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IN VIVO MEAL MODEL FOR THE EVALUATION OF AGENTS WHICH AFFECT THE ABSORPTION OF TRIGLYCERIDE AND CHOLESTEROL*

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Abstract—A meal model in which rats were trained to consume within 2 hr a high fat meal containing glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol was compared to the corn oil bolus model. In the meal model, dietary triglyceride was absorbed from the small intestine faster during the first 6–8 hr and more completely than intubated corn oil, as determined by analysis of intestinal contents, serum radioactivity, serum triglycerides, adipose tissue and liver lipids. The effects of Cholestyramine, Colestipol and Pluronic L-101 (1% dietary admixes) on these variables were evaluated for 5 days in the meal model. Lipid absorption during a 72-hr period was reduced by all compounds. The per cent excretion of glycerol tri[1-^{14}C]oleate was increased significantly by Pluronic L-101 (10-fold), Cholestyramine (5.7-fold) and Colestipol (2.7-fold). The excretion of [^{3}H]cholesterol was enhanced significantly by Cholestyramine (1.6-fold) and Colestipol (1.3-fold). The following observations were made 4 hr after the initiation of the meal. Pluronic L-101 increased significantly the retention of glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol in stomach (380 and 375 per cent, respectively), and of glycerol tri[1-^{14}C]oleate in the small intestine (1100 per cent). The percent of intestinal lipid remaining as triglyceride from the intestinal lumen was increased significantly by Pluronic L-101 (160 per cent). Pluronic L-101 reduced significantly [^{14}C]lipid and [^{3}H]cholesterol in liver; Cholestyramine and Colestipol suppressed only [^{3}H]cholesterol. In adipose tissue, Pluronic L-101 treatment reduced significantly [^{14}C]lipid content only; Cholestyramine and Colestipol suppressed selectively [^{3}H]cholesterol. After 5 days of treatment, only Pluronic L-101 treatment resulted in significantly reduced serum triglycerides (32 per cent), cholesterol (21 per cent) and glucose (15 per cent). These data suggest that this *in vivo* meal-feeding model provides a physiological technique for evaluating agents affecting lipid absorption.

The study of digestion and absorption of dietary lipids in animals and of the effects of various agents in these processes involves both invasive and noninvasive techniques. A frequently employed invasive technique is the cannulation of the thoracic duct for measurement of lymph flow and content [1–6]. Other methods involve the removal of stomach and intestine [7] or the preparation of intestinal loops [8] for lipid recovery after intragastric administration of liquid lipid test meals. Although providing excellent data, the invasive methods do not lend themselves easily to the evaluation of more than a few effectors of lipid absorption in a limited number of animals.

The commonly used noninvasive absorption technique is fat balance which relies on accurate food intake measurements since lipid absorption is determined by the difference between dietary lipid intake and fecal excretion [9–12]. Frequently, radioactive liquid lipid test meals are administered intragastrically to control accurately the amount of lipid ingested followed by a period of *ad lib.* feeding [13]. However, the presentation of triglycerides as either emulsified or nonemulsified test meals has marked effects on absorption in the small intestine [1, 6]. This was apparent in a method of measuring lipid absorption which monitored the appearance of serum triglycerides after the administration of a radiolabeled olive or corn oil bolus [14, 15]. Problems of adequate emulsification of the lipid in the small intestine and the resultant delay in appearance of triglyceride in serum were major drawbacks to the use of this method [15].

The present report describes a 2-hr meal-feeding model in rats which provides a useful, physiological *in vivo* method for evaluating potential inhibitors of triglyceride and cholesterol absorption utilizing the fat balance technique with radiolabeled lipid tracers. The method allows the kinetics of metabolism of dietary lipid to be followed by analysis of animal tissues. This model was shown to be superior to the corn oil bolus model. To assess the usefulness of the meal-feeding model in screening for agents which affect the absorption of triglyceride and cholesterol, Pluronic L-101, Cholestipol and Cholestyramine, agents known to affect lipid absorption, were evaluated. Pluronic L-101 was shown to affect primarily triglyceride absorption, whereas Colestipol and Cholestyramine affected both cholesterol and trigylceride absorption.

MATERIALS AND METHODS

Animals and dietary treatment. Female rats (Charles River CD Strain, 180–200 g) were housed individually in wire-bottomed cages in a temperature- (22°) and light-regulated [12 hr light (6:00 a.m. to 6:00 p.m.) and 12 hr dark] room. Rats had free

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access to a commercial diet (Purina Rodent Chow, Ralston Purina Co., St. Louis, MO) and water.

Two weeks prior to experimentation, rats were fasted overnight and then trained to consume within 2 hr a 10% corn oil meal containing 0.01% cholesterol. Experiments in this laboratory have shown that this level of dietary cholesterol (0.01%) does not perturb the endogenous cholesterol pool. The 10% corn oil meal consisted of 60% glucose, 20% vitamin-free casein, 10% corn oil (Mazola), 5% salt mixture, 1% vitamin mixture and 4% cellulose [12]. Each rat received a food cup each morning at 8:00 a.m. The food cup was removed at 10:00 a.m. Within 10 days rats were able to consume sufficient amounts of this diet (approximately 12 g/day) within the allotted time to gain weight at nearly the normal rate.

