ABSTRACT

A study was made of the effect of the administration of reserpine and parachlorophenylalanine, an inhibitor of 5-hydroxytryptamine (serotonin; 5-HT), on the capacity of thyroid parafollicular cells to synthesize and store 5-HT. The two drugs were given to nonhibernating bats in doses which produced an equivalent degree of depletion of 5-HT from the thyroid. Tritiated 5-hydroxytryptophan, the precursor of 5-HT, was then given intravenously to assess the ability of parafollicular cell granules to take up and retain newly synthesized 5-HT. Reserpine, but not parachlorophenylalanine, decreased the amount of labeled 5-HT found in the thyroid and prevented autoradiographic labeling of parafollicular cell granules. Quantitative ultrastructural and stereological analysis demonstrated that the granules in untreated animals appeared to be nearly spherical prolate ellipsoids, with a uniformly electron-opaque inner matrix. In animals given reserpine, the axial ratio of the ellipsoidal granules increased greatly and a faint internal striation parallel to the long axis of the granules became apparent. Similar changes were not induced by parachlorophenylalanine. No other morphological changes in the thyroid epithelium were detected after administration of reserpine. This study confirms the association of 5-HT with the mature small secretory granules of thyroid parafollicular cells.
1968) and 5-HT synthesized from administered exogenous precursor have been associated with the characteristic secretory granules of parafollicular cells (Ericson, 1970; Nunez and Gershon, 1972). As a result of a quantitative electron microscope autoradiographic analysis, Nunez and Gershon (1972) concluded that tritiated 5-HT, synthesized in vivo from injected tritiated 5-HTP, was exclusively localized to mature secretory granules and was distributed around the periphery of these granules. They also found that parafollicular cells contain monoamine oxidase (MAO), an enzyme which degrades 5-HT. Since the conversion of 5-HTP to 5-HT probably occurs in the cytosol, and no organelles other than the granules were labeled at any time after injection of tritiated 5-HTP, Nunez and Gershon suggested that the secretory granules could take up cytoplasmic 5-HT. This mechanism would account for the observed kinetics of labeling, as well as the protection of newly synthesized 5-HT from catabolism by MAO.

The secretory granules of parafollicular cells are also thought to contain thyrocalcitonin (TCT), a hormone which lowers the concentration of calcium in the blood (Hirsch and Munson, 1969). This polypeptide hormone is probably synthesized in granular endoplasmic reticulum and concentrated and packaged with the formation of the secretory granules in the Golgi apparatus (Nunez et al., 1969). The transfer of material from endoplasmic reticulum to Golgi apparatus in parafollicular cells of bats appears to undergo a relative slowdown with respect to synthesis, during the prehibernating phase of the annual life cycle (Gershon and Nunez, 1970; Nunez et al., 1970). This leads to the accumulation of material in the granular endoplasmic reticulum, thereby giving rise to the formation of large intracisternal granules. Since these intracisternal granules never become labeled after injection of tritiated 5-HTP, Nunez and Gershon (1972) postulated that the characteristic of 5-HT uptake is a function of post-Golgi granules. Thus, the hypothesis has been formed that the mature secretory granules of parafollicular cells store two hormones, TCT and 5-HT. These would be considered to be added separately and consecutively to the granules. While the hypothesis postulates uptake of 5-HT by the granules from the cytosol, nothing is known about the nature of this process. Either a carrier-mediated transport of 5-HT into the granules or osmotic inactivation of 5-HT by binding to a macromolecule within the granule could account for the concentration of 5-HT.

The present study was undertaken in order to learn more about 5-HT uptake and storage in parafollicular cell granules. Two drugs were employed, both of which are known to deplete 5-HT. One drug, p-chlorophenylalanine (PCPA), inhibits tryptophan hydroxylase, the rate limiting step in 5-HT biosynthesis (Koe and Weissman, 1966; Lovenberg et al., 1967), and thus depletes 5-HT by blocking its formation from tryptophan. The other drug, reserpine, has been shown in many systems to interfere with storage of monoamines in granules, thereby leading to intracellular release of the amines and their catabolism by MAO (Shore, 1962; Carlsson, 1966). The two drugs were given to bats in doses adequate to produce an approximately equivalent degree of depletion of 5-HT from the thyroid. The ability of the cells to store tritiated 5-HT synthesized in vivo from administered tritiated 5-HTP was assessed biochemically. This parameter was also studied by autoradiography and served as an index of 5-HT retention in storage granules. Since reserpine but not PCPA blocked 5-HT retention, the submicroscopic structure of parafollicular cell granules was compared by electron microscopy in control, reserpine-treated, and PCPA-treated animals. Changes in the granular matrix or membrane were sought which might be correlated with an inability of the granules to take up and/or store 5-HT.

