TABLE III. Transferrin Distribution and Turnover in 3 Iron-Loaded (1-3) and 3 Control Rabbits (4-6).

Rabbit	Plasma vol (ml/kg)	Total body transferrin (mg/kg)	Transferrin extravascular: intravascular ratio	Fractional transferrin turnover (plasma pools/day)	Absolute transferrin turnover (mg/kg/day)
Iron-loaded					
1	32.9	148	1.34	.35	22.0
2	26.0	137	1.62	.40	20.3
3	27.9	120	1.43	.34	16.9
Normal					
4	24.9	198	1.60	.36	27.6
5	28.1	188	1.49	.35	26.2
6	26.2	183	1.68	.36	24.3

protein. The actual cells involved have not been established.

Summary. Iron overload was produced in 3 rabbits by intramuscular injection of 1,300 mg iron as iron-dextran, while 3 control rabbits received no treatment. Four years later, plasma iron and transferrin turnover and distribution in the main abdominal organs were studied using plasma-bound radioiron and ^{131}I-labeled rabbit transferrin. The iron-labeled rabbits when compared with the controls had increased non-heme iron concentrations in liver, spleen, kidney, stomach and intestine; most of the excess iron was in the livers which showed only slight increases of connective tissue around the portal tracts where most of the stainable iron was deposited. Iron-loading was associated with decreased plasma transferrin concentration and total body transferrin and decreased absolute turnover of transferrin. The distribution of transferrin between extravascular and intravascular spaces was the same in both groups of rabbits. Hence the decreased plasma transferrin level in the iron-loaded animals was probably due to lowered rates of synthesis.

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Absorption of 14C Triolein in the Bile Fistula Rat. (31061)

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Previous *in vitro* experiments(1) demonstrated that conjugated bile salts facilitate the esterification of fatty acids by rat small intestinal mucosa. Furthermore, analyses of thoracic duct lymph and portal blood *in vivo* (2,3) suggested that there was a decreased esterification of absorbed fatty acids by the small bowel mucosa of bile fistula rats. This possibly accounted for the portal transport

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of the long chain fatty acids from the small bowel in these animals(2,3).

The effect of bile deprivation on mucosal esterification of absorbed long chain fatty acid has now been further examined by feeding 14C triolein to rats either with a bile fistula or after a sham operation and estimating the incorporation of radioactivity into the free fatty acids and glyceride fractions in the small bowel mucosa. In addition we have observed the effect of bile deprivation on the hydrolysis of triglycerides.

Methods. Glyceride-1-14C triolein (Radio-Chemical Centre, Amersham, Bucks) was diluted with pure triolein (Hormel Institute). Chemical and radioactive purity was demonstrated by thin layer chromatography while hydrolysis by pancreatic lipase confirmed that the fatty acids in the 1 and 2 position were equally labelled. All radioactive materials were counted in a Nuclear Chicago liquid scintillation spectrometer and quenching was assessed by the Channels ratio method (4).

Thirteen male albino rats weighing 200-300 g were used. Seven had a sham operation and in 6 a bile fistula was fashioned by cannulating the common bile duct immediately after its bifurcation with a Portex 10 cannula. The cannula drained into a glass saddle(5) in the unrestrained rats which were allowed to drink 0.9% saline containing 0.04% KCl and 5% glucose. The amount of bile which drained varied between 8 and 15 ml in 12 hours. Rats draining less than 8 ml were discarded. The day after the operation each rat was given 0.2 ml (1 μC) of triolein by gastric intubation under light ether anaesthesia. Four hours later, they were killed with ether. The total lipid of the stomach and colon was extracted separately after digestion and saponification in 25 ml of 30% KOH and 25 ml methanol. The small gut was cut into 3 equal parts. Each tract was washed out with 20 ml of diethyl-ether and immediately dried by adding sodium sulphate. Previous work(6), showing that under such conditions hydrolysis of glyceride was immediately inhibited, was confirmed and histological sections of the mucosa showed it to be undamaged. The ether extract was filtered, dried and taken up in light petroleum. The small gut segments were homogenized in chloroform-methanol (2.1), and filtered 2-3 hours later. The chloroform phase, separated by adding water, was evaporated under nitrogen and the dried lipids redissolved in light petroleum. The lipid extracted from the small bowel lumen and the small bowel mucosa was separated on thin plates of silica gel. The spot identified after exposure to iodine vapor was scraped into 10 ml Bray's solution before assaying the radioactivity.

