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ABSORPTION OF ALIPHATIC HYDROCARBONS BY RATS

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SUMMARY

The percentage retention of aliphatic hydrocarbons from relatively simple mixtures administered intragastrically to rats is shown to be an inverse linear function of carbon number, at least for major components of the mixtures. Retention (intake *minus* excretion) is reduced by treatment with certain antibiotics or by feeding amounts of hydrocarbon in excess of 320 mg/kg body weight as a single dose. Bacterial degradation of paraffin in the feces, and recirculation of intact hydrocarbon in the bile apparently do not occur to a significant extent under the conditions tested.

The major, if not the only significant, site of absorption of ingested hydrocarbons in rats is the small intestine. No conspicuous difference in ability to take up hydrocarbons in everted sac experiments was seen between duodenum, jejunum and ileum, although duodenal sacs released hydrocarbon or its metabolic products into the serosal medium more rapidly than did sacs of ileum. The primary recipient of absorbed paraffin *in vivo* was the lymph, but there was some evidence for absorption of hexadecane or, perhaps more likely, its metabolic products, directly into the portal blood.

INTRODUCTION

Absorption of aliphatic hydrocarbons by mammals is of interest in view of recent reports that follicular lipidosis of the spleen and hydrocarbon accumulation in lymph nodes have been conspicuously increasing in humans since the 1940's[1]. A comparison of human meninges and meningiomas showed a higher content of paraffins in the latter[2], while aliphatic hydrocarbons have been isolated in small amounts from human arterial tissues and plaques[3]. Hydrocarbons isolated from human tissues have usually been tentatively attributed to ingestion of mineral oil[4,5] or occasionally, in part, to natural components of the diet[6,7].

Although it is now well established that liquid and solid hydrocarbons are absorbed from the gastrointestinal tract of mammals[8–12], and in fact may be absorbed as efficiently as triglycerides under certain conditions[8], there is very little quantitative data available on the relative absorption of different types of hydrocarbons. The absorption process itself is quite poorly understood, such that the only descriptive statements that can be made concerning it are: (a) emulsification of the hydrocarbon is apparently necessary for absorption[9], (b) at least some of the hydrocarbon is absorbed

intact into the lymph[8,10], and (c) some oxidation to fatty acid occurs during the absorption of *n*-alkanes of appropriate chain length[8].

Quantitative determination of the extent of absorption of hydrocarbons is difficult for a number of reasons. Balance studies are complicated by the possibility of bacterial degradation of paraffins in the feces, and by the fact that the extent to which absorbed paraffin is recirculated *via* the bile is not known. Attempts to measure absorption directly by cannulation of the intestinal or thoracic lymph ducts give minimum values due to diversion of a significant portion of the lymph flow into what had, prior to cannulation, been very minor lymph channels[8]. In addition, it has not been established whether hydrocarbons are absorbed exclusively into the lymph or divided between the lymph and portal circulation. The nutritional state of animals, possibly because of its effect on spontaneous emulsification, has a large effect on the absorption of paraffin; thus *n*-hexadecane administered in bulk to starved rats is not absorbed[9] while administration in olive oil solution results in considerable absorption[8]. Even *n*-nonacosane is absorbed if it is mixed into the diet of well-fed rats[12].

The following experimental results are presented in an attempt to clarify some of the outstanding questions about hydrocarbon absorption in the rat. Although these results do not relate directly to paraffinosis in humans, it is hoped that they may simplify speculations as to possible origins of hydrocarbons found in animal tissues[6].

