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Membranes of the Adrenal Medulla: a Comparison between the Membranes of the Golgi Apparatus and Chromaffin Granules

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SUMMARY

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A comparative biochemical study of the membranes of the Golgi apparatus and chromaffin granules is described. Chromaffin granule membranes were almost completely devoid of galactosyltransferase activity, a Golgi marker enzyme, and 5'-nucleotidase activity. High specific activities of these two enzymes were detected in the Golgi-rich fraction, which, in addition, showed a small content of dopamine β-hydroxylase, as indicated by electrophoretic, immunological, and chemical techniques. The specific activity of dopamine β-hydroxylase was 12 times greater in chromaffin granule membranes than in Golgi membranes. Gel electrophoresis also showed the presence in both types of membranes of a band (component C) of similar mobility. Component C was highly concentrated in Golgi membranes. The molar ratio of cholesterol to phospholipid and the lysolecithin content were greater in the granules than in the Golgi membranes. Greater specific activities of Mg^{2+}- and Ca^{2+}-dependent ATPases were detected in Golgi membranes. However, transphosphorylation from ATP to membranes was 16 times greater in the granules than in the Golgi membranes. The results are discussed in connection with the origin of chromaffin granules, and it is concluded that, if chromaffin granules are derived from the Golgi apparatus, a specific process of "membrane differentiation" must take place in order to explain the marked biochemical differences between the two types of membranes.

INTRODUCTION

During stimulation, the adrenal medulla releases the soluble content of the chromaffin granules to the cell exterior by a process of exocytosis, a mechanism which is well documented by morphological (1-3) and biochemical observations (4-8). After secretion the granule membranes remain within the cells as empty vesicles (7), but the ultimate fate of these granule membranes remains unknown. Similarly, the biogenesis of the chromaffin granules is not clearly understood. Morphological observations have suggested that chromaffin granules originate in the Golgi apparatus (1, 9). However, there is no biochemical evidence to support this hypothesis. In order to understand the series of events involved in the secretory process, the origin of the chromaffin granules must be determined. We have previously described a method for the isolation and characterization of a Golgi-rich fraction from the

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adrenal medulla (10, 11). The present paper is a continuation of these studies and describes a comparative biochemical study of the membranes of the chromaffin granules and those of the Golgi complex. If chromaffin granules originate in the Golgi apparatus without a process of membrane differentiation, the enzyme activities of membrane preparations from the two sources would be expected to be similar. A preliminary account of some of the findings has already been given (12).

MATERIALS AND METHODS

Subcellular fractionation of adrenal medulla. Bovine adrenal glands obtained from the slaughterhouse were kept on ice, and the medullae were separated from the cortices. Each medulla was homogenized in 4 volumes of ice-cold 0.3 M sucrose (pH 7.0), and the chromaffin granules and the Golgi-rich fractions were obtained by the density gradient centrifugation procedure described previously (10, 11). The protocol followed in the isolation of the different fractions is shown in Fig. 1. The details of the preparaion of gradient 2 are described in the legend to Fig. 3. The purity of the Golgi preparation obtained by this method, as evaluated from the specific activity of different marker enzmes, was 75–80% (10, 11).

Preparation of Golgi and chromaffin granule membranes. Golgi and granule preparations obtained by the above method were resuspended in ice-cold 5 mM Tris-HCl buffer (pH 7.0) for 30 min and centrifuged at 100,000 × *g* for 60 min. Resuspension and centrifugation were repeated three more times. The final pellets, containing the Golgi and the chromaffin granule membranes, were kept frozen and used within 1–2 days.

Lipid extraction and thin-layer chromatography. Golgi and granule membranes were extracted with chloroform–methanol (2:1) plus 0.25% HCl as described previously (13). Samples from the final lipid extracts were applied to silica gel G plates (0.25 mm thick), and the individual phospholipids were separated by a two-dimensional technique (13). The different spots were detected, and the phospholipids were identified as described elsewhere (13). Lysolecithin was eluted from the silica by washing the silica first with the same solvents used in the chromatography, and then with chloroform–methanol (2:1). The phosphorus content of the lipids was determined by the method of Ames (14). Recovery of the phospholipids from the plates was 88% ± 7% (mean ± SE, n = 10), and the results were expressed after correction for 100% recovery.

