

EFFECT OF FASTING AND FEEDING ON SYNTHESIS AND INTRACELLULAR TRANSPORT OF PROTEINS IN THE FROG EXOCRINE PANCREAS

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ABSTRACT

Frog exocrine pancreatic tissue was studied *in vitro* under conditions which maintain the differences between tissues from fasted and fed animals. Sodium dodecyl sulfate (SDS) gel electrophoresis after labeling with [¹⁴C]amino acids showed that feeding stimulated the synthesis of secretory proteins to the same relative degree as the overall protein synthesis.

The intracellular transport of secretory proteins was studied by electron microscope autoradiography after pulse-labeling with [³H]leucine. It was found that the transport route is similar under both feeding conditions. After their synthesis in the rough endoplasmic reticulum (RER), the proteins move through the peripheral elements and cisternae of the Golgi system into the condensing vacuoles. The velocity of the transport increases considerably after feeding. When frogs are fasted, the release of labeled proteins from the RER takes >90 min, whereas after feeding, this happens within 30 min. Comparable differences were observed for transport through the Golgi system.

The apparent differences between the frog and mammalian pancreas in the regulation of synthesis, intracellular transport, and secretion of proteins are discussed.

KEY WORDS frog · exocrine pancreas ·
feeding · protein synthesis ·
intracellular transport

In serous cells, secretory proteins are synthesized at the membrane-bound ribosomes of the rough endoplasmic reticulum (RER) and are subsequently transported along the well-known RER-Golgi system-secretory granule route. It has been shown that protein transport in these organelles is not affected by treatments that influence protein synthesis (3, 7, 18) or secretion (10, 20, 21), and that it depends on a continuous supply of energy (4, 8). This suggests that protein transport is not a

passive process driven by the pressure of newly synthesized protein molecules.

The effect of feeding on the velocity of intracellular transport of secretory proteins has not yet been studied in pancreatic tissue. In rats, synthesis and secretion have been reported to be affected only moderately by feeding (15). On the other hand, synthesis and secretion of proteins strongly increase in the frog pancreas after feeding (22, 25). Therefore, the exocrine cell of the frog pancreas seemed suitable for studying the process of intracellular transport in relation to different levels of synthesis and secretion.

In this article, we describe the transport of in

vitro synthesized proteins in exocrine pancreatic cells of fasted and fed frogs as studied by electron microscope autoradiography (EMA). Electrophoresis showed that no important qualitative differences in protein synthesis after fasting and feeding exist. Therefore, the EMA data of both feeding conditions could be compared with each other. In both cases, we found that protein synthesis mainly concerns secretory proteins, which are transported through the cell much more rapidly in fed frogs than in fasted frogs.

MATERIALS AND METHODS

Animals

Frogs (*Rana esculenta*), which weighed 20–40 g, were kept under conditions as previously described (22). They were force-fed 100 g of meat, 48 h (fasted animals) or 4 h (fed animals) before being killed. The pancreas was quickly removed and sliced into 0.5 × 0.5 mm fragments with a TC-2 Sorvall tissue sectioner (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.) in two perpendicular directions.

In Vitro Incubation

Pulse-labeling experiments were performed under conditions previously reported (24). Fragments of fasted and fed tissue were incubated by immersion in 2 ml of incubation fluid in conical flasks, which were gently shaken. 50 $\mu\text{Ci/ml}$ [^3H]leucine (L-4,5- ^3H]leucine, Radiochemical Centre, Amersham, England) was added. The final concentration of leucine was 2.5 μM . After 10-min incubation, the tissue fragments were placed on strips of absorbent paper tissue through which incubation fluid containing 0.16 mM nonlabeled leucine flowed, and the incubation was continued for 10 or 80 min. All incubations were done at 22°C under 95% $\text{CO}_2/5\%$ O_2 gas phase.

