SYNTHESIS AND MIGRATION OF PROTEINS IN THE CELLS OF THE EXOCRINE PANCREAS AS REVEALED BY SPECIFIC ACTIVITY DETERMINATION FROM RADIOAUTOGRAPHS

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ABSTRACT

Radioautographs of pancreatic acinar cells were prepared in rats and mice sacrificed at various times after injection of leucine-, glycine-, or methionine-H^3. Measurements of *radioactivity concentration* (number of silver grains per unit area) and *relative protein concentration* (by microspectrophotometry of Millon-treated sections) yielded the *mean specific activity* of proteins in various regions of the acinar cells. The 2 to 5 minute radioautographs as well as the specific activity time curves demonstrate protein synthesis in ergastoplasm. From there, most newly synthesized proteins migrate to and accumulate in the Golgi zone. Then they spread to the whole zymogen region and, finally, enter the excretory ducts. An attempt at estimating turnover times indicated that two classes of proteins are synthesized in the ergastoplasm: "sedentary" with a slow turnover (62.5 hours) and "exportable" with rapid turnover (4.7 minutes). It is estimated that the exportable proteins spend approximately 11.7 minutes in the Golgi zone where they are built up into zymogen granules, and thereafter 36.0 minutes as fully formed zymogen granules, before they are released outside the acinar cell as pancreatic secretion. The mean life span of a zymogen granule in the cell is estimated to be 47.7 minutes.

It is believed that, after injection of a labeled amino acid, the proteins which are being synthesized become labeled, and may then be detected in histological sections by means of radioautography (1).

The first sites to become radioactive after injection of a labeled amino acid should be those where protein synthesis actually takes place. The radioactivity which appears later at other sites may be due to migration of the newly synthesized proteins (1). In practice, however, it is often difficult to decide whether the radioactivity in a given region is due to protein synthesis or to migration.

It is known that, when a labeled substance (referred to as "precursor") gives rise to another substance (referred to as "product"), their specific activities, *i.e.* their radioactivity per unit weight, usually vary with time according to precise laws (2). Since these laws are reflected in the pattern of the specific activity time curves, such curves are helpful in deciding whether one substance is the precursor of another (2). The precursor-product relationship should also apply when a given substance migrates from one compartment to another, since the substance may be considered to be precursor in the first compartment, and product in the second. Therefore, specific activity measurements may help decide whether the radioactive proteins within a given compartment were synthesized there or came in by migration.

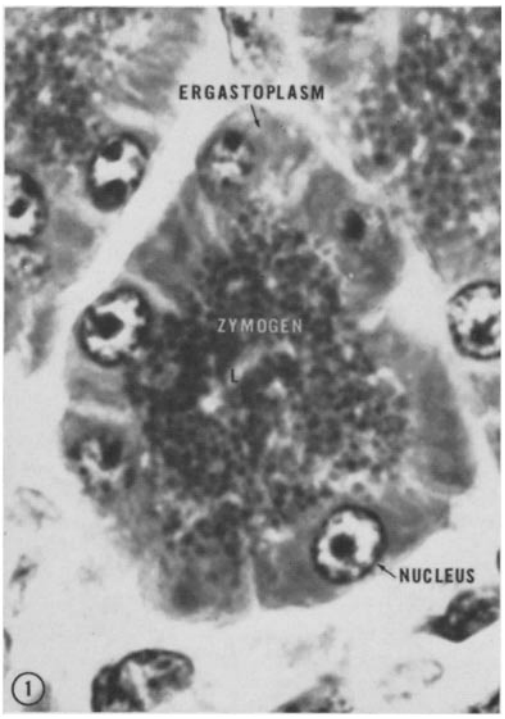


FIGURE 1

Pancreatic acinus of the rat stained with Masson's trichrome. This acinus is distinctly separated from surrounding acini. The basophilic (lighter appearing) ergastoplasm is seen to form a ring which almost completely encloses a central region of acidophilic (darker appearing) zymogen granules. The large nuclei, each containing one or more prominent nucleoli, are completely surrounded by ergastoplasm. Within the center of the zymogen granule region, an elongated light space indicates the acinar lumen (*L*) at the apices of the cells. × 2600.

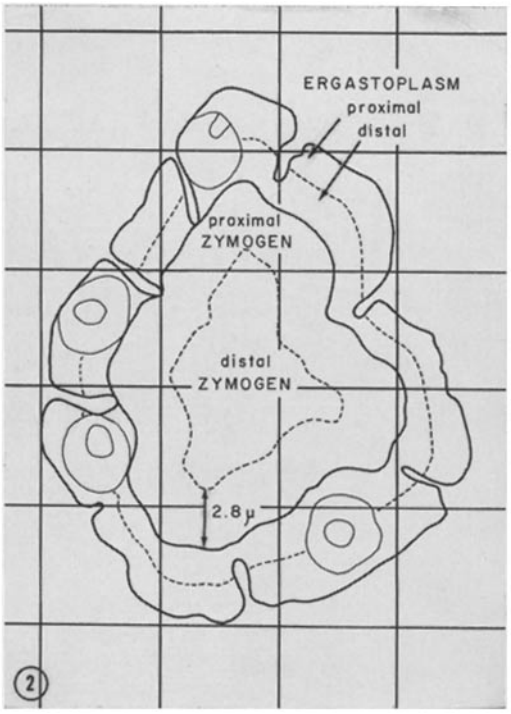


FIGURE 2

Outline drawing of the acinus shown in Fig. 1 to illustrate the preparation of the drawings used for the quantitative estimation of the number of silver grains and of the areas of each acinar region. The drawing shows the outline of the acinus (basement membrane), as well as the nuclei and the junction between the ergastoplasm and the group of zymogen granules (solid lines). The division of the ergastoplasm and zymogen granules into proximal and distal regions is indicated by the dashed lines. The dashed line dividing the ergastoplasm is placed midway between the basement membrane and the junction between the ergastoplasm and the zymogen granules, whereas the dashed line in the group of zymogen granules is drawn at an arbitrary distance from that junction (2.8 μ).

The acinus is shown in relation to the coordinate lines of the graph paper (1 × 1 inch). This relationship is identical with that obtained by photographing the graduations of a Whipple micrometer ocular grid (10 × 10 μ) superimposed on the acinus shown in Fig. 1. In practice, the location of each silver grain in the radioautograph was plotted on the drawing by using this system of coordinate lines. After the number of grains over each region were counted, the drawings were cut out and weighed. The relative weight of the paper making up each region was taken as a measure of the relative area of that region in the section.

Attractive hypotheses have been presented to account for the synthesis and secretion of enzymes in the acinar cells of the exocrine pancreas (recently reviewed in reference 3), but concrete evidence is scarce. Early radioautographic data with methionine-S^{35} showed that protein is rapidly synthesized in all acinar cells (1, 4–6) and is soon discharged into the excretory ducts (1, 6); Hansson observed radioactivity in all parts of the acinar cell and concluded that protein synthesis takes place throughout (6). Carneiro injected tritium-labeled leucine, glycine, and methionine, and sacrificed mice from 30 minutes to 45 days later; he reported an improved radioautographic resolution with the tritium label, but did not

describe migration within the pancreatic acinar cells (7). Nevertheless, we felt that it should be possible to trace the tritium label within the various structures of the acinar cells, provided that two further steps were taken. First, some of the animals should be sacrificed very early, that is, 10 minutes and eventually even 2 minutes after injection. Secondly, the specific activity of the proteins should be measured in the various structures of the acinar cells. Indeed, when we used this approach, a precise pattern for the synthesis and migration of the pancreatic enzymes became apparent.

MATERIALS AND METHODS

Main Experiment: Leucine-H³ in the Rat

Thirty-three young male rats, weighing 122 ± 6 grams, were fed on Purina Fox Chow *ad libitum*. Between 9 and 10 a.m. they received a single intraperitoneal injection of DL-leucine-4,5-H³ (New England Nuclear Corporation, Boston; specific activity, 29.1 mc/mM). The dose was defined by a radioactivity content of 2.5 μc for each gram of body weight. From the above specific activity, the amount of DL-leucine injected was calculated to be 11.2 μg per gram body weight. The animals were sacrificed under ether anesthesia by withdrawing blood from the abdominal aorta. This procedure was carried out 10 minutes after injection in 3 of the animals, whereas the remaining 5 groups, composed of 6 animals each, were sacrificed at 30 minutes, 4 and 36 hours, 7 and 30 days respectively after injection. The pancreas was fixed for 48 hours in Bouin fixative and routinely processed for histology. Sections 6 μ thick were stained with hematoxylin-eosin and radioautographed by the coating technique (8). The radioautographs were exposed for 15 days.

QUANTITATIVE RADIOAUTOGRAPHIC METHOD: Radioautographic density was estimated by counting the number of silver grains per unit area over various regions of the pancreatic acini. First, acini similar to the one seen in Fig. 1 were selected; these showed a clear separation from adjacent acini; the basophilic area at the base of the cells, hereafter referred to as ergastoplasm, was distinct, and arranged as a ring surrounding, more or less completely, a central region of acidophilic zymogen granules.

Then, each selected acinus was projected at a magnification of 2540 times and drawn on graph paper with the help of an ocular grid.[1] The acinar boundary was outlined, and the junction between ergastoplasm and zymogen granule area was drawn (Fig. 2). Those nuclei judged to be at the surface of the section were included in their proper position on the drawing.

The ergastoplasm was subdivided by a line drawn approximately parallel to its junction with the zymogen granule area. The line was placed halfway between this junction and the basement membrane of the acinar cells (Fig. 2). The two regions thus outlined were referred to as proximal ergastoplasm (near the basement membrane) and distal ergastoplasm (near the zymogen granules). It must be emphasized that the ergastoplasm region, as described above, comprises only the basophilic area at the base of the cells and does not include the small areas scattered between the zymogen granules (where the electron microscope shows rough surfaced sacs and ribosomes, as in the ergastoplasm itself (9)).

The zymogen granule area was also divided into proximal and distal regions by a line drawn parallel to its junction with the ergastoplasm and separated from it by an arbitrarily set distance of 2.8 μ (Fig. 2).

The radioautographic silver grains overlying the various acinar regions were localized using the ocular grid lines as coordinates, and recorded on the drawings, where the graph paper lines were used as the corresponding coordinates.[1] The numbers of silver grains drawn over the proximal and distal regions of both ergastoplasm and zymogen granules were then counted in all drawings. (The grains over the nuclei were omitted.)