Experimental 10% corn oil diets were prepared by mixing the radioactive lipid tracers and experimental drugs in the corn oil portion of the diet prior to mixing with the dry ingredients. The lipid tracers were added in the following amounts: glycerol tri[1-^{14}C]oleate, 0.2 μCi/g diet; and [^{3}H]cholesterol, 0.4 mCi/g diet. Samples of the diets were analyzed for radioactive content by extraction of lipid and scintillation counting, using the method described for tissue lipids. Experimental compounds were administered as a 1% dietary admix at the expense of the cellulose component.

Five-day 2-hr meal-feeding model. On the first day of the 5-day experimental period, control rats (ten per group) were fed the 2-hr meal containing radioactive tracers from a weighed food cup. A 2-hr meal-feeding regimen was chosen because it allowed a maximum amount of food to be consumed in the shortest period of time. Experimental rats (ten per group) were fed the radioactive diet which contained the experimental drugs as 1% dietary admixes. From day 2 to 4, rats were fed for 2 hr daily the non-radioactive 10% corn oil meal with the appropriate experimental drug. On day 5, rats were again fed a 2-hr radioactive diet containing drug. Each food cup was weighed before and after each meal and food consumptions were calculated. Spillages were collected daily and food consumptions were adjusted accordingly. The lipid and radioactivity ingested were calculated from food consumption. Feces were collected free of dietary contamination from day 1 until day 4 (72-hr period) and stored frozen.

Rats were decapitated 2 hr after the meal on day 5. Blood was collected on ice. Stomach, small intestine, liver and retroperitoneal adipose tissues were quickly removed and stored on ice. Contents of stomach and small intestine were collected by washing the tissues with saline. The pH of the washings was adjusted immediately to 2.0 with 5 N HCl to inactivate lipolytic enzymes. All tissues and washings were stored at −20°.

Corn oil bolus method. In a study comparing the absorption of dietary lipid administered as a 2-hr meal to the corn oil bolus model, rats (ten per group) were fasted overnight and then given a radioactive corn oil bolus (0.66 μCi glycerol tri[1-^{14}C]oleate/ml corn oil, 20 ml/kg) [15]. This corn oil dose was selected because it produced measurable elevations in serum triglycerides [14, 15]. The 2-hr meal-fed rats were treated as on the first day of the 5-day experiment described above. Rats that received corn oil were killed at 2-hr intervals after the first hour, while rats that were meal-fed were killed at 2-hr intervals beginning 1 hr after the end of the meal. Blood, liver, retroperitoneal adipose tissue, stomach and intestines were quickly dissected and chilled. Stomach and intestinal contents were collected as described above and all tissues were frozen at −20°.

Tissue analyses. Absorption of dietary lipid was determined by gravimetric analysis of fecal lipid based on the method of Miettinen *et al.* [16] as modified by Comai *et al.* [12]. After gravimetric determinations were performed, the radioactive lipids were determined in a Searle Analytic Mark III scintillation counter in a toluene based PPO–POPOP* scintillation mixture (LSC Complete, Yorktown Research, South Hackensack, NJ). Data are expressed as percent recovery of ingested [^{14}C]lipid and [^{3}H]cholesterol.

Serum, liver and adipose tissues were analyzed for lipids as described previously [15]. After lipid extraction of adipose and liver, aliquots were removed for the determination of radioactive lipids by scintillation counting using the procedure described above. Lipids were not isolated by class prior to scintillation counting.

Radiolabeled lipids were isolated from stomach and intestinal contents by extraction with hexane. The volumes of the contents were first adjusted to 30 ml with saline, and then extracted three times each with 10 ml hexane. The hexane layers were combined and concentrated under nitrogen. Aliquots were transferred to scintillation vials and radioactivity was determined as described for the fecal lipid. Additional aliquots of intestinal contents were removed for chromatographic separation of free fatty acids and triglycerides [17].

Materials. Glycerol tri[1-^{14}C]oleate (55 mCi/mmole) and [1α, 2α(n)-^{3}H]cholesterol (56 Ci/mmole) were purchased from Amersham, Arlington Heights, IL. The cholesterol and glucose test kits were from Abbott laboratories, Pasadena, CA; the triglyceride test kit was from CalBiochem, La Jolla, CA. Corn oil (Mazola) was purchased locally. Cholestyramine (pure resin) was provided by Mead Johnson, Evansville, IN, Colestipol by the Upjohn Co., Kalamazoo, MI, and Pluronic L-101 by BASF Wyandotte, Wyandotte, MI. All other chemicals were purchased from the Sigma Chemical Co., St. Louis, MO, and were of the highest quality available.

Statistical methods. All experiments were performed at least twice and data were processed for outliers [18]. A two-sided *t*-test was used to evaluate the experimental results [19].

RESULTS

Gastric emptying of dietary lipid. The effect of presenting dietary lipid as either a meal (2 hr, 10% corn oil) or an intragastric bolus of corn oil (20 ml/kg) on stomach emptying and intestinal content is shown

* PPO = 2,5-diphenyloxazole; and POPOP = 1,4-bis-2-(5-phenyloxazolyl)-benzene.