The capacity of the tissue to synthesize proteins in vitro was studied by incubating it under similar conditions as in pulse-labeling experiments, except that 5 $\mu\text{Ci/ml}$ [^3H]leucine was added (concentration, 0.16 mM) to the incubation fluid. After different time intervals, the incubation was stopped by washing the tissue in ice-cold medium containing 75 mM nonlabeled leucine. The tissue was homogenized, and trichloroacetic acid (TCA)-insoluble radioactivity was measured and expressed per microgram DNA (23).

For electrophoresis, tissue fragments were incubated for 30 min by immersion in a medium containing a ^{14}C -amino acid mixture (80 $\mu\text{Ci/ml}$ medium, sp act 45 mCi/milligram of carbon, Radiochemical Centre). Unlabeled amino acids were not added except for those that were either not present or were present in relatively low concentration in the ^{14}C -mixture, so that the concentration of all essential amino acids varied between 10 and 20% of the concentration usually added to the incubation medium (24).

SDS Polyacrylamide Gel Electrophoresis

Tissue was homogenized with a tightly fitting Teflon homogenizer in 10% sucrose, and insufficiently homogenized particles were removed by centrifuging at 500 g for 2 min. The supernate was taken as the tissue homogenate. A secretory granule-enriched fraction was pelleted from this homogenate by centrifuging it at 1,000 g for 10 min (6). SDS gel electrophoresis by the method of Laemmli (12) was carried out with samples of the homogenate and the secretory granule fraction. Each sample, containing ~100 μg of protein, was heated in 1% SDS and 2% 2-mercaptoethanol at 100°C for 3 min and applied to slab gels, which contained a 9–18% acrylamide gradient. After electrophoresis, the gels were stained with Coomassie Brilliant Blue for 2 h and destained at 40°C in a medium containing 250 ml of methanol and 75 ml of acetic acid per liter.

To have comparable amounts of radioactivity in samples of ^{14}C -amino acid-labeled tissue from fasten and fed animals, we mixed labeled homogenates of fed tissue in a ratio of 1:5 with a nonlabeled homogenate. Gels with radioactivity were dried under vacuum on filter paper and autoradiographed with a Kodak x-ray film type RP-Royal X-omat. To determine the radioactivity, we put parts of the dried gels in 1 ml of NCS (Amersham-Searle, Corp., Des Plaines, Ill.), heated them at 60°C for 16 h, and, after addition of a toluene-based scintillation fluid, we counted the gels in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Protein content was determined by the method of Lowry et al. (14). Amylase activity was measured by the method of Bernfeld (1) in a phosphate-buffered reaction mixture (pH 6.9) to which 0.1% Triton X-100 was added.

Electron Microscope Autoradiography

Details of this procedure are given elsewhere (23, 24). After incubation, tissue fragments were fixed in 1% OsO_4 (pH 7.4), and embedded in Epon 812. Gold-colored sections were covered with Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England) by the dipping method. The autoradiograms were developed in Phenidone (Geigy Chemical Corp., Ardsley, N. Y.) at 18°C for 1 min and studied with a Zeiss EM9A electron microscope. In micrographs ($\times 20,000$) taken from autoradiographs in the well-labeled peripheral zone of tissue blocks of three fasted and three fed frogs, silver grains (>400 per animal) were counted over the following structures of exocrine cells: RER, Golgi system (except condensing vacuoles), condensing vacuoles, secretory granules, mitochondria, and nucleus. We attributed grains found over the cytoplasmic matrix to the nearest of these structures. Relative labeling of a structure i was expressed as percentage of the total number of grains counted over the structures mentioned above. In the same micrographs, we determined the relative area occupied by i using the "point-hit" method (2). The

relative grain density over i was then obtained from the ratio:

