Intracellular Processing of Epidermal Growth Factor

I. ACIDIFICATION OF 125I-EPIDERMAL GROWTH FACTOR IN INTRACELLULAR ORGANELLES*  

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We previously reported that 125I-labeled epidermal growth factor is processed intracellularly to acidic macromolecules in Rat-1 fibroblasts. The present study defines the precursor-product relationship and localization of the processing steps to subcellular organelles by the use of a single isoelectric species of 125I-epidermal growth factor and Percoll gradient fractionation. The native pI 4.55 125I-epidermal growth factor was rapidly processed to a pI 4.2 species on or near the cell surface and in organelles corresponding to clathrin-coated vesicles, Golgi, and endoplasmic reticulum. This species was then processed to a pI 4.35 species in similar organelles. The pI 4.2 and 4.35 species were converted to a pI 4.0 species in dense, lysosome-like organelles. This species was ultimately degraded and exocytosed from the cell as low molecular weight products.

Previously this laboratory demonstrated that 125I-labeled epidermal growth factor was processed intracellularly to macromolecular derivatives of the same approximate size as EGF but which were more acidic (1). One of the acidic processed forms of EGF was associated with an organelle-rich fraction, as demonstrated by centrifugation of cell homogenates on Percoll gradients, whereas another later appearing species was not associated with organelles but appeared to be present in the cytoplasm.

A major problem in demonstrating the precursor-product sequence of EGF processing, however, was the presence of a number of isoelectric forms of 125I-EGF in the 125I-EGF employed in the experiments. Recently we demonstrated that iodination of EGF generates multiple species with differing isoelectric points which behave like EGF, but of which only one (pI = 4.55) corresponds to intact EGF (2).

The present study was undertaken to elucidate the order of processing of purified 125I-EGF to its macromolecular derivatives and to understand the cellular localization of those derivatives. Our results demonstrate that pI 4.55 EGF becomes processed to a pI 4.2 form at or near the cell surface and in membranous vesicles which cofractionate with markers for coated vesicles, Golgi, and endoplasmic reticulum. This form is subsequently processed to a pI 4.35 species in similar vesicles, followed by conversion to a pI 4.0 species in lysosome-like vesicles. It is the latter form of EGF which is ultimately degraded to low molecular weight compounds.

MATERIALS AND METHODS

Cell Culture—Rat-1 cells (3) were propagated in Dulbecco's modified Eagle's medium containing 10% calf serum and 2% newborn calf serum at 37 °C in a humidified 5% CO2/95% air atmosphere. Epidermal Growth Factor—EGF was prepared from mouse submandibular glands (4) and further purified to a single peak by high performance liquid chromatography as previously described (5) except that a gradient of 10-40% acetonitrile was generated over 90 min in a aqueous phase of 0.05 M acetic acid brought to pH 7.1 with triethylamine. The purified EGF was iodinated using chloramine-T (6) and further purified by preparative isoelectric focusing on agarose gels as previously described (1, 2). The pI 4.55 iodinated form of EGF was eluted from the gel and kept frozen at -20 °C until use. The specific activity of the 125I-EGF was approximately 500 μCi/μg.

Isoelectric Focusing—Isoelectric focusing was performed in 1% agarose slab gels as previously described (1, 2). The pH range of the gels was from 3.0 to 6.0. pH across the gel was measured with a micro pH probe (Microelectrodes, Inc.) or Isobol pI markers (FMC Corp.). Autoradiography of gels was performed as described (1).

Percoll Gradient Fractionation and Marker Determinations—Percoll gradient fractionation was performed as described previously (1) with the following modifications. Cells were disrupted by resuspending in 1.0 ml of SEAT buffer (1 mM EDTA, 10 mM acetic acid, 10 mM triethanolamine, pH 7.4, Ref. 7) and lysed by pipetting the suspension 20-40 times with a P1000 Pipetman (8). One ml of the cell extract was loaded onto 8.0 ml of 20% Percoll and centrifuged at 18,000 × g for 90 min in a Beckman type 40 fixed angle rotor.

Acid phosphatase, a marker enzyme for lysosomes, was assayed as described previously (1) using 0.15 ml of each fraction. UDP-galactosyl transferase activity, a marker enzyme for Golgi, was measured as described (9) using 0.05 ml of sample in a final volume of 0.1 ml.