MATERIALS AND METHODS

Materials

Squalane was obtained from General Biochemicals, Chagrin Falls, Ohio, pristane from Eastman (No. P7825), 8-hexadecyne (95 %) from Farchan Research Labs, Willoughby, Ohio, pentadecylcyclohexane (96 %), *n*-nonadecane (99 %), *cis* + *trans*-9-nonadecene (99 %), 1,13-tetradecadiene (97 %) and phytane (97 %) from Chemical Samples Co., Columbus, Ohio, *n*-paraffins C_{14}–C_{28} (99 %) and 1-octadecene from LaChat Chemicals, Inc., Chicago Heights, Ill. Streptomycin sulfate, chlortetracycline, sulfanilamide, monopalmitin, palmitic acid, chenodeoxycholic acid, and sodium taurocholate were from Sigma Chemical Co., St. Louis, Mo. All other reagents and solvents were the best grades available from Fisher Scientific Co., Raleigh, N. C.

Male C–D strain rats were supplied by Charles River Breeding Labs., and maintained on D & G Laboratory Diet (Price-Wilhoite Co., Frederick, Md.). Radioactivity was assayed using a Beckman LS-250 scintillation counter with a cocktail consisting of, per l, 4 g 2,5-bis[2-(5-*tert.*-butylbenzoxazolyl)]-thiophene (BBOT), 80 g naphthalene, 600 ml toluene, and 400 ml ethyleneglycol monomethyl ether. Counting efficiencies were estimated by the channels ratio method. *n*-[1-^{14}C]Hexadecane was obtained from Isotopes, Inc., Westwood, N. J., and purified by column chromatography on Florisil[13]. Solid supports and liquid phases for gas chromatography were supplied by Applied Sciences Laboratories, State College, Pa., or Supelco, Inc., Bellefonte, Pa.

Gas chromatography

Hydrocarbons were isolated as has been described previously[13] and analyzed on Hewlett–Packard Model 5750 and Varian Aerograph Model 1200 gas chromatographs equipped with hydrogen flame ionization detectors. Relative peak areas were closely proportional to mole percentages for the hydrocarbons used in this study. In all cases

the injection ports and detector blocks were maintained at 240 and 290°, respectively; helium was the carrier gas through 1/8-inch outer diameter stainless steel columns at 40–45 ml/min measured at the column outlet. The following column lengths and packings were used for qualitative and quantitative analyses: a, 1.5 m, 10% OV-1 on 100–120-mesh Gas Chrom Q; b, 3 m, 10% OV-17 on 80–100-mesh Supelcoport; c, 3 m, 10% OV-210 on 80–100-mesh Supelcoport; d, 1.7 m, 3% cyclohexanedimethanol succinate on 100–120 mesh Gas Chrom Q. Columns a–c were linearly programmed from 150 to 270° at 10°/min; Column d was operated isothermally at 150, 200, or 230°.

General methods

Bile was collected as described by KOTIN *et al.*[14] and cannulation of the intestinal lymph duct was according to BOLLMAN *et al.*[15]. Everted intestinal sacs were initially prepared as described by WILSON AND WISEMAN[16], but open-ended sacs as described by CLARKSON AND ROTHSTEIN[17] were more convenient for repeated sampling of the serosal fluid and were used in all of the experiments reported here. The serosal medium was rat serum diluted 1:1 with glucose–saline. The mucosal medium, into which air was continuously bubbled, was a 1:1 mixture of "hydrocarbon emulsion" and glucose–saline at pH 8.

"Hydrocarbon emulsion" was prepared as follows: An "artificial rat bile" was made having the following composition in mg/100 ml: sodium taurocholate, 1000; cholic acid, 70; chenodeoxycholic acid, 15; bovine serum albumin, 500; soy phospholipids (Azolectin), 50; monopalmitin, 50; and palmitic acid, 20. 100 ml of this mixture was combined with 2.5 g of anhydrous Na_2CO_3, 10 ml of oleic acid and 50 mg of hydrocarbon. For tracer studies the hydrocarbon was *n*-[1-^{14}C]hexadecane, 0.2 μC/mg for everted intestinal sac experiments. Gentle stirring of this mixture gave an emulsion that was stable for several hours.

