Conservation of Digestive Enzymes

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I. Introduction

In this review we summarize experiments whose implications were of great interest when they were first reported. They provided unexpected evidence that the conventional belief that every meal is digested by an entirely new complement of digestive enzymes is secreted by the pancreas into the small intestines with each meal is incorrect. The data suggested that instead of being completely degraded in the small bowel with the food they digest, a large fraction of the digestive enzymes secreted by the pancreas are absorbed and recycled in an enteropancreatic circulation (Fig. 1).

Not long after the original findings were reported (20, 48), the idea and the evidence were variously affirmed and questioned by additional experimentation (26, 37, 44, 74, 76–78). Disagreement ensued, and the subject became controversial (6, 42, 75, 79, 87–90, 94). This was not just because of the surprising nature of the observations, but also because they seemed to require that the proteins cross several cellular membranes in transit, a possibility viewed as unlikely if not impossible at the time. In addition, the exact chemical forms of the circulating proteins were not known. Were they modified, and if they so, in what way? And, finally, it turned out that the negative
studies were not failed attempts to reproduce the original observations, but different experiments altogether that neither proved the circulation hypothesis false nor explained the affirmative evidence in other ways. Given these uncertainties, research on the topic soon ended, leaving the existence of digestive enzyme conservation an unsettled matter, seemingly neither adequately confirmed nor adequately refuted. Indeed, the evidence has never been the subject of retrospective review and analysis. This is our intention here some 25 years after the initial discoveries. What did the evidence show, what didn’t it show, and what remains to be learned?

II. FOUNDATION OF THE TRADITIONAL VIEWPOINT: THE SINGLE PASS

It may seem surprising, but the traditional understanding that the pancreas produces an entirely new complement of enzymes with each meal is not based on direct experimental evidence that this is the case, or even on evidence that it is capable of such replacement. Nor is it based on proof that the digestive enzymes are completely degraded in the small intestines subsequent to their secretion, thereby requiring their replacement. What is known is that 95–99% or almost all digestive enzyme activity disappears as the chyme passes down the small bowel into the colon (8, 40, 51, 68). This has been assumed to be the consequence of enzyme degradation in transit and it alone.

This view is grounded in two presumptions. First, because mixtures of activated digestive enzymes, for example, from pancreatic juice or tissue homogenates, left standing at room temperature lose their activity over time, presumably due to auto- and heterocatalytic catalysis. The amino acid products are then absorbed across the intestinal epithelium into blood. An equivalent amount of amino acid is accumulated by pancreatic tissue, and the cycle is completed when these amino acids are incorporated into new proteins that are secreted by the pancreas in the ensuing period. According to this scheme, each meal is digested by a completely new complement of digestive enzymes synthesized during the interdigestive period. An alternative model that is supported by results discussed in this review. From this perspective, only a small fraction of the digestive enzymes secreted by the pancreas into the intestines is degraded during a single cycle of digestion (the degradative cycle is shown by narrow arrows on the right). Most is absorbed into blood, accumulated by the pancreas, and secreted during the next digestive cycle (shown by the broad arrows on the left). In this scheme, the pancreas does not produce a new complement of digestive enzymes for each meal but conserves and reutilizes these protein products.
The second presumption is that the epithelium of the small bowel is completely impervious to proteins. If this were true, then the only way that the digestive enzymes could escape the intestinal enclosure would be by their movement aborally into the large bowel. Because almost all activity is lost during this passage, some sort of inactivation, presumably due to proteolytic degradation, would have to occur. Throughout the 20th century there was confidence, bordering on certainty, that biomembranes were impermeable to protein molecules. Not only was there no direct experimental evidence for this conclusion, but there was considerable evidence to the contrary for the epithelium of the small intestines. It appeared to be permeable to a variety of protein molecules, including various digestive enzymes (1–4, 9, 13, 22, 32–34, 41, 52, 53, 66, 99–101). These results, however, were judged to be artifactual, pathological, quantitatively trivial, or exceptions to a general rule of impermeability. Whatever the evidence, the intestines of adult animals were widely believed to be impermeable to proteins, including digestive enzymes.

This conclusion was reinforced by a third presumption. It was believed that if digestive enzymes, particularly active proteolytic enzymes, entered the bloodstream, massive systemic pathology would result, including the uncontrolled “digestion” of the body’s organs. This would of course be life threatening, and because digestion normally occurs in the absence of such a cataclysm, it was safe to assume that digestive enzymes were not absorbed into blood.

However, two unavoidable facts argued against this conclusion. First, digestive enzymes, including various active proteolytic forms, were normal, even appreciable, constituents of blood (5, 7, 10, 11, 15, 17, 18, 36, 38, 39, 43, 73, 93, 96, 97, 98, 104). Although their presence might primarily reflect release directly from the pancreas into the bloodstream, their intestinal absorption could not be excluded. Indeed, it was suggested by the presence of active proteolytic digestive enzyme. Second, blood plasma contained a variety of enzyme inhibitors, particularly protease inhibitors, whose presence could, and indeed seemed designed to, prevent or mitigate pathological occurrences that might occur pursuant to the absorption of active enzyme. In fact, the second most abundant plasma protein after albumin was a trypsin inhibitor, α₁-antitrypsin.

It was the realization that digestive enzymes might be absorbed into blood that gave rise to the idea of an enteropancreatic circulation. It had been discovered that some of these proteins could be transported from the interstitial space of pancreatic tissue across the basolateral surface of its secretion cells (29, 30, 45, 47), and it was appreciated that material accumulated by these cells might subsequently be secreted into the gland’s duct system and enter the intestinal lumen along with endogenous digestive enzymes. As a consequence, if digestive enzymes could be absorbed from the intestines into the bloodstream, they could be taken up by the cells of the pancreas and be secreted into the intestines where they would be reutilized.

If such a process existed for substantial quantities of digestive enzymes, they might be recirculated in a manner similar to bile salts. Bile salts are released into the gut from the liver and gallbladder where they emulsify fat. After fat absorption takes place, the unneeded bile salts are absorbed across the ileum, reaccumulated by the liver, conserved, and secreted again with the next cycle of digestion. This is called the enterohepatic circulation. The liver only manufactures a small fraction of the bile salt pool de novo each day, and the possibility that digestive enzymes, which are far more energetically costly to manufacture, indeed are among the most costly biomolecules to synthesize, might be involved in a similar conservation process was intriguing.

The capacity to conserve digestive enzymes was first tested in the mid 1970s. Two types of experiment were carried out. In the first, the permeability of the relevant membranes (pancreatic and intestinal) to particular digestive enzymes was assessed in vitro. Did processes exist that would allow for a circulation? Second, given their existence, could circulation be demonstrated in intact animals?

