of concurrent dietary lipid digestion and absorption on intestinal absorption of the hydrocarbons. The first part of the investigation involved an *in vitro* analysis of the physico-chemical behaviour of the above substances in model intestinal content. The aim was to define factors which either favoured or hindered aqueous dispersion of the hydrocarbons which in turn might influence their bioavailability. Factors included the presence of bile acid micelles, extent of simulated triglyceride hydrolysis and degree of fatty acid saturation and chain length.

Once the *in vitro* data was collected, an *in vivo* analysis of the effect of concomitant fat feeding and assimilation of the substances by conscious, restrained rats was carried out. It was thought possible to characterize intraluminal factors which may modify the rate and extent of absorption of a PAH *in vivo* by administering the carcinogen in various lipid carriers. These vehicles were chosen with regard to their abilities to influence PAH behaviour in simulated intestinal content *in vitro*. Using bile fistula

rats permitted an assessment of the role of bile in hydrocarbon assimilation, and the importance of the interaction of bile with the particular lipids in promoting uptake of hydrocarbon. It was also considered of importance to determine the influence of triglyceride carrier volume on plasma levels of hydrocarbon because high fat intake is associated with increased tumour incidence. Any tendency for the compound to favour either the portal venous or lymphatic routes as determined by dose of DMBA were examined to assess the metabolic capability of the enterocyte.

In summary, the information gained by studying the physico-chemical behaviour of hydrophobic toxins *in vitro* were applied to an *in vivo* situation to define those factors which may either favour or hinder the appearance of lipophilic toxins in the mammalian system. There is a dearth of information on the intestinal absorption of such compounds and since the gastrointestinal tract is a major portal of entry into the body, it is vital to understand the factors which may maximize or minimize the absorption of such ubiquitous and powerful toxic agents.

MATERIALS AND METHODS

I. In vitro experimentation

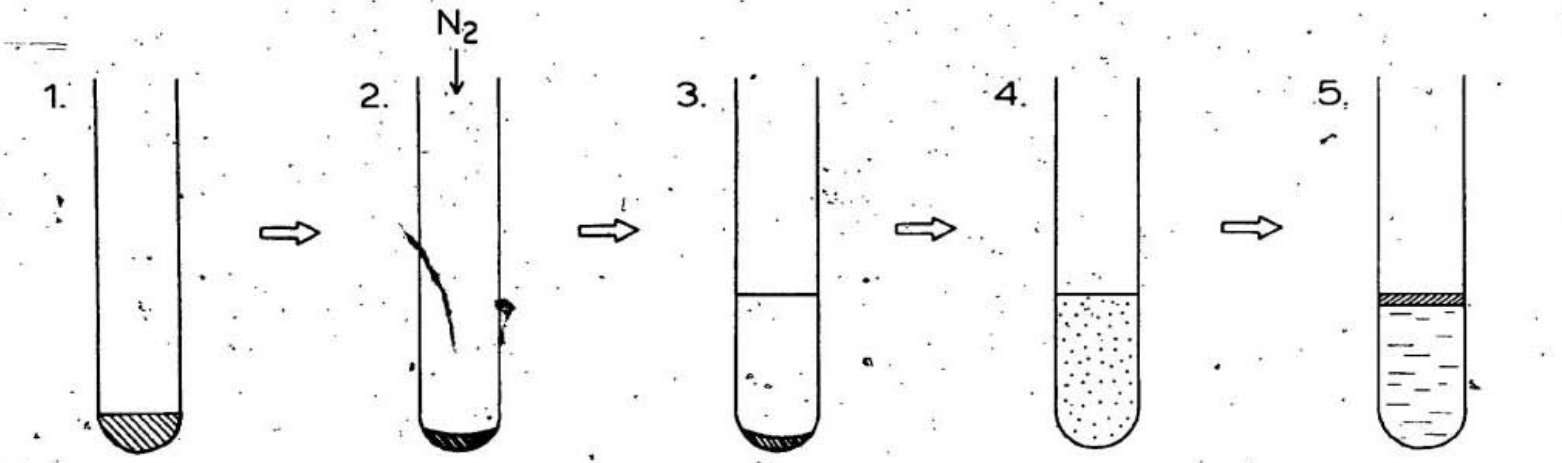
A. Experimental procedures

1. General method for preparation of micellar solutions

The basic procedure used in the preparation of micellar solutions for oil-micellar partition studies has been adapted from El-Gorab and Underwood (1973) and Takahashi and Underwood (1974) and is summarized in Fig. 3. The lipids used in each particular section of the study were combined in appropriate proportions in heptane; phosphatidylcholine was dissolved in chloroform; the hydrocarbon under investigation and its radiolabelled tracer (0.5 μCi ^{3}H or 0.13 μCi ^{14}C) were dissolved in toluene. Aliquots of these solutions were combined in glass vials, then the solvents were evaporated under a steady stream of nitrogen for 10 minutes to yield an oil droplet containing the hydrocarbon. As far as possible the hydrocarbon was protected from light. Buffered sodium taurocholate (NaTC) solution and 0.1 M sodium phosphate buffer (pH 6.3, 0.15 M Na^{+}) were added to give final test mixture volumes of 3.0 ml. The biphasic mixtures were then sonicated for exactly 1 minute with a Sonifier Cell Disrupter (Model W185, Branson Sonic Power Co.) equipped with a standard microtip at power output 3 to

Figure 3: Simplified *in vitro* methodology.

METHODS


1.
2.
N_2
3.
4.
5.
Lipids + hydrocarbon (^3H) in solvents
Evaporation: hydrocarbon in oil droplet
Addition of bile salt and buffer (pH 6.3, 0.15 M Na^+)
Emulsion: Incubation at 37° x 30 min.
Ultracentrifugation: lipid layer and micellar solution

yield homogeneous emulsions. These were then incubated at 37°C for 30 minutes and 50 µl samples removed for scintillation counting. The mixtures were transferred to 10 ml polycarbonate centrifuge tubes of the Oakridge type (Beckman Instruments Inc., Irvine, California) and then centrifuged in a Beckman type 75 Ti rotor at 106,000 x g_{av} (40,000 rpm) using a Beckman L2-65B ultracentrifuge for 18 hours at ambient temperature. Centrifugation produced a supernatant oily phase and a clear aqueous infranatant bile salt solution. Following aspiration of the oily phase, the infranatant phase was carefully drained using a fine pasteur pipette and analyzed for solubilized hydrocarbon by liquid scintillation counting of appropriate aliquots.

2. Micellar solubilization of hydrocarbons

The lipids used in this portion of the study were oleic acid (a long-chain monounsaturated fatty acid), 1-mono-olein, triolein and phosphatidylcholine. These were combined with the hydrocarbons (B(a)P, 3MC, DMBA or PCB) as described above. Appropriate volumes of a 0.1 M NaTC solution (0.15 M Na^+) and sodium phosphate buffer were added to the lipids in each flask to total 3.0 ml final volume. Final concentrations of the components were hydrocarbon 100 µM, triolein 1.13 mM, 1-mono-olein 2.5 mM, oleic acid 7.5 mM, phosphatidylcholine 0.68 mM and NaTC 0, 1, 2, 3, 4, 5, 6, 8, 10, or 12 mM. Subsequent procedures were as outlined in section 1 (General methods).

Hydrocarbon micellar:oil ratios at each NaTC concentration were calculated as

$$\frac{\text{DPM aqueous infranatant}}{\left(\text{DPM emulsion before centrifugation} - \text{DPM aqueous infranatant}\right)}$$

and represent the mean ± SEM for at least 4 replicate experiments. Data were also calculated as nmoles hydrocarbon solubilized in the micellar phase per bile salt concentration. Using these results, a partition ratio (moles hydrocarbon solubilized in the aqueous phase/mole of bile salt) was derived for each hydrocarbon by regression analysis of the data above the CMC of NaTC (these partition ratios correspond to the slope of the curves above the 4 mM inflection points in Fig. 5, p. 54).

3. Simulated triglyceride hydrolysis

Oleic acid, triolein and 1-mono-olein were combined to give final concentrations in 3.0 ml as follows:

triolein	20, 18, 16, 15, 5 mM
1-mono-olein	20 mM - [triolein (mM)]
oleic acid	2 x [1-mono-olein (mM)]

NaTC, phosphatidylcholine and DMBA concentrations were fixed at 12 mM, 0.68 mM and 100 µM, respectively. Conditions and procedures were as outlined above in section 1(General methods). Micellar:oil ratios and percentage of DMBA solubilized at each stage of simulated triglyceride hydrolysis were calculated and represent the mean ± SEM for 4 replicate experiments.

4. Lipid saturation and chain length

The 1-monoglyceride, triglyceride and fatty acid of either the octanoic ($C_{8:0}$), oleic ($C_{18:1}$) or linoleic ($C_{18:2}$) series together with phosphatidylcholine were the lipids studied. In this series of experiments the amount of triglyceride was increased from 1.13 mM to 5.0 mM to obtain a more definite lipid supernatant. Concentrations of other components were fatty acid 7.5 mM, monoglyceride 2.5 mM, phosphatidylcholine 0.68 mM and hydrocarbon (3MC, DMBA or PCB) 100 µM. Conditions and procedures were as outlined above in section 1 (General methods). Micellar solubilization of hydrocarbon was calculated as mean percentage solubilized (± SEM) for 8 replicate experiments.

5. Molar saturation ratio

A mixed micellar solution was prepared containing NaTC 18 mM, oleic acid 7.5 mM, 1-mono-olein 2.5 mM, phosphatidylcholine 0.68 mM in 0.1 M sodium phosphate buffer (pH 6.3, 0.15 M Na^+). Appropriate volumes of this solution were added in combination with sodium phosphate buffer to total 2.5 ml in flasks containing 10 mg crystalline DMBA (0.6 µCi ^{3}H-DMBA). Final NaTC concentrations were 0, 5, 6.7, 8.3, 10, 12, 15 mM. The sealed flasks were then sonicated in an ultrasonic water bath (Cole Parmer) for 1 hour at ambient temperature. Following this, they were shaken in the dark for 18 hours at 37°C then centrifuged for 30 minutes at 3,000 rpm (Sorvall GLC-2 Benchtop Centrifuge).

The supernatant solution was decanted and filtered through Whatman 42 filter paper to retain crystalline DMBA. Aliquots of 100 µl of the micellar solutions were removed for liquid scintillation counting to determine solubilized DMBA.

B. Liquid scintillation counting

Appropriate volumes of emulsion or micellar solution were combined with 10 ml of scintillation cocktail for direct scintillation counting in a Beckman LS 8100 Scintillation Counter. Quenching was corrected by means of an external standard to allow counts per minute (cpm) to be converted into absolute activity (dpm).

II. In vivo experimentation

A. Animals

Male Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (St. Constant, Quebec) and were maintained under standard light and temperature conditions. They were allowed free access to food (Purina Rat Chow, Ralston Purina Co.) and tap water until the time of operation by which time they weighed between 275 and 325 g.

B. Surgery and post-operative procedures

1. Anaesthesia

Diethylether vapour was used to induce and maintain anaesthesia. Minimal dosage and length of exposure to the anaesthetic was attempted for each animal. During the procedure each rat was closely observed for signs of respiratory distress or collapse and the ether was withdrawn and replaced as necessary.

2. Bile duct cannulation

The animal was placed in a dorsal recumbent position and its abdomen shaved. A laparotomy was performed. Bleeding along the incised linea alba was usually minimal and hemostasis, when necessary, was achieved by light pressure. The common bile duct was visualized by drawing

the duodenum to the left through the laparotomy wound. A catheter of polyethylene tubing (PE 10) was introduced into the common bile duct above its confluence with the pancreatic ducts. The common bile duct was ligated with 4-0 surgical silk immediately below the point of entry of the catheter. When bile flow in the catheter was judged to be adequate the catheter was secured in the duct by an additional ligature of 4-0 silk, and exteriorized through a stab wound in the right flank using a 20 g hypodermic needle as a trocar.

3. Duodenum cannulation

Following laparotomy a saline filled premature infant naso-gastric feeding tube was passed into the duodenum through a small incision 1 cm distal to the pylorus on its antimesenteric border. The cannula was fed in a caudal direction for 3 cm and secured with a drop of cyanobutylacrylate adhesive. Patency was checked by injecting 1 ml of normal saline into the duodenum and examining the site for leakage. The cannula was exteriorized through the laparotomy wound.

4. Tail artery cannulation

A 2 cm longitudinal incision was made on the ventral surface of the tail beginning 2 cm from the anus. Following blunt dissection of surrounding tissue, the ventral tail artery was visualized and ligated with 4-0 silk.

Heparinized PE 10 tubing was inserted into the vessel immediately proximal to the ligation and fed in a cephalad direction or about 4 cm. A 30 g hypodermic needle attached to a heparin-filled syringe was inserted into the free end of the catheter. The blood/heparin margin in the catheter was examined for pulsation to insure that the catheter was properly positioned. A 4-0 silk ligature held the catheter secure and the wound was closed with 2 sutures of 4-0 silk. A solution of heparinized saline was infused during the recovery period at a rate of 0.5 ml/h to deliver 10 I. U. heparin/h to prevent clotting.

5. Intestinal lymphatic cannulation

Following laparotomy the duodenum was reflected to the left and covered with warm saline soaked gauze. This exposed the superior mesenteric artery bordered rostrally by the superior (main) mesenteric lymphatic duct and caudally by the inferior mesenteric lymphatic duct. Using a 16 g hypodermic needle as a trocar, vinyl tubing filled with heparin was introduced into the abdominal cavity through the right flank. A curved capillary tube was passed under the vena cava and the right kidney. The end of the catheter was fed into the capillary tube which was then withdrawn back along its original route. This procedure placed the cannula level with and parallel to the main intestinal lymphatic duct. Overlying connective tissue was gently dissected away from the mesenteric artery and lymphatics and a 5-0 silk

ligature was passed below them. Both ends of the silk were held taut so that the lymphatics could be distinguished from surrounding tissue following puncture and subsequent collapse upon loss of lymph. The silk was left untied to avoid occlusion of the vessels.

The cannula (cut at a 45° angle) was introduced into a small cut on the superficial surface of the superior lymphatic and inserted in a caudal direction for about 5 mm. When consistent lymph flow was obtained, occasionally aided by gentle massage of the abdomen, a drop of adhesive was applied to the site of entry. The inferior lymphatic duct was snipped and glued to ensure that no auxiliary intestinal lymphatic drainage escaped the catheter. Infusion of duodenal saline in the post-operative period helped retard clot formation in the cannula by producing an adequate flow of rather dilute lymph. If clotting occurred, pulsing suction with a saline filled syringe often helped dislodge the clot. If this was impossible, the animal was discarded from the study. Animals exhibiting lymph in their abdominal cavities at necropsy were similarly rejected.

6. Closure

The abdominal organs were carefully replaced in the peritoneal cavity, with attention to the maintenance of correct intra-abdominal relations, particularly with the duodenum. The abdominal fascia and peritoneum together were

closed using a simple continuous suture pattern with 4-0 surgical silk. The skin layer was closed in a similar manner.

7. Post-operative maintenance

Immediately following surgery and prior to their recovery from anaesthesia, the rats were placed in Bollman-type restraint cages. These provided effective immobilization and prevented access to the cannulae. An overnight duodenal infusion of saline delivered at a rate of 2.5 ml/h prevented dehydration. Following overnight recovery, and prior to test meal administration, the animals were observed for a 30-minute period to ensure adequate flow of the fluid to be sampled (e.g. bile, lymph, blood). If this was not achieved, the animal was discarded from the study.

8. Sacrifice

Following the experiment the animals were given 30 mg of pentobarbital sodium intraduodenally. If death had not occurred within 15 minutes, the then anaesthetized animals were sacrificed by cervical dislocation. Animals which had exhibited abnormal performance (e.g. low flow rate or lack of chylomicra in lymph) and several animals chosen at random, were examined at necropsy for proper placement of catheters and leakage into the abdominal cavity. Rats with dislodged cannulae were rejected from the study.

C. Dosing and collections

1. Test meal preparation

Test meals were administered intraduodenally via the duodenal catheter . This route rather than intragastric administration was chosen as it obviated interanimal variations in gastric emptying patterns. The lipids used as vehicles were olive oil, safflower oil, MCT oil and triolein. Olive oil and safflower oil were obtained from local sources unable to supply compositional information. They were therefore analyzed by gas liquid chromatography to determine fatty acid compositions, which are given in Table 1 along with the composition of MCT oil, according to the manufacturer.

a) DMBA in lipid vehicle

A stock solution of DMBA in toluene was combined with a toluene solution of ^{3}H-DMBA in a glass vial. The solvents were evaporated under a gentle stream of nitrogen to dryness, leaving a known quantity of radiolabelled and unlabelled hydrocarbon in the bottom of the vessel. When using quantities of DMBA in excess of 10 mg the crystalline hydrocarbon was weighed directly into the tared vial in which ^{3}H-DMBA had previously been added and dried down. As far as possible, the compound was kept protected from light. A specific quantity of oil was then added and the mixture stirred gently in the dark for 16 to 18 hours. Prior to

Table 1. Fatty acid compositions of the triglycerides used as lipid test meals

Lipid vehicle	% of total fatty acids
Olive oil	
oleic ($C_{18:1}$)	80.1
linoleic ($C_{18:2}$)	8.9
palmitic ($C_{16:0}$)	10.3
Safflower oil	
oleic ($C_{18:1}$)	13.2
linoleic ($C_{18:2}$)	79.1
palmitic ($C_{16:0}$)	7.1
MCT oil	
less than C_8	3
octanoic ($C_{8:0}$)	71
decanoic ($C_{10:0}$)	23
greater than C_{10}	3

administration, the solution was visually examined for the presence of undissolved hydrocarbon and samples were removed for liquid scintillation counting.

b) DMBA in lipid-bile vehicle

The above procedure was followed then pooled rat bile was added to the DMBA and ^{3}H-DMBA solution so that the final oil to bile ratio was 2:1 v/v. This mixture was then vortexed for at least one minute to emulsify the preparation. When evidence of creaming or cracking (separation of the oil and aqueous phases) appeared, the mixture was again vortexed to maintain homogenization. Aliquots were removed for liquid scintillation counting.

c) DMBA biliary metabolites

Three donor rats equipped with biliary catheters were given a bolus injection of 50 µg DMBA (30 µCi ^{3}H-DMBA) in 0.1 ml ethanol via the tail vein. Bile was collected for 5 hours, pooled, then brought up to 18 mls with pooled rat bile containing no DMBA or derivatives. This resulting mixture containing radiolabelled metabolites of DMBA were taken for liquid scintillation counting prior to administration to recipient rats.

2. Test meal administration

Prior to administration of test meals samples of fluid (e.g. bile) were taken and used as controls. All dosing was

carried out using an appropriately sized syringe containing the test meal. A pre-determined volume of the test meal was drawn up in the syringe which was then weighed on a Mettler A30 analytical balance. The test meals were administered either as bolus doses or as infusions using Harvard infusion pumps (Harvard Parallel/Reciprocal Infusion/Withdrawal Pump: Series 940 or Harvard Compact Infusion Pump: Series 975). Using a separate syringe, the residual mixture in the catheter was rinsed in with 0.5 cc of saline or bile, depending on the experimental protocol. The empty syringe used to administer the test meal was then reweighed to determine the exact quantity given to each animal. Dosing procedures are summarized in Table 2.

3. Sample collection

a) Bile

Bile was collected at timed intervals into preweighed 7 ml glass vials which were partially stoppered to retard evaporation. The vials were then reweighed on a Mettler A30 analytical balance to determine the weight of bile excreted. Using a specific gravity value of 1.00, the bile weight (g) was equivalent to bile volume (ml) excreted. Aliquots were removed for liquid scintillation counting and total dpm's excreted per unit time were calculated.

Table 2. Procedures for intraduodenal test meal administration

Test meal	Approximate volume	Method of administration	Catheter rinse
DMBA in oil*	1.0 ml	infusion: 1.0 ml/h x 1 h	saline
DMBA in oil/bile	1.5 ml	infusion: 1.5 ml/h x 1 h	bile
DMBA in triolein	0.25, 0.5, 1.0 & 2.0 ml	bolus dose x 1 minute	saline
DMBA biliary products	3.5 ml	infusion: 1.0 ml/h x 1 h	bile

* olive oil, safflower oil or MCT oil

b) Plasma

Arterial blood (250 µl) was withdrawn into a heparinized 1 cc disposable syringe via the tail artery catheter at half-hour intervals. An arterial infusion of saline was maintained at a rate of 0.75 cc/h between collections to prevent clotting. The blood was expelled into heparinized 400 µl microfuge tubes then centrifuged for 2 minutes (Beckman 152 microfuge). Plasma volumes of 100 µl were removed for liquid scintillation counting.

c) Lymph

At half-hour intervals, lymph was collected into partially-stoppered preweighed vials containing sodium citrate as an anticoagulant. The vials with lymph were then reweighed to obtain lymph weight. Using a specific gravity of 1.00 (Barrowman, 1966), lymph weight (g) was equivalent to volume of lymph (ml) recovered per unit time. The lymph was vortexed and 100 µl aliquots were taken for liquid scintillation counting.

Ultracentrifugation of pooled lymph diluted 1:1 with normal saline enabled separation of chylomicra. The mixture was spun at 30,000 rpm for 30 minutes. The opaque supernatant containing chylomicra was aspirated then 100 µl aliquots of the clear infranatant phase were removed for liquid scintillation counting. Comparing dpm before and after centrifugation enabled calculation of the fraction of radiolabel confined to chylomicra.

$$\frac{\text{DPM lymph} - \text{DPM infranate}}{\text{DPM lymph}} \times 100 = \% \text{ in chylomicra}$$

D. Liquid scintillation counting

Duplicates of an appropriate volume of each sample to be analyzed were combined with 6 ml of liquid scintillation cocktail, then subjected to direct scintillation counting in a Beckman LS 8100 scintillation counter. Quenching was corrected by means of an external standard which allowed cpm to be converted to dpm. The dpm of each control sample were compared to the dpm of collections subsequent to the test meal.

E. Experimental procedures

In all instances experiments were performed on the first post-operative day and involved intraduodenal test-meal administration. Except where noted, an intraduodenal infusion of saline at 2.5 ml/h followed the test meal. Details of each experimental procedure are summarized in Table 3.

Procedure 1. Biliary excretion of radiolabel following ^{3}H-DMBA administration in 3 lipid vehicles; bile diversion

In animals with bile duct and duodenal cannulae a control bile sample was collected over a 20 minute period.

Table 3. Summary of *in vivo* experimental designs

Procedure	Test meal[a.] DMBA	µCi ^{3}H	Vehicle	Volume (ml)	Fluid sampled	Collection period	No. of animals
1	10 µg	2.5	olive oil	1.0	bile	0 - 24 h	6
	"	"	safflower oil	"	"	" "	"
	"	"	MCT oil	"	"	" "	"
2	10 µg	2.5	olive oil/bile	1.5	bile	0 - 24 h	6
	"	"	" " "	"	"	0 - 36 h	3
	"	"	safflower oil/bile	"	"	0 - 24 h	6
	"	"	MCT oil/bile	"	"	" "	"
3	10 µg	12.5	olive oil	1.0	plasma	0 - 6 h	6
	"	"	MCT oil	"	"	" "	"
4	26 µg	12.5	triolein	0.25	plasma	0 - 7 h	4
	"	"	"	0.5	"	" "	"
	"	"	"	1.0	"	" "	"
	"	"	"	2.0	"	" "	"
5	10 µg	12.5	olive oil/bile	1.5	bile + lymph	0 - 24 h	6
	10 mg	"	" " "	"	" "	" "	"
	20 mg	"	" " "	"	" "	" "	4
6	4 µg[b.]	2.5	pooled rat bile	3.5	bile	0 - 24 h	6

a. refer to Table 2 for details on intraduodenal administration of test meals

b. unidentified biliary derivatives of ^{3}H-DMBA administered; equivalent to 4 µg of parent DMBA

DMBA (10 µg; 2.5 µCi ^{3}H-DMBA) dissolved in 1.0 ml olive, safflower or MCT oil was infused intraduodenally at a rate of 1.0 ml/h. Bile was collected every 20 minutes for 7 hours, then a cumulative 17 hour collection was taken and analyzed for radiolabel.

