synthetic substrates known to be hydrolyzed by bovine trypsin, chymotrypsin, and carboxypeptidase. When activated with bovine trypsin, preparations from chicken pancreas hydrolyzed all of the synthetic substrates tested, and also exhibited proteolytic activity toward denatured hemoglobin. Unactivated preparations exhibited little or no activity toward proteins or synthetic substrates. Trypsin-like and chymotrypsin-like activities were inhibited by diisopropylphosphofluoridate, and by naturally occurring trypsin inhibitors from soybeans, lima beans, and pancreas. The optimum activity for hydrolysis of trypsin substrates was at pH 8.5 to 9.0, and for chymotrypsin substrates was from pH 8.3 to 8.7. The significance of comparative biochemical studies of proteolytic enzymes of different species is discussed.

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Specificity of an Intestinal Lipase for Monoglycerides.* (27638)

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In a recent study in this laboratory an enzyme was observed in rat intestinal mucosa which appeared specific for the hydrolysis of monoglycerides(1). Lipases in the succus entericus have been previously demonstrated to resemble the lipase of the pancreas in that they were active as hydrolytic enzymes for triglycerides(2). Nikkila *et al.*(3) demonstrated the clearing of olive oil emulsions with a saline extract of rat duodenum, and Schmidt *et al.*(4) described an enzyme in extracts of rat intestine which could promote the splitting of monoglycerides. More recently DiNella *et al.*(5) have demonstrated a lipase from hog duodenum which hydrolyzed tri-, di-, and monoglycerides at approximately equal rates. Hübscher and Clark(6) and Senior and Isselbacher(7) have observed hydrolysis of monoglycerides by rat intestinal mucosal fractions in studying the pathway of triglyceride synthesis. While there is ample evidence for the occurrence of a lipase in the small intestine, its exact role in fat digestion is not clear.

In this study an enzyme from the homogenized mucosa of the rat small intestine has been shown to hydrolyze monoglycerides of long chain saturated and unsaturated fatty

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TABLE I. Changes in Monoglyceride Content of Di- and Triglyceride Substrates as a Result of Incubation with Mucosal Enzyme Preparation.

		MG content of total lipid†			Enzymatic hydrolysis‡	
Substrate*	Glyceride	Initial wt (I), mg	Control (C), mg	Exp. (E), mg	Wt change of MG (I−E), mg	$\frac{I_{(MG)}-E}{\text{Total I}} \times 100$, %
TG	TG	129				
	MG	11	8	6	−5	−4
DG	DG	132				
	MG	8	8	12	+4	+3

* TG, DG and MG represent tri-, di-, and monoglyceride respectively.
† Control and experimental values were obtained after incubation of substrate alone and the active enzyme preparation, respectively.
‡ + and − indicate a gain or loss in content of this fraction. Differences in values are not significant.

acids to a much greater extent than the corresponding di- or triglycerides. However, the enzyme appears to have no specificity for either the α- or β-forms.

Materials and methods. Preparation of solutions. Emulsions of the glycerides, containing approximately 140 mg of substrate in 20 ml of Krebs-Ringer phosphate buffer (pH 6.6), were prepared by homogenizing the glyceride with the buffer in a Potter-Elvehjem or an Omni-Mixer homogenizer after addition of 0.1 ml of Tween 80 to stabilize the emulsion. Substrates employed included the following: β-monopalmitin prepared by the method of von Lohuizen and Verkade(8), α-monopalmitin prepared by the method of Hartman(9), α-monooleate (Myveral), purified dioleate (both oleates from Distillation Products, Inc), and highly purified triolein (Hormel Institute).

Preparation of enzyme and incubation procedures. Adult albino rats, which had been fed a stock diet *ad libitum,* were anesthetized with ether. The small intestine was washed out with pre-chilled Krebs-Ringer phosphate buffer (pH 6.6) *in situ* and then removed. The intestine was split longitudinally and the mucosa removed by scraping with a glass slide. The mucosa was homogenized in a Potter-Elvehjem homogenizer with sufficient buffer to give a concentration of 10% of the mucosal scrapings in the homogenate. Five ml of this homogenate were incubated with 20 ml of the appropriate glyceride emulsion in a Dubnoff shaker for 2 hr at 37°C. Emulsions without enzyme, and the mucosal homogenates without substrates, were treated in the same manner to serve as controls. All substrates were incubated in triplicate.