Blood was sampled through 25-gauge syringe needles inserted into either the right ventrical of the heart, the portal vein just below its point of branching, or a tail vein. Lymph was occasionally sampled with a syringe from the thoracic duct in 10–20 μl amounts.

Squalane as a marker for balance studies

Preliminary experiments revealed that 96–100% of squalane fed to rats could be recovered in feces collected for 4 days following administration. This was the case whether fed or fasted rats were used, and whether the squalane was administered by stomach tube in bulk or as a solution in corn oil, or fed as a mixture with the standard diet wafers. Holding the feces at room temperature or at 37° for up to 72 h after collection did not reduce the recovery of squalane, indicating the absence of bacterial attack. No squalane was detected in the 72-h urine of 400 g rats given 85-mg doses of squalane by stomach tube. Bile collected for 8 h and lymph collected for 5 h after administration of squalane did not contain detectable amounts of the hydrocarbon (*i.e.* less than 0.01 μg/ml). 72 h after the administration of squalane only 120 $\pm$ 10 μg of hydrocarbon, 0.14% of the dose, could be recovered from extracts of the entire gastrointestinal tract including its contents. Finally, when a mixture of squalane–*n*-octadecane –*n*-octacosane (1:1:1, by weight) was administered to a rat, the ratio of components in the feces was constant over a 72-h period. These observations supported the suggestion of MORGAN AND HOFMANN[18] that squalane should serve as an excellent oil-phase marker for balance studies *in vivo*.

Balance studies

Mixtures of hydrocarbons always including squalane were prepared involving three or four different hydrocarbons in an equal weights ratio. The mixtures were administered at various dose levels (0.8 ml/kg body weight unless otherwise indicated) to fed, 250 g rats by stomach tube. The rats were allowed food and water *ad libitum* while feces were collected twice daily and stored in chloroform until excretion of the hydrocarbon ceased (72–96 h).

Both the test feces and control feces to which had been added aliquots of the hydrocarbon mixture being tested were extracted and the hydrocarbons were analyzed by gas chromatography. The percentage of each hydrocarbon "retained" by the animals was considered to be 100 % *minus* the percentage excreted, and was calculated from the formula $R = [(A/S)_D—(A/S)_F)/(A/S)_D] \times 100\,\%$, where R = percentage retained, A/S = molar ratio of test hydrocarbon to squalane, D refers to the diet and F to the feces. Each hydrocarbon was tested in three animals and each feces sample was analyzed at least 3 times.

Recirculation in the bile

Bile was collected continuously for 7 h after subcutaneous injection of 5 mg of [1-^{14}C]hexadecane (5 μC) in 100 μl of corn oil or intravenous injection (leg vein) of 0.1 ml of rat serum saturated with [1-^{14}C]hexadecane (0.1 μC). The bile, collected over 15-min intervals, was assayed both for total ^{14}C content and content of ^{14}C in the hydrocarbon fraction after chromatography on Florisil[13]. Aliquots of bile were saponified, and the saponifiable lipids (fatty acids and bile acids) were assayed for ^{14}C. Cholesterol was isolated by preparative thin-layer chromatography in hexane–diethyl ether–acetic acid (85:15:1, by vol.).

Action of bacteria

Two experiments were performed to examine the possibility that bacterial action in the intestines or feces might influence the results obtained in these studies. Rats initially weighing 150 g were maintained for 5 days on a diet consisting of (a) pulverized D & G Laboratory Diet, (b) same as (a) but containing 1.0 % chlortetracycline·HCl, or (c) as (a) but containing 0.5 % sulfanilamide and 0.1 % streptomycin sulfate. A mixture of squalane, *n*-hexadecane, pristane, and pentadecylcyclohexane was then administered as for the balance studies and the rats were returned to their respective diets for an additional 72 h. The R values for the three test hydrocarbons were determined for each group of rats and compared.