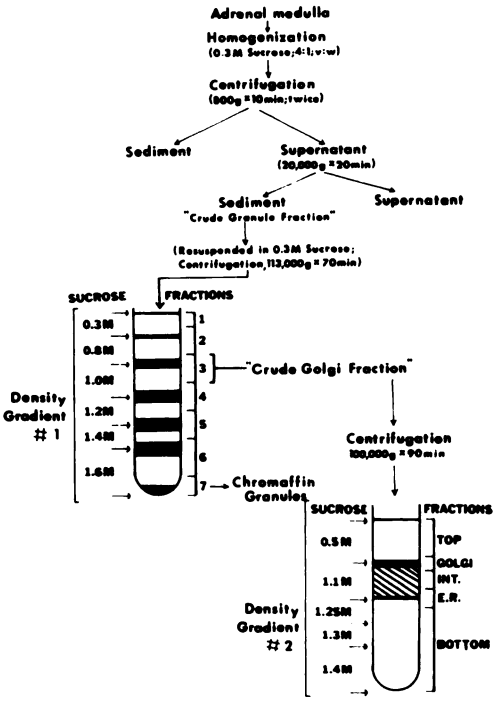

FIG. 1. *Flow diagram of method followed for isolation of chromaffin granules and Golgi-rich fraction from adrenal medulla*

More details are described in MATERIALS AND METHODS, in the legend to Fig. 3, and in a previous paper (11).

Chemical determinations. Samples from the different fractions were treated with trichloracetic acid (10%, final concentration), and the protein concentration in the different preparations was measured according to Lowry *et al.* (15), using bovine serum albumin as a standard. Mg^{2+}- and Ca^{2+}-dependent ATPases were determined by measuring the hydrolysis of [γ-^{32}P]ATP as described previously (16). Transphosphorylation from ATP to Golgi or granule

membranes was also determined using [γ-^{32}P]ATP (specific activity, 2 mCi/μmole) in the presence of 1.0 mM $MgCl_2$ (16). The ^{32}P radioactivity of the samples was measured with counting efficiencies between 85% and 92%, the results were corrected to 100% efficiency and expressed in micro- or nanomoles of P_i per milligram of protein, calculated on the basis of the specific activity of the added [γ-^{32}P]ATP. 5'-Nucleotidase was assayed by the method of Mitchell and Hawthorne (17). Dopamine β-hydroxylase was measured by the method described by Viveros *et al.* (18), using [*G*-^{3}H]tyramine hydrochloride (specific activity, 10 Ci/μmole) as substrate. UDP-galactose:*N*-acetylglucosamine galactosyltransferase was determined by the method of Fleischer *et al.* (19). The following modifications were introduced: (a) the concentration of uridine diphospho[*U*-^{14}C]galactose (specific activity, 1 μCi/μmole) was 0.05 μmole instead of 0.15; (b) Triton X-100 was present in the incubation medium at a concentration of 0.6%; and (c) the total incubation volume was 80 μl, and the addition of 6 μmoles of EDTA in 20 μl of distilled water (pH 7.0) terminated the incubations. The results were expressed as nanomoles of galactose transferred per milligram of protein per hour. Cholesterol was measured by the fluorometric method of Tishler and Bathish (20).

Polyacrylamide gel electrophoresis. SDS[3]-polyacrylamide gel electrophoresis was performed as described by Weber and Osborn (21). Samples (150–250 μg of protein) of the SDS-extracted membrane proteins were applied to 6% acrylamide gels containing 0.1% SDS. Nine-centimeter gels were used, and electrophoresis was carried out first for 20 min with a current of 5 mamp/tube and then continued for 7 hr with a current of 8 mamp/tube. The gels were stained for 1 hr with a solution of 0.1% Amido black in 7.5% acetic acid. Destaining was performed electrophoretically in 7.5% acetic acid.

