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Voluntary Food Consumption and Gastric Emptying in Rats Given Intravenous Triton WR-1339[1] (42268)

JAMES C. McPHERSON III* AND JAMES C. McPHERSON, JR.†

Department of Clinical Investigation, Dwight David Eisenhower Army Medical Center, Fort Gordon, Georgia 30905-5650, and †Department of Surgery, and Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912

Abstract. Intravenously administered Triton WR-1339, a nonionic surface active agent, has been used as an endogenous hyperlipemic agent since 1951. We expected Triton to increase food consumption to supply, at least partially, the energy and acetyl groups necessary for producing the hyperlipemic state. In this study, however, we observed that the rats injected intravenously with various dose levels of Triton decreased their voluntary food intake in a dose-related manner. Two other nonionic surface active agents, Tween 20 and Tween 80, given intravenously did not alter food intake. Further studies revealed that Triton WR-1339 administered intravenously 30 min before feeding by stomach tube resulted in a marked delay in the rate of gastric emptying which was also dose related. A delay in gastric emptying has previously been suggested as one mechanism that controls food intake. Tween 20 and Tween 80 did not alter the rate of gastric emptying. We suggest that the mechanism responsible for the decrease in voluntary food consumption in Triton WR-1339 injected rats may be due to the delay of gastric emptying in these animals.

In our studies on the treatment of fat embolism by administration of Triton WR-1339 (1), we observed a decrease in voluntary food consumption in those rats injected with Triton. Since Triton WR-1339 has long been recognized as a major endogenous hyperlipemic agent (2), we expected rats given Triton would increase their food consumption to supply, in part at least, the energy (ATP) necessary for synthesizing the elevated circulating lipoproteins as well as to supply the acetyl coenzyme A (CoA) units necessary for lipogenesis and cholesterogenesis whose rates are also increased by Triton (3). This expectation was based on the repeated observations of Adolph (4) that rats consume calories when fed nutritionally adequate diets of varying caloric density. In this study we have demonstrated that intravenous Triton WR-1339 decreases voluntary food consumption in the rat and we suggest a pharmacologic effect to explain this effect on food consumption by delaying gastric emptying. We have also compared Triton's effects with some other nonionic surface active agents.

Materials and Methods. *Animals.* Female (200 to 220 g) and male (330–350 g) rats raised in our colony from Carworth Farms CFN strain stock were employed. They were housed individually in suspended cages with one-half in wire-mesh bottoms and maintained in a temperature- and humidity-controlled room with a 12-hr light/dark cycle. Water and food were available *ad libitum.*

Surface active agents and food. The nonionic surface active agents (surfactants) Triton WR-1339 (Ruger Chemical Co., Inc., Irvington, N.J.) and Tween 20 and Tween 80 (J.T. Baker Chemical Co., Phillipsburg, N.J.) were dissolved in normal saline at a concentration of 10% (w/v). A commercial pelleted rat stock diet (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) was finely ground and fed in tared food cups (5) from the time of weaning through their use in this study.

Voluntary food consumption. Seven groups of 10 female rats (200–220 g) were injected

[1] The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, the National Research Council.

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intravenously (without anesthesia) via the tail vein daily between 9 and 10 AM Food consumption and body weights were measured for 24 hr prior to the first injection (Day 0) and daily thereafter. The volume of the surface active agents solution administered was adjusted daily for the body weight on that day so that the dose levels were constant throughout the study. All animals were sacrificed on the morning of Day 5 (after measuring the food consumption and body weight of Day 4). Any animal that lost weight between weighing on Day 0 and the morning of Day 1 was discarded as were any animals that scratched food out of the tared feeding cup (5) during the study. Each animal served as her own control, i.e., food consumption on Day 0 before any injections. Another control group (Group 1) consisted of 10 animals injected with saline (0.8 ml/100 g body wt, which was equivalent to the volume of an 800 mg/kg dose of the surface active agents). Triton WR-1339 was injected at dose levels of 100, 200, 400, and 800 mg/kg body wt daily (Groups 4–7). Tween 20 and Tween 80 were injected at a dose level of 400 mg/kg body wt daily (Groups 2 and 4). All animals were sacrificed under chloroform anesthesia at the end of Day 4 (i.e., morning of Day 5) and blood was obtained from the vena cava for determination of the serum cholesterol level (6) as a means of quantitating the hyperlipemic response to the surface active agents.