Procedure 2. Billiary excretion of radiolabel following ^{3}H-DMBA administration in 3 lipid vehicles; bile supplementation

Procedure 1 was modified slightly in which the test meal was a combination of oil and bile. An intraduodenal infusion of bile at 0.4 ml/h for 7 hours followed administration of the test substance. An additional 3 animals were monitored every half-hour for bile excretion of radiolabel from 7 to 23 h after the test meal and then cumulatively from 23 to 36 hours.

Procedure 3. Plasma levels of radiolabel in animals with intact enterohepatic circulations following ^{3}H-DMBA administration in 2 lipid vehicles.

An intraduodenal infusion of DMBA (10 µg; 12.5 µCi ^{3}H-DMBA) dissolved in olive or MCT oil was given to animals with duodenal and arterial cannulae. Plasma levels of radiolabel were monitored immediately before test meal administration then every 30 minutes for 6 hours following administration of the test meal.

Procedure 4. Plasma levels of radiolabel in animals with intact enterohepatic circulations following ^{3}H-DMBA administration in increasing volumes of triolein carrier.

A control sample of blood was taken then bolus test meals were given to animals with arterial and duodenal cannulae. Each animal received 100 nmoles DMBA (25.6 µg; 12.5 µCi ^{3}H-DMBA) in 0.25, 0.50, 1.0 or 2.0 ml triolein. Plasma levels of radiolabel were then monitored every 30 minutes for 7 hours.

Procedure 5. Lymphatic and biliary recovery of radiolabel following intraduodenal administration of 3 doses of DMBA in olive oil; bile supplementation

Control samples of lymph and bile were collected then DMBA was given at 3 dosage levels (10 µg, 10 mg, 20 mg; 2.5 µCi ^{3}H-DMBA) dissolved in olive oil with bile added. A bile in saline mixture (1:5) was then infused at a rate of 4.8 ml/h for 7 hours. Lymph and bile levels of radiolabel were monitored every 30 minutes for 7 hours, followed by a 17 hour and then a 30 minute collection. In the 17 to 24.5 hour period, a saline infusion of 2.5 ml/h was given.

Procedure 6. Biliary excretion of radiolabel following an intraduodenal infusion of biliary products of ^{3}H-DMBA.

A 3.5 h infusion of exogenous pooled biliary metabolites of ^{3}H-DMBA was delivered intraduodenally at a

rate of 1 ml/h. Endogenous bile was collected and monitored for radiolabel every half-hour for 6 hours, then cumulatively for an 18 hour period.

III. Materials

Aroclor 1242 (PCB; an isomeric mixture of polychlorinated biphenyls containing 42% chlorine by weight); Foxboro Canada Inc., LaSalle, Quebec.

Benzo(a)pyrene (B(a)P) 98% pure; Sigma Chemical Co., St. Louis, Missouri, U.S.A.

(1,3,6-^{3}H)-Benzo(a)pyrene (^{3}H-B(a)P), 20 Ci/mmole, 98.6% pure; New England Nuclear Canada Ltd., Lachine, Quebec.

Chloroform ($CHCl_3$), with 1% ethanol as preservative, Burdick and Jackson Laboratories Inc., Muskegon, Michigan, U.S.A.

7,12-Dimethylbenz(a)anthracene (DMBA); 98% pure; Sigma Chemical Co., St. Louis, Missouri, U.S.A.

(G-^{3}H)-7,12-Dimethylbenz(a)anthracene (^{3}H-DMBA), 43 Ci/mmole 92.4% pure; Amersham Corp., Oakville, Ontario.

Ether, anhydrous A.C.S., Fisher Scientific Limited, Don Mills, Ontario.

Heparin Sodium Injection U.S.P., 1000 U.S.P. units/ml; Allen & Hanburys Co., Toronto, Ontario.

Heptane; Eastman Kodak Co., Rochester, New York, U.S.A.

Linoleic acid (*cis*, *cis*-9,12-octadecadienoic acid) > 99% pure; Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Liquid scintillation cocktail; Aquasol-2 L.S.C. Cocktail; New England Nuclear Canada Ltd., Lachine, Quebec.

MCT oil (medium chain triglyceride oil, composition given in Table 1); Mead Johnson Canada Ltd., Candiac, Quebec,

3-Methylcholanthrene (3MC; 20-methylcholanthrene); Sigma Chemical Co., St. Louis, Missouri, U.S.A.

(G-^{3}H)-3-Methylcholanthrene (^{3}H-3MC), 35 Ci/mmole; Amersham Corp., Oakville, Ontario.

Monolinolein (1-monolinoleoylglycerol) > 99% pure; Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Monooctanoin (1-monooctanoylglycerol) > 99% pure; Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Monoolein (1-monooleoylglycerol) > 99% pure; Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Octanoic acid, > 99% pure Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Oleic acid (cis-9-octadecenoic acid), > 99% pure by GLC; Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Olive oil (composition given in Table 1); Diamond Brand Mfgrs., St. John's, Newfoundland.

Pentobarbital Sodium Injection (Somnotol^{R}), 65 mg/ml; M.T.C. Pharmaceuticals, Hamilton, Ontario.

Phosphatidylcholine (lecithin; 1,2-diacyl-sn-glycero-3-phosphocholine, egg source); Serdary Research Laboratories, London Ontario.

Polychlorinated biphenyls (PCB; see Aroclor 1242)

(U-^{14}C)-Polychlorinated biphenyls (^{14}C-PCB; an isomeric mixture of polychlorinated biphenyls containing 42% chlorine by weight; ^{14}C-Aroclor 1242), 24.4 mCi/mmole; New England Nuclear Canada Ltd., Lachine, Quebec.

Polyethylene (PE 10) tubing, external diameter 0.61 mm, internal diameter 0.28 mm, animal tested; Clay Adams, Parsippany, New Jersey, U.S.A.

Premature Infant Feeding Tube (Bardic), size 5 Fr., 15" length; C.R. Bard Ltd., Mississauga, Ontario.

Safflower oil (composition given in Table 1); Sunny Crunch Foods Ltd., Markham, Ontario.

Sodium chloride (NaCl) A.C.S., Fisher Scientific Ltd., Don Mills, Ontario.

Sodium phosphate buffer, 0.1 M (pH 6.3, 0.15 M Na^+)

Sodium phosphate, monobasic	1.07 g
Sodium phosphate, dibasic	0.32 g
Sodium chloride	0.16 g
Distilled water to	100.0 ml

Sodium phosphate, dibasic (Na_2HPO_4) A.C.S., Fisher Scientific Ltd., Don Mills, Ontario

Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_2O$) A.C.S., J.T. Baker Chemical Co., Phillipsburg, New Jersey, U.S.A.

Sodium taurocholate (NaTC; taurocholic acid, sodium salt), 98% pure; Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Surgical silk, 4-0, 5-0; Ethicon Inc., Somerville, New Jersey, U.S.A.

Toluene A.C.S.; Fisher Scientific Co., Don Mills, Ontario

Trilinolein (trilinoleoylglycerol), > 99% pure, Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Trioctanoin (trioctanoylglycerol), > 99% pure, Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Triolein (trioleoylglycerol), > 99% pure, Nu Chek Prep Co., Elysian, Minnesota, U.S.A.

Vinyl (SV31) tubing, external diameter 0.80 mm, internal diameter 0.50 mm, medical grade, Dural Plastics and Engineering, Dural, N.S.W., Australia.

IV. Statistical analysis and data presentation

The mean and standard error of the mean (SEM) were calculated for each group of results, both *in vitro* and *in vivo*. Graphical presentation of data has included standard error bars whenever possible unless at the expense of clarity. Student's t-test for unpaired values, or an analysis of variance where appropriate, were used to test statistical significance between groups of data within an experiment. Significance was established at $p \leq 0.05$ unless otherwise specified.

EXPERIMENTAL DESIGN AND RESULTS

I. Factors influencing hydrocarbon behaviour *in vitro*

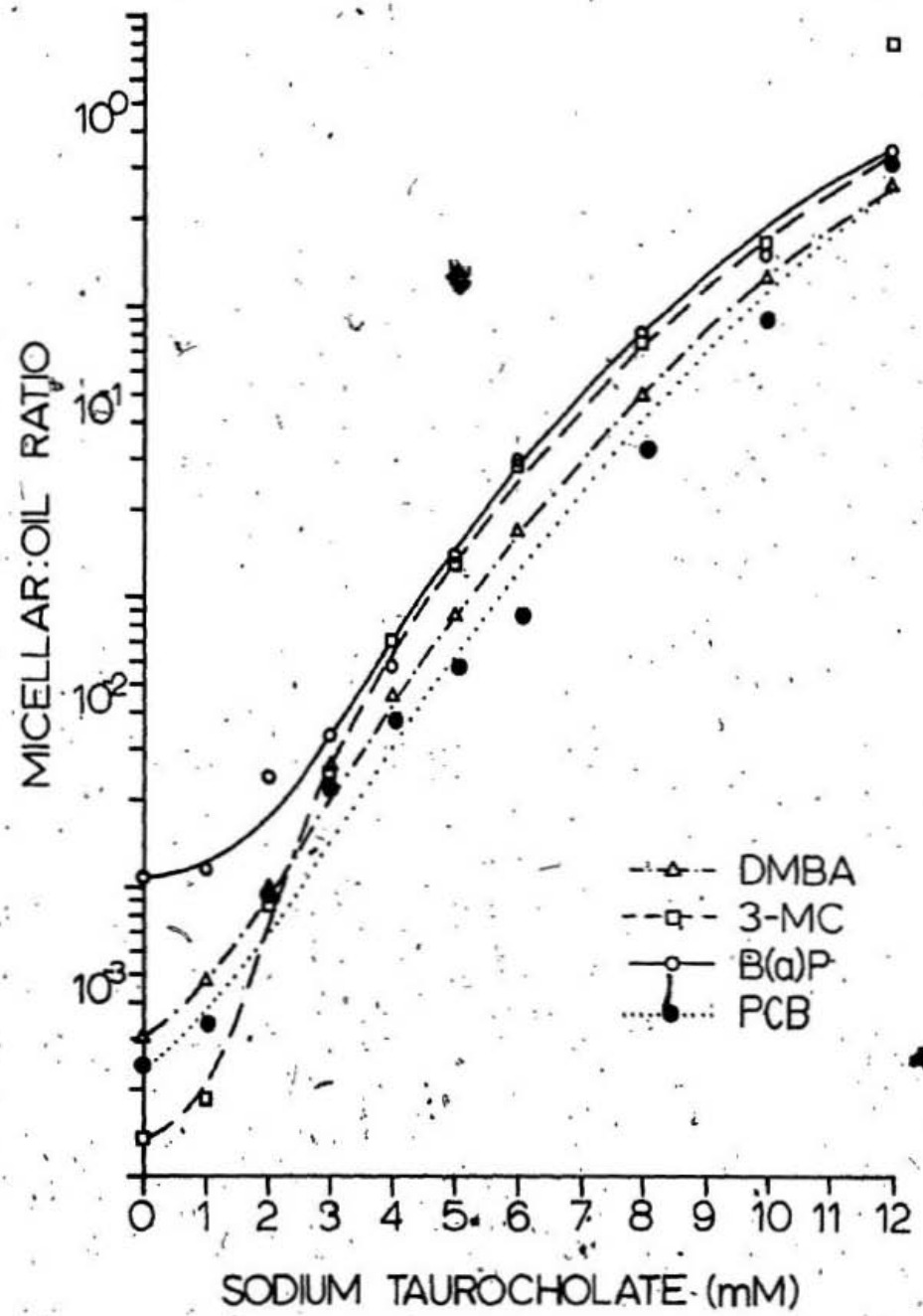
A. Introduction

The *in vitro* experimentation examined the physico-chemical behaviour of 4 xenobiotic hydrocarbons in model intestinal content. The systems used were usually biphasic and attempted to simulate post-prandial intestinal luminal conditions. Various components of each mixture were adjusted to determine possible factors influencing hydrocarbon partition from an oil into an aqueous phase and the ultimate micellar-aqueous solubilization of the non-polar compounds.

B. Micellar solubilization

This experiment involved examining the partition of the 4 hydrocarbons from an oil into a micellar phase as bile salt concentration was increased. The ability of NaTC to influence the micellar:oil ratios of B(a)P, 3MC, DMBA and PCB is shown in Fig. 4. The hydrocarbons exhibit similar micellar:oil ratios which are dependent upon bile salt concentration. At physiological bile salt concentrations there are no significant differences between the ratios for the 4 hydrocarbons ($p > 0.05$). The average supernatant:oil ratio increases from 0.005 ± 0.002 in the absence of NaTC to 4.3 ± 1.2 at 12 mM NaTC. Approximately half of the 300

Figure 4: Effect of NaTC concentration of the micellar:oil ratio of DMBA, 3MC, B(a)P and PCB in a two-phase system. Each point represents the mean for 4 determinations. Composition of the mixture in 0.1 M sodium phosphate buffer (pH 6.3, 0.15 M Na^+): triolein 1.13 mM, 1-monoolein 2.5 mM, oleic acid 7.5 mM, phosphatidylcholine 0.68 mM, hydrocarbon 100 µM, NaTC as specified.


MICELLAR:OIL RATIO
10^0
10^1
10^2
10^3
0 1 2 3 4 5 6 7 8 9 10 11 12
SODIUM TAUROCHOLATE (mM)
DMBA
3-MC
B(a)P
PCB

nmoles of each hydrocarbon present is found in the 3.0 ml of micellar phase at 10 mM NaTC and an average of 79 ± 4% is solubilized at 12 mM NaTC. Although of comparatively small volume (Ca. 30 µl), the oil phase manages to retain a significant proportion of the PAHs and PCB.

Figure 5 shows the quantitative relationship between solubilized hydrocarbon and NaTC concentration. At about 4 mM NaTC, there occurs an inflection point below which very little hydrocarbon is found. Above this 4 mM value, the amount of hydrocarbon solubilized in the micellar phase is directly proportional to the amount of bile salt present. Determination of the slopes of these curves by regression analysis allows calculation of a partition ratio for each compound in this system. These ratios are nearly identical and are given in Table 4. The values correspond to 1 molecule of hydrocarbon solubilized per approximately 100 molecules of NaTC in the biphasic system.

These derived molar partition ratios do not reflect the true capacity of NaTC micelles to solubilize hydrocarbon. Instead they are indicative of the ability of a typical bile salt, NaTC, to influence the partition of hydrocarbon between an oil and a micellar phase. A further experiment involved determining the micellar solubilizing capacity of mixed lipid-bile salt micelles for hydrocarbon in the absence of a competing lipid phase. Increasing concentrations of mixed lipid-NaTC micelles were added to an excess of crystalline DMBA, and solubilization of the

Figure 5: Effect of NaTC concentration on the aqueous solubilization of DMBA, 3MC, B(a)P and PCB in a two-phase system. Solubility expressed as nmoles hydrocarbon solubilized in 3.0 ml of an NaTC solution at specified concentrations. Each point represents the mean for 4 determinations. Above the 4 mM NaTC concentration, each curve was plotted by regression analysis of individual results and a correlation coefficient determined. Composition of the mixture in 0.1 M sodium phosphate buffer (pH 6.3, 0.15M Na^+): triolein 1.13 mM, 1-mono-olein 2.5 mM, oleic acid 7.5 mM, phosphatidycholine 0.68 mM, hydrocarbon 100 µM, NaTC as specified.

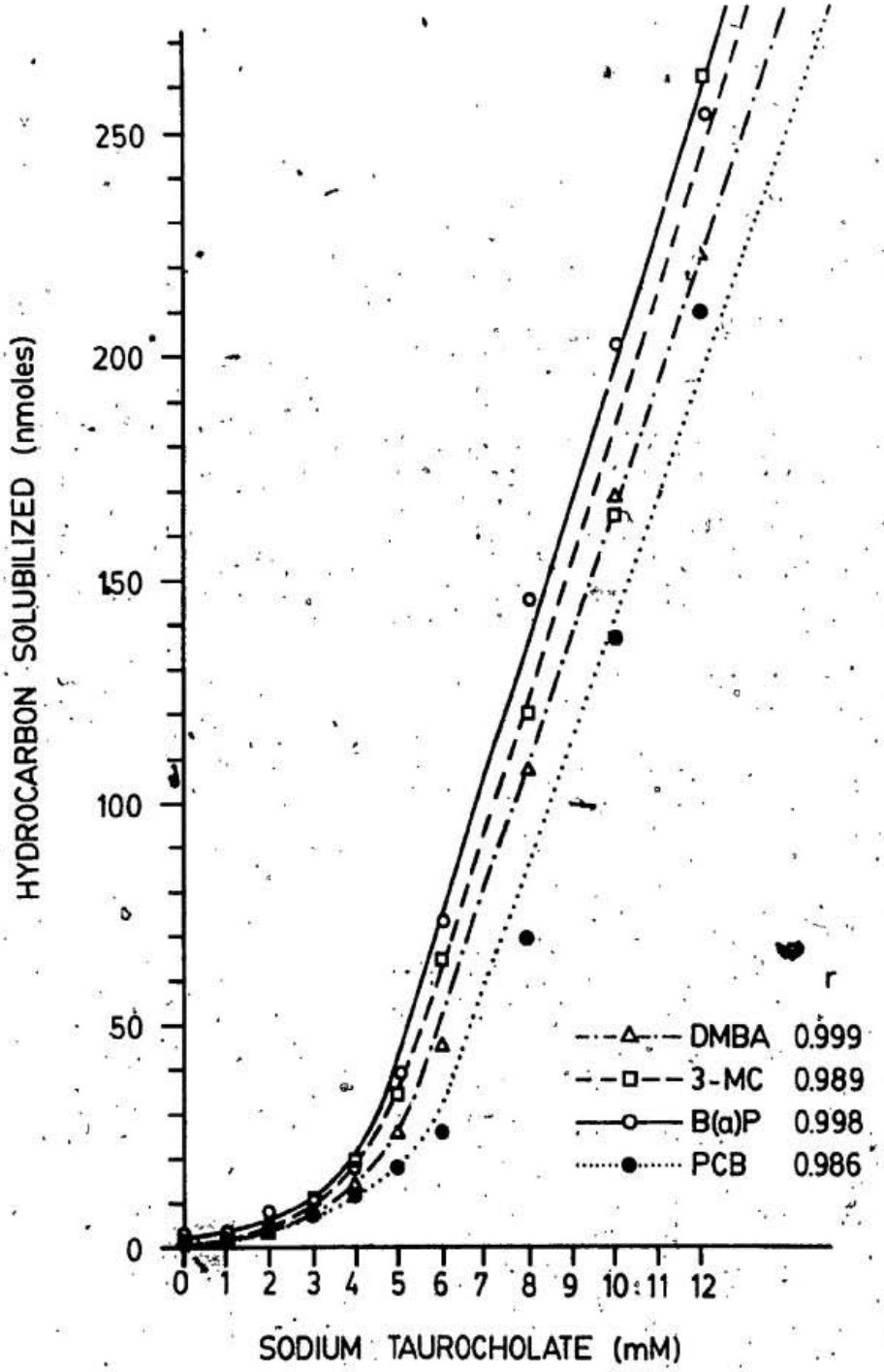

HYDROCARBON SOLUBILIZED (nmoles)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12
SODIUM TAUROCHOLATE (mM)
r
DMBA 0.999
3-MC 0.989
B(a)P 0.998
PCB 0.986

Table 4. Molar partition ratios of hydrocarbons in the biphasic system[a].

Hydrocarbon	Molar partition ratio (± S.D.)
B(a)P	1.04×10^{-2} ($\pm 8.20 \times 10^{-5}$)
3MC	1.04×10^{-2} ($\pm 8.80 \times 10^{-5}$)
DMBA	9.63×10^{-3} ($\pm 7.60 \times 10^{-5}$)
PCB	9.42×10^{-3} ($\pm 9.70 \times 10^{-5}$)

a. calculated by regression analysis from individual results summarized in Fig. 5 for bile salt concentrations above 4 mM.

hydrocarbon assessed. The resulting plot (Fig. 6), attained by regression analysis, has a slope of 0.162, equivalent to the saturation ratio (moles DMBA solubilized/mole NaTC). The reciprocal of the slope (6.2), indicates that approximately 6 molecules of NaTC in the presence of fatty acid and monoglyceride can solubilize 1 molecule of a representative PAH in the absence of a competing oil phase.

C. Lipids

1. Triglyceride volume

As shown in Table 5, as the triglyceride concentration in the biphasic system is increased, but the fatty acid, monoglyceride and bile salt concentrations remain constant, there is greater affinity for hydrocarbon in the oil phase. A study of the effect of simulated triglyceride hydrolysis on DMBA partition where long-chain triglyceride (triolein) is replaced by its digestion products was performed. Physiological bile salt (12 mM) and phosphatidylcholine (0.68 mM) concentrations remained constant. The results are depicted in Fig. 7.

When no monoolein or oleic acid is included in the system (ie. 20 mM triolein) 4.9 $\pm$ 0.4% of the DMBA is located in the micellar infranatant. The partition of DMBA into the micellar phase from the oil phase progresses as triolein is gradually replaced by monoolein and oleic acid. Significant increases in micellar solubilization are evident

Figure 6: Aqueous solubilization of excess DMBA by mixed NaTC micelles in the absence of a lipid phase. The curve was calculated by regression analysis and each point represents the mean ± SEM for 4 determinations. Composition of the mixture in 2.5 ml 0.1 M sodium phosphate buffer (pH 6.3, 0.15 M Na^+): DMBA 10 mg; NaTC concentrations varied as indicated from 0 - 15 mM. Oleic acid and 1-mono-olein were present according to a NaTC-fatty acid-monoglyceride ratio of 7.2:3:1.

