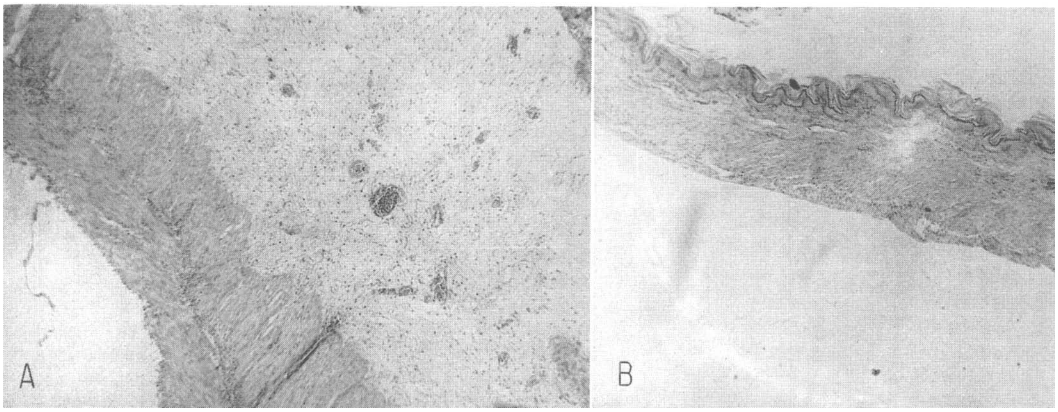

FIG. 3. Effect of COL on rumen of pylorus ligated animals. A. Rumen of untreated rat; tissues are distended by abundant edema and show marked inflammatory cell infiltration. 42 ×. B. Rumen of animal treated with COL. No edema, no inflammatory reaction. 42 ×.

men, a nonglandular structure, reacts differently than the corpus, which is the secretory portion of the stomach.

The production in the rat of ulcers in the pyloric portion following COL treatment and their expression in terms of "ulcer index" constitute the basis of an ulcer assay for steroids and will be the subject of a separate publication.

Summary. 1) Cortisol (COL) given at the daily dose of 10 mg inhibited to a large extent the development of rumenal ulcers in pylorus ligated rats. It also reduced markedly the incidence of gastric perforation. It is suggested that this protection is due to the antiphlogistic property of COL by which this hormone counteracts the initial inflammatory reaction in the rumen wall brought about normally by concentrated gastric juice. 2) At the same time, COL promoted formation of ulcers in the corpus of the stomach, whether or not the pylorus was ligated.

1. Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., Siplet, H., *Gastroenterol.*, 1945, v5, 43.
2. Haroutunian, L. M., Segal, H. L., *ibid.*, 1952, v21, 556.
3. Madden, R. J., Ramsburg, H. H., *ibid.*, 1951, v18, 128.
4. Tuerkischer, E., Wertheimer, E., *J. Endocrinol.*, 1945, v4, 143.
5. Welbourn, R. B., *Gastroenterol.*, 1953, v23, 356.
6. Gray, S. J., Ramsey, C. G., Villarreal, R., Krakauer, L. J., in: Selye, H., Heuser, G., *Fifth Ann. Report on Stress*, 1955, Acta Inc., Montreal, p138.
7. Remouchamps, L., *Ann. d'Endocrinol.*, 1954, v15, 949.

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Absorption of Free and Combined Fatty Acids.* (23924)

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Much evidence has accumulated to support two hypotheses that either fat must be either completely or only partially hydrolyzed to permit intestinal absorption(1). Recently, the latter of the two possibilities appears to have been more generally accepted. The reported rapid and selective absorption of labeled free fatty acids, when fed in small amounts mixed with a triglyceride(2), would make possible a transfer of mainly fatty acids across the intestinal cell wall as long as even

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small amounts of free fatty acids were present for absorption. Also some evidence has been presented to indicate that fed TG are absorbed to a greater extent as free FA than as glycerides(3).

To gain further insight into the major form involved in the absorption of fatty acids, an attempt has been made to follow rates of intestinal absorption of various glycerides and free fatty acids, and the effect upon absorption of their state in the intestinal tract. The finding by Mattson *et al.*(4) of appreciable amounts of the mono-, di-, and triglycerides as well as free fatty acids in the intestinal tract during fat absorption prompted the determination of the rate of absorption of each of these when fed separately. The state of emulsification and degree of hydrolysis of the intestinal lipids have been given such important roles in the absorptive process(5) that these two factors have been reexamined along with the effect of fat supplements which promote lipolysis.