NADPH-cytochrome c reductase, a marker for endoplasmic reticulum, was assayed as described (10) using 0.15 ml of sample in a final volume of 0.3 ml. Results are expressed as the change in absorbance at 553 nm at 37 °C for 5 min.

The presence of clathrin, a protein associated with coated vesicles or membranes, was determined by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Clathrin was purified from bovine brain as described by Keen et al. (11). Antibody to the mucose gradient-purified clathrin was prepared in rabbits by multiple intradermal injections of 0.5 mg of glutaraldehyde-treated clathrin in Freund's adjuvant. Untreated Rat-1 cells (20-100 nm Petri dishes) were fractionated by centrifugation in Percoll as described above. Fractions (0.5 ml) were incubated with 0.01 ml of anti-clathrin antisera for 1 h at room temperature, followed by the addition of 0.1 ml of formalin-fixed Staphylococcus aureus (IGGosorb, The Enzyme Center). The mixture was allowed to react for 15 min at room temperature. After centrifugation the pellet was washed in a buffer containing 150 mM NaCl, 5 mM EDTA, 0.25% gelatin, 0.05% Nonidet P-40, and 50 mM Tris-HCl, pH 7.4, and resuspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer (12). The samples were electrophoresed on a 5% polyacrylamide gel with a Tris-glycine/sodium dodecyl sulfate buffer essentially as described previously (12). Protein was stained with Coomassie blue R250 dye.

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The abbreviations used are: EGF, epidermal growth factor; HBSS, Hank's balanced salt solution; IEF, isoelectric focusing; BSA, bovine serum albumin.
RESULTS

To examine the processing of $^{125}$I-EGF, we exposed Rat-1 cells to purified $^{125}$I-EGF (pl = 4.55) for 10 min and then applied a cold EGF chase by adding a 1000-fold excess of unlabeled EGF. At times up to 4 h both surface-bound and internalized $^{125}$I-EGF were differentially removed by the method of Haigler et al. (13). Immediately after the $^{125}$I-EGF pulse, approximately 90% of the radioactivity had been internalized and was inaccessible to removal by acidic saline (Fig. 1A). Approximately 30 min after application of the cold chase the intracellular levels of $^{125}$I-EGF began to decline, and the decrease continued over the next several hours. By 3 h the total levels of cell-bound $^{125}$I-EGF had declined to less than 10% of the maximal values.

As a control for the ability of the acidic saline extraction procedure to discriminate external from internal $^{125}$I-EGF, cells were exposed to $^{125}$I-EGF for 4 h at 4 °C, rinsed exhaustively in ice-cold HBSS and then shifted to 37 °C prior to removal of external and internal $^{125}$I-EGF. When cells were incubated at 4 °C in the presence of $^{125}$I-EGF, greater than 90% of the $^{125}$I activity was eluted in the acidic saline solution, indicating the surface localization of $^{125}$I-EGF (Fig. 1B). Following warming of cells to 37 °C, the $^{125}$I activity quickly became internalized as manifested by its resistance to extraction in the acidic saline.

The isoelectric points of the $^{125}$I-containing extracts from the pulse-chase experiment shown in Fig. 1A were examined by gel isoelectric focusing and autoradiography (Fig. 2). The majority of the radioactivity which remained surface-bound at the end of the pulsing interval corresponded to intact $^{125}$I-EGF with a pl of 4.55 (lanes 1 and 2). Fifteen min after application of the cold chase, virtually no pl 4.55 $^{125}$I activity was recovered from the cell surfaces (lane 3). By the end of the 10-min $^{125}$I-EGF pulse, the internalized $^{125}$I activity was distributed between two forms: one representing unmodified EGF with a pl of 4.55, and the other with a pl of 4.2 (lane 4).

By 15 min after application of the cold chase, the internalized pl 4.55 $^{125}$I-EGF had decreased dramatically and became indetectable at later times (lanes 6–11). By 15 min after the cold chase, a new radioactive species with a pl of 4.35 appeared, and by 30 min after application of the cold chase a species of pl 4.0 appeared. Over the next several hours the total extractable $^{125}$I activity decreased (see Fig. 1A), and the decrease was evident in all the $^{125}$I-containing species (Fig. 2, lanes 6–11). The pl 4.0 species became the most prominent one beginning 60 min after the cold chase.