### III. MEMBRANE PERMEABILITY

#### A. Intestinal Epithelium

The ileum was found to be permeable to the two digestive enzymes that were tested, chymotrypsinogen and amylase, using chymotrypsinogen and amylase, using everted sacs of intestine (48) as well as pieces of intestine as membranes separating two halves of an incubation (Ussing) chamber (21) to measure transport. After labeled chymotrypsinogen was added to the medium bathing everted sacs of rabbit ileum (the mucosal compartment), it appeared in the internal medium of the sac (the serosal compartment) at a rate some 10 times that seen for a control protein, albumin (48). Similarly, amylase (55 kDa) appeared in the serosal medium of an Ussing chamber soon after being added to its mucosal compartment (21). In this case, the enzyme was transported at a rate two to three orders of magnitude greater than seen for the control molecule, the much smaller polysaccharide inulin (∼9 kDa). In this study, enzyme activity, rather than radioactivity, was followed to ensure that the molecule had not been degraded en route. Amylase was transported intact across the epithelium in both directions, but was preferentially absorbed by an active transport process (Fig. 2) (21).

It was possible to estimate the absorptive capacity of...
the intestines from these measurements. With the use of conservative assumptions (that transport only occurs across ileal epithelium and that the maximal rate possible was being observed under the particular conditions of the study), 50–65% of the amylase secreted by the rabbit pancreas in response to maximal stimulation would be absorbed over a comparable period of time (21). Thus, at least as a first-order approximation, the potential existed to transport sufficient quantities of amylase across the intestinal epithelium to support a significant conservation of this enzyme.

B. Transpancreatic Transport

Likewise, the potential for transport across the pancreatic epithelium, i.e., from the interstitial spaces of the gland to its duct system, was assessed. An organ culture preparation of rabbit pancreas was used for this purpose. The rabbit pancreas is extremely thin and can be studied whole in vitro without vascular perfusion (80, 86). The whole organ is removed from the animal and placed in an incubation chamber where it is bathed by a physiological salt solution. The gland’s exocrine secretion can be collected pure and undiluted by cannulating the pancreatic duct. As in the intestinal studies, amylase and chymotrypsinogen were the test molecules.

When radiolabeled forms of these enzymes were added to the medium bathing the gland, providing access to its interstitial fluid compartment, they appeared in ductal fluid over time. Like the ileal epithelium, the pancreatic epithelial layer was permeable to these proteins. Also, like the intestines, transport seemed to be selective. For example, labeled chymotrypsinogen was secreted at 20 times the rate observed for the control protein albumin (48). Again, the secretion of the exogenous material was also measured as enzyme activity (31) to demonstrate that the enzymes were transported across the gland intact and functional.

The rate of transpancreatic transport could be substantial, greatly surpassing basal rates of endogenous secretion. For example, maximal transpancreatic amylase secretion was some 15 times the rate of unstimulated endogenous secretion, or ~20% of a maximal endogenous response to pharmacological stimulants. For chymotrypsinogen, transpancreatic secretion was 7.5 times the unstimulated endogenous rate, or ~30% of a maximal response. This was in the absence of stimulants such as the hormone cholecystokinin (CCK) or acetylcholine that were found to greatly enhance transpancreatic transport as well as endogenous secretion (28, 48).

Transport across the pancreas was discovered to be the result of protein movement through the gland’s secretory epithelial or acinar cells, not extracellularly via paracellular shunts (31, 55–57, 92). For example, when chymotrypsinogen was added to the bathing medium several hours before applying a maximal cholinergic stimulus, the response elicited by the stimulant subsequently was more than three times the maximal response seen without pre-incubation (31) (Fig. 3). This salutary effect was the result of the prior uptake and storage of the exogenous enzyme by the secretion cells. If endogenous amylase pools were labeled with radioactive amino acids, the addition of unlabeled amylase to the medium bathing the gland not only reduced the specific radioactivity of the secreted amylase by some 90%, meaning that ~90% was of exogenous origin, but produced a large (72%) absolute reduction in the secretion of endogenous (labeled) protein (31). This was the result of the mixing of exogenous and endogenous enzyme in the cell and their subsequent competition for exit from it.

IV. CIRCULATION MEASURED IN SITU

A. Circulation of Intact Digestive Enzymes by Tracer Analysis

A variety of studies had established that intestinal and pancreatic epithelia were permeable to digestive enzymes and that this permeability was of sufficient magnitude to accommodate a conservation process. Although
the fact that the pertinent membranes were permeable suggested that circulation would occur given the opportunity, it was important to demonstrate in situ that it did. To test for circulation, labeled chymotrypsinogen was instilled into the upper duodenum of anesthetized rabbits, and secretory fluid was simultaneously collected from the cannulated pancreatic duct to monitor for its appearance (Fig. 4). Shortly after intestinal instillation, a small amount of labeled protein (48) (Fig. 4) was recovered in ductal fluid. Electrophoretic profiles established that the label was still associated with chymotrypsinogen and had not been reincorporated into the various endogenous proteins secreted by the gland.

B. Magnitude of Circulation

Although this confirmed the existence of an entero-pancreatic circulation, a different approach was needed to assess its magnitude and hence the possibility of conservation. To make such a determination, the secretion of digestive enzymes by the pancreas of anesthetized rabbits was followed in three different experimental situations (Fig. 5) (20). In the first situation, secretion was collected directly from the duct system of the gland via a catheter and permanently removed from the animal. Equal amounts of albumin were instilled into the intestines instead of the secreted enzymes. In the second experimental situation, after removal of a small aliquot for the measurement of its protein content, the secreted fluid was immediately returned to the intestinal lumen close to the site of its original secretion to follow its normal route. In the third situation, the secreted fluid was collected and injected into circulatory system via a large vein, rather than being allowed to enter the intestinal lumen. All of this was done in the continued presence of a maximal cholinergic stimulus to drive protein secretion.

In the absence of enzyme circulation, there was no reason to expect that the diversion of the secreted proteins from the animal would influence the subsequent rate of protein secretion by the gland. That would be determined by the cholinergic agonist acting locally on the secretion cells, and there was no basis for thinking that the removal of already secreted protein from the body would affect its presence. For the same reason, reintroducing the secreted enzymes into the intestines, or injecting them into the bloodstream, was not expected to affect
From the single pass perspective, whatever one might do to the material after it was secreted, removing it from the animal, letting it enter the duodenum, injecting it into blood, or anything else that we might think of, would not be expected to affect the ensuing rate of secretion.

On the other hand, if enzyme conservation occurred, under the right circumstances protein secretion would be altered by each of these manipulations. It would be reduced if sufficient circulating enzyme was removed from the animal by its diversion. Whereas the reinstillation or intravenous injection of the same material would ameliorate that reduction by reintroducing the diverted enzyme and allowing it to be recycled. Furthermore, at steady state, it would be immaterial whether the material had been added back to the intestinal lumen or injected directly into blood. The effect would be roughly the same either way, since only the location of reintroduction would have differed.

As it turned out, all three conditions, diversion, intestinal instillation, and intravenous injection, altered secretion by the gland and in the particular ways predicted by the circulation hypothesis. When the secreted material was diverted from the gut, protein secretion by the gland declined in a roughly exponential fashion (Fig. 5). Both reinstillation into the intestines and intravenous injection of the secreted material ameliorated this decline and to approximately the same degree (Fig. 5).