Experimental. Male white rats, weighing approximately 325 g, were fasted overnight and administered a lipid by tube. Either a triglyceride (TG) as olive oil, glycerol dioleate (DG)†, glycerol monooleate (MG), or free fatty acids (FA) prepared from olive oil, were given in amounts equivalent to the fatty acids in 0.3 ml of olive oil per sq dm of body surface. A similar amount of a mixture of 2 parts TG and 1 part of MG and FA, equal to that formed by the hydrolysis of 1 part of TG, was given in like manner. Blood samples were collected at intervals for chylomicron counts. The experiment was repeated with only ⅓ of the above intake of TG or FA. The areas under the curves obtained were used as relative measure of the rate of lipid absorption(6). In another experiment after a 48-hour fast, rats were administered either olive oil, or fatty acids prepared from it. The animals were sacrificed after 3 hours and separate recoveries were made of the unabsorbed lipid from the stomach and intestines, permitting calculations of relative rates of absorption(7) based either upon total ingested lipid or upon that presented for absorption. In a study of the effect of several supplements on fat absorption, 19 rats were placed in 5 groups. All rats were fasted and given 0.15 ml of olive oil per sq dm of body surface, and the intestinal contents were recovered 2 hours later by flushing the intestines with water. The washings were made up to uniform volumes and the optical density of each was determined as a measure of the state of emulsification of the fat present, according to the method of Mead *et al.*(8). The contained lipids were recovered and titrated with alkali to determine the extent of lipolysis of the recovered lipids. Recovery of fat from only the intestines again permitted the calculation of fat absorption on the basis of the lipid which had passed out of the stomach and was readily available for absorption. In the last part of this investigation, the rates of appearance of particulate fat in the blood were followed after giving 0.3 ml of olive oil, as in the first experiment, along with a single supplement of 2 ml of 0.1 M solutions of histidine, ethionine or glycine. The method of Balls *et al.*(9) was used to determine the effect of the supplements used on the rate of fat hydrolysis.

† The mono- and dioleate were generously supplied by the Distillation Products Industries.

Results. Rates of absorption of ingested TG and DG were similar when measured by the increase of particulate blood lipid (Table I). No increase in the rate of absorption was obtained after administration of a like amount of a mixture of TG, MG, and FA previously reported(5) to best promote emulsification

TABLE I. Relative Rates of Appearance of Particulate Blood Lipid after Feeding Glycerides or Fatty Acids.

No. rats	Lipid given*	Amt fed, ml/dm²	Area under chylo. curve† 1-3 hr	"t" values
10	TG	.30	99 ± 4	
10	DG	.32	92 ± 2	1.53
10	MG	.34	55 ± 3	9.08
13	FA	.30	70 ± 3	6.11
10	TG + MG + FA	.30	94 ± 2	.91
19	TG	.10	70 ± 2	
19	FA	.10	73 ± 1	1.40

* Tri-, di- and monoglycerides are designated by TG, DG, and MG respectively. The mixture contains 2 parts of TG and 1 part of its fatty acid (FA) equivalent of MG and FA.

† Stand. errors of mean and "t" values are included. All "t" values greater than 3 are statistically significant.

TABLE II. Absorption of Lipid Fed as Free and Combined Fatty Acids.

No. of rats	Lipid fed		Lipid recovered after 3 hr		Lipid absorbed	
	As	Amt, ml/dm²	Stomach	Intestine	Based on total fed	Based on avail. fat*
			%			
9	Olive oil	.1	11	23	66	74 ± 3
8	Oleic acid	.1	50	15	35	70 ± 4
12	Olive oil	.3	34	26	40	61 ± 4
9	Oleic acid	.3	62	14	24	63 ± 7

* Available fat is total ingested less that retained in the stomach. Stand. error of mean is included.

and absorption. The lower blood fat levels after ingestion at this level of intake of MG or free FA were the result of gastric retention (Table II). However, similar blood fat levels were obtained by this method after very low intakes of either FA or TG. Both MG(10) and FA at both levels of intake disappear from the intestinal tract as rapidly as TG if only lipid that has passed from the stomach is considered (Table II).

A comparison of the state of emulsification or degree of hydrolysis of intestinal fat with the per cent of lipid absorbed was made by obtaining the ratio of either optical density (D) or the per cent hydrolysis (H) to the percentage of available fat absorbed (Table III). Variations in the ratios in the latter are much smaller (Table III). To the extent that such variations may be used as a measure of a relationship, the hydrolysis of the fat in the intestine appears to be more related to the rate of absorption than to the state of emulsification of the lipid.