AREA OF THE ACINAR COMPONENTS: Because of variation in acinar size, the results of the grain counts in the various regions were not readily comparable from one acinus to another. A method

[1] Each selected acinus, as well as the 10 μ graduations of a stage micrometer, were photographed at a magnification of 550 times. The negative image of the micrometer lines was projected, using a photographic enlarger, onto graph paper ruled in 1 inch squares. The enlargement was so adjusted that the micrometer lines coincided with those of the graph paper. Thus, a final magnification was achieved such that 10 μ corresponded to 1 inch, which resulted in a magnification of 2450 times. Using a microscope with a Whipple ocular micrometer grid ruled in 10 μ squares, and an oil immersion objective, the acinus to be drawn was aligned in relation to the squares of the grid. With this as a guide, the negative photographic image of that acinus was projected onto the squared paper, and similarly aligned with the squares of the paper. The acinar outline was then traced onto the paper so that the drawn acinus had the same relation to the squares of the paper as the microscopic image of that acinus had to the superimposed squares of the ocular grid.

was therefore devised to express each result as the number of grains per unit area of the region. The relative surface area of the acinar regions in sections was measured by the paper cut-out method (10) and the number of grains was expressed per weight of paper cut-out. Multiplying this figure (number of grains per gram of paper) by the weight of a paper cut-out for an area outlined by projection of a 100 μ^2 grid (grams of paper per 100 μ^2) gave the number of grains per 100 μ^2. On the assumption that the loss of beta rays by absorption is the same were therefore pooled, whereas the data for proximal and distal zymogen regions were recorded separately (Table II).

RELATIVE CONCENTRATION OF MILLON-REACTIVE PROTEIN: The cytochemical Millon reaction, as described by Rasch and Swift (11), was used to measure the protein concentration in the various parts of the cell studied. Quantitative data were obtained by the use of a microspectrophotometer (12, 13) constructed by Watzka Inc., Montreal.

TABLE I

Concentration of Radioactivity (Number of Silver Grains per 100 μ^2 ± S.E.) in Proximal and Distal Ergastoplasm of Rats after a Single Injection of Leucine-H^3*

(Main experiment)

Region of ergastoplasm	Time after injection			
	10 minutes	30 minutes	4 hours	36 hours
Proximal	21.7 ± 1.7	20.8 ± 1.3	12.2 ± 1.5	8.5 ± 1.0
Distal	22.3 ± 2.6	22.3 ± 2.2	14.0 ± 1.5	6.9 ± 1.6

* Concentration of radioactivity was averaged from counts over 12 acini at the 10 minute, 4 hour, and 36 hour time intervals, and 24 acini at the 30 minute interval.

TABLE II

Concentration of Radioactivity (Number of Silver Grains per 100 μ^2 ± S.E.) in Each Acinar Region after Intraperitoneal Injection of Leucine-H^3 in the Rat*

(Main experiment)

	Time after injection			
	10 minutes	30 minutes	4 hours	36 hours
Ergastoplasm	21.2 ± 1.2	21.6 ± 1.5	13.2 ± 0.9	7.7 ± 1.0
Proximal zymogen	22.5 ± 2.4	50.2 ± 3.3	51.2 ± 5.9	8.8 ± 1.2
Distal zymogen	9.4 ± 2.4	21.0 ± 2.9	61.5 ± 3.9	6.5 ± 3.0

* The number of observations for each time point is the same as given for Table I.

in the various regions of pancreatic acini, the number of grains per 100 μ^2 is directly proportional to the amount of radioactivity present, and will be referred to as *concentration of radioactivity* (grains per 100 μ^2).

The actual counts were done using drawings of 4 acini from each of 3 animals in the 10 minute, 4 hour, and 36 hour groups and from each of 6 animals in the 30 minute group.

It was soon found that the concentration of radioactivity in proximal and distal ergastoplasm showed no significant difference (Table I) and, therefore, the entire ergastoplasm could be considered a homogeneous unit. The data for the ergastoplasm

For the Millon reaction, sections 5 μ thick were cut from the blocks of pancreas used for the preparation of the radioautographs. To avoid the loss of the sections during subsequent treatment, it was found useful to keep the slides in a 56°C oven for 24 hours after mounting. The sections were then deparaffinized and dipped in a saturated solution of lithium carbonate to remove the picric acid taken up during Bouin fixation. Following the procedure of Rasch and Swift (11) the sections were washed in distilled water, placed for 10 minutes at 40°C in a 5 per cent solution of mercuric acetate in 30 per cent trichloroacetic acid, and then for 1 hour at 30°C in a solution of the same composition to

which 0.05 per cent sodium nitrite had been added. Finally, the sections were washed for 10 minutes in each of 3 baths of 70 per cent ethanol, dehydrated in absolute ethanol, cleared in xylene, and mounted in a Cargille oil with a 1.584 refractive index (selected by matching the oils to the pancreatic acinar cell according to reference 13; Fig. 3). For the measurement of the non-specific absorption, blanks were prepared by placing pancreas sections for 1 hour in 30 per cent trichloroacetic acid containing 0.05 per cent sodium nitrite. The section thickness was

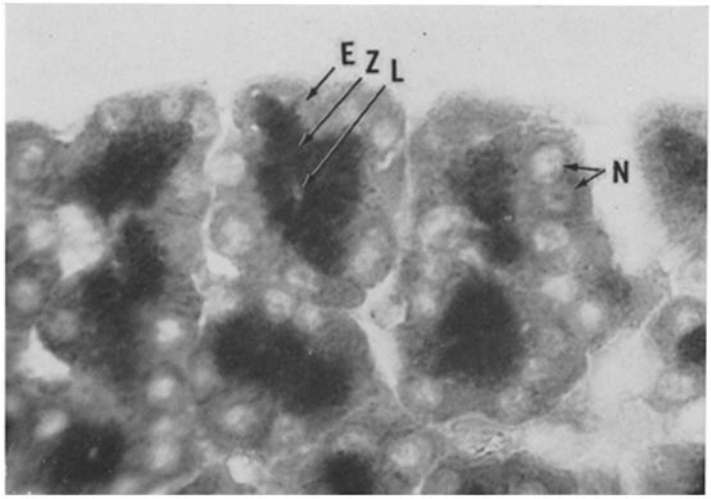


FIGURE 3

An area of the exocrine pancreas of the rat treated with the Millon reaction. Several acini are seen, in each of which the ergastoplasmic band (*E*) at the periphery of the acinus is much lighter than the central mass of darkly stained zymogen granules (*Z*). In the center of some acini, a light dot (*L*) indicates the acinar lumen. The nuclei (*N*) appear as light circles within the ergastoplasm. × 1000.

estimated in each specimen by measuring, with an ocular micrometer, the thickness of the edges of the folds (often present in Millon-treated sections), as indicated by Swift and Rasch (13).

The Millon reaction has been extensively studied both chemically and cytochemically (11). The first step in the reaction series involves the combination of mercury with the tryptophane and tyrosine residues of proteins; the second step involves the formation of the Millon chromophore at the sites of reaction. This reaction is quantitative, and the color intensity has been shown to increase in direct proportion to the amount of total protein present, in accordance with the Beer-Lambert laws.

Absorption curves for the Millon chromophores and for the blanks were prepared in serial sections of the same pancreatic acinus, care being taken to select homogeneous areas of ergastoplasm and zymogen granules. The total Millon absorption curve (Fig. 4, solid line) presents a high extinction in the ultraviolet, with a maximum at 350 mμ, and peaks at about 400 and 490 mμ which, according to Rasch and Swift (11), would coincide with the maximum of absorption for tryptophane and tyrosine solutions respectively. The blanks obtained by treatment with trichloroacetic acid and sodium nitrite indicate a net absorption in the ultraviolet, which extends in the visible range as far as 490 mμ (Fig. 4, dashed curve). Subtracting the blank from the total Millon extinction at each measured wavelength yields the corrected Millon extinction value (Fig. 4, dotted curve).

Many of the measured areas showed a non-homogeneous distribution of the Millon chromophores. To correct for any such distributional error, the two-wavelength method of Patau (14) was used. Two wavelengths, 545 and 590 mμ, were selected in homogeneous areas, such that the extinction at the first wavelength was half that at the other. The transmittance was computed for each wavelength in all measured acini. The tables of Mendelsohn (15) based on the work of Patau (14) and Ornstein (16) made it possible to calculate the extinction for such non-homogeneous areas. Since the extinction thus obtained was directly proportional to the concentration of the chromophore, it was used as a measure of the relative con-

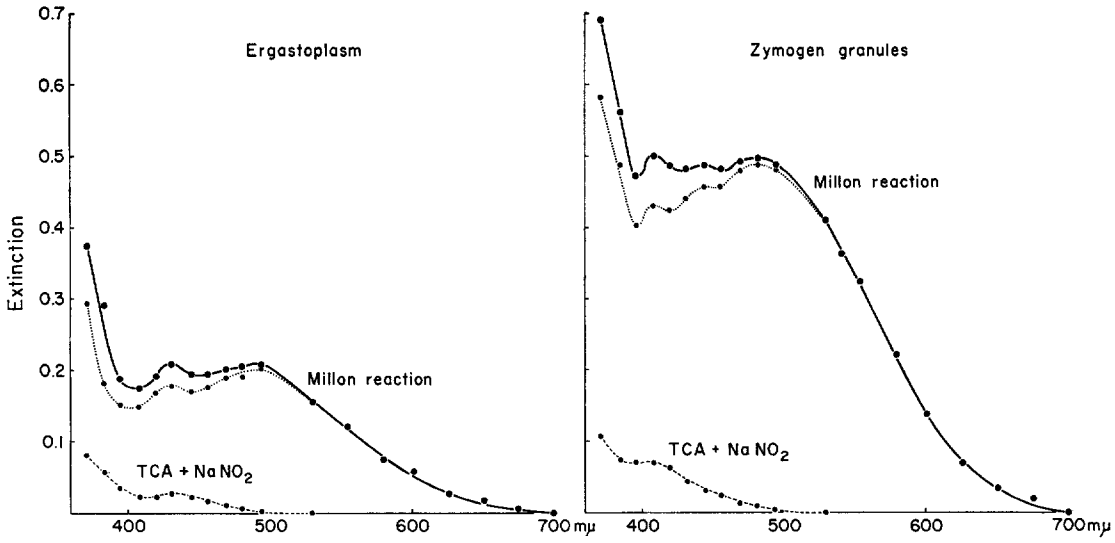


FIGURE 4

Absorption curves run over a homogeneous area of ergastoplasm (left) and of zymogen granule region (right) in an acinus of the exocrine pancreas. Solid curve: total Millon absorption curve. Dashed curve (TCA + $NaNO_2$): non-specific absorption of blanks obtained after treatment of sections with trichloroacetic acid and sodium nitrite. Dotted curve: corrected Millon absorption curve, obtained by subtracting the values of the dashed curve from those of the solid curve at each wavelength. At the wavelengths of 545 to 590 mμ, chosen for the determination of Millon absorption, the non-specific absorption is negligible.