Preparation of rabbit antibody against dopamine β-hydroxylase. Twelve aliquots (150 μg of protein each) containing chromaffin granule soluble proteins were subjected to polyacrylamide gel electrophoresis following the technique described by Davis (22). At the end of the run, two gels were stained and destained to visualize the different electrophoretic bands. The stained gels were matched to the other 10 gels, and the segments corresponding to the position of dopamine β-hydroxylase were cut out. Three of these segments were homogenized in 0.3–0.4 ml of 50 mM Tris-HCl buffer (pH 7.0). The homogenate was emulsified with an equal volume of complete Freund's adjuvant. Because of the small amount of dopamine β-hydroxylase available, the antigen was injected directly into the spleen. White New Zealand male rabbits, weighing 2.5–3.0 kg, were anesthetized with sodium pentobarbital (25 mg/kg of body weight given intravenously). A midline incision was made in the upper part of the abdomen, and the spleen was exposed. Aliquots containing 0.1 ml of the antigen solution were injected through a 28-gauge needle in different areas of the spleen. The total volume injected per spleen was 0.6–0.8 ml. The spleen was the returned to its anatomical position and the abdominal cavity was closed. Booster doses equivalent to the content of one gel segment were injected subcutaneously at 10-day intervals until a good antibody titer had developed. Antibodies could generally be detected 4–5 weeks after initial immunization.

Immunological techniques. The double immunodiffusion technique described by Ouchterlony (23) was used. Petri dishes (5.5 cm in diameter) contained 7 ml of the following mixture: 1% Agarose, 0.9% NaCl, 5% borate buffer (0.61% boric acid, 0.95% sodium tetraborate, and 0.44% NaCl, pH 8.5), and 0.01% trypan blue. The antibody and antigen solutions were placed in wells with a 30-μl capacity, and the Petri dishes were left at room temperature for the development of precipitin bands. When the anti-dopamine β-hydroxylase serum was tested against Golgi and chromaffin granule membranes, the membranes were extracted for 30 min with Lubrol PX (15 mg of detergent per milligram of membrane protein). The preparations were then centrifuged at 100,000 $\times$ *g*

[3] The abbreviation used is: SDS, sodium dodecyl sulfate.

for 60 min (24), and the supernatants thus obtained were used in the immunodiffusion tests.

Chemicals. The chemicals were obtained from the following sources: *N*-acetylglucosamine, 2-mercaptoethanol, AMP, ATP, and bovine serum albumin, from Sigma Chemical Company; acrylamide and *N,N'*-methylenebisacrylamide, from Eastman Kodak; Lubrol PX from CIL Chemicals; agarose, from Behring; and [*G*-^{3}H]tyramine hydrochloride and uridine diphospho[*U*-^{14}C]galactose, from New England Nuclear. [γ-^{32}P]ATP was synthesized in our laboratory as previously described (16).

RESULTS

Distribution of marker enzymes in Golgi and chromaffin granule membranes. As we have previously described, galactosyltransferase is a suitable marker enzyme for the Golgi apparatus of the adrenal medulla, since it is concentrated mainly in a fraction with the morphological characteristics of the Golgi apparatus (10, 11). The Golgi-rich fraction prepared by our procedure had a galactosyltransferase activity of 149 nmoles of galactose transferred per milligram of protein per hour (Table 1). This contrasts with the activity of 0.6 nmole/mg of protein per hour detected in chromaffin granule preparations (Table 1). The enzyme was detected in 18 of the 22 granule preparations tested. When the Golgi and chromaffin granule membranes were examined for their content of 5'-nucleotidase, marked differences were also observed. The specific activity of this enzyme was 3460 times greater in Golgi membranes than in granule membranes.

Dopamine β-hydroxylase is a suitable marker for chromaffin granule membranes; indeed, these structures show the highest enzyme specific activity among the different subcellular organelles of the adrenal medulla (25). Dopamine β-hydroxylase was also detected, both chemically and immunologically, in the membranes prepared from the Golgi-rich fraction. The double immunodiffusion test performed with both granule and Golgi membrane proteins solubilized by Lubrol PX showed a single precipitin line when allowed to react against rabbit anti-dopamine β-hydroxylase serum (Fig. 2). Furthermore, both precipitin lines fused, indicating immunological identity (Fig. 2). The specific activity of dopamine β-hydroxylase in Golgi membranes was $^{1}/_{12}$ of that observed in chromaffin granule membranes (Table 1). The subcellular distributions of galactosyltransferase and dopamine β-hydroxylase were also studied. Figure 3 shows the distribution of these two subcellular markers after resolution of the "crude Golgi preparation." This fraction contained, in addition to Golgi structures, smooth endoplasmic reticulum and some mitochondria and granule membranes. The procedure followed in the isolation and further resolution of the crude Golgi preparation is shown in Fig. 1 and has been described in detail in a previous paper (11). Two main bands (Golgi and endoplasmic reticulum), separated by an intermediate zone, were thus obtained (Fig. 3). The largest percentage of galactosyltransferase was, as expected (10, 11), recovered in the top layer of the gradient, a fraction which represents the final Golgi preparation. However, the distribution of glucose

