Intracellular Transport and Storage of Secretory Proteins in Relation to Cytodifferentiation in Neoplastic Pancreatic Acinar Cells

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ABSTRACT The pancreatic acinar carcinoma established in rat by Reddy and Rao (1977, *Science* 198:78–80) demonstrates heterogeneity of cytodifferentiation ranging from cells containing abundant well-developed secretory granules to those with virtually none. We examined the synthesis intracellular transport and storage of secretory proteins in secretory granule-enriched (GEF) and secretory granule-deficient (GDF) subpopulations of neoplastic acinar cells separable by Percoll gradient centrifugation, to determine the secretory process in cells with distinctly different cytodifferentiation. The cells pulse-labeled with [^{3}H]leucine for 3 min and chase incubated for up to 4 h were analyzed by quantitative electron microscope autoradiography. In GEF neoplastic cells, the results of grain counts and relative grain density estimates establish that the label moves successively from rough endoplasmic reticulum (RER) → the Golgi apparatus → post-Golgi vesicles (vacuoles or immature granules) → mature secretory granules, in a manner reminiscent of the secretory process in normal pancreatic acinar cells. The presence of ~40% of the label in association with secretory granules at 4 h postpulse indicates that GEF neoplastic cells retain (acquire) the essential regulatory controls of the secretory process. In GDF neoplastic acinar cells the drainage of label from RER is slower, but the peak label of ~20% in the Golgi apparatus is reached relatively rapidly (10 min postpulse). The movement of label from the Golgi to the post-Golgi vesicles is evident; further delineation of the secretory process in GDF neoplastic cells, however, was not possible due to lack of secretory granule differentiation. The movement of label from RER → the Golgi apparatus → the post-Golgi vesicles suggests that GDF neoplastic cells also synthesize secretory proteins, but to a lesser extent than the GEF cells. The reason(s) for the inability of GDF cells to concentrate and store exportable proteins remain to be elucidated.

Intracellular aspects of the process of protein secretion have been unraveled in the adult pancreatic exocrine acinar cell by the classical autoradiographic studies of Caro and Palade (4) and Jamieson and Palade (20–22). These studies have identified six successive steps in the secretory process namely, synthesis, segregation, intracellular transport *via* the Golgi complex, concentration, intracellular storage of discharge (for detailed discussion of this process see references 18 and 27). The existence of such a secretory process in a variety of highly differentiated eucaryotic cell types is now well documented (5, 6, 8, 15, 18, 27, 47). Information regarding the acquisition of various steps of the secretory process during cytodifferentiation is, however, lacking. The studies with developing embryonic rat pancreas have provided a greater understanding of the sequential events involved in the histogenesis, the differentiation of the secretory apparatus, and the regulation of expression of pancreas specific genes in this organ (24, 28, 42). Further delineation of intracellular and molecular events such as the secretory process is complicated by the co-emergence of ductal and islets cells in this very small fetal organ where numerous transitional events occur during 14–21-d gestation. This makes it difficult to isolate pancreatic acinar cells and their protodifferentiated precursors for detailed studies. The transplantable pancreatic acinar cell carcinoma of rat established by Reddy and Rao (30) provides

THE JOURNAL OF CELL BIOLOGY · VOLUME 96 APRIL 1983 949–960
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a suitable model system for analyzing certain aspects of cellular differentiation and exocrine pancreas specific gene expression in neoplasia.

The pancreatic acinar cell carcinoma demonstrates a spectrum of cellular differentiation ranging from cells that contain abundant secretory granules to those devoid of secretory granules (31). Recently, we have separated two subpopulations of neoplastic cells from the dissociated pancreatic carcinoma cell population (3). One neoplastic cell subpopulation termed secretory granule-enriched fraction (GEF) contains highly differentiated acinar cells which essentially resemble mature normal pancreatic acinar cells; the second subpopulation designated secretory granule-deficient fraction (GDF) contains cells with immature cytodifferentiation (3). Despite this phenotypic variation, immunofluorescence studies suggested that neoplastic acinar cells with or without secretory granules contain variable amounts of at least six secretory proteins (14). It is pertinent, therefore, to investigate how genetic information is elaborated and processed by the two populations of neoplastic pancreatic acinar cells. Here we examine in detail the secretory process by electron microscope autoradiography, in both the differentiated GEF and undifferentiated GDF neoplastic pancreatic acinar cell subpopulations.

MATERIALS AND METHODS

Chemicals

L-[3,4,5-^{3}H]leucine (specific activity 56.8 Ci/mM) was obtained from ICN Pharmaceuticals (Irvine, CA), class IV collagenase (172 U/mg) and soybean trypsin inhibitor (STI) from Worthington Biochemical Corp. (Freehold, NJ), bovine serum albumin (BSA) from Sigma Chemical Co. (St. Louis, MO), and Percoll from Pharmacia Fine Chemicals (Uppsala, Sweden). Hanks' balanced salt solution (HBSS) and minimal essential medium (MEM) without L-leucine and L-glutamine were purchased from Grand Island Biological Company (Grand Island NY). Ilford L-4, a nuclear research emulsion, was purchased from Polysciences, Inc. (Paul Valley Industrial Park, Warrington, PA). Formvar in ethylene dichloride was obtained from Ladd Research Industries (Burlington, VT). All other chemicals were reagent grade.

Transplantation of Pancreatic Acinar Carcinoma

Small 1- to 2-mm fragments of pancreatic acinar carcinoma (30) were transplanted i.p. into the abdominal mesentery of weanling male F344 rats as previously described (3). These implants grew for the most part as single, large encapsulated tumors between 3 to 5 wk. The tumor-bearing animals were starved for 24 h before killing.

Preparation of Single Cell Suspensions

Acinar carcinoma fragments were prepared at room temperature in Krebs' Ringer Biocarbonate (KRB) buffer containing 14 mM glucose, 2.5 mM Ca^{++}, 1.2 mM Mg^{++}, 0.1 mg STI, a complete L-amino acid supplement, 100 U penicillin/ml, 100 μg streptomycin/ml, and 0.2% (wt/vol) BSA (3, 44), and dissociated into single cells using EDTA chelation and collagenase digestion (15-min first digestion and 45-min second digestion) procedure (1) as previously described (3). Viability of the dissociated acinar carcinoma cells was monitored by their ability to exclude trypan blue.

Separation of GEF and GDF Neoplastic Cells

Dissociated pancreatic acinar carcinoma cells were fractionated on Percoll:HBSS gradients as previously described (3). GEF and GDF neoplastic cell populations were diluted to a final concentration of 5 × 10^6 cells/ml for use in autoradiographic studies. For general morphologic studies an aliquot of each cell fraction was processed for electron microscopy.

Pulse-labeling and Chase

GDF and GEF subpopulations were diluted to 5 × 10^6 cells/ml and preincubated in MEM with 0.4 mM leucine for 15 min at 37°C. The cells were then centrifuged and washed in MEM without L-leucine and gently resuspended into 10-ml volume (5 × 10^6 cells/ml) of prewarmed MEM minus leucine. This suspension was then added to 2.5 mCi of [^{3}H]leucine lyophilized in a siliconized flask resulting in a final concentration of 250 μCi of radio-labeled precursor/ml cell suspension and incubated for 3 min at 37°C. Immediately after 3-min pulse the cell suspension was pelleted through the prewarmed chase medium containing a BSA cushion and 4.4 mM [^{1}H]leucine. The cell pellet was resuspended in KRB buffer with 4.4 mM [^{1}H]leucine and 0.2% BSA and chased for 5, 10 ,20, 30, 60, 120, 180, and 240 min. At the end of the pulse and at each chase interval, 1.0-ml aliquots of cell suspension were added to ice-cold (4°C) chase medium, centrifuged, and fixed in 2.5% glutaraldehyde after three washes. Cells were postfixed in 1% OsO_4, dehydrated, stained en bloc for 60 min in 0.5% uranyl acetate, and embedded in Epon as previously described (3).

Preparation of Autoradiograms

For light microscope autoradiography, 0.5 μm-thick sections were cut from the Epon-embedded blocks and processed as previously described (31). For electron microscope autoradiography, ultrathin sections displaying pale gold interference color were cut and mounted on 100- and 200-mesh Formvar coated copper grids and coated with a monolayer of Ilford L-4 emulsion. The grids were exposed for up to 4 wk and developed for 2 min in D-19 at 18–20°C, washed in double distilled water, and fixed for 1 min in 20% sodium thiosulfate/2% potassium metabisulfite.

Analysis of Autoradiograms

SAMPLING OF CELLS: Autoradiograms were photographed on a JEOL 100-CX microscope at a primary magnification of × 7,000, and printed at a final magnification of × 17,500. Each micrograph included one cell, and where necessary a montage of prints was made to include all grains overlying a particular cell profile. Only cell profiles possessing a nucleus and/or the Golgi region were sampled. A total of 400–1,300 grains were examined for each time interval for each subpopulation of cells examined by high resolution electron microscopic autoradiography. Under the conditions used in this study the resolution of the autoradiographic method is 1,450 Å as determined by Salpeter and Bachmann (35). The location of each developed grain was determined according to the simple grain density method (35–37). Briefly, for each grain localized on a micrograph for each time interval a series of concentric circles on a plastic overlay was fitted over each silver grain. The center of the best fit circle was then taken as the autoradiographic grain center. The grain compartment over which the grain center fell was then reported and scored on the electron micrograph.

