lowing deprivation of food, decreases at 3 days and increases again between 7 and 11 days of starvation. In fed rabbits, intraperitoneal injections of 1.0 g l-tryptophan/kilogram body weight induces LTP activities of 10 μmoles/g/hour but in rabbits previously starved for 36 hours or 7 days, a similar injection induces twice as much LTP as in fed animals. Liver tryptophan pyrrolase activity of starved rabbits returns to normal values within 2 days following refeeding.

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Effect of Tween 80 on Cholestyramine-Induced Malabsorption.* (29542)

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Cholestyramine, a quaternary ammonium anion exchange resin with a styrene-divinylbenzene skeleton, has been shown to be an effective bile acid sequestering agent. Administered by mouth, it forms a nonabsorbable complex with bile acids in the intestinal tract (1,2). In human subjects this interruption of the enterohepatic cycle of bile acids results in a lowering of serum total cholesterol(3), and at higher dose levels in significant steatorrhea(4). In view of the intestinal intraluminal detergent role of bile salts in the emulsification of lipids, it seemed of interest to study the effects of ingestion of a surface active agent on the steatorrhea induced in man by large doses of cholestyramine.

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Polyoxyethelyene sorbitan monooleate (Tween 80), a partial ester of a fatty acid, is a nonionic surface active agent. It has been shown to be non-toxic in man(5). It has no effect on the motility of the small intestine(6), or on the serum total cholesterol (7). It does not affect fat absorption in normal adults(8), and in bile fistula dogs(9). Administration of Tween 80 has been shown to increase fat absorption in patients with steatorrhea secondary to sprue, regional ileitis, and gastrojejunostomy(10).

Methods. Three healthy female adults, aged 22-26 were maintained for 3 to 4 weeks on a constant, homogenized liquid formula diet providing 40 calories per kg body weight per day. Forty per cent of the calories was derived from corn oil (90-100 g of fat/day), 43% from dextrose, and 17% from casein. Cholestyramine was given as a suspension in

TABLE I. Effect of Tween 80 on Cholestyramine Induced Malabsorption: Fecal Fat, Nitrogen, and Bile Acid Excretion in 3 Subjects Maintained on a Constant Formula-Type Diet.

	Week				Change with cholestyramine over control period	Change with Tween 80 over cholestyramine period
	1	2	3	4		
Subject 1:						
Fat (g/day)	6.5	2.8	2.1		+4.4 (210%)	-3.7 (132%)
Nitrogen (g/day)	.9	.4	.5		+ .4 (80%)	- .5 (100%)
Cholestyramine	+	+				
Tween 80		+				
Subject 2:						
Fat (g/day)	3.6	14.4	5.5		+ 10.8 (300%)	- 8.9 (160%)
Nitrogen (g/day)	.7	1.8	1.2		+ 1.1 (160%)	- .6 (50%)
Bile acids (mg/day)	109	1103	162		+994 (911%)	-941 (581%)
Cholestyramine		+	+			
Tween 80			+			
Subject 3:						
Fat (g/day)	5.5	15.2	1.2	1.5	+ 14.0 (700%)	- 9.7 (176%)
Nitrogen (g/day)	.5	1.6	1.0	.75	+ .6 (60%)	- 1.1 (220%)
Bile acids (mg/day)	265	1930	70	50	+1860 (2660%)	-1665 (630%)
Cholestyramine	+	+				
Tween 80	+			+		

Four-day pooled sample of feces. Subjects taking cholestyramine 30 g/day and Tween 80 6 g/day.

water, 30 g daily in 4 divided doses. Six g per day of Tween 80 were administered in 4 doses. The study was divided into weekly periods, and the order of medication varied (Table I).

Ninety-six hour fecal collections were started on day 3 of each period, collected in 95% ethanol, and homogenized for 30-45 minutes. Determination of fecal fat was performed by the method of Van der Kamer *et al*(11), and fecal nitrogen by a macro-Kjeldahl procedure. After rehomogenization with saturated ammonium carbonate, bile acids were measured by an adaptation(12) of an ion exchange chromatography procedure for separation of fecal bile acids(13). In specimens from one subject, the bile acids in the column eluates were quantified individually by silicic acid glass paper chromatography (14). I^{131} triolein absorption test and prothrombin time were done in the routine clinical manner.

To study the possibility that Tween interfered with the capacity of cholestyramine to bind bile salts, an *in vitro* experiment was performed in which cholestyramine was exposed to an aqueous solution of C^{14}-labelled sodium taurocholate and sodium cholate. Similar experiments were performed in the presence of an excess amount of Tween 80. The sequence of incubations is shown in the first 2 columns of Table II.

Results. The effects of cholestyramine and cholestyramine plus Tween 80 on excretion of fat, nitrogen, and total bile acids in the 3 subjects maintained on a constant formula-diet are given in Table I. During the control periods daily fecal fat excretion was less than 3.6 g, fecal nitrogen was less than 1.0 g, and fecal bile acid excretion was 109 mg and 70 mg daily in subjects 2 and 3. When 30 g per day of cholestyramine were administered there was a 2- to 7-fold increase in fecal fat, a 60-160% rise in nitrogen excretion, and a 9- and 27-fold rise in fecal bile acids. Nitrogen excretion, although increased during cholestyramine administration, did not exceed 2.0 g per day. Administration of Tween 80 with the cholestyramine resulted in a decrease (132-176%) in fecal fat excretion. During this period, in subjects 2 and 3, the fecal bile acid excretion fell strikingly to control values. Fecal nitrogen also was reduced significantly.

The results in one subject in whom the fecal bile acids were individually characterized are presented in Table II. Deoxycholic acid was the predominant bile acid excreted

TABLE II. Effects of Addition of Tween 80 on Binding Capacity of Cholestyramine *in vitro*.