TABLE 1

Specific activity of marker enzymes

Samples of Golgi and chromaffin granule membranes were assayed for the enzyme activities as described in MATERIALS AND METHODS. Galactosyltransferase, 5'-nucleotidase, and dopamine β-hydroxylase activities are expressed in terms of galactose transferred, P_i released, and octopamine formed, respectively. Values are the means and standard errors of the number of experiments shown in parentheses.

Fraction	Galactosyltransferase	5'-Nucleotidase	Dopamine β-hydroxylase[a]
	nmoles product/mg protein/hr		
Golgi	149 ± 7 (16)	2080 ± 60 (6)	4.9 ± 0.2 (6)
Chromaffin granules	0.61 ± 0.11 (22)	0.60 ± 0.24 (5)	60.1 ± 1.7 (8)

[a] Data from Trifaró and Duerr (11).

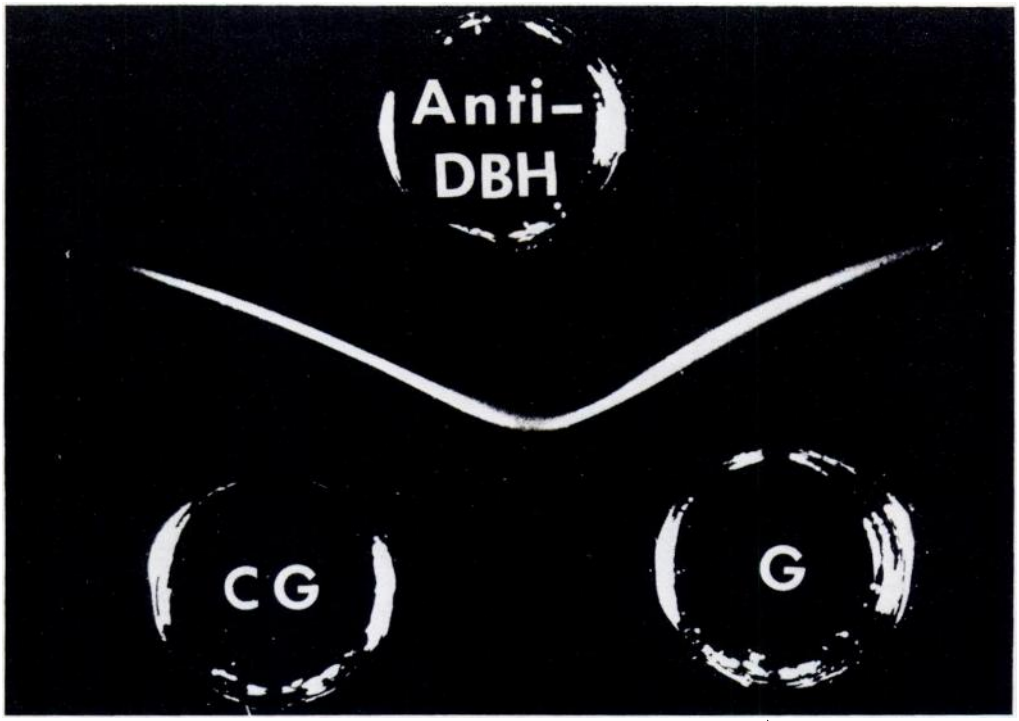

FIG. 2. *Double immunodiffusion test between anti-dopamine β-hydroxylase serum and membrane proteins of Golgi and chromaffin granules*

Membrane proteins from Golgi and chromaffin granules were extracted with Lubrol PX as indicated under MATERIALS AND METHODS. Twenty-five microliters of rabbit anti-dopamine β-hydroxylase serum were placed in the top well, and equal volumes of Lubrol-extracted membrane proteins from Golgi (G; 60 μg of protein) and chromaffin granules (CG; 12 μg of protein) were placed in the bottom wells. The plate was left at room temperature for 48 hr before the photograph was taken. In both cases there were single precipitin bands which fused completely in the central area, forming the so-called "reaction of complete identity."