$$\frac{\text{relative labeling of } i}{\text{relative area of } i}$$

RESULTS

Identification of Newly Synthesized Proteins

Frog pancreas homogenate was electrophoresed and protein banding was compared with those of a zymogen granule fraction. This fraction was enriched in zymogens, as measured by amylase activity per mg protein, by 70%. In electropherograms of the homogenate (Fig. 1*a*), numerous faint and some distinct bands (1, 2, 4, and 6) were visible, which were much less clearly present in the secretory granule fractions (Fig. 1*b*). These bands most likely represent nonsecretory proteins. Secretory proteins were located in bands 3, 5, and the complex 7, which dominate in the secretory

granule fraction. Previous work showed that most of the proteins synthesized in the frog pancreas after feeding are transportable proteins (23, 24), and autoradiographs of electropherograms of ^{14}C -amino acid labeled fed tissue (Fig. 1*c*) show that most of the radioactivity is in bands 3, 5, and 7. This also indicated that secretory proteins were located in these bands.

For this autoradiographic study, it was important to know whether the proportions of radioactivity incorporated into secretory and nonsecretory proteins change after feeding. Therefore, autoradiographs of electropherograms from ^{14}C -amino acid-labeled tissue of fasted and fed animals were compared (Fig. 1*c* and *d*). This revealed no detectable shifts in the nature of newly synthesized proteins. Quantification of the radioactivity in bands 3, 5, and 7 showed that they contained almost similar percentages of the radioactivity under both conditions. Hence, in our EMA experiments, silver grains represented similar proteins after fasting and feeding. Approx. 50% of the radioactivity was found in bands 3, 5, and 7. Approx. one-half of the remaining activity was found diffusely spread over the lower regions of the gels. A part of this label probably belongs to proteins partly broken down during the tissue processing.

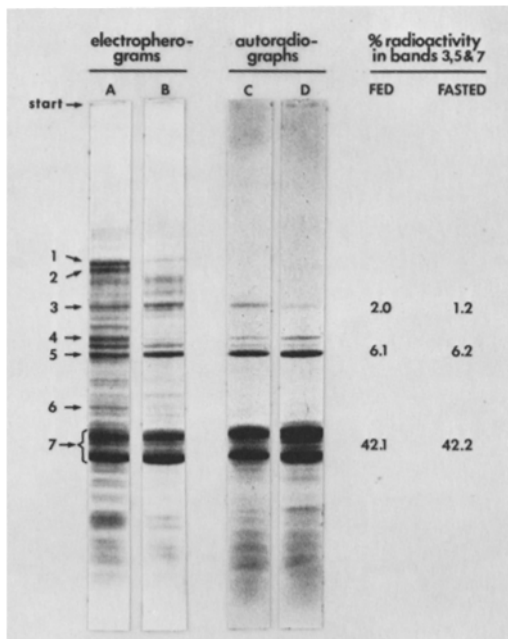


FIGURE 1 Electropherogram of pancreatic tissue homogenate (A) and secretory granule fraction (B). C and D are autoradiographs of electropherograms of tissue homogenate from fed and fasted frogs respectively, labeled in vitro with ^{14}C -amino acid mixture. The relative parts of radioactivity in bands 3, 5, and 7, which are the predominant proteins in the secretory granule fraction, are given at the right. For details, see Material and Methods section. The figure represents the data of one of two experiments with identical results.

Intracellular Transport

Intracellular protein transport was studied in tissue of fasted and fed frogs by EMA localization of proteins pulse-labeled with ^3H -leucine in vitro for 10 min, followed by chase periods of 10 or 80 min (indicated below as 10 + 10 and 10 + 80 min). During this procedure, the tissue remained well-preserved (24) and the difference in protein synthesizing capacity persisted during the 90 min of incubation (Fig. 2). Hence, within this period the system was supposed to reflect quite well the situation in vivo. Longer incubation periods appeared injurious to the tissue, and its capacity to incorporate ^3H -leucine was impaired.