For the second experiment, fresh feces from control rats were made into a paste with either 0.9 % NaCl or Trypticase Soy Broth and incubated with 50 mg (5 μC) of *n*-[1-^{14}C]hexadecane per 5 g of feces at 37°. Three samples were incubated under nitrogen, three in air, and three were immediately extracted as zero-time controls. *n*-Hexadecane was chosen for this experiment as it would be expected to be near the optimum chain length for attack by hydrocarbon-oxidizing bacteria[19]. The percentages of ^{14}C converted to fatty acid over periods up to 72 h were estimated by saponification of the extracts and assay of the saponifiable and nonsaponifiable fractions for radioactivity. The nonsaponifiable fractions were chromatographed on Florisil to determine the percentage of ^{14}C remaining as hydrocarbon.

Site of absorption

Rats weighing approx. 300 g were anaesthesized with pentobarbital and injected with 0.1 ml of hydrocarbon emulsion containing 5 μC of *n*-[1-^{14}C]hexadecane each. The emulsion was injected either (a) into the stomach, which was tied off with a suture at the duodenum, (b) into the duodenum with the small intestine tied off at stomach and caecum, (c) into the caecum tied off both at ileum and colon, and (d) into the colon tied off at the caecum. Blood was collected at intervals from the tail vein, and its content of ^{14}C taken to be a relative measure of absorption from the various gastrointestinal sites.

Absorption from the small intestine was examined in more detail using everted sacs (3 cm) of duodenum, jejunum, and ileum. Samples of serosal medium were taken through teflon tubes attached to syringes, and assayed for total radioactivity at hourly intervals. After 3-h incubation, the sacs were rinsed in saline, then hexane, and extracted with chloroform–methanol (2:1, v/v) to determine the amounts of radioactivity taken up by the tissues. To simplify the controls required, sacs of all three intestinal regions were incubated simultaneously in the same beaker of mucosal medium (described above).

Lymph versus portal circulation

Rats weighing about 180 g were anaesthesized with pentobarbital and 0.2-ml aliquots of hydrocarbon emulsion containing 10 μC of *n*-[1-^{14}C]hexadecane each were injected into the duodena. Each duodenum was tied off between the puncture and the previous location of the tip of the syringe needle to avoid leakage. Samples of thoracic lymph (20 μl), blood from the portal vein (0.5 ml) and blood from the heart (0.5 ml) were taken at intervals up to 1 h after injection of the emulsion. Each sample was assayed for radioactivity and the disint./min per ml compared. A higher concentration of ^{14}C in the portal blood than in blood from the heart was tentatively considered to reflect absorption of hydrocarbon or its metabolic products directly from the intestines into the portal circulation.

RESULTS

The effects of hydrocarbon structure on percentage retention by fed rats are summarized in Fig. 1. The percentage retention of *n*-alkanes showed an inverse linear relationship to chain length that was describable by the regression line: (percentage retained) = $115.9 - 3.94 \times$ (number of carbon atoms). This line had a correlation coefficient of -0.995, standard error of estimate $Sy \cdot x = 3.30$, $t = 30.85$ and $P < 0.001$ (ref. 20). Paraffins having more than 29 carbon atoms thus would not be absorbed to a significant extent under these conditions. If the regression equation can be applied to low carbon numbers, hydrocarbons with fewer than 5 carbon atoms (gases at 37°) should be absorbed quantitatively.

The branched, cyclic and unsaturated hydrocarbons examined in this study, when compared to the corresponding *n*-saturated hydrocarbons of the same total number of carbon atoms, all gave $P > 0.9$ (2-tailed *t*-test) or no statistically significant difference in percentage retention. Thus carbon number appeared to be the determining factor in retention under these conditions.

The effect of dose on percentage retention was similar for all of those hydrocarbons retained to a sufficient extent to permit detection of small differences, and is

illustrated for *n*-octadecane in Fig. 2. The percentage retained was constant between 60 and approx. 320 mg/kg rat, but fell off above this level. The retention was only 70 % of maximum at a dose of 530 mg/kg rat.