6-phosphatase, a marker for the smooth endoplasmic reticulum, was quite different (11). The specific activity of glucose 6-phosphatase in the endoplasmic reticulum fraction was 10 times greater than in the Golgi fraction (11). The greatest amount of dopamine β-hydroxylase was contained in the intermediate zone of the gradient (Fig. 3). When the specific activities of these enzymes were plotted, differences in their subcellular distributions were also observed (Fig. 3). The Golgi layer showed the highest dopamine β-hydroxylase specific activity (Fig. 3).

ATPase activities. The membranes of different subcellular organelles of the adrenal medulla, as well as other tissues, contain ATPases which are involved in diverse energy-dependent processes. The chromaffin granule membrane is especially rich in Mg^{2+}-dependent ATPase (16, 26), and under certain conditions it is possible to demonstrate transphosphorylation from ATP to granule membranes (16, 27, 28). Therefore both Golgi and granule membranes were tested for their ATPase activities. Mg^{2+}- and Ca^{2+}-dependent ATPase activities were greater in Golgi than in chromaffin granule membranes (Table 2). However, transphosphorylation from ATP, a process which is Mg^{2+}-dependent (27), was 16 times greater in the granule membranes than in the Golgi membranes.

Cholesterol and phospholipid content. Both types of membranes were also compared for their lipid content. Chromaffin granule membranes were the richest in cholesterol (Table 3), and, as expected from this finding, the molar ratio of cholesterol to phospholipid was higher in granule than in Golgi membranes (Table 3). The phospholipid content, although higher

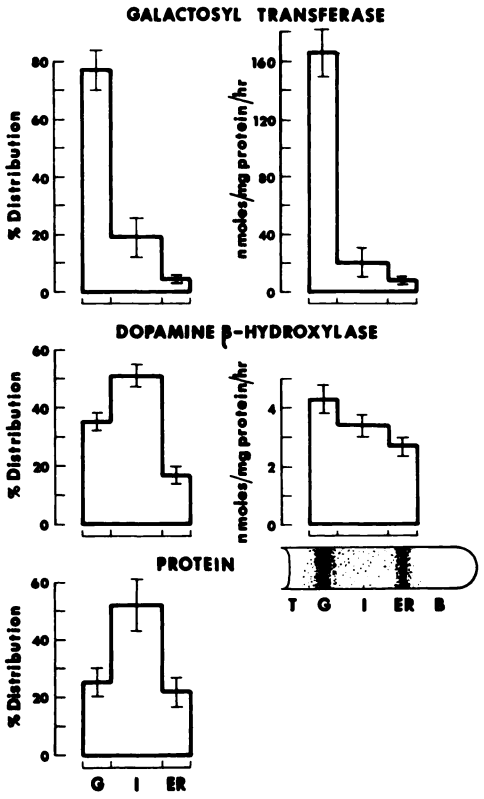

FIG. 3. *Subcellular distribution of galactosyltransferase and dopamine β-hydroxylase*

The "crude Golgi fraction" was prepared as described previously (10, 11), following the protocol shown in Fig. 1, and adjusted to 1.1 M by adding sucrose; this was measured with the aid of a refractometer (11). The suspension (10 ml) was layered on top of a discontinuous sucrose gradient of the following composition (from top to bottom): 6 ml, 6 ml and 8 ml of 1.25, 1.3, and 1.4 M sucrose (pH 7.0). Then 6 ml of 0.5 M sucrose were layered on top of the preparation. The tube was centrifuged at 100,000 × *g* for 90 min. Five fractions (top, T; Golgi, G; intermediate, I; endoplasmic reticulum; ER; bottom, B) were separated. Fractions G, I, and ER were assayed for protein, galactosyltransferase, and dopamine β-hydroxylase. Fractions T and B contained no galactosyltransferase or dopamine β-hydroxylase activity. The specific activities of galactosyltransferase and dopamine β-hydroxylase are expressed as nanomoles of galactose transferred or octopamine formed per milligram of protein per hour, respectively. The values represent the means and standard errors of six different experiments.

in granule membranes, was not significantly different from the values found for the Golgi membranes. However, granule membranes showed the usual (13%) large content of lysolecithin, in contrast to the low values (3%) observed in Golgi membranes (Table 3).