A markedly accelerated rate of *in vitro* hydrolysis of olive oil by lipase in the presence of histidine has been reported by Yamamoto (11). Similar results were obtained with histidine and ethionine but not with glycine. As measured by increases in particulate blood fat, only the fat supplements which were found to increase the speed of fat hydrolysis were associated with significant increases in the rates of fat absorption (Table IV).

Discussion. Although most data from labeling technics have been interpreted as showing that not more than 50% of the TG is completely hydrolyzed before absorption, only traces of TG have been found to be absorbed unchanged(12,13). If this is true, a complete hydrolysis of half of the TG would necessarily be associated with a partial hydrolysis of most of the remaining TG and necessitate the absorption of most of the total FA as free FA.

The reported slower absorption of free FA than TG(2,14) can be attributed at least in part to a slower passage of FA from the stomach when more than very small amounts are ingested. The slightly greater amounts of FA recovered from the intestine after feeding an excess of FA with a fat was said by Borg-

TABLE III. Comparison of Variabilities in Ratios of Optical Densities or Degree of Hydrolysis of Fat in the Intestine with Fat Absorption.

Group No.	No. of animals	Intest. contents: Optical density (D)	Intest. contents: Fat hydrol. (H)	Available fat* absorbed (A)	D†/A	H/A
			%	%		
I	4	.465	43	73	.64	.59
II	4	.481	43	64	.75	.67
III	4	.683	38	57	1.20	.67
IV	3	.371	45	70	.53	.64
V	4	.590	55	80	.74	.69
		Avg ± stand. dev.			.77 ± .25	.65 ± .04
		Stand. dev. × 100/av			39	6

* Absorption values based on recoveries of available fat—total fed less that retained in the stomach.
† Optical density values × 100.

TABLE IV. Effect of Histidine, Ethionine and Glycine Supplements on Fat Hydrolysis and Absorption.

No. rats	Fat supplement As	Fat supplement Hydrol. ratio*	Area under chylo. curve 1-3 hr†	"t" value
9	None	1.0	94 ± 4	
10	Histidine	2.5	136 ± 3	3.38
10	Ethionine	2.0	141 ± 6	7.98
7	Glycine	1.0	96 ± 6	

* Ratio of hydrolysis of lipid with .02 M supplements as compared with that of none.

† Stand. error of mean included. All "t" values over 3 are significant with a P value of less than .01.

ström(2) and Mattson *et al.*(4) to indicate a limited absorption of FA. In this study, the smaller amounts of lipid found in the intestine after feeding only FA, as compared with that after TG ingestion (Table II), suggests that FA are readily absorbed when available for absorption. Undoubtedly, small amounts of free FA when fed as such are absorbed at least as rapidly as the FA of the readily hydrolyzable TG and DG (Tables I, II).

The constancy of the contents of the lumen, almost regardless of the form in which the fatty acid is ingested(4), certainly cannot be accepted as proof of the absorption in any one form. It could well be a means of preventing any unphysiological effect of excessive amounts of free FA in the intestine. Normally, instead of flooding the gastrointestinal tract with large quantities. of free acid, a small amount of FA is slowly released by a continuing hydrolysis and constantly removed by absorption. A selective absorption(2) of any FA present could promote the transfer of most of the lipid in that form.

The importance of the role of FA in the absorptive process is suggested by 4 findings in this study. (1) The free FA appear to be at least as rapidly absorbed as are those of TG (Table II) if only the amounts actually available for absorption are considered. (2) The percentage of FA in the intestines bears a much less variable relationship to rate of absorption than degree of emulsification (Table III). (3) An attempt to promote emulsification by the presence of an ideal mixture of fat with MG and FA, according to Frazer(5), did not hasten the absorptive rate (Table I). (4) Furthermore, fat supplements which accelerate the action of the lipolytic enzyme are associated with marked increases in rate of absorption of the ingested fat (Table IV). This finding supports the suggestion(15) that a direct relationship exists between rate of hydrolysis and speed of absorption.

Indisputable evidence is not available regarding the exact quantities of the various forms in which the fatty acids are absorbed. Results of this study are in agreement with the suggestion that the major part of the fat absorbed from the intestinal lumen may be in the form of the unesterified FA.

Summary. Evidence is presented which suggests that free FA are at least as rapidly absorbed as those of the readily hydrolyzable glycerides when the rate is measured either by increased blood fat levels or by disappearance of available lipid from the intestinal tract. The absorptive rate appears to be more related to the degree of hydrolysis of the intestinal lipid than to its state of emulsification. Conditions which promote lipolysis increase the rate of fat absorption. These findings support the hypothesis that the major part of the fat absorbed probably is in the form of free FA.