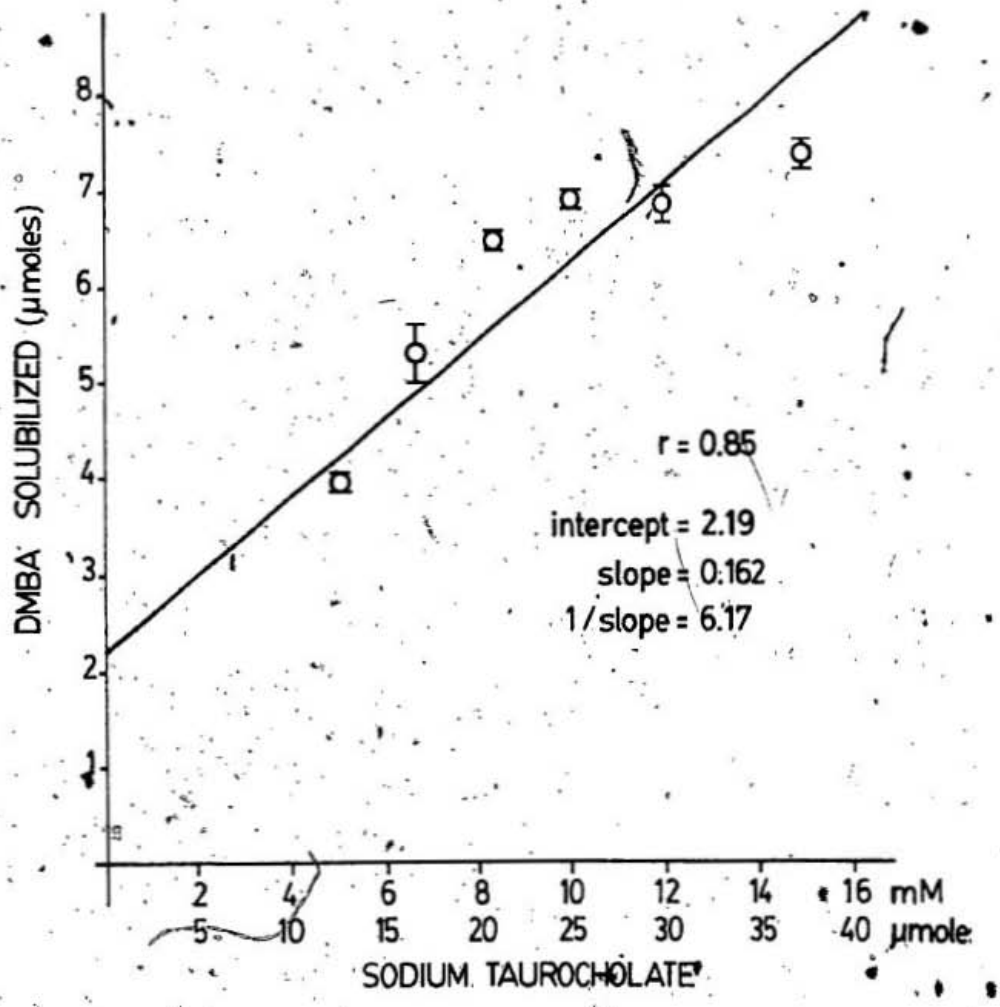

DMBA SOLUBILIZED (µmoles)
8
7
6
5
4
3
2
1
r = 0.85
intercept = 2.19
slope = 0.162
1/slope = 6.17
2 4 6 8 10 12 14 16 mM
5 10 15 20 25 30 35 40 µmole
SODIUM TAUROCHOLATE

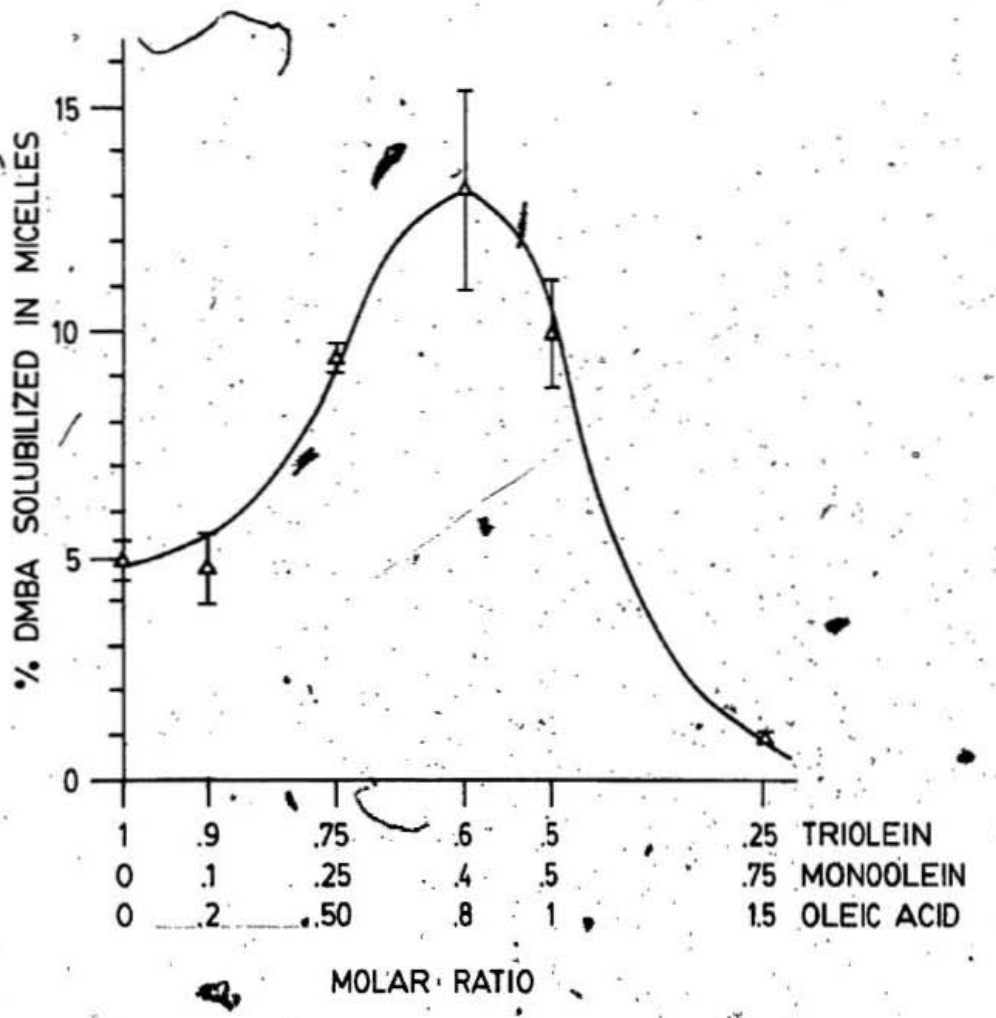
Table 5. Effect of triolein concentration on the partition of DMBA, 3MC and PCB between an oil phase and a mixed micellar solution [a].

Triolein concentration (mM)	Percentage of 300 nmoles of hydrocarbon solubilized in 3.0 ml aqueous		
	DMBA	3MC	PCB
1.13	74 ± 4	87 ± 4	71 ± 7
5.00	21 ± 3	20 ± 1	25 ± 2

a. at fixed bile salt, monoolein and oleic acid concentrations of 12 mM, 2.5 mM and 7.5 mM, respectively (n = 4).

Figure 7: Influence of simulated triglyceride hydrolysis on the partition of DMBA from a lipid phase into a mixed NaTC micellar solution. Partition expressed as percentage of DMBA (± SEM) present in the aqueous phase (n=4). Total DMBA present in 3.0 ml = 300 nmoles (100 μM), NaTC 12 mM, phosphatidylcholine 0.68 mM. Lipid components varied as follows:

Triolein	20, 18, 16, 15, 5 mM
1-Mono-olein	20 mM - [Triolein (mM)]
Oleic acid	2 x [1-Mono-olein (mM)]

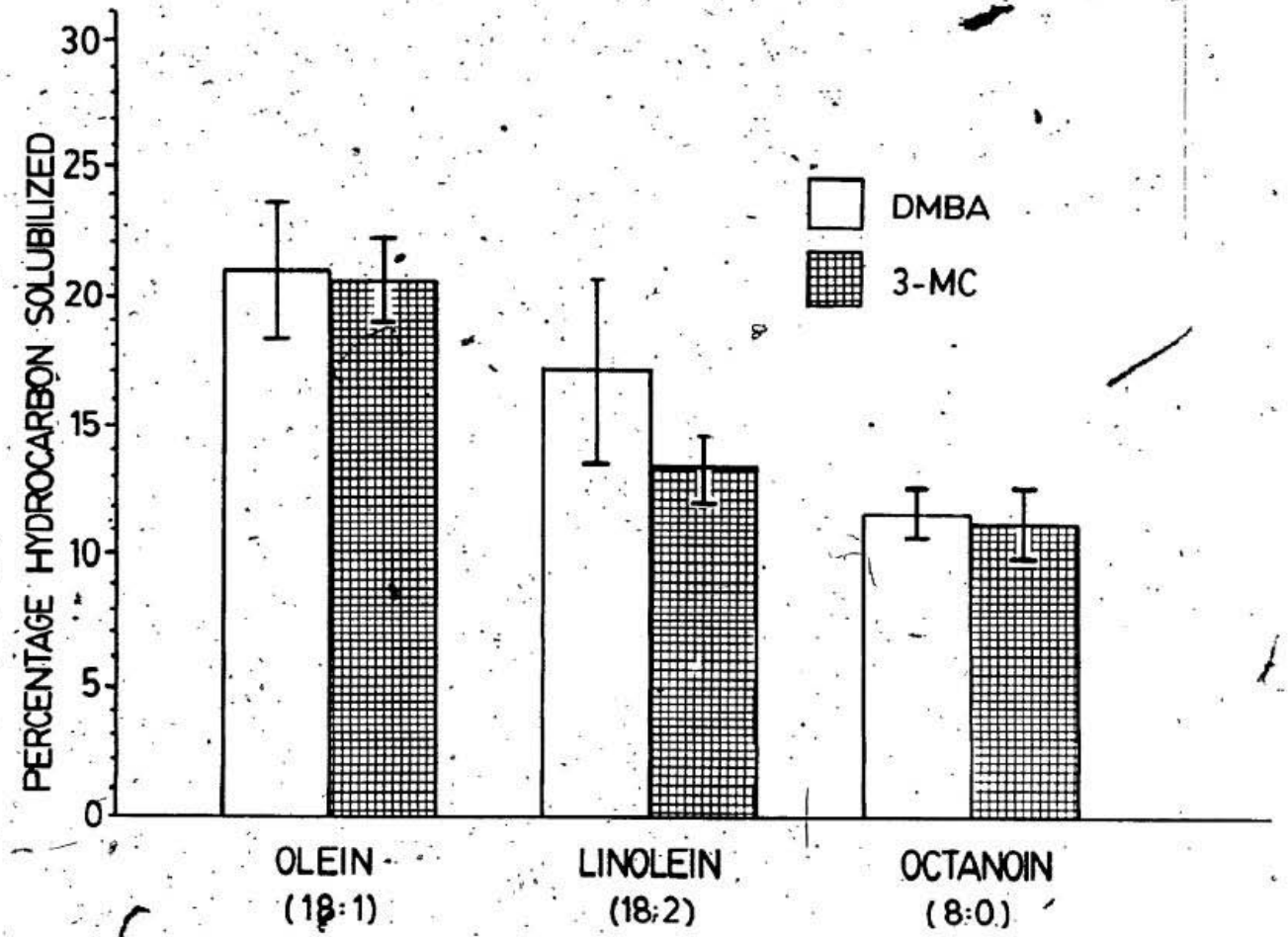

% DMBA SOLUBILIZED IN MICELLES
15
10
5
0
1 .9 .75 .6 .5 .25 TRIOLEIN
0 .1 .25 .4 .5 .75 MONOOLEIN
0 .2 .50 .8 1 1.5 OLEIC ACID
MOLAR RATIO

from 25 to 50% "hydrolysis" as compared to the absence of lipid digestion products ($p < 0.05$). Maximal micellar solubilization occurs at 40% "hydrolysis" (i.e. 12 mM triolein, 8 mM monoolein, 16 mM oleic acid) where 13 $\pm$ 2% of total DMBA is present in the mixed micellar solution. After this 40% "hydrolysis" point DMBA found in the aqueous phase decreases to reach 0.9 $\pm$ 0.1% at 75% "hydrolysis" (5 mM triolein, 15 mM monoolein, 30 mM oleic acid); significantly less than hydrocarbon solubilized at 0% "hydrolysis" ($p < 0.05$).

2. Fatty acid saturation and chain length.

This experiment used fatty acids, monoglycerides and triglycerides of 3 lipid classes to examine possible influences of lipid type on the oil:micellar partition of hydrocarbons. The lipids were chosen on the basis of their fatty acid saturation and chain length. The oleic series represented long-chain monounsaturated lipids ($C_{18:1}$); the linoleic series, long-chain polyunsaturated lipids ($C_{18:2}$) and the octanoic series, medium-chain saturated lipids ($C_{8:0}$). The abilities of the various lipid classes to influence the oil:micellar partitions of the PAHs, DMBA and 3MC, are shown in Fig. 8. DMBA is solubilized in the micellar phase of the octanoic system in an amount only 55% of that seen with the oleic system (11.6 $\pm$ 1.0% vs 20.9 $\pm$ 2.7%; $p < 0.05$). Differences in the micellar solubilization

Figure 8: Influence of fatty acid saturation and chain length on the partition of DMBA and 3MC from a lipid phase into an NaTC micellar phase. Partition is expressed as percentage of hydrocarbon (± SEM) present in the aqueous phase (n=8). Total DMBA or 3MC present in 3.0 ml ≅ 300 nmoles (100 μM); NaTC 12 mM, fatty acid 7.5 mM, monoglyceride 2.5 mM, triglyceride 5.0 mM, phosphatidylcholine 0.68 mM.


PERCENTAGE HYDROCARBON SOLUBILIZED
30
25
20
15
10
5
0
DMBA
3-MC
OLEIN
(18:1)
LINOLEIN
(18:2)
OCTANOIN
(8:0)

of DMBA between the linoleic system and the oleic or octanoic systems are not statistically significant ($p > 0.05$).

The oleic system solubilizes 3MC to the same extent as it does DMBA (20.5 ± 1.3%). Solubilization of 3MC is significantly greater with the oleic system than with either the octanoic or linoleic lipid mixtures ($p < 0.05$). Again, micellar solubilization of this PAH in the medium-chain system to an extent of 11.2 ± 1.4% is only 54% of that solubilized in the oleic mixture and is nearly identical to the amount of DMBA solublized. The linoleic mixture solubilizes significantly less 3MC than does the oleic system ($p < 0.05$).

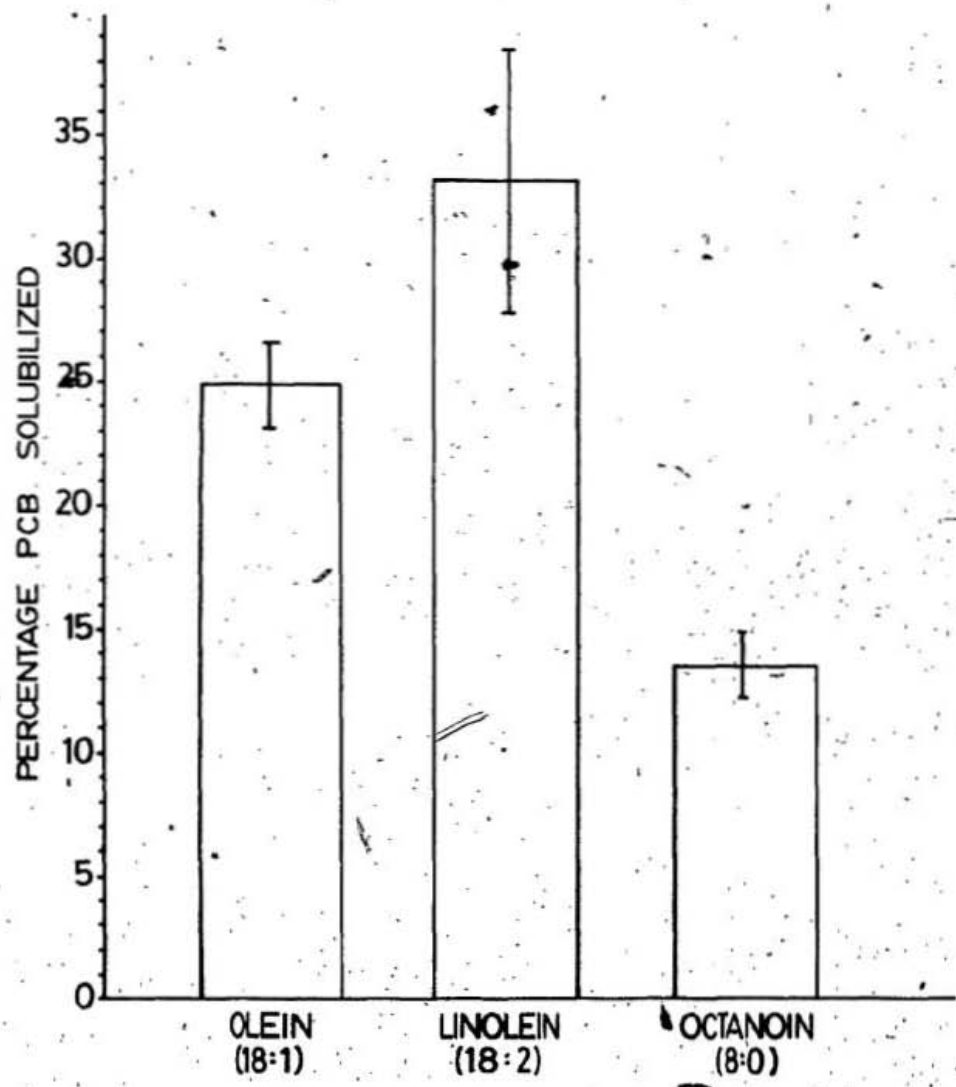
The effect of fatty acid saturation and chain length on the oil:aqueous partition of PCB is shown in Fig. 9. Likewise for this compound, the amount present in micellar solution with the octanoic system is only 54% of that observed using the oleic mixture (13.5 ± 1.3% vs 24.9 ± 1.7% solubilized; $p < 0.05$). In contrast to the PAH data, the polyunsaturated linoleic system promotes the aqueous partition of PCB more than the oleic system but this effect is not significant.

II. Factors influencing DMBA bioavailability in vivo

A. Introduction

As shown in Results I, distribution patterns of PAHs and PCB between phases in simulated intestinal content can

Figure 9: Influence of fatty acid saturation and chain length on the partition of PCB from a lipid phase into an NaTC micellar solution. Partition expressed as percentage of hydrocarbon (± SEM) present in the aqueous phase (n=8). Total PCB in 3.0 ml = 300 nmoles (100 μM); NaTC 12 mM, fatty acid 7.5 mM, monoglyceride 2.5 mM, triglyceride 5.0 mM, phosphatidylcholine 0.68 mM.


PERCENTAGE PCB SOLUBILIZED
0
5
10
15
20
25
30
35
OLEIN
(18:1)
LINOLEIN
(18:2)
OCTANOIN
(8:0)

be influenced by the state of lipid in the system. Not only is the amount of unhydrolyzed triglyceride influential in hydrocarbon oil:aqueous partition, but lipids of different fatty acid chain length will promote partition into the aqueous phase to various degrees. On the basis of insight gained in the *in vitro* situation, *in vivo* experiments were devised to examine the role of intraluminal conditions in governing the bioavailability of a PAH co-administered with dietary lipid. The lipids chosen as vehicles are representatives of 3 lipid categories: olive oil as a prototype of long-chain monounsaturated fat, safflower oil for a long-chain polyunsaturated fat and MCT oil representing the medium-chain saturated lipid class. Fatty acid compositions of these oils are given in Table 1 (p. 34).

B. Lipid class and bile participation

1. Biliary recovery

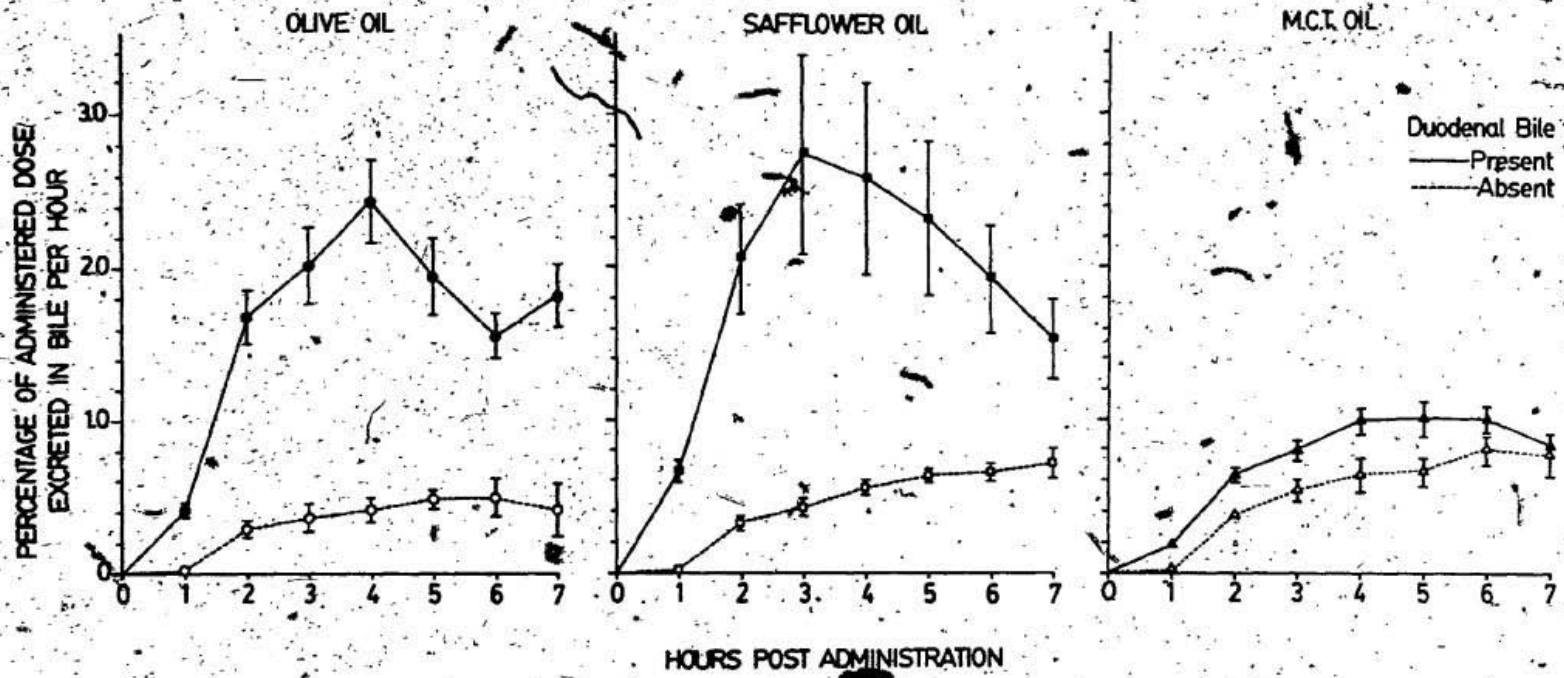
It is known that following systemic entry of a PAH, the majority of an administered dose is rapidly and efficiently excreted in the bile. As well, because biliary excretion of a radiolabeled sample is simple to monitor, it has been chosen as an index of the relative bioavailability of ^{3}H-DMBA following intraduodenal administration in different lipid vehicles. A major disadvantage with this method, however, extends from the complete diversion of bile

(containing bile acids crucial to normal fat digestion and absorption) from the intestinal lumen. To maintain a physiological intraluminal situation, in certain experiments, bile was coadministered with hydrocarbon in its lipid vehicle. Comparing these results with animals totally devoid of intraduodenal bile, it was possible to assess the role of bile in hydrocarbon bioavailability from different classes of dietary fat (c.f. Methods II, E; procedures 1 and 2).

Figure 10 shows the 7 hour pattern at hourly intervals of biliary radiolabel recovery following ^{3}H-DMBA administration in the 3 oils, both with and without bile supplementation. In the absence of duodenal bile, recovery patterns of ^{3}H are similar from olive, safflower and MCT oil. The rate of ^{3}H biliary excretion remains relatively constant between 2 and 7 hours following instillation with no obvious peaks occurring. The largest hourly recovery of radiolabel occurs in the 5-6 hour period for MCT and olive oils and in the 6-7 hour period for safflower oil. The highest hourly recovery is found for MCT oil (0.80 $\pm$ 0.1% of the given dose/h) but this is not significantly different from the largest values for either long chain oil ($p > 0.05$).

Upon inclusion of exogenous bile with MCT oil there is a slight yet insignificant increase in peak biliary excretion of radiolabel to 1.08 $\pm$ 0.12% of the administered dose recovered per hour which occurs in the 4-5 h post-

Figure 10: Hourly excretion of radiolabel in bile following intraduodenal ^{3}H-DMBA administration in olive oil, safflower oil or MCT oil, each with and without the coadministration of exogenous bile. Biliary excretion of radiolabel was monitored at regular intervals for 7 hours following the test meal. Each point represents the mean (± SEM) for 6 animals.


OLIVE OIL
SAFFLOWER OIL
M.C.T. OIL
PERCENTAGE OF ADMINISTERED DOSE EXCRETED IN BILE PER HOUR
30
20
10
0
0 1 2 3 4 5 6 7
HOURS POST ADMINISTRATION
Duodenal Bile
Present
Absent

infusion period. Unlike its effect observed with MCT, exogeneous bile profoundly enhances the recovery of radiolabel from both long-chain fats which have very similar patterns and peaks of recovery ($p < 0.05$). Peak recoveries from both long-chain oils are advanced to within 4 hours of administration and increase in magnitude 4- and 5-fold for safflower and olive oil, respectively ($p < 0.05$). Peak hourly appearance of radiolabel using the MCT/bile mixture is only 40% and 44% of peaks attained with bile-supplemented safflower and olive oils, respectively ($p < 0.05$).

