duce an increase of amylase activity and intermediate concentrations cause first an increase in activity with time and then a decrease. This indicates that the process is more complicated than just that of the ionic binding of protamine to amylase with a masking of the active site or sites. Caldwell and her co-workers(9) have shown that the free amino groups of hog pancreatic amylase are essential to its activity but no reference in the literature to the role of carboxyl groups can be found.

The observation that heparin added to protamine before amylase prevents the inhibition by protamine, but will not reverse the inhibition if added after protamine and amylase have been allowed to react, could perhaps be explained in several ways. The most likely explanation is that some irreversible change takes place in the amylase in the presence of, or after combining with protamine.

Summary. Protamine, which at low concentrations caused slight enhancement of the activity of hog pancreatic amylase, was inhibiting at higher concentrations. This inhibition could be prevented by heparin but heparin would not reverse the inhibition once established. Heparin alone produced some enhancement of amylase activity. It is postulated that part of the explanation for the inhibiting effect of protamine is the combination with negatively charged side chains on the amylase followed by some irreversible change in the amylase.

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Fat Digestion and Absorption in the Proximal Intestine of the Dog.* (27267)

LEON K. KNOEBEL (Introduced by E. E. Selkurt)
Department of Physiology, Indiana University School of Medicine, Indianapolis

Most investigations which deal with the digestive processes of the gastrointestinal tract utilize animals in acute experiments in which a test meal is fed, the animal is killed after a certain time, and the gastrointestinal contents are analyzed for the products of digestion under consideration. This approach simply supplies information regarding digestion at one particular time following feeding. Furthermore, a quantitative study of absorption cannot be made unless unabsorbed material from the entire gastrointestinal tract and feces is recovered. Another approach to the problem of digestion and absorption is the use in chronic experiments of animals with fistulas established in various regions of the intestine. Intestinal contents can be quantitatively collected from a fistula during the entire passage of a test meal through the intestine and analyses of the material so collected give data regarding the overall result of the activity of the digestive enzymes. In addition, absorption can be quantitatively evaluated, since the material recovered from a fistula represents that which was not absorbed to the point of collection. Jejunal fistula dogs were used in this study to investigate the proximal intestinal digestion and absorption of a common dietary triglyceride, cottonseed oil. Data of general interest concerning how the gut functions as chyme passes through the intestinal tract were also obtained.

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Methods. Maydl jejunostomies(1) were prepared in 2 dogs by transecting the jejunum

TABLE I. Digestion* and Absorption of Cottonseed Oil by Jejunal Fistula Dogs.

Dog No.	No. of runs	Time of run (hr)	Vol recovered (ml)	Fat absorbed (%)	Monoglycerides (%)	Free fatty acids (%)	Di- and triglyceridest (%)
1	13	7.9 ± .1‡	788 ± 35	69.9 ± 4.3	6.5 ± .4	29.3 ± 2.2	64.4 ± 2.4
2	13	9.9 ± .7	370 ± 29	55.0 ± 7.0	10.6 ± .8	25.7 ± 3.4	63.7 ± 3.9

* All values represent percentage composition by weight.

† Combined diglycerides and triglycerides were determined by difference between other fractions and 100%.

‡ Mean ± stand. error of mean.

approximately 50 cm below the ligament of Treitz and anastomosing the proximal to the distal end of the gut 10 cm below the point of transection by an end-to-side anastomosis. The distal end was passed through a stab wound in the belly wall, sutured firmly in place to the fascia, and the mucosa was turned out and down by stitching the submucosa to the skin. Because peristalsis is away from the external opening, food and secretions ordinarily did not pass to the outside through the fistula. Movement of the intestine is not impaired by the operation and both dogs remained in excellent health during the studies. Following a 24-hour fast, the dogs were comfortably secured in a stall and a double-lumen rubber catheter inserted into the fistula in such manner that a latex balloon tied to the end of the catheter, and which could be inflated through one lumen, was just distal to the jejunal anastomosis. After a preliminary aspiration, a test meal consisting of 25 g cottonseed oil, 25 g casein, and 25 g sucrose was fed and intestinal contents were quantitatively withdrawn under slight negative pressure through the other lumen into a 100 ml graduated cylinder immersed in an ice bath. The collecting cylinder was replaced every 30 minutes, the volume of each fraction measured, pH determined by means of a Beckman potentiometer, and the contents diluted with 4 times their own volume of a mixture of 50-50 ethyl ether-ethyl alcohol. Certain criteria were chosen to indicate when a run had come to end; *i.e.*, when the upper gastrointestinal tract was devoid of food. These were (1) a fraction collected over a half-hour period should be of small volume and free of solids, (2) this collection should give a negative Benedict's test, and (3) the fraction should have a pH approaching that of the pre-feeding period or if, as in most cases, no sample was obtained before feeding the test meal, pH of the final collection should approach or exceed neutrality. The run was considered terminated when 2 successive samples met these requirements. All samples for each run were pooled and total fat isolated as described previously(2). Extent of lipolysis was determined by free fatty acid and monoglyceride assays. Free fatty acids were titrated in 95% ethanol with alcoholic 0·1 N KOH to the phenolphthalein endpoint and monoglycerides were determined by the iodometric technic of Pohle and Mehlenbacher (3). That part of the recovered lipid not accounted for as free fatty acids and monoglycerides was assumed to consist of diglycerides and triglycerides. Percentage fat absorption was calculated by the following equation:

$$\frac{\text{g fat fed} - \text{g fat recovered}}{\text{g fat fed}} \times 100.$$

Results and discussion. Each dog was used in 13 runs and the data obtained, some of which have been previously presented(2), are given in Table I. Relatively long periods of time were required for complete transit of unabsorbed chyme to the fistulous opening. The average total volume of contents collected from Dog 2 was only approximately one-half that recovered from Dog 1. This large variation can probably be accounted for by the fact that Dog 1 was the larger of the two and willingly drank the 50 ml of water which was offered every 2 hours during a run, whereas Dog 2 often refused to drink. This variation seemed to have no effect on digestive and absorptive processes.

That the proximal intestine is capable of absorbing fat quite efficiently is shown by the fact that in both dogs considerable amounts of fat were absorbed over this relatively short length of intestine. This is in accord with observations on humans(4) in which absorp-

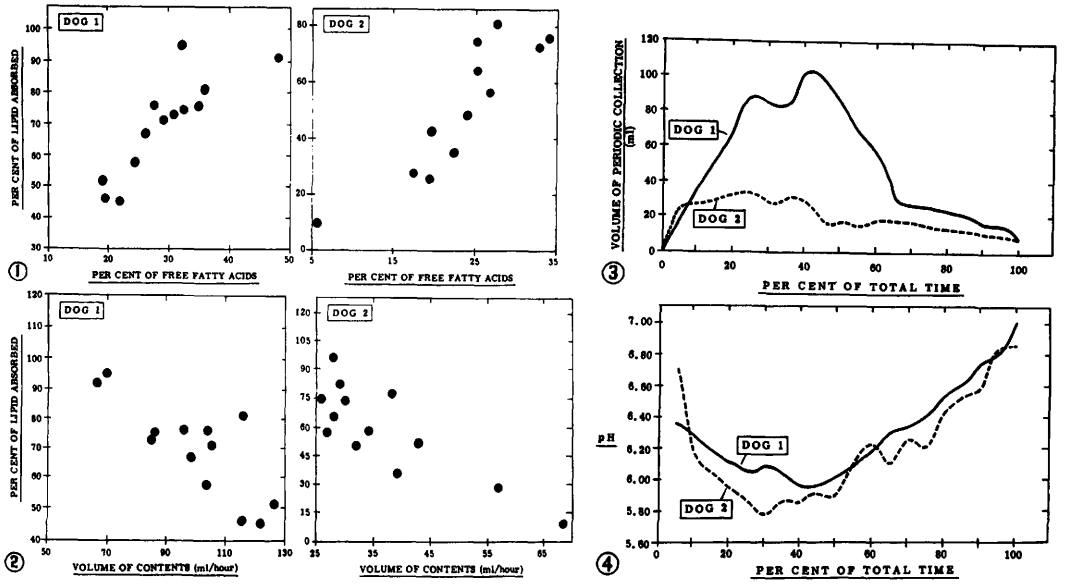

FIG. 1. Relationship of percentage of lipid absorbed to percentage of free fatty acids in unabsorbed lipid in jejunal fistula dogs fed cottonseed oil test meals.

FIG. 2. Relationship of percentage of lipid absorbed to volume of jejunal contents collected from jejunal fistula dogs fed cottonseed oil test meals.

FIG. 3. Volumes of intestinal contents collected from jejunal fistula dogs during complete gastric evacuation of cottonseed oil test meals.