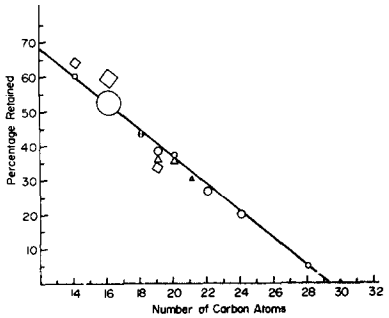

Fig. 1. Relative retention of hydrocarbons by rats. Circles represent *n*-alkanes, squares indicate unsaturates, and triangles refer to branched or cyclic species. The size of each figure is a representation of the standard deviation. The following compounds are indicated by □ and △: C_{14}, 1,13-tetradecadiene; C_{16}, 8-hexadecyne; C_{18}, 1-octadecane; C_{19}, pristane (△) and 9-nonadecene (□); C_{21}, pentadecylcyclohexane; C_{30}, squalane.

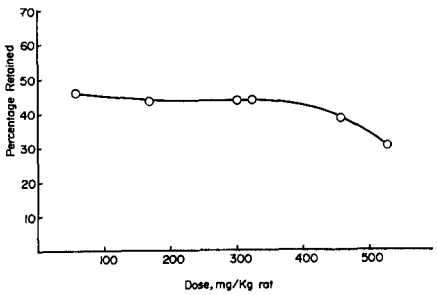

Fig. 2. Effect of dose on retention of *n*-octadecane. Administration of *n*-octadecane as a 1:1 mixture with squalane as described under *Balance studies* in the text.

In the course of these experiments it was observed that the relative amount of squalane in a mixture of hydrocarbons had no detectable effect on the retention of the other components. Thus doses of squalane between 50 and 500 mg/kg had no effect on the retention of a constant 200 mg/kg dose of *n*-octadecane. A situation resembling competition did occur, however, between two hydrocarbons both of which were well retained. If mixtures having the composition squalane–hexadecane–octadecane ($1:X_1:X_2$, by weight) where $X_1 + X_2 = 2$, X_1 = amount of hexadecane and X_2 = amount of octadecane, were fed, the percentage retentions of the two paraffins fit the regression equation above only when $X_1 \simeq X_2$. For $X_2 \gg X_1$, the retention of octadecane fit the regression equation but that of hexadecane was well below the line.

This phenomenon was not a true, simple competition. Any absorbed hydrocarbon was retained as well from an equal weights mixture of six components as from one of three compounds, even though its percentage of the total was very different in the two test mixtures. We did not make a detailed study of this interaction in the present work, but it would appear that the regression equation above should not be strictly applied to the very minor components of a mixture of hydrocarbons, as their retention will be less than that predicted by the equation. The discrepancy is not large; in the example above $[X_2]/[X_1] = 7$ when the retention of *n*-hexadecane is reduced to 50 % of the value predicted by the regression equation.

As reported elsewhere[7], radioactivity was detected in the bile within 15 min after subcutaneous or intravenous injection of *n*-[1-^{14}C]hexadecane. The peak concentration of radioactivity was reached within 45–60 min, but no radioactivity chromatographing with hydrocarbon on Florisil, or silicic acid[7], could be detected in bile collected for 7 h after injection of labeled hydrocarbon. All of the radioactivity recovered was associated with water-soluble materials, saponifiable lipids, and cholesterol. The relative amount of radioactivity associated with water-soluble materials

varied from 40 to 60 % of the total during the 7-h period, but the distribution of ^{14}C among lipid fractions was not significantly variable, 90 ± 1 % being in saponifiable lipids (fatty acids and bile acids), 10 ± 1 % in cholesterol. Thus recirculation of intact hydrocarbon in the bile would not be expected to influence the results of the balance studies.