Expanded pictures of the 7-hour radiolabel biliary excretion patterns showing 20 minute excretion patterns from olive and MCT oil are given in Fig. 11 and Fig. 12, respectively. Figure 13 depicts the mean biliary radiolabel excetion profile for 3 animals taken from 7 to 23 hours post-administration of ^{3}H-DMBA in olive oil with bile. This portion of the study was carried out to determine if there were any subsequent peaks in excretion following the one observed between 3 and 4 hours. It can be seen that excretion of radiolabel declines steadily after the initial 3 hour climb and plateaus at about 18 hours following the test meal.

Cumulative recovery of radiolabel under the above conditions is shown in Table 6 which depicts recovery on an hourly basis for 7 hours and in Fig. 14 which shows cumulative 24 hour recovery of radiolabel in bile. With bile exclusion, the long-chain vehicles give similar

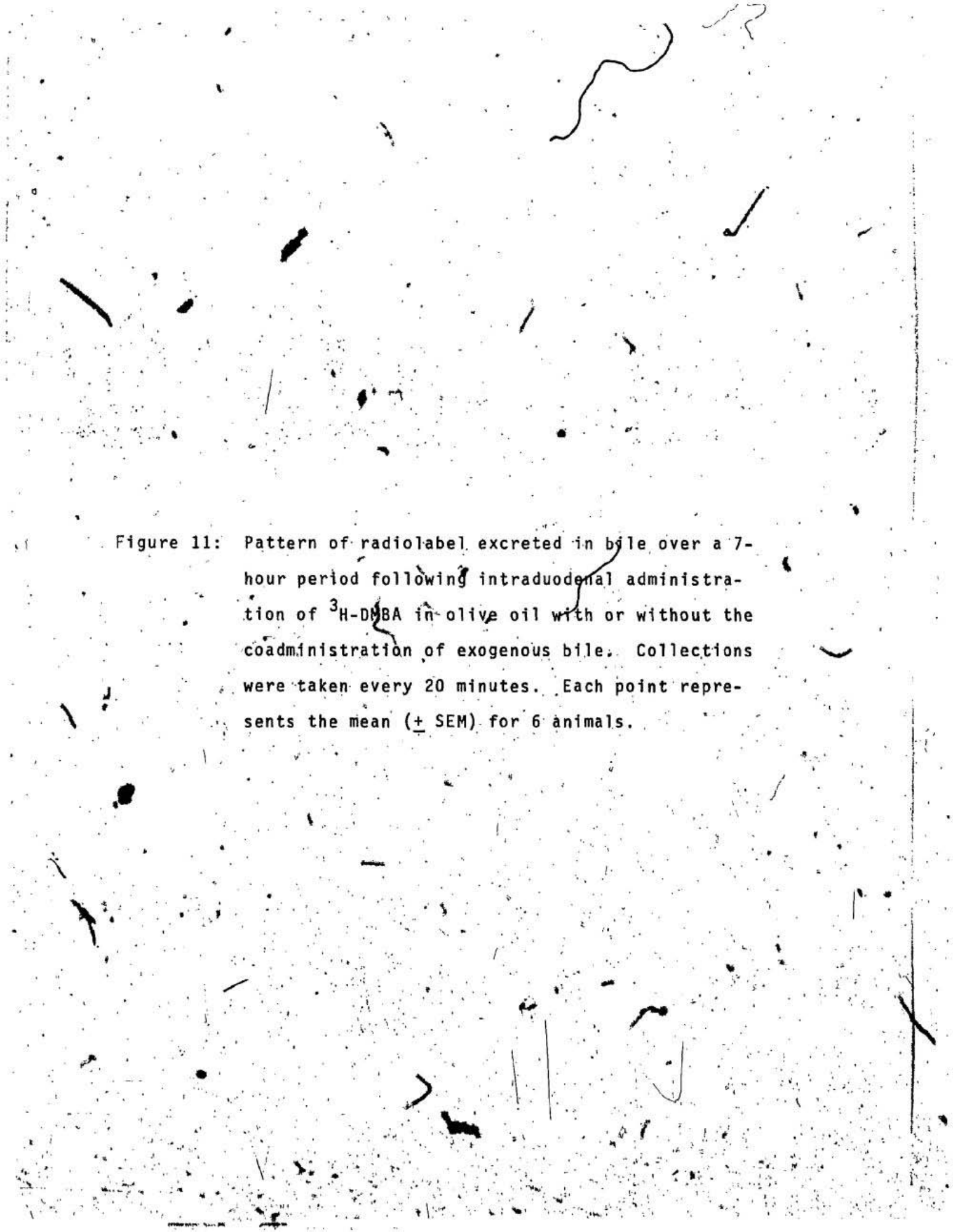


Figure 11: Pattern of radiolabel excreted in bile over a 7-hour period following intraduodenal administration of ^{3}H-DMBA in olive oil with or without the coadministration of exogenous bile. Collections were taken every 20 minutes. Each point represents the mean (± SEM) for 6 animals.

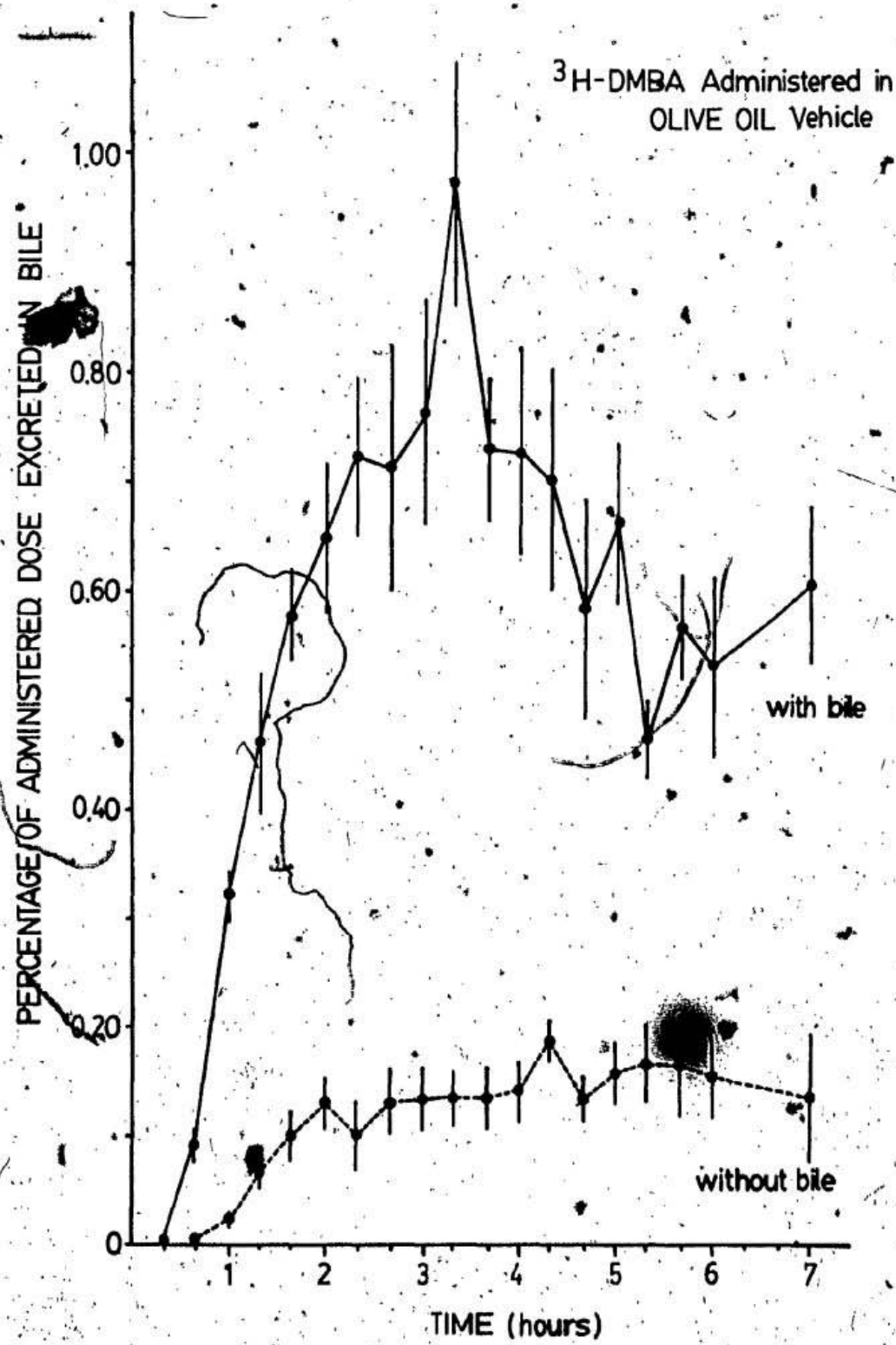

^{3}H-DMBA Administered in
OLIVE OIL Vehicle
PERCENTAGE OF ADMINISTERED DOSE EXCRETED IN BILE
1.00
0.80
0.60
0.40
0.20
0
with bile
without bile
1
2
3
4
5
6
7
TIME (hours)

Figure 12: Pattern of radiolabel excreted in bile over a 7-hour period following intraduodenal administration of ^{3}H-DMBA in MCT oil with or without the coadministration of exogenous bile. Collections were taken every 20 minutes. Each point represents the mean (± SEM) for 6 animals.

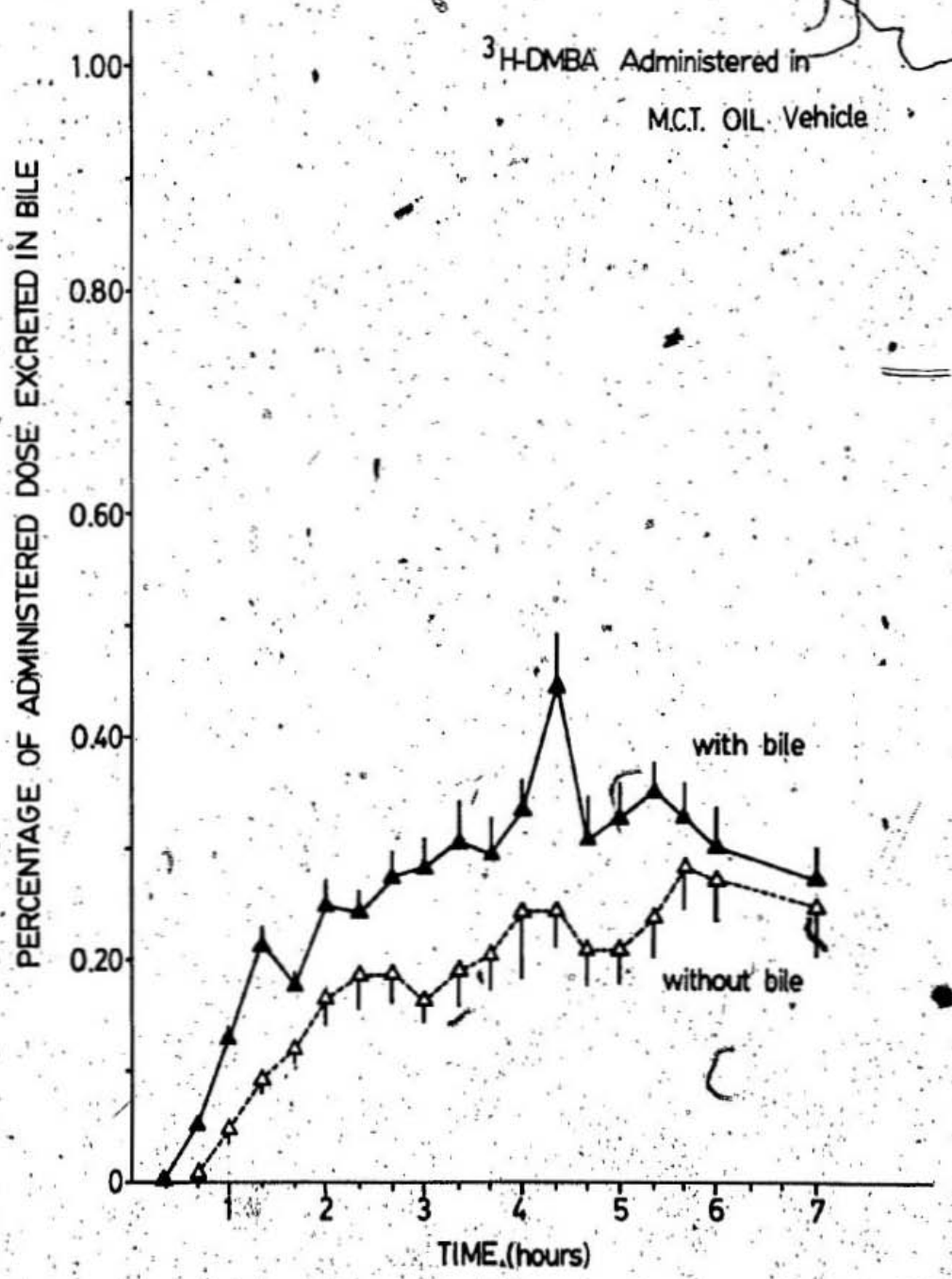

^{3}H-DMBA Administered in
M.C.T. OIL Vehicle
PERCENTAGE OF ADMINISTERED DOSE EXCRETED IN BILE
1.00
0.80
0.60
0.40
0.20
0
with bile
without bile
1
2
3
4
5
6
7
TIME (hours)

Figure 13: Pattern of radiolabel excreted in bile from 8 to 23 hours following intraduodenal administration of ^{3}H-DMBA in olive oil with the co-administration of exogenous bile. Excretion of radiolabel was monitored every 30 minutes. Each point represents the mean for 3 animals.

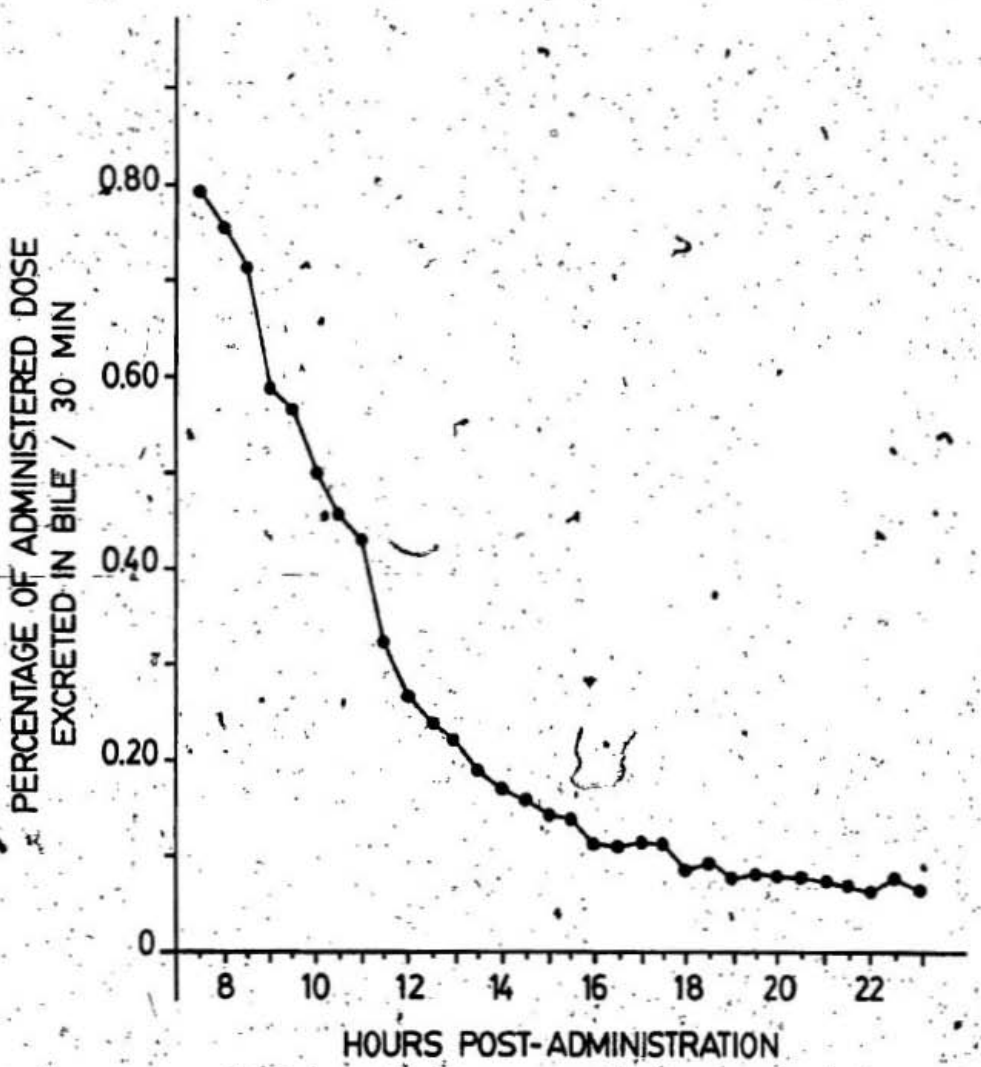

PERCENTAGE OF ADMINISTERED DOSE
EXCRETED IN BILE / 30 MIN
0.80
0.60
0.40
0.20
0
8
10
12
14
16
18
20
22
HOURS POST-ADMINISTRATION

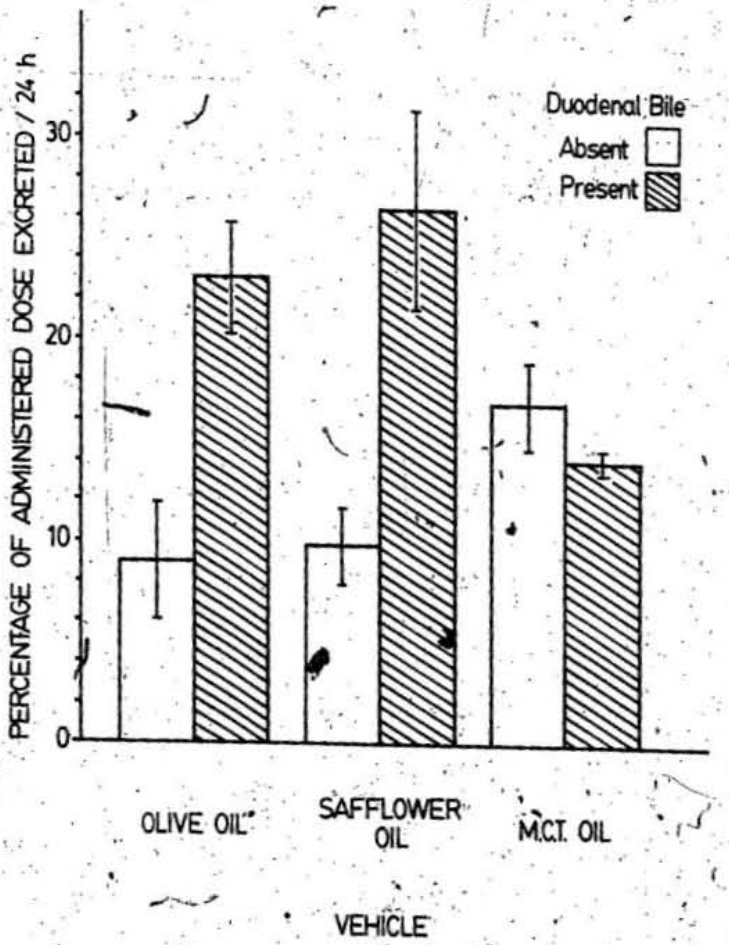
Table 6. Hourly cumulative recoveries of biliary radiolabel over 7 hours following ^{3}H-DMBA administration in different lipid vehicles with and without luminal bile [a,b].

Hours	Olive oil		Safflower oil		MCT oil	
	alone	bile	alone	bile	alone	bile
1	3.66×10^{-2} ($\pm 6.2 \times 10^{-3}$)	0.418 ($\pm 3.4 \times 10^{-2}$)	1.95×10^{-2} ($\pm 1.04 \times 10^{-2}$)	0.654 ($\pm 8.0 \times 10^{-2}$)	5.64×10^{-2} ($\pm 1.3 \times 10^{-2}$)	0.183 ($\pm 1.7 \times 10^{-2}$)
2	0.333 ($\pm 6.3 \times 10^{-2}$)	2.11 ($\pm$ 0.20)	0.340 ($\pm 4.5 \times 10^{-2}$)	2.71 ($\pm$ 0.44)	0.433 ($\pm 4.7 \times 10^{-2}$)	0.820 ($\pm 5.8 \times 10^{-2}$)
3	0.698 ($\pm$ 0.118)	4.31 ($\pm$ 0.45)	0.764 ($\pm 9.6 \times 10^{-2}$)	5.46 ($\pm$ 1.07)	0.968 ($\pm$ 0.116)	1.62 ($\pm$ 0.12)
4	1.11 ($\pm$ 0.23)	6.75 ($\pm$ 0.68)	1.31 ($\pm$ 0.12)	8.03 ($\pm$ 1.65)	1.61 ($\pm$ 0.21)	2.59 ($\pm$ 0.19)
5	1.59 ($\pm$ 0.28)	8.70 ($\pm$ 0.87)	1.94 ($\pm$ 0.16)	10.35 ($\pm$ 2.11)	2.26 ($\pm$ 0.29)	3.57 ($\pm$ 0.29)
6	2.07 ($\pm$ 0.37)	10.23 ($\pm$ 0.93)	2.59 ($\pm$ 0.20)	12.28 ($\pm$ 2.44)	3.06 ($\pm$ 0.38)	4.66 ($\pm$ 0.35)
7	2.49 ($\pm$ 0.54)	12.09 ($\pm$ 0.98)	3.26 ($\pm$ 0.29)	16.79 ($\pm$ 3.81)	3.80 ($\pm$ 0.48)	5.48 ($\pm$ 0.41)

a. recoveries expressed as percentage of administered dose

b. each value represents the mean ($\pm$ SEM) for 6 determinations

Figure 14: Total biliary excretion of radiolabel in bile for a 24-hour period following intraduodenal ^{3}H-DMBA administration in olive oil, safflower oil or MCT oil, each with and without the coadministration of exogenous bile. Each value represents the mean ($\pm$ SEM) for 6 animals.


PERCENTAGE OF ADMINISTERED DOSE EXCRETED / 24 h
30
20
10
0
Duodenal Bile
Absent
Present
OLIVE OIL
SAFFLOWER OIL
M.C.T. OIL
VEHICLE

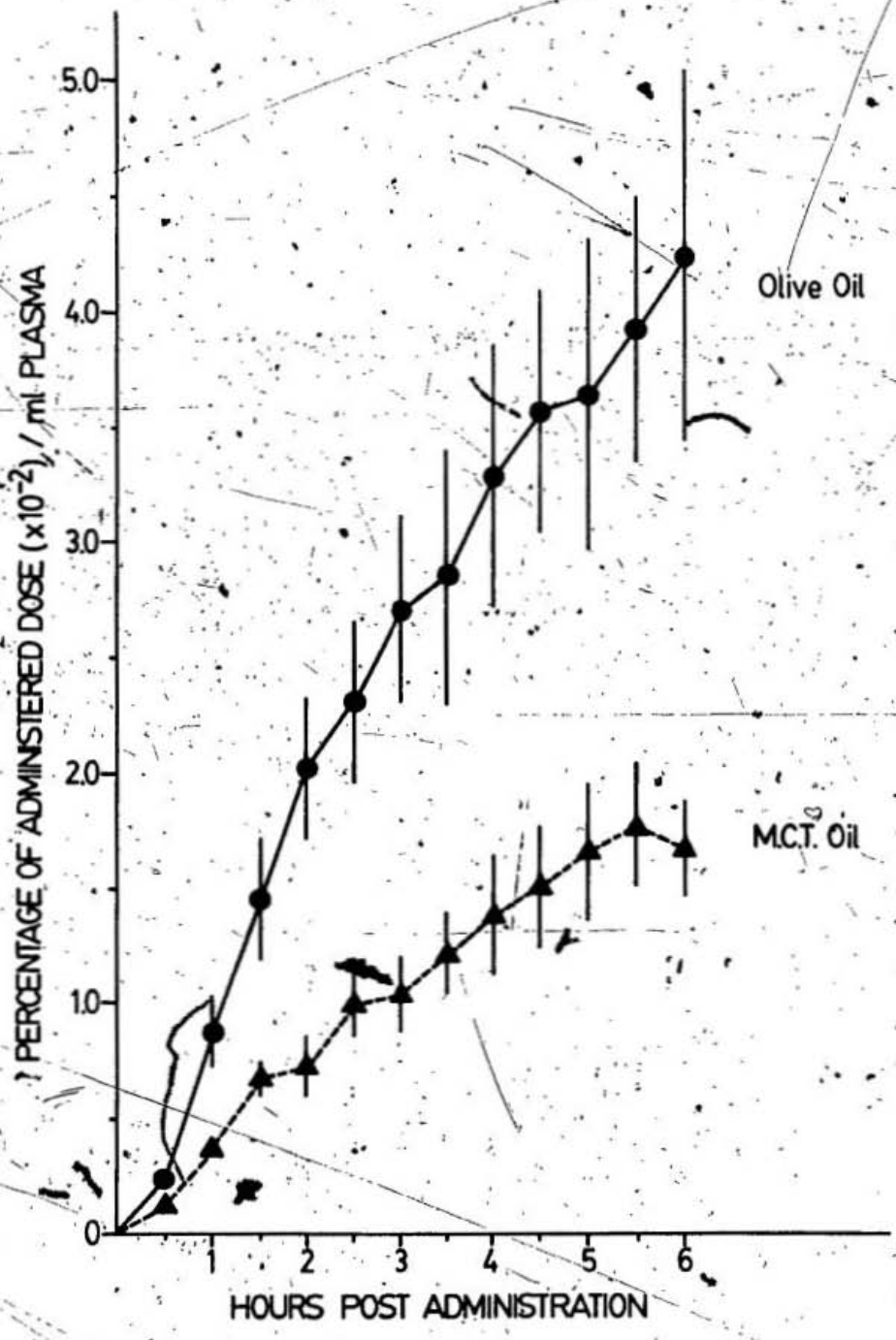
recoveries of biliary radiolabel; 9.0 ± 3.0% for olive oil and 9.8 ± 1.9% of the administered dose recovered from safflower oil in a 24 hour period. The corresponding value for MCT oil is significantly greater at 16.9 ± 2.2% than from either long chain oil ($p < 0.05$).