The kinetics of the progressive alteration in intracellular $^{125}$I species was examined in order to ascertain potential precursor-product relationships. The radioactivity in each of the four $^{125}$I-containing species from the preceding experiment (i.e. pl 4.55, 4.35, 4.2, and 4.0; Fig. 2) was determined by excision of the bands from the dried gel and $\gamma$ counting of the radioactive bands (Fig. 3). The time course of appearance and disappearance of the $^{125}$I-containing species was consistent with the conclusion that $^{125}$I-EGF (pl 4.55) became processed to the pl 4.2 species (lane b, Fig. 3) which in turn became processed to the pl 4.35 (lane a) and 4.0 (lane c) species. The
pl 4.0 species appeared to be the longest lived of the intracellular $^{125}$I-containing molecules.

The precursor-product relationship of the different species of intracellular $^{125}$I-EGF derivatives was investigated by preparing them in pure form and testing their ability to bind and be processed by cells. Cells were exposed to $^{125}$I-EGF for 1 h at 37°C, at which time the cells were extracted successively in acidic saline (to remove surface-bound $^{125}$I) and in 0.05 N HCl (to remove intracellular $^{125}$I activity). The extracts were dialyzed, concentrated, and separated by preparative IEF. The radioactive bands located on the gel were excised and eluted. Each of the eluted species refocused as a single band by IEF (Fig. 4).

The abilities of $^{125}$I-EGF and each of the internalized forms of $^{125}$I-EGF to bind to cells were compared (Table I). The pl 4.2 species showed a greater percentage of binding than the parental $^{125}$I-EGF, whereas both the pl 4.35 and 4.0 species showed much less binding.

The ability of the pl 4.2 intracellular species to be internalized and processed was tested by exposing cells to the purified iodinated pl 4.2 species at 37°C for 60 min. The cell-bound $^{125}$I activity was extracted in 0.05 N HCl and examined by IEF (Fig. 5, lane A).

Most of the activity was present in the pl 4.2 form, but some of the pl 4.3 and 4.0 species had been produced. When a parallel set of cells was similarly exposed to the pl 4.2 species for 60 min at 37°C but then chased with unlabeled EGF for 30 min, there was an increase in the relative composition of the pl 4.0 species (Fig. 5, lane B). The pl 4.35 species was also present after the 1-h chase. These data support the conclusion drawn from the kinetic data of Fig. 3 that the pl 4.2 species of $^{125}$I-EGF, which is formed early after exposure of the cells to $^{125}$I-EGF, is the precursor of both the pl 4.35 and pl 4.0 processed forms of $^{125}$I-EGF.

### Figure 4: Purification of internalized $^{125}$I-EGF species

Five confluent 10-cm plates of Rat-1 cells were washed and exposed successively to 4.0 ml of binding media containing 2 x 10⁶ cpm/ml of the pl 4.55 species of $^{125}$I-EGF for 1 h at 37°C. The surface-bound $^{125}$I-EGF was removed from each plate by washing with 5 ml of 0.5 M NaCl, pH 2.5. Internalized $^{125}$I-EGF was eluted for 30 min in 5 ml of 0.05 N HCl. The sample was dialyzed against water for 2 days and purified by isoelectric focusing as described under "Materials and Methods." The radioactive bands located on the gel were excised and eluted. Each of the eluted species refocused as a single band by IEF. The autoradiograph was prepared as described under "Materials and Methods." The radioactive bands located by autoradiography, and the appropriate gel slices were eluted in 1 ml of 0.1% BSA in water. An aliquot of the sample eluted from each gel slice was refocused, and the purity of the isolated species was examined by autoradiography. Lane 1, pl 4.55 species. Lane 2, pl 4.35 species. Lane 3, pl 4.2 species. Lane 4, pl 4.0 species.

### Table I

**Binding of intracellular $^{125}$I-EGF derivatives**

Internalized $^{125}$I-EGF species were purified as described in the legend to Fig. 4. Binding ability of each species was tested by exposing Rat-1 cells (1.7 x 10⁶ cells/35-mm culture dish) to 10,000 ± 2,000 cpm/ml/dish of the pl 4.55, 4.2, and 4.0 species and 4,600 cpm/ml/dish of the pl 4.35 species at 4°C for 4 h. Plates were incubated at 37°C for 30 min in 0.5 ml of binding media. Groups A, B, and C: following the indicated treatments, "surface" radioactivity was removed by incubation in 0.5 N NaCl, pH 2.5, for 4 min and "internalized" radioactivity was eluted for 30 min in 0.05 N HCl.