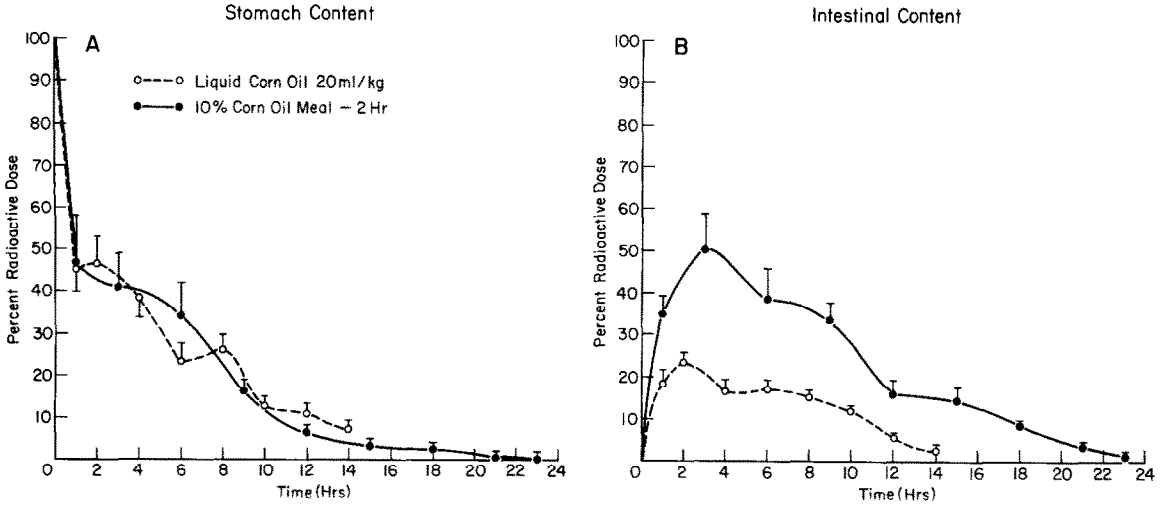

Fig. 1. Stomach and intestinal contents of rats receiving liquid corn oil boluses or high fat meals. The corn oil bolus group (20 ml/kg) received 2.6 μCi glycerol tri[1-^{14}C]oleate, and the high fat meal-fed rats consumed 12.9 g diet corresponding to 2.4 μCi glycerol tri[1-^{14}C]oleate. Rats were killed at 2-hr intervals. Zero time for the corn oil bolus was immediately following intubation. Zero time for the meal-fed rats was immediately following the 2-hr meal. Contents from stomachs and intestines were extracted and then analyzed for [^{14}C]lipid. Values are the means of each experimental group (ten rats per group). The bar represents one standard error. Key: panel A, stomach contents; panel B, intestinal contents; liquid corn oil bolus (O– – –O); and 10% corn oil meal (●——●).

in Fig. 1. There were no significant differences in the rate of stomach emptying at any time point when lipid was administered either as a corn oil bolus or in a meal. Within 1 hr after administration of the liquid corn oil and 1 hr after the completion of the 2-hr meal, more than 50 per cent of the lipid in the stomach had emptied, followed by a slower emptying rate over the following 12-hr period (Fig. 1, panel A).

The recovery of lipid from the small intestinal contents showed significant differences in intestinal processing of the meal lipid and the corn oil bolus lipid (Fig. 1, panel B). A significantly greater percentage of the radioactive lipid was recovered from rats fed the 2-hr meal. Unlike the intestinal contents from the meal-fed rats, the intestinal contents from rats receiving the corn oil bolus were poorly emulsified at early time points (1–4 hr), and unemulsified corn oil was found in the large intestine. At the later time points (10 and 12 hr), some anal leakage of corn oil due to improper emulsification was observed in agreement with earlier observations and made fat analysis of fecal matter impossible [2, 15].

Appearance of lipid in serum. Serum lipid radioactivity and serum triglycerides were monitored (Fig. 2). Their time courses during the experiments were very different in the 2-hr meal-fed rats compared with rats given the corn oil bolus. The lipid radioactivity in the serum paralleled the serum triglyceride profile for the liquid corn oil-fed rats. In the 2-hr meal-fed rats, serum lipid radioactivity increased rapidly within 3 hr, and then plateaued at approximately 1 per cent of the radioactive dose (Fig. 2, panel A). In rats receiving the corn oil bolus, radioactivity increased more slowly, reached a peak value at 10 hr, and then rapidly declined (Fig. 2, panel A). Serum triglycerides of the meal-fed rats increased almost 2-fold following the meal, and then gradually diminished to nearly normal values (Fig. 2, panel B). The serum triglycerides of rats receiving the corn oil bolus exhibited a markedly different profile (Fig. 2, panel B). Serum triglycerides gradually increased over 6–8 hr after the administration of corn oil. The peak value (400 mg/100 ml) for serum triglycerides was reached at the 10 hr time point, a value 8-fold higher than the fasting value.

Appearance of radiolabeled lipid in liver and adipose tissue. The lipid from the 2-hr meal-fed rats appeared quickly in liver, followed a smooth curve reaching a maximum at 9 hr (2 per cent of dose), and then gradually declined as metabolism occurred (Fig. 3, panel A). In rats receiving the corn oil bolus, the appearance of radiolabeled lipid in liver was delayed. At approximately the 8 hr time point, the percentage of dose recovered in livers of rats receiving the corn oil bolus was equal to that of rats receiving the 2-hr meal. However, in contrast to the meal-fed rats, after the 8 hr time point livers from rats receiving the corn oil bolus continued to accumulate radiolabeled lipid.