The efficiency of circulation could be estimated by comparing the rate of secretion with diversion to that after readministration of the secreted material (either intestinal instillation or vascular injection). When the amount readministered was plotted against the increase in pancreatic secretion, input was related to output by a linear function with a slope of 0.92 (Fig. 6). That is, the efficiency of circulation was 92%. In accordance with the theory, the data fit the same function whether the material was added to the intestines or injected into the bloodstream.

Perhaps the most convincing observation was that protein secretion by the pancreas could be restored when pancreatic fluid was injected into blood. This made no sense in terms of a single pass mechanism. Why would digestive enzyme in blood increase the rate of protein secretion? The fact that the effect of intestinal instillation and intravenous injection were comparable seemed to rule out the possibility that digestive enzymes in the intestines had stimulated secretion by means of some sort of intestinal nervous or hormonal action.

FIG. 5. The bulk circulation of digestive enzyme. Top: drawing shows three experimental conditions: 1) pancreatic secretion (open circles) diverted from the animal via a catheter, 2) pancreatic secretion returned to the duodenum after a sample is removed for measurement of its protein contents, and 3) pancreatic secretion injected intravenously. Intestinal instillation and intravenous injection were carried out every 30 min. A cholinergic agonist (methylcholine chloride) and the peptide hormone secretin were injected at half-hourly intervals for the duration of the experiment, starting at time 0. Bottom: graph shows the amount of protein in pancreatic secretion after the 3 different treatments. The instillation or intravenous injection of the secreted protein (2 and 3) enhanced subsequent protein secretion two- to threefold compared with the diverted control (1). [Modified from Goetze and Rothman (20).]
Circulation seemed to be a process of substantial magnitude for the particular conditions of study. We underline this phrase to stress the importance of appreciating the circumstances in which circulation was examined. The results demonstrated, and only demonstrated, that when secretion is driven by maximal cholinergic stimulation for some 5 h in animals that were previously fasted overnight, circulation was substantial. A period of maximal, prolonged stimulation was chosen to ensure depletion of tissue enzyme contents, and the animals were fasted overnight to ensure the presence of substantial enzyme stores in the pancreas initially and to provide a more or less food-free intestinal tract.

One would not expect to see a similar effect in any and all circumstances, notably in the absence of substantial and prolonged stimulation of secretion to mobilize large amounts of product for circulation, or in the absence of an appropriate fast to maximize pancreatic enzyme contents and provide an empty small bowel. In other conditions, for example during unstimulated secretion or after short periods of stimulation, the amount circulating might be small and difficult to measure. And if the animals were not fasted, the presence of food in the intestines might delay circulation in an uncharacterized, meal-dependent manner as food substrate competed with the absorptive process for the enzymes.

C. Effect on the Plasma Pool

Although these experiments provided convincing evidence for bulk circulation, they did not actually follow the movement of the secreted products through the relevant compartments, most importantly into and through blood. This was subsequently shown in awake rats. As in the rabbit studies just discussed, rats were given a maximal cholinergic stimulus for an extended period of time (3 h), after which the distribution of amylase in various organs and tissues of the body was assessed and compared with that observed before stimulation (61). Amylase distribution was appraised by measuring the enzyme’s activity, rather than by means of its physical separation or by radioactive labeling, to ensure that the distribution of active enzyme was being surveyed.

The cholinergic stimulant was known to produce a significant reduction in the amylase content of the rat pancreas and to increase amylase secretion into the intestines by at least an order of magnitude over unstimulated rates (81). Under these conditions, the decrease in the enzyme contents of the gland is roughly equal to the amount it secretes. If recirculation occurred efficiently, then reducing the tissue content of digestive enzyme should add approximately the same amount of protein to the circulating pools and should be measurable there.

After an overnight fast (again, to maximize tissue content and minimize food in the small bowel), over 99% of the amylase activity in the rat was found in three compartments: the pancreas with 92.1%, the intestines (mucosa plus contents) with 5.9%, followed by the plasma (plus interstitial fluid) with 1.3% (61). How did things change after a 3-h course of stimulation? According to the single pass hypothesis, the amylase lost from the pancreas should be found exclusively in the intestines, primarily in the small intestines, minus whatever degradation had taken place.

Remarkably, after 3 h of continuous highly augmented secretion into the small intestines, no significant elevation in amylase activity was seen at this location (Fig. 7). That copious amounts of the enzyme had entered the small bowel could be confirmed by large increases in amylase activity seen in the cecum (two orders of magnitude above controls levels) and colon (61). Why wasn’t there a comparable, or even a greater increase, in the small intestines? This could be explained in three ways. The secreted amylase might have simply passed into the

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**FIG. 7.** Change in the percentage of the whole body distribution of amylase activity attributable to the pancreas, small intestine (intestines), and blood plus interstitial fluid (blood) after 3 h of cholinergic stimulation (depletion, stippled bars) and a subsequent 2-h period of recovery (solid bars). Note that at 3 h (depletion) amylase lost from the pancreas is in great part found in blood, not the small intestines. Similarly, the recovery of pancreatic amylase content is accompanied by an equivalent loss of amylase from blood. [Modified from Miyasaka and Rothman (61).]
large bowel from the small intestines, as the elevated levels there might suggest. Alternatively, it might have been degraded or otherwise inactivated in the small intestines. Finally, it may have been absorbed.

As it happened, it was clear that the missing amylase had not simply passed through. Although enzyme levels in the cecum and colon were greatly elevated, they still only represented a tiny fraction of the activity found at the same time in the small bowel even without any elevation (<1%) (61). Nor did it seem likely that degradation accounted for the missing amylase activity. Although the rate of amylase degradation in the small intestines is unknown, it must be far lower than its rate of secretion. How else could the enzyme be of use in digestion? Whatever its rate, if we assume that degradation is increased in proportion to the increase in amylase secreted, we would nonetheless expect to see a substantial, indeed a proportional increase in amylase activity. And, of course, however much degradation may have occurred, how do we explain the fact that increases at least proportionately as great as those seen in the large bowel and cecum were not observed in the small intestines?

This left the third possibility. Amylase was absorbed. This could be assessed simply by looking for the missing amylase in blood or in tissues that might be accessed through the bloodstream. When plasma levels were measured, much of the missing amylase activity was found. Plasma and interstitial fluid amylase now represented ~13% of the total amylase contents of the body, an order of magnitude increase above the ~1% seen before stimulation. It was now the second largest amylase pool after the pancreas, and more than double the intestines (61). Most importantly, the increase accounted for most of the amylase that had been secreted by the pancreas (Fig. 7).

Because it was known that amylase is also secreted directly from the pancreas into blood (28, 60), the elevated blood levels might have been the consequence of this process, not the absorption of enzyme. But this made little sense, since in this case we would have to conclude that almost all of the amylase secreted by the gland after cholinergic stimulation had been directed into the bloodstream, not the gut, and this was observably false (28, 81).

