Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat

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Abstract This study assessed the effect of concomitant lipid absorption on the bioavailability and lymphatic transport of benzo(a)pyrene (BP), a carcinogenic polycyclic aromatic hydrocarbon (PAH). Conscious, male Sprague-Dawley rats, equipped with biliary and mesenteric lymphatic catheters received intraduodenally a dose of 0.4 μmoles ^{3}H-labeled BP completely dissolved in either 50 μmoles or 500 μmoles of olive oil. Diversion of mesenteric lymph allowed biliary and urinary excretion of ^{3}H to be used as an indirect measurement of relative ^{3}H portal transport. Total radiolabel recovered in a 24-hr period in each group was 20.0 ± 2.6% of the ^{3}H dose given in 50 μmoles of oil, and 17.0 ± 1.0% of the ^{3}H dose administered in 500 μmoles of oil. In animals receiving the low-fat test meal, 79.4 ± 1.4% of the recovered radiolabel was found in bile; the corresponding value for the high fat dose was 78.5 ± 2.6%. Thus a tenfold variation in the mass of the carrier vehicle (triglyceride oil) did not significantly effect the disposition of BP, and portal, not lymphatic transport, was the major route of post-absorptive transport. Although the chylomicrons produced from both fat doses were initially contaminated with BP, within 1–1.5 hr the radioactivity in lymph began to drop such that by 3 hr in the animals fed high fat, the chylomicrons were essentially free of BP. These results show that the rat enterocyte quickly adapts to PAH-contaminated dietary fat, even during the assimilation of a single dose of fat. Presumably, during the post-absorptive synthesis of chylomicrons from pre-chylomicrons, BP is metabolized and removed from the triglyceride oil droplets.—**Laher, J. M., M. W. Rigler, R. D. Vetter, J. A. Barrowman, and J. S. Patton.** Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat. *J. Lipid Res.* 1984. **25:** 1337–1342.

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Although it has repeatedly been proposed that there is a positive relationship between colorectal and breast cancer incidence and fat consumption (1–3), firm epidemiological evidence remains elusive. The gastrointestinal tract is often exposed to dietary carcinogens (4) including the ubiquitous polycyclic aromatic hydrocarbons (PAHs), which occur in charcoal-broiled meat (5, 6), vegetable oils (7), and many other foods (8). Possibly high fat diets simply carry high loads of carcinogens. In animal experiments, however, increased levels of dietary fat cause an increase in tumorogenesis following administration of a chemical carcinogen (9, 10). Thus dietary fat, by nutritional and/or some other means, enhances the survival and growth of tumors.

In addition to the possible cancer-promoting effects of fat, fat can markedly increase the bioavailability of hydrophobic molecules such as PAHs (11) (R. D. Vetter, M. C. Carey, and J. S. Patton, unpublished observations). Hydrocarbons, such as octadecane, are readily absorbed in the presence of triolein, and the extent of absorption is proportional to their concentration in the triglyceride carrier (12). The extent of lymphatic transport of cholesterol is also correlated with the amount of triolein vehicle (13). Trace lipid bioavailability is at least partially dependent upon conditions in the intestinal lumen such as the formation of mixed lipid-bile salt micelles (14). PAHs are mixed micellar solutes in vitro (15), and in the rat the presence of bile salts and long-chain partial glycerides in the lumen maximizes 7,12-dimethylbenz(a)anthracene uptake (16).

Once absorbed, hydrophobic compounds usually gain direct access to the systemic circulation by means of lymphatic transport, while polar substances are carried in the portal vein leading to possible first-pass metabolism by the liver. It has generally been assumed that following ingestion in lipid vehicles, PAHs are predominantly, if not exclusively, transported in lymph (17–19) as parent compound-solubilized in the triglyceride cores of chylomicrons (20). We have tested this assumption by employing biliary and mesenteric lymph catheters. We varied dietary fat content to investigate the possibility that increased lipid feeding enhances the bioavailability and also lymphatic transport of one PAH, benzo(a)pyrene

Abbreviations: BP, benzo(a)pyrene; PAH, polycyclic aromatic hydrocarbon.