Polyacrylamide gel electrophoresis of membrane proteins. Electron microscopic studies of the Golgi-rich fraction prepared by our method revealed the presence of platelike center regions, an extensive system of peripheral tubules, and vesicles (10, 11). The small diameter of these structures would suggest a large ratio between membrane and soluble proteins. Hypotonic lysis of these structures released 27.3% ± 1.6% (n = 18) of the total protein, a figure which is smaller than that obtained for chromaffin granule soluble proteins (71.5% ± 1.7%, n = 29). Proteins from both membrane preparations were subjected to gel electrophoresis following the technique described by Weber and Osborn (21), using SDS as a solubilizing agent. The results showed 6–8 and 16–18 bands of different electrophoretic mobilities for the granule and Golgi proteins, respectively (Fig. 4). When the electrophoretic bands of these two kinds of membranes were compared, there were at least two components present in both preparations: first, Golgi membranes showed a small band of mobility similar to that of granule dopamine β-hydroxylase (component A, Fig. 4); second, another band (component C) was always present on electrophoresis of granule membrane proteins, which was highly concentrated in the Golgi membranes (Fig. 4). Similar results were obtained when the SDS-extracted membrane proteins were separated by the method of Bartlett and Smith (29), or when membrane proteins were solubilized with Lubrol PX (24) and electrophoresis was carried out following the method described by Davis (22). Furthermore, using the method of Barlett and Smith (29), it was also possible to observe that component A of the Golgi proteins was formed by two bands of very close electrophoretic mobility. One of these two bands was probably dopamine β-hydroxylase.

DISCUSSION

The results described in this paper indicate that there are marked biochemical differences between the membranes of the

TABLE 2

Ca^{2+}- and Mg^{2+}-dependent ATPase activities and transphosphorylation from ATP to Golgi and chromaffin granule membranes

Samples (200 μg of protein) of Golgi and chromaffin granule membranes were assayed for ATPase and transphosphorylation activities as described in MATERIALS AND METHODS. Values are the means and standard errors of the number of experiments shown in parentheses.

Fraction	Ca^{2+}-ATPase	Mg^{2+}-ATPase	Transphosphorylation from ATP
	μmoles P_i/mg protein/hr		*nmoles P_i/mg protein/hr*
Golgi	4.4 ± 0.4 (6)	7.4 ± 0.8 (6)	2.2 ± 0.6 (10)
Chromaffin granules	0.8 ± 0.2 (6)	3.9 ± 0.4 (6)	34.5 ± 4.5 (10)

TABLE 3

Cholesterol and phosholipid content in Golgi and chromaffin granule membranes

Cholesterol and phospholipids were extracted and measured as described in MATERIALS AND METHODS. Aliquots of the phospholipid extract were further resolved by thin-layer chromatography. The lysolecithin spot was identified, eluted, and assayed as described in the text. Values are the means and standard errors of six experiments.

Fraction	Cholesterol	Phospholipid	Ratio of cholesterol to phospholipid	Lysolecithin	
	μmoles/mg protein	*μmoles/mg protein*		*μmole/mg protein*	*% total phospholipid*
Golgi	0.48 ± 0.08	1.17 ± 0.23	0.42 ± 0.02	0.036 ± 0.003	3.1 ± 0.3
Chromaffin granules	0.92 ± 0.07	1.57 ± 0.21	0.58 ± 0.04	0.212 ± 0.028	13.4 ± 1.9

Golgi apparatus and those of the chromaffin granules.