SELECTION OF GRAIN COMPARTMENTS: Cytoplasmic grain compartments were modeled essentially as outlined by of Salpeter and Farquhar (36). The modified grain compartments selected were as follows: first is compartment 1, rough endoplastic reticulum (RER), which was divided into (*a*) RER/IN representing grains overlying the RER lumen and limiting membrane and (*b*) RER/OUT representing a 1-HD (half-distance) rim around all RER profiles. Next is compartment 2, the Golgi region, which includes the Golgi cisternae and the large Golgi vacuoles (this compartment was divided into three regions: [*a*] GOL/C, the Golgi cisternae, [*b*] GOL + ER, a 2-HD rim of RER on the forming [*cis*] face of the Golgi [see Ehrenreich et al. (7) for further description], and [*c*] GOL + CV, a 2-HD rim of condensing vacuoles located at the maturing [*trans*] face [or secretory face] of the Golgi apparatus). Compartment 3 is the post-Golgi vesicles, which is divided into (*a*) PGV/IN, condensing vacuoles proper, and (*b*) PGV + CYT, a 2-HD rim of surrounding cytoplasm. Compartment 4 is secretory granules, which is divided into (*a*) ZG/IN, grains with centers above secretory granules, and (*b*) ZG + CYT, a 2-HD rim of cytoplasm surrounding secretory granules. Compartment 5 is the plasma membrane and a 2-HD rim (2HDPM), compartment 6 the nucleus, compartment 7 the cytoplasmic matrix excluded by other compartments, compartment 8 the mitochondria, compartment 9 lysosomes and vacuoles associated with phagocytosis, and compartment 10 miscellaneous elements of the cytoplasm not included in compartments 1 through 9. During the allocation of grains to specific compartments partial credit was given whenever a grain center fell within more than one of the above defined compartments. The "grain percent" was determined as outlined by Salpeter and McHenry (37). The delegation of grains to specific cellular compartments was devised such that the shielded zones were added to the primary compartment to yield one grain total for these pooled compartments. Specifically, the shielded pooled zones for the RER (RER/IN and RER/OUT = RER total), the Golgi complex (Golgi/CV + Golgi/ER + Golgi/C = Golgi total), post-Golgi vesicles (PGV/IN and PGV/OUT = PGV total), and secretory granules (ZG/IN and ZG/OUT = ZG total) permitted an adequate quantitative comparison of GEF and GDF cells.

MORPHOMETRIC ANALYSIS TO DETERMINE AREAS OCCUPIED BY VARIOUS COMPARTMENTS: The point counting method of Weibel et al. (45) was employed to determine the percent area occupied by each compartment. For

this study a transparent overlay was placed over each micrograph and points by intersection overlying the various subcellular compartments were scored using a lattice where *d* equaled 1 cm. The percent area was determined as previously described (3). The consistency of the determinations at various chase intervals took into consideration the factors outlined by Weibel et al. (45). Within experiment groups there was no significant difference in the analysis of variance for the intra group volume density data.

DETERMINATION OF GRAIN DENSITY: The grain density was determined by dividing the grain percent by its respective area percent for an individual compartment.

RESULTS

Morphology of Pancreatic Acinar Carcinoma Cells

Two different preparations of neoplastic acinar cells were investigated by high resolution electron microscopic autoradiography: GEF cells consisting of neoplastic cells that show abundant secretory granules and prominent Golgi apparatus and closely resemble normal pancreatic acinar cells; and GDF cells consisting of morphologically undifferentiated carcinoma cells that are devoid of zymogen granules. The GDF cells differ from GEF cells in nuclear position, nuclear/cytoplasmic ratio, and amount of stored secretory product in the form of mature zymogen granules (3). Both GEF and GDF neoplastic cell subpopulations have been isolated by subjecting the freshly dissociated neoplastic cell population of Percoll gradient centrifugation (3).

Differentiation of Cells Sampled

The cellular differentiation and degree of zymogen maturation of the component cells of the two different preparations used for autoradiographic study were investigated by morphology and morphometry. Table I shows both the number of granule enriched and granule-deficient cells contained in GEF and the number contained in GDF cells. GEF cells show a high degree of zymogen maturation and >90% of the cells sampled in this experimental group were highly differentiated granule-enriched cells. GDF cells show a much lower level of phenotypic zymogen maturation as evidenced by the virtual absence of secretory granules. This preparation of carcinoma cells consists of >85% granule-deficient carcinoma cells and the <15% granule containing cells included in GDF reveal few, small secretory granules.

Area Percent Values

The area of the two enriched subpopulations occupied by each of the nine organelle compartments defined in Materials and Methods is given in Table II. The percentage of total cell area occupied by various cell organelles depicted in Table II

TABLE I

Analysis of Extent of Differentiation of Subpopulations of Neoplastic Pancreatic Acinar Cells Used for Autoradiographic Studies

Cell group* studied	Highly differentiated granule-enriched cells (%)	Poorly differentiated granule-deficient cells (%)	Total number of cells
GDF	47 (11.2)	450 (88.8)	507
GEF	470 (93.8)	31 (6.2)	501

* The GDF and GEF subpopulations of acinar carcinoma cells isolated on Percoll gradients were classified according to the extent of cytodifferentiation as described by Becich and Reddy (3).

TABLE II

Percentage of Total Cell Area Occupied by Various Cellular Compartments in Isolated Subpopulations of Pancreatic Acinar Carcinoma

Compartment	Granule-deficient fraction	Granule-enriched fraction
RER	27.6 ± 1.7	24.9 ± 1.8
Golgi complex	2.2 ± 0.3	4.1 ± 0.6
Post-Golgi vesicles	7.2 ± 0.7	9.4 ± 0.7
Secretory granules	0.6 ± 0.1	15.4 ± 1.6
Plasma membrane zone	7.0 ± 0.7	7.8 ± 0.7
Nucleus	42.0 ± 2.4	23.3 ± 2.2
Cytoplasmic matrix	5.7 ± 0.8	6.2 ± 0.8
Mitochondria	7.1 ± 0.4	8.2 ± 0.5
Miscellaneous + lysosomes	0.4 ± 0.1	0.6 ± 0.4

Mean area percent values represent the average of 9 time points analyzed for each of the four experimental cell groups. A total of 450 micrographs were analyzed for each group. The values are mean ± standard deviation.

represents an average volume density of the nine time points analyzed for each group. Actual grain density determinations were however, based on the area percent distribution of a given cell organelle at the individual time points. The variations in area percent determinations observed at the individual time points are represented statistically in Table II.

Electron Microscopic Autoradiographic Analysis of Intracellular Transport

INCORPORATION OF [^{3}H]LEUCINE IN GEF AND GDF SUBPOPULATIONS SEPARATED ON PERCOLL: As previously described (3), both GEF and GDF cells incorporate [^{3}H]leucine to a comparable extent as visualized by light microscope autoradiography (Fig. 1 *a* and *b*). At the end of the pulse period, autoradiographic grains appear randomly distributed over the nucleus and cytoplasm in both GEF and GDF cells (Fig. 1 *a* and *b*). At the electron microscope level, immediately postpulse, the majority (~47% for GEF and ~37% for GDF) of autoradiographic grain centers was localized directly over or within 1-HD of the RER (Tables III and IV). The number of grains associated with nucleus and 2-HD of the plasma membrane in GDF cells appears higher than that in GEF cells, but a significant grain density difference was noted only for the plasma membrane compartment ($P < 0.01$).

INTRACELLULAR TRANSPORT IN GEF SUBPOPULATION: The GEF neoplastic pancreatic acinar cells transport newly synthesized proteins similar to that observed in dissociated normal pancreatic acinar cells (Table III; Fig. 2). Results of grain counts clearly demonstrated the peak specific activity of the isotope in the RER at 0 and 5 min of chase incubation (grain percent 46.6 and 43.6; and grain density 1.7 and 1.5, respectively). With increasing periods of chase incubation the level of specific activity in RER fell to basal level by 30 and 60 min (grain density 0.2–0.5). The grain density in the Golgi complex increased to a maximum of 8.2 at 20 min of chase incubation (grain percent 35.3) with the peak of activity probably occurring between 10 and 20 min postpulse.

Fig. 3 shows transport of radio-labeled proteins in GEF at 10, 20, and 30 min of chase incubation. At 10 min of chase (Fig. 3 *a*) autoradiographic grains are seen near transitional elements of RER and have begun to concentrate in the Golgi complex. By 20 min (Fig. 3 *b*) the majority of label in GEF cells is over the cisternae of the Golgi complex. Transfer of radio-labeled proteins to the *trans* face of the Golgi to post-