Gastric emptying. Male rats, 330–350 g body wt, housed similarly to the females, were fasted for 48 hr. These animals referred to as fasted rats were tube fed 10 ml of a 15% sucrose solution in tap water after 8, 24, and 30 hr of fasting. With this technique, the fasting stomach contents were less than the fasting stomach contents of rats prepared by the method of Lippmann (7). The solutions and dose levels of the various surface active agents were the same as those used in the food consumption study. The dose administered was based on the prefasted body weight because animals of the same initial body weight did not always have the same fasted body weights. The surfactant or saline (controls) solutions were injected into the tail vein 30 min before feeding. Five milliliters of a tube feeding diet (Tube Feeding Diet, Modified, Rat, ICN Nutritional Biochemicals, Life Sciences Group, Cleveland, Ohio), prepared as previously described (8), was fed to the rats by stomach tube. Three 5-ml aliquots of each batch of diet were dried to constant weight as a measure of the weight of the diet actually fed (average weight of the three aliquots). Groups of 10 animals per dose level were sacrificed at 0, 1, 2, 4, and 8 hr; however, not all time intervals were studied with all dose levels (15 different groups of 10 rats were used, see Table III). A 2-hr postfeeding time interval seemed the most appropriate interval to demonstrate a significant effect with this diet. The animals were sacrificed under chloroform anesthesia. The abdomen was rapidly opened using a midline incision and a silk ligature placed first around the pyloric-duodenal junction and tied. The esophagus was then ligated just above the stomach. The stomach was removed and placed in a −20°C freezer. After frozen solid (2–4 hr or overnight), the stomach was moistened with tap water, split along the greater curvature with a razor blade, the solid contents shelled out into a tared container, any remaining food or ice crystals were added to the container with the aid of a spatula (9), and the stomach contents dried to a constant weight in a 105–110°C oven. The rate of gastric emptying was measured by calculating the ratio of the weight of the recovered diet (dried) to the weight (dried) of the 5-ml aliquot of the fed diet.

Statistical analysis. Statistical analysis of our data was performed by our computer center using a Student's *t* test program.

Results. *Voluntary food consumption.* The effect of Tween 20, Tween 80, and Triton WR-1339 at various dose levels on voluntary food consumption is shown in Table I and compared both to their own control on Day 0 and with the saline-injected control group. There were no significant effects on food consumption in the Tween 20- and Tween 80-injected animals. Triton at all dose levels significantly depressed food consumption on at least 1 day compared to its own control (Day 0) and most dose levels were significantly depressed compared to the saline group of the same day. The effect of Triton was dose related.

The effect of the various surfactants on body weight followed the change in food consumption and is, therefore, not shown in the table. No animal or group had a significant decrease in food consumption without a loss of body

TABLE I. VOLUNTARY FOOD CONSUMPTION AS INFLUENCED BY INTRAVENOUSLY ADMINISTERED NONIONIC SURFACE-ACTIVE AGENTS (in g/day ± SD)

IV injections (mg/kg)	Day 0	Day 1	Day 2	Day 3	Day 4
Saline	15 ± 1	13 ± 2	13 ± 2	14 ± 2	14 ± 2
Tween 20, 400	13 ± 2	14 ± 2	13 ± 4	13 ± 3	13 ± 2
Tween 80, 400	14 ± 3	13 ± 3	12 ± 2	13 ± 4	14 ± 2
Triton, 100	16 ± 2	14 ± 2	12 ± 2[b]	12 ± 2[e]	13 ± 2[b]
Triton, 200	14 ± 1	12 ± 3[f]	11 ± 3[f]	11 ± 3[c,e]	11 ± 2[b,d]
Triton, 400	14 ± 2	10 ± 2[a,d]	7 ± 3[a,d]	6 ± 3[a,d]	7 ± 3[a,d]
Triton, 800	16 ± 1	6 ± 2[a,d]	4 ± 1[a,d]	4 ± 2[a,d]	4 ± 2[a,d]

[a] Significantly different from saline control of same day ($P < 0.001$).
[b] Significantly different from saline control of same day ($P < 0.01$).
[c] Significantly different from saline control of same day ($P < 0.05$).
[d] Significantly different from Day 0 of the same dose ($P < 0.001$).
[e] Significantly different from Day 0 of the same dose ($P < 0.01$).
[f] Significantly different from Day 0 of the same dose ($P < 0.05$).

weight. Also, no animal or group that had a marked loss of body weight continued to consume normal amounts of food.

Table II shows the effect of surfactant treatment on the serum levels of cholesterol at the end of Day 4 compared to the saline control group. The hyperlipemic response to the various surfactants and dose levels was quantified by measuring the change in the serum cholesterol level. The sera from all rats treated with 100 mg/kg Triton daily were clear and the cholesterol levels were moderately increased compared with those of the saline group (82% increase). Other dose levels of Triton resulted in a visible lipemia and markedly elevated cholesterol levels (1325 to 3000% increase) which were dose related.