TABLE I. Distribution of Radioactivity (Mean %) in Small Gut Segments.

	Contents		Mucosa	
Segment	Normal	Bile fistula	Normal	Bile fistula
1	6.7	3.8	13	8
2	16.3	18.5	39	27
3	77	78	47	59

From these experiments one may assess the amount of triolein absorbed, its distribution in the small gut, the degree of luminal hydrolysis and the proportion of glyceride resynthesized in the mucosa. The significance of the difference of the mean was assessed by the t test.

Results. Absorption has been expressed as a percentage of the dose which has left the stomach, because this varied from animal to animal. The mean absorption in normal rats was 81% and in the bile fistula rats 37%.

The distribution of radioactivity in different parts of the gut (Table I) did not differ between normal and bile fistula rats either with respect to intestinal contents or the mucosa. In both groups most of the luminal radioactivity was recovered in the distal third of the small gut (Table I), although the scatter was wide. The distribution of mucosal radioactivity was also similar in both groups and was present predominantly in the 2nd and 3rd segment (Table I).

There was a significantly greater amount of triglyceride and less FFA and monoglyceride in the contents of the distal third of the small gut (Table II); the mean values reflected this trend in other segments but the differences were not significant.

In the first small bowel segment of the

TABLE II. Radioactivity in FFA and Glyceride Fraction in Lumen of 3 Small Gut Segments (Mean %).

	1		2		3	
Segment	N	BF	N	BF	N	BF
FFA	31.6	17	35	23.3	70	27*
MG	9.6	6.8	5.6	7.9	11.9	3.7*
DG	6.7	10.8	3.3	17.5*	9.7	19.1
TG	46.7	64.3	58.6	50.6	7.4	36.7*

* $P \leq .01$ (BF *vs* N).
N = normal. BF = bile fistula.

sham operated animals there was a significantly greater incorporation of label into the mucosal (monoglyceride + phospholipid) fraction (28.6%) as compared with the second and third segment (12.8 & 9.2%). Separation on silicic acid columns showed that most of the radioactivity of this fraction was in fact phospholipid. Less radioactivity was incorporated into the mucosal triglyceride of the 2nd and 3rd segments of the bile fistula as compared with the controls, and in all segments a significantly greater proportion of the radioactivity was present in the mucosal FFA and diglyceride fraction in the bile fistula animals.

Discussion. The relatively good absorption of fat in the absence of bile has been noted previously(7,8) and demonstrates that bile is not obligatory for triglyceride absorption although it facilitates it. Although traditionally bile is thought to be important in triglyceride lipolysis no difference was noted in the absorption of free acid and triglyceride in bile fistula animals(7,8,9) and no difference in the degree of luminal hydrolysis of triglyceride was found between normal and bile fistula dogs(9). This discrepancy with our work may not be due to species variation but to technical differences in extracting the luminal lipids; indeed we found that unless immediate ether washing of the lumen was used the difference between the normal and bile fistula animals was masked as lipolysis continued if, *e.g.*, the lumen was washed with ice cold saline. Whether these differences in lipolysis are of physiological significance is difficult to assess. The reason why most of the significant differences were found in the third segment of small bowel is related to the large amount of lipid which stagnates there.