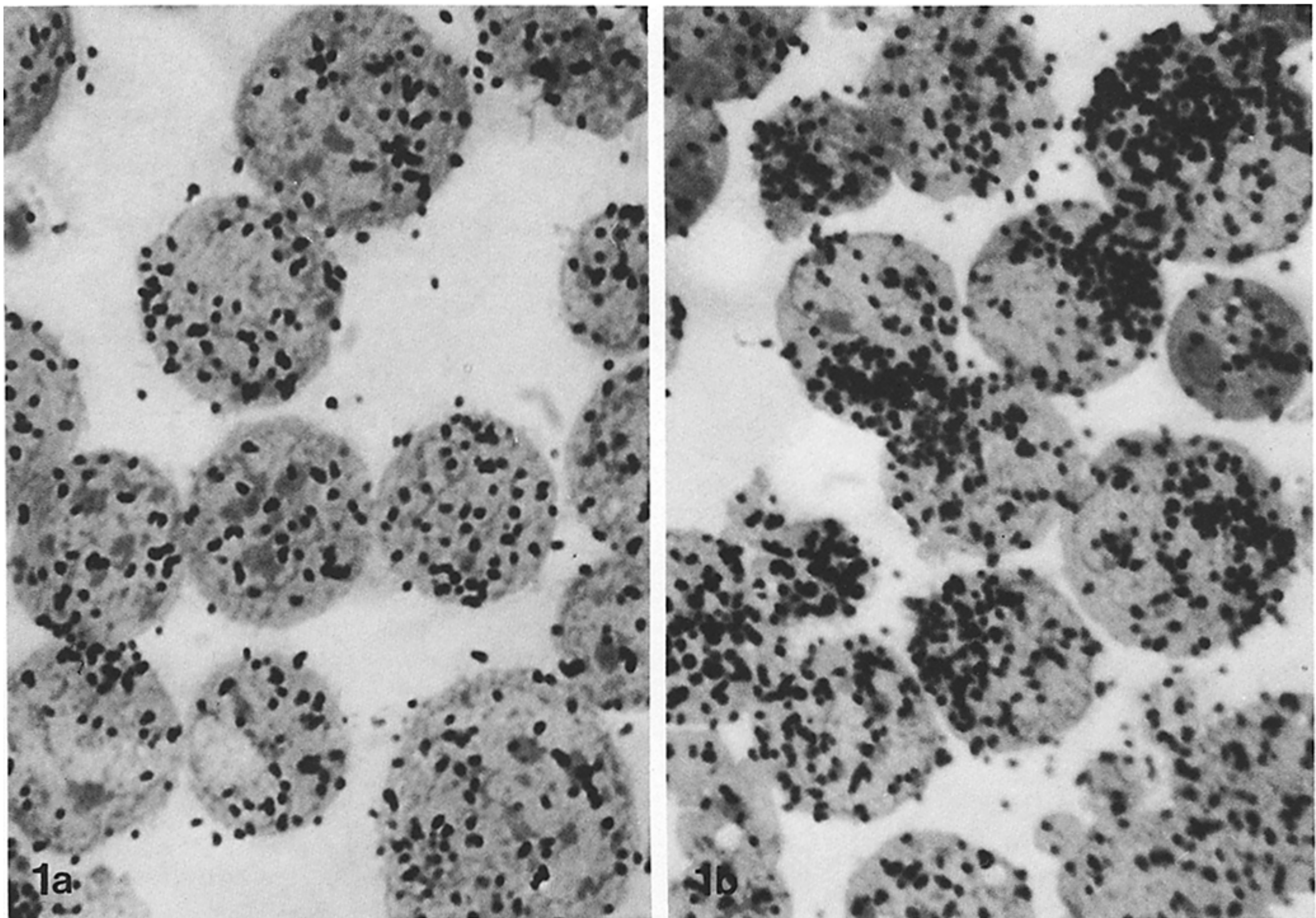


FIGURE 1 Light microscope autoradiograms of secretory granule-deficient (*a*) and secretory granule-enriched (*b*) subpopulations of neoplastic pancreatic acinar cells separated on Percoll gradient. The cells were pulsed for 3 min with [^{3}H]leucine and fixed immediately postpulse; the autoradiographic grains are uniformly distributed over the cells. The presence of numerous secretory granules and the polarity make it difficult to appreciate the distribution of autoradiographic grains in this black and white photograph of secretory granule enriched cells (*b*). × 1,800.

Golgi vesicles is seen at 30 min postpulse (Fig. 3*c*). Little retention of radioactivity is seen in the Golgi complex after 60 min of chase incubation (Fig. 4*a*) indicating efficient transport of newly synthesized protein. Concentration of grains over post-Golgi vesicles begins at 10 min postpulse and reaches a maximum at 60 min (Fig. 4*a*) (grain percent 38.2, grain density 4.02 at 60-min chase). The label over post-Golgi vesicles does not return to basal levels (0.2 at 5 min) even by 240 min postpulse incubation indicating retention of radioactivity in these vesicles. Concentration of label in secretory granules was first evident at 60 min of chase incubation (Fig. 4*a*), reaching a level of 41% at 240 min at chase. GEF cells contain a large amount of pre-existing secretory granules; the incorporation of newly synthesized secretory proteins into this pool of stored protein led to labeling of several, but not all secretory granules (Fig. 4*b*). At all time points analyzed mitochondrial and nuclear labeling remained relatively constant, but the observed grains were higher than those derived from studies using normal pancreatic acinar epithelium (2). Plasma membrane labeling remained constant through 60 min of chase; thereafter there was a slight increase in grain density that coincided with a peak in transport to post-Golgi vesicles and zymogen granule compartments.

INTRACELLULAR TRANSPORT IN GDF SUBPOPULATION: At the end of a 3-min pulse, the majority of (~75% of the radioautographic grains) were associated with cytoplasm of these granule-deficient pancreatic acinar carcinoma cells (Fig. 5 and Table IV). The results of grain counts expressed as the percentage of total grains over a given compartment at each time point shown in Table IV and Fig. 5 demonstrate the transport of proteins from RER to the Golgi complex. Concentration of radioactivity in the Golgi complex of GDF occurs at levels comparable to those of GEF cells (grain density of 10.2 at 10 min and 8.3 at 20 min), with the peak activity occurring closer to 10 min of chase incubation (Figs. 5 and 6*a*). This is somewhat earlier than that observed for GEF cells. By 30-min chase (Fig. 6*b*) transport of newly synthesized protein to post-Golgi vesicles is evident and by 60 min (Fig. 7) the grain density in the Golgi complex dropped to 1.7 from its peak level of 10.2. Because these neoplastic cells are devoid of secretory granules the resolution of the post-Golgi transport, i.e., concentration and storage steps of the secretory process of label, was not possible, although there was a rise in the number of autoradiographic grains in post-Golgi vesicles at 30- and 60-min postpulse. By 240 min, a considerable number of autoradiographic grains appeared over the nucleus and the remainder over profiles of RER, mitochondria, and plasma membrane (Table IV). At this interval, only a small percentage of autoradiographic grains were associated with secretory elements of these cells (the Golgi complex, post-Golgi vesicles, etc.). The

TABLE III

Intracellular Distribution of Newly Synthesized Proteins as Visualized by Electron Microscope Autoradiography in Isolated Granule-enriched Cells from Pancreatic Acinar Carcinoma

Compartment	% Distribution of autoradiographic grains at incubation times post-pulse*								
	0 min	5 min	10 min	20 min	30 min	60 min	120 min	180 min	240 min
RER	46.6 (1.7)	43.6 (1.5)	24.9 (1.1)	19.2 (0.7)	12.8 (0.5)	5.9 (0.2)	5.0 (0.2)	5.0 (0.2)	5.2 (0.2)
Golgi complex	7.9 (1.7)	12.1 (3.9)	25.7 (6.7)	35.3 (8.2)	19.5 (4.6)	8.7 (2.1)	3.6 (0.7)	5.9 (0.9)	2.2 (0.6)
Post-Golgi vesicles	0.9 (0.1)	1.8 (0.2)	6.5 (0.7)	4.2 (0.5)	25.4 (2.7)	38.2 (4.0)	20.3 (1.9)	14.8 (1.6)	12.7 (1.3)
Secretory granules	6.8 (0.6)	4.2 (0.3)	8.1 (0.5)	14.2 (0.8)	12.6 (0.8)	17.1 (1.1)	35.4 (2.1)	37.2 (2.4)	41.0 (2.7)
Plasma membrane zone	9.6 (1.1)	10.5 (1.4)	11.6 (1.7)	9.5 (1.3)	11.7 (1.5)	11.8 (1.5)	17.1 (1.8)	15.3 (2.0)	15.2 (2.0)
Nucleus	20.4 (0.8)	16.8 (0.8)	14.9 (0.6)	10.6 (0.5)	13.3 (0.6)	12.0 (0.5)	11.2 (0.6)	14.9 (0.6)	13.3 (0.5)
Cytoplasmic matrix	2.7 (0.5)	3.7 (0.5)	3.0 (0.5)	2.7 (0.4)	0.9 (0.1)	1.6 (0.2)	2.8 (0.5)	2.2 (0.4)	2.8 (0.6)
Mitochondria	4.6 (0.6)	6.3 (0.7)	4.7 (0.6)	3.7 (0.5)	3.4 (0.4)	4.6 (0.6)	4.3 (0.5)	3.2 (0.4)	6.0 (0.7)
Miscellaneous + lysosomes	0.5 (1.0)	0.9 (1.8)	0.7 (1.6)	0.4 (3.5)	0.4 (0.7)	0.0 (0.0)	0.2 (0.3)	1.4 (2.8)	1.6 (1.0)

* Values in parentheses are grain density data. Underlined values represent maximum concentrations of radio-label in that compartment.

TABLE IV

Intracellular Distribution of Newly Synthesized Proteins as Visualized by Electron Microscope Autoradiography in Isolated Granule-deficient Cells from Pancreatic Acinar Carcinoma

Compartment	% Distribution of autoradiographic grains at incubation times post-pulse*								
	0 min	5 min	10 min	20 min	30 min	60 min	120 min	180 min	240 min
RER	37.6 (1.3)	36.5 (1.3)	28.3 (1.0)	22.6 (0.7)	18.0 (0.6)	16.3 (0.6)	13.0 (0.5)	10.4 (0.4)	9.6 (0.4)
Golgi complex	7.0 (4.0)	10.8 (4.4)	20.0 (10.2)	16.0 (8.3)	15.0 (6.9)	4.3 (1.7)	3.6 (1.5)	3.2 (1.2)	1.5 (0.7)
Post-Golgi vesicles	2.3 (0.3)	2.6 (0.3)	3.3 (0.5)	2.5 (0.4)	16.6 (2.3)	22.6 (3.3)	21.5 (3.0)	20.7 (2.4)	12.9 (1.8)
Secretory granules	0.4 (0.6)	0.8 (0.8)	0.0 (0.0)	0.5 (0.9)	0.4 (0.6)	0.6 (1.2)	0.4 (0.6)	0.4 (0.6)	1.5 (2.2)
Plasma membrane zone	17.2 (2.4)	18.2 (2.1)	13.0 (2.0)	13.9 (2.4)	12.7 (2.0)	14.8 (2.0)	15.2 (2.2)	19.6 (2.8)	15.3 (2.1)
Nucleus	24.6 (0.6)	20.9 (0.5)	26.4 (0.6)	36.2 (0.9)	30.3 (0.7)	34.7 (0.8)	36.2 (0.8)	38.7 (0.9)‡	50.2 (1.2)‡
Cytoplasmic matrix	4.1 (0.6)	2.8 (0.6)	3.0 (0.6)	2.5 (0.4)	1.5 (0.3)	0.8 (0.2)	3.0 (0.5)	0.9 (0.2)	1.2 (0.2)
Mitochondria	6.2 (0.9)	6.9 (0.9)	5.3 (0.7)	5.5 (0.7)	5.4 (0.8)	5.7 (0.9)	6.8 (1.0)	5.7 (0.8)	6.9 (1.0)
Miscellaneous + lysosomes	0.4 (1.0)	0.5 (1.4)	0.8 (1.9)	0.3 (0.6)	0.1 (0.2)	0.2 (0.8)	0.3 (0.7)	0.4 (0.7)	0.8 (1.8)

* Values in parentheses are grain density data. Underlined values represent maximum concentrations of radio-label in that compartment.
‡ Significantly different from 0 min ($P < 0.01$).