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(BP), and thereby the amount of unchanged BP available to remote sites in the body.

MATERIALS AND METHODS

Preparation of test meals

BP (98% pure) was obtained from Sigma Chemical Co., St. Louis, MO. [G-^{3}H]BP was purchased from Amersham/NEN and its radiopurity was found to be 98% by TLC. Highly refined olive oil and oleic acid (99% pure) were obtained from Sigma Chemical Co.

A stock solution of BP completely dissolved in olive oil was prepared by combining cold crystalline BP and oil in a vial in which ^{3}H-labeled BP had been added previously. The final stock solution contained per 50 μmol of olive oil (~50 μl), 0.4 μmol (100 μg) of BP (12.5 μCi of ^{3}H-labeled BP). This represents about 10% of the total BP-solubilizing capacity of olive oil at 23°C (21). A molecular weight of 882 was used for olive oil. The mixture was shaken in the dark under nitrogen for 24 hr, then kept protected from light and under nitrogen until used.

The low-fat test meal was composed of a 50-μmol (~50 μl) aliquot of BP stock solution, 9 μmol of oleic acid and 950 μl of 0.9% NaCl in distilled water. The high-fat meal consisted of 50 μmol of BP stock solution, 450 μmol of olive oil, 87 μmol of oleic acid, and 713 μl of 0.9% NaCl in distilled water. The mixtures were emulsified by sonication (Sonifier Cell Disruptor, Model 185, Branson Sonic Power Co., equipped with a standard microtip at power output 5). Samples of the mixtures were removed for liquid scintillation counting, then known volumes of the mixtures were quickly drawn up into syringes for administration.

Animal surgery

Male Sprague-Dawley rats weighing 300 g were purchased from Sprague-Dawley Laboratories (Chicago, IL) and maintained on Purina Rat Chow (4.5% fat, <5% fiber) (Ralston Purina Co.). They were allowed free access to food and water until the time of operation. Anesthesia was induced and maintained with ether. Following a ventral midline incision, the main small intestinal lymph duct was cannulated using SV31 vinyl tubing (Dural Plastics and Engineering, Dural N.S.W. Australia, ext. diam. 0.80 mm, int. diam. 0.50 mm) after the method of Turner and Barrowman (22). The common bile duct was cannulated above its confluence with the pancreatic ducts with polyethylene tubing (Intramedic PE 10, Clay Adams, Parsippany, NJ, ext. diam. 0.61 mm, int. diam. 0.28 mm) and secured with 4.0 silk ligatures. Both cannulae were exteriorized through stab wounds in the right flank. A saline-filled premature infant naso-gastric feeding tube (size 5 Fr., C.R. Bard Ltd., Mississauga, Ontario) was passed into the duodenum close to the pylorus. The catheter was secured with cyanobutylacrylate adhesive and its patency was checked. It was exteriorized through the abdominal wound which was then closed in two layers with silk sutures.

The animals were placed in Bollman-type restraint cages and allowed to recover for 24 hr. A continuous intraduodenal infusion of 0.9% NaCl at 3.4 ml hr^{-1} via constant infusion syringe pump maintained hydration and, by promoting intestinal lymph flow, minimized clot formation. The 24-hr recovery period insured that the small bowel was clean (23) prior to infusion. During the first 5 hr of this recovery period, bile was collected and kept under refrigeration. Any animal exhibiting abnormal bile or lymph flow during the recovery period was discarded from the study. Eleven successful catheterizations were made, but three were discarded. (One had abnormally low lymph flow, and the infusion pump did not engage properly with two others.)

Experimental procedure

Control samples of lymph and bile were taken over 30 min. An intraduodenal pulse of 0.5 ml of rat bile was given, followed by a 1-min infusion of either test meal. This was then washed in with 0.5 ml of rat bile. Lymph and bile samples were collected into tared vials at 30-min intervals over the next 7 hr. Lymph clotting within the vial was prevented with sodium citrate. During the initial 7-hr collection period the animals received an infusion of bile:saline (1:4 v/v) at 3.4 ml hr^{-1}, after which time they were switched to infusions of saline alone and 17-hr bile and lymph collections were taken. Following this 24 hr experimental period, the animals were killed by sodium pentobarbital overdose (Somnitol, M.T.C. Pharmaceuticals, Hamilton, Ontario), the abdomen was opened, and the patency of the cannulae was checked.