Galactosyltransferase has been previously used as a marker for Golgi membranes in subcellular fractionation studies carried out in testes, liver, and hypophysis (30–33). Furthermore, work from our laboratory has shown that this enzyme also has a high specific activity in the Golgi-rich fraction isolated from the adrenal medulla (10, 11). In contrast, little galactosyltransferase activity was detected in some of the granule preparations. This activity could occur if the granules were contaminated less than 0.4% with Golgi membranes. Therefore the granules seem to have no significant indigenous galactosyltransferase. Similarly, there were only traces of 5′-nucleotidase in chromaffin granule membranes. There is now enough evidence to believe that 5′-nucleotidase, an enzyme used by many investigators as a marker for plasma membranes, is also a conspicuous component of the Golgi membranes (11, 34).

The Golgi-rich fraction of the adrenal medulla showed the presence of a small amount of dopamine β-hydroxylase, as indicated by the electrophoretic, immunological, and chemical techniques used. However, it remains to be determined whether or not this enzyme is a true component of the Golgi membrane. The different patterns of distribution in the density gradient between dopamine β-hydroxylase and galactosyltransferase indicate that the presence of dopamine β-hydroxylase in Golgi membranes may be due, at least in part, to contamination with chromaffin granule membranes. However, all the above experiments cannot rule out the possibility that a small amount of dopamine β-hydroxylase is indigenous to Golgi membranes, especially if the granules originate in the Golgi apparatus. Furthermore, it should be remembered that dopamine β-hydroxylase is a glycoprotein (35, 36) and that the Golgi complex is the place where the glycosidation reactions necessary for the synthesis of glycoproteins occur (37). Immunohistochemical studies carried out on intact medullae may indicate whether or not dopamine β-hydroxylase is a conspicuous component of Golgi membranes,

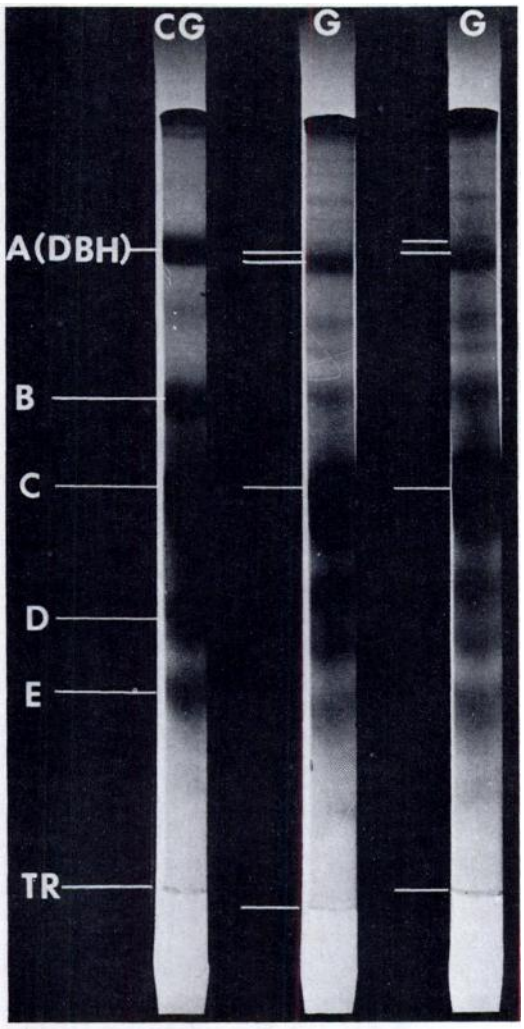

FIG. 4. *Polyacrylamide gel electrophoresis of Golgi (G) and chromaffin granule (CG) membranes*

Aliquots from granule (175 μg of protein) and Golgi (175 and 210 μg of protein for the middle and right gels, respectively) membrane proteins solubilized by SDS were applied to 6% polyacrylamide gels containing 0.1% SDS. Electrophoresis was performed as described in MATERIALS AND METHODS. The direction of migration was from top to bottom, which was the anode. TR indicates the position of the tracking rings.

and these studies are presently being performed in our laboratory.