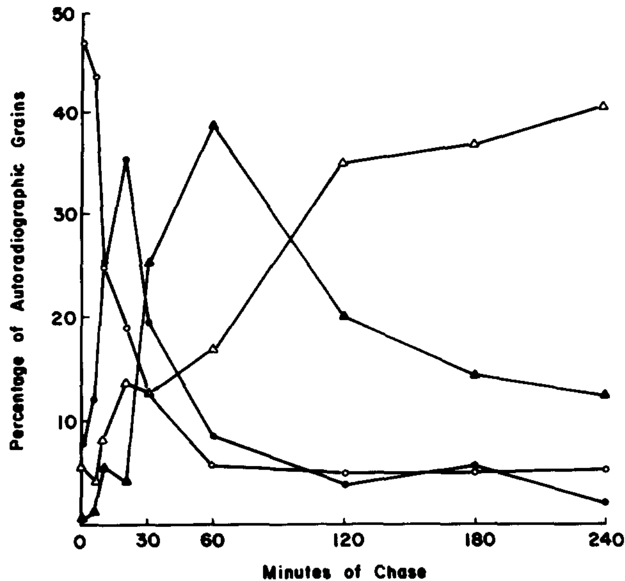


FIGURE 2 Intracellular transport of pulse-labeled secretory proteins in secretory granule-enriched subpopulation of neoplastic pancreatic acinar cells (based on data presented in Table III). A definite wavelike movement of pulse-labeled secretory proteins from RER (○) → the Golgi complex (●) → post-Golgi vesicles (▲) → secretory granules (△) is evident in these well differentiated neoplastic cells. The transport of label to the secretory granules is clarified in this neoplastic cell subpopulation which displays nearly terminal cytodifferentiation.

grain density of the nucleus in GDF cells was 0.5 at 5-min postpulse and increased about twofold (to 2.2) at 240 min ($P < 0.01$). In contrast, the grain density over the nucleus of GEF cells did not show a significant change at any time point (0.8–0.5). The difference in the grain density of the nucleus of GEF (0.5) and GDF (1.2) at 240 postpulse, however, was statistically significant ($P < 0.01$).

Viability of GEF and GDF Cells at 240-min Chase

The viability was >90% at 240 min of chase incubation as determined by exclusion of the vital dye trypan blue. In addition, in both GDF and GEF groups several mitotic profiles were observed. In GDF cells in mitosis, autoradiographic grains were present over chromosomal profiles as well as cytoplasmic elements. Mitotic profiles were also seen in some GEF cells at 240 min of chase incubation. In these mitotic cells there was evidence of concurrent transport of secretory proteins; several autoradiographic grains are discerned over secretory granules. In the mitotic cells of both GDF and GEF there was autoradiographic evidence of incorporation of [^{3}H]leucine into nonexportable proteins.

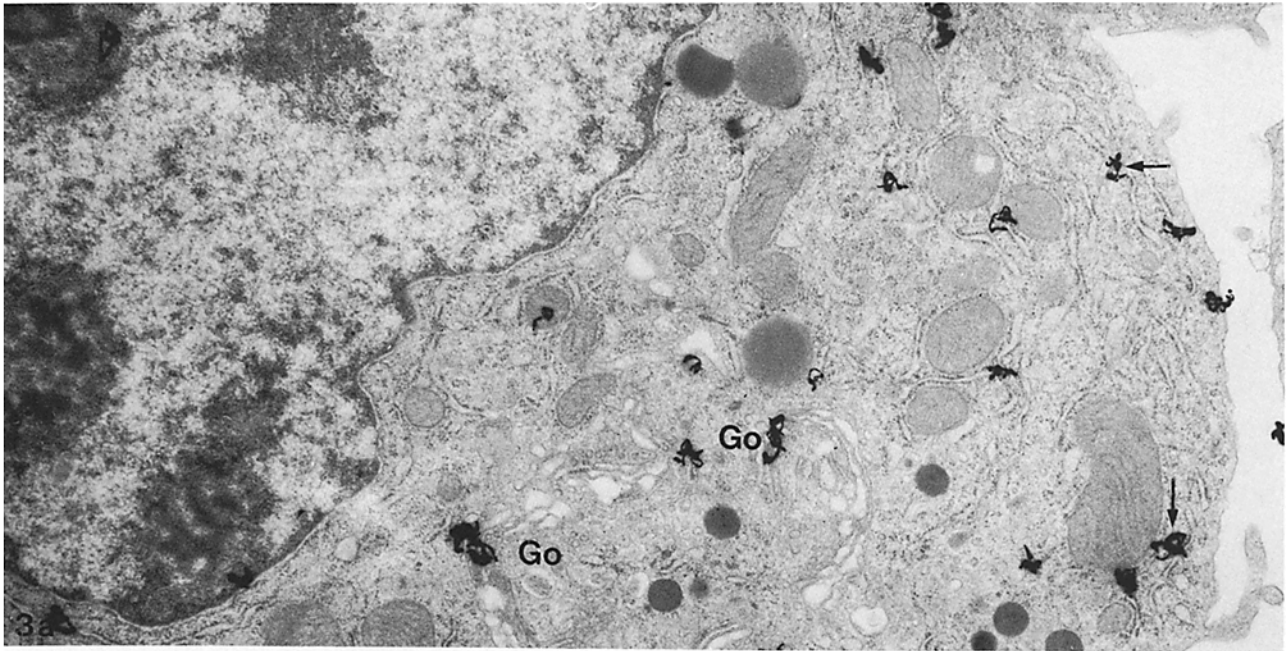
DISCUSSION

There is considerable impetus to investigate the intracellular synthetic and degradative processes involved in cellular differentation during embryonic development and in neoplastic transformation and progression (10, 11, 25, 26, 29, 40–42). An increased understanding of these processes appears necessary in order to gain insight into the mechanisms regulating ontogeny and the evolution of cellular heterogeneity in neoplasia (10, 11, 26, 40–42). The cellular diversity encountered in neoplasms provides an important biological phenomenon for the elucidation of intracellular processes and mechanisms of differentiation. Crucial stages in differentiation can be studied in tumor models such as the murine teratoma (39), Friend leukemia (12), melanoma (10), and pancreatic acinar carcinoma (30), because they exhibit distinctive biochemical and/or morphological markers that characterize the extent of cellular differentiation. In the present study, we investigated, by quantitative electron microscope autoradiography, the process of intracellular transport and storage of exportable proteins in relation to cytodifferentiation in relatively homogeneous GEF and GDF neoplastic cell subpopulations isolated from the heterogeneous pancreatic acinar carcinoma cells by isopyknic Percoll gradient centrifugation (3). These two subpopulations reveal distinct differences in cytodifferentiation particularly in nuclear: cytoplasmic ratio, secretory granule content, and degree of structural polarity (3). Whether these cell types represent sequential stages of differentiation from a transformed "stem cell" or derived from transformed mature acinar cells which require immature phenotypic characteristics as a result of continued mitosis (i.e., differentiation) remains unclear (31).

To our knowledge no investigations have been concerned specifically to determine the secretory pathway and the timetable of intracellular transport in cells during the process of cytodifferentiation. Analysis of the GEF cells following pulse-chase electron microscope autoradiography established that these cells transport newly synthesized protein destined for export in a vectorial manner reminiscent of normal pancreatic acinar cells (2, 4, 20, 27). The data show that the pulse-labeled protein in GEF cells moves successively from RER to the Golgi apparatus and from there to the post-Golgi vesicles (vacuoles or immature granules) and eventually to mature secretory granules as in a variety of other cells that store their secretory product (5, 6, 8, 15). Transfer of label to the Golgi apparatus occurs very rapidly in these GEF cells; by 10-min postpulse, ~26% of the total grains is associated with the Golgi apparatus, reaching a peak of ~35% by 20-min postpulse. The kinetic data demonstrate that movement of secretory proteins into the post-Golgi vesicles commences rapidly because by 10-min postpulse ~6% of the label is in association with this compartment. Movement of label into secretory granules becomes evident by ~20-min postpulse and continues even up to 4-h postpulse. The persistence of high residual levels of radioactivity in the post-Golgi vesicles at 4-h postpulse suggests a possible defect involving condensation of secretory proteins in this region. The possibility that this residual label might represent a nonsecretory protein, however, cannot be excluded.

The majority of label associated with RER moves very rapidly (i.e., drained within 30 min) in these GEF neoplastic acinar cells. Our results further indicate that ~40% of the label at 4-h postpulse is in association with secretory granules. This value is comparable to that observed by Amsterdam and