TABLE III

*Concentration of Millon-Reactive Protein (Extinction Value) in Pancreatic Acinar Cells of Rats, As Measured Microspectrophotometrically**

Animal no.	Time	Extinction ± S.E.		
		Ergastoplasm	Zymogen region	Ratio zym./erg.
1	10 min.	0.146 ± 0.013	0.360 ± 0.014	2.47 ± 0.24
2	30 min.	0.129 ± 0.012	0.338 ± 0.017	2.62 ± 0.29
3	4 hr.	0.152 ± 0.024	0.370 ± 0.027	2.43 ± 0.44
4	36 hr.	0.146 ± 0.006	0.358 ± 0.016	2.45 ± 0.16
5	7 days	0.143 ± 0.016	0.368 ± 0.022	2.57 ± 0.45
Mean		0.143 ± 0.033	0.359 ± 0.043	2.51 ± 0.64

* Measurements were made on 10 acini from each of 5 rats. In each selected acinus, 4 readings were recorded, 2 over the zymogen granule region and 2 over the ergastoplasm, care being taken to avoid nuclei.

centration of Millon reactive protein (Table III).

SPECIFIC ACTIVITY CALCULATION: The specific activity of the proteins in the three regions was expressed as the ratio:

$$\frac{\text{concentration of radioactivity (number of grains per unit area)}}{\text{relative concentration of Millon reactive protein (extinction)}}$$

and was calculated for the proteins of the ergastoplasm and of the proximal and distal zymogen granule regions.

Corroborative Experiments

LEUCINE-H^3 AT EARLY TIME INTERVALS IN THE RAT: The results obtained in the main experiment were extended to time intervals earlier than 10 minutes after leucine-H^3 injection. Eight

TABLE IV

Concentration of Radioactivity (Number of Silver Grains per 100 μ^2 ± S.E.) in Each Acinar Region, at the Early Time Intervals after Intraperitoneal Injection of Leucine-H^3 in the Rat*

(Corroborative experiment at early time intervals)

	Time after injection			
	2 minutes‡	5 minutes	10 minutes	30 minutes
Ergastoplasm	(21.5 ± 2.4)	15.7 ± 1.7	19.3 ± 2.1	31.5 ± 3.9
Proximal zymogen	(10.9 ± 2.2)	10.1 ± 1.8	27.6 ± 4.5	71.5 ± 12.6
Distal zymogen	(4.0 ± 2.2)	3.8 ± 1.3	6.1 ± 1.3	18.4 ± 5.8

* Concentration of radioactivity was averaged from counts over 8 acini at each time interval.
‡ The figures at 2 minutes were unduly high because the choice of acini was restricted to the few radioactive ones.

TABLE V

Concentration of Radioactivity (Number of Silver Grains per 100 μ^2 ± S.E.) in Proximal and Distal Ergastoplasm of Mice after a Single Injection of Labeled Amino Acids*

Glycine-H^3

(3.5 μc/gm body weight)

Region of ergastoplasm	Time after injection		
	5 minutes	15 minutes	30 minutes
Proximal	21.6 ± 2.7	26.3 ± 2.7	28.4 ± 3.4
Distal	18.3 ± 2.8	28.6 ± 2.9	31.8 ± 4.6
Methionine-H^3 (3.5 μc/gm body weight)			
Proximal	14.7 ± 1.1	28.6 ± 3.7	44.3 ± 4.0
Distal	10.8 ± 1.6	30.7 ± 3.4	47.9 ± 2.3

* Concentration of radioactivity was averaged from counts over 12 acini in the glycine-H^3 experiment, and over 24 acini in the methionine-H^3 experiment.

rats conforming to the specifications given above were injected intraperitoneally with a single dose of 2.5 μc (9.2 μg) per gram of body weight of DL-leucine-4,5-H^3 (New England Nuclear Corporation, Boston; specific activity, 35.7 mc/mM) and sacrificed in pairs at 2, 5, 10, and 30 minutes respectively after the injection. The pancreas was fixed in Bouin fluid, and the 5 μ sections were radioautographed as above. The preparations were exposed for 35 days. Concentration of radioactivity was measured in 4 acini from each animal (Table IV).

GLYCINE- AND METHIONINE-H^3 IN THE MOUSE: Adult male mice, weighing 25 to 30 grams, each received a single intraperitoneal injection of 3.5 μc (17.0 μg) or 5 μc (24.4 μg) per gram of body weight of glycine-2-H^3 (New England Nuclear Corporation, Boston; specific activity, 15.4 mc/mM) or 3.5 μc (18.7 μg) of DL-methionine-H^3 (generally labeled; Radiochemical Centre, Amersham, England; specific activity, 28.0 mc/mM). The mice were sacrificed in pairs at 5, 15, and 30 minutes respectively after injection, and pancreas sections were radioautographed as above. Following a 21 day exposure, the concentration of radioactivity was measured in 12 acini from each animal.

In the mouse, as in the rat, the proximal and distal ergastoplasm yielded equal values (Table V). Again, the two parts of the ergastoplasm were combined in recording the results (Tables VI and VII). Protein concentration for specific activity determination was measured as above in 5 acini from each of 4 of the glycine-H^3–injected mice.

TABLE VI

Concentration of Radioactivity (Number of Silver Grains per 100 μ^2 ± S.E.) in Each Acinar Region after Intraperitoneal Injection of Glycine-H^3 in Mice*

	Time after injection				
	5 minutes		15 minutes		30 minutes
	3.5 μc	5 μc	3.5 μc	5 μc	3.5 μc
Ergastoplasm	20.3 ± 2.6	18.8 ± 2.3	27.2 ± 2.4	24.8 ± 3.0	30.5 ± 3.5
Proximal zymogen	14.5 ± 2.0	16.6 ± 1.8	32.0 ± 2.8	26.1 ± 2.9	54.8 ± 6.9
Distal zymogen	8.1 ± 2.9	7.1 ± 2.2	11.6 ± 1.4	10.0 ± 2.4	44.2 ± 8.8

* Concentration of radioactivity was averaged from counts over 12 acini at each time interval.

TABLE VII

Concentration of Radioactivity (Number of Silver Grains per 100 μ^2 ± S.E.) in Each Acinar Region after Intraperitoneal Injection of Methionine-H^3 in Mice*

	Time after injection		
	5 minutes	15 minutes	30 minutes
Ergastoplasm	13.0 ± 1.1	29.4 ± 3.5	45.8 ± 3.1
Proximal zymogen	12.4 ± 0.9	34.9 ± 2.4	61.9 ± 4.5
Distal zymogen	3.9 ± 0.8	17.0 ± 4.6	46.3 ± 5.4

* Concentration of radioactivity was averaged from counts over 24 acini at each time interval.

RESULTS

The pancreatic acini of rats and mice are chiefly composed of groups of cells containing zymogen granules, the acinar cells. In addition, the odd centroacinar cell (similar to and continuous with the duct cells) may also be seen, but was considered infrequent enough to be neglected in this investigation.

It was stated above that the acinar cells are composed of a basophilic base, referred to as ergastoplasm and containing the cell nucleus, and of an acidophilic apical region mainly composed of zymogen granules. The Golgi apparatus is within the zymogen granule region, though it makes contact with the edge of the ergastoplasm (Figs. 25 and 27).

When a whole acinus is considered (Fig. 1), it is seen that cell limits are not distinct. The ergastoplasm of the adjacent cells makes up a basophilic ring, which encloses the group of zymogen granules. The acinar lumen is small and barely, if at all, visible, appearing then as a light dot (Fig. 3) or an irregular slit (Fig. 1).

Main Experiment: Leucine-H^3 in the Rat

Radioautographs prepared between 10 minutes and 36 hours after leucine-H^3 injection showed photographic silver grains in the emulsion overlying every acinus throughout the entire section of the pancreas from each animal. The intensity of the reaction appeared to be fairly similar in all acini at any given time interval, except for a few strongly labeled ones located along the peritoneal surface and at the periphery of some of the lobules. These were not used for counting.

At 10 minutes (Fig. 5) and 30 minutes (Fig. 6) after injection, silver grains were located over the ergastoplasm and the proximal zymogen region, but only rare and scattered reactions were seen over the distal zymogen region. At 30 minutes, the grains observed in the proximal zymogen region were more or less clustered, usually at a short distance from the distal pole of the nucleus (Fig. 6, upper arrow). This was particularly clear in radioautographs exposed 67 instead of 15 days, in which each cell showed a discrete mass of

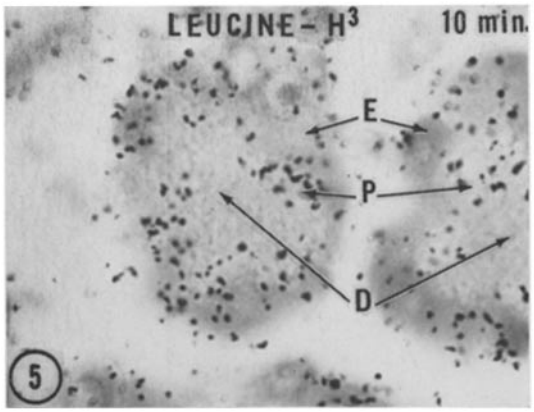
grains in the proximal zymogen region (Figs. 26, 28, *C*).