The profile of radiolabeled lipid accumulation in adipose tissue was different from that observed in liver in that lipid accumulation continued after 12 hr in the 10% corn oil meal-fed rats and appeared to be increasing after 14 hr in the liquid corn oil rats (Fig. 3, panel B). Although the rate of entry of radioactive lipid into the retroperitoneal adipose tissue of rats receiving the 2-hr meal appeared to be 6-fold greater than rats receiving the corn oil bolus, when corrected for specific activity these differences were less marked.

Pluronic L-101, Cholestyramine and Colestipol evaluated in the 5-day 2-hr meal-feeding model. When administered as 1% dietary admixes, the three compounds produced significant reductions in dietary lipid absorption (Table 1). Control rats absorbed

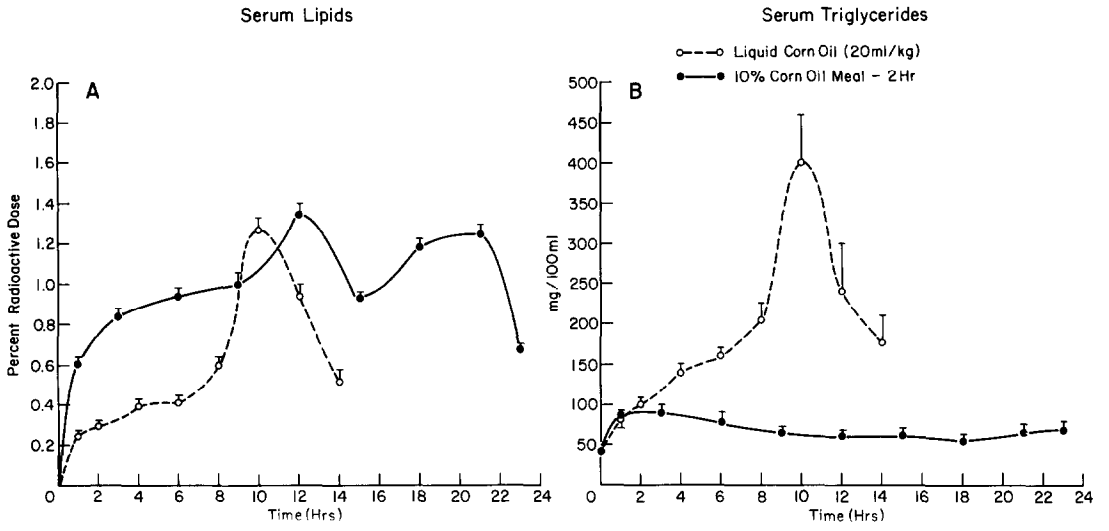

Fig. 2. Serum lipids and triglycerides from rats receiving liquid corn oil or high fat meal. Rats were those from Fig. 1. Values are the means of each experimental group (ten rats per group). The bar represents one standard error. Panel A: serum radioactivity. Serum radioactivity was determined by scintillation counting and expressed as a percentage of the dose administered. Panel B: serum triglycerides. Serum triglycerides were determined by an enzymic method [15]. Key: liquid corn oil bolus (O- - -O); and 10% corn oil meal (●——●).

96.5 ± 0.5 per cent of the dietary fatty acid when determined by the fat balance technique, while rats treated with Pluronic L-101, Colestipol and Cholestyramine absorbed 91.7 ± 0.8, 94.7 ± 1.1 and 92.4 ± 0.8 per cent of the dietary fatty acids, respectively. The percentage of the glycerol tri[1-^{14}C]oleate recovered in the feces of control rats was very small (1 per cent of the dose) indicating almost complete absorption (99 per cent) of the glycerol tri[1-^{14}C]oleate, in agreement with the nonradioactive fat balance technique. Colestipol produced a 2.7-fold increase in [^{14}C]lipid excretion, and Cholestyramine produced a 5.7-fold increase; both were significant. The greatest effect on [^{14}C]lipid excretion was produced by Pluronic L-101 (a 10-fold increase over control).

Increases in [^{3}H]cholesterol excretion were also observed (Table 1). Control rats absorbed approximately 72 per cent of the [^{3}H]cholesterol administered. As expected, rats treated with Colestipol or Cholestyramine had significantly reduced [^{3}H]cholesterol absorptions, 63.2 ± 3.9 and 54.8 ±

