Effect of Tween 80 on Intestinal Bile Salt Absorption *in vitro*.* (29543)

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The synthetic surface active detergent, polyoxyethylene sorbitan monooleate (Tween 80) has been used in sprue, regional ileitis and after gastrectomy in an attempt to increase the absorption of fat from the intestine(1). Studies from this laboratory in subjects given large doses of cholestyramine, a bile acid binding resin, have shown that Tween 80 decreases the experimental steatorrhea induced by this agent(2). The reduction in fecal fat excretion in these subjects was, however, associated with a pronounced fall in excretion of bile acids in the feces. These observations suggested that Tween 80 might enhance bile acid absorption by the small intestinal mucosa. The present studies were designed to assess the effect of Tween 80 on small intestinal bile salt absorption *in vitro*.

Methods. Commercial preparations of cholic acid† and taurine‡ were re-crystallized twice before use. Cholic-acid-C^{14}§ was found to be over 99% pure by thin layer chromatography on silicic acid. Sodium taurocholate was a gift of Eli Lilly Co., Indianapolis and C^{14} labeled taurocholate was prepared by the method of Norman(3) and purified by preparative thin layer chromatography on silicic acid(4). Assays of C^{14} radioactivity were made in a Packard liquid scintillation spectrometer.

Experiments were performed in albino rats of 250 to 350 g weight whose bile duct has been cannulated for 24 hours in order to divert bile from the intestine. The animals were killed by a blow on the head and everted intestinal sacs were prepared by the method of Wilson and Wiseman(5). Ileal sacs were taken from the area just proximal to the ileocecal junction and jejunal sacs from intestine just distal to the ligament of Treitz. Jejunal and ileal sacs were approximately 5 cm in length. The solution used on serosal and mucosal sides were oxygenated Krebs bicarbonate buffer with a glucose concentration of 200 mg per 100 ml and half the usual concentration of calcium. Sodium taurocholate-C^{14} was present in a concentration of 0.2 mM in the presence or absence of 1.0 mM Tween 80. The sacs were filled with 0.8 ml and incubated in 10 ml of test solution for one hour in a Dubnoff shaking incubator at 37°C. The flasks were gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide.

At completion of the incubation an aliquot of serosal and mucosal solutions was assayed for radioactivity. The tissue bile salts were extracted and radioactivity measured as described before(4).

The uptake of sodium taurocholate and sodium cholate 0.2 mM by everted slices of jejunum and ileum from a Krebs bicarbonate medium after 30 minutes' incubation was also measured(4). Bile salt absorption by these tissue slices was calculated per gram of tissue water in the presence of Tween 80 at concentrations of 0 to 4 mM.

Results. Following incubation of everted ileal sacs, the final concentration of taurocholate in the serosal medium was greater than that on the mucosal surface indicating that transport of this bile salt had occurred against a concentration gradient (Table I). At the same time the concentration of taurocholate in ileal tissue water was calculated to be 3- to 4-fold greater than the final concentration in the mucosal medium exceeding that in the serosal medium. Thus, it would appear that the rate of tissue uptake was more rapid than the rate of transfer across

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‡ Distillation Products Industries, Rochester, N. Y.

§ New England Nuclear Corp., Boston, Mass.

TABLE I. Effect of Tween 80 on Transport of Taurocholate-C^{14} by Everted Sacs of Rat Intestine.

	Tween 80 1 mM	No. of exp	Serosal/mucosal concentration	Tissue concentration μmoles per g tissue water	Tissue/mucosal concentration
Jejunum	—	7	.85 (.032)*	.17 (.005)*	.87
	+	8	.84 (.04)	.17 (.020)	.87
Ileum	—	6	2.17 (.45)	.51 (.06)	3.02
	+	6	2.70 (.20)	.56 (.06)	3.56

* ( ) ± Standard error of mean.

TABLE II. Effect of Tween 80 on Accumulation of Taurocholate-C^{14} and Cholate-C^{14} by Everted Slices of Rat Intestine.

	Concentration of Tween 80 mM	Taurocholate-C^{14} accumulation		Cholate-C^{14} accumulation	
		No. of exp	μMoles per g tissue water	No. of exp	μMoles per g tissue water
Jejunum	0	6	.11 (.007)*	3	.21 (.007)*
	.2	6	.12 (.012)	3	.21 (.007)
	1.0	5	.10 (.011)	3	.21 (.014)
	4.0	6	.09 (.007)	3	.17 (.011)
Ileum	0	6	.83 (.042)	5	.97 (.042)
	.1	5	.89 (.036)	4	.95 (.078)
	.2	6	.94 (.08)	4	.98 (.070)
	.4	5	.77 (.042)	4	.84 (.071)
	1.0	6	.72 (.049)	4	.78 (.075)
	4.0	6	.67 (.042)	4	.70 (.051)

In each experiment 2 slices were incubated in duplicate.
* ( ) Standard error of mean.

the serosa. No such concentration of taurocholate was detected in tissue or serosal medium in the jejunum.