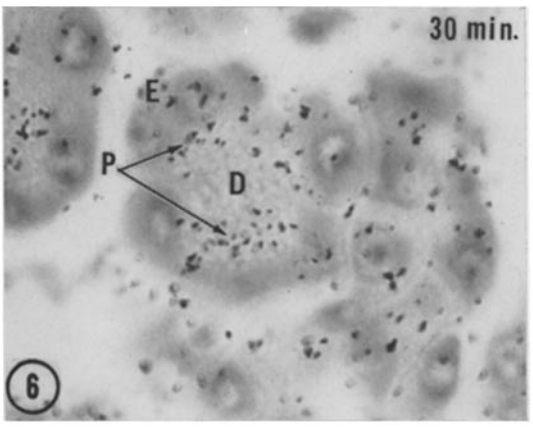
By 4 hours after the injection, the reaction was intense over the distal zymogen region (Fig. 7).

The reaction over the acini was weak at 36 hours, and extremely weak at 7 and 30 days after injection. The preparations obtained at 7 and 30 days were not investigated further.

The luminal content of the *excretory ducts* was not overlaid by silver grains at 10 minutes. A few grains were seen at 30 minutes (Fig. 8) and many at 4 hours (Fig. 9).

Measurements of the *concentration of radioactivity* (Table II) showed it to be high in the ergastoplasm and proximal zymogen region at the early





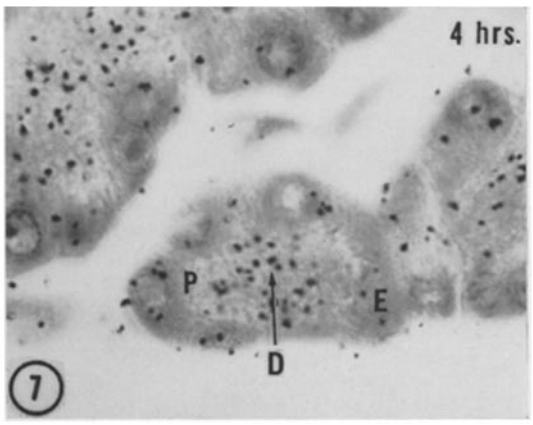


FIGURE 5 TO 7

Hematoxylin-eosin–stained radioautographs of pancreatic acini of the rat after intraperitoneal injection of leucine-H³. *Main experiment*, 15 day exposure. With this stain, the ergastoplasm appears dark and the zymogen granule region light. × 1300.

FIGURE 5

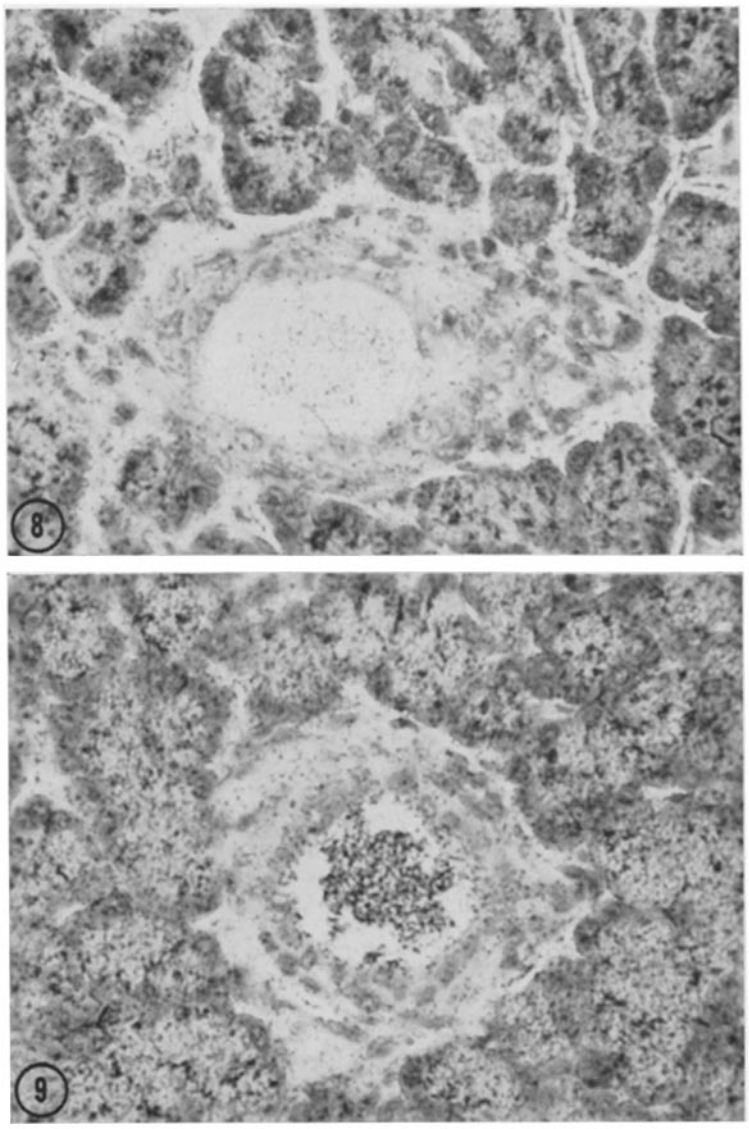
Acinus 10 minutes after leucine-H³ injection. The silver grains are located over the darker staining ergastoplasm (*E*) and over the proximal zymogen region (*P*). The distal zymogen region (*D*) shows no reaction.

FIGURE 6

Acinus 30 minutes after leucine-H³ injection. The ergastoplasm (*E*) shows some reaction, but more grains are now located over the proximal zymogen region (*P*), where the grains may be arranged into clusters. Again, the distal zymogen region (*D*) shows no reaction.

FIGURE 7

Acinus 4 hours after leucine-H³ injection. While some reactions persist over the ergastoplasm (*E*), most reactions are distributed over the proximal (*P*) and distal (*D*) zymogen regions.



FIGURES 8 AND 9

Hematoxylin-eosin–stained radioautographs of the excretory ducts of the rat pancreas after leucine-H^3 injection, 67 day exposure. × 500.

FIGURE 8

Pancreatic duct, 30 minutes after leucine-H^3 injection. The material in the lumen of this duct is slightly radioactive. The surrounding acini show dark clusters of silver grains, which, under higher power, can be localized to the proximal zymogen region.

FIGURE 9

Pancreatic duct, 4 hours after leucine-H^3 injection. The material within the lumen is now intensely radioactive. The radioautographic reactions over the surrounding acini are mostly distributed over the entire zymogen granule region.

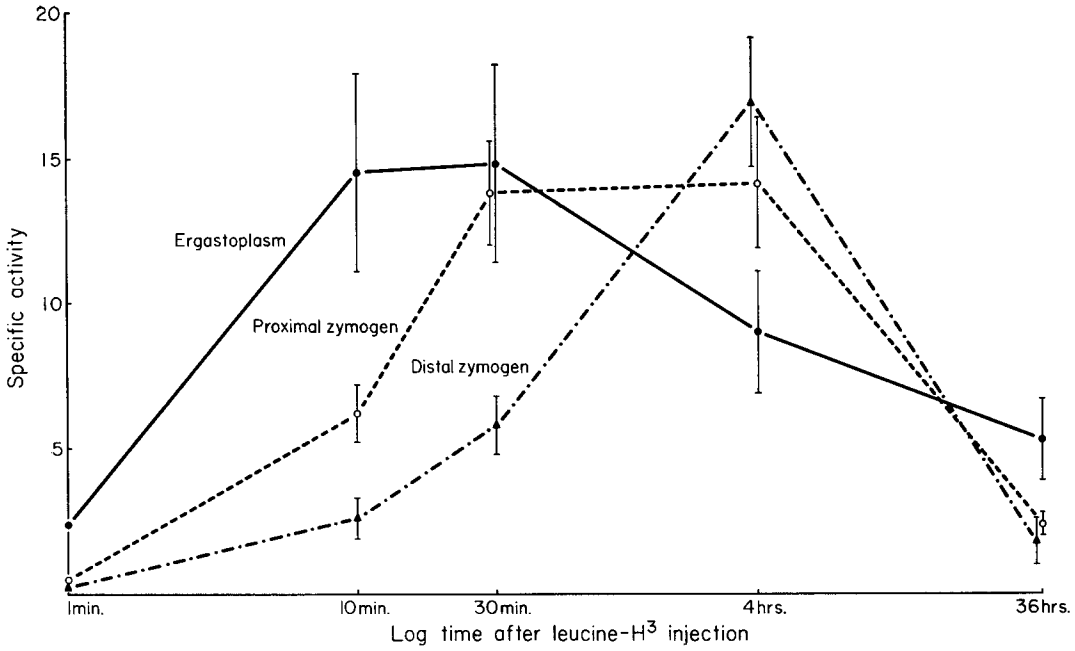


FIGURE 10

Specific activity of the proteins in the ergastoplasm (solid circles), the proximal zymogen region (open circles), and the distal zymogen region (solid triangles), plotted against the log of the times after injection of leucine-H^3 into rats. Main experiment.

Each point shows the specific activity and standard error for each time interval in the three regions of the acinus. (The 1 minute points were determined graphically by extrapolation on a non-logarithmic scale from 0 to 10 minutes, since, when x is very small, log x may be considered to be almost equal to x.) The peak of specific activity is reached first in the ergastoplasm, then in the proximal, and finally in the distal zymogen regions.

intervals, whereas it increased slowly in the distal zymogen region with a peak occurring at 4 hours.

The *relative protein concentration* measured in Millon-treated sections (Fig. 3) was the same in the proximal and distal zymogen regions, but was 2.49 ($\pm$0.64) times greater in these regions than in the ergastoplasm (Table III).

The *specific activity time curves* of the proteins were then calculated for the three regions (Fig. 10). A peak was reached in the ergastoplasm at 10 to 30 minutes after injection. Between 30 minutes and 4 hours a peak occurred in the proximal zymogen region. The distal zymogen region achieved maximum specific activity at 4 hours.