The occurrence of circulation was further validated by what happened when the stimulant was withdrawn. Two hours after its withdrawal, the percentage of amylase in the pancreas had recovered to values close to those found in fasted rats (61) and once again contained ~90% of the total in the body. This rapid replenishment was consistent with microscopic studies in which recovery in the volume density (the volume of the cell occupied) ofzymogen granules (the structures in which the digestive enzymes are stored) occurred over roughly the same period of time for the same conditions in the same species (81). Whatever the rate of recovery, as pancreatic levels of amylase increased, its level in plasma fell in a roughly reciprocal fashion (61) (Fig. 7). That is, the rate at which amylase disappeared from blood was correlated with its reappearance in the pancreas. It was not distinct from it, as we might expect if we were dealing with two unrelated events, for example, if the disappearance of amylase from blood was the consequence of its breakdown in the liver or its excretion via the kidney, while its appearance in the pancreas was due to the synthesis of new amylase.

V. ATTEMPTS AT CORROBORATION

Taken together, the observations just outlined provided a compelling case for the conservation and circulation of digestive enzymes. It was not long before the phenomenon was corroborated (24, 37). However, almost as quickly there were reports that questioned its existence (44, 74, 78). As noted at the outset, these negative observations were not failed attempts to reproduce the findings that we have just discussed, such as the effect of the diversion, reinstitution, and intravenous injection of secreted protein on pancreatic function, but entirely different experiments that focused in particular on one prediction of the circulation hypothesis. For circulation to occur, enzyme in blood must be taken up by and transported across pancreatic tissue into the gland’s duct system. Two subsidiary hypotheses, or what were thought to be subsidiary hypotheses, of this understanding were tested.

A. Pancreatic Arteriovenous Concentration

Difference

The first hypothesis was that if uptake occurs, digestive enzymes in plasma should be extracted during the passage of blood through the pancreatic capillary bed. As a consequence, they should be present at lower concentrations in venous blood leaving the gland than in arterial blood entering it. When such measurements were made, venous blood had a higher, not a lower, amylase concentration (44). This meant that the enzyme was being released from the pancreas into blood, not withdrawn from it, as predicted. From this observation it was concluded that digestive enzymes are not taken up by the pancreas from blood and as such cannot cross the gland. Of course, if they cannot cross the gland, then their circulation through the pancreas is not possible.

However, what was observed only applied to the particular conditions that were examined. One could not generalize, as was done, from it to all states and circumstances. Given that digestive enzymes equilibrate across the basolateral membrane of the acinar cell (29, 30, 45), it could not be concluded that there would always be a net movement of amylase from tissue to blood whatever the physiological state. This turned out to be particularly
important since the researchers had infelicitously chosen to look for net amylase extraction from blood under the most unfavorable conditions possible. They examined fasted dogs under general anesthesia. In this circumstance, the pancreas does not secrete significant quantities of digestive enzymes into the intestines. The amount of enzyme secreted and made available for circulation would be expected to be one to two orders of magnitude less than during active secretion. Hence, given the existence of an ongoing release of digestive enzymes from the pancreas into blood (28), it was not surprising that a net uptake of enzyme by the pancreas was not seen. The judgement that there would never be a net uptake of amylase by pancreatic tissue because of what was observed in this particular circumstance was like concluding that because most passengers on the New York subway are heading from the Boroughs to Manhattan at 8 A.M. that this would also be true at 5 P.M.

B. Intravenous Injection of Radiolabeled Digestive Enzyme

The second prediction was that if radiolabeled digestive enzymes were injected into the systemic circulation they would be accumulated by the pancreas and subsequently found in its exocrine secretion. Two groups (74, 76–78) tested this hypothesis, and both reported that little if any of the injected material was secreted by the gland for the times and circumstances they examined. Like the workers who measured arteriovenous concentration difference, they concluded that transport of digestive enzymes across the pancreas does not occur and hence that their circulation through this gland is not possible.

The supposition seemed to be that if the circulation hypothesis was correct, then most, or at least a large percentage, of the injected material would appear forthwith in exocrine secretion as the gland rapidly cleared the plasma of the labeled protein, much like the kidney clears it of glucose. One group thought that the recovery of 3–4% of the injected material in pancreatic secretion was too small to be considered meaningful.

The use of radiolabeled enzymes in these studies was predicated on the understanding that the radioactive material “traced” the movement of the bulk of unlabeled digestive enzyme; that is, if little labeled enzyme crossed the pancreatic epithelium, then the same could be said of unlabeled digestive enzymes of endogenous origin. This is called tracer kinetics, and for it to apply two things must be known. First, the labeled material must have equilibrated with the unlabeled form of the substance in all relevant compartments, in this case in the various extracellular pools of the body. Second, the ratio of the labeled to the unlabeled material, referred to as specific radioactivity (SRA), in each of these compartments must be known. Only then can we calculate how much unlabeled material is moved for a given amount of radioactivity. Such knowledge was lacking in these studies and consequently it could not be assumed, as it was, that the radiolabeled material traced the movement of endogenous substances. The fact that only small, seemingly negligible quantities of labeled protein appeared in pancreatic secretion said nothing about the movement of unlabeled protein.

On entering the systemic circulation, either as a result of intravenous injection or absorption, marker molecules like the labeled digestive enzymes that were used, first equilibrate with their endogenous forms in plasma, and, where they exit the capillary beds, in the various interstitial fluid compartments of the body. That is to say the labeled digestive enzymes would not be “attracted” to the pancreas as if it were some sort of magnet but would be dispersed broadly throughout the body. Only after this broad distribution takes place can there be any sizable clearance by the pancreas.

As a consequence, one would expect that only a small percentage of the labeled material would end up in the interstitial spaces of the pancreas initially and be available for uptake and transport across the glandular epithelium. We can estimate how much from the mass of the pancreas in relation to the total soft tissue mass of the body, assuming that the size of the interstitial space is a relatively constant proportion of the fractional mass of all soft tissues and that most capillary beds are equally permeable to these substances. Calculated in this way, the interstitial space of the pancreas would contain \( \sim 0.15\% \) of the injected aliquot after its initial equilibration throughout the extracellular spaces of the animal. The actual number might be somewhat smaller or larger depending on the suitability of the assumptions used in making the calculation, but even if it were an order of magnitude larger, only a small percentage of the injected material would be found in the interstitial spaces of the pancreas initially. Given a value of 0.15%, finding 3–4% of the injected label in pancreatic secretion is impressive.

In any event, as said, only after its broad dispersion throughout the body can the labeled protein be effectively redistributed to the pancreas and other tissues that might accumulate it. If, for the sake of estimation, we assume that this redistribution occurs by the same diffusive and flow processes that led to the initial distribution, then it would occur more slowly to the degree that the exogenous (labeled) material is diluted by its endogenous (unlabeled) forms in blood and the various interstitial spaces. The greater the dilution, the longer redistribution would take.

For the case at hand, we would expect a considerable dilution because the concentration of digestive enzymes in the relevant endogenous compartments is substantial, in the tens to hundreds of nanograms per milliliter range.
The amount of labeled protein moved per mole of total digestive enzyme would be reduced in proportion to this dilution. For example, given the same forces driving movement, if the ratio of labeled to unlabeled protein (SRA) was reduced by two orders of magnitude during an initial period of equilibration of say 10 min, it would take 1,000 min, or ~16 h, for redistribution to occur. Dilution by its unlabeled form would in this sense “trap” the labeled material in the animal’s extracellular spaces. Consequently, it is not justified to conclude, as was done, that because little tracer surfaced in pancreatic secretion in the short run, that more would not be found there in due course.