Preincubation	Incubation	Sodium taurocholate uptake, mg	Sodium cholate uptake, mg
	Cholestyramine + bile salt	38.5	35.0
	Idem + Tween 80	38.7	34.6
Cholestyramine + Tween 80	+ Bile salt	37.2	33.8
Cholestyramine + bile salt	+ Tween 80	40.0	34.8
Tween 80 + bile salt	+ Cholestyramine	36.5	33.9

Incubation and preincubation performed at 37° for one hour. Incubation mixtures contained 50 mg of carbon 14 labeled bile salt, 15 mg Tween 80 and 15 mg of cholestyramine in 10 ml.

during all experimental periods. During the cholestyramine period, a marked increase in deoxycholic and lithocholic acid excretion occurred. With addition of Tween 80 there was a distinct fall in total bile acid excretion, particularly marked in lithocholic and deoxycholic acids. Cholic acid, although present as 12% of the total fecal bile acids during the control period, was not detected during the 2 subsequent experimental periods. Chenodeoxycholic acid excretion rose slightly during the cholestyramine period, although its percentage contribution to the total bile acids actually fell. However, the addition of Tween 80 resulted in both absolute and relative increase in excretion of chenodeoxycholic acid.

In the subjects presented in Table I, following oral administration of I^{131} triolein, the 4-day fecal radioactivity was 8.2%, 18% and 29% during the cholestyramine period and 4.6%, 2.4% and 4.8% when Tween 80 was added. Prothrombin activity remained normal.

The results of the *in vitro* uptake of taurocholate and cholate by cholestyramine are shown in Table II. Tween 80 did not prevent the uptake of bile salts by the resin when incubated together or following preincubation with the resin.

Discussion. The role of bile acids in absorption of fat is due in part to their ability to form micellar complexes with lipids. The anion exchange resin, cholestyramine, has been shown to be capable of binding bile acids in the intestinal tract, thus lowering intraluminal bile acid concentration and causing an increase in their fecal excretion. In the subjects studied, administration of cholestyramine in doses of 30 g per day induced a marked rise in fecal bile acid excretion, mild steatorrhea, and a slight increase in fecal nitrogen. The simultaneous administration of the synthetic detergent Tween 80 to the subjects was associated with a return of fecal fat, fecal radioactivity, and nitrogen excretion to normal. These results suggested that Tween 80 substituted for the emulsifying action of bile acids. However, there was a concomitant striking reduction in fecal bile acid excretion.

The mechanism by which Tween 80 affected fecal bile acid excretion in the presence of cholestyramine is difficult to assess. There was no significant effect *in vitro* of Tween 80 on the bile acid binding properties of cholestyramine. A direct effect of Tween 80 on production or excretion of bile acids by the liver has not been investigated, but this would seem unlikely as a cause for the marked reduction in fecal bile acid excretion despite the continued administration of cholestyramine.

As seen in Table III, the effect of Tween 80 on the individual bile acids in the stools of the subject was to reduce strikingly the fecal excretion of deoxycholic and lithocholic acids and increase slightly the excretion of chenodeoxycholic acid. Deoxycholic and lithocholic acids are products of bacterial degradation of cholic and chenodeoxycholic acids, respectively(15). The latter bile acids are produced in the liver and have been termed "primary" bile acids. The availability of primary bile acids for bacterial degradation in the large bowel is influenced by the efficiency of absorption of these bile acids from the small intestine.

If Tween 80 had altered the bacterial flora and prevented bacterial degradation of the

TABLE III. Distribution of Individual Fecal Bile Acids in a Subject Maintained on a Constant Formula-Type Diet.

Bile acid	Control		Cholestyramine		Tween 80 + cholestyramine	
	mg/Day	% Total	mg/Day	% Total	mg/Day	% Total
Cholic	11.5	10.6	0	0	0	0
Chenodeoxycholic	18.6	17.0	30.6	2.7	72.1	44.5
Deoxycholic	65.8	60.4	537.5	48.7	79.8	48.3
Lithocholic	13.0	12.0	535.1	48.6	10.3	6.2
Total	108.9	100.0	1103.2	100.0	162.2	100.0

Four-day pooled sample of feces. Subject taking cholestyramine 30 g/day and Tween 80 6 g/day.

primary bile acids in the colon this would have resulted *inter alia* in an increase in fecal excretion of chenodeoxycholic and cholic acids. Actually, the administration of Tween 80 in the presence of cholestyramine was associated with a reduction in total bile acid excretion, due entirely to decreases in fecal output of secondary bile acids. Little inhibition of growth of enteric bacteria has been noted in the presence of synthetic nonionic detergents(16).

Tween 80 may have enhanced the absorption of bile acids from the small intestine despite the presence of cholestyramine. Although experiments of transport of taurocholate and cholate by everted sacs and slices of rat small intestine failed to show an increase in bile salt transport in the presence of Tween 80(17) such results *in vitro* do not preclude the possibility that an enhanced bile transport may occur *in vivo*. Direct evidence for this phenomenon is not available from the present study. Of interest, however, was the finding that Tween 80 was capable of increasing the absorption of another steroidal compound, spironolactone, *in vivo*(18). Greater bile acid absorption in the subjects in this study might be expected to induce inhibition of the conversion of hepatic cholesterol to bile acids. Such inhibition would then result in reduction in the intestinal concentration of bile acids available for ion exchange binding with cholestyramine.

Summary. Tween 80, a synthetic detergent, was shown to affect cholestyramine-induced malabsorption in man by reducing fecal output of fat, bile acids, nitrogen, and I^{131} triolein. Possible mechanisms of action are discussed.

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