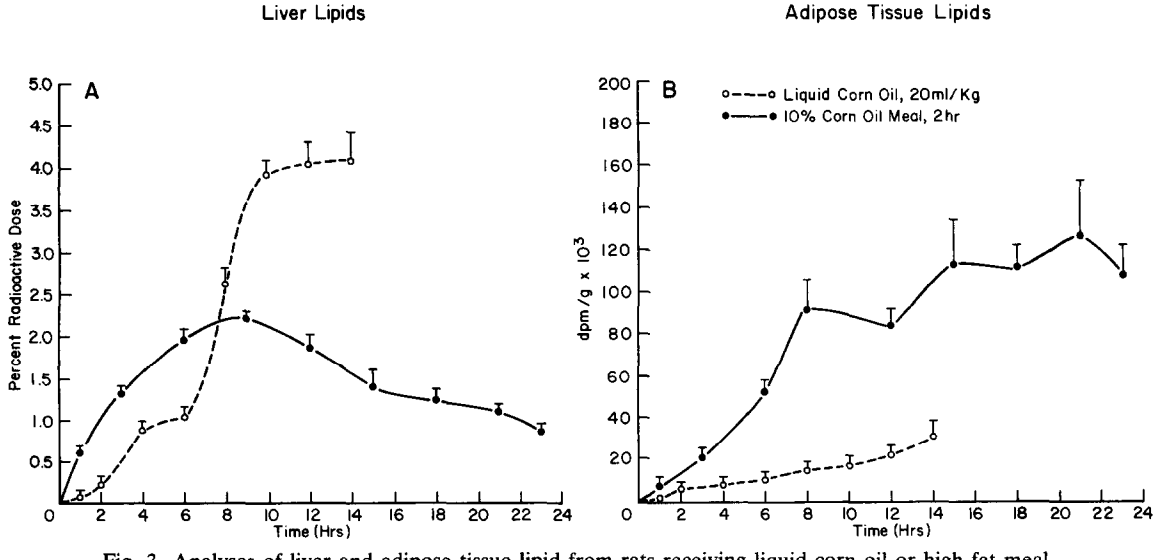

Fig. 3. Analyses of liver and adipose tissue lipid from rats receiving liquid corn oil or high fat meal. Rats were those from Fig. 1. [^{14}C]Lipids were isolated after saponification, and radioactivity was determined by scintillation counting. Values are the means of each experimental group (ten rats per group). The bar represents one standard error. Panel A: liver lipid. Data are reported as the percentage of the administered radioactive dose. Panel B: adipose tissue. Data are expressed as d.p.m. × 10^3 per g tissue. Key: liquid corn oil bolus (O- - -O); and 10% corn oil meal (●——●).

Table 1. Dietary lipid absorption in the 5-day meal model

Treatment*	Dose (mg/kg)	Dietary fatty acid absorption† (%)	[^{14}C]Fatty acid absorption‡ (%)	[^{3}H]Cholesterol absorption§ (%)
Control		96.5 ± 0.5	99.0 ± 0.2	72.1 ± 1.7
Pluronic L-101	610	91.7 ± 0.8‖	89.9 ± 0.8‖	67.7 ± 1.9
Colestipol	650	94.7 ± 1.1	97.3 ± 0.5‖	63.2 ± 3.9‖
Cholestyramine	660	92.4 ± 0.8‖	94.3 ± 1.5‖	54.8 ± 2.9‖

* Rats (ten per group) trained to consume a 10% corn oil meal within 2 hr were fed diets containing either Pluronic L-101, Cholestipol or Cholestyramine during a 5-day period. On day 1, glycerol tri[1-^{14}C]oleate (0.2 μCi/g diet) and [^{3}H]cholesterol (0.4 mCi/g diet) were contained in the diets. Feces were collected for 72 hr. Dietary fat absorption was determined gravimetrically [12, 16]. Radioactive lipids were determined by scintillation counting.

† Determined by the fat balance technique. Values are means ± S.E.

‡ Determined by fecal elimination of [1-^{14}C]fatty acid. Values are means ± S.E.

§ Determined by fecal elimination of [^{3}H]cholesterol. Values are means ± S.E.

‖ Significantly different from control, $P \leq 0.05$.

2.9 per cent, respectively. Pluronic L-101 did not affect the absorption of [^{3}H]cholesterol.

Stomach contents of rats treated with Cholestyramine, Colestipol and Pluronic L-101 were analyzed for glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol 2 hr after the completion of the meal (Fig. 4, panel A). Pluronic L-101-treated rats retained a significantly greater amount (350 per cent) of glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol in their stomachs than control rats or rats treated with either Cholestyramine or Colestipol. Cholestyramine-treated rats retained a slightly greater amount (150 per cent) of glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol in their stomachs when compared with controls. Colestipol appeared to have enhanced stomach emptying. There were no significant changes in food consumption when Pluronic L-101, Cholestyramine or Colestipol was added to the diets. All groups consumed an average of 11.8 ± 0.2 g of diet.

The observation that Pluronic L-101 significantly retarded stomach emptying of the meal lipid led to the analysis of lipid in small intestinal contents (Fig. 4, panel B). There was a significant increase in the [^{14}C]lipid retention (11-fold over control) in the small intestinal contents of rats treated with Pluronic L-101. The content of [^{3}H]cholesterol was also increased significantly (4.5-fold) over control. Cholestyramine produced smaller but not significant increases in both [^{14}C]lipid and [^{3}H]cholesterol retention in small intestine (2.8-fold over control). Colestipol appeared to be without effect on intenstinal contents lipid.

The free fatty acids and triglycerides of the [^{14}C]lipid intestinal contents were separated by thin-