<table>
<thead>
<tr>
<th>Lane</th>
<th>pl of the EGF derivative</th>
<th>cpm specifically bound</th>
<th>Bound/free</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.55 (parental EGF)</td>
<td>1027 ± 70</td>
<td>0.114</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>1492 ± 73</td>
<td>0.178</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>290 ± 23</td>
<td>0.030</td>
</tr>
<tr>
<td>4</td>
<td>4.3</td>
<td>218 ± 14</td>
<td>0.047</td>
</tr>
</tbody>
</table>

*Corrected to 10,000 cpm added.

### Figure 5: Intracellular processing of the pl 4.2 species

The purified pl 4.2 species (50,000 cpm/0.5 ml/dish) was added to 35-mm dishes of Rat-1 cells in binding media, and the cells were incubated at 37°C for 60 min. Lane B, the pulse was followed by the addition of 2 μg of unlabeled EGF for 30 min at 37°C. In both lanes A and B, cells were washed 6 times in cold HBSS with BSA; cell surface radioactivity was removed in 0.5 M NaCl, pH 2.5, and internalized $^{125}$I-EGF was eluted in 0.05 N HCl for 30 min. The internalized $^{125}$I-EGF samples were dialyzed against water for 2 days, concentrated, and focused. The autoradiograph was prepared as described under "Materials and Methods."

### Table II

**Formation of the pl 4.2 species at the cell surface**

Rat-1 cells were exposed to 2 x 10⁶ cpm/0.5 ml/35-mm dish of purified pl 4.55 $^{125}$I-EGF in binding medium for 2 h at 4°C. Cells were then washed 6 times in HBSS with BSA. Group B: cultures were returned to 4°C for 2 h in 0.5 ml of binding media. Group C: cultures were incubated at 37°C for 30 min in 0.5 ml of binding media. Groups A, B, and C: following the indicated treatments, "surface" radioactivity was removed by incubation in 0.5 N NaCl, pH 2.5, for 4 min and "internalized" radioactivity was eluted for 30 min in 0.05 N HCl.

<table>
<thead>
<tr>
<th>Lane</th>
<th>&quot;Surface&quot; $^{125}$I-EGF activity</th>
<th>&quot;Internalized&quot; $^{125}$I-EGF activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6,425</td>
<td>3,650</td>
</tr>
<tr>
<td>B</td>
<td>37,150</td>
<td>4,330</td>
</tr>
<tr>
<td>C</td>
<td>2,900</td>
<td>34,780</td>
</tr>
</tbody>
</table>

"Surface" = 0-4°C, 2 h-rinse-extraction
"Internalized" = 37°C, 30 min-extraction

### Figure 6: Isoelectric focusing of surface-bound $^{125}$I-EGF

Samples described in the legend to Table II were dialyzed against water for 2 days, concentrated, focused, and autoradiographed as described under "Materials and Methods." Lane A, sample from protocol A, eluted in 0.5 N NaCl, pH 2.5. Lane B, sample from protocol B, eluted in 0.5 N NaCl, pH 2.5. Lane C, sample from protocol B, media sample.
We determined whether the pI 4.2 form of $^{125}$I-EGF, which is the main component associated with intracellular organelles (1), could be produced at the cell surface (i.e. prior to internalization via vesicles). Cells were exposed to $^{125}$I-EGF for 2 h at 4 °C, rinsed well in HBSS, and divided into 3 groups. One group of cells was immediately incubated in acidic saline (to remove surface $^{125}$I activity) followed by 0.05 N HCl (to remove internalized $^{125}$I activity). Approximately 95% of the cell-bound $^{125}$I activity was surface-bound (Table II, protocol A). A second set of cells was returned to 4 °C in fresh medium for 2 h to permit diffusion of surface-bound $^{125}$I activity. Over 40% of the surface-bound activity diffused from the cells during the postincubation and little if any $^{125}$I activity entered the cells (Table II, protocol B). A third set of cells was shifted to 37 °C to ensure that the internalization mechanism remained intact in these cells. In these cells over 90% of the cell-bound $^{125}$I activity was intracellular and inaccessible to the acidic saline rinse (Table II, protocol 3).

Examination of the acidic saline extract (surface-bound $^{125}$I activity) immediately after the binding of $^{125}$I-EGF at 4 °C (Table II, protocol A) revealed not only the pI 4.55 $^{125}$I-EGF, but the pI 4.2 species as well (Fig. 6, lane A). Excision and counting of the $^{125}$I activity demonstrated that the pI 4.2 species represented 55% of the total activity in the sample. The $^{125}$I activity which remained on the cell surfaces after the 4 °C postincubation (protocol B, Table II) contained both pI 4.2 and 4.55 species, but the latter species predominated (Fig. 6, lanes B and C).