Treatment with antibiotics reduced the retention of the hydrocarbons tested to the same extent in both groups of treated rats. The retention data for the treated rats (Groups b and c combined) fit the regression line: (percentage retained) = 61.9 — 2.64 × (number of carbon atoms), with a correlation coefficient of — 0.979, $Sy \cdot x$ = 5.12, t = 9.60 and $P < 0.001$. No oxidation of n-[1-^{14}C]hexadecane to radioactive fatty acid could be detected in any of the fecal incubation experiments. The recovery of ^{14}C in the hydrocarbon fraction averaged 98.4 ± 1.0 % in all cases. The significance of these somewhat conflicting results is discussed later.

Fig. 3 shows the recoveries of ^{14}C in blood from the tail vein of rats injected with radioactive n-hexadecane at various gastrointestinal sites. It is apparent from these results that the small intestine is by far the major site of paraffin absorption. Results of the everted sac experiment appear in Table I. All portions of the small intestine appeared equally competent relative to uptake of hydrocarbon from the mucosal medium, although ileum may have had a slight edge for uptake into the cells while duodenum and jejunum both exceeded ileum in passing ^{14}C into the serosal fluid. These results are quite different from those obtained using labeled fatty acid[21], where uptake was much greater in proximal than in distal small bowel. This may reflect a requirement for the jejunal/duodenal esterifying enzymes for the absorption of fatty acids[21] but not for absorption of hydrocarbons.

The concentrations of ^{14}C in thoracic lymph, portal blood, and blood from the heart of rats injected intraduodenally with labeled hydrocarbon emulsion are shown in

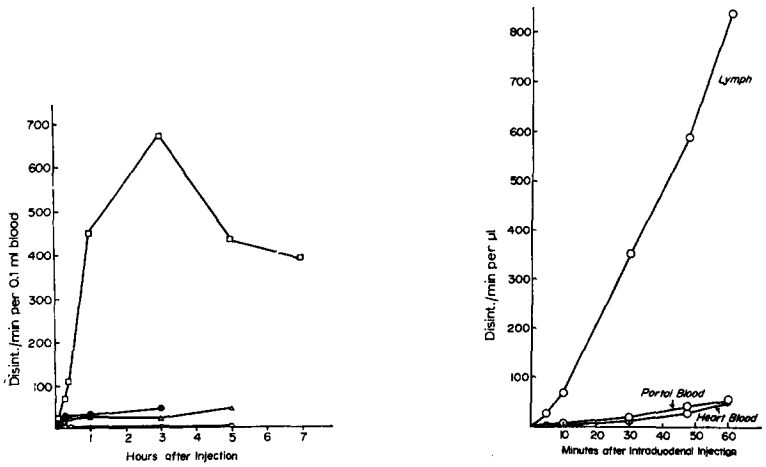

Fig. 3. Concentration of ^{14}C in the blood of rats after gastrointestinal injection of n-[1-^{14}C]hexadecane. Hydrocarbon emulsion containing 5 μC was injected into stomach (○); duodenum (□); caecum (△); colon (●). For details see text.

Fig. 4. Appearance of ^{14}C in body fluids after intraduodenal injection of emulsified n-[1-^{14}C]hexadecane. 5 μC injected in 0.1-ml emulsion.

TABLE I

UPTAKE OF ^{14}C BY EVERTED INTESTINAL SACS

Uptake by 3-cm lengths containing 0.5 ml of serosal medium and suspended in 200 ml of mucosal medium containing 10 μC (50 mg) of *n*-[1-^{14}C]hexadecane.

Source	*Disint./min per µl serosal medium**			*Disint./min* $\times 10^{-5}$ *in tissues after 3 h*	*Total uptake*** *(disint./min* $\times 10^{-5}$*)*
	1 h	*2 h*	*3 h*		
Duodenum	129	308	452	1.014	1.47
Jejunum	48	117	172	1.329	1.50
Ileum	25	61	89	1.718	1.81

* All values in the table are $\pm$ 1% S. D., three determinations.
** Sum of Columns 3 and 4, with Column 3 corrected to 0.5 ml.

