LOCALIZATION AND CHARACTERIZATION OF CARBOHYDRATES IN ADRENAL MEDULLARY CELLS

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ABSTRACT

The localization and characterization of carbohydrates in adrenal medullary cells were studied by histochemical and cytochemical methods. Adrenaline (A)- and noradrenaline (N)-storing granules were argentaphobic when ultrathin sections of Araldite-embedded medullae were stained according to the periodic acid-thiocarbohydrazide-silver proteinate technique of Thiéry. A small amount of glycogen in the form of single β-particles as well as lysosomes were, however, visualized by this technique. The entire core of the A granules was markedly positive after ultrathin sections of glutaraldehyde-fixed, glycol methacrylate (GMA)-embedded medullae were stained with phosphotungstic acid (PTA) at a low pH (0.3). The N granules, in contrast, were mostly unreactive. In the A cells, PTA stained a large part of the Golgi complex, whereas in the N cells the Golgi complex was mostly unstained. In both cell types, the cell coat, lysosomes, and multivesicular bodies reacted to PTA. The periodic acid-Schiff (PAS) technique showed A but not N granules in semithin sections of GMA- or Araldite-embedded medullae. The PTA and PAS stains were abolished by acetylation, restored by saponification, unchanged by methylation, and greatly diminished by sulfation. In ultrathin sections of GMA- or Araldite-embedded medullae incubated with colloidal iron according to various techniques, the cell coat and lysosomes of both cell types were stained, unlike all the other cytoplasmic organelles. These results indicate that A granules and the Golgi complex of A cells, unlike the same structures in N cells, are rich in glycoproteins which are probably not acidic.

The presence of a small amount of glycogen in adrenal medullary cells has been established both biochemically (41, 66) and morphologically (14). Very little attention, however, has been paid to the possibility that more complex carbohydrates also may exist in these cells. The discovery of 0.5% glucosamine by weight, 1.1% galactosamine by weight, and 2% neutral sugars by weight (orcinol reaction) in bovine adrenal medullary granules has suggested that the protein characteristic of the granules is, in fact, a glycoprotein (59). However, interpretation of the results obtained on adrenal medullary cells with the periodic acid-Schiff (PAS) technique differs among investigators. Lillie (32) has reported that the granules react positively to PAS after chromate fixation. Pearse (43) and Coupland (9, 11), however, have stated that the PAS reaction is negative in adrenal medullary cells. Hopwood (22) attributed the weak PAS positivity of these cells (cryostat sections) to PAS-positive lipids and proteins. Other workers interpreted the PAS reaction as being due to either glycogen (27, 40) or catecholamines (19). The aim of the present study was to investigate, by various
cytochemical and histochemical methods, the localization and characteristics of glycogen and complex sugars in adrenal medullary cells.

MATERIALS AND METHODS

Female Sprague-Dawley rats (200-250 g) were used for the light and electron microscope studies, and female hamsters (100-150 g) from the Lakeview Hamster Colony, Newfield, N. J., for the light microscope investigations.

Electron Microscopy

In most animals the adrenals were perfused with fixatives while in some they were fixed by immersion. In the former case, the lower thoracic aorta was cannulated under ether anesthesia and the adrenals were perfused first with 20-40 ml of Locke-Ringer solution and then either with 2% glutaraldehyde buffered with cacodylate-HCl (0.1 M at pH 7.1) or 10% formaldehyde (prepared from paraformal) containing 2% calcium acetate (38). In the latter case, small fragments of medullae were rapidly cut and put into 2% osmium tetroxide (OsO₄) buffered with S-collidine (5). All the specimens were washed for three 15-min periods in cacodylate buffer to which 2% sucrose had been added. They were then left in the buffer for 12 h. The hamsters were treated similarly but their adrenals were perfused with glutaraldehyde only. Postfixation for 1 h in 2% OsO₄ buffered with Veronal acetate was done only with some fragments of rat or hamster medullae which had been previously fixed with glutaraldehyde. Some fragments of rat or hamster medullae fixed with glutaraldehyde were not postosmicated.