Corroborative Experiments

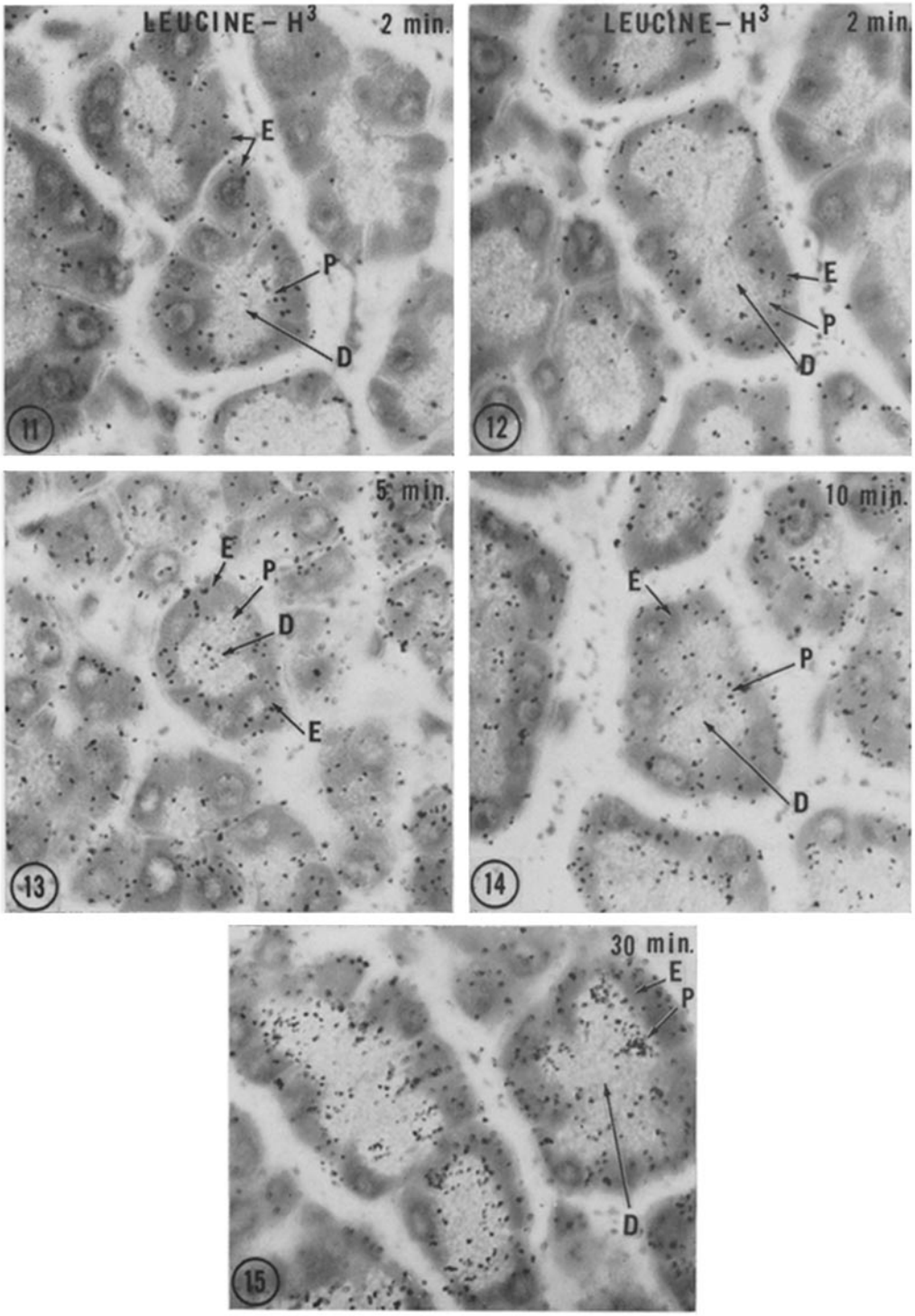
LEUCINE-H^3 AT EARLY INTERVALS IN THE RAT: Since, at 10 minutes after leucine-H^3 injection, reactions were seen over both ergastoplasm and proximal zymogen granules, it was decided to sacrifice animals at even earlier intervals and explore whether the localization would then be restricted to one of the two regions.

At 2 minutes, that is, very early after injection, only a few acini showed reactions. In those acini, the silver grains were almost exclusively localized over the ergastoplasm, with only the odd grain over the zymogen granules (Figs. 11 and 12).

By 5 minutes after injection, all acini were labeled; most grains were again located over the ergastoplasm (Fig. 13). Scattered grains, found over the proximal zymogen region at 5 minutes, increased in number at 10 minutes after injection (Fig. 14).

The pattern at 30 minutes was similar to that observed at the same time interval in the main experiment, with clusters of grains over the proximal zymogen region (Fig. 15).

The *concentration of radioactivity* (Table IV) gradually increased between 5 and 30 minutes


LEUCINE – H3
2 min.
E
P
D
11
LEUCINE – H3
2 min.
E
P
D
12
5 min.
E
P
D
E
13
10 min.
E
P
D
14
30 min.
E
P
D
15

in the three components of pancreatic acini. The mean values at 2 minutes were unduly high because the choice of acini was restricted to the few that were radioactive.

The *specific activity time curves* were calculated from the values in Table IV, and the extinction values obtained for the main experiment. The curves were drawn without the 2 minute figures, since these could not be considered a true mean, as the choice of acini was restricted to the few radioactive ones (Fig. 16). A high specific activity was seen in the ergastoplasm at the very early time intervals, thus indicating that the incorporation of the administered radioactivity occurs first in this region.

GLYCINE- AND METHIONINE-H^3 IN THE MOUSE: Five minutes after injection of either glycine- or methionine-H^3 into mice, all acini showed a radioautographic reaction. At this time, the reaction was, again, chiefly localized over the ergastoplasm (Figs. 17 and 19; Table V), whereas at 15 and particularly 30 minutes after injection (Figs. 18 and 20) the concentration of radioactivity was high in the proximal zymogen region. At 30 minutes, the grains were often clustered in a supranuclear position (Figs. 18 and 20, *P*) and a small amount of radioactivity was present in the material within the lumen of the pancreatic ducts.

When the *relative protein concentration* was measured in the mouse pancreas treated for the Millon reaction, the mean extinction value calculated from 80 microspectrophotometric measurements was 0.147 ± 0.018 in the ergastoplasm and 0.345 ± 0.042 in the zymogen granules.

The *specific activity time curves* observed with glycine- and methionine-H^3 in mice (Figs. 21 and 22) showed a pattern similar to that observed with leucine-H^3 in rats (Fig. 16).

DISCUSSION

Newly Synthesized Proteins as the Cause of the Radioautographic Reactions over the Pancreas

The conditions of tracer methodology require that the dose of labeled substance injected be small enough not to produce significant radiation damage and not to upset the normal physiological balance. The dose of radioactivity used in most of these experiments (2.5 μc/gm body weight) was selected after previous experiments had shown that the bone marrow and other organs appeared qualitatively normal after injection of 5 μc/gm body weight (7). As for the amount of

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FIGURES 11 TO 15

Hematoxylin-eosin–stained radioautographs of pancreatic acini of the rat after leucine-H^3 injection. Corroborative experiment, 35 day exposure. × 1000.

FIGURES 11 AND 12

Pancreatic acini 2 minutes after leucine-H^3 injection. The reactions are confined to the basophilic ergastoplasm (*E*), with the proximal (*P*) and distal (*D*) zymogen regions showing very little radioactivity. At this early time interval, the majority of acini (upper right in both figures) show no reactions.

FIGURES 13 AND 14

Acini 5 and 10 minutes respectively after leucine-H^3 injection. At both intervals, all acini are reactive. A fairly strong reaction is located over the ergastoplasm (*E*). At 5 minutes (Fig. 13), few reactions are seen over both proximal (*P*) and distal (*D*) zymogen regions. The reactions over the zymogen granules at 10 minutes (Fig. 14) are mainly over the proximal zymogen region (*P*).

FIGURE 15

Acini 30 minutes after leucine-H^3 injection. A slightly heavier reaction is seen over the ergastoplasm (*E*) than at the previous time intervals. However, the most intense reaction is now over the proximal zymogen region (*P*), where clusters of grains are distinct. The distal zymogen region (*D*) shows no reaction.

amino acid injected, it may be recalled that in the two experiments using leucine-H^3, the doses were 9.2 and 11.2 μg respectively of labeled leucine per gram of body weight. Since the blood plasma of the rat contains about 20 μg of free leucine per milliliter (17–20) and liver, brain, muscle, and spleen contain 100, 32, 42, and 184 μg of free leucine per gram, respectively (18), the injection of 9.2 to 11.2 μg of leucine-H^3 per gram of body weight was thought not to upset the normal balance. Rather, the leucine-H^3 would mingle with, and randomly label, the circulating leucine. The

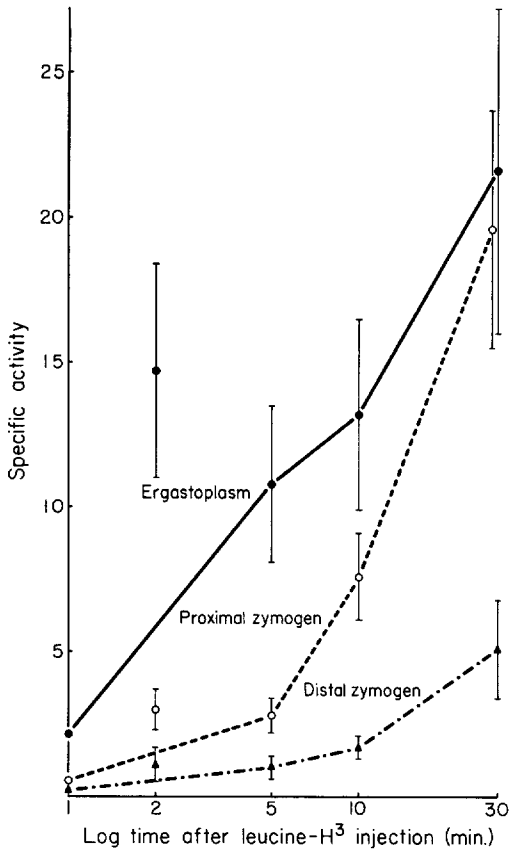


FIGURE 16

Specific activity of the proteins in the corroborative experiment with leucine-H^3 in the rat. Details as in Fig. 10. The curves are drawn without the 2 minute values, because these could not be considered true means. At these early times after injection, the specific activity of the ergastoplasm is higher than that of the proximal zymogen region, which in turn is higher than the specific activity in the distal zymogen region.