**MATERIALS AND METHODS**

**Animals**

The bats used in this study were *Myotis lucifugus*. All were adults and were of either sex. Active bats were caught in the prehibernating state in September. Hibernating bats were collected in March and were transported over ice to the laboratory. These latter animals were kept in the cold (4°C) during the course of the experiment. Active and hibernating animals were all obtained from an abandoned mine in New Jersey. Bats in the arousal phase of their yearly cycle were captured in April as they emerged from the caves where they had been hibernating. These animals were obtained from central Illinois.

**Drugs**

Reserpine (Serpasil; Ciba Pharmaceutical Co., Summit, N. J.) was administered intraperitoneally in a total volume of 0.1 ml. Ten active bats, ten hiber-
Injection volume was 0.2 ml and the dose was 300 mg/kg of reserpine. These four animals were used for autoradiography and were given tritiated 5-HTP (see below) on the day after administration of reserpine. 

PCPA was given intraperitoneally as a suspension. The suspension was prepared as follows: PCPA in saline was dissolved by adding 5 N NaOH to reach pH 10. One drop of polyoxyethylene (20) sorbitan monoocte was added and the material was reprecipitated as a fine suspension with HCl. Final injection volume was 0.2 ml and the dose was 300 mg/kg. PCPA was given to ten active bats, and ten bats in the arousal phase of their annual life cycle. Both of these groups were killed the next day. Six hibernating bats were aroused artificially, kept at room temperature for 24 h, and given 250 mg/kg PCPA (base) as the methyl ester. This latter material is soluble and was given intraperitoneally in saline solution. These six animals were given tritiated 5-HTP and were used for autoradiography on the day after administration of PCPA. Equal numbers of animals were given distilled water and served as controls for each group of PCPA- or reserpine-treated animals.

Tritiated 5-HTP (3.3 Ci/mmole; Amersham/Searle Corp., Arlington Heights, Ill.) was given intravenously or, in some experiments where autoradiography was not performed, intraperitoneally. For most studies, 500 μCi were given to each animal. For electron microscope autoradiography the dose was 5 mCi. In addition to the PCPA- and reserpine-treated animals mentioned above, nine artificially aroused, but otherwise untreated animals were also given tritiated 5-HTP and served as controls. All animals were killed 4 h after administration of tritiated 5-HTP.

Biochemical Determinations

The concentration of 5-HT in the thyroid was measured by the spectrophotofluorometric method of Snyder et al., (1965). Procedures were essentially the same as have been described previously (Nunez and Gershon, 1972). Trinititated compounds present in the thyroid 4 h after administration of tritiated 5-HTP were identified and assayed utilizing thin layer chromatography. Thyroids were homogenized and extracting in 2.0 ml of 70% ethanol at 4°C with constant agitation for 24 h. Ethanol extracts about 95% of the labeled 5-HT (Gershon and Altman, 1971). The precipitated protein was removed by centrifugation and the supernatant was saturated with nitrogen, frozen, and dried. The material was re dissolved in 0.1 ml of 50% ethanol, spotted onto cellulose thin layers (Eastman chromatogram, Eastman Kodak Co., Rochester, N. Y.), and developed in butanol:acetic acid:water (4:1:5). Authentic 5-HT, 5-HTP, and 5-hydroxyindole acetic acid (5-HIAA) were added as carriers. Each sample spot was also flanked by spots of standard solutions of the three indoles. These standards were run in parallel with the experimental samples. Standard compounds were located by spraying developed chromatograms with Ehrlich's reagent. In preliminary experiments developed chromatograms were divided into 4-mm strips which were scraped into liquid scintillation vials containing 2 ml of 70% ethanol. After shaking overnight, 15 ml of liquid scintillation cocktail (Aquasol; New England Nuclear Corp., Boston, Mass.) was added to the vials and the samples were counted. Essentially all of the radioactivity migrated as either 5-HT, 5-HTP, or 5-HIAA. In subsequent experiments, therefore, these three compounds were located by reference to parallel standards, and the radioactivity attributable to each was determined. In each experiment a portion of the original ethanolic extract was removed before concentration and chromatography and was counted. The proportions of the total radioactivity attributable to 5-HT, 5-HTP, or 5-HIAA were determined by thin layer chromatography. The total amount of each of these labeled compounds in the thyroid was thus estimated by multiplying the total radioactivity by the proportion that each labeled compound contributed to the radioactivity recovered from the chromatograms.