Moreover, the appearance of labeled protein in pancreatic tissue does not guarantee its immediate secretion. Exogenous digestive enzymes taken up by the acinar cell mix with its endogenous stores (31) just as they do in the extracellular spaces of the body and are diluted by them. Except that the dilution in this case is far greater because the pancreas contains these proteins at very high concentrations (mM). In any event, once again the labeled protein would be “trapped,” unable to leave the acinar cell in any quantity due to its severe competitive disadvantage in relation to the far more abundant unlabeled enzyme reserves. In fact, even the complete turnover of the cellular enzyme pool would not guarantee its expulsion, because it would remain, at all times, at a severe competitive disadvantage for exit from the cell. Its release could only be ensured if the gland was fully depleted of its protein contents during active, stimulant-induced exocrine secretion.

Although it seemed unremarkable to these workers, when they made observations over an extended period, a substantial redistribution of the labeled material to the pancreas was actually seen, just as predicted by the circulation hypothesis (Table 1) (76). Shortly after injection of the radiolabeled enzyme (15 min), the pancreas contained a low concentration of labeled protein compared with a variety of other tissues. For example, the liver contained about an order of magnitude more per unit tissue mass. However, with the passage of time, the amount in the pancreas increased greatly, indeed magnitudinally, while that in other tissues either decreased or only increased modestly (Table 1). By 7 h after the injection, the pancreas had the highest concentration of any tissue examined, and within an hour its contents had increased some 24-fold and were higher than that found in the liver. The second largest increase was for intestinal tissue (Table 1), which presumably for the most part comes from the pancreas. If we factor this in, at 7 h the increase was 43-fold.

The authors thought that the presence of labeled protein in the pancreas was due to the uptake of labeled amino acids previously released from the injected protein elsewhere in the body and its subsequent incorporation into the endogenous protein pool of the gland. Their evidence for this conclusion was that when they collected secretion from the pancreatic duct of rats after the intravenous injection of a mixture of radiolabeled guinea pig digestive enzymes, only radiolabeled rat protein was found in secretion as estimated by two-dimensional gel electrophoresis.

Although the amount of labeled protein in secretion was small and the distinction between rat and guinea pig spots in the electrophoretogram uncertain in some respects, at least some of the labeled protein appeared to comigrate with unlabeled rat proteins, suggesting that they might also be rat proteins. Two measurements were needed to validate this interpretation. The first was a control in which small amounts of labeled guinea pig protein (similar to the amount of labeled protein found in secretion) would be admixed with larger amounts of unlabeled rat digestive enzyme (the same amount placed on the gel in the experiment) and subjected to electrophoretic separation. In this case, where small amounts of labeled guinea pig proteins were known to be present, were distinct guinea pig spots seen and were rat spots devoid of label? The second measurement is to show that all cellular label is associated with rat protein and that guinea pig protein is not in the acinar cell. Neither measurement was provided then or subsequently, and as a result the authors’ claim was not experimentally substantiated.

There was another issue. As in the studies of arteriovenous concentration difference, the researchers had only studied the unstimulated glands of anesthetized animals. As said, under these conditions, the pancreas only secretes small amounts of protein. Thus once again tissue uptake as well as transpancreatic transport was being sought in a setting in which we would expect both to be minimal.

Finally and significantly, accepting their results as presented, they neither explained away nor otherwise

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**TABLE 1. Tissue levels of radioactivity after injection of [$^{35}$S]methionine-labeled pancreatic exocrine proteins into the blood circulation**

<table>
<thead>
<tr>
<th>Organ or Tissue</th>
<th>TCA-Insoluble Radioactivity, dpm/100 mg tissue $\times 10^{-2}$</th>
<th>Change From 15 Min, %</th>
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<td></td>
<td>15 min</td>
<td>60 min</td>
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<td>Kidney</td>
<td>311</td>
<td>170</td>
</tr>
<tr>
<td>Lung</td>
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<td>24</td>
</tr>
<tr>
<td>Spleen</td>
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<td>47</td>
</tr>
<tr>
<td>Liver</td>
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<td>48</td>
</tr>
<tr>
<td>Skeletal muscle</td>
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<td>5</td>
</tr>
<tr>
<td>Intestine</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>72</td>
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</tbody>
</table>

Times (in min) refer to time after injection. [Modified from Rohr and Scheele (76).]
accounted for what at the time was the already substantial published evidence, some of which we have discussed, that digestive enzymes are degraded in the small bowel along with their substrates (the ingested food and the other digestable contents of the small intestines) was not the result of the direct measurement of degradation and its correlation to the loss of enzymatic activity. If degradation is the fate of all digestive enzyme secreted into the bowel subsequent to the ingestion of a meal, then such measurements would show that over time the disappearance of enzymatic activity in various aboral locations was accompanied by the concomitant and quantitatively equivalent appearance of small peptides and amino acids liberated by that degradation.

Making such measurements admittedly presents significant problems, but an attempt was made by researchers to assess the situation in a simpler way. They compared observed levels of enzyme activity in the intestines to the amount of the enzyme bound in a radioimmunoassay (40). As enzyme is degraded, the peptide fragments produced by degradation would either be unable or become increasingly unable to bind to the antibody. The parallel loss of enzymatic activity and immunoreactivity would be consistent with the loss of activity by enzyme degradation. Perhaps this can be appreciated with a counter example. If enzymatic activity disappeared, but not immunoreactivity, we would have to conclude that some event other than degradation had produced the reduction in enzyme activity.

The researchers who carried out this work understood that the parallel disappearance of enzymatic activity and immunoreactivity could be the consequence of enzyme absorption as well as degradation. In this case, as enzyme is absorbed both activity and immunoreactivity would also disappear. Consequently, the authors concluded from their measurements that secreted digestive enzyme was degraded, not absorbed, but careful examination of their data reveals something else.

Experiments were carried out on human subjects after the ingestion of a meal comprised solely of starch. Enzymatic activity and immunoreactivity of lipase and trypsin, and the activity of amylase, were measured in aspirates from different portions of the gut taken at different times. As expected, with the passage of time the presence of the three enzymes in the upper small bowel, or duodenum, first increased and then decreased, returning at variable rates over a 3- to 6-h period to values similar to those seen before the meal. But almost everything else that was observed was not this straightforward.

As the chyme moved aborally, through the jejunum to the ileum, only 1% of the lipase activity was still present, but over 20% of the trypsin and some 75% of the amylase made the passage without being either degraded or absorbed. The authors explained these large differences as being due to variations in the resistance of the different enzymes to degradation and additionally for amylase, to the protective effect of its binding to the starch substrate, although they presented no evidence to support either suggestion. The observed differences might just as well have reflected differences in the rate and location of the absorption of the three enzymes. In any event, and significantly, it turned out that for all of the enzymes, what passed into the lower small bowel was apparently intact or almost intact enzyme, not substantially degraded products.

If one looks at the concentration of enzyme (amount per unit volume of aspirate), although lipase concentration decreased by some 97% between the duodenum and ileum as measured by activity, its immunoreactivity was retained. That is, the enzyme appeared to be essentially intact or at least sufficiently intact to react with its antibody. Trypsin, on the other hand, had become less immunoreactive but retained its enzymatic activity, suggesting nondegradative structural change. Finally, amylase concentration increased.