With MCT oil as the vehicle for DMBA, duodenal bile fails to enhance biliary recovery in comparison to the oil alone ($p > 0.05$) with only 14.1 ± 0.6% of the given dose excreted in bile in 24 hours. Recoveries from olive and safflower oil are profoundly increased to 23.1 ± 2.7% and 26.6 ± 4.9%, respectively. These represent significantly greater recoveries from any of the oils alone or from the MCT oil/bile combination used as the test mixture ($p < 0.05$).

2. Plasma recovery

A series of experiments were performed to validate the premise that biliary excretion of radiolabel can be used as a relative index of the bioavailability of intraduodenally administered hydrocarbon. Test meals of ^{3}H-DMBA in olive oil or MCT oil were infused intraduodenally to rats with intact enterohepatic circulations. Plasma levels of radiolabel were then monitored at regular intervals (c.f. Methods II, E; procedure 3). Figure 15 shows the relative plasma levels of radiolabel attained following each test meal. Throughout the study period olive oil achieves mean plasma levels of radiolabel more than double those using the medium-chain vehicle ($p < 0.05$). Peak appearance of

Figure 15: Plasma appearance of radiolabel following intra-duodenal administration of ^{3}H-DMBA in olive oil or MCT oil to animals with intact enterohepatic circulations. Plasma levels were monitored every 30 minutes for 6 hours following the test meals. Each point represents the mean (± SEM) for 6 animals.


5.0
4.0
3.0
2.0
1.0
0
PERCENTAGE OF ADMINSTERED DOSE (x10⁻²) / ml PLASMA
1
2
3
4
5
6
HOURS POST ADMINISTRATION
Olive Oil
M.C.T. Oil

radiolabel is not apparently achieved by 6 h following administration in olive oil but may be achieved before this time with the medium-chain lipid. Peak levels of radiolabel seen with MCT oil are equivalent to 6.9 ± 1.0 p moles DMBA/ml plasma, whereas maximal levels attained with olive oil in the 6 hour period correspond to 16.5 ± 3.2 p moles/ml.

C. Enterohepatic recycling

Because DMBA metabolites are extensively excreted in the bile there is a strong possibility of some subsequent reabsorption. Rat biliary metabolites of ^{3}H-DMBA were collected and pooled. These radiolabelled DMBA derivatives were then infused intraduodenally to recipient rats and subsequent biliary excretion of radiolabel was monitored (c.f. Methods II, E; procedure 6). Table 7 expresses the mean cumulative percentage of the administered dose re-excreted in the bile following the infusion. The recipient rats absorb and re-excrete radiolabel quickly and at 24 hours from commencing the infusion, 32.8 ± 2.8% of the administered radioactivity is recovered in the bile.

D. Lipid carrier volume

These experiments were carried out to determine if the volume of carrier triglyceride concomitantly absorbed with DMBA could influence the amount of hydrocarbon absorbed. A constant quantity of ^{3}H-DMBA was administered

Table 7. Cumulative recovery of biliary radiolabel following administration of radiolabelled biliary products of ^{3}H-DMBA [a,b].

Time (min)	Cumulative excretion of biliary radiolabel
0	2.37 (± 0.70)
30	3.30 (± 0.97)
60	4.43 (± 1.20)
90	5.57 (± 1.40)
120	6.86 (± 1.61)
150	8.17 (± 1.84)
1230	32.81 (± 2.76)

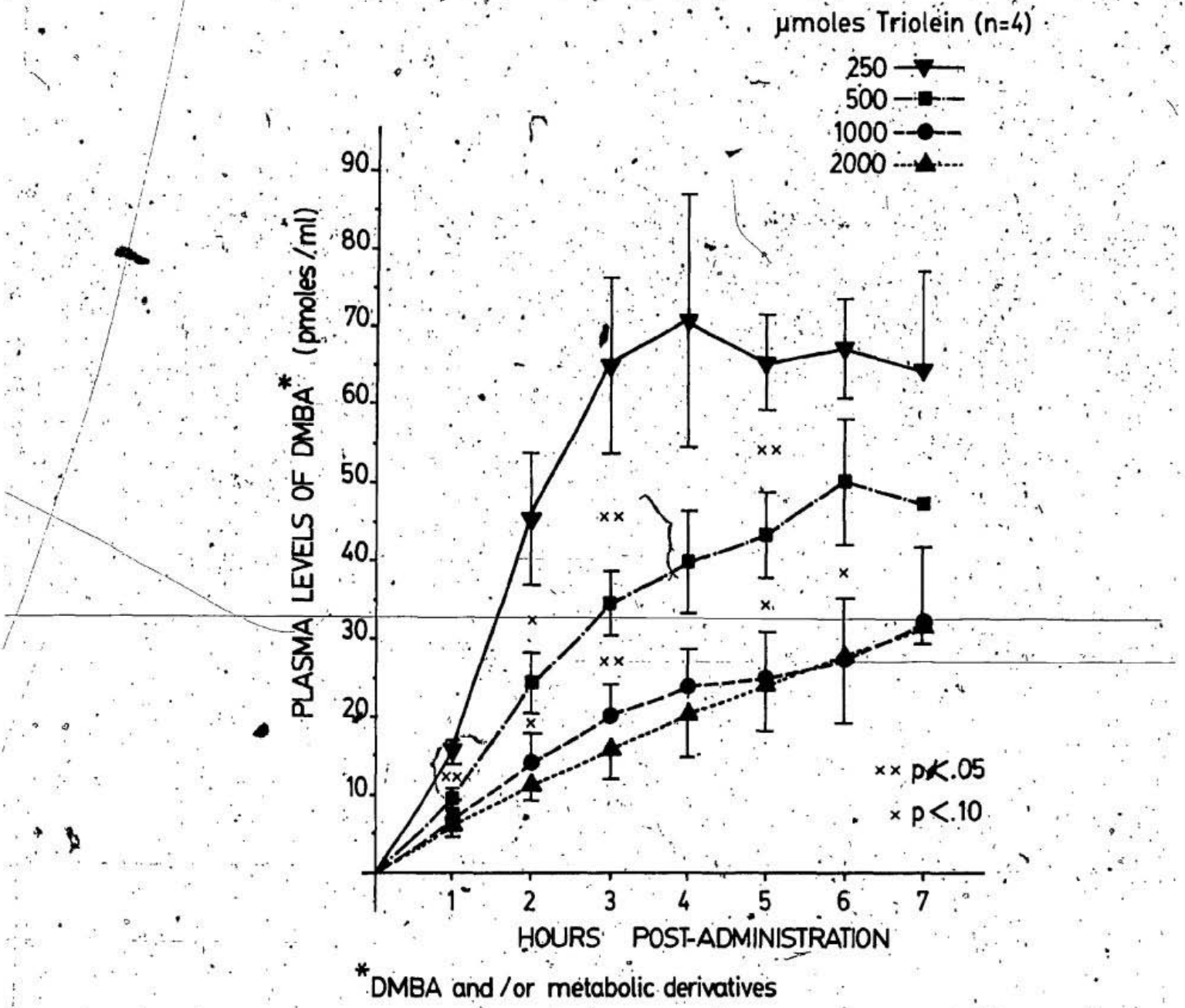
a. infusion period of 210 minutes; t=0 at 210 minutes from commencing infusion

b. recovery expressed as percentage of administered dose; each value represents the mean (± SEM) for 6 determinations

intraduodenally in increasing volumes of a triolein test meal and then plasma levels of radiolabel were monitored for 7 hours (c.f. Methods II, E; procedure 4). It is found that levels of plasma radiolabel are inversely related to triolein volume (Fig. 16). Levels attained following administration in 500 μmoles of triolein are consistently greater than those observed with 1000 or 2000 μmoles of lipid but consistently less than those achieved with a 250 μmole dose of triolein. The 250 μmole dose of lipid produces significantly greater ($p < 0.05$) plasma radiolabel levels at all time points when compared to the 2000 μmole dose, and also the 1000 μmole dose (except at 7 hours where $p < 0.10$). The relatively low amounts of plasma radiolabel observed with 1000 and the 2000 μmole amounts of triglyceride are nearly identical ($p > 0.05$) throughout the 7 hour study period.

The most rapid rise in plasma radiolabel is observed following the smallest dose of triolein, with 500 μmoles of lipid producing a more modest rate of increase. Peaks of radiolabel may be discerned at 4 hours and 6 hours post-administration for the 250 μmole and 500 μmole triolein doses, respectively. With the larger amounts of lipid there are small but persistent hourly increases in plasma radiolabel with no leveling trend yet apparent at 7 hours post-administration.

Figure 16: Plasma levels of radiolabel derived from ^{3}H-DMBA following intraduodenal administration in different amounts of triolein carrier to rats with intact enterohepatic circulations. Plasma radiolabel was monitored every hour for 7 hours following the test meal. Each point represents the mean (± SEM) for 4 animals.



*DMBA and/or metabolic derivatives

E. Lymphatic vs portal transport: effect of hydrocarbon dose

To assess the major route of transport of DMBA from the small intestine, animals with main intestinal lymphatic and biliary catheters were infused with olive oil-bile test meals containing 3 different doses of hydrocarbon (c.f. Methods II, E; procedure 5). It was presumed that with total lymphatic diversion, radiolabel excreted in bile had gained access to the system via the portal blood. As portal blood is generally considered responsible for the carriage of predominantly polar compounds, and the lymphatic system is mainly concerned with the transport of non-polar substances, it is likely that the distribution of radiolabel between the two systems will roughly reflect the degree the metabolism of the xenobiotic. The DMBA doses used (10 µg, 10 mg and 20 mg) were chosen in an attempt to find a dose of hydrocarbon which would exceed the metabolizing capacity of the intestinal mucosa, and alter the lymph:bile ratio of radiolabel. An assay of the fraction of lymph responsible for radiolabel transport was also undertaken. The relative recoveries of radiolabel in bile and in lymph from the 3 doses were calculated, as well as the relationship of bile or lymph flow rate with their respective radiolabel levels to the overall recovery of radiolabel in either compartment.

1. Recovery patterns

a. 10 µg DMBA

Figure 17 shows the 7-hour patterns of lymph and bile flow and their transport of radiolabel (representing parent DMBA and/or metabolites) following a 10 µg dose of DMBA. Following test meal administration bile flow increases from 0.24 ± 0.03 ml/30 min to plateau at approximately 0.37 ± 0.02 ml/30 min (with fluctuations between 0.29 and 0.42 ml/30 min). Radiolabel is detected in bile within 30 minutes of beginning test meal infusion with levels increasing throughout the entire 7 hour study period. Concentrations of radiolabel attained at the end of this period are equivalent to 271 ± 62 ng DMBA/ml bile.

Lymph flow rises dramatically 2 hours subsequent to the test meal from 0.65 ml/30 min to reach a mean rate of 1.28 ± 0.05 ml/30 min. Fluctuations in flow rate occur throughout the study period. No or only trace quantities of radiolabel are detected in lymph 30 minutes from beginning the test meal, by contrast with the early appearance of biliary radioactivity. By two hours post-administration, lymphatic radiolabel concentrations climb rapidly and appear to correspond with the appearance of chylomicra. Concentrations peak at 3 hours achieving levels equivalent to 31.9 ± 5.5 ng DMBA/ml lymph. Lymphatic radiolabel then declines to levels at 6 to 7 hours which are approximately 50% of peak levels.

Figure 17: Relative recoveries of radiolabel in bile and lymph over 7 hours following administration of 10 µg ^{3}H-DMBA in olive oil with luminal bile.

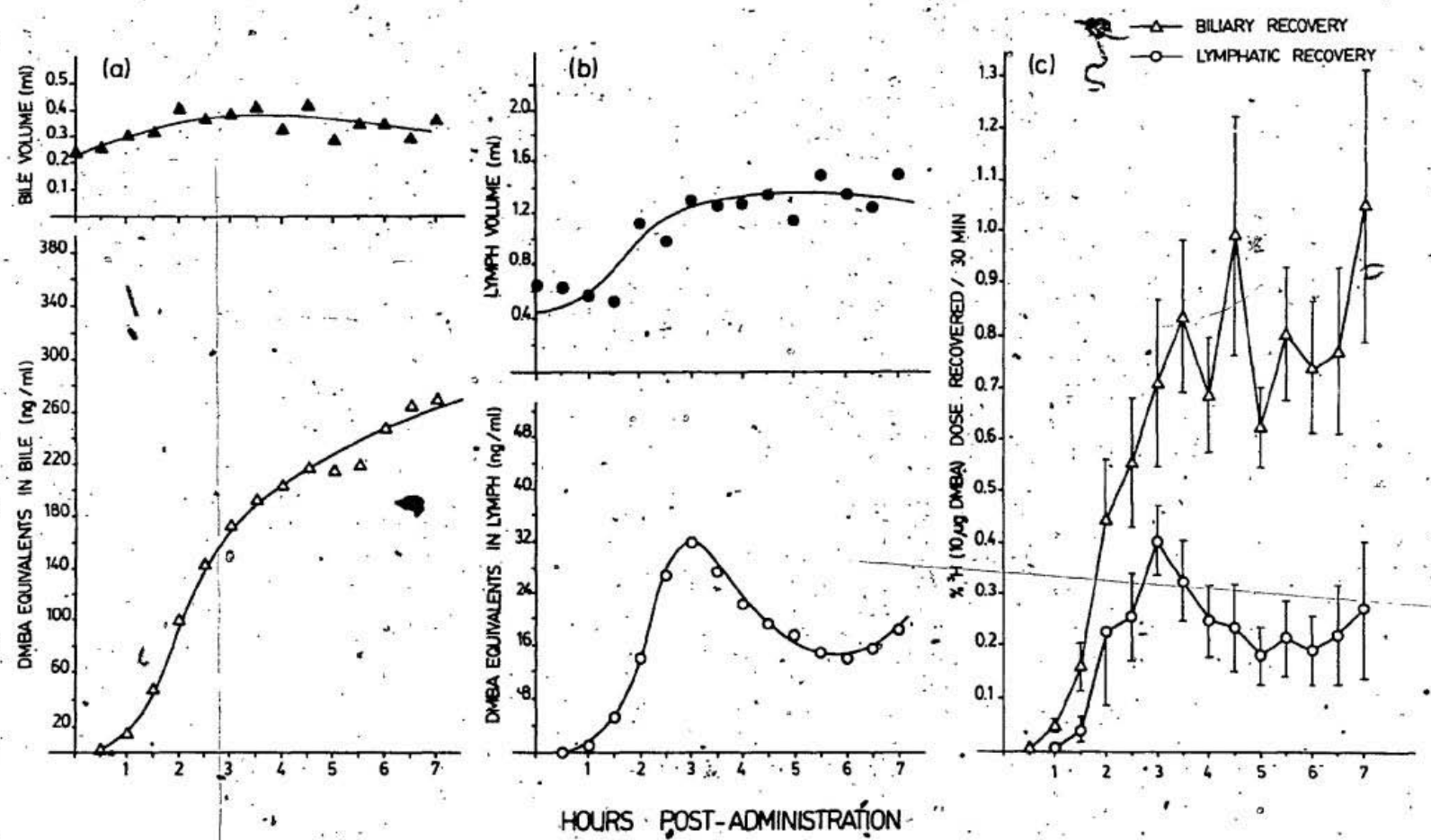
(a) Bile volume and DMBA concentration*

(b) Lymph volume and DMBA concentration*

(c) Total recoveries of radiolabel in bile and in lymph as percentage of administered radiolabel.

Each point represents the mean for 6 determinations. Vertical lines represent the SEM which are shown for (c) only.

*Concentrations of DMBA are derived from measurement of radiolabel only and therefore may include the presence of metabolites.


(a)
BILE VOLUME (ml)
0.5
0.4
0.3
0.2
0.1
DMBA EQUIVALENTS IN BILE (ng/ml)
380
340
300
260
220
180
140
100
60
20
1 2 3 4 5 6 7
(b)
LYMPH VOLUME (ml)
2.0
1.6
1.2
0.8
0.4
DMBA EQUIVALENTS IN LYMPH (ng/ml)
48
40
32
24
16
8
1 2 3 4 5 6 7
HOURS POST-ADMINISTRATION
(c)
BILIARY RECOVERY
LYMPHATIC RECOVERY
% ³H (10 µg DMBA) DOSE RECOVERED / 30 MIN
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1 2 3 4 5 6 7

Actual recoveries of radiolabel are a product of both concentration in and volume of bile or lymph for each 30 minute period. During the 7 hour post-test meal period, lymphatic radiolabel recovery remains considerably lower than corresponding radiolabel retrieved from bile. Although biliary concentrations of radiolabel rise steadily, marked fluctuations in actual quantities recovered are evident and correspond to alterations in bile flow. Variations in lymph flow, although not without effect, do not exhibit as profound an influence on lymphatic radiolabel recovery which more closely parallels lymphatic radiolabel concentration patterns.

b. 10 mg DMBA

The 7 hour radiolabel recovery patterns in lymph and bile following a 10 mg dose of DMBA are shown in Fig. 18. Bile flow increases following the test meal to plateau at about 0.37 $\pm$ 0.01 ml/30 min from a pre-infusion rate of 0.31 $\pm$0.02 ml/30 min. Within 30 minutes from beginning the test meal, radiolabel is detected in bile and peaks at 164 $\pm$ 22 µg DMBA equivalents/ml bile over the next 3.5 hours. Thereafter concentrations gradually decline to 130 $\pm$ 25 µg DMBA equivalents/ml at 7 hours post-administration.

Mean lymph flow increases markedly following instillation of the test meal from 0.58 $\pm$ 0.2 ml/30 min pre-infusion to reach a plateau of roughly 1.13 $\pm$ 0.14 ml/30 min. Only trace quantities of radiolabel are detected in

Figure 18: Relative recoveries of radiolabel in bile and lymph over 7 hours following administration of 10 mg ^{3}H-DMBA in olive oil with luminal bile.

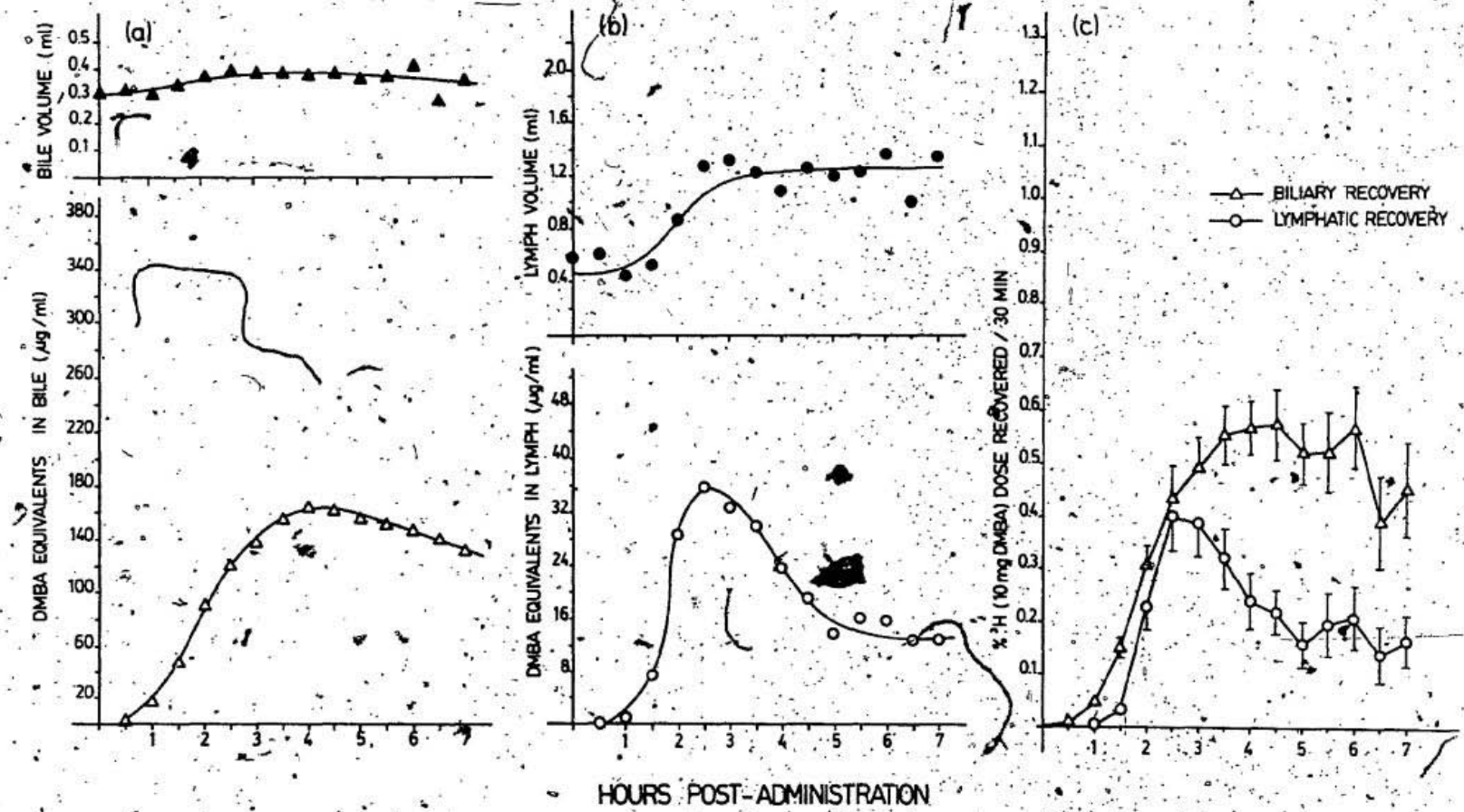
(a) Bile volume and DMBA concentration*

(b) Lymph volume and DMBA concentration*

(c) Total recoveries of radiolabel in bile and in lymph as percentage of administered radiolabel.

Each point represents the mean for 6 determinations. Vertical lines represent the SEM which are shown for (c) only.

*Concentrations of DMBA are derived from measurements of radiolabel only and therefore may include the presence of metabolites.