FIGURE 3 Autoradiograms of secretory granule-enriched neoplastic pancreatic acinar cells separated on Percoll gradient, pulsed with [^{3}H]leucine for 3 min and chased for 10 (*a*), 20 (*b*), and 30 min (*c*). At the end of 10-min chase period the label is associated with RER (arrows) and the Golgi stacks (*Go*). At 20- and 30-min postpulse the movement of pulse through long stacks of Golgi cisternal elements (*Go*) is discerned. *a*, × 16,000; *b*, × 17,500; *c*, × 27,000.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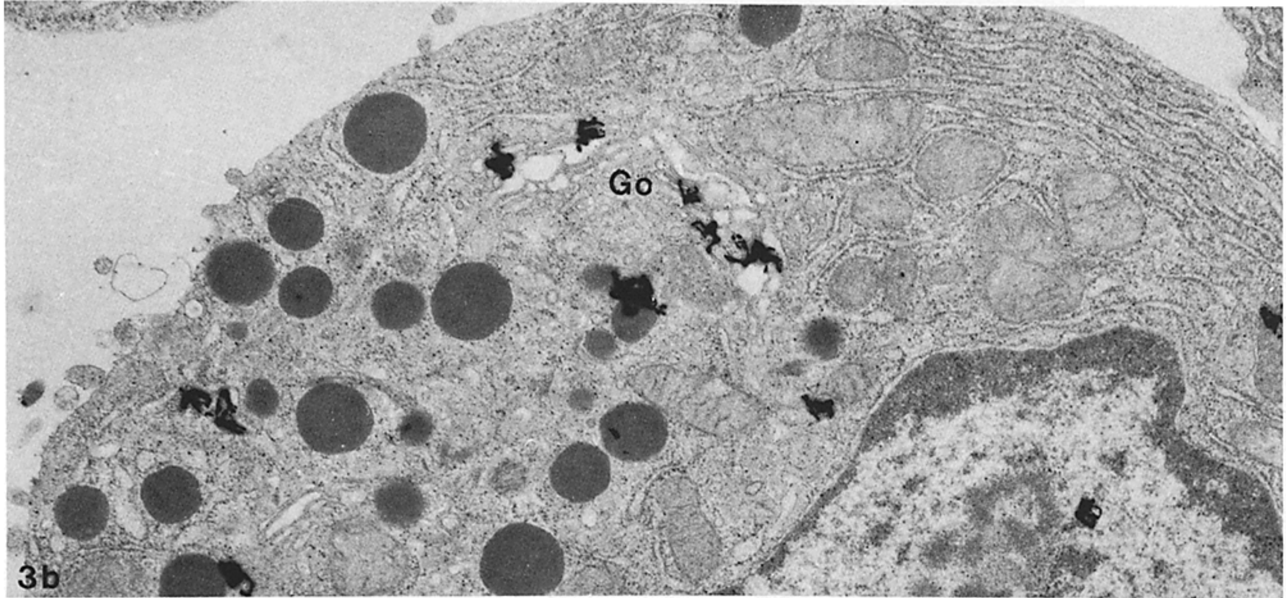
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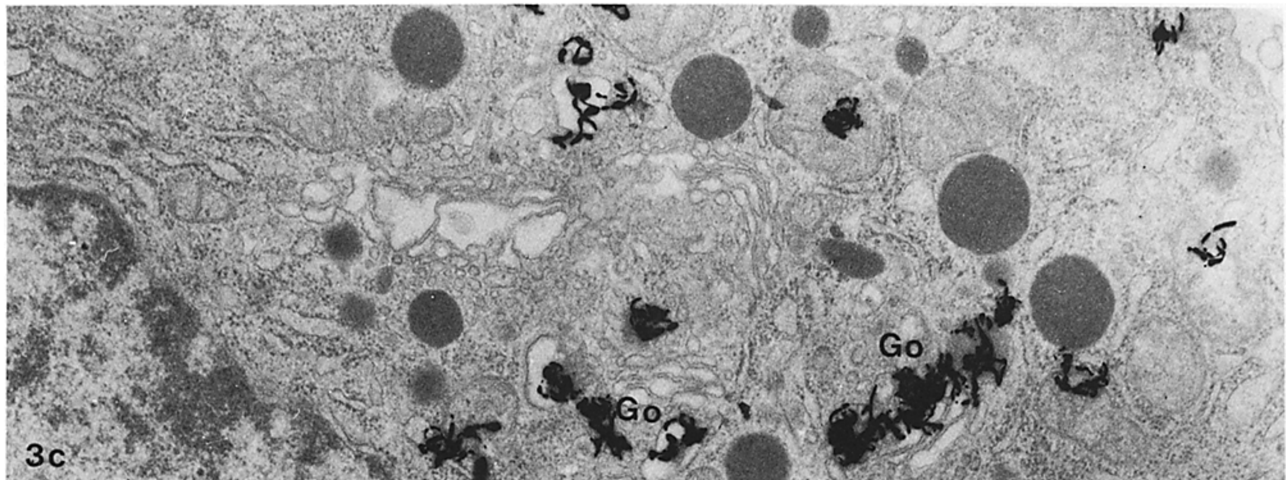
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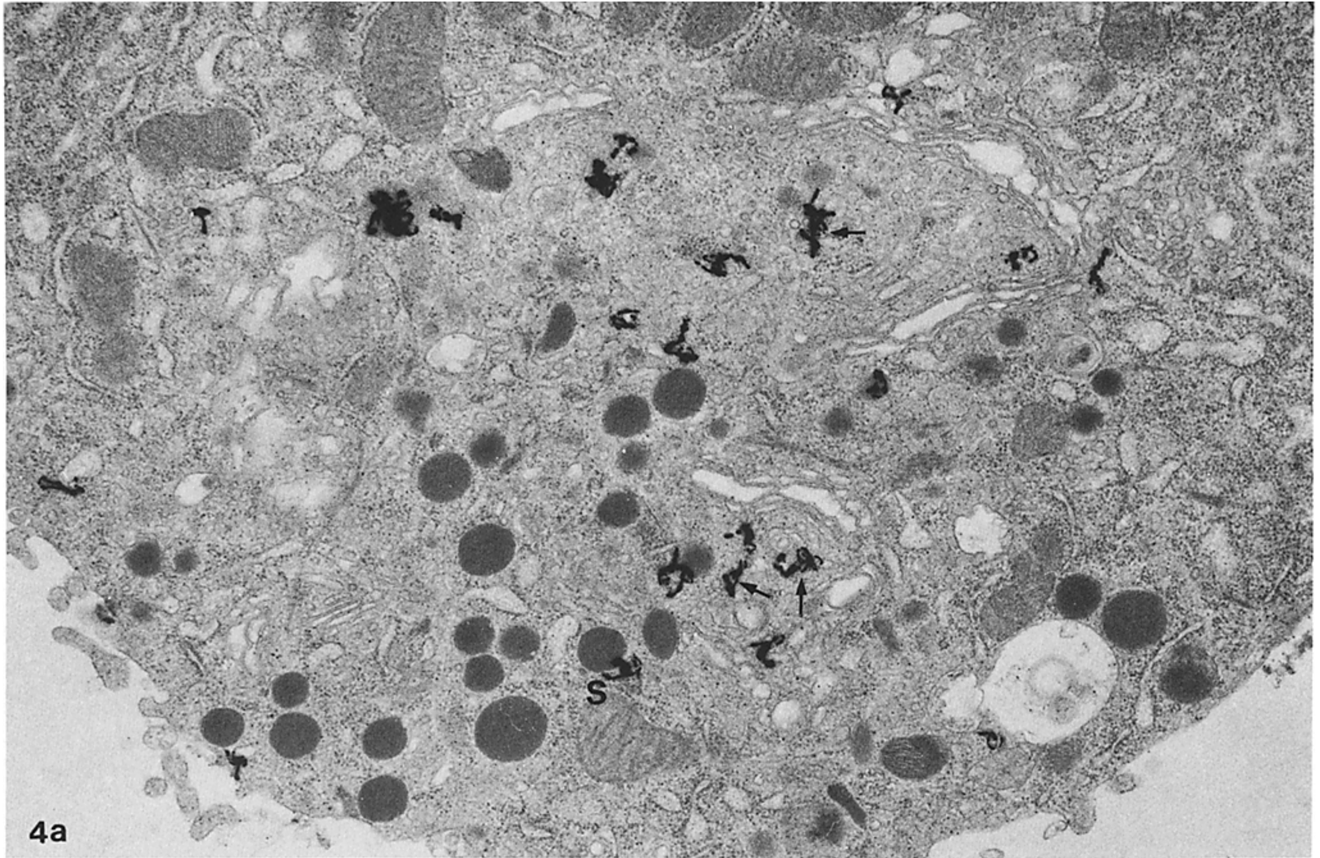
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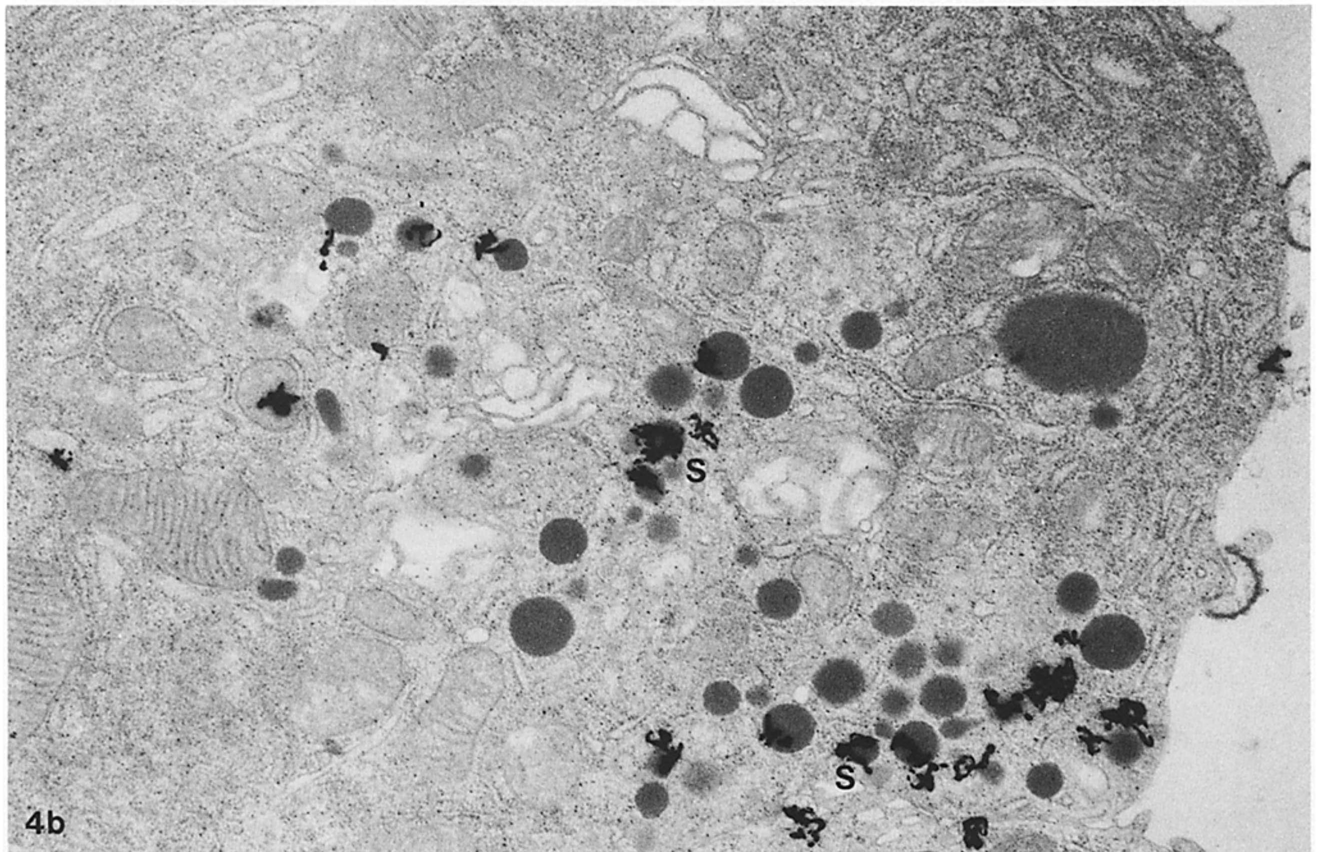
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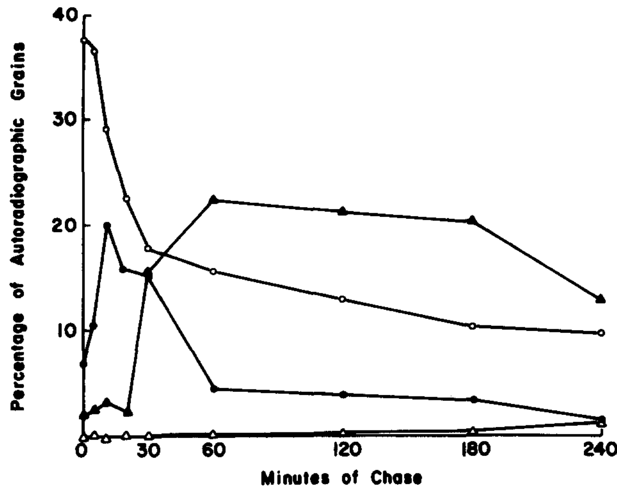