Fig. 4. After a slight lag the concentration of ^{14}C in the lymph increased almost linearly as did that in the blood. At all sampling times, the portal blood showed a very slightly higher concentration or ^{14}C than the blood from the heart. This would be consistent with absorption of some of the labeled hydrocarbon and/or its metabolites directly into the portal circulation. The blood ^{14}C differences are significant in terms of non-quantitative "sign test"[22], but are not statistically significant in terms of a 2-tailed "*t*" ($P>0.01$ in all cases). For this reason, no estimate of the relative transport of ^{14}C through portal and lymphatic routes is made at the present time.

DISCUSSION

The retention (intake *minus* excretion) of hydrocarbons observed in this study was well within the range expected from balance type of experiments in the literature. FRAZER *et al.*[9] observed absorption of from 11 to 43% of a paraffin oil (composition unspecified) depending on the degree of emulsification achieved. EL MADI AND CHANNON[23] found an apparent retention of between 23 and 75% of *n*-hexadecane fed to rats over a 3-week period. The percentage retention in their experiments depended to some extent on both dosage and mode of administration.

Higher apparent retentions are usually observed when hydrocarbons are administered as an integral component of the diet. STETTEN[24] reported that only 4% of *n*-hexadecane fed at a level of 0.75% in a well-balanced diet to rats for 5 days was recovered in the feces. KOLATTUKUDY AND HANKIN[12] observed an apparent absorption of 25% of *n*-nonacosane fed at a level below 0.3% of a semi-solid diet for 5 days. They indicated[12] that a lower degree of absorption resulted when the hydrocarbon was administered by stomach tube in corn oil. The absolute values for percentage retention in Fig. 1 of the present paper must therefore be applied only to the administration of hydrocarbons by stomach tube to fed rats in a single dose.

Our failure to detect microbial attack on hydrocarbon in rat feces is in agreement with the findings of MITCHELL AND HÜBSCHER[25]. They were unable to detect oxidation of *n*-hexadecane to fatty acid by mixed cultures of guinea pig intestinal flora. These negative results, and our finding that *n*-hexadecane apparently does not

recirculate intact in the bile, should increase confidence in the interpretations of the present and past balance studies in the literature.

There are several possible interpretations of the decreased hydrocarbon retention caused by treatment with the antibiotics. It is conceivable that some portion of the intestinal flora is capable of metabolizing hydrocarbons *in situ* but not in feces (present work) or *in vitro* culture[25]. Secondly, the antibiotic treatment may have caused sufficient diarrhea to reduce lipid absorption. This is considered unlikely in the present case, as neither the fecal mass nor consistency differed noticeably between the treated and control groups. Finally, since interference with normal protein synthesis is associated with the actions of streptomycin and tetracyclines[26,27], it is possible that the antibiotics reduced the availability of lipases essential for the production of emulsified hydrocarbon. This last possibility appears deserving of future study.

Since excretion of hydrocarbon continued for at least 3 days following an intragastric dose, it would appear that the entire small intestine was exposed to significant levels of material. Since all regions of the small intestine were roughly of equal competence at absorbing hydrocarbon in the everted sac experiments, it is probable that absorption continues normally throughout the small intestine. We were able to detect squalane in feces as early as 5 h after intragastric administration, while the peak concentration of radioactivity in the lymph was observed 3 h after intraduodenal injection of *n*-[1-^{14}C]hexadecane. McWEENY[8], who carried out studies with lymph-cannulated rats, found absorption of *n*-hexadecane continuing for at least 60 h after intragastric administration. These observations further support the supposition that normal absorption of hydrocarbons occurs throughout the small intestine.