The authors believed that they could exclude absorption from consideration because the disappearance of immunoreactivity and enzymatic activity did not occur in parallel for either lipase or trypsin. As said, they understood that for every absorbed molecule both its enzymatic activity and immunoreactivity should disappear from the gut in concert, and this was not seen. However, this explanation holds only if one does not consider alterations to the molecules that might occur before their absorption. For example, given that nondegradative chemical modification takes place in the intestines, altered, but still intact, molecules might be absorbed as well as or even in preference to the unaltered molecule.

In the final analysis, one could not conclude from this study that the disappearance of enzyme activity was the result of degradative processes alone, as was done. Not only didn’t the data provide reason to choose between degradative and absorptive explanations for the disappearance of enzyme activity, remarkably there was no proof of enzyme degradation at all. In fact, the maintenance of enzyme activity (trypsin and amylase) or immunoreactivity (lipase) in the ileum seemed to argue against
it. What was observed was exactly what would be expected if absorption had taken place: the disappearance of enzyme from the bowel in the continued presence of intact digestive enzyme within it.

VI. RATE OF SYNTHESIS OF DIGESTIVE ENZYMES COMPARED WITH THEIR RATE OF SECRETION

Another way to approach the question of the conservation of digestive enzymes is to assess the capability of the exocrine pancreas to manufacture sufficient quantities of these proteins to replace those it secretes (88). If the gland is not capable of meeting this need, then a conservation of digestive enzymes is not merely suggested, but required. Over time, shortfall would build on shortfall, deficiency on deficiency, until the gland was depleted of its enzyme contents and unable to respond to stimuli.

The most common method of measuring protein synthesis, following the rate of incorporation of radioactive amino acids into protein molecules, although useful for comparing synthetic rate under different conditions is not satisfactory for quantitative measurements of the sort needed. There are, however, other ways to assess the sufficiency of synthesis. Next we consider three approaches, each dependent on different variables.

First we must settle on a value for protein secretion to compare to estimates of synthetic capacity. As we have already discussed in another context, the question is not whether synthesis is capable of replacing what is secreted under some circumstances, but under all circumstances, particularly when rates of secretion are high and the gland is partially or fully depleted of its enzyme contents, as is frequently the case. The important question is whether under these conditions the recovery of tissue digestive enzyme content is due solely to new synthesis?

Using data from fasted anesthetized rats (25, 61, 81), and extrapolating to daily rates of secretion, we estimate the amount of protein secreted by the pancreas to be between 70 and 140 mg protein·g tissue\(^{-1}\)·day\(^{-1}\). We have assumed that secretion occurs at low or unstimulated rates for 75% of the day and at highly elevated rates for 25%. Under these conditions, tissue digestive enzyme stores would turn over, in one way or another, once to twice daily based on the observed digestive enzyme contents of 70 mg protein/g tissue in rats after an overnight fast (25). This range is conservative, that is, biased toward the possibility of synthetic sufficiency. Actual 24-h rates of secretion in awake animals fed ad libitum would likely be larger, not smaller.

A. Basal Rate of Protein Secretion

One way to estimate the rate of synthesis is from the rate of secretion seen in the relative absence of exogenous nervous and hormonal stimuli, that is, “unstimulated” or “basal” secretion. Under these circumstances tissue contents remain unchanged despite ongoing low levels of secretion. As such, what is being secreted is being quantitatively replaced. If replacement is due to new synthesis, then the rate of secretion is equal to the rate of synthesis.

If a significant fraction of new digestive enzyme does not successfully negotiate the posttranslational phase of production, that is, is degraded without ever being secreted, this method underestimates the true synthetic rate. However, this is not the rate in which we are really interested. Rather, what we want to know is how much new protein is available to be secreted to replace previously released protein.

In the fasted anesthetized rat, secretion in the absence of exogenous stimuli ranges from ~0.25 to 1.0 mg protein·g tissue\(^{-1}\)·h\(^{-1}\) (81). Tissue levels of enzyme are constant at rates of secretion in this range, and hence they provide a good estimate of the “effective” rate of protein synthesis, the amount of new protein available for secretion under these conditions. On the basis of these values, between 6 and 24 mg protein are manufactured each day per gram of tissue, or some 4–35% of the amount secreted (Table 2). With the assumption of the highest estimate for synthetic rate and lowest for daily secretion, synthesis is still only about one-third of that needed.

<table>
<thead>
<tr>
<th>TABLE 2. Estimates of the rate of protein synthesis by the exocrine pancreas</th>
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<tr>
<td>Based On:</td>
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<tr>
<td>------------------------------</td>
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<tr>
<td>Protein · g tissue(^{-1}) · day(^{-1})</td>
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<tr>
<td>Basal secretion</td>
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<tr>
<td>Product of ribosome number and time for synthesis of peptide chain</td>
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<tr>
<td>Oxygen consumption</td>
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<tr>
<td>Stimulant enhanced</td>
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<tr>
<td>Rate of secretion</td>
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Percent of tissue pool per day is based on 70 mg protein/g tissue (that found in rat pancreas after an overnight fast). Basal secretion is based on the equivalence of basal secretion to the effective rate of synthesis. Product of ribosome number and time for synthesis of peptide chain is based on 10\(^6\) attached ribosomes per cell and a synthetic rate of 3 min/chain. Oxygen consumption compares oxygen consumption to the cost of peptide bond synthesis. Stimulant enhanced refers to elevations over basal rate (given in “basal secretion”) of 50% for 5 h (based on the experimental literature). Rate of secretion is given for comparison. It is based on 75% at the basal rate (0.25–1.0 mg · g\(^{-1}\) · h\(^{-1}\)) and 25% at 10–20 mg · g\(^{-1}\) · h\(^{-1}\) (estimated from data in the rat, Refs. 25, 61, 81).
B. Enhancing Synthesis

Could this shortfall be made up by increases in the rate of synthesis after periods of active secretion? The evidence does not support this possibility. There are numerous reports, in an abundant literature (for example, Refs. 12, 23, 26, 35, 54, 62–65, 70, 103), of elevations in synthetic rate after the administration of stimulants of secretion. However, the effect is modest, generally in the range of 25–50%. Sometimes no enhancement is seen or even a decrease, whereas on the other hand, the largest increase ever reported is about a doubling (54).

Whatever value we might settle on, it would not be nearly enough to meet the need. We should understand that without boosting synthetic rate, even a gland that is only slightly depleted of its enzyme contents during periods of active secretion would be unable to recover what it lost whatever the rate of synthesis might be. When secretion returns to low, “unstimulated” rates after a period of active secretion, protein manufacture once again equals the rate of secretion, and consequently no accumulation of enzyme can occur.