FIGURE 5 Intracellular transport of pulse-labeled secretory proteins in secretory granule-deficient neoplastic pancreatic acinar cell subpopulation (based on data presented in Table IV). Wavelike movement of label from RER (○) → the Golgi complex (●) → post-Golgi (▲) vesicles is evident. The absence or paucity of ultrastructurally identifiable secretory granules (△) precludes the delineation of the concentration and storage steps of the secretory process at this stage of cytodifferentiation.

Jamieson (2) in their studies on dispersed normal pancreatic acinar cells, suggesting that these well-differentiated GEF cells retain the essential regulatory controls involved in the secretory process. The immunocytochemical demonstration of 10 enzymes in all secretory granules and the Golgi apparatus of GEF neoplastic acinar cells (Bendayan, M., M. J. Becich, and J. K. Reddy, manuscript in preparation) similar to that seen in normal pancreas (23), further suggests that these well-differentiated neoplastic cells retain the ability to express pancreatic specific gene function. This level of synthesis of exportable proteins is remarkable considering the fact that GEF cells are neoplastic and that a sizable proportion of this subpopulation is in DNA-replicative phase at any given period (3, 31). The presence of several of these cells in mitosis at 4-h postpulse and the autoradiographic evidence of label in secretory granules of such mitotic cells observed in the present study suggest that the processing of exportable proteins is not interrupted during cell division.

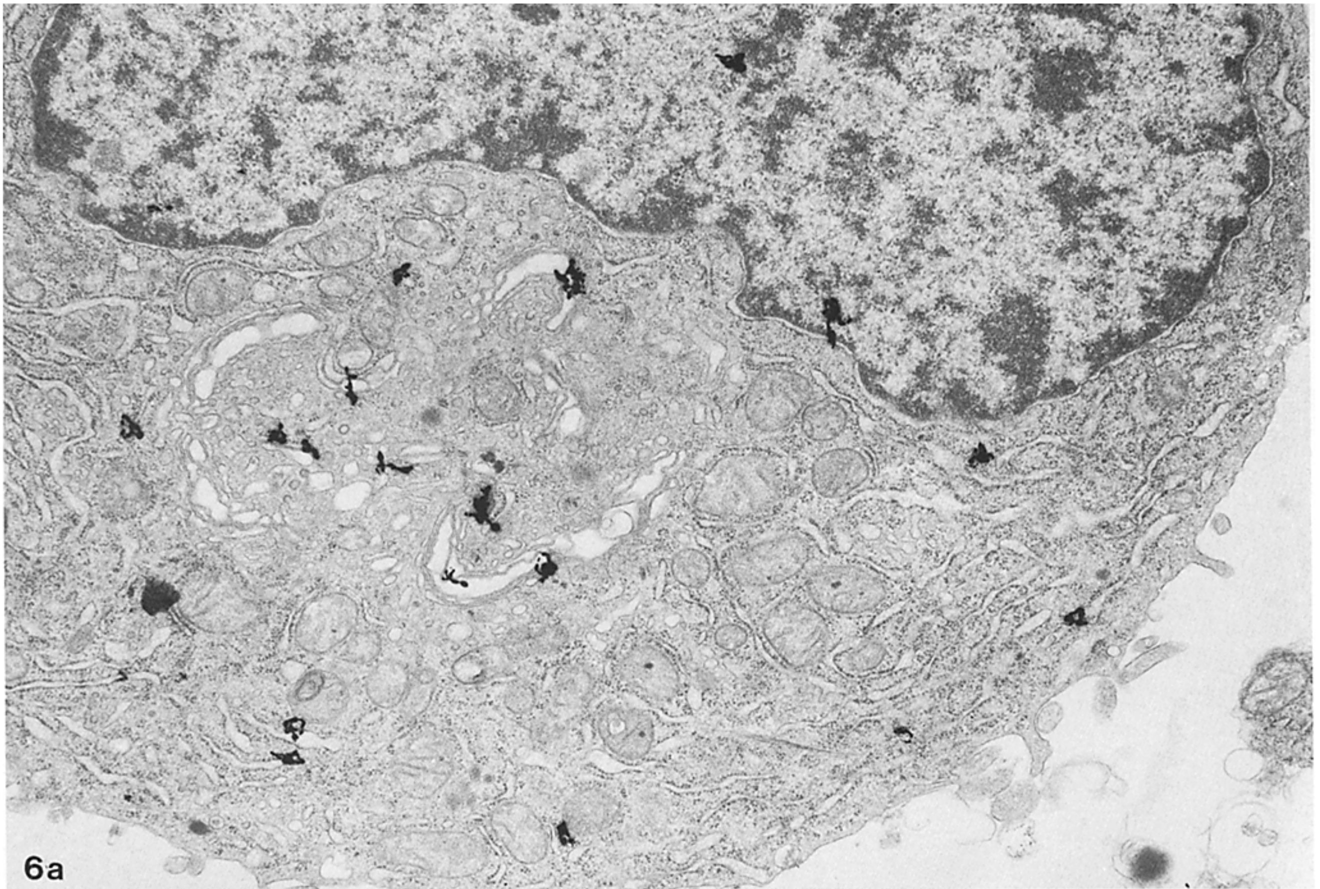
In the GDF neoplastic pancreatic acinar cells, secretory granules are virtually absent. The Golgi apparatus in these cells is less developed when compared to that present in GEF cells (3, 31). At the end of the 3-min pulse ~38% of the label is seen over the RER in these GDF cells. Transport to the Golgi apparatus takes place relatively rapidly because by 10-min postpulse ~20% of the label is seen in association with this organelle. The data also indicate that the drainage of RER is slower in GDF cells; however, the drainage of the Golgi apparatus is essentially complete by 60-min postpulse as in GEF cells. Our results also indicate that the relative amount of the peak label in the Golgi and the post-Golgi vesicles in GDF cells is less than that present at these intracellular sites in GEF cells. The present autoradiographic results do not provide additional insight into the fate of proteins transported into the post-Golgi vesicles through the Golgi apparatus because of the absence of typical condensing vacuoles and secretory granules in these GDF cells.

The RER synthesizes a variety of proteins (bonefide secretory or exportable proteins, lysosomal enzymes, and membrane proteins) that have different intracellular destinations (18). The plasma membrane glycoproteins synthesized in the RER pass through the Golgi apparatus and transported to the cell periphery for insertion with kinetics similar to those reported for secretory proteins (18). The present autoradiographic study of GDF neoplastic cells does not, however, distinguish between secretory and nonsecretory component of the label seen in the Golgi and post-Golgi vesicles. The relatively marginal immunocytochemical labeling of secretory enzymes in the Golgi apparatus of these GDF cells (Bendayan, M., M. J. Becich, and J. K. Reddy, manuscript in preparation) provides only an equivocal support for the assumption that at least part of the label is destined for export. Accordingly, on the basis of the present autoradiographic data and somewhat limited immunocytochemical evidence, we tentatively conclude that GDF cells synthesize secretory proteins which move from the RER → the Golgi apparatus → the post-Golgi vesicles but do not store the secretory product in recognizable mature granules.

The autoradiographic data also demonstrate that at 4-h postpulse only ~15% of the label in GDF cells is in association with the Golgi, the post-Golgi vesicles, and secretory granules, whereas these three compartments in GEF cells account for ~55% of the label. Calculation of total grain density per cell as a function of incubation time postpulse in GEF and GDF revealed that these subpopulations retain the same proportion (i.e., ~72% and ~80%, respectively) of the labeled macromolecules at 4-h postpulse. These data suggest that GDF cells may at least secrete constitutively a portion of the labeled macromolecules. At present, the reasons for the inability of these GDF cells to concentrate and store secretory proteins are not readily apparent, though two possibilities can be considered.