Golgi membranes have the larger Mg^{2+}-dependent ATPase activity of the two types of membranes compared. However, transphosphorylation from ATP, a Mg^{2+}-dependent activity, was 16 times greater in the granule than in the Golgi membranes. Another difference in the ATP-cleaving activity of these two membranes was the presence in the Golgi fraction of greater Ca^{2+}-dependent ATPase activity. We do not know the role of these enzymes in the Golgi membrane of the adrenal medulla. However, this is not a unique finding, since Ca^{2+}-ATPase has been reported in Golgi fractions isolated from another tissue (38).

When the lipid contents of the two membranes were compared, it was found that the total phospholipid content of the Golgi membranes, although somewhat smaller than that found in granule membranes, was similar to the content reported for the Golgi membranes isolated from liver (39). In agreement with previous observations from our and other laboratories (25, 40), the content of lysolecithin in chromaffin granules was very high. In contrast, the concentration of this phospholipid in Golgi membranes was 25% of that found in granule membranes; the content of lysolecithin in the Golgi membranes from the adrenal medulla is similar to that reported for the Golgi preparations isolated from liver (41). Moreover, the concentration of lysolecithin in the Golgi membranes is a little higher than in mitochondria and microsomal membranes (42), but lower than that reported for plasma membranes (43). The other lipid component measured was cholesterol, and the granule membranes were the richest in this lipid. Consequently the molar ratio of cholesterol to phospholipid was greater in granule membranes than in Golgi membranes.

Treatment of the Golgi and granule preparations with hypotonic solutions released 2.6 times more soluble protein from the granules than from the Golgi structures. This difference is probably due to the large diameter of the chromaffin granules compared with the vesicles and tubules of the Golgi-rich fraction (11). The larger the diameter of the vesicles, the greater should be the ratio of soluble to membrane proteins. SDS-polyacrylamide gel electrophoresis of the granule membrane proteins revealed a pattern of bands

similar to that previously described (24, 44). Furthermore, the use of gels containing either SDS or Lubrol PX showed the presence of six to eight bands, components A, B, and C being the three main bands. Component C, which was highly concentrated in Golgi membranes, was detected using three different electrophoretic techniques in both granule and Golgi preparations. The presence of component C in Golgi membranes was not due to contamination of the Golgi structures by granule membranes. If this had been the case, large amounts of lysolecithin and dopamine β-hydroxylase would have been detected in Golgi membranes. Therefore component C may represent a protein indigenous to Golgi membranes. It is not yet known whether component C is a structural protein common not only to both types of structures but to other organelles of the adrenal medulla as well.

In order to understand the steps involved in the secretory process, a question related to the biogenesis of the chromaffin granules should be answered: Are chromaffin granules derived from the Golgi apparatus? If they are, and because our results showed marked biochemical differences between the two types of membranes, the possibility exists that the granules originate from the Golgi by a specific process of "membrane flow and membrane differentiation." If this were the case, the cell would have to possess the information and the tools to introduce or to remove a new protein, lipid, or enzyme from the membranes during the process of membrane flow. Such an idea would explain the absence of galactosyltransferase and 5'-nucleotidase and the presence of large amouns of cholesterol, lysolecithin, and dopamine β-hydroxylase in granule membranes. Furthermore, some proteins (structural proteins), for example, component C, would remain intact during this process of membrane differentiation and serve as the backbone for the membranes. There is yet another possibility; that is, that granules are formed in the Golgi apparatus, but very early in the development of the cell, and that their membranes, which may have a very low turnover, are reused many times during the secretory cycle. In such a case the Golgi apparatus would have specialized in the mature cell and would be involved in glycosidation of the cellular products, for example, dopamine β-hydroxylase, which would be released upon stimulation. Then synthesis *de novo* of granule membranes might occur only in cases of strong, sustained adrenal medullary stimulation, as during insulin shock. Thus the increase over the control values in the content of dopamine β-hydroxylase in the adrenal medulla during the recovery period which follows an insulin shock (45) could be interpreted as synthesis *de novo* of storage vesicles.

In conclusion, our results, showing marked biochemical differences between the membranes of the Golgi complex and those of the chromaffin granles, suggest that, if chromaffin granule membranes are derived from the Golgi complex, the granule membranes must undergo significant biochemical changes and that an important and very selective cellular mechanism (membrane differentiation) might control and regulate these changes.

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