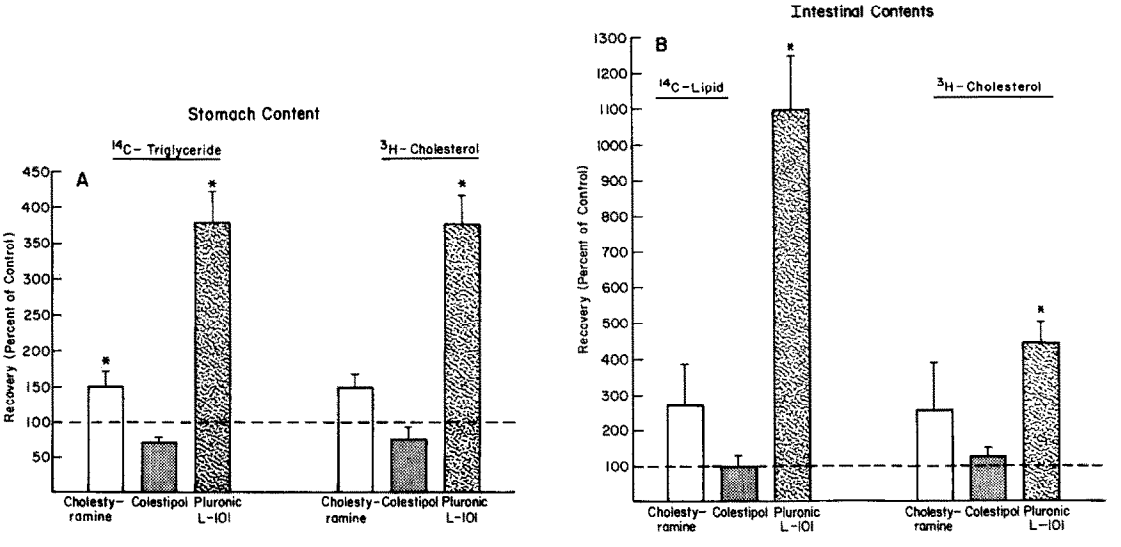

Fig. 4. Stomach and intestinal lipid recovery from rats consuming diets containing Pluronic L-101, Cholestyramine or Colestipol. Glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol were isolated from stomach and intestinal contents of rats killed 2 hr after the meal. Values are the means of the experimental groups expressed as per cent of control. The bar represents one standard error. Significance ($P \leq 0.05$) is indicated by an asterisk. Panel A: stomach content. Recovery of lipid in control rats was 11.5 ± 1.2 per cent of the administered dose of both glycerol tri[1-^{14}C]oleate (2.1 μCi) and [^{3}H]cholesterol (4.2 mCi). Panel B: intestinal content. Recovery of [^{14}C]lipid in control rats was 0.42 ± 0.06 per cent of the dose and that of [^{3}H]cholesterol, 0.97 ± 0.10 per cent of the dose.

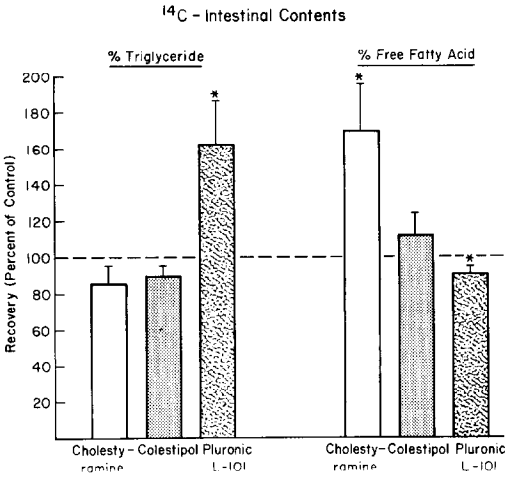

Fig. 5. Chromatographic separation of intestinal content of triglyceride and free fatty acid. After acidification and extraction with hexane, lipids were separated on silica gel impregnated paper and developed in isooctane–acetic acid (500:1). The percentages of free fatty acid and triglycerides were calculated from the total radioactivity. Control samples contained 12.0 ± 1.0 per cent triglyceride and 57.1 ± 1.1 per cent free fatty acid. Cholestyramine samples contained 10.2 ± 1.4 per cent triglyceride and 90.0 ± 1.3 per cent free fatty acid. Colestipol samples contained 10.8 ± 1.4 per cent triglyceride and 66.7 ± 1.7 per cent free fatty acid. Pluronic L-101 samples contained 19.8 ± 2.8 per cent triglyceride and 52.2 ± 2.2 per cent free fatty acid. Data are expressed as per cent of control. The bar represents one standard error. Significance ($P \leq 0.05$) is indicated by an asterisk.

layer chromatography. In control samples, more than 50 per cent of the [^{14}C]lipid appeared as free fatty acids. In animals treated with Pluronic L-101 a significantly greater amount of [^{14}C]lipid appeared as triglycerides (160 per cent of control) (Fig. 5). At the same time, the fatty acid present was reduced significantly, suggesting an inhibition of the pancreatic lipase. Cholestyramine, which produced a modest increase in accumulation of intestinal [^{14}C]lipids, did not appear to have an inhibitory effect on hydrolysis of intestinal glycerol tri[1-^{14}C]oleate; the [^{14}C]free fatty acid content, however, was increased significantly (1.6-fold) over control. Colestipol, which produced no changes in total [^{14}C]lipid of intestinal contents, showed no variation from control in the ratio of free fatty acids to triglycerides.

Liver lipids from rats treated with Pluronic L-101 exhibited significant decreases in [^{14}C]lipid (40 per cent) and [^{3}H]cholesterol (28 per cent) levels as compared to control rats (Fig. 6, panel A). Livers from rats treated with either Cholestyramine or Colestipol showed no changes in [^{14}C]lipid content, but significant decreases in [^{3}H]cholesterol content: 47 per cent reduction for Cholestyramine and 40 per cent reduction for Colestipol (Fig. 6, panel A).

As expected from the significant decrease in dietary fat absorption produced by Pluronic L-101, there was a significant reduction (30 per cent) in the accumulation of [^{14}C]lipid in adipose tissue (Fig. 6, panel B). There was no change in the [^{3}H]cholesterol content of adipose tissue from Pluronic L-101-treated rats. The reverse situation was observed in adipose tissue from rats treated with either Cholestyramine or Colestipol. No significant changes were found in the [^{14}C]lipid content of adipose tissue. However, similar and significant reductions were found in the [^{3}H]cholesterol content of rats receiving Cholestyramine (40 per cent) and Colestipol (38 per cent).