(a)
BILE VOLUME (ml)
0.5
0.4
0.3
0.2
0.1
DMBA EQUIVALENTS IN BILE (µg/ml)
380
340
300
260
220
180
140
100
60
20
1
2
3
4
5
6
7
(b)
LYMPH VOLUME (ml)
2.0
1.6
1.2
0.8
0.4
DMBA EQUIVALENTS IN LYMPH (µg/ml)
48
40
32
24
16
8
1
2
3
4
5
6
7
HOURS POST-ADMINISTRATION
(c)
BILIARY RECOVERY
LYMPHATIC RECOVERY
% ³H (10 mg DMBA) DOSE RECOVERED / 30 MIN
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1
2
3
4
5
6
7

lymph 30 minutes after beginning administration whereas biliary appearance at this time is significant. Peak concentations of 35.8 µg DMBA equivalents/ml lymph were seen at 2.5 hours post-feeding. The subsequent decline in concentration is initially rapid to reach a plateau at levels about one-third of peak values during the latter phase of the study period.

Actual recoveries of radiolabel in bile are consistently greater than corresponding lymphatic values. Between 2 and 3 hours post-administration (near peak lymphatic radiolabel recovery) there is no significant difference between lymphatic and biliary recoveries ($p > 0.05$). The subsequent decrease in lymphatic radiolabel accompanies an increase in biliary recovery during the next 3 hours. Recovery in bile begins to decline in the 6 to 7 hour post-administration period. Fluctuations in actual biliary radiolabel result from deviations in bile flow and are not attributable to erratic alterations in concentration which decline at an even rate.

c. 20 mg DMBA

Total transport of radiolabel in bile throughout the 7-hour study period is significantly greater than corresponding lymphatic transport (Fig. 19). Actual recovery of radiolabel in bile peaks at 4.5 hours post-administration which is equivalent to 370 ± 80 µg DMBA/ml bile. Following this, biliary levels of radiolabel assume a

Figure 19: Relative recoveries of radiolabel in bile and lymph over 7 hours following administration of 20 mg ^{3}H-DMBA in olive oil with luminal bile.

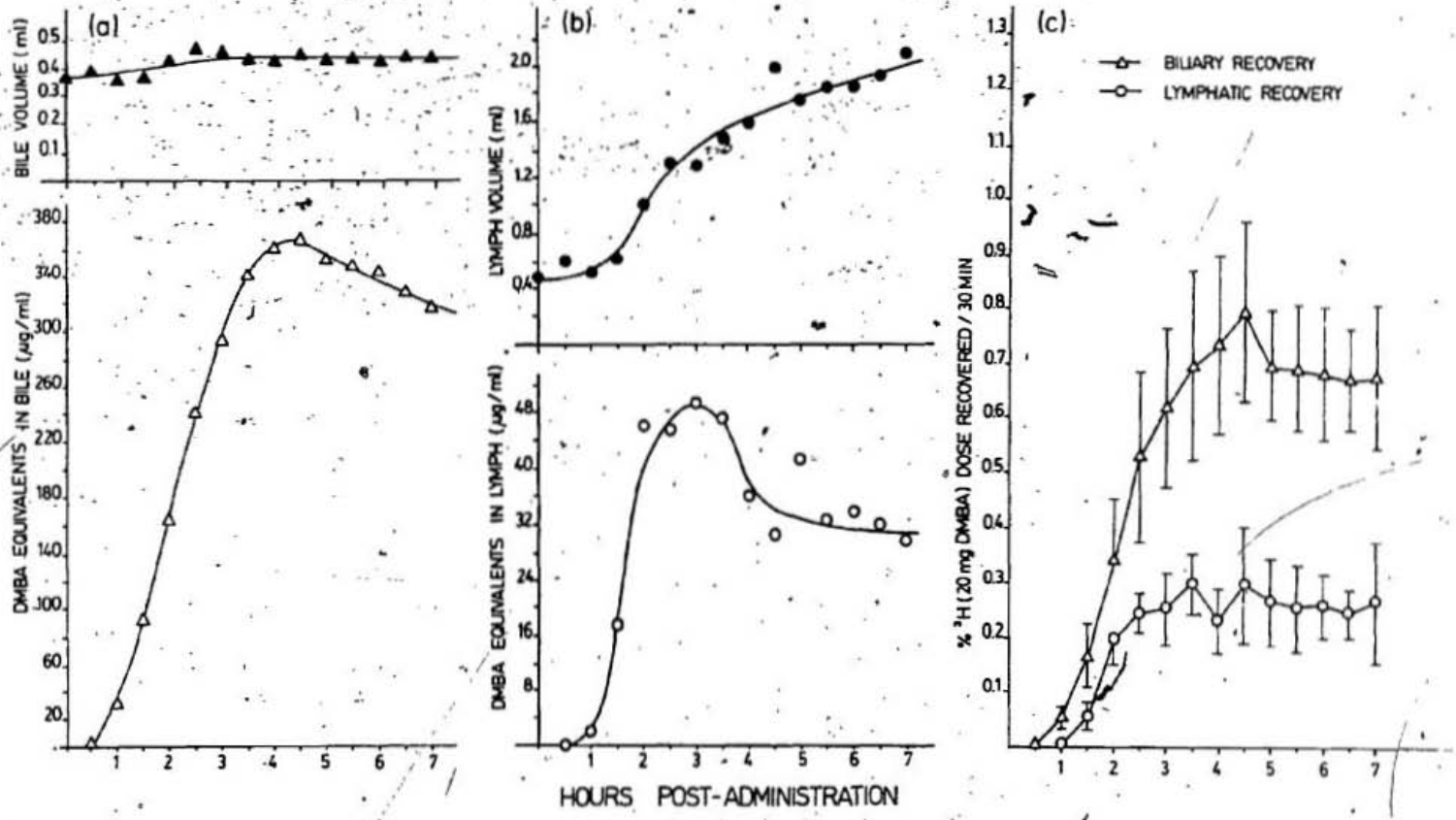
(a) Bile volume and DMBA concentration*

(b) Lymph volume and DMBA concentration*

(c) Total recoveries of radiolabel in bile and in lymph as percentage of administered radiolabel.

Each point represents the mean for 6 determinations. Vertical lines represent the SEM which are shown for (c) only.

*Concentrations of DMBA are derived form measurements of radiolabel only and therefore may include the presence of metabolites.


(a)
BILE VOLUME (ml)
DMBA EQUIVALENTS IN BILE (μg/ml)
(b)
LYMPH VOLUME (ml)
DMBA EQUIVALENTS IN LYMPH (μg/ml)
HOURS POST-ADMINISTRATION
(c)
BILIARY RECOVERY
LYMPHATIC RECOVERY
% ³H (20 mg DMBA) DOSE RECOVERED / 30 MIN

gentle decline attributable to reduced concentrations of radiolabel and slight fluctuations in flow rate.

Peak lymph concentrations of radiolabel (corresponding to 49.4 ± 15.8 µg DMBA/ml lymph) occur at 3 hours post-feeding. This, however, is not reflected in a peak for actual recovery of radiolabel in lymph. Accompanying the subsequent decline in radiolabel concentration is a steady increase in lymphatic flow rate from 1.31 ± 0.28 ml/30 min concomitant with the peak to over 2 ml/30 min at 7 hours post-administration. Total transport of radiolabel in lymph, therefore, plateaus from 3 to 7 hours post-administration.

2. Total recovery

Figure 20 illustrates the cumulative 24-hour recovery of radiolabel derived from bile and lymph with the relative distribution of radioactivity between these compartments at each level of hydrocarbon. Total recoveries as percentage of administered dose are not significantly different between the 3 doses ($p > 0.05$), although recovery from the 10 mg dose as compared to the 10 µg and 20 mg doses was somewhat less ($p < 0.01$).

In a 24-hour period, the percentages of radiolabel recovered in the lymph from the 10 µg, 10 mg and 20 mg doses are 4.35 ± 1.12%, 3.32 ± 0.36% and 4.62 ± 1.11% of the administered doses, respectively. Biliary radioactivity accounts for the greatest proportion of retrieved radiolabel.

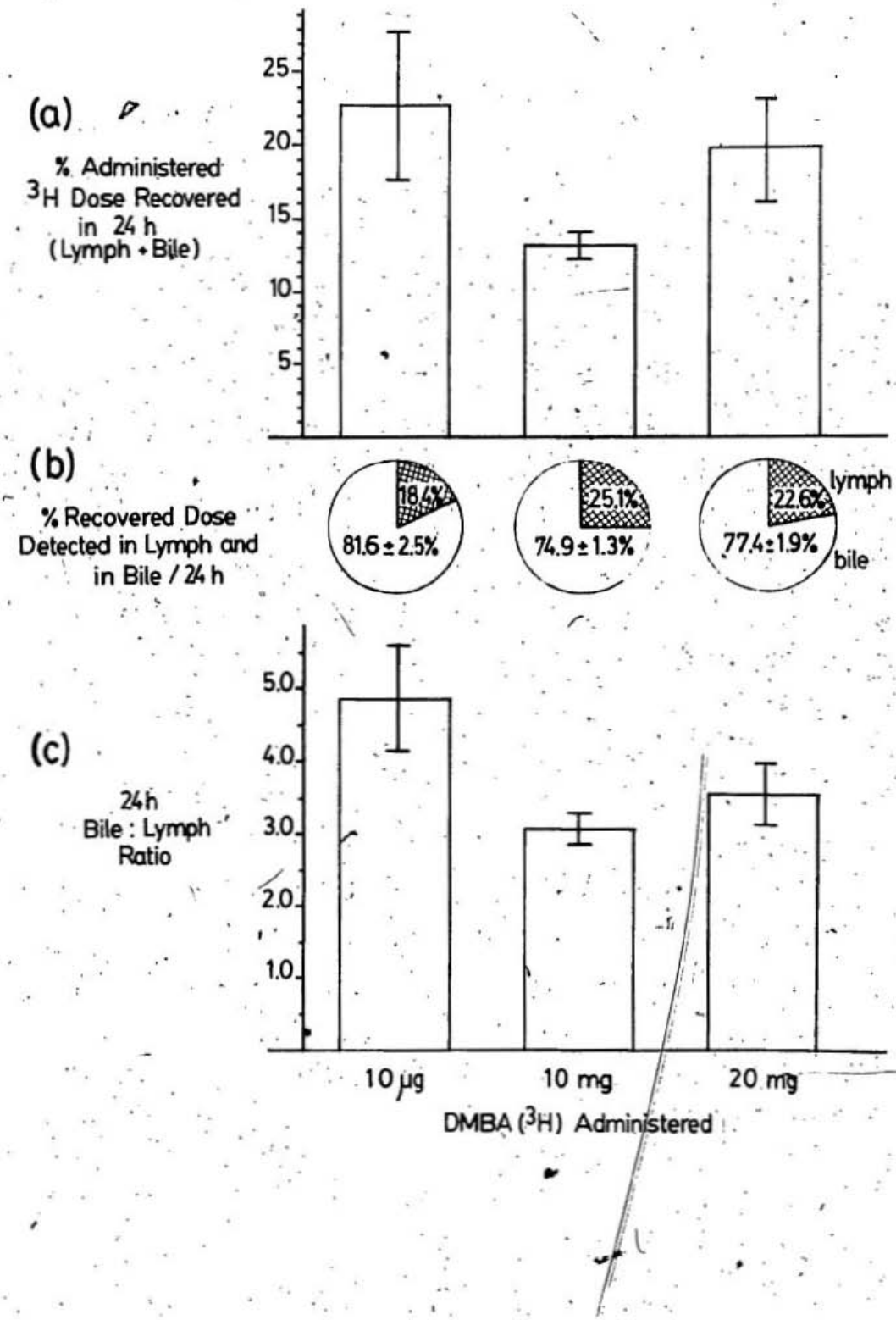
Figure 20: Twenty-four hour recoveries of radiolabel in bile and lymph following intraduodenal administration of ^{3}H-DMBA at 3 dosage levels in olive oil with bile supplementation.

(a) Total radiolabel recovery from bile plus lymph as percentage of administered dose

(b) Distribution of recovered radiolabel between lymph and bile

(c) Bile : lymph ratios of radiolabel

Each value represents the mean (± SEM) for at least 4 determinations.


(a)
% Administered
^{3}H Dose Recovered
in 24 h
(Lymph + Bile)
25
20
15
10
5
(b)
% Recovered Dose
Detected in Lymph and
in Bile / 24 h
18.4%
81.6 ± 2.5%
25.1%
74.9 ± 1.3%
22.6%
77.4 ± 1.9%
lymph
bile
(c)
24 h
Bile : Lymph
Ratio
5.0
4.0
3.0
2.0
1.0
10 µg
10 mg
20 mg
DMBA (^{3}H) Administered

regardless of the dose of hydrocarbon given. The 10 mg dose exhibits a significantly lower bile:lymph ratio and percentage of recovered dose present in bile than did the 10 µg dose of DMBA ($p < 0.05$). Values obtained for the 20 mg dose are not significantly different from either the 10 µg or 10 mg dose of hydrocarbon ($p > 0.05$).

3. Detection in chylomicra

As significant levels of lymph radioactivity are only obtained once lymphatic turbidity is observed following the lipid test meals, it was decided to assay the chylomicron fraction of lymph for the presence of radiolabel. Samples of lymph were pooled then ultracentrifuged to separate the chylomicra. Radiolabel retained in the clear infranatant was compared to the level obtained prior to centrifugation. Following removal of the chylomicra only 14.0 ±1.3% of the original radiolabel remained in the infranate. Separation of the chylomicra extracted 86% of the radioactivity from the lymph.

4. Lymphatic contribution to biliary recovery

To gain insight into the biliary excretion of lymph-derived radiolabel, comparisons of total radiolabel recovery in animals with and without lymphatic fistulae were made. All animals received 10 µg DMBA with ^{3}H-DMBA in an olive oil plus bile vehicle. Animals with bile fistulae alone were monitored for biliary excretion of radiolabel and those with

both bile and lymph fistulae had both these fluids monitored and total recovery calculated (c.f. Methods II, E; procedures 2 and 5). A comparison of the two groups is shown in Table 8. Employing Students t-test at any of the time intervals studied, it is evident that biliary recovery in animals with intact intestinal lymphatics does not significantly differ from total recovery (lymph plus bile) in rats with lymphatic diversion.

Table 8. Total recovery of radiolabel from animals with bile fistulae only or from animals with bile and lymph fistulae [a,b].

Fistylae created	Cumulative % of administered dose recovered		
	0 - 7 h	7 - 24 h	0 - 24 h
Bile (n=6)	12.09 (± 0.98)	11.26 (± 2.59)	23.06 (± 2.73)
Bile + lymph (n=6)	11.26 (± 2.18)	10.70 (± 3.18)	22.74 (± 5.01)

a. recovery of 10 µg dose of ^{3}H-DMBA given in olive oil with bile supplementation

b. each value shows the mean (± SEM)

DISCUSSION OF RESULTS

I. Factors influencing hydrocarbon behaviour in vitro

A. Micellar solubilization of hydrocarbons

Non-polar solutes possess an understandably high affinity for a lipid medium. Falk and Kotin (1963) studied the partition of B(a)P between a hydrophilic (dilute plasma protein solution) and a hydrophobic (lipid) medium. When originally present in the aqueous phase the hydrocarbon slowly equilibrated between the aqueous and lipid phases. No transfer occurred, however, when B(a)P was originally present in the lipid solution which retained all the hydrocarbon.

The ability of bile salts to promote aqueous solubilization of non-polar substances is recognised as an important characteristic (Carey and Small, 1970). Efficient intestinal absorption of hydrophobic compounds (e.g. cholesterol) requires solubilization into bile salt micelles as one of several steps involved in lipid assimilation (Hofmann, 1968). During fat digestion, intestinal lipids partition between an oil phase containing mainly higher glycerides and a bile salt micellar phase containing monoglyceride and fatty acid (Hofmann and Borgström, 1962, 1964). Since bile salt lipid micelles are generally thought to be of great importance as a medium for delivery of trace

nutrient lipids to the absorptive epithelial cells of the small intestine, and since similar considerations probably apply to lipophilic xenobiotics, this thesis examines factors governing the distribution of PAH and PCB between an oil phase and an aqueous phase. The simulated intestinal system is modelled after that used by El-Gorab and Underwood (1973) at physiological pH (6.3) containing concentrations of fatty acid, monoglyceride, triglyceride, phospholipid and sodium ion likely to be found post-prandially in the small intestine. The majority (about two-thirds) of monoglyceride present in the luminal contents of the small bowel after a meal is in the form of 2-monoglyceride (Hofmann and Borgström, 1964). Hofmann (1963b) has shown that 1-monoolein and 2-monoolein are solubilized identically by bile salt solutions and it is therefore expected that the use of 1-monoglyceride would not significantly alter the physico-chemical properties of the model system.

The mixed micellar system, which was generated progressively from a mixed lipid phase by increasing the concentration of bile salt, efficiently transferred the PAH and PCB compounds from an oil to an aqueous phase. The micellar:oil ratios of the 4 hydrocarbons examined at 12 mM NaTC were about 1000-fold greater than the system devoid of bile salt (Fig. 4, p. 51). As shown in Fig. 5 (p. 54) partition of the hydrocarbons from the oil into the micellar phase is dependent upon bile salt concentration. Only trace amounts of the 4 hydrocarbons were detected in the micellar

phase below 4 mM NaTC. This concentration corresponds to the critical micellar concentration (CMC) of 4.2 mM for NaTC in a system containing monoolein and 0.15 M sodium ion (Hofmann, 1963a; Carey and Small, 1972). Above this value, the solubilization of each hydrocarbon was directly proportional to NaTC concentration suggesting that aqueous solubilization of PAHs and PCBs is directly dependent on bile salt micellar formation.

The principle of "true micellar solubilization" proposes that each micelle must contain at least one molecule of solubilizate. Carey and Small (1970) estimate that NaTC micelles under physiological circumstances contain about 15 bile salt molecules. The present partition ratios given in Table 4 (p. 55) of 1 PAH or PCB molecule per 100 NaTC molecules indicate that the principle of "true" micellar solubilization is not strictly followed. It must be remembered, however, that these ratios are not saturation ratios. Each partition ratio is a measurement of relative affinity of the hydrocarbon for the lipid phase versus the hydrophilic core of the micelle. Past attempts to calculate the volume of the micellar phase have not been successful (e.g. Borgström, 1967) and therefore calculation of a true partition coefficient for the systems does not seem possible at present.

To determine the capacity of mixed micelles to solubilize a representative PAH (DMBA) in the absence of a competing lipid phase, a molar saturation ratio was

determined utilizing an excess of DMBA and solutions with increasing concentrations of mixed NaTC micelles. The apparent saturation ratio for DMBA (moles DMBA solubilized/mole of bile salt) was calculated to be 0.162 which corresponds to about 6.2 molecules of NaTC in the presence of 3.5 polar lipid molecules (e.g. oleic acid and mono-olein) as necessary to promote the aqueous solubilization of 1 DMBA molecule. This observation in the mixed micellar system is consistent with the principle of "true" micellar solubilization but is in contrast to results from earlier studies for polycyclic hydrocarbons in pure micellar systems. In simple bile salt micelles, Norman (1960) found that about 1000 molecules of bile salt were required to solubilize 3 molecules of 3MC. Ekwall (1951) obtained somewhat similar results for other PAHs ranging from 63 molecules of bile salt per DMBA molecule to 385 bile salt molecules for each solubilized 3MC molecule. It is difficult to suggest an explanation for these early solubilization phenomena. Carey and Small (1970), while finding these observations at variance with "true" micellar solubilization, have proposed other factors may be involved such as change in water structure due to the presence of detergent molecules, etc. It is likely that the expanded lipophilic cores of the micelles in the present study can accommodate more hydrocarbon than could the pure micelles in the earlier works and that these compounds are micellar solutes in mixed rather than pure bile salt micelles. This observation is substantiated by the work of Borgström (1974)

who noted that the solubility of octadecane in 8 mM taurodeoxycholate of pH 6.3 was increased more than 10-fold upon the addition of 7.2 mM 1-monoolein. This aspect of micellar solubilization capacity shall be dealt with in greater depth in a later section of this chapter.

The enhanced solubilization of the PAHs and PCB once the CMC of NaTC was attained coupled with the capacity of one mixed micelle to accommodate at least one molecule of solute indicates that these hydrocarbons are true bile salt micellar solutes in a physiological situation. As the compounds exhibited similar partition ratios in the in vitro system, it is possible that they will have comparable behaviours in intestinal content. It is also likely that in vivo, the 6 to 10 mM bile salt concentrations found in the intestinal lumen (Hofmann, 1965) will be capable of effectively solubilizing xenobiotic hydrocarbons of the PAH and PCB classes.

B. Lipids

1. Triglyceride volume

Because DMBA, 3MC, B(a)P and PCB demonstrate similar behaviours in the bile salt lipid mixture of the preceeding section, one compound (DMBA) was selected to determine the influence of simulated triglyceride hydrolysis on hydrocarbon oil: micellar partition (Fig. 7, p. 61). When both fatty acid and monoglyceride are excluded from the system, with triglyceride and lecithin as the only lipids present,

approximately 5% of the 100 μM DMBA partitions into the micellar phase. The micellar solubility of triolein is small (Borgstrom, 1967) but the solubilization of lecithin in micelles is well established (Carey and Small, 1972). That mixed phospholipid-bile salt micelles are capable of solubilizing the hydrocarbons is evident. With reference to the poor micellar solubilities of PAHs in pure bile salt micelles (Ekwall, 1951, Norman, 1960) the inclusion of phospholipid in this study suggests a capacity of mixed micelles to carry hydrocarbon.

Studies performed on the micellar solubilization are in accord with this premise. Although pure micelles can promote solubilization of cholesterol, it is known that the addition of lecithin increases the cholesterol solubilization capacity of pure bile salt micelles (Carey and Small, 1970). Carey (1982) has proposed an interesting model for this phenomenon. Whereas cholesterol is solubilized within the hydrophilic interiors of mixed micelles, he suggests that simple bile salt micelles adsorb cholesterol molecules on their exterior (hydrophilic) surface.

As triolein is replaced by its digestion products increasing quantities of DMBA partition into the micellar phase, reaching a maximum of 13% of the hydrocarbon in the aqueous phase at 40% "hydrolysis" (figure 7, page 61). It appears that the increased micellar volume, by expansion of

the phospholipid-bile salt micelles with monoolein and oleic acid is responsible for further partition of DMBA into the micellar phase of the system. Beyond 40% "hydrolysis", a greater proportion of DMBA is retained in the lipid phase. This is particularly evident at 75% "hydrolysis" where the ability of bile salt micelles to solubilize triglyceride digestion products is apparently exceeded. A substantial oil phase, visible following ultracentrifugation is able to retain greater than 99% of the DMBA.

Using the present experimental method, a lipid phase is continually present. In a normal physiological situation, however, a constant diminution of an oil phase will occur. Fatty acid and monoglyceride generated from the triglyceride would be rapidly absorbed by the enterocytes and therefore be unavailable for expansion of the oil phase. From Table 5 (p. 59), it is evident that depletion of triglyceride content at fixed oleic acid and monoolein concentrations significantly promotes hydrocarbon partition from an oil into an aqueous micellar solution. It seems reasonable to assume that <u>in vivo</u> aqueous dispersal of hydrocarbon accompanies the generation of a mixed micellar solution from a non-dispersible oil phase.

2. Fatty acid saturation and chain length

The addition of monoolein or oleic acid to bile salt solutions has been shown to enhance the micellar solubilization of various non-polar solutes such as

azobenzene (Hofmann, 1963a) and hexadecane (Savary and Constantin, 1967). The solubility of cholesterol in bile salt solutions was found to increase dramatically and in linear proportion to the amount of oleic acid and/or monoolein present in the system (Simmonds et al. 1967). Borgström (1967) demonstrated that cholesterol partitioned more in favour of an aqueous phase when the fatty acid species was oleic rather than linoleic acid. This is consistent with the present observations regarding the effect of saturation on the partition of DMBA and 3MC between the oil and micellar phases (Fig. 8, p. 64). Conversely, PCB appeared to favour aqueous solubilization when the polyunsaturated rather than the monounsaturated fatty acid and monoglyceride were present (Fig. 9, p. 67).