Gastric emptying. The effect of the various surfactants on gastric emptying is compared to the control rate after saline injection in Table III. The zero time recovery rate of 5 ml of the diet was 97 ± 2%. Tween 20, Tween 80, and the 100 mg/kg dose level of Triton WR-1339 did not significantly alter the rate of gastric emptying and these had very little effect on food consumption (Table I). All other dose levels of Triton delayed the rate of gastric emptying at all time intervals studied. The effect of Triton is dose related as was the effect of Triton on voluntary food consumption (Table I).

Discussion. Rats have long been a favorite laboratory animal for the study of factors which affect voluntary food consumption, in part at least, because they are known to consume calories rather than food weight or volume when they are fed nutritionally adequate diets of varying caloric density (4). Triton WR-1339 has been used as a hyperlipemic agent for over 30 years (2); however, we have found no quantitative reports of its effect on food consumption. Triton has been reported to not affect food consumption in several species (10–13) except in one long-term study (14). Andersen and Dietschy (15) noted that rats injected with a 1000 mg/kg per day dose of Tri-

TABLE II. BLOOD CHOLESTEROL LEVEL AS INFLUENCED BY INTRAVENOUSLY ADMINISTERED NONIONIC SURFACE-ACTIVE AGENTS (mean ± SD)

				Triton WR-1339			
	Saline	Tween 20 (400 mg/kg)	Tween 80 (400 mg/kg)	(100 mg/kg)	(200 mg/kg)	(400 mg/kg)	(800 mg/kg)
Cholesterol (mg%)	87 ± 21	85 ± 7	76 ± 7	158 ± 29[a]	1240 ± 280[a]	2470 ± 340[a]	2700 ± 300[a]

[a] Significantly different from saline control ($P < 0.001$).

TABLE III. EFFECT OF NONIONIC SURFACE ACTIVE AGENTS ON GASTRIC EMPTYING (RATIO = RECOVERED DIET WEIGHT [DRIED] ÷ WEIGHT [DRIED] OF 5 ml OF FED DIET)

IV injection (mg/kg)	Ratio ± SD				
	Zero time	1 hr	2 hr	4 hr	8 hr
Saline	0.97 ± 0.02	0.72 ± 0.04	0.44 ± 0.08	0.15 ± 0.08	0.02 ± 0.04
Tween 20, 400			0.47 ± 0.13		
Tween 80, 400			0.43 ± 0.09		
Triton, 100			0.41 ± 0.07		
Triton, 200			0.53 ± 0.07[a]		
Triton, 400		0.78 ± 0.05[b]	0.59 ± 0.08[c]	0.23 ± 0.08[a]	
Triton, 800		0.93 ± 0.08[c]	0.71 ± 0.09[c]		0.25 ± 0.12[c]

[a] Significantly different from saline group at the same time interval ($P < 0.05$).
[b] Significantly different from saline group at the same time interval ($P < 0.01$).
[c] Significantly different from saline group at the same time interval ($P > 0.001$).

ton for 3 days decreased food intake, but did not quantitate this response. In view of its hyperlipemic effect (2, 3, 10–13, 15), which was confirmed in this study (Table II), we expected the rats to increase their voluntary food consumption at a time when they should need the extra calories (see introduction). The opposite effect, i.e., a decreased food intake, was observed (Table I). The mechanism(s) which is responsible for this effect is not readily apparent. It does not appear to be a general effect of nonionic surface active agents because Tween 20 and Tween 80 did not affect food intake. There are several mechanisms that have been proposed with experimental support to control appetite [for reviews of some of these see Refs. (16, 17)]. We find no reports involving Triton that would indicate Triton affects any of the receptor mechanisms or neurohormonal mechanisms in appetite control. An effect of Triton on one or more of these mechanisms cannot be ruled out, however, by the methods used in this study.

Our study demonstrates that intravenously administered Triton WR-1339, but not Tween 20 and Tween 80, results in a significant delay in the rate of gastric emptying. This is a rapid response since it is evident as soon as 1 hr after feeding, the shortest time interval we studied, and it has a long duration (still evident after 8 hr). This delay in gastric emptying may explain the decreased food intake without involving the appetite receptors or neurohormonal receptors. Because all animals received the same diet and all received an intravenous injection, the only difference being Triton in the intravenous solution, it appears that Triton WR-1339 exerts a pharmacological response on gastric emptying in the rat and that this response is dose related (Table III). A delay in gastric emptying has been suggested to control food intake (18) which is the mechanism suggested for the effect of Triton on voluntary food consumption in this study.

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