Exogenous Emulsifiers and Fat Absorption.* (19764)

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The rather general acceptance in the recent past of Frazer's(1) partition hypothesis seems to have strengthened the idea that emulsifying agents should hasten the rate of the absorption of fat from the intestine. Such an effect might be the result of a greater emulsification which may either promote the speed of absorption of the finely divided particulate fat or permit a more rapid action of the lipolytic enzymes.

The findings of several investigators could be interpreted as evidence supporting this effect of emulsifiers. An increased rate of fat absorption has been obtained when the ingested fat is supplemented with lecithin(2-4), which has an emulsifying action. Becker *et al.*(5) reported Tween 80 to affect fat absorption differently in young and aged people, but their results seemed to indicate a faster rate of fat absorption in both groups. Tween 80 has been found by Jones *et al.*(6) to promote the absorption of fat and fat soluble substances by patients with steatorrhea. However, they stated that in the normal subject "the absorption of dietary fat is so nearly complete that the addition of such an agent to the diet would seem to be of negligible value." An increased rate of fat absorption has also been reported to occur when an emulsified fat is instilled in the ligated rat intestine.

Earlier studies of Holt *et al.*(8) gave no evidence that the normal infant found any difficulty in intestinal emulsification of dietary fat. The results of Jones *et al.* were not confirmed by Badenoch, Johnson and their collaborators(9) and the latter group reported Tween 20 to be ineffective in reducing the fecal fat of prematures and of infants with steatorrhea. Recently, Shoshkes *et al.*(10) found absorption of fat to be unaffected by the presence of 20% lecithin or Tween 80, amounts greatly in excess of that required to make excellent emulsions by mechanical means.

With this somewhat conflicting evidence, it seemed wise to include some of the synthetic emulsifiers in our studies of the various factors influencing the rate of fat absorption. The effect of a monoglyceride† and of a number of the fatty acid esters of some sorbitol or alkylene oxide derivatives‡ on this rate has been determined at different levels of fat intake and emulsifying supplement. Several of the latter emulsifying agents were included in these studies because they have slightly different physical properties. Also an attempt has been made to learn whether these substances influenced either gastrointestinal motility or fat splitting, since such actions might affect the rate of fat absorption.

Experimental. The groups of animals used were all male or female albino rats with weights ranging from 150 to 500 g. As checks upon each other, two methods, the chylomicrograph and a modified Cori technic, were used to determine the rate of fat absorption.

A number of investigators(4,5,11,12) have shown the former method can be used to determine the relative amount of fat in the blood and that the area under the curve obtained gives a similar measure of the rate of the various processes involved. After a 24-hour fast, the rats were given 0.05 ml of olive oil per square decimeter of body surface with or without the supplement of 6 or 20% of the emulsifier. The small amount of fat permit-

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† Generously supplied by Distillation Products, Inc., Rochester, N. Y.

‡ Atlas Powder Company trade marks and chemical descriptions:

Tween 80—polyoxyethylene (20) sorbitan monooleate

Myrj 45—polyoxyethylene (8) stearate

Span 60—sorbitan monostearate

Tween 60—polyoxyethylene (20) sorbitan monostearate

TABLE I. Effect of Supplements of Various Emulsifiers on Blood Fat Levels after Fat Ingestion (Chylomicrographs).

No. rats	Fat supplement	Chylomicron count Fasting	1 Hr	1½ Hr	2 Hr	3 Hr	Area under curve	"t" value
8	Saline	13	36	41	42	44	83 ± 6	
10	Tween 80 6%	15	41	47	46	45	91 ± 3	1.285
8	" 20%	8	47	44	51	53	98 ± 6	1.696
	Saline	6	69	43	44	40	92 ± 5	
	Myrj 45 6%	4	58	51	49	36	95 ± 5	.392
10	Span 60 6%	4	63	48	51	49	102 ± 8	1.184
	Tween 60 6%	5	64	46	45	55	100 ± 5	1.223
	Monooleate 6%	4	66	55	41	44	97 ± 8	.538
35	Saline	8	48	52	52	44	99 ± 4	

Including the stand. error of the mean and Fisher's "t" value for areas under the curve.

ted a more accurate counting of the chylomicrons and the use of a more physiological intake of fat. The supplements given approximated the levels used by Jones, Becker, and Stare in their studies. The method followed was that previously used in this laboratory(4). An eyepiece micrometer disc (5 mm square divided into 100 squares) replaced the Whipple disc, and from the 4 or more fields of 100 squares counted, the average number of chylomicrons in 10 squares were the counts recorded.