Only the specimens fixed in glutaraldehyde alone were embedded in glycol methacrylate (GMA) as already described (7), i.e., according to the method of Rosenberg et al. (53) as modified by Leduc and Bernhard (31). All the other specimens were embedded in Araldite. For routine electron microscopy, ultrathin sections of either Araldite- or GMA-embedded medullae were cut on an LKB ultramicrotome with glass knives and stained with uranyl acetate and lead citrate (7, 25).

Cytochemistry

Thiery's Method: The periodic acid-thiocarbohydrazide-silver proteinate method of Thiéry (63, 64) was used for the demonstration of periodate-reactive vicinal glycols on tissues fixed either in glutaraldehyde alone, or in combination with OsO₄, and embedded in Araldite as already described (7, 25).

Low pH Phosphotungstic Acid (PTA)-Hydrochloric Acid Method: This technique (34, 44, 48) was used for the visualization of hydroxyl groups in complex carbohydrates. Ultrathin (silver to gray) sections from glutaraldehyde-fixed, GMA-embedded tissues were floated on a 1% solution of PTA in 1 N HCl (pH 0.3) for 8-10 min, then rapidly washed in distilled water (7, 25).

Triple Staining: Some of the GMA-embedded tissues were sequentially stained, i.e., after PTA staining as above, they were examined under a Philips 200 electron microscope and counterstained with uranyl acetate and lead citrate, as described elsewhere (25, 26).

Colloidal Iron (CI) Method: Three variants of this technique were used for the detection of acidic groups in complex carbohydrates. In the first variant, ultrathin sections of GMA-embedded medullae were floated for 1½-3 h, as described previously (25, 30, 37), on a dialyzed CI solution, prepared according to the method of Rinehart and Abul-Haj (52).

In the second variant, the CI solution was prepared according to the method of Curran et al. (15). Small fragments (less than 1 mm³) of adrenal medullae, fixed in glutaraldehyde, in OsO₄, or in Formal-calcium, were placed in the iron solution at pH 1.6 and constantly agitated for 72 h (42) at room temperature. The samples were embedded in Araldite.

In the third variant (38, 67), fragments of adrenal medullae, fixed in glutaraldehyde, with OsO₄ or Formal-calcium, were cut at 30 µm, either in a cryostator with a Smith-Farquhar tissue sectioner, and stained for 30 min to 3 h at room temperature with dialyzed iron, prepared according to the method of Rinehart and Abul-Haj (52). After staining, the sections were embedded in Araldite. Controls for the second and third variants were incubated in a solution of 4 parts of 1.7 M aqueous magnesium chloride and 1 part of concentrated glacial acetic acid for 30 min to 3 h (38).

PAS Reaction in Semithin Sections: Semithin sections of glutaraldehyde-fixed hamster and rat medullae, embedded in GMA, were stained according to the PAS technique with or without diastase pretreatment.

FIGURE 1 Section of adrenal medulla fixed in glutaraldehyde and OsO₄, embedded in Araldite, and stained with uranyl acetate and lead citrate. A cells show a nucleus (N), mitochondria (m), a few cisternae of rough endoplasmic reticulum with ribosomes (RER), Golgi saccules and vesicles (G), a multivesicular body (MVB), and several granules (g) of variable electron opacity. The scale marker in this and subsequent electron micrographs equals 0.5 µm. × 14,000.

FIGURE 2 Section of adrenal medulla treated as in Fig. 1. N cells exhibit nucleus (N), mitochondria (m), lysosomes (L), residual bodies with lipid droplets (LI), and irregular, highly electron-opaque granules (g) with content occupying only a portion of the space delimited by their membrane. × 14,000.
Semifine sections of hamster and rat medullae, fixed in glutaraldehyde alone or in combination with OsO₄ and embedded in Araldite, were also stained in the same manner after removal of the plastic (35).

**CHEMICAL MODIFICATION OF THE REACTIVITY OF ADRENAL MEDULLARY GRANULES TO PTA OR PAS:** Small fragments (less than 1 mm³) of rat adrenal medullae, fixed in glutaraldehyde, were incubated for various periods in the presence of chemicals before being embedded in GMA. These specimens were constantly agitated during incubation. Semifine sections (1-2 μm) were mounted on glass slides and stained with PTA, as described above. The methods (acetylation, acetylation followed by saponification, methylation, and sulfation) that were employed have been described by McManus (36), Spicer et al. (62), and, as applied to electron microscopy, by Marinozzi (34) and Rambourg (49).