The relative labeling of the cell structures in autoradiographs is shown in Table I. In fasted animals, after 10 + 10 min, almost 70% of the silver grains was still over the RER and 14% was found over the Golgi system. After 10 + 80 min, 40% of the label was found over the RER and 37% over the Golgi system. Because of the poor contrast in the autoradiographs, especially in fasted tissue, we could not discern substructures in the Golgi system other than the condensing

vacuoles (Fig. 3). After 10 + 80 min, condensing vacuoles contained only a minor part of the label and the number of silver grains over the secretory granules was not higher than after 10 + 10 min.

The EMA results from fed tissue corresponded well with those reported previously (24). There was more progress in transport of labeled proteins after feeding than after fasting at both incubation times studied. At 10 + 80 min, 50% of the silver grains were found over condensing vacuoles and secretory granules (Fig. 4).

After feeding, the relative areas occupied by some of the cell structures in the section changed. The relative areas for Golgi system, condensing vacuoles, and nucleus were larger in fed tissue than after fasting, while the area occupied by secretory granules was smaller in fed tissue. These changes are in good agreement with those observed in freshly fixed tissue (22).

For cell structures with different relative areas

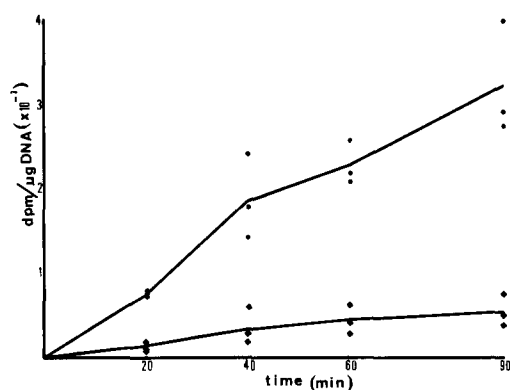


FIGURE 2 Incorporation of [³H]leucine in TCA-insoluble substances of fasted (squares) and fed (dots) frog pancreas, during incubation in vitro on absorbent paper tissue. The lines are drawn through the means of three values from different experiments at each time indicated.

in fasted and fed tissue, the relative grain density (Table II) is not proportional to the relative labeling given in Table I. At 10 + 10 min in fed tissue, and 10 + 80 min in fasted tissue, the relative labeling of the Golgi system was equal, but the relative grain density is much higher in the latter case because of the smaller relative area of the Golgi system after fasting. Correspondingly, after fasting only a small part of the silver grains was found over condensing vacuoles, and these occupied a very small area in sections, so that the relative grain density over them was high.

After fasting as well as after feeding, labeling is most prominent in the peripheral regions of the tissue blocks (see also reference 24). Within these regions in fasted tissue, the degree of labeling differed from cell to cell (Fig. 3). In contrast to the regional difference, this cellular variance was accompanied by differences in the progress of the radioactivity through the cell. This mainly appeared as a relatively stronger labeling of the condensing vacuoles in the highly labeled cells after 10 + 80 min. Concentrations of silver grains over secretory granules, however, were never observed at that time in fasted animals, and labeling of the Golgi system was consistent, irrespective of the cellular variance in [³H]leucine incorporation. Highly labeled cells were rather scarce, but their contribution to the grain counts was relatively large. Therefore, it must be realized that the transport progress, given in Table I for fasted animals, is not characteristic of the majority of the cells, in which transport speed decreased even more.

DISCUSSION

In frog tissues involved in the production of digestive enzymes, feeding stimulates protein synthesis considerably (25, 26). We did not find

TABLE I
Percentages of Autoradiographic Grains (\pm SEM) over Cell Structures of Fasted and Fed Frog Exocrine Pancreas after 10-min Pulse-Labeling with [³H]Leucine In Vitro and 10- or 80-min Chase Incubation