The second method of this study made use of the Cori technic as modified by Deuel *et al.*(13). The determination of the rate of fat absorption by a recovery of unabsorbed fat after an interval for absorption has been found satisfactory by a number of investigators(7,14). The ages or sex of the animals did not influence this absorption rate. The rats were fasted 48 hours and given by tube 0.15 or 0.30 ml of Sudan IV stained olive oil per square decimeter of body surface. After a 2- or 3-hour period for absorption the rats were sacrificed and the distance the stained fat had passed along the intestine as well as the total intestinal length measured. Finally, the fat was recovered, weighed and the free fatty acids titrated. This procedure permitted not only an estimation of the per cent of fat absorbed and the rate of this process, but also a relative measure of gastrointestinal motility and fat splitting as affected by the various supplements given.

Results and discussion. The chylomicrographs, obtained in 2 experiments when the fat was given alone or with either 6 or 20% of the exogenous emulsifiers, are shown in Table I. Although the area under the curve for the first group receiving only the fat appears to be much smaller than that of those receiving the 20 or 6% of Tween 80, the differences are not statistically significant. Wide individual differences in this particular control group (fat alone), and a mean control value of 99 on 35 rats involved in all studies about this time, would indicate that the differences should not be significant. A later experiment confirmed this conclusion. That similar results were obtained when supplements of 6% Myrj 45, Span 60, Tween 60 and glyceryl monooleate were given with the fat may be seen in Table I. The similar peaks and areas under the curves indicated comparable fat absorption in all groups. Hence, the use of this method gave no evidence of any change in the rate of the absorption of the fat when it was supplemented with any of the emulsifiers.

The results obtained by the modified Cori technic are included in Table II. In the first experiment, there was no significant change in the per cent of fat absorbed or in the rate of its absorption over a 2-hour period when 0.15 ml of fat per square decimeter of body surface was supplemented with 20% Tween 80 or glyceryl monooleate. Similar results were obtained when twice as much fat was supplemented with 6% of the previously mentioned exogenous emulsifiers and the absorptive period extended to 3 hours. The latter changes permitted a comparison of our values with those of Deuel and co-workers when they fed cottonseed oil. Good agreement was ob-

TABLE II. Absorption and Splitting of Fats, and Intestinal Motility as Affected by Various Supplements of Emulsifiers (by Recovery).

No. rats	Fat supplement	Fat absorbed		"t" value	% of free fatty acids recovered	% of length of intestine traversed
		%	mg/dm²/hr			
16*	Saline	33.9	21.6 ± 1.7		17.5	80
8*	Tween 80 20%	32	22.9 ± 1.6	1.067	—	90
8*	Monooleate 20%	36.8	25.6 ± 2.5	2.068	16.5	81
8	Saline	44.3	40.3 ± 2.9		17	85
8	Myrj 45	47.8	43.9 ± 3	.860	15.9	94
9	Span 60 6%	48.8	44.6 ± 1	1.263	12	85
7	Tween 60	42.3	39 ± 3.6	.284	12.5	92

* 2 hr absorptive period after ingestion of .15 ml of fat per dm² of body surface; all others, 3 hr and .30 ml of fat.

served. Again, there was no evidence of an effect upon the rate of fat absorption when it was determined by the recovery method instead of from changes in the blood fat levels.

The amounts of free fatty acids in the recovered fat and the relative length of the intestine traversed appear to be unaffected by the presence of these amounts of the emulsifiers. These results are in agreement with previous, and as yet unpublished, data from similar tests. Thus, the evidence suggests no change in the fat splitting or intestinal motility in the animals given the fat and supplement, as compared with a control group receiving the fat alone.