As it happens, during periods of active secretion the enzyme content of pancreatic tissue declines substantially, sometimes dramatically. For example, after 3 h of highly augmented secretion in the awake rat, tissue content decreases by ~50% (25, 61). For synthesis to account for the recovery under such circumstances, it must be greatly elevated. The rate of recovery would be proportional to the increase in synthetic rate. For example, if secretion was elevated by an order of magnitude in response to a meal, as commonly occurs, synthesis would have to be increased by an order of magnitude for recovery to occur in the same time frame as loss from the gland. If synthesis only increased by 50%, a relatively high value, it would take 20 times as long to replace what was lost as to lose it. More particularly, if secretion was magnitudinally elevated for 3 h and returned to the unstimulated state immediately thereafter, it would take 60 h, or 2.5 days, to replace that lost.

Even this is unrealistically optimistic, because it assumes that synthetic rate remains elevated for 60 h until recovery is complete, and this is not seen. Synthetic enhancement occurs primarily in the presence of stimulants, that is, during periods of tissue depletion, not recovery, and in any event only lasts for a few hours. For the example given above, the rat pancreas loses ~35 mg of its digestive enzyme contents (50%) after 3 h of active secretion (25, 61). If the rate of synthesis were elevated by 50%, and secretion returned to its unstimulated rate with removal of the stimulant, synthesis would only have provided about a milligram of new protein, or just a few percent of the 35 mg needed for the recovery of tissue contents. Even if the elevation in synthesis lasted well beyond withdrawal of the stimulant, say five times as long, or 15 h, only 5 mg of new protein would be produced. For recovery to be complete in this case, synthesis would have to remain elevated for more than 4 days. Obviously, this is far too long for animals like rats and humans that feed daily or more frequently.

The replenishment of tissue contents normally occurs within a few hours of the cessation of active secretion. For the circumstances described above in rats, it takes just 2 h to reach levels that are roughly equivalent to those seen in fasted animals (25, 61). Even if it took 15 h, or a full 24 h, synthesis could not account for restocking at the increases in rate that have been observed.

C. Ribosomes

Another means of estimating the rate of synthesis is to calculate the product of the time it takes to manufacture a single peptide chain and the number of ribosomes actively engaged in the task, since proteins are manufactured in a one to one correspondence to ribosomes. It takes several minutes to produce single peptide chains in eukaryotic cells (49, 50, 67, 69, 71, 106). More particularly, it takes ~3 min to manufacture a molecule of pancreatic ribonuclease or amylase (67, 71). The number of ribosomes in the secretion (acinar) cells of the pancreas engaged in digestive enzyme synthesis can be estimated by counting those attached to the endoplasmic reticulum in electron micrographs. This number is thought to be the same, or almost the same, as the number of digestive enzyme chains being manufactured based on the understanding that 1) digestive enzymes are produced exclusively on ribosomes attached to the membranes of the endoplasmic reticulum (ER); 2) all of these ribosomes are actively engaged in protein manufacture; and finally 3) the great majority are busy manufacturing digestive enzymes, not other proteins such as membrane proteins, proteins destined for the ER itself or for organelles such as lysosomes. These discernments are widely believed to be true, but whether they are or are not, by presuming them true, we bias our estimate toward higher synthetic rates.

The ER is a striking feature of the pancreatic acinar cell and occupies some 20% of the cell’s volume (16). It appears to be comprised of numerous elongated, ribosome-studded membrane sacs that are stacked upon each other. By making some simple assumptions about the arrangement of ribosomes on the membranes and how the ER sacs fill the volume of the cell they occupy, the number of ribosomes present can be calculated. Specifically, and to further bias our estimate toward higher synthetic rates, we assume that the membranes are arranged in orderly rows and that the ribosomes attached to them are packed densely in a rectilinear array, separated from each other by the diameter of a single ribosome. We chose a 60-nm spacing between sacs based on what is
seen in electron microscopic images. Given this geometry, there are about a million attached ribosomes per cell. If the stacking of ER membrane is not this regular or the placement of ribosomes on their surface not this dense, or both, as appears to be the case, then this is an overestimate, and hence overestimates synthetic rate, again biasing the result in favor of synthetic efficiency.

With the use of $3\text{ min}$ as the time required to synthesize an average digestive enzyme chain, $20$ protein molecules would be manufactured per hour per attached ribosome assuming that there is no significant delay between the manufacture of one chain and the next on a given ribosome (any delay would of course reduce the rate of synthesis). Given a million attached ribosomes per cell, $\sim 1.2 \times 10^{16}$ protein molecules$^{-1}\cdot g^{-1}\cdot h^{-1}$ would be manufactured. Applying Avogadro’s number and converting to mass (assuming an average molecular mass of $30$ kDa for the digestive enzymes), $1$ g of tissue is capable of producing $\sim 0.67$ mg of digestive enzymes per hour. Given the various assumptions made in making this calculation, half this value, or $0.34$ mg$^{-1}\cdot h^{-1}$, is probably a more realistic, but still a generous, estimate. This amounts to $8–16$ mg protein synthesized$\cdot$day$^{-1}\cdot g^{-1}$, or about $6–24\%$ of the amount we estimate is secreted per day (Table 2). Once again, there is a substantial shortfall. Even if we use our highest estimate for synthesis and lowest for secretion, the shortfall is fourfold.

D. Energy Requirement

A final way to judge synthetic rate is to ask whether the pancreas can support the replacement of the digestive enzyme pool from an energetic perspective? That is, does it generate enough energy to replace what it loses by new synthesis? As said, protein synthesis is among the most costly biological reactions. The energy required to form the peptide bond has been estimated to be $\sim 3.5$ kJ/g protein (58, 59, 72, 95, 102). The pancreas’ available energy has been measured experimentally as oxygen consumption (14, 19). Metabolism is primarily oxidative in this gland, and values for oxygen consumption reflect its energy utilization relatively well. Thus, knowing the cost of synthesis and the available energy, we can estimate how much protein can be manufactured by the pancreas per unit time, assuming, incorrectly of course, that all of the energy is used for this purpose.

Oxygen consumption in the pancreas is $\sim 0.01–0.02$ ml$\cdot$g tissue$^{-1}\cdot$min$^{-1}$ at standard temperature and pressure in mammals (14, 19) or $14–28$ ml$\cdot$oxygen$\cdot$g tissue$^{-1}\cdot$24 h$^{-1}$. With the assumption that this in the main reflects glucose oxidation, $1.2$ J would be produced for every milliliter of oxygen consumed, and as a consequence, the pancreas would generate somewhere between $17$ and $34$ J$\cdot$day$^{-1}\cdot$g tissue$^{-1}$. This value is roughly in proportion to the gland’s mass as a percentage of the total mass of the animal (59), indicating that at least in the rat, the pancreas is about as metabolically active as other tissues on average.

To replace the $70$ mg$\cdot$g tissue digestive enzyme pool of the fasted rat once a day would require $238$ J or almost $10$ times the available energy. Thus putting the cell’s other needs aside, $\sim 10\%$ of the digestive enzyme pool can be replaced daily, or $\sim 5–10$ mg protein can be synthesized per gram of tissue each day, or $\sim 4–15\%$ of our estimate of the daily rate of secretion (Table 2). With the assumption of the highest estimate for synthesis and the lowest for secretion, we would still have a shortfall of some sevenfold.