**IN VITRO STUDIES:** Tests were performed with aqueous solutions (1/1,000) of adrenaline bitartrate (K. & K. Laboratories, Inc., Cleveland, Ohio) and noradrenaline bitartrate (K. & K. Laboratories) to find out if the results obtained by staining tissues could be reproduced in vitro. For this purpose, the solutions were first mixed with 2% glutaraldehyde buffered with cacodylate-HCl (0.1 M at pH 7.1). As expected (68), a very fine, pale yellow precipitate formed rapidly in the noradrenaline but not in the adrenaline solution. No further change was produced in the noradrenaline-glutaraldehyde solution by mixing it with 1% PTA in 1 N HCl (pH 0.3), but a fine, brown precipitate appeared in the adrenaline solution. C1, prepared according to the method of Curran et al. (15) or Rinehart and Abul-Haj (52), induced a heavy, brown precipitate in both catecholamine-glutaraldehyde solutions. The same effect was noted when adrenaline or noradrenaline alone was mixed with either C1 preparation.

**RESULTS**

**Ultrastructural Morphology of Adrenal Medullary Granules**

Fine sections of glutaraldehyde- and OsO₄-fixed medullae, stained with uranyl acetate and lead citrate, revealed two types of cells. As already described in the rat (9, 10, 12, 16, 65), the majority were identified as adrenaline-storing cells (A cells) since they contained round granules of moderate and variable electron opacity with a finely granular core and an inconstant peripheral halo beneath their limiting membrane (Fig. 1). These granules were randomly distributed in the hyaloplasm but were less numerous in the paranuclear, Golgi zone. In contrast, the noradrenaline-storing cells (N cells) were characterized by granules with an extremely electron-opaque core which often occupied only a small portion of the space delimited by the limiting membrane (Fig. 2). Some of these cores were round, others ovoid or irregular, and still others crescent shaped.

**Ultrastructural Cytochemistry**

**A AND N GRANULES:** All the granules, whether of the A- or N-storing type, were argentaphobic when stained by Thiéry's method, even after 5 days of exposure to thiocarbohydrazide (Table I). The A granules, after fixation in glutaraldehyde alone or in glutaraldehyde and OsO₄, were of moderate density. The N granules, in contrast, were highly electron-opaque, irrespective of postfixation in OsO₄. This electron opacity was inherent to the granules since it was observed after fixation in glutaraldehyde alone, without any staining, and was increased by OsO₄ postfixation. With Thiéry's method, the size and shape of both types of granules were similar to those noted after staining with uranyl acetate and lead citrate (Figs. 3 and 4). The A granules always reacted positively to PTA (Figs. 5 and 7): they appeared as round, uniformly dense structures, some of which were intensely stained while others were less so. They contrasted with the pale, unstained background of hyaloplasm and mitochondria. Some of the N granules were totally unstained, others showed only a very weak reaction, but a few paranuclear granules were moderately stained.

**FIGURE 3** Section of adrenal medulla fixed in glutaraldehyde and OsO₄, embedded in Araldite, and stained according to Thiéry's technique (float on thiocarbohydrazide for 24 h). Fine, highly electron-opaque particles of glycogen are scattered between unstained organelles: nucleus (N), mitochondria (m), and A granules (g). The cell coat on the plasmalemma (arrow) and on the surface of an endothelial cell (double arrow) shows no reaction. × 11,000.