It appears important that it be established whether the physical effect of an increased emulsification of the fat in the intestine of the normal animal plays a role in promoting fat absorption. Our results from the use of 2 methods are in complete agreement with those from Stare's laboratory—that the absorption of orally fed fat is unaffected by the presence of emulsifiers, even when greatly in excess over that needed to make excellent emulsions by mechanical means. Factors, such as splitting and fat passage along the intestine, that might affect such absorption also appear unchanged. It seems logical to suppose that under normal conditions, a sufficient emulsification of the fat would naturally occur in the normal intestine to permit the maximum absorption of the fat. If this be true, then use of exogenous emulsifiers as fat supplements should not affect the rate of fat absorption in the normal subject. Such reasoning is in accord with our findings.

If it be established that emulsifier supplements do not promote fat absorption, then the effect of lecithin must be otherwise explained. The fact that both lecithin and choline(4) appear to increase the rate of fat absorption would suggest that one or both are involved in some chemical mechanism required for fat absorption. Thus it appears that the presence of substances that may promote changes in the physical state of the fat do not increase the rate of the absorptive process in the normal animal, while those that may enter into the chemical actions involved do promote fat absorption.

Summary. As determined by changes in the blood fat levels and by recovery of fat from the intestine after an absorptive period, the rate of fat absorption in normal, orally fed rats appears to be unaffected by 6 or 20% supplements of a number of exogenous emulsifiers. The lack of changes in the degree of fat splitting and intestinal motility which could affect fat absorption are in complete agreement with the above finding. The action of lecithin in promoting fat absorption seems best explained by other than its emulsifying action.

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Effects of Single Intravenous Injections of Pituitary Growth Hormone to Normal Adult Men.* (19765)

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Highly purified preparations of pituitary growth hormone† are capable of inducing consistent effects in laboratory animals. Thus growth hormone accelerates the growth rate of both hypophysectomized and normal animals(1); induces a permanent state of diabetes mellitus in certain species(2),‡ increases the ketosis of the fasting state(3); decreases total body fat(4); and diminishes the urinary excretion of sodium and potassium(5). In contrast this hormone has, in general, been ineffective in man. Recently, however, positive nitrogen and phosphorus balance has been reported with growth hormone in a case of hypopituitarism(7), and hyperglycemia of short duration has been observed in a patient with pancreatic islet cell tumor(8).

It is the purpose of this paper to report briefly experiments which demonstrate consistent, rapidly occurring effects of a highly purified growth hormone preparation§ in man.

Methods. Eight subjects were studied, 7 normal men (ages 20-29) who were ambulatory except during the experiment, and a 42-year-old patient with cancer of the stomach and malnutrition. Five of the normal subjects were admitted to the Metabolic Ward of the Royal Victoria Hospital for 4 days. Dietary intakes were not controlled before or during the course of the experiments. On day 1 no food was taken after 6 p.m. The bladder was emptied at midnight and 500 cc of water administered orally. On day 2 urines were collected at 6 a.m. and hourly thereafter until 1 or 2 p.m. Amino acid nitrogen(11), glucose, phosphorus and creatinine (Evelyn modification of Folin-Wu) determinations were done on these specimens. 250 cc of water were given at 6, 7, 10 and 11 a.m. and 12 noon to insure adequate urine volumes but the fast was continued until the last urine was collected. Beginning at 8:15 a.m. on day 2, 320 cc of an amino acid solution (1.24% amino N) was administered intravenously at a constant rate over a period of 26 minutes. Venous blood samples were drawn without stasis and the following determinations done: amino acid

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† Hereafter referred to as growth hormone.

‡ Raben and Westermeyer(6) reported that they had obtained a purified growth hormone preparation which did not exhibit diabetogenic properties in 3 dogs.

§ Generously supplied by Dr. Leonard Mitchell of Frank W. Horner Ltd., Montreal. This preparation was isolated from beef pituitaries by the Wilhelmi method(9). Its biological activity is equal to that of the electrophoretically homogeneous preparation of Li(10). It is very low in ACTH, TSH, gonadotropin and pitressin activity by biologic assay.

‖ Kindly supplied by Dr. J. H. Laurie of Merck Co. Ltd., Montreal. This solution contains 12 amino acids; 10 are the l-form, 1 the d-l (tryptophane).