 Autoradiographic Studies

Tissues used for autoradiography were fixed either by an initial perfusion through the heart (for electron microscopy) or by immersion (for light microscopy) in hypertonic 6.5% glutaraldehyde (containing 9% sucrose and 0.1 M phosphate buffer, pH 7.4). This fixative preserves tritiated 5-HT but permits residual 5-HTP and metabolites of 5-HT to wash out (Gershon and Ross, 1966 a). Fixation in glutaraldehyde continued for 2-4 h. Tissues were postfixed in 0.067 M cacodylate-buffered 1% osmium tetroxide (pH 7.3) containing 9% sucrose for 1 h. Subsequently, specimens were dehydrated and embedded in epoxy resin (Epon 812). Thin and semithin (0.5 μm) sections were cut with a Sorvall MT-2 ultramicrotome.

For light microscope autoradiography, semithin sections mounted on glass slides that had been dipped in a chromium alum-gelatin adhesive solution (Gershon and Ross, 1966 b). Ilford L-4 photographic emulsion, diluted 1:1, was applied by dipping. Slides were exposed for 3 wk in a dry at-
mosphere of 100% CO₂ at room temperature. After exposure, slides were developed in Kodak “D-19” for 4 min at 68°F. After photographic processing, most slides were viewed unstained by phase-contrast microscopy. A few slides were stained with a 1% aqueous solution of toluidine blue (pH 9.0). For electron microscope autoradiography, thin sections were picked up on copper grids coated with Formvar. Grids were then coated with Ilford L-4 photographic emulsion, diluted 1:6 by dipping. The grids were subsequently exposed for 4 wk in a dry atmosphere of 100% CO₂. After exposure, grids were developed in Kodak “Microdol-X” for 5 min. After photographic processing, the sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in a Philips EM 300 electron microscope. Autoradiographs were analyzed according to the procedure suggested by Rogers (1971). First, labeled organelles were identified by the method of Williams (1969). Circles were drawn around each silver grain, the center of which was the midpoint of the largest axis of the grain. The diameter of the circles was 1.5 times the half distance (HD; the distance from a radioactive line within which 50% of developed grains fall; Salpeter et al., 1969). For the conditions of the present study (Ilford monolayer, medium gold sections, Microdol X development), Salpeter et al. (1969) have determined the HD to be 1,650 Å. Potential isotope sources within the circles were scored as single items (organelle filled the circle), junctional items (two or more organelles within the circle), or compound items (organelles such as parafollicular cell granules, too small to fill a circle). Attribution of grains to the components of junctional or compound items was based on the frequency of association of grains found for the component organelles which also occurred as single items. A regular grid of circles identical in size to those used in scoring grains was used to determine the relative tissue area occupied by each cellular component. The frequency with which cellular components occurred in the circles of the grid was noted. In order to determine if grain distribution was random, grid circle and grain frequency distributions were compared by a χ² test. The frequency of occurrence of an organelle in the grid circles was its “effective area”. Relative activity of each organelle was determined by dividing its grain count by its effective area. After this analysis, curves of distribution of grains around parafollicular cell granules were constructed (Salpeter et al., 1969).

**Electron Microscopy**

For routine electron microscopy, methods used were similar to those described above for autoradiography, except that tissues were fixed for 2-4 h in 0.067 M cacodylate-buffered 6.25% glutaraldehyde and postfixed for 1 h in 0.067 M cacodylate-buffered osmium tetroxide, both at pH 7.3. Sucrose was omitted from these solutions (Nunez and Gershon, 1972).