These contradictory observations, in spite of otherwise similar behaviours in the simulated intestinal systems, are difficult to interpret at present but may be related to chemical structure and stereochemical "fit" into the lipid cores of the mixed micelles. DMBA and 3MC, both PAH's, might have a better "fit" when the oleic lipids are incorporated in the micelles. The PCB compound, of a different chemical class, might be better accommodated in the mixed micelles when the polyunsaturated lipids are present. It is also possible that the hydrocarbons have different affinities for the non-dispersed triglyceride supernatant, with PCB tending to be retained more tightly by a polyunsaturated triglyceride than are the PAH's.

Fatty acids and monoglycerides of medium chain length form micelles with a significantly poorer capacity to solubilize the hydrocarbons than the corresponding long chain fatty acids and monoglycerides (Figs. 8 & 9, p. 64 and 67). This influence of fatty acid chain length was very similar in magnitude for all 3 hydrocarbons. The oleic system was able to solubilize DMBA, 3MC and PCB to nearly twice the extent which the octanoic mixture achieved. Similarly, Takahashi and Underwood (1974) found that aqueous solubilization of α-tocopherol was increased when lipids of medium-chain length were incorporated into a bile salt micellar system but this effect was 3-to 7-fold less than for a corresponding long-chain mixture. If the hydrocarbons do undergo solubilization in the lipid cores of the mixed micelles, then perhaps greater expansion of the micellar hydrophobic interior by long-chain lipids is responsible for the observed differences.

It must also be remembered that medium-chain fatty acids and monoglycerides have an inherent water solubility. Borgstrom (1967) demonstrated that fatty acid chain length will determine their oil: micellar distribution. At pH 6.3 octanoic acid was almost completely in molecularly dispersed form, preferring true solution to micellar solubilization. In contrast, long-chain fatty acids have very low water solubility and their presence in aqueous solution was attributed to micellar solubilization. It therefore seems likely that not only do long chain fatty acids and

monoglycerides create mixed bile salt micelles with larger lipophilic interiors; but by virtue of their obligatory micellar solubilization, form more mixed micelles with bile salts than do medium-chain lipids, and are therefore able to promote hydrocarbon micellar solubilization to a larger degree.

II. Factors influencing DMBA bioavailability *in vivo*

A. Choice of relative bioavailability monitoring method

The two primary methods employed in this thesis to measure the relative bioavailability of ^{3}H-DMBA following intraduodenal administration to male Sprague-Dawley rats were biliary radiolabel excretion and plasma radioactivity. Monitoring radiolabel in the bile was chosen because biliary excretion is considered the main route for elimination of these compounds in rodents. Metabolites appear quickly in the bile following an intravenous dose of B(a)P (Kotin et al. 1959; Boroujerdi et al. 1981; Chipman et al. 1981a), DMBA (Levine, 1974; Khanduja, 1981) or various other PAH's (Harper, 1959) and account for the majority of the administered dose. For example, Falk and Kotin (1969) have reported that following an i.v. dose of ^{14}C-B(a)P, greater than 60% of administered radioactivity was subsequently excreted in the bile in a 4 hour period.

Biliary excretion also allows a relative assessment of bioavailablity on a cumulative basis and not merely relative tissue levels at a particular time. As well as being an easy substance to harvest, (see Methods, p.28), collection of bile with fluid replacement should not severely interfere with the normal physiology of the animal or influence the distribution volume of compound. Male rats are able to metabolize and excrete B(a)P, and presumably other PAH's, in the bile more extensively than are females (Falk and Kotin, 1969; Levine, 1970). As this thesis deals with male rats exclusively this difference between the sexes is not applicable.

Plasma radiolabel was also monitored and used both to confirm the bile data and to study absorptive patterns in an animal model with an uninterrupted enterohepatic circulation. Due to the limited blood volume of the rat (65 ml/kg) there is a restriction on the size and number of samples which can be drawn. Plasma levels do, however, provide a reliable index of systemic bioavailability and therefore cannot be overlooked.

B. Lipid class and bile participation

Three different dietary oils were used as vehicles for the administration of ^{3}H-DMBA. Olive oil and safflower oil represented long-chain monounsaturated and long-chain polyunsaturated fats, respectively. MCT oil was chosen as a

medium-chain saturated fat. (For fatty acid compositions of these triglycerides refer to Table 1, p. 34). Creation of a biliary fistula in an animal diverts bile from the duodenum permitting the assessment of the importance of bile in the absorption of hydrocarbon.

DMBA absorption is rapid as significant biliary appearance of radiolabel occurred within 30 minutes of intraduodenal administration in most animals. Daniel et al. (1967) noted the biliary excretion of radiolabel following intragastric administration began at about 1 hour after instillation of ^{3}H-DMBA. This delay probably reflects gastric emptying, obviated by intraduodenal administration in the present work.

With no luminal bile present, 7 hour excretory patterns of biliary radioactivity are very similar following administration in the 3 lipid vehicles (Fig. 10, p. 71). Cumulative 24 hour biliary recoveries of radiolabel are nearly identical from the two long-chain oils but significantly greater biliary recovery of radiolabel follows administration of hydrocarbon in MCT oil. A medium chain lipid phase would be depleted more rapidly than a long-chain viscous isotropic phase.

In the absence of bile, long-chain triglyceride absorption proceeds but is impaired (Gallagher et al. 1965; Morgan and Borgstrom, 1969; Knoebel, 1972). Under these conditions DMBA absorption from a long-chain vehicle is markedly hindered. This may be attributed to the lack of a mixed lipid-bile salt micellar phase to solubilize

hydrocarbon and provide transport to the absorptive enterocyte membrane. Also contributing to this effect could be the presence of unabsorbed lipid in the intestinal lumen which would withhold the hydrocarbon from the absorbing epithelium. Falk and Kotin (1963) noted that following intraduodenal administration of B(a)P in a lipid medium, biliary recovery of radiolabel was only a fraction of that recovered when a protein solution was employed as a vehicle. As these authors made no reference to bile acid supplementation, the relatively low recovery from the lipid carrier probably reflects poor lipid absorption and absence of a micellar phase, both favouring hydrocarbon retention in the lumen.

Upon the addition of bile to either olive or safflower oil, permitting long-chain fat digestion and absorption to proceed in a physiological manner, biliary excretion of radiolabel dramatically increases. The magnitude of the 4 to 5-fold increase in peak excretion is indicative of the ability of the digestive tract to rapidly absorb and eliminate these compounds. In the case of medium-chain triglyceride, whose absorption is not impaired by the absence of bile (Bennett Clark and Holt, 1968), luminal bile has little effect on the absorption of DMBA. Similarly, in studying α-tocopherol absorption, Davies et al. (1972) found that whereas cholestyramine (which binds bile acids) did not influence the percentage of vitamin absorbed from a medium-chain lipid, it significantly reduced recovery from a long-

chain triglyceride. It is possible that these observations are attributable to the relatively poor capacity of medium-chain lipid-bile salt micelles to solubilize PAH's, as discussed earlier. As well, the water solubility of the lipids and their ready uptake by the enterocyte prevents the creation of an effective micellar phase in the intestinal lumen (Bennett, Clarke and Holt, 1968) impeding delivery of DMBA to the enterocyte.

AIthough bile acids increase intestinal permeability, this effect does not appear to be solely responsible for increased uptake of hydrocarbon in the presence of bile. Rose and Nahrwold (1982) noted an increased loss of both DMBA and inulin from rat and guinea pig colonic loops upon inclusion of bile acid in the infusate. The magnitude of loss was greater for DMBA than inulin indicating that increased permeability is not the only factor. It is likely that these and the present results reflect the importance of micelles in DMBA absorption for greater absorption ocurred using long-chain fat than medium-chain lipid in the present study although the amount of bile infused was kept constant.

As there are no pronounced peaks in biliary radiolabel excretion in situations where no substantial micellar phase is created, PAH uptake probably involved relatively inefficient absorption in proximal regions of intestine resulting in distal involvement in absorption. Such phenomena are know to occur under conditions which compromise lipid absorption (Simmonds, 1976).

In the absence of bile the major barrier to passive lipid absorption, namely diffusion through the aqueous bulk of the intestinal lumen and unstirred water layer becomes increasingly rate limiting as polarity of the lipid molecule decreases. Whereas the mucosal uptake of polar lipids such as short- and medium- chain fatty acid is not notably influenced by the absence of bile, absorption of an insoluble lipid such as cholesterel is reduced by a factor of 150, becoming virtually abolished (Thomson & Dietschy, 1981). The results of the present experiments demonstrate that there is appreciable biliary excretion of radioactivity following intraduodenal administration of DMBA in the total absence of duodenal bile. This finding, although presently unexplained, can be compared with the fact that the absorption of cholesterol and lipovitamins is markedly impaired by the diversion of bile (Hofmann, 1968).

With bile supplementation, cumulative 24-hour bile radiolabel recoveries using long-chain oils as vehicles are nearly double those observed from MCT oil. In animals with intact bile ducts, plasma radiolabel levels are also twice as great from olive oil as compared to the medium-chain fat (Fig. 15, p. 84). These findings are in accord with studies by Dao (1969) who found olive oil and sesame oil (both long chain fats) superior to trioctanoin in promoting the absorption of orally ingested 3-MC in mice. Cholesterol uptake is also markedly enhanced in direct proportion to increasing fatty acid chain length of the carrier triglyceride (Sylvén and Borgström, 1969b). It may be

argued that the enhanced bioavailability seen with long-chain vehicles in the present work is attributable to lymphatic transport. However, as will be discussed later in this thesis, the amount of radiolabelled DMBA actually transported in intestinal lymph is not sufficient to explain the magnitude of the observed differences, and factors within the lumen itself are responsible for altering DMBA uptake.

The above results are most likely related to the better ability of long-chain lipids, in the presence of bile, to maintain non-polar solutes in a "hydrocarbon continuum" until they are eventually absorbed. It is feasible that the lipid domain created during long-chain triglyceride digestion and absorption could literally "feed" DMBA to the enterocyte, while medium-chain fat, with or without bile, may tend to leave the PAH stranded in the lumen, possibly clinging to other hydrophobic components in the diet. Possibly another mechanism may also be contributing to the enhanced uptake with the bile-long chain fat test meal. Feldman and Cheng (1975) have postulated that specific interactions of the enterocyte membrane with bile salt and long chain fatty acid or monoglyceride are necessary for mucosal uptake of trace lipid solutes. It is premature to attribute the observed results of the present study to such a mechanism but it is an attractive hypothesis worthy of further study.

It should be noted, in contrast to the present study, that the presence of long-chain polyunsaturated fats has been shown to hinder the absorption of fat-soluble vitamins instilled in micellar solutions into intestinal loops (Hollander, 1981). This has been attributed to an increased affinity of the vitamins for the hydrophobic interiors of the micelles resulting in decreased partitioning from micellar core to enterocyte membrane. It is difficult to compare experiments employing closed loops, however, with ones such as the present where the entire intestine is involved in absorption and all micellar components, including bile acids, are permitted a chance for absorption. Gallo-Torres et al. (1978) observed the bioavailablity of vitamin E to be greater from an emulsion of tripctanoin than from one of triolein following intragastric instillation. It is possible that the small amount of lipid administered by these authors (200 µl) combined with very rapid absorption of MCT might contribute to the observed differences.

C. Enterohepatic recycling

It is well known that metabolic activation is responsible for conversion of relatively inert PAH compounds to cytotoxic, mutagenic and carcinogenic intermediates (for reviews see Gelboin, 1980; Levin et al. 1982). Hepatic mixed-function oxidases have a notorious reputation for involvement in the bioactivation of PAHs such as DMBA

(DiGiovanni and Juchau, 1980) and intestinal mixed-function oxidases are probably similarly involved. Proximate carcinogens of B(a)P have been detected in recirculating bile and mutagenic metabolites of this PAH are known to undergo enterohepatic circulation in the rat (Chipman et al. 1981a). Following intravenous administration of ^{3}H-DMBA, 96-97% of radioactivity subsequently excreted in the bile is present as polar metabolites (Levine, 1974) with glucuronide conjugates of diols and dihydrodiols as the main metabolic species (Khanduja et al. 1981). It is highly probable that certain biliary products of DMBA, like those of B(a)P, possess great toxic potential, and it is therefore important to assess the extent of their enterohepatic recycling.

The existence of an enterohepatic circulation for this hydrocarbon becomes apparent in a comparison of radiolabel patterns between animals with or without biliary fistulae. In animals with intact bile ducts peak plasma radiolabel is not attained even 6 hours following a test meal of ^{3}H-DMBA in olive oil (Fig. 15, p. 84). In contrast, peak biliary excretion in bile fistulae animals after the same test meal with bile supplementation occurs within 4 hours (Fig. 11, p. 74). Rat biliary products of DMBA are rapidly absorbed and re-excreted in the bile of recipient rats following intraduodenal infusion. The recycling is extensive with at least a third of an administered dose available for reabsorption (Table 7, p. 86).

Levine (1974) showed that the appearance of biliary radioactivity was achieved more rapidly following

intravenous administration of DMBA metabolites than with DMBA itself, leading to the hypothesis that metabolism is the rate limiting process in the excretion of PAH in the bile. The present study concurs with this assumption as significant levels of radiolabel appeared in the bile at a faster rate following biliary product administration than parent compound. It cannot be ignored, however, that polar metabolites will be more rapidly dispersed throughout an aqueous intestinal lumen than parent hydrocarbon, suggesting luminal dispersion may be an alternative rate limiting factor.

It is possible that the rapid and extensive enterohepatic circulation which DMBA undergoes can lead to increased systemic exposure of potential carcinogens. An assessment of the efficiency with which the liver extracts these substances from portal blood and an analysis of their presence in gastro-intestinal lymph would be important in determining the risk associated with the recycling of metabolites. It has been shown that a fraction of the recycled metabolites of B(a)P are voided in the urine (Peacock, 1940; Chipman et al. 1981b), implying that a proportion of PAH biliary metabolites do attain systemic distribution and may be available to inflict damage at extrahepatic sites.

D. Lipid carrier volume

As mentioned ealier, under favourable conditions in the upper intestinal lumen, lipid absorption can proceed with

striking efficiency. If conditions become less suitable causing decreased lipid uptake, sites in the distal bowel become involved in absorption (Simmonds, 1976). Such a phenomenon has been reported for oleic acid and monoolein (Knoebel, 1972). Due to distal bowel involvement in absorption of these lipids when bile salts were absent from the lumen, there was no marked alteration in overall uptake in comparison to infusion of micellar lipids.

Plasma levels of radiolabel vary in inverse relation to triolein volume during a 7 hour period following intraduodenal administration of a constant dose of ^{3}H-DMBA in increasing amounts of carrier. The surge in plasma radiolabel accompanying 250 μmoles of triolein is in marked contrast to the sluggish rise observed with the 1000 μmole and 2000 μmole doses of lipid. Efficient digestion and absorption of the small volume of triolein and the resultant loss of a luminal lipid microenvironment to retain DMBA is the most plausable explanation. Relatively incomplete absorption of the larger doses of lipid during the same time frame could conceivably withhold hydrocarbon from the absorptive surface (Jandacek, 1982).

Whereas plasma peaks in radiolabel can be discerned using smaller doses of carrier, levels increase in a persistent fashion with increasing vehicle volume, which might indicate distal bowel involvement in hydrocarbon absorption. Normally, fat is delivered to the duodenum at a slow rate by controlled gastric emptying. In the present

experiment large bolus doses of lipid may overwhelm the solubilization capacity of bile salts entering the lumen. Consequently, lipid absorption may not be complete even 7 hours after administration.

In this study, the 7 hour observation period may not be sufficient to fully assess the influence of vehicle volume on the uptake of hydrocarbon. A comparison of areas under the curves for a longer time frame (e.g. 24 hours) would be helpful in reducing this problem. As well, the extensive enterohepatic circulation observed from DMBA will tend to maintain high levels of plasma radiolabel that may not be attributable entirely to initial absorption of parent hydrocarbon. These complications may be circumvented in future studies by measuring biliary excretion of radiolabel, thereby interrupting the enterohepatic circulation and also allowing quantitative determination of biliary radiolabel excretion in a cumulative manner.

E. Lymphatic versus portal transport: effect of hydrocarbon dose

Following absorption of a compound its route of systemic entry is largely determined by its polarity, with hydrophobic compounds predominantly favouring transport in lymph and hydrophilic substances mainly partitioning towards portal blood.

The ability of the enterocyte to convert a non-polar hydrocarbon into a hydrophilic derivative may define its

subsequent extracellular transport. Since PAHs have been in the diet for millenia it is not surprising that intestinal epithelial cells are capable of their metabolism, although studies on the ability of the liver to metabolize PAHs have usually eclipsed similar investigations on extrahepatic tissues.

Metabolizing capabilities of gastrointestinal epithelial cells towards PAHs have been demonstrated in the rat (Wattenberg et al. 1962; Chhabra et al. 1974; Stohs et al. 1977) and human (Autrup, 1982). Although metabolic activity is found in all regions of rat intestine, highest activity is located in the proximal small intestine with diminished activity in distal regions (Wattenberg et al. 1962). Within the epithelium, the midvillus region exhibits the greatest localization of metabolic activity (Porter et al. 1982) with essentially no enzyme activity located in cells of the crypts, stroma or muscle layers of the intestine (Wattenberg et al. 1962). Water soluble metabolites of B(a)P are rapidly formed using everted rat intestinal sacs (Hietanen, 1980) and isolated rat intestinal epithelial cells (Stohs et al. 1977) which are mainly phenol's, dihydrodiols and oxides largely conjugated to glucuronic acid. Similar metabolic patterns are seen in cultured human colon and duodenum except that conjugates are predominantly sulphate esters and glutathione conjugates (Autrup, 1982).

Radioactivity has been detected in both rat portal blood (Bock et al. 1979) and lymph (Daniel et al. 1967; Janss and Moon, 1970; Rees et al. 1971; Kamp and Neumann, 1975) following luminal instillation of radiolabelled PAHs. As the lymphatic appearance of radiolabel in these studies is associated with relatively large PAH doses (e.g. 10-20 mg), it is feasible that the enterocyte possesses a metabolic threshold for PAHs where excess hydrocarbon, escaping metabolism, will be transported in gastrointestinal lymph when administered in an oil vehicle.

In studies using rat jejunum in situ, Bock et al. (1979) instilled radiolabelled B(a)P in a solvent intraluminally and monitored appearance of free hydrocarbon and metabolites in portal blood. It was found that within 30 minutes of instillation, portal blood contained 40% of administered radioactivity with greater than 90% of this present as metabolites. The majority of the hydrocarbon remained in the lumen with only 2 to 5% of total radioactivity associated with the mucosa, indicating that absorbed PAHs are rapidly passed from the cell. Findings in the present study are in accord with this observation. Significant levels of radiolabel are observed in bile (an indirect measurement of portal transport) prior to lymphatic appearance of radioactivity following intraduodenal administration of ^{3}H-DMBA to rats with lymphatic fistulae. At no point does the lymphatic recovery of radiolabel exceed recovery in bile despite heroic increases in DMBA dosage.

This pattern indicates that this hydrocarbon first gains access to the system via the portal blood, probably reflecting the capacity of the enterocytes to metabolize PAH carcinogens.

PAH transport in lymph is usually associated with the chylomicron fraction (Daniel et al. 1967; Janss and Moon, 1970; Kamp and Neumann, 1975) where it is believed they exist predominantly as free compound. Grubbs and Moon (1973) found parent DMBA dissolved in the triglyceride core of chylomicra following gastric administration of the hydrocarbon in sesame oil. In the present study, measurable quantities of lymphatic radioactivity are concomitant with the visibly milky appearance of lymph laden with chylomicra. Peak concentration in lymph occurs at 3 hours post administration in the olive oil vehicle, a period associated with high total lymphatic triglyceride transport (Turner, 1978). Fractionation of the lymph confirms the existence of nearly all the radiolabel in the chylomicra.

Nearly identical patterns of radiolabel recovery in lymph are obtained for 10 µg and 10 mg doses of DMBA. Relatively transient peaks in lymphatic radiolabel are accompanied by continually increasing biliary radiolabel concentrations. The explanation for mounting biliary radiolabel even after lymphatic radiolabel (and presumably peak absorption rate) has declined is not immediately obvious and may or may not be related to absorptive phenomena. Perhaps systemic entry of hydrocarbon occurs faster than biliary elimination and the patterns reflect a

lag time in biliary excretion. It is also possible that distal bowel involvement in DMBA absorption is responsible for the maintenance of relatively high biliary excetion, subsequent to the initial rapid DMBA and triglyceride lymphatic transport proximally. Possibly, a decrease in the lymph chylomicron population eliminates the alternate route. None of these explanations is satisfactory, however, as the patterns are dosage independent. Sampling of portal venous blood using an isolated intestinal loop in an animal equipped with a lymphatic catheter would help further define the lymphatic/venous partitioning patterns of radiolabelled DMBA and/or derivatives.

Concentrations of radiolabel in bile tend to steadily increase at the 10 µg dose (Fig. 17, p. 93) but plateau and begin a slight decline at the larger doses (Figs. 18 and 19, p. 96 and 99). Levine (1974) has proposed that metabolism is the rate-limiting step in the biliary excretion mechanism for DMBA. Similarly Kotin et al. (1959) noticed a threshold for B(a)P biliary elimination (150 µg i.v.) beyond which a plateau in excretion occurred. These authors suggested that excess PAH becomes available for extrahepatic deposition. Following an injection of B(a)P to rats (Peacock, 1940), hydrocarbon was rapidly distributed to adipose tissue, the CNS and mammary glands, which did not begin to diminish until up to 6 hours following the injection despite constant biliary excretion. Perhaps in this study, larger doses of PAH are deposited at remote sites once absorbed, but as they

probably enter the system as polar metabolites and not as parent PAH this explanation awaits confirmation.

In the past, much emphasis has been placed on lymphatic PAH transport largely attributable to studies monitoring thoracic duct lymph. Janss and Moon (1970) reported 40% recovery of radiolabel in a 24 hour period following an intragastric dose of 20 mg ^{3}H-DMBA. A 10 to 20% recovery followed a 10 mg dose of B(a)P (Rees et al. 1970). Kamp and Neumann (1975) observed that of a 5 mg/kg dose of 3MC, 9% was recovered in lymph but with a 10-fold increase in dose 2.5% was retrieved in 24 hours.

Approximately 4% of DMBA at each dosage in the present study is recovered in the intestinal lymph in a 24 hour period, which is comparable to the 5% recovery value obtained by Daniel et al. (1967) following 5 µg to 2 mg doses. These comparatively low recoveries in contrast to those cited above are difficult to interpret at present due to lack of methodological information. It is likely, however, that the lymph sampled (intestinal vs thoracic) may play a factor in the observed differences. Hepatic lymph is a major component of thoracic duct lymph with estimates of its contribution to the 10 ml/day thoracic flow in the rat varying from about 2 ml/day (Yoffey and Courtice, 1970) to 5 ml/day (Barrowman and Granger, 1981). However, more significant differences in the methodology must be present as the higher recoveries of lymphatic radiolabel (e.g. 40%) in previous works, exceed the total recovery of radiolabel

from lymph plus bile in the present study. Clearly additional studies are required to determine lymphatic transport of hydrocarbon as a fraction of total hydrocarbon absorption.

Total recoveries of hydrocarbon do not significantly differ between doses of hydrocarbon, nor does the fraction in each compartment significantly change with hydrocarbon burden. Because the fraction of a 20 mg DMBA dose transported in lymph is almost identical to that recovered with a 10 µg dose, it is unlikely that PAH load is a factor in determining lymphatic recovery. Although the reason for constant fraction absorbed is not clear, the data is reminiscent of that obtained for cholesterol by Sylven and Borgstrom (1968). These authors found that almost constant fractions (about 0.4) of cholesterol doses ranging from a trace to 100 µ moles were recovered in thoracic duct lymph. Similarly, sitosterol transport in lymph was largely independent of the dose administered (Sylven and Borgstrom, 1969a).