Sample analysis

Radioactivity. Bile and lymph samples were weighed and 50-μl aliquots were removed and combined with 10 ml of liquid scintillation cocktail (ScintiVerse II, Fisher Scientific Co., Fairlawn, NJ). Urine volume over the entire experimental period was measured and 50-μl samples were analyzed for radioactivity. Radioactivity was determined in a Beckman LS8100 liquid scintillation counter with quench correction by means of an external standard.

Lymph lipid content. Percentage transmittance of each lymph sample was used as a relative index of lipid content. Transmittance of 50 μl of lymph diluted to 1.5

ml with distilled water was measured at 540 nm in a Hitachi Spectrophotometer Model 100-40. To correlate lymph turbidity with lipid content, total lipid analyses of lymph samples were performed on chromarods with a TH 10 Iatroscan according to Harvey and Patton (24) using a standard composed of olive oil (90.35%), egg phosphatidylcholine (9.55%), and BP (0.1%). Rat lymph (400 μl) was extracted in 5 ml of ethyl acetate overnight at 5°C. Samples were then centrifuged at 2120 *g* for 5 min in a clinical centrifuge. A sample volume of 4 ml was removed, evaporated to dryness with a stream of N_2, and then reconstituted with 2 ml of $CHCl_3$–MeOH 2:1. A 5-μl sample was spotted per chromarod and then chromatographed in a solvent system composed of ethyl ether–NH_4OH 100:1.

Separation of chylomicrons. Samples of lymph from a rat that had received a high-fat test meal were pooled, diluted 1:1 with 0.9% NaCl solution, and vigorously mixed. Aliquots were taken for liquid scintillation counting. The lymph mixtures were ultracentrifuged for 30 min in a Beckman type 75Ti rotor at 30,000 rpm in a Beckman L2-65B ultracentrifuge. The lipid supernatant was gently aspirated and samples of the clear infranatant were taken for liquid scintillation counting.

Determination of BP in lymph. Samples of lymph (500 μl) from a rat that had received a high-fat test meal were extracted three times with ethyl acetate and the extracts were dried down under a steady stream of nitrogen. Following redissolution in $CHCl_3$, the samples were spotted on silica gel TLC plates and developed in benzene using BP as a reference. The plates were divided into fractions and analyzed for radioactivity by scraping sections and combining them with liquid scintillation cocktail.

RESULTS

The profile of lymph turbidity over time following either a low-fat (50 μmol) or high-fat (500 μmol) test meal of BP in olive oil is depicted in **Fig. 1.** Whereas the four animals receiving the high dose of fat produced opaque lymph from 1–7 hr post-administration, the four receiving 50 μmol of oil produced only slightly turbid lymph at 1 hr post-feeding, after which transparent lymph was collected. Turbidity continued to decline in the high-fat group between 7 and 24 hr (data not shown). **Fig. 2** shows that the turbidity of the lymph is roughly proportional to its total lipid content. Regression analysis of % transmittance and total lipid content showed a correlation coefficient of −0.989.

The appearance of radioactivity in lymph from both groups of animals at 1 hr post-feeding (**Fig. 3**) was simultaneous with the production of chylomicrons (Fig. 2). The patterns of lymphatic radiolabel recovery were similar in the high- and low-fat groups; radioactivity peaked at 1–1.5 hr but then rapidly declined. After approximately 3 hr, radiolabel levels plateaued at less than 10% of peak values. Over 90% of the radiolabel appearing during the peak lymph ^3H recovery from one randomly chosen high-fat recipient rat cochromatographed with pure BP. Following this the proportion of radiolabel present as BP gradually decreased and was 65% at 5.5 hr after the test meal. Flotation of chylomicrons in the peak lymph samples from the same high-fat test meal recipient rat removed 88% of the radioactivity. In contrast, at the end of the 7-hr sampling period, only 15% of the radioactivity was removed by ultracentrifugation, although lymph turbidity at this time remained high. Of the BP radioactivity that remained in suspension at 7 hr (85%), >65% cochromatographed with BP. After centrifugation, similar amounts of radioactivity (ca. 600 dpm) were found in the supernatant at all times; at longer times, however, this activity became a greater proportion of the total.