Further cellular fractionation was achieved by Percoll gradient centrifugation using a recently described cell lysis technique (7). This technique allows the separation of dense lysosome vesicles (acid phosphatase activity, Fig. 7C) from the membrane and/or vesicles that are associated with enzyme markers for endoplasmic reticulum (NADPH-cytochrome c reductase, Fig. 7E) and Golgi components (UDP-galactosyl transferase, Fig. 7D) and from the clathrin marker (Fig. 7B) and plasma membrane marker (Fig. 8A).

Rat-1 cells were pulse-labeled with $^{125}$I-EGF at 37 °C for 5 min, followed by rapid washing of the cells and incubation at 37 °C for the indicated times. The cells were harvested and fractionated by Percoll gradient centrifugation, and the $^{125}$I activity in each fraction was determined. The $^{125}$I activity was...
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FIG. 8. Percoll gradient analysis of 125I-EGF. Rat-1 cells in 10-cm dishes were washed once in Dulbecco’s modified Eagles’s medium and incubated at 4 °C for 2 h in binding medium containing 5 × 10⁶ cpm/plate of the pH 4.55 species of 125I-EGF. Cells were washed 6 times with cold HBSS with BSA, the media were replaced with warm binding media, and the cultures were incubated at 37 °C for the indicated times. Cultures were harvested and processed for Percoll gradient fractionation as described under “Materials and Methods.” The radioactivity in fractions (0.25 ml) was measured in a Tracor γ counter. A–E, cultures were at 37 °C for 0, 5, 10, 20, and 45 min, respectively. L, fractions containing the highest activity of acid phosphatase, the lysosomal marker; M, fractions corresponding to mem-

braneous organelle markers (plasma membrane, Golgi, clathrin-coated vesicles, and endoplasmic reticulum).

FIG. 9. Isoelectric focusing of Percoll gradient-purified 125I-EGF. Samples indicated by the arrows in Fig. 8A were centrifuged at 100,000 × g for 1 h to remove the Percoll. The supernatant was dialyzed against water for 2 days, concentrated, and focused as described under “Materials and Methods.” Lane A, the fraction indicated by the arrow in Fig. 8A, representing plasma membrane-associated radioactivity. Lane B, the fraction indicated by the arrow in Fig. 8B. Lane C, the fraction indicated by the arrow in Fig. 8E, which co-elutes with the marker enzyme for lysosomes.

initially concentrated in a peak with a density of 1.037 g/ml (Fig. 8A). This peak was the only 125I-containing peak observed when cells were incubated at 4 °C (data not shown), and therefore, probably represents plasma membrane-bound EGF. The 125I activity associated with the top of the gradient in A corresponded to the position of nonbound 125I-EGF (data not shown). Throughout the period of the chase (Fig. 8, B–E) the 125I activity shifted progressively to higher densities and at 45 min was mostly associated with fractions containing lysosomes (Fig. 8E).

To determine the isoelectric point of the 125I-EGF species associated with fractions corresponding to separated subcellular organelles, the fractions indicated with an arrow were centrifuged at 100,000 × g to remove the Percoll after which the supernatants were dialyzed, concentrated, and separated by isoelectric focusing (Fig. 9). The radioactivity associated with the plasma membrane fraction consisted of the pH 4.55 species (76% of total radioactivity) and a small amount of the pH 4.2 species (16%, Fig. 9A). The vesicle fraction from the 5-min time point contained the pH 4.55, 4.35, and 4.2 species (75, 5, and 13%, respectively). At 45 min, the fraction corresponding in position to lysosomes contained 77% of the radioactivity at pH 4.0, 9% at 4.35, and 10% at 4.2.