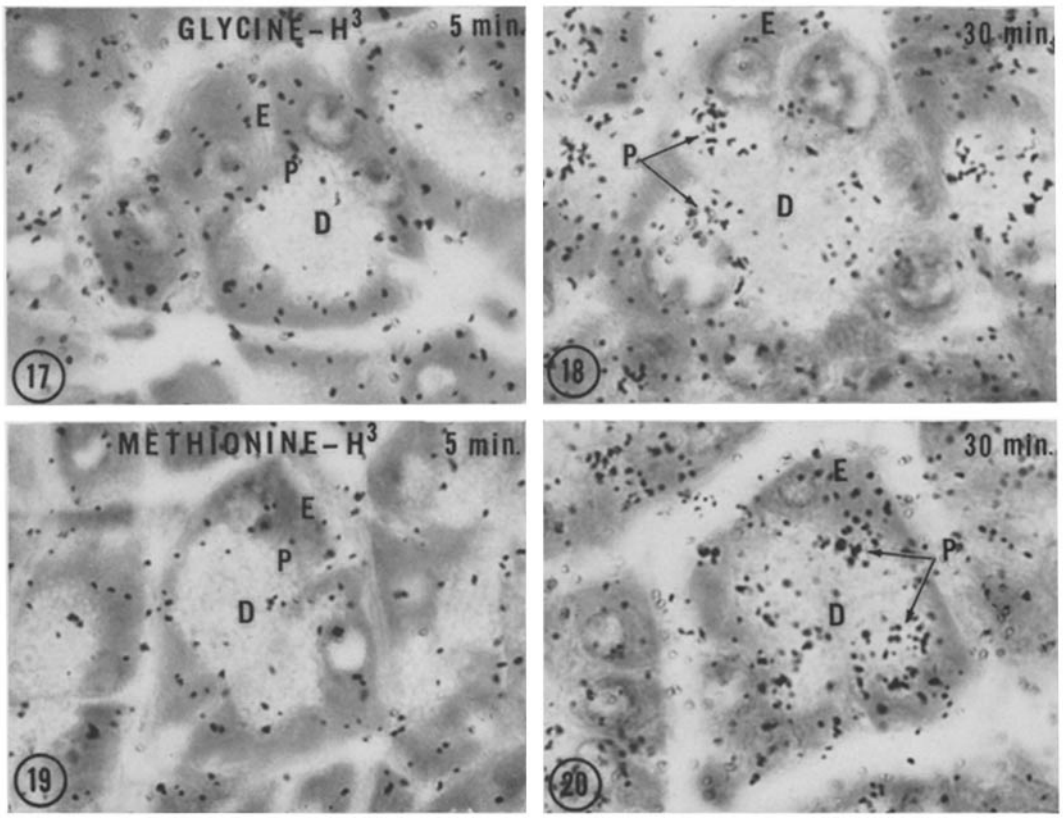
doses of glycine- and methionine-H^3 used in the mouse experiments were of the same order of magnitude. Therefore, the behavior of each one of these labeled amino acids should provide a true picture of the behavior of the corresponding non-labeled amino acid in the body.

After injection into an animal, free leucine—taken as an example of the behavior of the three amino acids—rapidly leaves the circulation (21) to be used by organs and tissues as a precursor of cholesterol (22) or, after combination with the so-called transfer RNA (23), as a building block for proteins. This protein synthesis occurs within or on the ribosomes (24–27).

Of the three labeled substances found in tissues after injection of labeled leucine, the free leucine itself was shown to be washed out by Bouin fixative (28), and cholesterol was likely to be extracted by dioxane, alcohol, and xylol, but proteins should be retained throughout the histological procedure (28).

However, the conclusion that the radioactivity present in our sections is due to amino acid residues of proteins can be safely reached only if two other possibilities are excluded. First, some amino acids, for example glycine, can serve as precursors of nucleic acids (29). Secondly, evidence presented by Godin indicates that amino acids can be bound to preexisting proteins by salt linkage (30). These possibilities were examined by Droz and Warshawsky (28, 31) in mice given leucine-C^{14}. Numerous histological sections were cut from the pancreas, pooled, and fractionated by standard biochemical procedures. The nucleic acids were removed by treatment with hot trichloroacetic acid (95°C). Then, salt-linked amino acids were extracted with sodium hydroxide and the proteins were reprecipitated. It was found that 91 to 94 per cent of the radioactivity in the pooled histological sections was in the protein fraction. The remainder was distributed in the nucleic acid and sodium hydroxide fraction, but even this may have been due to contamination by radioactive protein. Indeed, in another type of experiment with tissue sections containing labeled leucine (mounted on histological slides), no loss of radioactivity was found whether nucleic acids were removed with hot trichloroacetic acid (32) or salt-linked amino acids were removed with alkaline solutions (28).

The conclusion reached was that the radioactivity in our sections of pancreas is present as



FIGURES 17 TO 20

Hematoxylin-eosin–stained radioautographs of pancreatic acini after glycine-H^3 and methionine-H^3 injection. Corroborative experiments in the mouse. × 1000.

FIGURES 17 AND 19

Pancreatic acini 5 minutes after injection of glycine-H^3 and methionine-H^3, respectively. At this early time interval, with both amino acids, reactions are present mainly over the basophilic ergastoplasm (*E*). The proximal zymogen regions (*P*) show a few grains, but no reaction is present over the distal zymogen regions (*D*).

FIGURES 18 AND 20

Pancreatic acini 30 minutes after injection of glycine-H^3 and methionine-H^3, respectively. Whereas reactions are still present over the ergastoplasm (*E*) in both figures, the heaviest reactions are now over the proximal zymogen regions (*P*). Again, clusters of silver grains may be seen over this region. The distal zymogen regions (*D*) show few reactions.

labeled amino acids held in protein by bonds which are firm enough to suggest peptide linkage.

Many proteins are known to be present in pancreatic acinar cells (3). Some of these, the precursors of the digestive enzymes—amylase, lipase, proteases, and ribonuclease—may be referred to as "exportable" proteins. Others, the "sedentary" proteins, are those which remain within the cell and are turned over *in situ*. Thus, the recorded specific activities will be an average of the specific activities of all the constituent proteins.

Site of Protein Synthesis in the Acinar Cells of the Pancreas

A common practice in studies of pancreatic secretion is to fast the animals for some time and feed them just before use, in the hope of inducing

all acinar cells to secrete actively (33, 34). However, in our experience, fasting may produce untoward effects in rats and mice (irritability, gastrointestinal hemorrhages, etc.), and, therefore, pilot experiments were designed to find out if all acinar cells are actively secreting in rats fed *ad libitum*. Such rats were injected with a labeled amino acid (methionine-H^3) at four different times of the day (2 a.m., 8 a.m., 2 p.m., and 8 p.m.). At each time, two animals were sacrificed 30 minutes, and two others, 3 hours after injection; and the pancreas was sectioned and radioautographed. In the animals sacrificed at 30 minutes, all the acini showed the presence of radioactive protein, which was arranged in a pattern similar to the one recorded at the 30 minute time interval, as shown in Fig. 20. Hence, it became evident that the acinar cells of the pancreas were elaborating proteins whatever the time of day at which the animals were sacrificed. Furthermore, in all animals sacrificed 3 hours after injection, there was radioactivity not only in the acini, but in the material present within the lumen of the excretory ducts. Therefore, the acinar cells of the rat produce pancreatic secretion throughout the day. These findings might be related to the habits of rats and mice, which are known to eat quite frequently; and indeed, the rat stomach contains food at whatever time of day it is examined (35). In any case, it was clear that, in the rat pancreas, both protein synthesis and secretion go on continuously. When planning the present experiments, it was therefore decided that the animals would not be fasted, but fed *ad libitum*. The results should then give a picture of what is going on under physiological conditions.

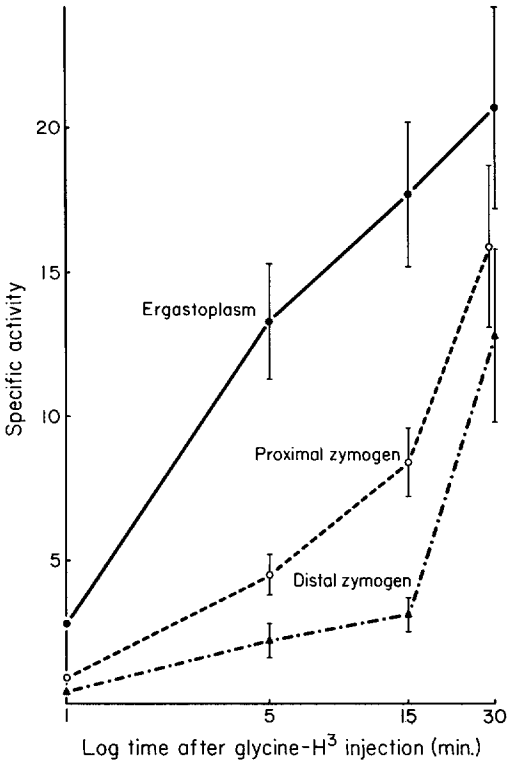


FIGURE 21

Specific activity of proteins in the corroborative experiment with glycine-H^3 in the mouse. Details as in Figs. 10 and 16.

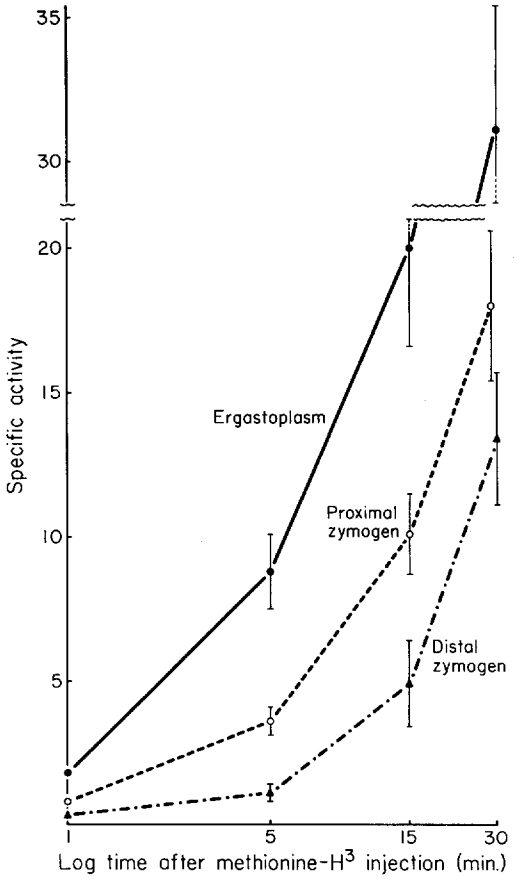


FIGURE 22

Specific activity of proteins in the corroborative experiment with methionine-H^3 in the mouse. Details as in Figs. 10 and 16.