	10-min chase			80-min chase		
	Fasted \pm SEM	Fed \pm SEM	P*	Fasted \pm SEM	Fed \pm SEM	P*
RER	68.63 \pm 3.25	42.87 \pm 1.16	<0.001	40.40 \pm 2.95	20.97 \pm 1.42	<0.005
Golgi system	14.15 \pm 2.36	39.30 \pm 1.04	<0.001	37.30 \pm 3.80	22.23 \pm 2.27	<0.001
Condensing vacuoles	0.65 \pm 0.25	1.07 \pm 0.12	NS	6.13 \pm 2.26	22.07 \pm 1.32	<0.005
Secretory granules	4.98 \pm 0.94	5.17 \pm 1.59	NS	6.80 \pm 1.82	25.70 \pm 1.14	<0.001
Mitochondria	5.65 \pm 0.44	4.50 \pm 0.35	NS	4.17 \pm 0.74	3.23 \pm 0.56	NS
Nucleus	5.93 \pm 0.44	5.97 \pm 0.38	NS	5.27 \pm 0.37	5.53 \pm 0.75	NS

* P values here and in Table II show the level of significance for differences between data from fasted and fed animals. No significance (NS) was attributed P > 0.1.

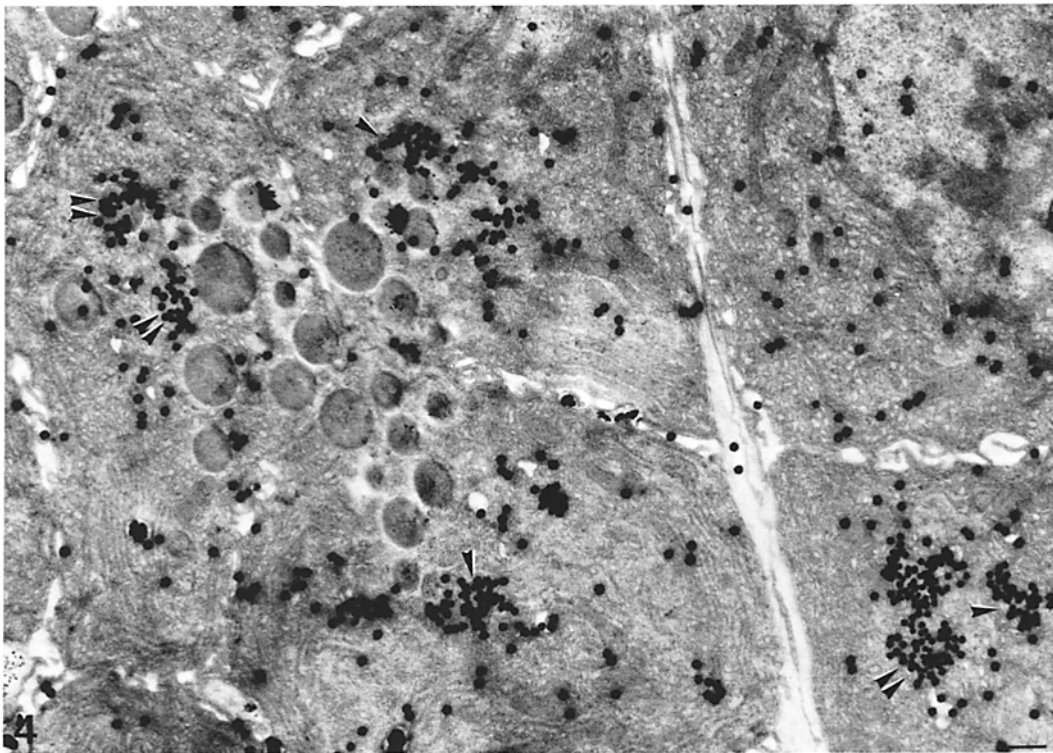
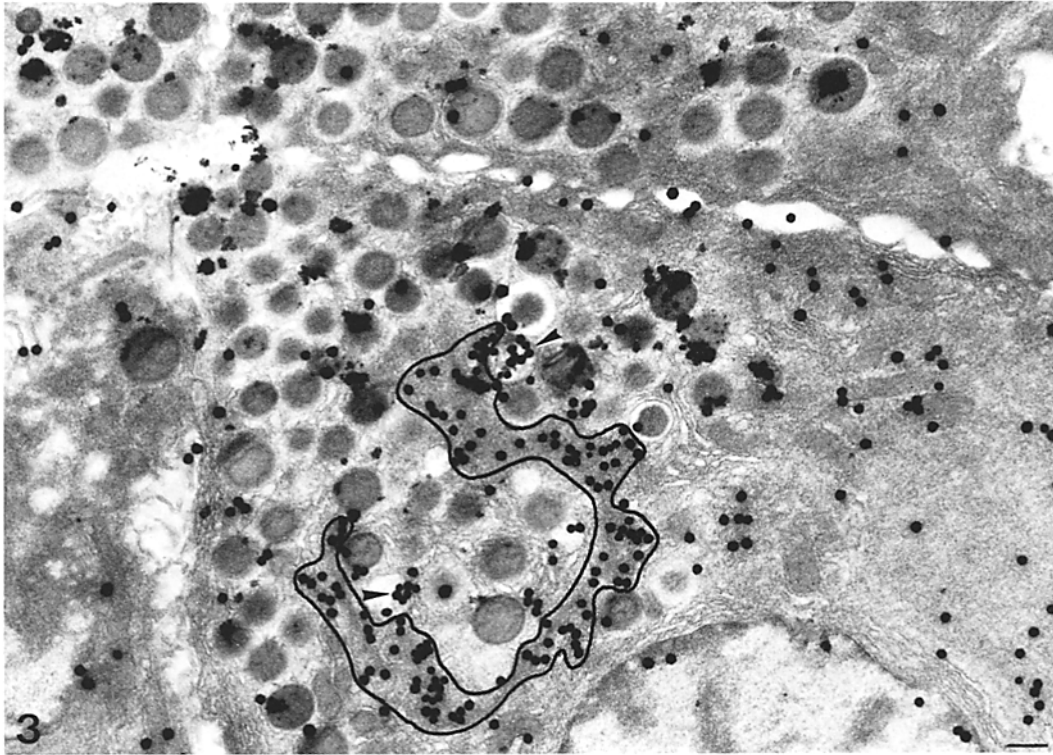