**RESULTS**

The concentration of 5-HT in the bat thyroid has been found to fluctuate widely as the animals pass through the phases of their annual life cycle (Nunez and Gershon, 1972). As far as 5-HT is concerned, the cycle can be divided into three such phases, the active period, hibernation, and arousal. The mean and 95% confidence limits of the 5-HT level in the active period is 4.6 ± 0.4 μg/g (nine determinations on 90 bats), during hibernation is 10.2 ± 1.9 μg/g (six determinations on 60 bats), and at arousal is 10.3 ± 3.9 μg/g (four determinations on 40 bats). Control values for the 5-HT concentration in pooled bat thyroids, used as pairs for the determination of the effect of reserpine and PCPA (Fig. 1), were 4.48 μg/g in early September (active period; 20 bats), 8.26 μg/g in early March (hibernation; ten bats) and 12.80 μg/g in mid-April (arousal; ten bats). The effect of treatment with reserpine or PCPA on the concentration of 5-HT in the thyroid at these times of the year is shown in Fig. 1. Note that chronic treatment with small doses of reserpine during the active period appears more effective than a single large dose in depleting 5-HT. The 5-HT store in the thyroid at arousal from hibernation seems to be more resistant to reserpine and PCPA than during the normally active or homeothermic period. 1 day after single doses of reserpine (5 mg/kg) or PCPA (300 mg/kg), given during the active period, the degree of 5-HT depletion was about the same.

4 h after tritiated 5-HTP was injected into bats, 32.2 (±1.6 SE) percent of the recovered radioactivity was present as tritiated 5-HT, 33.5 (±16.3 SE) percent as tritiated 5-HTP, and 34.3 (±12.5 SE) percent as tritiated 5-HIAA. The latter two compounds are readily washed out of tissues while tritiated 5-HT remains bound. Autoradiography confirmed that tritiated material was almost exclusively located in parafollicular cells (Figs. 2, 3, 4). The turnover of labeling in
The effect of reserpine and PCPA on the concentration of 5-HT in the bat thyroid during the three phases of the annual life cycle. Values were measured 1 day after a single injection of 5 mg/kg of reserpine or 900 mg/kg of PCPA. The asterisk indicates the effect of 0.5 mg/kg of reserpine given daily for 8 days. Thyroids from ten animals were pooled in each group. Values are shown as the percent of the 5-HT concentration found in paired control animals killed at the same time (see text).

The distribution of silver grains is shown in Fig. 2. Note that follicular cells and luminal colloid (Fig. 4) as well as brown fat surrounding the thyroid (Fig. 5) do not concentrate the label. Analysis of electron microscope autoradiographs by the method of Williams (1969), for which 1,000 grains were tabulated, gave the following relative specific activities (percent grains/effective area) of various cell components: granular endoplasmic reticulum-0.4; Golgi apparatus, 1.1; nucleus, 0.4; parafollicular cell granules, 12.9; mitochondria, 0.2; cytosol, 0.1. This distribution of silver grains is significantly different (p < 0.001) from the distribution that would have been predicted from the areas of the micrograph occupied by the various cellular components. Areas were measured for this comparison by point count planimetry (Elias et al., 1971). Thus, label appears primarily associated with parafollicular cell granules. This association was confirmed by analyzing the distribution of grains around the granules according to the method of Salpeter et al., (1969). The distribution of grains found in this study did not differ from that found previously 1 h after injection of labeled 5-HT (Nunez and Gershon, 1972), and corresponded to the distribution that would have been predicted if the labeled source acted as a hollow circle with a radius of 1 HD (1,650 Å). Thus, tritiated 5-HT appears in this type of experiment to be most concentrated around the periphery of the parafollicular cell granules.

These experiments were repeated in animals treated with reserpine or PCPA. Reserpine decreased the average amount of tritiated 5-HT found in the thyroid 4 h after injection of tritiated 5-HTP by 82.2% (p < 0.01; eight animals). PCPA, on the other hand, increased the average amount of tritiated 5-HT found under the same conditions by 139.1% (p < 0.02; 11 animals). Reserpine also decreased the mean ratio of labeled 5-HT to 5-HTP to 46% of control (p < 0.05; eight animals). This ratio was unchanged from control in animals receiving PCPA.

The effect of the drugs on the storage of 5-HT was also apparent in autoradiographs prepared from thyroids removed 4 h after injection of labeled 5-HTP. Parafollicular cells were labeled in animals injected with PCPA (Fig. 6) but no labeled cells could be found in thyroids from animals treated with reserpine (Fig. 7). In fact, counts of labeled cells revealed an increase in the animals given PCPA. The number of labeled cells increased from 2,012 (±68 SE) cells/mm² to
2,433 (±12 SE) cells/mm² (sections were exposed for 3 wk; 1,067 cells were counted from four sections each of control and PCPA-treated animals; p < 0.001). Thus, reserpine, but not PCPA, does appear to interfere with storage of 5-HT.