Since the radioactive material in the histological sections was shown to consist of protein, the structures in which radioactivity first appeared after injection of labeled amino acids must be the sites where proteins are being synthesized. However, this is true only if the pancreas is examined very soon after injection, since at later times proteins might have migrated out of these sites. In the main experiment, radioactive proteins were observed at 10 minutes after leucine-H^3 injection in both the ergastoplasm and the proximal zymogen region (Table II; Fig. 5) and we wondered if synthesis had taken place in both regions. The problem was approached in two ways: (*a*) by examining animals sacrificed earlier than 10 minutes after injection and (*b*) by considering the specific activity time curves of the pancreatic proteins in the various parts of the acinar cell.

As early as 2 and 5 minutes after leucine-H^3 injection into the rat (Table IV; Figs. 11 to 13) and 5 minutes after glycine- or methionine-H^3 injection into the mouse (Tables VI and VII; Figs. 17 and 19), labeled proteins were mainly present in the ergastoplasm. The distribution of radioactivity was uniform throughout this region (Tables I and V). It follows that protein synthesis must be taking place throughout the ergastoplasm.

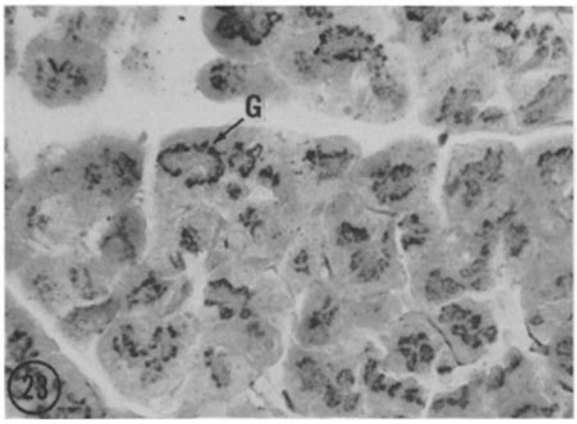
The synthesis of labeled protein presumably continues in the ergastoplasm at least as long as labeled amino acids are available in the circulation, that is, until the 30 minute interval or some time thereafter (21). Therefore, the presence of labeled proteins in the proximal zymogen region could be explained by either one of two alternatives: migration from the ergastoplasm or synthesis *in situ* (at a slower rate than in the ergastoplasm). The specific activity data were used to examine this problem.

It should be realized that use of specific activity data involves the assumption that the three regions under consideration are functionally homogeneous cell compartments. (A discussion of the validity of this assumption may be found in the Appendix to this paper.) It is known that, if a substance A gives rise to a substance B, then first, the specific activity of A is initially greater than that of B; second, the time curve of B reaches a maximum at the intersection with the time curve of A; and, third, the specific activity of B is thereafter greater than that of A (see Appendix and reference 2). Inspection of Fig. 10 showed a relatively high specific activity in the ergastoplasm at the early time intervals, thus confirming that protein is being synthesized in this region. Furthermore, the proximal zymogen proteins had a low specific activity at first, and their time curve reached a maximum at about the point where it cut that of ergastoplasmic proteins. Therefore, the conditions required for the ergastoplasmic proteins to be the precursors of the proximal zymogen proteins were fulfilled. It thus appeared that the ergastoplasm alone was the site of protein synthesis.

Let us compare this conclusion with biochemical observations. Morris and Dickman in 1960 (36) injected valine-C^{14} into mice, and ultracentrifuged the homogenized pancreas into separate fractions, including the microsomes (consisting of sacs of the endoplasmic reticulum with the attached ribosomes) and zymogen granules. Ribonuclease was then extracted from each fraction. The radioactivity of the enzyme in the microsomes was high at 5 minutes and reached a maximum at 15 minutes, whereas in the zymogen granules it rose slowly and became maximal only after 120 minutes. Similarly, Siekevitz and Palade, who in 1960 (37) injected leucine-C^{14} into guinea pigs, separated several pancreatic fractions—including the ribosomes themselves and the zymogen granules—and extracted α-chymotrypsinogen from each. The radioactivity of the enzyme rose first in the ribosomes and later in the zymogen granules. They concluded that pancreatic enzymes are synthesized within the ribosomes and then migrate to the zymogen granules. Since most of the ribosomes of the acinar cell are contained within the ergastoplasmic region, this conclusion is consistent with our finding of protein synthesis there. (The rare grains seen in the zymogen regions at 2 to 5 minutes after injection may be due to proteins synthesized in the few ribosomes scattered between zymogen granules (9).)

Assuming that the ergastoplasmic proteins give rise to proximal zymogen proteins, we may use the early specific activity data to calculate an estimate of their turnover time, that is, the time for the replacement of an amount of protein equal to that present (see Table I of Appendix). The turnover time of ergastoplasmic proteins was found to be short (4.7 minutes in the main experiment), indicating that the migration of these proteins toward the proximal zymogen region takes place very soon after or even during their formation.

The short turnover time of ergastoplasmic proteins suggests that very little if any of the rapidly replaced labeled proteins should be left in this region by 4 and especially 36 hours. However, a fair amount of radioactivity was still present. This indicated that, in addition to the rapidly turned over proteins, the ergastoplasm contains some proteins with a slow rate of replacement. Calculations based on the part of the curves extending from 4 to 36 hours (Fig. 10) yielded a turnover time of 62.5 hours for the slowly replaced proteins of the ergastoplasm. Presumably, the more rapidly turned over proteins are the "exportable" ones, which are secreted as digestive enzymes, whereas the proteins with a lower turnover rate would be the "sedentary" proteins used for the maintenance of the cell's own metabolism.



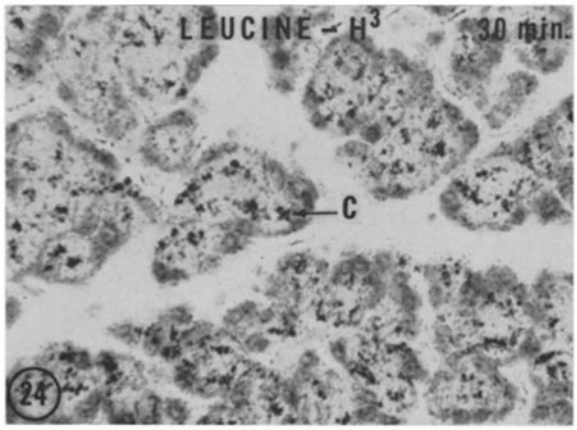


FIGURE 23

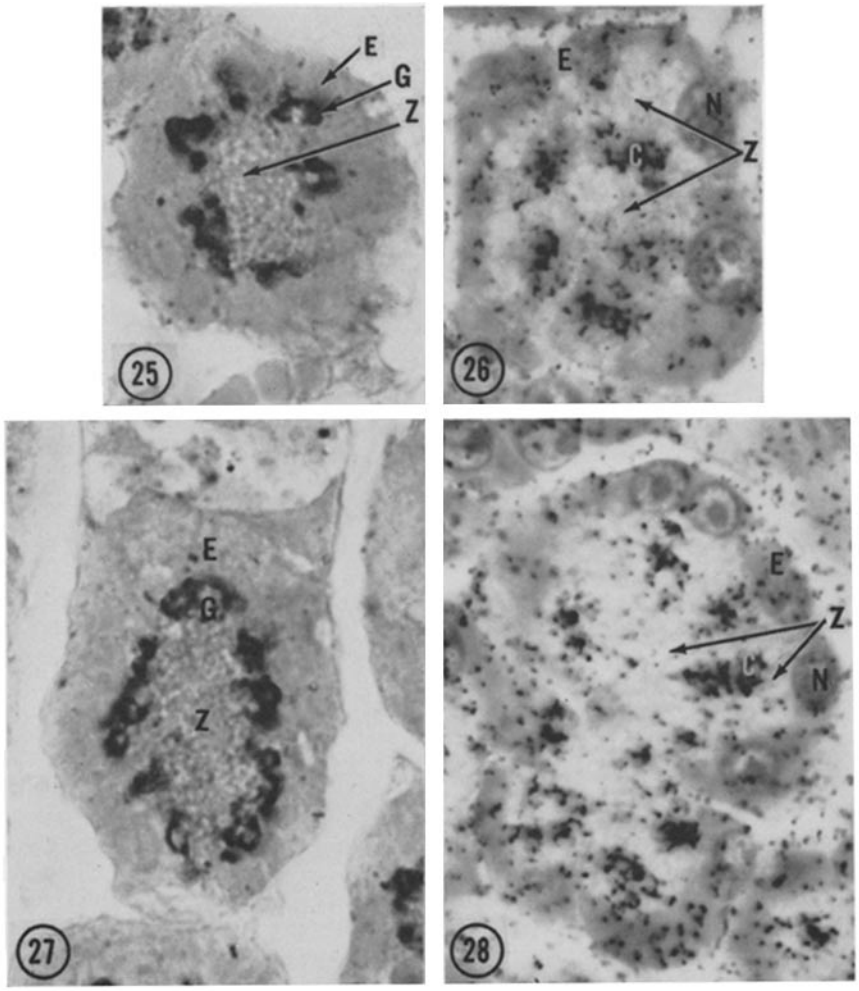
Exocrine pancreas of the rat stained with osmium tetroxide–zinc iodide to demonstrate the Golgi apparatus. Each acinus contains a group of darkly stained, osmiophilic Golgi elements. These elements often make up an interrupted ring, as shown at the arrow *G*. × 350.

FIGURE 24

Hematoxylin-eosin–stained radioautograph of an area of the ras pancreas 30 minutes after leucine-H^3 injection, 67 day exposure. Clusters of silver grains (*C*) are seen in the acini. Comparing this micrograph with Fig. 23 suggests that the clusters of silver grains are also arranged as an interrupted ring, which is, however, less distinct than in the Golgi stain (Fig. 23). × 350.