The cumulative 24 hour bile:lymph ratio of radiolabel may give the most reliable profile of distribution patterns between different doses of hydrocarbon as inter-animal variations in absorptive capacity can be ignored. The 10 mg dose of DMBA provides a significantly lower bile:lymph ratio than did the 10 µg dose. As would be expected, a smaller dose of PAH would be more readily metabolized and available for portal transport. Conversely, with an increasing

hydrocarbon burden, the metabolizing capacity of the epithelium may be overwhelmed and excess hydrocarbon leaves the cell in the lymph. It is apparent, however, that the portal vein is responsible for the majority of PAH transport, and not the lymphatics as previously supposed. Again, this is probably a reflection of the overwhelming metabolic capacity of the gastrointestinal epithelium which converts these compounds to polar derivatives.

It cannot be overlooked that diet can have a profound influence on the PAH metabolizing capability of intestinal epithelial enzymes. Of particular concern to the present study is the presence of exogenous inducer in a normal laboratory rat diet, specifically Purina Rat Chow, as determined by Wattenberg (1971). Starvation and low-fat diets are associated with rapid depletion in enzyme activity (Wattenberg et al. 1962). Extrapolation to the present study is difficult, for although the rats had been maintained on a Purina diet until the time of the study the animals were fasted for nearly 24 hours prior to test meal administration. Starvation for as little as one day has been associated with nearly a complete loss in enzyme activity (Wattenberg, 1971). In light of the rapid and significant transport of DMBA (or metabolites) in portal blood, it is likely that the animals have retained a significant degree of PAH metabolizing capability.

PAHs transported in the lymphatics have a potentially wider systemic distribution than those bound for the liver

in portal blood. Compounds travelling in lymph escape first pass metabolism by hepatocytes and enter the systemic circulation directly. Conceivably parent hydrocarbon thus becomes available for deposition at remote sites such as the mammary gland following disassembly of chylomicra (Grubbs and Moon, 1973). Results of the present study show that excreted biliary radiolabel following a 10µg dose of ^{3}H-DMBA in animals with intact lymphatic systems, is equal to total recovery of radiolabel (from lymph plus bile) in animals with biliary and lymphatic fistulae. This suggests that hydrocarbon entering the body in association with chylomicra is ultimately destined for hepatic extraction and excretion in the bile and only a small fraction of lymph-derived PAH is deposited, and perhaps retained, in remote tissues.

SUMMARY

This thesis examines intraluminal factors governing the bioavailability of PAH carcinogens. Information gained by studying the physico-chemical behaviour of hydrophobic xenobiotics *in vitro* was applied to an *in vivo* situation to define those factors which may either favour or hinder the appearance of lipophilic toxins in the mammalian system.

The initial studies were undertaken to determine the physico-chemical behaviour of 3 hydrocarbon carcinogens (B(a)P, 3MC and DMBA) and a PCB compound in simulated intestinal content. The results indicate that all these compounds behave very similarly in intestinal content and are micellar solutes, being readily dissolved in mixed bile salt-partial glyceride-fatty acid micelles. It appears that mixed micelles containing glycerides and fatty acids are better able to solubilize hydrocarbon than pure bile salt micelles. Partition of PAH from an oil into a mixed micellar system improves as the triglyceride phase is replaced by its hydrolysis products. Micelles containing long-chain monoglycerides and fatty acids are better able to solubilize hydrocarbon than micelles containing medium-chain lipids. Degree of fatty acid saturation does not significantly influence partition of hydrocarbon.

The second portion of the study examined the absorption of DMBA in the rat. The liver appears to be very efficient at clearing this compound whereupon radiolabel is subsequently excreted in the bile and can be used as an

index of relative absorption. In rats with bile fistulae DMBA is absorbed from the small bowel despite the absence of luminal bile. This contrasts with lipovitamins and cholesterol which show a virtually absolute dependence on bile salts for their absorption. Under these circumstances, absorption from a medium-chain oil is greater than that from a long-chain triglyceride vehicle reflecting the efficient absorption of medium-chain lipid in the absence of bile.

When bile is replaced in the duodenum, DMBA intestinal absorption and biliary excretion are greatly enhanced from a long chain vehicle, regardless of fatty acid saturation. When DMBA is fed in a medium-chain oil, bile has no significant effect in enhancing the absorption as judged by biliary excretion. An assessment of absorption in animals with intact bile ducts by monitoring arterial plasma levels confirms this observation. Following intraduodenal administration of ^{3}H-DMBA, considerably greater plasma levels of radiolabel are achieved with long-chain than with medium-chain lipids. The presence of unabsorbed fat in the digestive tract severely retards hydrocarbon uptake as significantly higher plasma levels of radiolabel are achieved in a 7 hour period in inverse relation to the volume of triglyceride carrier. These results suggest that factors favouring the creation of a continuous lipophilic microenvironment (with a substantial micellar phase) which can solubilize hydrocarbon in the aqueous intestinal lumen will also promote uptake of PAHs. There is also extensive

absorption and re-excretion of biliary metabolites of DMBA. Thus when a dose of hydrocarbon is given to an animal, there is the potential for extensive, repetitive enterohepatic recycling of the biliary metabolites.

Once in the enterocyte, a PAH can gain access to the system by transport in both intestinal lymph and portal blood. Radiolabel associated with lymph is almost exclusively confined to the chylomicron fraction, suggesting that it is present as a non-polar compound probably as parent hydrocarbon. Conversely, portal blood, which transports hydrophilic substances most likely carries metabolic derivatives generated by the enterocyte. Biliary excretion of radiolabel in animals with lymphatic fistulae can be used as an indirect assessment of portal transport, and is 3- to 4-fold greater than lymphatic transport. This suggests that these compounds are largely metabolized by the enterocytes prior to systemic access. The fraction of administered DMBA recovered remains largely unchanged, despite a 2000-fold increase in dosage. The fraction of absorbed hydrocarbon carried in lymph or portal blood is not significantly altered with varying doses but an increased lymph:portal partition is observed with a larger burden of DMBA.

Essentially all of the hydrocarbon transported in lymph is destined for eventual excretion by the biliary system. Although some deposition at remote sites no doubt also

occurs, it can be considered as only a fraction of the oral dose. Whether the amount remaining is sufficient to promote carcinogenesis remains to be seen.

Autrup, H. (1982) Carcinogen metabolism in human tissues and cells. Drug Metab. Rev. 13: 603-646.

Barrowman, J.A. (1966) The flow and composition of lymph in the cisterna chyli of the rat. Ph.D. Thesis. University of London

Barrowman, J.A. (1983) Intestinal absorption of fat-soluble vitamins: physiology and pharmacology. In: Intestinal absorption. Handbook of experimental pharmacology. Edited by Csaky, T. Springer-Verlag, Heidelberg. In press.

Barrowman, J.A. and Granger, D.N. (1981) Hepatic lymph. In: Hepatic circulation in health and disease. Edited by Lautt, W.W. Raven Press, New York, pp. 137-150.

Baum, E.J. (1978) Occurrence and surveillance of polycyclic aromatic hydrocarbons. In: Polycyclic hydrocarbons and cancer, Vol. 1. Edited by Gelboin, H.V. and T'so, P.O.P. Academic Press, New York, pp. 45-70.

Bennett, S. (1964) Intestinal absorptive capacity and site of absorption of fat under steady state conditions in the unanaesthetized rat. Q. J. Exptl. Physiol. 49: 210-218.

Bennett Clark, S. and Holt, P.R. (1968) Rate-limiting steps in steady state intestinal absorption of trioctanoin-1-^{14}C. J. Clin. Invest. 47: 612-623.

Berg, J.W. (1975) Diet. In: Persons at high risk of cancer. Edited by Fraumeni, J.F. Academic Press, New York, pp. 201-224.

Blumer, M. (1976) Polycyclic aromatic compounds in nature. Sci. Amer. 234: 35-45.

Bock, K.W., v. Clausbruch, U.C. and Winne, D. (1979) Absorption and metabolism of naphthalene and benzo(a)pyrene in the rat jejunum in situ. Med. Biol. 57: 262-264.

Borgström, B. (1967) Partition of lipids between emulsified oil and micellar phases of glyceride-bile salt dispersions. J. Lipid Res. 8: 598-608.

Borgström, B. (1974) Fat digestion and absorption. In: Biomembranes, Vol. 4B. Edited by Smyth, D.H. Plenum Press, New York, pp. 555-620.

Borgström, B. (1977) Digestion and absorption of lipids. Int. Rev. Physiol. 12: 305-323.

Boroujerdi, M., Kung, H., Wilson, A.G.E. and Anderson, M.W. (1981) Metabolism and DNA binding of benzo(a)pyrene in vivo in the rat. Cancer Res. 41: 951-957.

Campbell, A.D., Horwitz, W., Burke, J.A., Jelinek, C.F., Rodricks, J.V. and Shibko, S.I. (1977) Food additives and contaminants. In: Handbook of physiology, Section 9: Reactions to environmental agents. Edited by Lee, D.H.K. Am. Physiol. Soc., Bethesda, Maryland, pp. 167-179.

Carey, M.C. (1982) The enterohepatic circulation. In: The liver: biology and pathobiology. Edited by Arias, I., Popper, H., Schachter, D. and Shafritz, D.A. Raven Press, New York, pp. 429-465.

Carey, M.C. and Small, D.M. (1970) The characteristis of mixed micellar solutions with particular reference to bile. Am. J. Med. 49: 590-608.

Carey, M.C. and Small, D.M. (1972) Micelle formation by bile salts. Physical-chemical and thermodynamic considerations. Arch. Intern. Med. 130: 506-527.

Chhabra, R.S., Pohl, R.J. and Fouts, J.R. (1974) A comparative study of xenobiotic-metabolizing enzymes in liver and intestine of various animal species. Drug. Metab. Disp. 2: 443-447.

Chipman, J.K., Hirom, P.C., Frost, G.S. and Millburn, P. (1981a) The biliary excretion and enterohepatic circulation of benzo(a)pyrene and its metabolites in the rat. Biochem. Pharmacol. 30: 937-944.

Chipman, J.K., Frost, G.S., Hirom, P.C. and Millburn, P. (1981b) Biliary excretion, systemic availability and reactivity of metabolites following intraportal infusion of [^{3}H]benzo(a)pyrene in the rat. Carcinogenesis 2: 741-745.

Daniel, P.M. Pratt, O.E. and Prichard, M.M.L. (1967) Metabolism of labelled carcinogenic hydrocarbons in rats. Nature 215: 1142-1146.

Dao, T.L. (1969) Studies on mechanism of carcinogenesis in the mammary gland. Prog. Exp. Tumour Res. 11: 235-261.

Davies, T., Kelleher, J., Smith, C.L., Walker, B.E. and Losowsky, M.S. (1972) Effect of therapeutic measures which alter fat absorption on the absorption of α-tocopherol in the rat. J. Lab. Clin. Med. 79: 824-831.

Dietschy, J.M. (1968) Mechanisms for the intestinal absorption of bile acids. J. Lipid Res. 9: 297-309.

DiGiovanni, J. and Juchau, M.R. (1980) Biotransformation and bioactivation of 7,12-dimethylbenz(a)anthracene (7,12-DMBA). Drug Metab. Rev. 11: 61-101.

Dipple, A. (1983) Formation, metabolism and mechanism of action of polycyclic aromatic hydrocarbons. Cancer Res. (Suppl) 43: 2422s-2425s.

Ekwall, P. and Setälä, K. (1948) On the solubilization of carcinogenic hydrocarbons by association colloids. Acta Chem. Scnad. 2: 733-739.

Ekwall, P., Setälä, K. and Sjöblom, L. (1951) Further investigations on the solubilization of carcinogenic hydrocarbons by association colloids. Acta Scand. 5: 175-189.

El-Gorab, M. and Underwood, B.A. (1973) Solubilization of β-carotene and retinol into aqueous solutions of mixed micelles. Biochim. Biophys. Acta 306: 58-66.

Falk, H.L. and Kotin, P. (1963) Chemistry, host entry and metabolic fate of carcinogens. Clin. Pharmacol. Ther. 4: 88-103.

Falk, H.L. and Kotin, P. (1969) Pesticide synergists and their metabolites: potential hazards. Ann. N.Y. Acad. Sci. 160: 299-313.

Farber, E. (1982) Chemical carcinogenesis: a biologic perspective. Am. J. Path. 106: 271-296.

Feldman, E.B. and Cheng, C.Y. (1975) Mucosal uptake in vitro of cholesterol from bile salt and surfactant solutions. Am. J. Clin. Nutr. 28: 692.

Gallagher, N., Webb, J. and Dawson, A.M. (1965) The absorption of ^{14}C oleic acid and ^{14}C triolein in bile fistula rats. Clin. Sci. 29: 73-82.

Gallo-Torres, H.E., Ludorf, J. and Brin, M. (1978) The effect of medium-chain triglycerides on the bioavailability of vitamin E. Internat. J. Vit. Nutr. Res. 48: 240-249.

Gelboin, H.V. (1980) Benzo(a)pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed function oxidases and related enzymes. Physiol. Rev. 60: 1107-1166.

Gray, J.I. and Morton, I.D. (1981) Some toxic compounds produced in food by cooking and processing. J. Human Nutr. 35: 5-23.

Grubbs, C.J. and Moon, R.C. (1973) Transport of orally administered 9,10-dimethyl-1,2-benzanthracene in the Sprague-Dawley rat. Cancer Res. 33: 1785-1789.

Harper, K.H. (1959) The intermediary metabolism of polycyclic hydrocarbons Brit. J. Cancer 13: 718-731.

Hietanen, E. (1980) Oxidation and subsequent glucuronidation of 3,4-benzopyrene in everted intestinal sacs in control and 3-methylcholanthrene pretreated rats. Pharmacol. 21: 233-243.

Hofmann, A.F. (1963a) The function of bile salts in fat absorption. Biochem. J. 89: 57-68.

Hofmann, A.F. (1963b) The behaviour and solubility of monoglycerides in dilute, micellar bile-salt solution. Biochim. Biophys. Acta 70: 306-316.

Hofmann, A.F. (1965) Clinical implications of physicochemical studies on bile salts. Gastroenterol. 48: 484-494.

Hofmann, A.F. (1968) Functions of bile in the alimentary canal. In: Handbook of physiology, Section 5: Alimentary canal. Edited by Code, C.F. Am. Physiol. Soc., Bethesda, Maryland, pp. 2507-2533.

Hofmann, A.F. (1976) Fat digestion: the interaction of lipid digestion products with micellar bile acid solutions. In: Lipid absorption: biochemical and clinical aspects. Edited by Rommel, K. and Goebell, H. MTP Press, Lancaster, pp. 3-18.

Hofmann, A.F. and B. Borgström (1962) Physico-chemical state of lipids in intestinal content during their digestion and absorption. Fed. Proc. 21: 43-50.

Hofmann, A.F. and B. Borgström (1964) The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. J. Clin. Invest. 43: 247-257.

Hollander, D. (1981) Intestinal absorption of vitamins A, E, D and K. J. Lab. Clin. Med. 97: 449-462.

Jandacek, R.J. (1982) The effect of nonabsorbable lipids on the intestinal absorption of lipophiles. Drug. Metab. Rev. 13: 695-714.

Janss, D.H. and Moon, R.C. (1970) Absorption of intragastrically administered 7,12-dimethylbenzanthracene. Fed. Proc. 29: 817.

Kamp, J.D. and Neumann, H.-G. (1975) Absorption of carcinogens into the thoracic duct lymph of the rat: aminostilbene derivatives and 3-methylcholanthrene. Xenobiotica 5: 717-727.

Khanduja, K.L., Dogra, S.C. and Sharma, R.R. (1981) Glucuronic acid conjugates of metabolites of 7,12-dimethylbenz(a)anthracene excreted through bile ducts in rat. Ind. J. Exp. Biol. 19: 375-378.

Knoebel, L.K. (1972) Intestinal absorption in vivo of micellar and non-micellar lipid. Am. J. Physiol. 223: 255-261.

Kotin, P., Falk, H.L. and Busser, R. (1959) Distribution, retention and elimination of C^{14}-3,4-benzpyrene after administration to mice and rats. J. Natl. Cancer Inst. 23: 541-555.

Levin, W., Wood, A., Chang, R., Ryan, D., Thomas, P., Yagi, H., Thakker, D., Vyas, K., Boyd, C., Chu, S., Conney, A. and Jerina, D. (1982) Oxidative metabolism of polycyclic aromatic hydrocarbons to ultimate carcinogens. Drug Metab. Rev. 13: 555-580.

Levine, W.G. (1970) The role of microsomal drug metabolizing enzymes in the biliary excretion of 3,4-benzpyrene in the rat. J. Pharmacol. Exp. Ther. 175: 301-310.

Levine, W.G. (1974) Hepatic uptake, metabolism, and biliary excretion of 7,12-dimethylbenzanthracene in the rat. Drug Metab. Disp. 2: 169-177.

Lijinsky, W. and Shubik, P. (1964) Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. Science 145: 53-55.

Lo, M.-T. and Sandi, E. (1978) Polycyclic aromatic hydrocarbons (polynuclears) in foods. Residue Rev. 69: 35-86.

MacMahon, M.T. and Thompson, G.R. (1970) Comparison of the absorption of a polar lipid, oleic acid and a non-polar lipid, alpha-tocopherol from mixed micellar solutions and emulsions. Eur. J. Clin. Invest. 1: 161-166.

McCarthy, R.D. (1964) Mammalian metabolism of straight-chain saturated hydrocarbons. Biochim. Biphys. Acta 84: 74-79.

Mes, J., Davies, D.J. and Turton, D. (1982) Polychlorinated biphenyl and other chlorinated hydrocarbon residues in adipose tissue of Canadians. Bull. Environm. Contam. Toxicol. 28: 97-104.

Morgan, R.G.H. and Borgström, B. (1969) The mechanism of fat absorption in the bile fistula rat. Q. J. Exp. Physiol. 54: 228-243.

National Research Council (1982) Executive summary of the committee on diet, nutrition and cancer. Nutr. Today 17 (4): 20-25.

Norman, A. (1960) The beginning solubilization of 20-methylcholanthrene in aqueous solutions of conjugated and unconjugated bile acid salts. Acta Chem. Scand. 14: 1295-1299.

Patton, J.S. (1981) Gastrointestinal lipid digestion. In: Physiology of the gastrointestinal tract. Edited by Johnson, L.R. Raven Press, New York, pp. 1123-1146.

Patton, J.S. and Carey, M.C. (1979) Watching fat digestion. Science 204: 145-148.

Peacock, P.R. (1940) Biophysical factors influencing the absorption and distribution of benzpyrene, and their bearing on the mechanism of carcinogenesis. Am. J. Cancer 40: 251-254.

Pocock, D.M.-E. and Vost, A. (1974) DDT absorption and chylomicron transport in rat. Lipids 9: 374-381.

Porter, C.W., Dworaczyk, D. and Gurtoo, H.L. (1982) Biochemical localization of aryl hydrocarbon hydroxylase in the intestinal epithelium of the rat. Cancer Res. 42: 1283-1285.

Rees, E.D., Mandelstam, P., Lowry, J.Q. and Lipscomb, H. (1971) A study of the mechanism of intestinal absorption of benzo(a)pyrene. Biochim. Biophys. Acta 225: 96-107.

Rose, R.C. and Nahrwold, D.L. (1982) Bile acids: effects on absorption of 1,2-dimethylhydrazine and 7,12-dimethylbenz(a)anthracene in the colon of the rat and guinea pig. J.N.C.I. 68: 619-622.

Safe, S. Robertson, L.W., Safe, L., Parkinson, A., Bandiera, S., Sawyer, T. and Campbell, M.A. (1982) Halogenated biphenyls: molecular toxicology. Can. J. Physiol. Pharmacol. 60: 1057-1064.

Sallee, V.L. and Dietschy, J.M. (1973) Determinants of intestinal mucosal uptake of short- and medium-chain fatty acids and alcohols. J. Lipid Res. 14: 475-484.

Savary, P. and Constantin, M.J. (1967) Sur la solubilisation micellaire de l'hexadecane et son passage dans la lymphe thoracique du rat. Biochim. Biophys. Acta 137: 264-276.

Shabad, L.M. (1980) Circulation of carcinogenic polycyclic aromatic hydrocarbons in the human environment and cancer prevention. J.N.C.I. 64: 405-410.

Shiau, Y-F. (1981) Mechanisms of intestinal fat absorption. Am. J. Physiol. 240: G1-G9.

Simmonds, W.J. (1976) Uptake of fatty acid and monoglyceride. In: Lipid absorption: biochemical and clinical aspects. Edited by Rommel, K. and Goebell, H. MTP Press, Lancaster, pp. 51-61.

Simmonds, W.J., Hofmann, A.F. and Theodor , E. (1967) Absorption of cholesterol from a micellar solution: intestinal perfusion studies in man. J. Clin. Invest. 46: 874-890.

Sims, P. (1980) The metabolic activation of chemical carcinogens. Br. Med. Bull. 36: 11-18.

Stafford, R.J., Donovan, J.M., Benedek, G.B. and Carey, M.C. (1981) Physical-chemical characteristics of aqueous duodenal content after a fatty meal. Gastroenterol. 80: 1291.

Stohs, S.J., Grafström, R.C., Burke, M.D. and Orrenius, S. (1977) Benzo(a)pyrene metabolism by isolated rat intestinal epithelial cells. Arch. Biochem. Biophys. 179: 71-80.

Sylvén, C. and Borgström, B. (1968) Absorption and lymphatic transport of cholesterol in the rat. J. Lipid Res. 9: 596-601.

Sylvén, C. and Borgström, B. (1969a) Absorption and lymphatic transport of cholesterol and sitosterol in the rat. J. Lipid Res. 10: 179-182.

Sylvén, C. and Borgström, B. (1969b) Intestinal absorption and lymphatic transport of cholesterol in the rat: influence of the fatty acid chain length of the carrier triglyceride. J. Lipid Res. 10: 351-355.

Takahashi, Y.I. and Underwood, B.A. (1974) Effect of long and medium chain length lipids upon aqueous solubility of α-tocopherol. Lipids 9: 855-859.

Thomson, A.B.R. and Dietschy, J.M. (1981) Intestinal lipid absorption: major extracellular and intracellular events. In: Physiology of the gastrointestinal tract. Edited by Johnson, L.R. Raven Press, New York, pp. 1147-1220.

Tulliez, J.E. and Bories, G.F. (1978) Metabolism of a n-paraffin, heptadecane, in rats. Lipids 13: 110-115.

Turner, S.G. (1978) Intestinal lymph flow and protein transport during digestive activity. Ph.D. thesis, University of London.

Wattenberg, L.W. (1971) Studies of polycyclic hydrocarbon hydroxylases of the intestine possibly related to cancer: effect of diet on benzpyrene hydroxylase activity. Cancer 28: 99-102.

Wattenberg, L.W., Leong, J.L. and Strand, P.J. (1962) Benzpyrene hydroxylase activity in the gastrointestinal tract. Cancer Res. 22: 1120-1125.

Westergaard, H. and Dietschy, J.M. (1974) Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. J. Clin. Invest. 54: 718-732.

Westergaard, H. and Dietschy, J.M. (1976) The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. J. Clin. Invest. 58: 97-108.

Wickizer, T.M. and Brilliant, L.B. (1981) Testing for polychlorinated biphenyls in human milk. Pediatrics 68: 411-415.

Williams, R.T. and Millburn, P. (1975) Detoxication mechanisms - the biochemistry of foreign compounds. In: Physiological and pharmacological biochemistry. Edited by Blaschko, H.K.F. Butterworth, London, pp. 211-266.

Wilson, F.A. and Dietschy, J.M. (1972) Characterization of bile acid absorption across the unstirred water layer and brush border of the rat jejunum. J. Clin. Invest. 51: 3015-3025.

Wilson, F.A., Sallee, V.L. and Dietschy, J.M. (1971) Unstirred water layers in intestine: rate determinant of fatty acid absorption from micellar solutions. Science 174: 1031-1033.

Wilson, R.D., Ziprin, R.L., Clark, D.E. and Elissalde, M.H. (1982) Absorption of pentachlorophenol by the ovine lymphatic system: a technical note. Vet. Hum. Toxicol. 24: 12-15.

Yoffey, J.M. and Courtice, C.F. (1970) Lymphatics, lymph and the lymphomyeloid complex. Academic Press, London.