Since the storage of 5-HT in parafollicular cell granules and the antagonism of this storage by reserpine were confirmed, changes induced by reserpine in the fine structure of the granules were looked for. The animals given PCPA in which 5-HT was depleted but in which the granular storage mechanism remained intact served as a control for this study. Changes found in both PCPA-treated and reserpinized tissues might be attributable to the loss of 5-HT. However, changes found in tissue from animals given reserpine but not also in those given PCPA might be related to the specific action of reserpine on the granular storage mechanism.

Reserpine produced two changes in the morphology of the bat thyroid. Both of these were related to parafollicular cell granules and were not seen after PCPA. The shape of the granular profiles became more elliptical and a faint internal striation parallel to the long axis of the granule became apparent. Compare Fig. 8 (control) and Fig. 9 (PCPA) with Figs. 10 and 11 (reserpine). The internal striations in the granules from reserpine-treated animals can be seen better in the gallery shown in Figs. 12 a–12 d. The change in the form of the granular profiles was studied quantitatively by measuring the ratio of major to minor axes of the granules. The major axis was taken as the length of the longest diameter of the granule. The minor axis was taken as the width of the granule measured at its widest point along a line perpendicular to the major axis. Measurements were made of 100 consecutively encountered granules in micrographs of parafollicular cells of control, reserpine-treated, and PCPA-treated animals. The mean ratio of major/minor axes (axial ratio) of granules in the reserpine group,
1.64 ± 0.08 SE, was significantly greater than that of either untreated animals, 1.12 ± 0.01 SE (p < 0.001), or animals given PCPA, 1.15 ± 0.02 SE (p < 0.001). The PCPA group did not differ significantly from the untreated animals (p > 0.25). However, not all granular profiles were changed by reserpine. A histogram showing the distribution of axial ratios of individual granules is plotted in Fig. 13. In this figure, the spread of ratios in animals given reserpine can be compared with those of control and PCPA-treated animals. When the cumulative frequency percentage of these ratios was plotted, the resulting curves were similar to those given by Elias and Pauly (see Elias et al., 1971; p. 182) for prolate rotatory ellipsoids of equivalent axial ratios. Thus, these data are compatible with the view that reserpine causes an increase in axial ratios of granules which are normally prolate ellipsoids. However, the technique does not permit one to determine whether all or only some of the parafollicular cell granules are affected by the drug.

Changes in thyroid ultrastructure induced by reserpine seemed to be limited to the ones described above. No changes were found in the large, intracisternal granules that accumulate during the prehibernating period (Fig. 14). Other organelles of parafollicular cells also appeared unchanged as did follicular cells, mast cells, and other connective tissue cells, as well as vascular endothelium (Fig. 15).

DISCUSSION

In the initial phase of this study the association of tritiated 5-HT (synthesized in vivo from tritiated 5-HTP) with parafollicular cell granules was confirmed. These observations conformed to the pattern found in a previous study (Nunez and Gershon, 1972). Fewer labeled cells were found in the thyroid 4 h after injection of tritiated 5-HTP than at 1 h (Fig. 2). However, free radioactive 5-HT persist in the blood for up to 2 h after its intravenous injection (Gershon and Ross, 1966 a). Therefore, 4 h after injection of label was selected as the time point for detailed study and for the determination of drug effects.

Doses of PCPA and reserpine were determined which were approximately equipotent in depleting 5-HT (Fig. 1). These doses of the two compounds differed in their ability to prevent storage of tritiated 5-HT. In animals given reserpine, much less radioactive 5-HT was retained by the thyroid 4 h after injection of tritiated 5-HTP than in control or PCPA-treated animals. This inability to store 5-HT was reflected in the failure of tritiated 5-HTP to label, autoradiographically, parafollicular cells in the thyroid glands of animals treated with reserpine. On the other hand, under the same conditions, treatment with PCPA actually increased retention of labeled 5-HT by the thyroid over that of controls. This increase was also reflected in more parafollicular cells becoming labeled in autoradiographs prepared from PCPA-treated animals.