Sera from control rats and the three treatment groups were analyzed for triglyceride, cholesterol and glucose (Fig. 7). Only Pluronic L-101 produced significant decreases in all three parameters after 5 days of administration. Serum triglycerides were reduced by 35 per cent, serum cholesterol by 20 per cent and glucose by 10 per cent. Cholestyramine produced an increase in serum triglycerides (25 per cent) which was not significant. A similar increase in cholesterol (24 per cent) was significant. Serum glucose remained unaffected. Colestipol produced no significant changes in these three serum parameters.

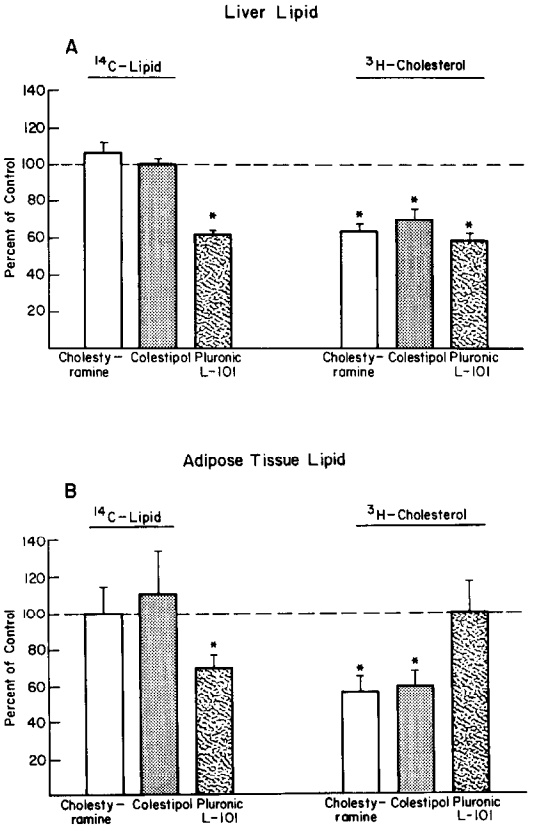

Fig. 6. Liver and adipose tissue lipids from rats receiving Cholestyramine, Colestipol and Pluronic L-101. [^{14}C]Lipid and [^{3}H]cholesterol were determined after tissue saponification. Results are expressed as per cent of control. The bar represents one standard error. Significance ($P \leq 0.05$) is indicated by an asterisk. Panel A: liver lipids. Control livers contained 0.75 ± 0.03 per cent of the ^{14}C-dose and 2.1 ± 0.1 per cent of the [^{3}H]cholesterol dose. Panel B: adipose tissue lipid. Control adipose tissue contained 82.8 ± 10.8 × 10^3 d.p.m. [^{14}C]lipid/g and 12.3 ± 1.4 × 10^4 d.p.m. [^{3}H]cholesterol/g.

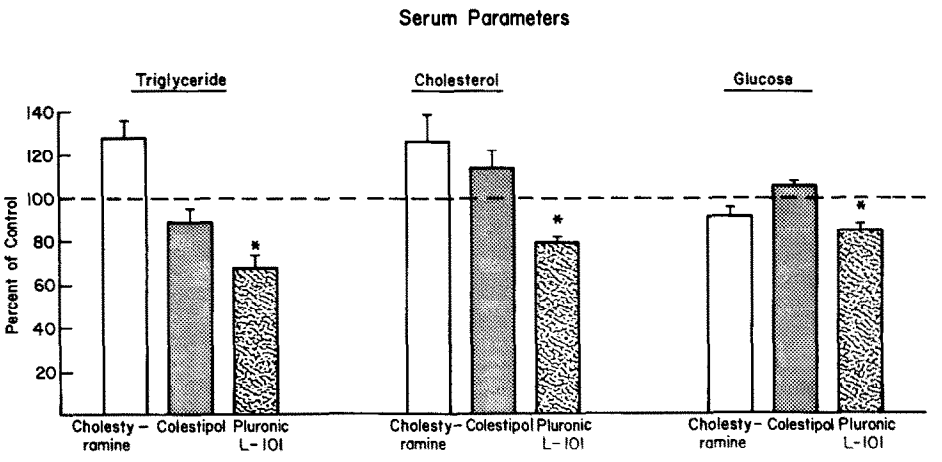

Fig. 7. Analyses of sera from rats treated with Cholestyramine, Colestipol and Pluronic L-101. The results are expressed as per cent of control. The bar represents one standard error. Significance ($P \leq 0.05$) is indicated by an asterisk. The control rat serum triglyceride value was 114 ± 10 mg/100 ml; serum cholesterol, 83 ± 5 mg/100 ml; and serum glucose, 172 ± 8 mg/100 ml.