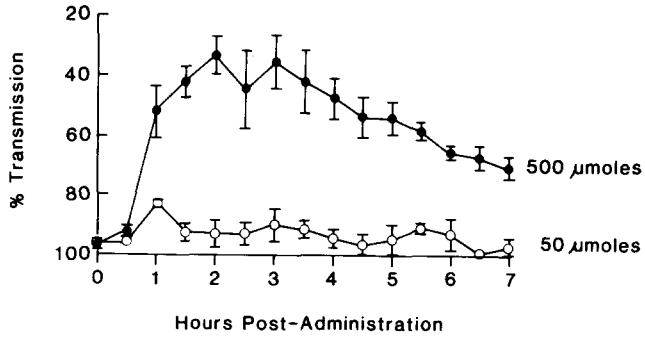

Fig. 1. Percentage transmittance of mesenteric lymph taken at 30-min intervals from rats receiving intraduodenally 50 μmol of olive oil (○) or 500 μmol of olive oil (●) at time = 0. Each point shows the mean ± SEM (n = 4).

At all time points, with both doses of olive oil, biliary radiolabel recovery exceeded lymphatic radiolabel recovery (Fig. 3). Between 7 and 24 hr biliary radioactivity continued to decline (data not shown). Maximal biliary excretion of ^3H occurred an hour later than time of maximal lymphatic radiolabel levels in each group. In magnitude, peak biliary recovery of radiolabel was approximately double that of the corresponding peak lymph value. Following maximal biliary ^3H excretion, levels of ^3H in bile declined at a gradual rate with the high-fat meal and more steeply with the low-fat dose.

Cumulative 24-hr recovery of ^3H in bile, lymph, and urine corresponded to 20.0 ± 2.6% of the BP given with 50 μmol of oil and 17.0 ± 1.0% of the BP in the 500 μmol of olive oil test meal. Of the radiolabel recovered after the low-fat meal, 79.4 ± 1.4% was detected in bile, 11.7 ± 2.0 in lymph, and 8.9 ± 1.4% in

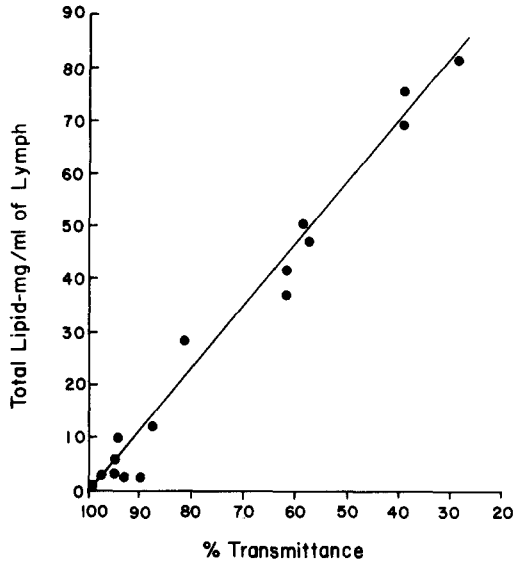

Fig. 2. The correlation between turbidity of lymph and the total lipid concentration of lymph.

urine; corresponding values from the group receiving the large oil dose were 78.5 ± 2.6% from bile, 12.5 ± 2.5% in lymph, and 9.1 ± 1.9% in urine. For the 24-hr recovery period, the ratios of biliary plus urinary radiolabel to lymphatic radiolabel were 8.3 ± 1.5:1 and 8.2 ± 1.9:1 for the low and high lipid meals, respectively.