Since the flow of blood through the portal vein is many times greater than the flow of lymph through the thoracic duct[15,28], the very small difference in ^{14}C concentrations in the two blood sources of Fig. 4, if real, could reflect a significant absorption directly into the portal circulation. However, since (1) the differences observed were not statistically significant, (2) it is by no means certain that any differences would solely reflect direct absorption, and (3) the chemical nature of the excess ^{14}C-labeled material in the portal blood could not be determined, it is necessary to conclude that the possibility of direct absorption of hexadecane and/or its metabolites into the portal blood is an open question requiring future study.

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REFERENCES

1 J. K. BOITNOTT AND S. MARGOLIS, *Federation Proc.*, 25 (1966) 200.
2 C. E. CAIN, O. E. BELL, JR., H. B. WHITE, JR., L. L. SULYA AND R. R. SMITH, *Biochim. Biophys. Acta*, 144 (1967) 493.
3 F. GAZZARRINI AND B. NAGY, *Arch. Biochem. Biophys.*, 113 (1966) 245.
4 J. BOITNOTT AND S. MARGOLIS, *Bull. Johns Hopkins Hosp.*, 118 (1966) 402.
5 J. BOITNOTT AND S. MARGOLIS, *Bull. Johns Hopkins Hosp.*, 118 (1966) 414.
6 A. F. LIBER AND H. G. ROSE, *Arch. Pathol.*, 83 (1967) 116.
7 P. W. ALBRO AND L. FISHBEIN, *Life Sci.*, 9 (1970) 729.
8 D. McWEENY, Ph. D. Thesis, University of Birmingham, 1957.
9 A. C. FRAZER, J. H. SCHULMAN AND H. C. STEWART, *J. Physiol.*, 103 (1944) 306.

10 K. BERNHARD, *Fette Seifen*, 55 (1953) 160.
11 A. G. EBERT, C. R. SCHLEIFER AND S. M. HESS, *Federation Proc.*, 24 (1965) 546.
12 P. E. KOLATTUKUDY AND L. HANKIN, *J. Nutr.*, 90 (1966) 167.
13 P. W. ALBRO AND J. C. DITTMER, *Biochemistry (ACS)*, 8 (1969) 394.
14 P. KOTIN, H. L. FALK AND R. BUSSER, *J. Nat. Cancer Inst.*, 23 (1959) 541.
15 J. L. BOLLMAN, J. C. CAIN AND J. H. GRINDLAY, *J. Lab. Clin. Med.*, 33 (1948) 1349.
16 T. H. WILSON AND G. WISEMAN, *J. Physiol.*, 123 (1954) 116.
17 T. W. CLARKSON AND A. ROTHSTEIN, *Am. J. Physiol.*, 199 (1960) 898.
18 R. G. H. MORGAN AND A. F. HOFMANN, *J. Lipid Res.*, 11 (1970) 231.
19 H. B. LUKINS AND J. W. FOSTER, *Z. Allg. Mikrobiol.*, 3 (1963) 251.
20 F. E. CROXTON, *Elementary Statistics with Applications in Medicine and the Biological Sciences*, Dover Publications, Inc., New York, 1953.
21 J. B. RODGERS, JR. AND W. BOCHENEK, *Biochim. Biophys. Acta*, 202 (1970) 426.
22 C. MACK, *Essentials of Statistics for Scientists and Technologists*, Plenum Press, New York, 1967, p. 129.
23 M. A. H. EL MADI AND H. J. CHANNON, *Biochem. J.*, 27 (1933) 1487.
24 D. STETTEN, JR., *J. Biol. Chem.*, 147 (1943) 327.
25 M. P. MITCHELL AND G. HÜBSCHER, *European J. Biochem.*, 7 (1968) 90.
26 T. D. BROCK, *Federation Proc.*, 23 (1964) 965.
27 M. E. SHILS, *Clin. Pharmacol. Therap.*, 3 (1962) 321.
28 L. SAPIRSTEIN, *Circulation Res.*, 4 (1965) 689.