FIGURE 3 Autoradiographs of tissue of fasted frog after 10-min pulse-labeling with [^3H]leucine and 80-min chase incubation. Silver grains are especially concentrated over the Golgi system (*outlined*). Some label is also present over condensing vacuoles (arrowheads). Note that labeling intensity is lower in the neighboring cells. Bar, 1 μm . $\times 5,500$.

FIGURE 4 Autoradiograph of fed tissue after the same treatment as in Fig. 3. Here the majority of the silver grains is located over condensing vacuoles (arrowheads) and secretory granules (double arrowheads). Bar, 1 μm . $\times 5,500$.

TABLE II
Relative Density of Silver Grains* (\pm SEM) over Various Cell Structures in Autoradiographs of Frog Exocrine Pancreas

	10-min chase			80-min chase		
	Fasted \pm SEM	Fed \pm SEM	P	Fasted \pm SEM	Fed \pm SEM	P
RER	1.10 \pm 0.04	0.69 \pm 0.05	<0.005	0.62 \pm 0.05	0.34 \pm 0.02	<0.005
Golgi system	2.96 \pm 0.25	6.08 \pm 0.51	<0.001	9.11 \pm 0.58	3.01 \pm 0.03	<0.001
Condensing vacuoles	0.90 \pm 0.32	0.72 \pm 0.16	NS	11.34 \pm 3.38	10.52 \pm 1.09	NS
Secretory granules	0.44 \pm 0.08	1.02 \pm 0.18	NS	0.60 \pm 0.18	5.28 \pm 1.16	<0.01
Mitochondria	1.01 \pm 0.18	0.86 \pm 0.09	NS	0.84 \pm 0.13	0.64 \pm 0.08	NS
Nucleus	0.43 \pm 0.01	0.37 \pm 0.09	NS	0.39 \pm 0.05	0.33 \pm 0.07	NS

NS, no significance.