Only 2.3 and 2.1% of the total fed benzo(a)pyrene dose made it into the lymph of the low-fat and high-fat groups, respectively. Concentrations of BP in lymph fat at 1 hr post-feeding were 0.08% and 0.4% of the initial concentrations in the low-fat and high-fat diets, respectively. Thus the intestine exhibits great efficiency in the removal of BP from dietary fat.

DISCUSSION

The experimental model used in this study provides a means for assessing the contribution of the intestinal lymphatic system to the systemic delivery of intraduodenally administered BP. With complete lymphatic diversion, the portal vein provides the only route for systemic entry of a substance upon absorption by the enterocyte. Following intravenous administration, radiolabeled PAHs are efficiently cleared from the circulation by the liver and excreted in the bile as metabolites (25, 26), providing a reliable index of bioavailability. This model also prevents the complication of enterohepatic recycling of biliary metabolites, which is extensive with BP (26).

This study demonstrated that varying the mass of the carrier vehicle (triglyceride oil) by one order of magnitude did not significantly affect the disposition of benzo(a)pyrene in the rat. Actually, the total combined 24-hr recovery of radioactivity (bile + lymph + urine) was slightly less in the high-fat animals (17.0 ± 1.0%) than in the low-fat animals (20.0 ± 2.6%). Mirvish et al.

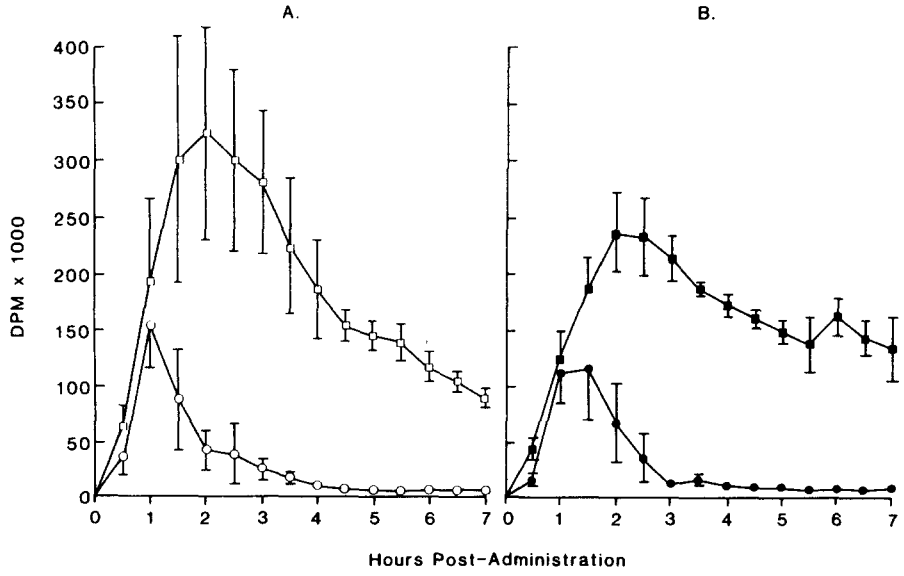

Fig. 3. A. DPM recovered in bile (□) and lymph (○) over 30-min periods following intraduodenal administration of 100 μg of BP (12.5 μCi of ^{3}H-labeled BP) in 50 μmol of olive oil (n = 4). Each point shows the mean ± SEM. B. DPM recovered in bile (■) and lymph (●) over 30-min periods following intraduodenal administration of 100 μg of BP (12.5 μCi of ^{3}H-labeled BP) in 500 μmol of olive oil (n = 4). Each point shows the mean ± SEM.

(27) noted a small increase in fecal BP excretion in rats when dietary corn oil was increased from 5% to 30%, which suggests that high-fat doses may retard BP absorption. Results obtained in a preliminary study of [^{3}H]7,12-dimethylbenz(a)anthracene absorption demonstrate an inverse relationship between arterial plasma levels of radiolabel and carrier triglyceride mass during the first 7 hr after intraduodenal administration (J. M. Laher and J. A. Barrowman, unpublished observations), a pattern which may be attributed to a delay in absorption with increased lipid load over the study period.