Extractions and analysis of digest. After incubation, 5 ml of ethyl ether were added to each digestion mixture, to limit further isomerizaton of the monoglycerides(10). The lipids were recovered by extraction with chloroform-methanol (2:1) as described by Folch *et al.*(11). The lipid extract was washed 4 times with equal volumes of water to remove the free glycerol and the bulk of the Tween 80, and the washed extract was filtered into a tared flask. Some of the Tween was not removed by this washing as evidenced by a greater weight recovery than lipid added. However, during the separation of the glycerides in the silica gel column(12), the Tween present remained on the column. To prevent isomerization of monoglycerides occasioned by high temperatures, the solvent was removed under reduced pressure at temperatures which did not exceed 40°C, and the flasks were placed in a vacuum desiccator until constant weights were obtained. The extracted lipids were dissolved in chloroform and appropriate aliquots were used for analysis of the lipids. The α- and β-monoglyceride contents were determined by the periodate method of Pohle *et al.*(13) as modified for micro samples by Martin(10). The relative amounts of the various glycerides were determined by separation on a silica gel column (12).

Results and discussion. The α- and β-forms or total monoglyceride content of the lipids

TABLE II. Effect of Mucosal Enzyme on Composition of Various Monoglyceride Substrates.*

Substrate	Isomeric form	MG content of lipid extract: Initial wt of isomers (I), mg	Control (C), mg	Exp. (E), mg	Enzymatic hydrolysis of α- or β-MG: Wt loss (C−E), mg	Enzymatic hydrolysis of α- or β-MG: $\frac{C-E}{C} \times 100$, %	Total loss of MG: $\frac{(I-E)}{I} \times 100$, %
Mucosal homogenate without substrate	α	0		0			
	β	0		0			
α-Monoolein	α	140	129	85	44	34	39
	β	0	10	0	10		
β-Monopalmitin	α	7	26	25	1	4	37
	β	133	114	63	51	45	
α- and β-MG mixture	α	64	70	49	21	30	50
	β	76	71	21	50	70	

* Abbreviations and conditions are as described in Table I.

from the various digestion mixtures, before and after incubation, are shown in Tables I and II. If this enzyme preparation should aid in the hydrolysis of triglycerides as does pancreatic lipase, some free fatty acids, di- and monoglycerides would be expected to be released. Similarly from diglycerides, one would expect monoglycerides and free fatty acids to accumulate. No significant increase in total monoglyceride content was obtained as a result of enzyme action on either of these glycerides (Table I). This is in agreement with earlier results in which little or no fatty acids were freed(1). The relatively pure α- and β-monoglycerides were appreciably hydrolyzed by this enzyme. No appreciable loss of either of these 2 glycerides occurred when incubated alone or with a boiled enzyme preparation.

Interpretation of the results with the monoglycerides (Table II) is complicated by gradual isomerization of the α- and β-forms into an equilibrium mixture, previously reported (14) to be 9 parts of the α- to 1 part β-isomer. This conversion has been suggested as a nonenzymatic transformation in the lumen of the intestine during digestion of fats(15). To determine the extent of this conversion and to correct for it, incubations without the enzyme were employed(4). There is some difference as seen in the last 2 columns of Table II whether the results are expressed in terms of the disappearance of the individual monoglyceride or in terms of the disappearance of the total monoglyceride. Approximately 39% of the α-monoglyceride and 37% of the β-monoglyceride appeared to be split by the enzyme preparation when they were incubated individually.

The findings from the hydrolysis of a mixture of α- and β-monoglycerides, involving approximately equal amounts of the two forms, are difficult to explain. Nevertheless, in view of the nature of an expected change toward an equilibrium mixture, containing 9:1 α- to β-forms, a greater loss of the β-form should be expected with its greater isomerization to the α-form, and consequently less apparent hydrolysis of the α-form since it is being formed as well as being hydrolyzed. The exact extent of hydrolysis of α- and β-mono-

TABLE III. Chromatographic Separations of Lipids after Incubation with and without Enzyme.*

Substrate		Relations of individual glycerides after fractionation TG	DG	MG	Enzymatic hydrolysis of substrate Total change, %
α-Monoolein	Control (C), mg	15	9	120	
	Exp. (E), mg	23	4	62	−48
	Wt change, mg	+8	−5	−58	
	% of MG‡	+6	−3	−48	
α-Monopalmitin	Control (C), mg	17	5	103	
	Exp. (E), mg	19	8	61	−41
	Wt change, mg	+2	+3	−42	
	% of MG‡	+2	+2	−41	
Triolein†	Control (C), mg	123	17	8	
	Exp. (E), mg	119	25	10	−3
	Wt change, mg	−4	+8	+2	
	% of TG‡	−3	+7	+2	

* Abbreviations and conditions are as described in Table I. Recoveries ranged from 95 to 103%.