E. Summary

Although calculations such as these always present uncertainties, and must to some degree be considered speculative, it is hard to envision errors that would be of sufficient magnitude to invalidate the conclusion that synthesis alone is not able to replace secreted protein under circumstances in which active secretion occurs. The calculations are based on published and well-documented experimental measurements, and moreover, to mitigate error, wherever possible we have tried to choose values that would maximize synthetic rate and minimize secretory capacity to bias our numbers toward synthetic sufficiency. The fact that three independent means of estimating the rate of synthesis, each depending on different variables, yield quite similar results ($5–10$, $8–16$, and $6–24$ mg$\cdot$g$^{-1}\cdot$day$^{-1}$), suggests that the rate of synthesis in these glands is within this range. If so, this is far less than any reasonable estimate for the rate of secretion from actively secreting glands. The conclusion that synthesis is unable to replace what is secreted is reinforced by the fact that to restock depleted glands by means of synthesis alone requires an enhancement, in either magnitude or duration, of synthetic rate that is well beyond anything that has been observed.

Whatever uncertainty there may be, these estimates cannot be taken, either singly or together, as support for the view that the gland is capable of meeting its need for digestive enzymes solely by new synthesis. To the contrary, they provide support for the conservation of digestive enzymes and buttress the experimental evidence for such a process presented above.

VII. MEMBRANE PROTEIN TRANSPORT

In section I we mentioned that some of the initial hesitancy to accept the existence of a conservation of digestive enzymes was due to the widely held belief that biological membranes were impermeable to protein mol-
ecules. Because conservation required the transport of digestive enzymes across both intestinal and pancreatic epithelia, minimally across four separate membranes, conservation seemed unattainable. Vesicle mechanisms could have been invoked; however, the properties of transport that were observed, for example, its equilibrating nature, suggested direct membrane permeation.

Since the first reports of a membrane permeability to protein molecules in the pancreas about 30 years ago (46), a large body of experimental evidence has been published establishing the direct transport of many different protein molecules across as many biological membranes. Beyond providing evidence that such processes exist, these studies have made it clear that membrane protein transport is ubiquitous and central to cellular life. Without these processes to sort proteins to their various assigned locations, to the mitochondrion, the nucleus, the peroxisome, the chloroplast, as well as to allow proteins to exit the cell, cellular life as we know it would not exist. Movement occurs variously by the passage of proteins through large membrane channels, through the lipid barrier, or by means of interactions with special membrane “carrier” proteins. We will not review this evidence here; however, we direct readers to older reviews that deal with membrane transport that were observed, for example, its equilibrating nature, suggested direct membrane permeation.

VIII. FORMS OF CIRCULATING PROTEINS

Hesitancy to accept the existence of an enteropancreatic circulation was also due to the complete lack of knowledge of the molecular forms of the circulating species. For example, were they proenzymes, active enzymes, inactivated enzymes, or all of these? And if molecules were modified, how and where did this happen? This also posed a chicken and egg problem. Before it made any sense to try to determine the forms of the circulating species, one had to be convinced that circulation actually occurred. And if the fact that nothing was known about the forms of the circulating species engendered skepticism about circulation itself, there seemed no point in attempting to determine the nature of events that did not occur.

Consequently, research along these lines was not carried out, and all we have is speculation as fodder for future work. We can start such speculation by asking whether each and every proteolytic enzyme is activated immediately after its secretion into the intestinal tract in any and all physiological circumstances? As noted, there is no evidence that this is the case, only widely held belief. Knowing the circumstances of activation might be a first step in helping us understand the relative roles of pro- and activated enzymes.

Active enzymes might be temporarily inactivated or otherwise transformed in the intestines before their circulation. In this regard, the pancreas secretes substantial amounts of enzyme inhibitors, most notably pancreatic trypsin inhibitor, along with its digestive enzymes. We have no idea what, if anything, these inhibitors do in the intestines. Certainly, trypsin inhibitor might inactivate trypsin. But if so, to what end? Of course, there are many other potential means of chemically altering the digestive enzymes, including, but not limited to, covalent modifications. For example, they might be phosphorylated, sulfonated, chlorinated, or leader or activation peptides reattached in some way. The experiments by Layer et al. (40) discussed above provide proof that some sort of nondegradative modification occurs in the intestinal lumen to whatever purpose.

Modification may also occur pursuant to transport across the intestinal epithelium, or after the proteins enter the bloodstream. As discussed, protease inhibitors are present at high concentrations in plasma, and we would expect active proteolytic enzymes to bind to them, thereby reducing the risk of a generalized proteolytic degradation. Perhaps these inhibitors also serve as “carrier” molecules presenting the circulating enzymes to the pancreas. Finally, when the enzymes return to the pancreas, they may be chemically modified before their secretion. Although it is thought that each digestive enzyme has a particular established structure before its secretion, and that whatever variations exist reflect the presence of gene-based isoenzymes, work with mass spectrometry suggests a multiplicity of forms of amylase and other digestive enzymes with only slightly different masses (105). Moreover, and significantly, the forms in secretion are not identical to those in the zymogen granule.

IX. CONSERVATION OF OTHER DIGESTIVE ENZYMES

It is natural to ask whether other digestive enzymes, like salivary amylase or gastric pepsin, might be conserved? After all, the same issues apply. Although there has been very little research to explore these possibilities, Miyasaka and Rothman, in studies discussed above (61), found that when blood levels of pancreatic amylase were greatly elevated subsequent to stimulation of pancreatic enzyme secretion in rats, the amylase contents of both the parotid and submandibular glands were also greatly elevated, with the parotid gland elevated by two orders of magnitude. This remarkable uptake of pancreatic amylase from blood suggests the possibility that subsequently it might be secreted into the mouth, though measurements of secretion were not made. In a related study, Fredette and Rothman (unpublished data) found that the administration of isoproterenol to rabbits to stimulate the secre-
tion of amylase into the gland’s duct system also leads to a large increase in plasma amylase levels. However, when amylase secreted into the mouth was prevented from traveling down the gastrointestinal tract by means of an esophagostomy, plasma amylase levels fell by ~50%.

X. CONCLUSIONS

As we reexamined the evidence for a conservation of digestive enzymes, we found it no less compelling than we did 25 years ago. Likewise, we found the studies that questioned its existence as incomplete as they seemed to us all those years ago. Add to this the calculations of synthetic sufficiency presented here for the first time, and it seems that the conservation of digestive enzyme does not merely occur but is a necessary agency.

In the most general terms, conservation has much to recommend it. Cells and organisms do not have unlimited amounts of energy to carry out their various life functions, and being able to do with less, being more efficient, has crucial selective advantage and can be an important determinant in the evolution of species. The traditional single pass view of digestion in which a completely new complement of digestive enzymes is manufactured for each meal has the curious consequence of requiring the organism to be particularly wasteful in its expenditure of energy to manufacture these costly molecules to meet its needs for sustenance, perhaps uniquely so among its metabolic and physiological processes, when just the opposite would seem desirable. As such, mechanisms that avoid the need to manufacture an entirely new batch of digestive enzymes for each meal, such as is the case with bile salts, would seem to provide an important advantage for an organism, particularly when food is scarce, as it often is in nature.

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