**FIGURE 4** Section of several N cells treated as in Fig. 3. Electron-opaque glycogen particles are as scarce as in Fig. 3. The nucleus (N), mitochondria (m), and cell coat on the plasmalemma (arrow) are unstained. The N granules (g) show inherent electron opacity and no staining reaction. × 9,000.
# Table 1

**Cytochemistry and Histochemistry of Adrenal Medulla**

| Embedding | Fixative | Staining | A* | N | A | N | A | N | A | N | A | N |
|-----------|----------|----------|----|---|---|---|---|---|---|---|---|---|
| A. Cytochemistry of rat adrenal medulla | | | | | | | | | | | | |
| Araldite | Glutaraldehyde, OsO₄ | Periodic acid, thio-carboxydradize (TCH), silver proteinate | 0 | 0 | +++ | +++ | 0 | 0 | 0 | 0 | 0 | 0 | +++ | +++ |
| Araldite | Glutaraldehyde, OsO₄ | TCH, silver proteinate | 0 | 0 | +++ | +++ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Araldite | Glutaraldehyde, OsO₄ | Periodic acid, silver proteinate | 0 | 0 | 0 to + | 0 to + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Araldite | Glutaraldehyde | Periodic acid, TCH, silver proteinate | 0 | 0 | ++ | ++ | 0 | 0 | 0 | 0 | 0 | 0 | +++ | +++ |
| Glycol meth-acrylate | Glutaraldehyde | PTA-hydrochloric-acid (pH 0.3) | ++ to +++ | 0 to + | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ | 0 | 0 |
| Glycol meth-acrylate | Glutaraldehyde | Dialyzed CI (pH 1.6) | 0 | 0 | + | + | 0 | 0 | 0 | 0 | ++ | ++ | 0 | 0 |
| Araldite | Glutaraldehyde§ | Dialyzed CI (pH 1.6) | 0 | 0 | ++ | ++ | 0 | 0 | 0 | 0 | ++ | ++ | 0 | 0 |
| B. Histochemistry of semithin sections of rat and hamster adrenal medulla (PAS) | | | | | | | | | | | | |
| Glycol meth-acrylate | Glutaraldehyde | PAS | ++ | | | | | | | | | | | |
| Glycol meth-acrylate | Glutaraldehyde | Diastase plus PAS | ++ | | | | | | | | | | | |
| Araldite | Glutaraldehyde | PAS | ++ | | | | | | | | | | | |
| Araldite | Glutaraldehyde | Diastase plus PAS | ++ | | | | | | | | | | | |

+ = weak; ++ = moderate; +++ = strong reaction.
*A, adrenaline-storing cell; N, noradrenaline-storing cell.
† Ultrathin sections were floated for 3 h on a dialyzed CI solution.
§ Sections were cut with a Smith-Farquhar tissue sectioner and stained with dialyzed CI.
FIGURE 5  Section fixed in glutaraldehyde, embedded in glycol methacrylate, and stained with PTA-hydrochloric acid. Several A cells show nuclei (N), densely stained granules, lysosomes (L), and a cell coat. Whenever two cells are close to one another, their cell coats seem to fuse. The mitochondria (m) are unstained. × 7,000.

FIGURE 6  Part of an N cell, treated as in Fig. 5, showing nucleus (N) and granules (g). Some of the granules (arrow) are weakly positive. Only a few sacculles and vesicles of the Golgi complex (G) are stained. × 25,000.
granules, in close proximity to the Golgi complex, reacted as intensely as the A granules (Figs. 6 and 8). To eliminate any possibility of mistaking one type of cell for the other, some sections were sequentially stained with PTA (viewed through the electron microscope) and then with uranyl acetate and lead citrate (triple staining). Under these conditions, the A granules retained the shape and electron opacity they had displayed when exposed to PTA alone (Fig. 9), whereas the N granules exhibited their characteristic dense, irregular core (Fig. 10), thus permitting easy differentiation between the two cell types. In GMA-embedded material, neither A nor N granules showed any inherent opacity. They were not stained by any of the CI preparations (Figs. 11–14), but the N granules showed, after glutaraldehyde fixation, the usual inherent opacity characteristic of Araldite embedding (Fig. 11).