The mucosal lipid analyses suggest that normally there are differences in the way absorbed fat is dealt with in different segments of the gut. The high proportion of radioactivity in the phospholipid of the first segment may partially account for the surprising recent observation that within 1 hour of feeding stearic acid 30% of the mucosal radioactivity was in the phospholipid fraction(10). Under the conditions of these experiments the bolus of lipid may have only reached the proximal small bowel. On the other hand the greater proportion of FFA in the distal segment of the normal animals may accound for the greater proportion of dietary fat in lymph FFA late (8 hours) after feeding when presumably the ileum is the main site of fat absorption.

The decreased incorporation of radioactivity into mucosal triglyceride and greater radioactivity in the FFA and diglyceride support the suggestion that there is a defect in mucosal esterification of long chain fatty acids in the absence of bile. Recently Kerns and Borgstrom(11) reported similar results. They analyzed the proximal small bowel mucosa in bile fistula and normal rats after feeding free oleic acid; but their results might be explained by a different adherence of luminal fat to the mucosa in the bile fistula rats. This would be an unlikely explanation for our results for in our experiments the luminal fat contained a greater proportion of triglyceride in the bile fistula animals than in the sham operated animals.

The possible reason for this effect of bile deficiency on mucosal lipid metabolism is obscure, especially as it is thought that bile salts are mainly absorbed in the ileum and do not enter the jejunal mucosal cell(12).

TABLE III. Radioactivity in FFA and Glycerides in Mucosa of 3 Small Gut Segments (Mean %).

	1		2		3	
Segment	N	BF	N	BF	N	BF
FFA	8.7	19.1*	4.5	24.2*	15.3	31.7
Mg + PL	28.6	11.1	12.8	12.3	9.2	13.2
Dg	7.1	15.1*	5.2	15.0*	6.3	11.2*
Tg	53.5	50.1	76.2	44.5*	68.0*	42.5

* $P < .05$ (N *vs* BF).
N = normal. BF = bile fistula.

Summary. Glyceride triolein-1-14C was fed to bile fistula and sham operated rats which were killed after 4 hours. In the bile fistula animals there was a diminished absorption and decreased lipolysis of the triolein. Analysis of small gut mucosal lipid showed a greater proportion of radioactivity in the FFA and lower glycerides of the bile fistula animals than of the controls. This supports the concept that bile facilitates mucosal esterification of absorbed long chain fat.

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Alteration of Hepatic Protein Synthesis in Acute Uremia. (31062)

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Muscle wasting and protein undernutrition frequently appear as complications in patients with uremia. These disturbances may be due in part to the nutritional deficiency of the diets usually prescribed to reduce the blood urea nitrogen level (BUN). However, some evidence suggests that azotemic patients may not metabolize protein normally. Thus, in nitrogen balance studies(1,2), uremic individuals appear to require higher than normal protein intakes to maintain nitrogen balance. Impaired absorption of dietary nitrogen cannot explain the negative balance since it usually is accompanied by increased urinary nitrogen excretion. These findings could indicate either reduced utilization of nitrogen for protein synthesis or accelerated protein breakdown.

Recently developed techniques with cell-free systems(3) permit controlled study of protein synthesis *in vitro*. The present investigation using such a system was undertaken to examine the effect of uremia on protein synthesis. Results indicate that incorporation of leucine into protein by cell-free liver preparations is altered in uremic rats.

Materials and methods. Male rats of the Walter Reed strain, weighing 200 to 250 g, were used as experimental animals. They were separated into 3 experimental groups: normal fasting controls, normal non-fasting controls, and uremic fasting test animals.

The rats were maintained on Purina chow and tap water *ad libitum* preceding the experimental period. All animals in each group were anesthetized with 15 mg sodium pentobarbital given intraperitoneally. Uremia was induced in the test group by ligation of the penis. The animals were placed in individual cages and the normal fasting control and the uremic fasting test groups were given water but no food. The non-fasting controls were allowed both food and water *ad libitum*.

Approximately 48 hours after anesthesia the rats were sacrificed by decapitation. Blood was drained into beakers containing ethylenediaminetetracetate as anticoagulant and aliquots were taken and saved for subsequent