Tween 80 increased the ileal transport of taurocholate into the serosal compartment and tissue slightly but the results were not significant ($p>0.1$). There was no effect of Tween 80 on jejunal transport of bile salts.

When the tissue uptake of cholate-C^{14} and taurocholate-C^{14} was measured in everted intestinal slices, concentration again occurred in the ileum but not in the jejunum (Table II). Addition of Tween 80 at concentrations less than 0.4 mM enhanced taurocholate uptake slightly but not significantly, and had no effect on cholate transport. Concentrations of 1.0 mM and 4.0 mM Tween 80 resulted in a slight but consistent reduction in the ileal accumulation of both conjugated and unconjugated bile salts.

There was no effect of Tween 80 on uptake of bile salts by the jejunum. No histologic changes in the intestinal mucosa were observed after incubating the tissue in medium containing the Tween 80.

Discussion. This study confirms previous observations(4) that the rat distal ileum transports bile salts against a concentration gradient *in vitro* and that this mechanism is not present in the jejunum. The non-ionic detergent, Tween 80, does not enhance active transport of bile salts by either everted ileal sacs or slices, nor does it affect the passive transport that occurs in the jejunum. In our previous studies(2) subjects were given 6 g of Tween 80 daily. Less than 5% of Tween 80 is absorbed(6) so that the concentrations of the detergent in ileal contents can be estimated to reach 3.0 to 5.0 mM. This concentration of Tween 80 depressed the uptake of both taurocholate and cholate by ileal slices. The reason for this effect is not clear. In contrast to cationic and anionic surface active agents, Tween 80 has been found not to be toxic to the intestinal mucosa(7). No histologic damage to the intestinal slices was detected. It has been shown that Tween 80 has an effect on the metabolism of hamster

intestinal mucosa, greatly increasing the utilization of glucose and production of lactate in a similar *in vitro* preparation(8). It would seem unlikely however, that stimulation of glycolysis would inhibit bile salt uptake.

Summary. Studies on the effect of Tween 80 on bile salt absorption *in vitro* do not support the hypothesis that this surface active detergent enhances intestinal absorption of bile salts. At high concentrations of Tween 80 inhibition of absorption occurred.

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Effect of Toxic Doses of L-Thyroxine on Tissue Water, Electrolytes and Plasma Protein in Rats. (29544)

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The metabolic stimulating effect of thyroid hormones has long been recognized. The functional capacity and biochemical composition of the heart, liver and skeletal muscle were found to be altered in hyperthyroidism(1-3). In thyrotoxicosis, changes in the heart are characterized by an increase in rate and strength of contraction, an elevated cardiac output and development of myocardial hypertrophy. Among other pathological conditions in human subjects or animals, liver glycogen depletion and muscular weakness are most commonly seen(4). The purpose of this work was to study and reevaluate the toxicity of L-thyroxine as concerned with electrolyte and water distribution patterns in various organs as well as the changes in plasma protein fractions of rats.

Methods and materials. The 25 male Sprague-Dawley rats used in this study weighed from 250-350 g. All animals were kept in separate cages and maintained on Purina chow *ad libitum.* Thirteen rats received a daily subcutaneous injection of 0.5 ml of 0.02 N NaOH containing 0.5 mg of L-thyroxine, whereas 12 rats were injected with the same alkaline solution without thyroxine. Administration of thyroxine was continued for 24 days when one of the experimental rats died. At 25 days, the rectal temperature of all surviving animals was measured with a thermistor probe and was followed by injection of an anesthetic dose of Na pentobarbital intraperitoneally (40 mg/kg in 1.5% solution). Blood samples were taken from the dorsal aorta. As soon as an adequate amount of blood was withdrawn into a heparinized syringe, the ventricles of the heart, random muscle samples of both hind limbs and the whole liver were immediately taken for biochemical analyses.

Hematocrit was determined by a microcapillary technique. Plasma was separated from the red blood cells by centrifugation at $4500 \times g$ for 10 min. Glucose concentration was measured immediately in plasma and packed red blood cells according to Nelson-Somogyi's method(5,6). Total plasma protein was determined(7) and the individual protein fractions were separated by paper electrophoresis(8). Plasma and tissue Na^+ and K^+ were determined in a Perkin-Elmer flame