Migration of Proteins in the Pancreatic Acinar Cell

While, between 10 and 30 minutes after injection, there was either no increase, or only a moderate one, in the specific activity of the ergastoplasmic proteins (Figs. 10, 16, 21, and 22), there was a greater increase in that of the *proximal zymogen* proteins. By 30 minutes, clusters of silver grains over a restricted portion of the proximal zymogen region of the acinar cell indicated a concentration of labeled protein (Figs. 24, 26, and 28). Since the clusters reminded us of the Golgi region, as stained by classical methods, we decided to compare the radioautographic preparations with sections of pancreas stained by osmium tetroxide–zinc iodide for the detection of the Golgi apparatus (38). As shown in Figs. 23, 25, and 27, the osmiophilic Golgi elements or dictyosomes (diagrammatically seen in Fig. 29) appeared in sections as dark C or V shapes, which in three dimensions are presumed to resemble plates or shells with their concavity facing distally. Zymogen granules were found only on the distal side of the osmiophilic elements (Figs. 25 and 27). In contrast, zymogen granules were seen on both the distal and proximal sides of the grain clusters



FIGURES 25 AND 27

Both figures show a pancreatic acinus of the rat stained with osmium tetroxide–zinc iodide to demonstrate the osmiophilic elements of the Golgi apparatus. In both figures, the osmiophilic elements of the Golgi apparatus (*G*), appearing as C-, V-, or circular-shaped bodies, can be seen forming a distinct, interrupted ring enclosing the mass of zymogen granules (*Z*). Outside the ring of Golgi elements is the homogeneous appearing ergastoplasm (*E*). × 1300.

FIGURES 26 AND 28

Hematoxylin-eosin–stained radioautographs of a pancreatic acinus of the rat 30 minutes after leucine-H^3 injection, 67 day exposure. The dark band at the periphery of the acinus is the ergastoplasm (*E*). Within it, the light mottled area is the group of zymogen granules (*Z*). Probably because of different fixations, the ergastoplasm looks smaller and the zymogen granule region larger in the radioautographs than in the Golgi preparations (compare to Figs. 25 and 27). The silver grains are arranged into clusters (*C*) located somewhat distal to the nuclei (*N*). The grain clusters are arranged as an interrupted ring in each acinus. Note that zymogen granules are seen on the proximal and distal sides of the grain clusters (*Z*, Fig. 28), as well as lateral to them (*Z*, Fig. 26).

Photographs prepared by Mr. A. Graham by superimposing a negative at the level of the silver grains, over a separate negative at the level of the tissue section. × 1300.

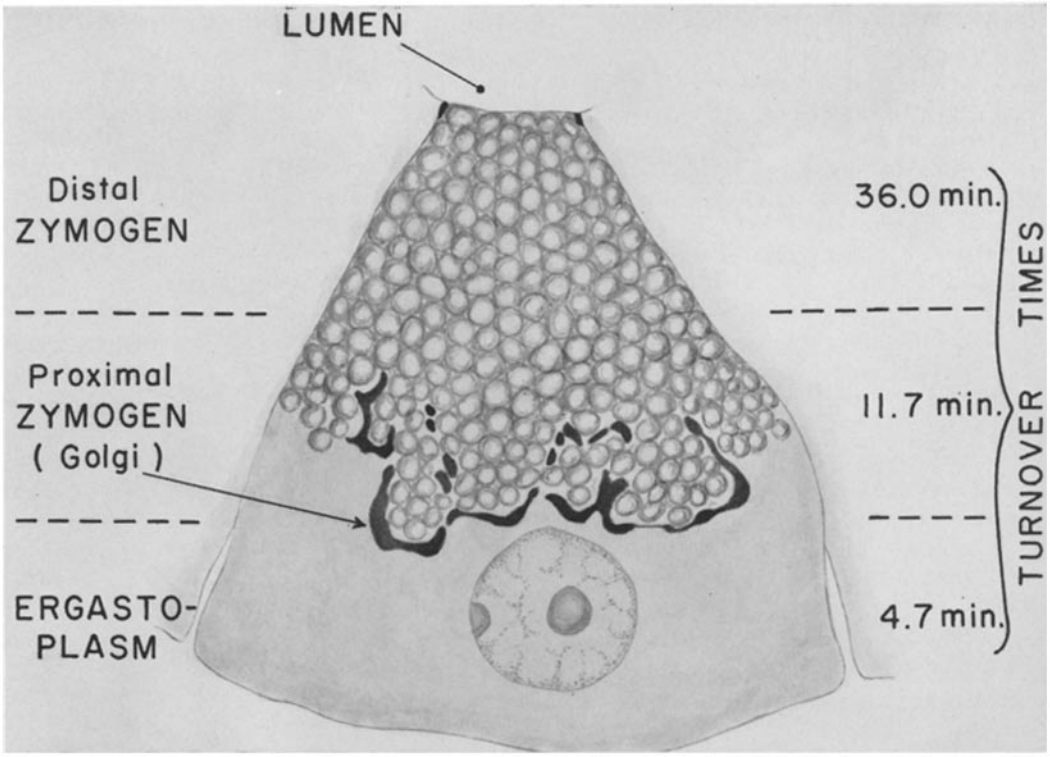


FIGURE 29

Diagrammatic drawing of a pancreatic acinar cell of the rat, as stained by the osmium tetroxide–zinc iodide method for the Golgi apparatus. The ergastoplasm (homogeneous gray), containing the nucleus, is seen at the base of the pyramidal cell. The osmiophilic elements of the Golgi apparatus are located at the junction between the ergastoplasm and the zymogen granules, with some elements between the granules. The zymogen granules, making up the remainder of the cell, extend to the apical lumen. The division of the cell into ergastoplasm and proximal and distal zymogen regions is indicated at the left of the figure. The estimated turnover times of the proteins in each region is shown at the right of the figure.

observed in 30 minute radioautographs (Figs. 26 and 28). In other words, the grain clusters did not seem to correspond exactly to the osmiophilic elements, but rather to an area located just distal to them. It was therefore concluded that the proteins synthesized in the ergastoplasm had migrated to a position just distal to the osmiophilic Golgi elements, but still within the proximal zymogen region.

That the next step in protein migration was to the *distal zymogen* region was suggested by the abundance of radioactivity in this region at 4 hours after injection (Figs. 7 and 10). Indeed, since at that time no significant amount of free labeled leucine is still available (21), the radioactivity which accumulates there must be due to migration of previously labeled proteins. Furthermore, the specific activity time curves for proximal and distal zymogen proteins fulfilled the requirement for the former to be the precursor of the latter (Fig. 10; note that the respective 4 hour values were not statistically different). Finally, it was clear that all the labeled proteins within the excretory ducts (Fig. 9) must have passed through the part of the cell in contact with either the central acinar lumen or intercellular canalicules, that is, usually through the distal zymogen region. Briefly then, most labeled proteins must have migrated from the proximal to the distal zymogen region of the acinar cell (Fig. 7) before being secreted into the duct system.

An estimate of the turnover time was calculated

at the early time intervals, that is, for the rapidly turning over (exportable) proteins, in both the proximal and distal zymogen regions. The results in the main experiment were 11.7 and 36.0 minutes, respectively, for the proteins in the two regions (Fig. 29). While the other experiments were of shorter duration and made use of fewer animals, the turnover times of proximal zymogen proteins were of the same order of magnitude (see Table I of Appendix).

Since the rapidly turning over (exportable) proteins of the acinar cell must be the pancreatic enzymes, it is concluded that the elaboration and secretion of these enzymes occur in several steps (Fig. 29): synthesis in the ergastoplasm; migration first to a position just distal to the osmiophilic elements of the Golgi apparatus, and from there to the rest of the zymogen granule region; finally, release as pancreatic secretion. Adding up the turnover times for the three regions of the pancreatic acinar cell (see Appendix) yields the mean life span of exportable protein molecules in the cells, that is, 52.4 minutes.

Comparison of the Results with Current Theories of Secretion

Current theories on synthesis of proteins in the cytoplasm imply a key role of the ribosomes (24, 27, 36, 37), and it has already been pointed out that the protein synthesis observed in the ergastoplasm of the pancreatic cell (Figs. 11, 17, and 19) may be referred to the abundant ribosomes present. Furthermore, the newly synthesized proteins migrating to the apical region of the cells (Fig. 7) must be within the zymogen granules which are piled up there and in which such proteins have been detected by biochemical methods (36, 37, 39). How then are the newly synthesized proteins taken up into zymogen granules?

The arrangement, in a sequential series, of pictures obtained with the light microscope (40) or the electron microscope (41–43) has led to the conclusion that zymogen granules are formed in the Golgi apparatus. The role of this apparatus would be to "package" the enzymes into zymogen granules (40–44), but the evidence is rather weak.

The first direct demonstration of Golgi involvement in the handling of pancreatic proteins may be found in an early report on the present results (45, 46). A comparable conclusion was reached in work published soon after by Caro (47). This author prepared radioautographs of electron microscope sections 20 minutes after injection of leucine-H^3 into a guinea pig and observed radioactivity in the Golgi zone, chiefly in vacuoles partly filled with heterogeneous material (47). This work has now been confirmed with several animals (48).

The Golgi region is known to consist of at least two parts: (*a*) the osmiophilic elements (also known as dictyosomes or "externum"), which are seen with the electron microscope to be composed of stacks of flattened, smooth surfaced sacs; and (*b*) usually on their distal side, the osmiophobic component ("internum," capable of accumulating neutral red vacuoles), which the electron microscope shows to be composed of vacuoles of various sizes (41–43). In the present work, the proteins synthesized in the ergastoplasm were shown to accumulate in a portion of the proximal zymogen region which is slightly distal to the osmiophilic elements (Figs. 25 to 28). This region seems to correspond to the Golgi vacuoles of the osmiophobic component. The recently synthesized proteins would migrate from the ergastoplasm to these Golgi vacuoles.

Since the evidence indicated that the proteins spreading from the Golgi zone to the apical region of the cells are within zymogen granules, the concentration of protein in the Golgi zone may be attributed to formation of zymogen granules there (45, 46), a conclusion in line with classical hypotheses (40–43, 49) as well as with Caro's work (47).

The life span of a zymogen granule in the cell would thus include the time taken by its formation in the Golgi zone, plus the time spent in the apical region of the cell until it is released to the acinar lumen as pancreatic secretion. Adding up the turnover time of the proximal and distal zymogen regions (see Appendix) should therefore yield the mean life span of a zymogen granule, that is, 47.7 minutes.

This work was done with the support of grants from the Medical Research Council of Canada and from the Banting Research Foundation.
Dr. Droz is a Fellow of The Rockefeller Foundation.
Received for publication, July 3, 1962.

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