While it is known that the gastrointestinal lymphatics are the major route for transport of nutrient lipids (28) and trace lipid-soluble compounds such as DDT (29), the present work shows that, in the conscious rat, the lymphatics play only a limited role in the systemic entry of BP, regardless of concurrent lipid transport. Following ^{3}H-labeled BP administration over a 24-hr study period, combined radiolabel recovery from the urine and bile was about eight times greater than lymphatic ^{3}H in both high- and low-fat groups of rats. Patterns of BP lymphatic radiolabel recovery were similar in both groups and occurred mainly during the first 2 hr after the test meal. This transient "pulse" of radiolabel was simultaneous with the production of chylomicrons by the enterocyte as reflected in lymphatic turbidity and lipid content. During this phase BP was found primarily as unchanged hydrocarbon in association with chylomicrons, probably solubilized within their triglyceride cores, however, at concentrations less than 1% of the initial BP concentrations in the dietary oils. Although large quantities of lipid-laden chylomicrons continued to appear in the lymph of high-fat recipient rats for at least 7 hr, BP transport dropped dramatically after 1–2 hr, and was essentially the same as in animals producing lymph low in lipid (i.e., those receiving 50 μmol of olive oil). These observations indicate that, although initial PAH lymphatic transport may require chylomicrons, a large flux of chylomicrons is not needed and increased lipid transport in lymph does not promote increased BP transport.

It is also apparent that, following a short delay, the rat intestine becomes capable of quantitatively separating absorbed BP from fat. This suggestion is supported by the prolonged 2–7 hr biliary excretion of radiolabel in levels which dwarf those of lymph, despite continued elevation of lymph lipid flow. The lower peak and more gradual decline in biliary ^{3}H excretion with the high-fat meal probably reflects a more prolonged absorption seen with an increased lipid burden. Biliary radiolabel is probably derived from portal venous transport of BP derivatives, most likely polar metabolites. With either lipid burden the enterocyte seems able to quickly differentiate between nonpolar substances destined for lymphatic transport in chylomicrons (e.g., triglyceride, cholesterol) and those to be diverted to the portal vein, probably following conversion to more polar substances. Rapidly inducible BP hydroxylase activity is found throughout the intestinal tract (30–32). Water-soluble metabolites of BP are rapidly formed using everted rat intestinal sacs (33) and isolated rat intestinal epithelial cells (34). Thirty minutes after instillation of ^{3}H-labeled BP in an acetone solution into a closed intestinal loop, 40% of the administered radiolabel was recovered in portal blood (35). Of this amount, greater than 90% was present as polar BP metabolites. Fasting and semisynthetic diets, however, can cause the complete disappearance of intestinal monooxygenase activities (36). Thus, in this study, the BP that initially entered the lymph may have done so because of low monoxygenase levels that occurred following the 24-hr post-operative fast.

Although the site where BP is separated from dietary fat in the rat intestine is uncertain, in the killifish BP that is dissolved in dietary triglyceride follows the fat through the processes of digestion, dispersion, absorption, and resynthesis to reappear inside the cell once again inside triglyceride droplets (unpublished observations). Benzo(a)pyrene is then metabolized, separated from the fat, and finally appears in the fish's gallbladder (unpublished observations). Extrapolation of fish data to the rat must be done with caution. However, if BP is also coassimilated with fat in the rat, then the BP must be separated from the intracellular fat droplets during their migration from the apical region of the cell through the endoplasmic reticulum (ER) to the Golgi complex where they become complete chylomicrons (37). During this lipid processing in the rat enterocyte, the fat droplets are enveloped by smooth ER membranes (38, 39), and it is here that separation of BP from fat probably occurs via the BP-metabolizing enzymes of the smooth ER.

In summary, this study showed that the bioavailability and extensive intestinal metabolism of BP were similar when fed in low and high doses of fat and that the minor partition of BP into lymph was independent of the mass of lymph fat. Since PAHs like BP are sequestered in dietary fats (7), a high-fat diet may be a high-carcinogen diet. The implications of this study are that the intestine is the major organ affected by dietary PAHs and that lymphatic export of fat-soluble PAH procarcinogens can be minimized by maintaining an induced monooxygenase system in the intestine.