LYSOSOMES, RESIDUAL BODIES, AND MULTIVESICULAR BODIES: In both A and N cells, the lysosomes were intensely silver positive as were the nonlipidic portions of the residual bodies. The multivesicular bodies were unstained by Thierry’s technique, whereas lysosomes and residual bodies were moderately reactive after exposure to silver proteinate alone. All of these structures were positively stained by the PTA technique. Some lysosomes were characterized by a more or less complete rim of densely stained material in an unstained core (Fig. 5); other lysosomes, presumably residual bodies, showed lightly stained, irregular areas with interlying, presumably lipidic, unstained zones. The multivesicular bodies characteristically displayed a markedly electron-opaque matrix with clear vesicles (Fig. 7). Some of these structures were also stained by all three CI techniques and every fixation procedure. With regard to the intensity of iron deposition and ultrastructural preservation, the best results were obtained in

FIGURE 7  Section of an A cell treated as in Fig. 5. An extensively stained Golgi complex shows a stepwise increase in staining intensity from the paranuclear (N) to the paragranular (g) face. Multivesicular body (MVB). × 41,000.

FIGURE 8  Paranuclear (N) area of an N cell treated as in Fig. 5. A few stained granules (g) are visible. Lysosomes (L) × 51,000.
FIGURE 9  Section of an A cell sequentially stained by the PTA method, uranyl acetate, and lead citrate. The structures normally stained by PTA, such as the granules (g) and Golgi complex (G), are visible as are those usually stained by uranyl and lead, such as the nucleus and mitochondria (m) $\times$ 35,000.

FIGURE 10  Section of an N cell stained sequentially as in Fig. 9. The N granules (g) with their irregular contours are now clearly visible. The mitochondria (m) and cell coat (arrow) are also stained $\times$ 13,000.
TABLE II
Chemical Modification of the PAS and PTA Reactivity of Adrenal Medullary Granules

| Treatment*         | PAS positivity (light microscopy) | PTA positivity (electron microscopy) |
|--------------------|----------------------------------|--------------------------------------|
|                    | A granules | N granules | A granules | N granules |
| Control            | ++         | 0          | ++ to +++  | 0 to +     |
| Acetylation        | 0          | 0          | 0          | 0          |
| Acetylation plus saponification | ++         | 0          | ++ to +++  | 0 to +     |
| Methylation        | ++ to +++  | 0 to +     | ++ to +++  | 0 to +     |
| Sulfation          | +          | 0          | +          | 0          |

+, weak; ++, moderate; ++++, strong reaction.

* Small fragments of adrenal medullae were exposed to the various treatments before being embedded in glycerol methacrylate. Semithin sections were then stained according to the PAS technique, while ultrathin sections were stained with PTA-hydrochloric acid.

tissues fixed in glutaraldehyde, cut with a Smith-Farquhar tissue sectioner, and exposed to dialyzed Cl. In lysosomes, iron was deposited irregularly in the core. In presumptive residual bodies, only irregular, presumably nonlipidic areas showed iron deposits. Iron deposition was more intense in the lysosomes (Fig. 14) after OsO₄ fixation but the ultrastructure, particularly of the granules, was not well preserved.

CELL COAT: The surface or cell coat was barely visible after Araldite-embedded tissues were stained with uranyl acetate and lead citrate (Figs. 1 and 2) or according to the Thiéry technique (Fig. 3) with 5 days of exposure to thiocarbohydrazide. However, it was effectively shown by PTA as a dark, very narrow line apposed on the external surface of each cell. Apparent fusion of the cell coats occurred whenever two cell surfaces came close to each other (Fig. 5). Membrane interdigitations were frequent between cells of the same type but were never observed between different kinds of cells. A cell coat was also visible on the luminal surface of capillary endothelial cells. The best results as regards staining with CI were again obtained after glutaraldehyde fixation, in tissues cut with the Smith-Farquhar sectioner. The CI...
precipitate was generally better defined and more intense after Formol-calcium or OsO₄ fixation (Figs. 13 and 14). With both PTA and CI, the cell coat of endothelial cells was thicker and more electron opaque than that of the A or N cells (Figs. 11–14). The cell coat was not visible with any of the fixatives in control tissues incubated with magnesium chloride.