FIG. 4. pH of intestinal contents collected from jejunal fistula dogs during complete gastric evacuation of cottonseed oil test meals.

tion of fat has been shown to be complete in the proximal 100 cm of jejunum, but is at variance with results obtained on dogs(5) in which the distal intestine was reported to be the major site of fat absorption. Average values reveal that the unabsorbed fat contained moderate amounts of free fatty acids and monoglycerides, with the bulk of this fat consisting of di- and triglycerides. Similar values have been reported for fat recovered in acute experiments from the jejunal contents of dogs fed cottonseed oil triglycerides(6) and for fat recovered in chronic experiments from jejunal fistula dogs fed olive oil(7).

When individual runs were considered, it was obvious that the extent to which cottonseed oil triglycerides were hydrolyzed to free fatty acids and monoglycerides varied considerably in the same dog. Furthermore, a relationship was evident between the percentage of free fatty acids in the recovered lipid and the percentage of fat absorbed. Fig. 1 shows these values plotted against each other. It is apparent that more fat was absorbed when the concentration of free fatty acids in the intestinal lipid was increased. The same relationship held true for monoglycerides. These results support the suggestion(8,9) that a relationship exists between rates of lipolysis and of fat absorption. The implication of such a relationship is that fat is better absorbed when it is more completely digested. However, it is conceivable that both the extent of digestion and of absorption of fat in these experiments are mutually dependent on another function, rather than on each other. For example, both fat digestion and absorption were found to be related to the volumes of contents collected per unit time during the runs. Fig. 2 shows the percentage of fat absorbed plotted *vs.* the average volume of contents collected per hour for each run for both dogs. It is apparent that the absorption of fat was diminished when the rate of collection of contents was increased. This was also the case for lipolysis. Although there are alternative explanations for these relationships, one possible interpretation is that accelerated gastrointestinal motility could cause a rapid movement of chyme through the intestine with the result that fat and other substances have less opportunity to be digested and ab-

sorbed; *i.e.*, relatively large volumes of poorly digested and unabsorbed contents would be collected per unit time. Consequently, although it is possible that rate of fat absorption is directly dependent on degree of intraluminar hydrolysis, this conclusion cannot be drawn from experiments in which no assessment is made of other variables which might affect digestion and absorption in the same direction.

Data obtained on the volumes of individual collections of intestinal contents were evaluated for both dogs. The volumes recovered at each 30-minute interval for each run were plotted on the basis of 100% of the average total collection time. Average volumes were then determined for every 5% time interval and final values are plotted in Fig. 3. A large proportion of the contents was collected when a run was 50% completed. Relatively large volumes of contents passed through the intestine during this time, after which these gradually diminished to very low levels until the stomach was empty.

The pH of the intestinal contents of both dogs was also plotted against the percentage of average total collection time (Fig. 4). The pH of the contents recovered at the beginning of a run was slightly on the acid side of neutrality and fell to a value somewhat lower than 6.0 when a run was approximately 50% completed, rose during the remainder of a run, and finally approached neutrality. The maintenance of intestinal content pH within a small range during the entire course of a run is in accord with results(7) obtained from jejunal fistula dogs in which the pH of jejunal contents was reported to be very constant between 6.0 and 6.5 on different days after feeding fat-containing test meals. In addition, the pH of human duodenal and jejunal contents was found to be very constant around 6.0 as measured by continuous recording of pH during passage of a fatty test meal through these regions(4). The observation that the intestinal contents were acid during the entire passage of chyme through the intestine is important when it is realized that the water-soluble soaps of fatty acids can exist in only small amounts in an acid medium(10). This does not support the theory(11) that soap formation plays an important role in absorption of fatty acids.

Summary. Two jejunal fistula dogs were fed a mixed test meal containing cottonseed oil triglycerides as the source of fat. Quantitative collection of material not absorbed during the complete transit of chyme to the fistulous opening revealed that cottonseed oil triglycerides were efficiently absorbed (69.9% and 55.0%) in the proximal intestine. Analysis of unabsorbed fat for free fatty acids and monoglycerides showed this lipid to be moderately well digested. Although absorption was increased when digestion was more complete, both the extent of digestion and absorption of fat could have depended primarily on variations in other gastrointestinal processes. Consequently, it cannot be concluded from these experiments that fat absorption is enhanced by increased lipolysis. The importance of soap formation in fat absorption is minimized by the fact that intestinal contents were almost always acid during the course of collection.

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