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REFERENCES

1. Wynder, E. L. 1979. Dietary habits and cancer epidemiology. *Cancer.* **43:** 1955–1961.
2. Correa, P. 1981. Epidemiological correlations between diet and cancer frequency. *Cancer Res.* **41:** 3684–3689.
3. Kinlen, L. J. 1983. Fat and cancer. *Br. Med. J.* **286:** 1081–1082.
4. Ames, B.N. 1983. Dietary carcinogens and anti-carcinogens. *Science* **221:** 1256–1264.
5. Lijinsky, W., and P. Shubik. 1964. Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science.* **145:** 53–55.
6. Gray, J. I., and I. D. Morton. 1981. Some toxic compounds produced in food by cooking and processing. *J. Human Nutr.* **35:** 5–23.
7. Kolarovic, L., and H. Traitler. 1982. Determination of polycyclic aromatic hydrocarbons in vegetable oils by caffeine complexation and glass capillary gas chromatography. *J. Chromatogr.* **237:** 263–272.
8. Howard, J. W., and T. Fazio. 1980. Review of polycyclic aromatic hydrocarbons in foods. *J. Assoc. Off. Anal. Chem.* **63:** 1077–1104.
9. Dao, T. L., and P. C. Chan. 1983. Effect of duration of high fat intake on enhancement of mammary carcinogenesis in rats. *J. Nat. Cancer Inst.* **71:** 201–206.
10. Kritchevsky, D., and D. J. Fink, editors. 1981. Workshop on Fat and Cancer. *Cancer Res.* **41:** 3681–3826.
11. Falk, H. L., and P. Kotin. 1963. Chemistry, host entry and metabolic fate of carcinogens. *Clin. Pharmacol. Ther.* **4:** 88–103.
12. Borgström, B. 1974. Fat digestion and absorption. *In* Biomembranes. Vol. 4B. D. H. Smyth, editor. Plenum Press, New York. 555–620.
13. Sylvén, C., and B. Borgström. 1968. Absorption and lymphatic transport of cholesterol in the rat. *J. Lipid Res.* **9:** 596–601.
14. Hofmann, A. F. 1968. Functions of bile in the alimentary canal. *In* Handbook of Physiology. Section 5: Alimentary Canal. C. F. Code, editor. American Physiological Society, Bethesda, MD. 2507–2533.
15. Laher, J. M., and J. A. Barrowman. 1983. Polycyclic hydrocarbon and polychlorinated biphenyl solubilization in aqueous solutions of mixed micelles. *Lipids.* **18:** 216–222.
16. Laher, J. M., G. A. Chernenko, and J. A. Barrowman. 1983. Studies of the absorption and enterohepatic circulation of 7,12-dimethylbenz(a)anthracene in the rat. *Can. J. Physiol. Pharmacol.* **61:** 1368–1373.
17. Janss, D. H., and R. C. Moon. 1970. Absorption of intragastrically administered 7,12-dimethylbenzanthracene. *Federation Proc.* **29:** 817 (Abstract).
18. Rees, E. D., P. Mandelstam, J. Q. Lowry, and H. Lipscomb. 1971. A study of the mechanism of intestinal absorption of benzo(a)pyrene. *Biochim. Biophys. Acta.* **225:** 96–107.
19. Kamp, J. D., and H-G. Neumann. 1975. Absorption of carcinogens into the thoracic duct lymph of the rat: aminostilbene derivatives and 3-methylcholanthrene. *Xenobiotica.* **5:** 717–725.
20. Grubbs, C. J., and R. C. Moon. 1973. Transport of orally administered 9,10-dimethyl-1,2-benzanthracene in the Sprague-Dawley rat. *Cancer Res.* **33:** 1785–1789.
21. Patton, J. S., B. Stone, C. Papa, R. Abramowitz, and S. H. Yalkowsky. 1984. Solubility of fatty acids and other hydrophobic molecules in liquid trioleoylglycerol. *J. Lipid Res.* **25:** 189–197.