† Obtained from Nutritional Biochemicals Corporation.

‡ From $\frac{C-E}{\text{total MG}} \times 100$ or from $\frac{C-E}{\text{total TG}} \times 100$.

glycerides would be difficult to determine since conditions which inhibit their interconversion would probably inhibit enzymic action. The total hydrolysis of the mixture was 50% which is probably not different from the results with each isomer alone.

The results of chromatographic separation into the various glycerides of the lipids extracted after triolein, α-monoolein and α-monopalmitin were incubated with the enzyme preparation are shown in Table III. The apparent hydrolysis of these glycerides, as indicated by the decrease in substrate content, is in agreement with values obtained by both the periodate method(10,13) in Table II and the fatty acids freed as previously determined(1).

Mattson and Beck(14) and Savary and Desnuelle(16) have shown that pancreatic lipase is specific for hydrolysis of the α-fatty acid esters bonds in the triglyceride molecule and propose the mechanism to be triglyceride → 1,2-diglyceride → 2-monoglyceride. The presence of a monoglyceride lipase in mucosa of the small intestine, specific for this resulting β-monoglyceride, would aid in completing the digestion of triglycerides, finally releasing free fatty acids and glycerol. However, this appeared not to be the case in this study, since the intestinal enzyme had the ability to hydrolyze both the α- and β-monoglycerides to a comparable extent (about 40%). With both monoglycerides present in the intestinal contents after fat meals, an enzyme, which could attach either form, would seem desirable. The possibility of 2 enzymes, one for α-monoglycerides and another for the β-monoglycerides, cannot be ruled out.

The enzyme obtained by the present method seems to differ in specificity from those previously described(2,3,5), excepting the enzyme described by Schmidt *et al.*(4), Hübscher and Clark(6) and Senior and Isselbacher(7). The first authors studied hydrolysis of α-monoglyceride, but did not examine the specificity for other glycerides. They were more specifically interested in an enzyme which hydrolyzed cephalin and found this enzyme in high concentration in rat mucosa, but in negligible amounts in mucosa of the rabbit, calf, and dog. This species specificity might explain the differences in specificity of the enzyme in the present study and the enzyme of the mucosa of the duodenum of the hog as described by DiNella *et al.*(5). Hübscher *et al.*(6) and Senior *et al.*(7) did not determine the specificity of the enzyme for higher glycerides or β-monoglycerides.

The exact role of this enzyme is difficult to delineate. In the preparation employed in

this study, the enzyme appears to specifically aid in the hydrolysis of both α- and β-monoglycerides. Since several investigators, Reiser *et al.*(17) with the aid of thoracic duct cannulated animals and Skipski *et al.*(18) from *in vivo* studies, have reported that monoglycerides can be absorbed intact, this enzyme may serve some role within the cell.

Summary. An enzyme present in homogenates of rat intestinal mucosa has been found to hydrolyze long chain saturated and unsaturated fatty acid monoglycerides, but little, if any of either the di- or triglycerides. The α- and β-forms of these monoglycerides appear to be hydrolyzed to a similar extent by this enzyme preparation. The relation of this enzyme to intestinal digestion and absorption of fat is discussed.

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Normal Feed Consumption of Female Sprague-Dawley-Rolfsmeyer Rats.* (27639)

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As an introduction to a study of food restriction on thyroxine secretion rates (TSR) of female Sprague-Dawley-Rolfsmeyer rats, it became necessary to determine mean and range of feed consumption of a standard diet under *ad libitum* conditions. Data on feed consumption of other strains during various states of activity with diets containing various amounts of energy have been reported(1,2,3). Data are presented on *ad libitum* feed consumption of this strain under standardized temperature and management.

Materials and methods. Twenty-four nulliparous mature female rats averaging 265 g, placed in 4½″ × 8″ × 4½″ metabolism cages so constructed as to prevent coprophagy, were allowed several days to become accustomed to feed and water sites. Temperature was maintained at 78 ± 1°F and the rats were artificially illuminated during daylight hours. Purina Lab Chow with an energy value of 4.41 calories per gram and 23.4%

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