(*a*) There is a defect in the synthesis, either qualitative or quantitative, and/or packaging of secretory proteins in these GDF cells. Recently, Iwanij and Jamieson (16, 17) compared the secretory proteins synthesized and discharged by the heterogeneous population of neoplastic pancreatic acinar cells with that of adult rat pancreas, as well as pancreatic rudiments of embryonic rat. They noted that a group of basic polypeptides, such as proelastase, basic chymotrypsinogen, and ribonuclease, were greatly reduced or absent in tumor cell secretion and concluded that the secretory protein patterns of the acinar tumor cells most closely resembles that of day-19 embryonic rat pancreas (17). However, it is appropriate to note that secretory protein pattern of a highly purified fraction of secretory granules from the pancreatic acinar carcinoma appears essentially similar to that of secretory granules isolated from adult rat pancreas (Hansen, L. J., M. K. Reddy, and J. K. Reddy, manuscript in preparation). These studies suggest that

FIGURE 4 Autoradiograms of secretory granule-enriched neoplastic acinar cells after 60 (*a*) and 240 min (*b*) of chase incubation. At 60 min, the stack of Golgi cisternae no longer contains appreciable quantity of the label, but the small vesicles in the inner face of the Golgi complex are labeled (arrows). Some label is seen associated with secretory granules (S). By 240 min of chase incubation, the label is concentrated over secretory granules. Very little label is found over the Golgi complex. *a*, × 20,000; *b*, × 18,000.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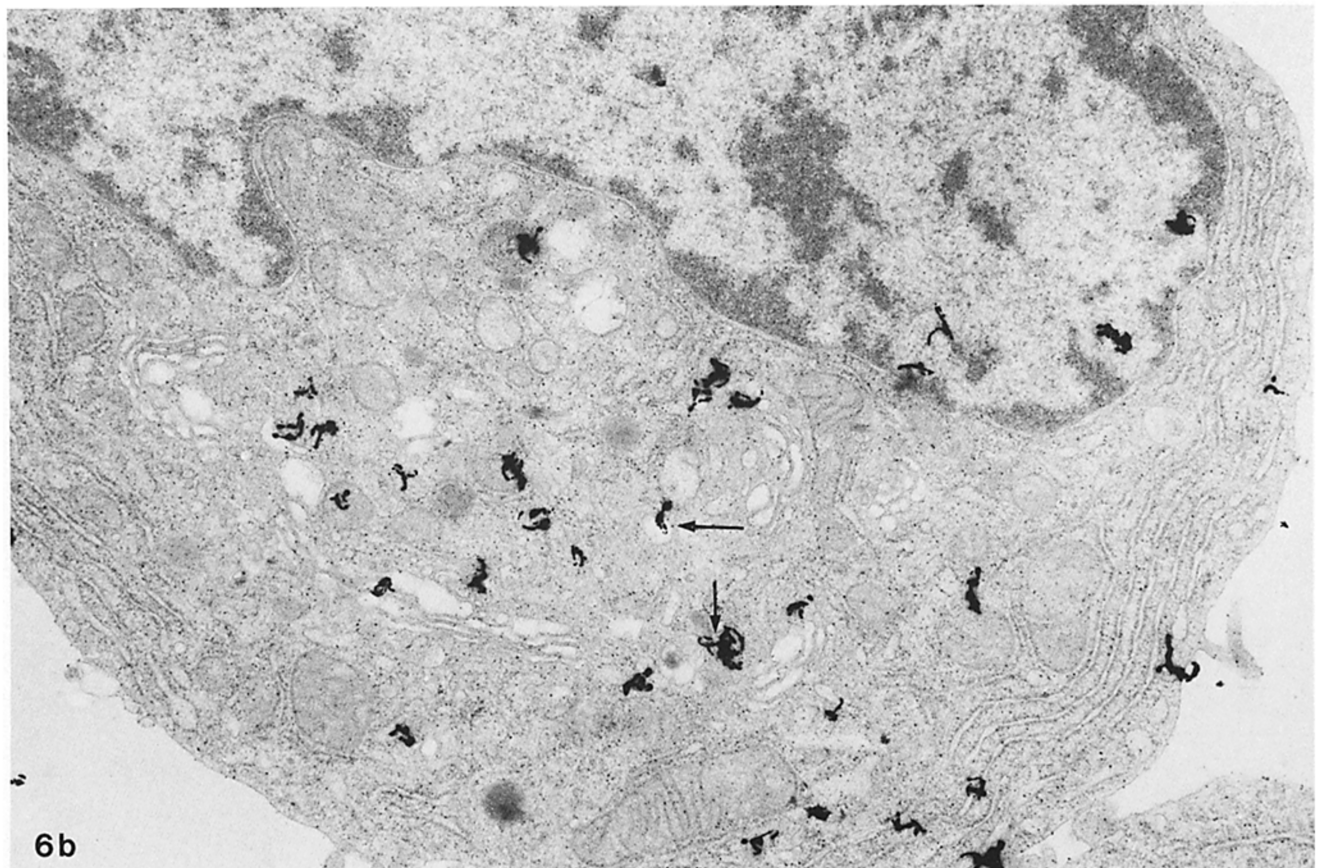
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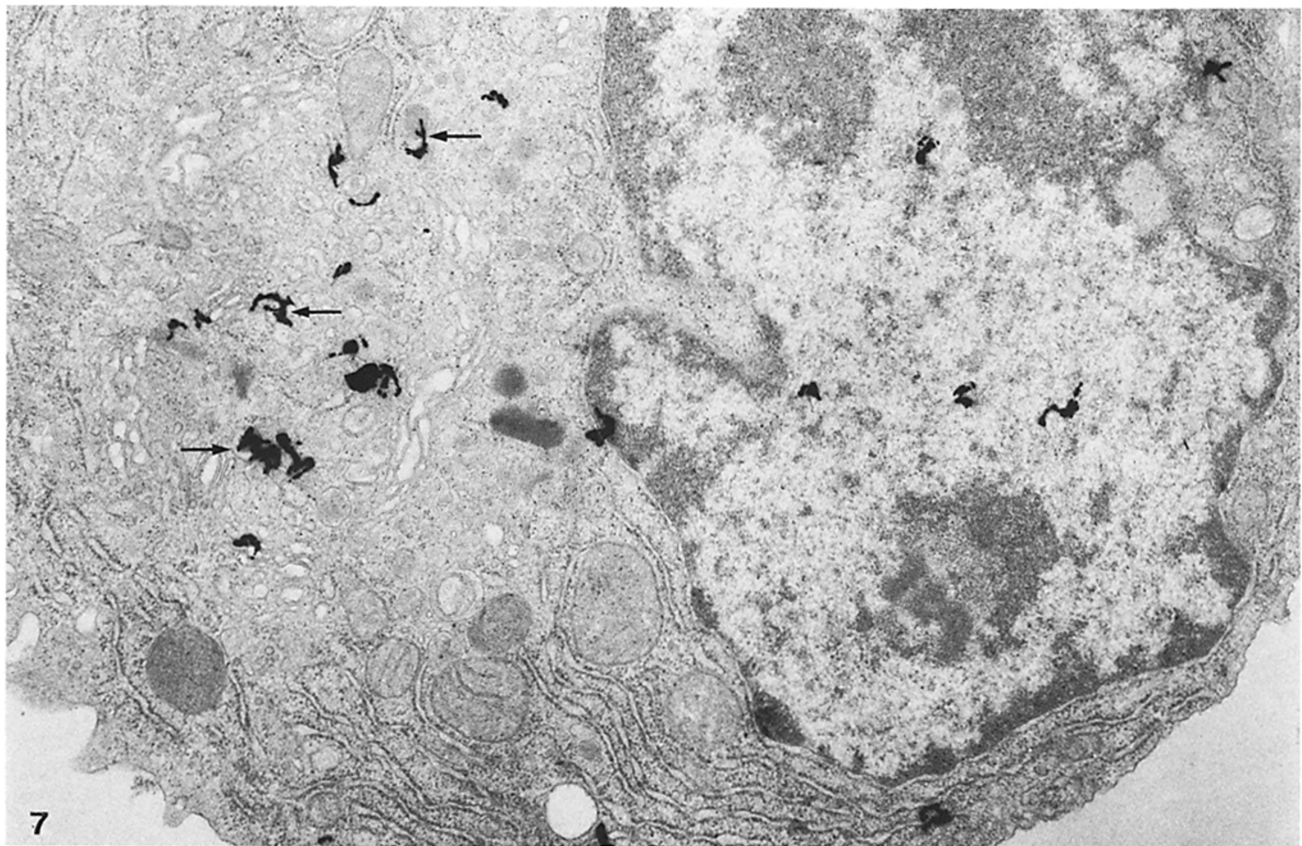

FIGURE 7 Autoradiograms of secretory granule-deficient neoplastic pancreatic acinar cells after 60 min of chase incubation. At 60-min postpulse, the label is not present over the Golgi complex; instead, the autoradiographic grains appear over small vesicles in the post-Golgi region (arrows), some containing electron dense material. × 20,000.

secretory protein expression in individual tumor cell subpopulations (e.g., GEF and GDF) may vary considerably. The GDF cells contain substantially lower amounts of secretory product when compared to GEF cells as judged by the amylase/DNA ratio of 0.45 and 3.3, respectively (3). The absence of secretory granules in GDF cells, therefore, may be due to substantially lower levels of synthesis of the secretory proteins and/or to a defect in the yet to be elucidated concentration mechanism(s) in the Golgi or the post-Golgi vesicles (13, 18, 27). For example, differences in the synthesis of sulfate-containing macromolecules (33, 34, 46) in the Golgi elements of the GDF and GEF neoplastic cells might play a significant role.

(*b*) The newly synthesized secretory proteins in these GDF cells bypass the typical storage step and are rapidly discharged due to a functional defect. It is pertinent to note that Jamieson and Palade (22) demonstrated that in secretagogue-stimulated normal pancreatic acinar cells the secretory proteins are concentrated and packaged in numerous small storage vesicles in a normal fashion, but they are rapidly discharged. Preferential release of newly synthesized prolactin in mammotrophs has been described (43).

Additional studies, therefore, are necessary to determine whether one or more of the possibilities considered above contribute to the difference in the secretory process between GDF and GEF neoplastic pancreatic acinar cells. Analysis by two-dimensional PAGE of the [^{35}S]methionine-labeled total (9) and secretory proteins (38) synthesized by these two subpopulations will be of value in this regard.

The pancreatic acinar carcinoma cells and fragments have been shown to respond to several secretagogues and discharge protein at approximately one-fifth the rate determined for the normal pancreas lobules (16, 44). The decreased responsiveness of acinar carcinoma cells is attributable to cellular heterogeneity and to the higher levels of nonexportable protein synthesis, particularly in GDF cells. The reduced secretory response may be due to differences in cell surface receptors for secretagogues in this heterogeneous tumor cell population. In conclusion, this transplantable pancreatic acinar carcinoma provides an important model system for the investigation of factors that modulate cytodifferentiation and gene expression (16, 17, 19, 32). Study of differentiation in these neoplastic cells may provide a better understanding of both abnormal and normal differentiation.