22. Turner, S. G., and J. A. Barrowman. 1977. Intestinal lymph flow and lymphatic transport of protein during fat absorption. *Q. J. Exp. Physiol.* **62:** 175–180.
23. Ruckebusch, M., and J. Fioramonti. 1975. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. *Gastroenterology.* **68:** 1500–1508.
24. Harvey, H. R., and J. S. Patton. 1981. Solvent focusing for rapid and sensitive quantification of total lipids on chromarods. *Anal. Biochem.* **116:** 312–316.
25. Falk, H. L., and P. Kotin. 1969. Pesticide synergists and their metabolites: potential hazards. *Ann. N.Y. Acad. Sci.* **160:** 299–313.
26. Chipman, J. K., P. C. Hirom, G. S. Frost, and P. Millburn. 1981. The biliary excretion and enterohepatic circulation of benzo(a)pyrene and its metabolites in the rat. *Biochem. Pharmacol.* **30:** 937–944.
27. Mirvish, S. S., P. Ghadirian, L. Wallcave, C. Raha, S. Bronczyk, and J. P. Sams. 1981. Effect of diet on faecal excretion and gastrointestinal tract distribution of unmetabolized benzo(a)pyrene and 3-methylcholanthrene when these compounds are administered orally to hamsters. *Cancer Res.* **41:** 2289–2293.
28. Barrowman, J. A. 1978. Physiology of the Gastrointestinal Lymphatic System. Cambridge University Press, Cambridge.
29. Pocock, D. M-E., and A. Vost. 1974. DDT absorption and chylomicron transport in rat. *Lipids.* **9:** 374–381.
30. Wattenberg, L. W., J. L. Leong, and P. J. Strand. 1962. Benzopyrene hydroxylase activity in the gastrointestinal tract. *Cancer Res.* **22:** 1120–1125.
31. Porter, C. W., D. Dworacyzk, and H. L. Gurtoo. 1982. Biochemical localization of aryl hydrocarbon hydroxylase in the intestinal epithelium of the rat. *Cancer Res.* **42:** 1283–1285.
32. Aitio, A., H. Vainio, and O. Hänninen. 1972. Enhancement of drug oxidation and conjugation by carcinogens in different rat tissues. *FEBS Lett.* **24:** 237–240.
33. Hietanen, E. 1980. Oxidation and subsequent glucuronidation of 3,4-benzopyrene in everted intestinal sacs in control and 3-methylcholanthrene-pretreated rats. *Pharmacology.* **21:** 233–243.
34. Stohs, S. J., R. C. Grafstrom, M. D. Burke, and S. Orrenius. 1977. Benzo(a)pyrene metabolism by isolated rat intestinal epithelial cells. *Arch. Biochem. Biophys.* **179:** 71–80.
35. Bock, K. W., U. C. V. Clausbruch, and D. Winne. 1979. Absorption and metabolism of naphthalene and benzo(a)pyrene in the rat jejunum in situ. *Med. Biol.* **57:** 262–264.
36. Wollenberg, O., and V. Ullrich. 1980. The drug monooxygenase system in the small intestine. *In* Extrahepatic Metabolism of Drugs and Other Compounds. T. E. Gram, editor. SP Medical and Scientific Books, 267–276.
37. Friedman, H. I., and B. Nylund. 1980. Intestinal fat digestion, absorption and transport. A review. *Am. J. Clin. Nutr.* **33:** 1108–1139.
38. Sjöstrand, F. S., and B. Borgström. 1967. The lipid components of the smooth-surfaced membrane-bounded vesicles of the columnar cells of the rat intestinal epithelium during fat absorption. *J. Ultrastruct. Res.* **20:** 140–149.
39. Sabesin, S. M., and S. Frase. 1977. Electron microscopic studies of the assembly, intracellular transport and secretion of chylomicrons by rat intestine. *J. Lipid Res.* **18:** 496–511.