GOLGI COMPLEX: In Araldite-embedded adrenal medullae, stained according to Thiery's technique, the Golgi complexes appeared to be identical in both A and N cells although fewer granules were present in the Golgi area of the latter, and the Golgi zone of the former blended more intimately with the remainder of the cytoplasm. None of the Golgi components was reactive to this technique. PTA revealed large, well-structured, positively stained Golgi complexes in the A cells (Fig. 7) and only a few positively stained saccules in the N cells (Fig. 6). In the A cells the positively stained saccules and vesicles occupied a relatively large area of the paranuclear space. In several instances, a stepwise increase in the staining intensity could be seen, from the forming paranuclear face to the mature paragranular face (Fig. 7). This was not observed in the N cells.

GLYCOGEN: Monoparticulate or B-type glyco- gen could be demonstrated after only 30 min of exposure to thiocarbohydrazide. The granules were scanty, tiny, spherical, and distributed singly between the organelles (Figs. 3 and 4). They were not stained when the periodic acid or thiocarbohydrazide step was omitted. Since glycogen was extracted (49), there was no reaction to PTA.

SEMITHIN SECTIONS OF GMA-OR ARALDITE-EMBEDDED MEDULLAE STAINED ACCORDING TO THE PAS TECHNIQUE: Rat tissues, fixed in glutaraldehyde alone or postfixed in OsO₄ and embedded in Araldite, showed PAS-positive cells in the center of the medullae. Constantly PAS-negative cells formed a rim in a paracortical position. A similar situation was noted in semithin sections of glutaraldehyde-fixed, GMA-embedded hamster medullae.

CHEMICAL MODIFICATION OF THE REACTIVITY OF MEDULLARY CELLS TO PTA (ELECTRON MICROSCOPY) AND PAS (LIGHT MICROSCOPY): Acetylation almost completely prevented the staining of all cellular structures normally demonstrated by PTA (Fig. 16). Reactivity was fully restored by saponification after acetylation (Fig. 17). Methylation did not alter the density of various cellular organelles normally stained by PTA (Fig. 18). Sulfation markedly decreased but did not completely abolish the reaction of PTA. The reactivity of A and N cells to PAS followed exactly the same pattern: acetylation totally prevented staining by PAS which was restored by saponification; methylation slightly increased the PAS positivity of A cells while sulfation virtually abolished it (Table II).

DISCUSSION

Direct staining of hydroxyl groups in complex carbohydrates has been achieved, with a high degree of specificity, with PTA (at a low pH) in GMA-embedded sections of glutaraldehyde-fixed tissues (33, 34, 44, 45, 48, 50). Complete agree-
ment between the PTA and PAS methods has already been reported (45, 51, 59). However, other studies (47, 56, 57) have cast doubt on the validity of the PTA technique for the detection of complex carbohydrates and indicated that PTA is an anionic stain for certain positive groups of proteins. The fact that some polysaccharides (viz., glycogen) do not show up in PTA-stained sections would be an argument against the specificity of the staining reaction, no one, to our knowledge, has disproved that, in fine sections of glutaraldehyde-fixed, GMA-embedded tissues, or in tissue sections obtained by ultracryotomy, PTA at a low pH reveals complex carbohydrates which, light microscopically, are PAS positive. Glycogen, which is not stained because it is dissolved, is intensely reactive in fine sections obtained by ultracryotomy (2, 28).

It is extremely unlikely that the staining of A granules is due, as it is in vitro, to a precipitation reaction between PTA and adrenaline. Previous studies (13, 65, 69) have revealed that although noradrenaline is precipitated in the granules by glutaraldehyde, adrenaline is not. In fact, most of the medullary adrenaline can be recovered from the fixatives and alcohols used for dehydration (12, 13). As previously shown (60, 65), adrenaline reacts in vitro to ammoniacal silver and diazonium but, in sections, A granules are not stained by these substances because adrenaline escapes from them during and after fixation in glutaraldehyde and several other aldehydes. An unsubstituted amino group seems to be essential for an interaction between catecholamines and some aldehydes (1, 60). The precipitation of CI by both adrenaline and noradrenaline in vitro, with no staining of either A or N granules in tissue sections incubated with CI, also indicated total absence of a parallelism between in vitro and cellular reactivity. The fact that such procedures as acetylation, saponification, methylation, and sulfation influence not only the staining of A granules but also (and in an identical manner) that of other cellular structures (e.g., the cell coat, whose glycoprotein nature is well established) suggests that A granules possess a glycoprotein component which is reactive not only to PTA but also to PAS. The effects of these procedures on the PTA and PAS staining of various cell organelles are in agreement with previous findings (34, 36, 49, 62).