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1. Deuel, H. J., Jr., *The Lipids—Their Chemistry and Biochemistry,* Interscience Publishers, New York 1955, v2, 137.

2. Borgström, B., *Acta Physiol. Scand.,* 1952, v25, 291, 328.

3. Borgström, B., Tryding, N., Westöö, G., *ibid.,* 1957, v40, 241.

4. Mattson, F. H., Benedict, J. H., Beck, L. W., *J. Nutr.,* 1954, v52, 575.

5. Frazer, A. C., *Physiol. Rev.,* 1946, v26, 103.

6. Burr, W. W., Jr., Dunkelberg, C., McPherson, J. C., Tidwell, H. C., *J. Biol. Chem.,* 1954, v210, 531.

7. Deuel, H. J., Jr., Hallman, L., Leonard, A., *J. Nutr.,* 1940, v20, 215.

8. Mead, J. F., Bennett, J. R., Decker, A. B., Schoenberg, M. D., *ibid.,* 1951, v43, 477.

9. Balls, A. K., Matlack, M. B., Tucker, I. W., *J. Biol. Chem.,* 1937, v122, 125.

10. Tidwell, H. C., Johnston, J. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 150.

11. Yamamoto, T., *J. Biochem.* (Japan), 1951, v38,

147, 277.

12. Reiser, R., Dieckert, J. W., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 649.

13. Dowse, C. M., Saunders, J. A., Schofield, B., *J. Physiol.*, 1956, v134, 515.

14. Reiser, R., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 666.

15. Reiser, R., Bryson, M. J., Carr, M. J., Kuiken, K. A., *J. Biol. Chem.*, 1952, v194, 131.

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Cerebrospinal Fluid Electrolytes after Death. (23925)

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While isolated data on electrolytes in postmortem cerebrospinal fluid have been reported (1,3,5), a systematic study of the principal ions has not been available. The present report gives analyses of 157 postmortem cerebrospinal fluids and includes values for cations, anions and pH.

Materials and methods. The material consisted of 131 male bodies, average 57 years old, of white and colored race, autopsied on the average 10½ hours after death. A second series was used for pH determinations only and consisted of 26 cases, 62 years average age, examined after an average 5½ hour postmortem interval. To obtain data, which may be valid as "normals," cases were selected on the basis of pathological changes presumably unrelated to electrolyte metabolism. Cerebrospinal fluid was removed by cisternal puncture and, if free of gross contamination by blood or brain tissue, was used after centrifugation. The following analytical procedures were employed: flame photometry for sodium and potassium(4); Clark-Collip's method for calcium(2); Denis-Briggs' method for magnesium(2); Van Slyke-Hiller's modification of Sendroy's method for chlorides(2). The CO_2 values were previously published(5) and obtained by Van Slyke's gas volumetric method(2). Measurements of pH were performed at 37.5°C (within 60 min. after removal) with a Beckman pH meter and a blood electrode assembly provided with rubber cap, through which the spinal fluid was injected directly from syringe or after centrifugation under oil.

Results. Average and range of cations, anions and pH in postmortem cerebrospinal fluid 10½ and 5½ hours after death respectively are compiled in Table I and compared to corresponding antemortem values. It will be noted that there is a marked increase of potassium and inorganic phosphorus, a moderate increase of magnesium and a slight to moderate decrease of sodium, chlorides and HCO_3 after death, while calcium values approach closely those found during life. There is, however, more or less marked scattering of all values over wide ranges. The postmortem values of pH are considerably lower than antemortem figures.

The acid-base equilibrium in postmortem cerebrospinal fluid as seen in Table II shows fair agreement of the total milliequivalents of cations with the antemortem figure due to the fact that the sodium deficit is compensated by the potassium increase. On the other hand, there is a large deficit of 24 milliequivalents of anions due to the decrease of chlorides and HCO_3 ions in postmortem cerebrospinal fluid and insufficient compensation by the increase of phosphorus. This result causes an apparent shift of the acid-base equilibrium to the side of base.

Discussion. Mason, Klyne and Lennox(3) first observed the marked postmortem rise of cerebrospinal potassium, which however, failed to show close correlation with time after death except on a statistical basis. These findings were borne out by the present investigation, and here again a constant time relation was absent in regard to postmortem changes of sodium, chlorides and phosphorus. The increase of potassium and the decrease of sodium and chlorides are obviously due to autolytic changes of cell membranes with resultant greater permeability, so that intra-