We thank Nancy Starks for typing this manuscript and Saeed A. Qureshi and Barbara Ann Dappert for technical assistance. We thank Dr. James D. Jamieson, Yale University School of Medicine and Dr.

FIGURE 6 Autoradiograms of secretory granule-deficient neoplastic pancreatic acinar cells separated on Percoll gradient, pulsed with [^{3}H]leucine for 3 min and chase incubated for 10 (*a*) and 30 min (*b*). At 10-min chase, the label is over the RER and the long stacks of the Golgi complex; by 30 min, the label is seen over the post-Golgi vesicles that are present at the *trans* face of the Golgi complex (arrows). *a*, × 13,500; *b*, × 16,000.

Lionel J. Rosenzweig, University of Minnesota, for their excellent suggestions.

This investigation was supported by National Institutes of Health grant CA 23055 from the National Cancer Institute.

Received for publication 17 September 1982, and in revised form 4 January 1983.

REFERENCES

1. Amsterdam, A., and J. D. Jamieson. 1974. Studies on dispersed pancreatic exocrine cells. I. Dissociation technique and morphologic characteristics of separated cells. *J. Cell Biol.* 63:1037–1056.
2. Amsterdam, A., and J. D. Jamieson. 1974. Studies on dispersed pancreatic exocrine cells. II. Functional characteristics of separated cells. *J. Cell Biol.* 63:1057–1073.
3. Becich, M. J., and J. K. Reddy. 1982. Separation and characterization of neoplastic cell subpopulations of a transplantable rat pancreatic acinar carcinoma. *Cancer Res.* 42:3729–3740.
4. Caro, L. G., and G. E. Palade. 1964. Protein synthesis, storage, and discharge in the pancreatic exocrine cell. *J. Cell Biol.* 20:473–493.
5. Case, M. 1978. Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. *Biol. Rev.* 53:211–354.
6. Castle, J. D., J. D. Jamieson, and G. E. Palade. 1972. Radioautographic analysis of the secretory process in the parotid acinar cell of the rabbit. *J. Cell Biol.* 53:290–311.
7. Ehrenreich, J. H., J. M. Bergeron, P. Siekevitz, and G. E. Palade. 1973. Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. *J. Cell Biol.* 59:45–72.
8. Farquhar, M. G., J. J. Reid, and L. W. Daniell. 1978. Intracellular transport and packaging of prolactin: a quantitative electron microscope autoradiographic study of mammotrophs dissociated from rat pituitaries. *Endocrinology.* 102:296–311.
9. Fey, S. J., R. Bravo, P. M. Larsen, J. Bellatin, and J. E. Celis. 1981. [^{35}S]Methionine labeled polypeptides from secondary mouse kidney fibroblasts: coordinates and one dimensional peptide maps of some major polypeptides. *Cell Biol. Inst. Rep.* 5:491–500.
10. Fidler, I. J., and I. R. Hart. 1982. Biological diversity in metastatic neoplasms: origins and implications. *Science (Wash. DC).* 217:998–1003.
11. Foulds, L. 1954. The experimental study of tumor progression: a review. *Cancer Res.* 14:327–339.
12. Friend, C., and J. R. Haddad. 1960. Tumor formation with transplants of spleen or liver from mice with virus-induced leukemia. *J. Natl. Cancer Inst.* 25:1279–1289.
13. Hand, A. R., and C. Oliver. 1981. The Golgi apparatus: protein transport and packaging in secretory cells. *Methods Cell Biol.* 23:137–153.
14. Hansen, L. J., M. Mangkornkanok/Mark, and J. K. Reddy. 1981. Immunohistochemical localization of pancreatic exocrine enzymes in normal and neoplastic pancreatic acinar epithelum of rat. *J. Histochem. Cytochem.* 29:309–313.
15. Howell, S. L., M. Kostianousky, and P. E. Lacy. 1969. Beta granule formation in isolated islets of langerhans: a study by electron microscopic radioautography. *J. Cell Biol.* 42:695–705.
16. Iwanij, V., and J. D. Jamieson. 1982. Biochemical analysis of secretory proteins synthesized by normal rat pancreas and by pancreatic acinar tumor cells. *J. Cell Biol.* 95:734–741.
17. Iwanij, V., and J. D. Jamieson. 1982. Comparison of secretory protein profiles in developing rat pancreatic rudiments and rat acinar tumor cells. *J. Cell Biol.* 95:742–746.
18. Jamieson, J. D. 1981. Summary and perspectives. *Methods Cell Biol.* 23:547–558.
19. Jamieson, J. D., D. E. Ingber, V. Muresan, B. E. Hull, M. P. Sarras, M.-F. Maylie-Pfenninger, and V. Iwanij. 1981. Cell surface properties of normal, differentiating, and neoplastic pancreatic acinar cells. *Cancer.* 47:1516–1525.
20. Jamieson, J. D., and G. E. Palade. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. Transport to condensing vacuoles and zymogen granules. *J. Cell Biol.* 34:597–615.
21. Jamieson, J. D., and G. E. Palade. 1968. Intracellular transport of secretory proteins in the pancreatic exocrine cell. IV. Metabolic requirements. *J. Cell Biol.* 39:589–603.
22. Jamieson, J. D., and G. E. Palade. 1971. Synthesis, intracellular transport, and discharge of secretory proteins in stimulated pancreatic exocrine cells. *J. Cell Biol.* 50:135–158.
23. Kraehenbühl, J. P., L. Racine, and J. D. Jamieson. 1977. Immunocytochemical localization of secretory proteins in bovine pancreatic exocrine cells. *J. Cell Biol.* 72:406–423.
24. MacDonald, R. J., M. M. Crerar, W. F. Swain, R. L. Pictet, and W. J. Rutler. 1981. Pancreas-specific genes: structure and expression. *Cancer.* 47:1497–1504.
25. Nicolson, G. L., K. W. Brunson, and I. J. Fidler. 1978. Specificity of arrest, survival, and growth of selected metastatic variant cell lines. *Cancer Res.* 38:4105–4111.
26. Nowell, P. C. 1976. The clonal evolution of tumor cell populations. Acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression. *Science (Wash. DC).* 194:23–28.
27. Palade, G. 1975. Intracellular aspects of the process of protein synthesis. *Science (Wash. DC).* 189:347–358.
28. Pictet, R. L., W. R. Clark, R. H. Williams, and W. J. Rutter. 1972. An ultrastructural analysis of the developing embryonic pancreas. *Dev. Biol.* 29:436–467.
29. Pierce, G. B., and W. F. Cox, Jr. 1978. Neoplasms as caricatures of tissue renewal. *In* Cell Differentiation and Neoplasia. G. F. Saunders, editor. Raven Press, New York. 58–66.
30. Reddy, J. K., and M. S. Rao. 1977. Transplantable pancreatic carcinoma of the rat. *Science (Wash. DC).* 198:78–80.
31. Reddy, J. K., M. S. Rao, J. R. Warren, S. A. Qureshi, and E. I. Christensen. 1980. Differentiation and DNA synthesis in pancreatic acinar carcinoma of rat. *Cancer Res.* 40:3443–3454.
32. Reddy, J. K., M. K. Reddy, L. J. Hansen, and S. A. Qureshi. 1980. Secretion granules of transplantable pancreatic acinar carcinoma of rat. *Biochem. J.* 188:921–924.
33. Reggio, H. A., and G. E. Palade. 1978. Sulfated compounds in the zymogen granules of the guinea pig pancreas. *J. Cell Biol.* 77:288–314.
34. Rosenzweig, L. J., and M. G. Farquhar. 1980. Sites of sulfate incorporation into mammotrophs and somatotrophs of the rat pituitary as determined by quantitative electron microscopic autoradiography. *Endocrinology.* 107:422–431.
35. Salpeter, M. M., and L. Bachman. 1972. Autoradiography. *In* Principles and Techniques of Electron Microscopy. M. A. Hayat, editor. Van Nostrand Reinhold Co., New York. 2:220–278.
36. Salpeter, M. M., and M. G. Farquhar. 1981. High resolution analysis of the secretory pathway in mammotrophs of the rat anterior pituitary. *J. Cell Biol.* 91:240–246.
37. Salpeter, M. M., and F. A. McHenry. 1973. Electron microscopic autoradiography: analyses of autoradiograms. *In* Advanced Techniques in Biological Electron Microscopy. J. K. Koehler, editor. Springer-Verlag, New York. 113–152.
38. Scheele, G. A. 1982. Two-dimensional electrophoresis in basic and clinical research as exemplified by studies on the exocrine pancreas. *Clin. Chem.* 28:1056–1061.
39. Stevens, L. C., and C. C. Little. 1954. Spontaneous testicular teratomas in an inbred strain of mice. *Proc. Natl. Acad. Sci. USA.* 40:1080–1087.
40. Till, J. E., and E. A. McCulloch. 1980. Hemopoietic stem cell differentiation. *Biochim. Biophys. Acta.* 605:431–459.
41. Uriel, J. 1979. Retrodifferentiation and the fetal patterns of gene expression in cancer. *Adv. Cancer Res.* 29:127–175.
42. Van Nest, G. A., R. J. MacDonald, R. K. Raman, and W. J. Rutter. 1980. Proteins synthesized and secreted during rat pancreatic development. *J. Cell Biol.* 86:784–794.
43. Walker, A. M., and M. G. Farquhar. 1980. Preferential release of newly synthesized prolactin granules is the result of functional heterogeneity among mammotrophs. *Endocrinology.* 107:1095–1104.
44. Warren, J. R., M. J. Trump, J. K. Reddy, and M. J. Becich. 1982. Carbamylcholine stimulation of protein secretion in pancreatic acinar carcinoma of rat. *Cancer Lett.* 15:245–253.
45. Weibel, E. R., G. S. Kistler, and W. F. Scherle. 1966. Practical stereological methods for morphometric cytology. *J. Cell Biol.* 30:23–38.
46. Young, R. W. 1973. The role of the Golgi complex in sulfate metabolism. *J. Cell Biol.* 57:175–189.
47. Zagury, D., J. W. Uhr, J. D. Jamieson, and G. E. Palade. 1970. Immunoglobulin synthesis and secretion. II. Radioautographic studies of sides of addition of carbohydrate moieties and intracellular transport. *J. Cell Biol.* 46:52–63.