DISCUSSION

When compared with the corn oil bolus method, the 2-hr meal-feeding model appears to be a more physiological method for evaluating agents which affect lipid absorption. Although the amounts of corn oil administered as either the bolus or the 10% corn oil meal differed, the shapes of the stomach emptying curves were nearly identical, indicating that the greater amount of corn oil in the stomachs of the rats given the corn oil bolus did not delay stomach emptying. One hour after the intragastric administration of corn oil about 55 per cent of the corn oil had emptied from the stomachs while 1 hr after the completion of the 2-hr meal, also, 55 per cent of the consumed corn oil had emptied from the stomachs. It is possible that other water-soluble nutrients in the 10% corn oil meal prevented the immediate discharge of corn oil into the duodenum. The percentage of the radioactive dose in the small intestines of rats receiving the corn oil bolus was smaller than in rats receiving the 10% corn oil meal. This difference may be due to the loss of corn oil through anal leakage or to the enhanced absorption of the liquid corn oil. The latter is unlikely since the increase in lipids in serum liver and adipose tissue was greater during the first 6–8 hr in the meal-fed rats compared to the rats receiving the liquid corn oil bolus. Others have suggested that the poor or delayed absorption of oily mixtures may be due to reduced lipolysis in the duodenum because of inadequate emulsification [2].

The appearance of radiolabeled lipid in serum followed the same time course as the appearance of serum triglycerides in circulation (Fig. 2) when the lipid was administered as liquid corn oil. The differences in the appearance of radiolabeled lipid and triglyceride in serum of rats receiving the 10% corn oil meal may be due to the recycling of the label into other lipids at longer durations. This observation was reported earlier by Stanley and Thannhauser [20] and was used as an alternative to chemical or gravimetric measurement of lipid assimilation. Unfortunately, the correlation of the serum level of radioactive lipid to the excretion of dietary fat was very poor, indicating that the fat balance technique (gravimetric analysis) remained a better screening method for demonstrating absorption [21]. Because the 2-hr meal-feeding model utilizes both glycerol tri[1-^{14}C]oleate and [^{3}H]cholesterol, potential errors in gravimetric analysis of fecal fat due to measurement of unsaponified lipid and carry-over of non-absorbable drugs can be avoided. The 2-hr meal-feeding method is attractive because the large number of animals needed for statistical analyses can be managed easily. Additionally, potential effectors of lipid absorption can be administered conveniently as dietary admixes, thus assuring the presence of the effector during the absorption process.

Two of the compounds evaluated in the 2-hr meal-feeding method were Cholestyramine and Colestipol, hypocholesteremic agents which act by sequestration of lumenal bile salts, leading to enhanced hepatic bile salt synthesis from cholesterol [11, 22, 23]. The significant decrease of dietary cholesterol absorption caused by both compounds stems from the fact that for absorption cholesterol must be in a micellar solution composed of monoglyceride, free fatty acids and bile salts [24]. Removal of bile salts from the lumen by Cholestyramine or Colestipol would markedly disrupt micelle formation and lead to the significant reduction in dietary cholesterol absorption and the milder but significant reduction in triglyceride absorption observed in the present study (Table 1) [23]. While decreasing lipid absorption, these agents appeared to have little effect on stomach emptying or intestinal transit of meal lipid at the 2 hr post-meal time. The decrease in fat absorption produced by Cholestyramine was greater than that produced by Colestipol at equivalent doses (Table 1). This can be traced to the greater percentage of free fatty acids in the intestinal contents of rats treated with Cholestyramine (Fig. 5). Since both drugs are bile salt sequestering resins, the chemical nature of Cholestyramine may have a higher affinity for fatty acids or exert an effect on micelle

structures or the intestinal epithelium to prevent the absorption of free fatty acids.

The third agent evaluated in the 2-hr meal-feeding model was Pluronic L-101. The pluronic compounds are nonabsorbable polymers composed of polyoxyethylene (hydrophilic) units and polyoxypropylene (hydrophobic) units whose physical properties depend on the percentages of hydrophilic and hydrophobic units present in the molecule [25]. Pluronic L-101 has the following properties: 10% hydrophilic units and 90% hydrophobic units, good wetting characteristics, nonsoluble in water and a molecular weight of 3600.

Various pluronics have been described in biological systems as inhibitors of fat emboli [26], as plasma expanders [27] and as inhibitors of lipoprotein lipase [28]. Recently, several pluronics (L-31, L-61, L-81 and L-121) were shown to reduce significantly but nonselectively the absorption of triglycerides and cholesterol, and to reduce the serum cholesterol level of rats made hypercholesteremic by dietary excess of cholesterol [13]. In the study reported here, Pluronic L-101 reduced significantly the absorption of triglyceride (10-fold over control) while affecting cholesterol absorption only slightly (Table 1).

The mechanism of reduction of triglyceride absorption by Pluronic L-101 appeared not to involve bile salt sequestering. The intestinal contents of rats treated with Pluronic L-101 contained a higher percentage of triglycerides and a lower percentage of free fatty acids than controls, indicating an inhibition of pancreatic lipase (Fig. 4, panel B). In other studies to be reported, the inhibitory effect of Pluronic L-101 on pancreatic lipase has been confirmed, and significant body weight reductions in rats due to enhanced fecal fat elimination have been observed. Another action of Pluronic L-101 may be a delay in gastric emptying, as indicated in Fig. 4, panel A. This delay could account for the significant decreases in serum cholesterol and glucose seen in rats treated with Pluronic L-101 (Fig. 7).

The 2-hr meal-feeding model provides a useful, physiological *in vivo* method for evaluating potential inhibitors of triglyceride and cholesterol absorption, utilizing the fat balance technique with radiolabeled lipid tracers. The method allows the kinetics of metabolism of dietary lipid to be followed by analysis of animal tissues.

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