* Grain densities were calculated from assays per animal of the relative labeling of the cell structures and the relative area occupied by them in the autoradiographs used.

important changes in the nature of the proteins synthesized in the pancreas. Thus, in the frog pancreas the rates of synthesis of secretory and nonsecretory proteins appear to remain proportional during the feeding cycle. This differs from the situation in other exocrine tissue (13, 16, 17) in which stimulation of protein synthesis affects secretory proteins preferentially. We found only 50% of the radioactivity in the main secretory proteins. However, minor bands may have been overlooked and the 25% of the label found in the front region of the gels probably represents proteins, including zymogens, that were partly degraded during the procedure in vitro. Hence, the part of the radioactivity incorporated in secretory proteins is most likely >50%, and the electrophoresis data do not conflict with EMA observations on this tissue which showed that at least 70% of the radioactivity is incorporated into transportable proteins (24).

Pancreatic tissue of the frog has been shown to be easily damaged by incubation in vitro. The incubation procedure applied in pulse-labeling experiments was the least harmful of the methods used. It did not affect intracellular transport, because the transport velocity in fed tissue in vitro was similar to that observed in vivo (24). Transport velocity has not been established in fasted tissue in vivo. However, because the fine structure and the rate of protein synthesis do not change considerably during 90 min of in vitro incubation, we assume that for fasted tissue also, the results in vitro essentially reflect the situation in vivo.

In previous work, we already described the route of intracellular transport in exocrine pancreatic cells of fed frogs (23, 24). As in many other serous tissues, transportable proteins appeared to be transported from the RER through peripheral elements and cisternae of the Golgi

system into condensing vacuoles. Our present results show that, after fasting, proteins follow a similar route but travel much slower. After feeding, drainage of pulse-labeled secretory proteins from the RER was completed within 30 min (24). Then, the relative labeling of the RER was decreased to a basal level of ~20%. After fasting, even at 10 + 80 min the RER still contained 40% of the label. The electrophoresis experiments revealed no detectable differences in the nature of the proteins synthesized in fed and in fasted tissue. We estimate, therefore, that about half of the RER label at 10 + 80 min represents radioactive secretory proteins. Hence, in fasted tissue, release of pulse-labeled secretory proteins from the RER is still not finished at 90 min.

The relative labeling of RER and Golgi system is similar after 10 + 10 min in fed and at 10 + 80 min in fasted animals. This implies that transport in the fed condition is $\frac{\frac{1}{2} \times 10 + 80}{\frac{1}{2} \times 10 + 10} = 5-6$ times faster than in the fasted frogs. Feeding caused a corresponding stimulation of protein synthesis (25). The similarity of grain distribution in both cases shows that the inhibition of protein transport occurs along the entire route and is not restricted to a particular point, such as found in the guinea pig pancreas after the application of energy-blocking agents (8). Shortage of energy probably does not underlie inhibition of protein synthesis and transport in frogs because ATP levels in the frog pancreas are similar in either feeding conditions.¹

In mammals, short periods of fasting (1 or 2 d) have only moderate influence on the exocrine

¹ ATP was assayed (5) in pancreatic tissue of six fasted and six fed animals, which contained, respectively, 0.161 ± 0.008 and 0.159 ± 0.021 μ mol ATP/mg DNA (\pm SEM).

pancreatic cell: synthesis (15, 19) and intracellular transport (10, 21) continue at almost maximal rates, and considerable fasting secretion has been demonstrated (11). In the frog, fasting induces stagnation of these three processes, and this is accompanied by ultrastructural changes that are not usually encountered in the mammalian pancreas after fasting (22). It appears, therefore, that the frog pancreas differs mainly from the mammalian pancreas by its ability to inhibit the three processes during fasting. This ability of the frog tissue may be related to its poikilothermic character; because synthesis, intracellular transport, and secretion of proteins are energy dependent (8, 9), variable activities of these processes may be essential for the energy-saving physiology of the frog.

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