The weak PAS reaction of adrenal medullary cells in cryostat sections is perhaps due to the glycoprotein content of both types of cells. The absence of a positive PAS reaction after fixation in various agents and embedding in paraffin can be ascribed to a loss of their glycoprotein material under these conditions. It is well-known that the content of adrenal medullary granules is extremely fragile so that proteins are lost from chromaffin granules whenever they are exposed to a host of fixatives (21, 22). The conservation of proteins by glutaraldehyde is in keeping with the strong cross-linking effect of this fixative (23, 54).

The results obtained with Thierry's method have not been systematically compared with the PAS and PTA stains. According to Thierry (63, 64), these results depend upon the duration of exposure to thiocarbohydrazide: 30–40 min are sufficient for glycogen, 24–48 h are adequate for mucopolysaccharides, and 48–72 h are suitable for glycoproteins. This technique, however, appears to be much less sensitive than the PTA method: special tubuloreticular structures observed in certain lymphocytes are silver negative when stained according to Thierry's technique but they also react to PTA (55); the cell coat in rat (25) and human (24) myocardium is stained by the latter but not by the former method. Moreover, the silver positivity of N granules, when exposed to ammoniacal silver (6) or silver methenamine (8), is not due to their glycoprotein content. Nevertheless, in agreement with previous studies, Thierry's technique reveals that glycoprotein is scarce in the adrenal medulla (14) and cannot possibly account for the selective PAS positivity of A cells. Some of the PTA and PAS positivity of adrenal medullary cells could be due to the acid phosphatase present in these granules (20). Although acid phosphatase has not been purified from adrenal medullary cells, it may be assumed, from other studies (17, 29), to be a type of glycoprotein.

The presence of acidic groups in the complex carbohydrates of the cell coat, lysosomes, and residual bodies of adrenal medullary cells is in agreement with similar findings in other cell types (15, 30, 50, 61, 67). The technique best suited for ultrastructural preservation, at least in our study, was the one in which tissues fixed in glutaraldehyde were cut with a Smith-Farquhar sectioner and exposed to dialyzed CI. As in platelets (38), the reactivity of lysosomes was enhanced by OsO₄ fixation. The absence of a reaction to CI by A and N granules as well as by the Golgi complex of both types of cells does not necessarily imply a total lack of acidic groups in the glycoproteins of...
adrenal medullary cells. It might be due to an acidic content that is too low to be detected by the CI technique. In fact, this is probably the case with the Golgi complex which is known to synthesize acidic carbohydrates in several cell types (50, 61).

Electron microscope, autoradiography, and cytochemistry studies on different cells have demonstrated that the Golgi complex (part of which is evidenced by appropriate stains for glycoproteins) is involved in the synthesis of complex carbohydrates later found in the cell coat (3), lysosomal system (4), and secretory products (18, 39, 46). The same mechanism probably applies to adrenal medullary A and N cells, as judged by the relative size of their PTA-positive Golgi complexes. When glycoproteins are present in large quantities in A granules, the Golgi area which is stained by PTA (and presumably is active in the synthesis of glycoproteins) is much larger than in N cells where the granules are sparsely and irregularly stained. The presence of stained paranuclear granules in N cells raises the possibility that relatively immature granules might contain more glycoprotein material than mature ones.

The results of this investigation are in agreement with previous biochemical studies (59) which, as already stated, showed that chromaffin granules contain 2% of neutral sugars. Since these biochemical analyses were performed on a mixture of A and N granules, our results indicate that A granules may have close to 4% of neutral sugars. Whether the presence of a carbohydrate-protein complex plays a role in the uptake, binding, and release of adrenaline remains to be determined. In any event, our results reveal a simple and reliable method to differentiate between A and N cells at the ultrastructural level.

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