DISCUSSION

Previously we showed that when 125I-EGF was bound to cells at 37 °C, it became processed to macromolecular derivatives which were of the same approximate size and immunoreactivity as precursor EGF but which were more acidic as determined by isoelectric focusing and ion exchange chromatography (1). A major obstacle in elucidating the precursor-product relationships of the EGF derivatives was the evolution of 125I-labeled EGF species of differing isoelectric points during the iodination procedure. Recently we demonstrated the production of at least 5 isoelectric forms of 125I-EGF following either chloramine-T- or Iodogen-facilitated iodination (2). All 5 of these forms demonstrated binding and processing to more acidic derivatives. However, only one of these forms, which comprised 30% of the total iodinated EGF, corresponded to intact 125I-EGF and had a pH of 4.55, the isoelectric point of noniodinated EGF. The other species were apparently degradation artifacts generated by the iodination
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The functional implications of EGF endocytosis and processing have yet to be elucidated. Fox and Das (25) have proposed that endocytosis is required for the mitogenic activity of EGF. Lysosomotropic amines, such as methyamine and ammonium chloride, inhibit both processing of 125I-EGF to the pl 4.0 species and biological activities of EGF such as the induction of DNA synthesis (26) and ornithine decarboxylase activity. However, there has not been a direct demonstration that either a product of EGF or its receptor is involved in the transmission of an EGF-induced biological response. Purification and testing of processed EGF derivatives in vitro may provide the means with which to test the hypothesis that an intracellular product of EGF is involved in the generation of a biological response.

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REFERENCES


procedure. The pl 4.55 form of 125I-EGF was purified by preparative IEF and was employed in the experiments reported in this paper.

The processing of pl 4.55 EGF to its pl 4.2 derivative occurred at least partly at the cell surface, prior to clustering and internalization, since it was formed at 4 °C and was freely diffusible from cell surfaces. Under these conditions there was little or no detectable internalization of surface-bound EGF. In the accompanying paper (14), we have demonstrated that the pl 4.2 derivative is lacking the COOH-terminal arginine, indicating the possibility that a surface-associated hydrolytic enzyme specific for the COOH terminus may be involved in this processing step.

Beginning about 25 min after exposure to 125I-EGF (or 15 min after the cold chase) the pl 4.2 species began to be processed to the pl 4.0 and 4.35 species. The precursor-product relationships were demonstrated by the quantitative determination of the 125I-labeled bands excised from the IEF gels (Figs. 2 and 9) and by experiments which showed that cells incubated in purified pl 4.2 species processed it to both 4.35 and 4.0 species (Fig. 5). The possibility that the pl 4.2 species was first processed to the 4.35 species and later processed to the 4.0 species was suggested by the earlier appearance of the 4.35 species in pulse-chase experiments (Figs. 2 and 3). However, at least some of the pl 4.2 species may be processed directly to the 4.0 species, since 1 h after application of the cold EGF chase the pl 4.35 species had disappeared, whereas the pl 4.0 species continued to be generated. This could be explained, however, by a lack of accumulation of the pl 4.35 species due to rapid rates of processing to the pl 4.0 species.

Conversion of the pl 4.35 species occurred in membranes or vesicles with a density of 1.043 g/ml. These membrane fractions include clathrin-coated membranes or vesicles, Golgi, or endoplasmic reticulum as determined by marker proteins or enzymes (Fig. 7). Conversion to the pl 4.0 species, however, was associated with dense lysosome-like vesicles (Figs. 7-9).

Several lines of evidence utilizing ligands conjugated to fluorescein, electron-dense, or radioactive compounds have demonstrated that EGF and other polypeptide ligands are rapidly internalized into coated vesicles after binding to their cell surface receptors (15-18). In the case of EGF (19), as well as several other ligands (20-23), the clathrin coat of the endocytic vesicles is immediately lost and the ligand is transported intracellularly via uncoated vesicles, termed "receptosomes" (20). Employing a conjugate of EGF and horseradish peroxidase, Willingham and Pastan (19) reported that the EGF-containing receptosomes accumulate in the perinuclear Golgi region 10-13 min after warming KB cells to 37 °C, coinciding temporally with our demonstration of the organelle-associated nature of the pl 4.2 processed form of EGF. By 15 min, the EGF-horseradish peroxidase began to be delivered to lysosomal elements located near the Golgi system. Using Percoll gradient fractionation, similar progression of 125I-EGF through vesicles migrating with Golgi markers to lysosome-like vesicles has been reported (8, 24). This pathway of endocytosis is supported by our data which demonstrated that processed EGF was first associated with gradient fractions containing clathrin and Golgi markers and was later associated with lysosomes. We have observed similar pathways of EGF endocytosis and EGF processing in cultured mouse and human fibroblasts.

Intracellular processing of epidermal growth factor. I. Acidification of 125I-epidermal growth factor in intracellular organelles.
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