These results are consistent with the known pharmacology of the two agents. The action of reserpine could be explained by the drug's blocking the protective storage mechanism for 5-HT in parafollicular cell granules. If 5-HT could not be bound in the cells, tritiated 5-HT, synthesized from labeled 5-HTP, would be catabolized by MAO (Nunez and Gershon, 1972). The ability of the tissues to retain tritiated 5-HT would thus be lost, as has also been found to be the case for tritiated norepinephrine (Muscholl, 1960). In contrast, PCPA did not seem to affect the storage mechanism. The drug prevents endogenous conversion of tryptophan to 5-HTP (Koe and
Figures 12 a–d Higher magnification electron micrographs of parafollicular cell granules from a control animal and from an animal treated with reserpine (one injection, 5 mg/kg). A faint light linear pattern of striations can be discerned in the center of the granules from the animals given reserpine (Figs. 12 a and 12 c) parallel to the long axis of the granules. These granules are elliptical in profile. In (Fig. 12 d), also from an animal treated with reserpine, the granular profile is round, but an internal substructure may be seen. No internal substructure can be discerned in the round control granule (Fig. 12 b) X Fig. 12 a, × 100,000; Figs. 12 b and 12 c, × 80,000; Fig. 12 d, × 120,000.

Weissman, 1966) but the administration of exogenous 5-HTP, as in the current experiments, effectively short circuits this enzymatic blockade. The additional tritiated 5-HT retention and parafollicular cell labeling in the PCPA-treated animals could be explained if some 5-HT storage sites were saturated in untreated animals. Partial depletion of 5-HT through inhibition of its biosynthesis would thus increase the storage capacity for newly synthesized 5-HT by freeing 5-HT binding sites which had previously been occupied.

Reserpine but not PCPA changed the morphology of the parafollicular cell granules. Since PCPA failed to mimic this action of reserpine the

Figures 8–11 Electron micrographs showing portions of parafollicular cells from control and drug-treated animals.

Figure 8 Control animal. The secretory granules have a homogeneous dense core and are mostly round or slightly ovoid in profile. × 30,000.

Figure 9 Animal treated with PCPA. The parafollicular cell granules are similar to those of control animals. They have a homogeneous matrix and are mostly round or slightly ovoid in profile. × 30,000.

Figure 10 Animal treated with reserpine (5 mg/kg). Many secretory granules now exhibit elongate elliptical profiles (arrows). Other granules are still round in profile. × 30,000.

Figure 11 Animal treated with reserpine (0.5 mg/kg given daily for 8 days). Many granules have elliptical profiles and a faint light striation can just be distinguished in the central region of the granular matrix (arrowhead). × 30,000.
morphologic change does not reflect simply a loss of 5-HT from the granules. The degree of 5-HT depletion in the two instances was the same. It is also likely that the action of reserpine is specific. Changes were confined to the parafollicular cell small granules which are the site on which reserpine must act in order to block 5-HT storage.

No toxic effects, such as the accumulation of lipid or reversible mitochondrial degeneration that have been seen in cardiac muscle after large or repetitive doses of reserpine (Hagopian et al., 1972), were found. Nor were changes seen in the large, rough membrane-enclosed granules of parafollicular cells which, even in untreated animals, are unable to take up tritiated 5-HT (Nunez and Gershon, 1972).

The alterations induced by reserpine, a change in granular form to become more ellipsoidal and the appearance of internal striations, suggest that the drug may act on the granular matrix. Alterations of the limiting membrane were not observed. Thus, reserpine's action on the thyroid may be to block amine binding inside of mature parafollicular cell granules. If so, the observation would support the view that 5-HT concentration within the granules is due to osmotic inactivation by internal binding of 5-HT to form high molecular weight aggregates, rather than to carrier-mediated transport of 5-HT by the granular membrane.

Such osmotic inactivation appears to be involved in the concentration of catecholamines in chromaffin granules of the adrenal medulla (DaPrada et al., 1971; Kirshner and Kirshner, 1969) and the storage of 5-HT in specific organelles in platelets (Berneis et al., 1969). Thus, osmotic inactivation by formation of high molecular weight aggregates has been proposed as a general mechanism involved in storage of biogenic monoamines (DaPrada et al., 1971). Mucopolysaccharide-protein complexes in amine storage granules have also been found in rat platelets and mast cells (Åborg and Uvnäs, 1971). Thus, it seems highly
FIGURES 14 and 15  Electron micrographs prepared from active, prehibernating bats treated with reserpine (5 mg/kg).

FIGURE 14  A portion of a parafollicular cell is shown which contains large granules enclosed by a rough membrane studded with ribosomes (arrow). These intracisternal granules are mainly round in profile and have a homogeneous matrix. They cannot be distinguished from similar granules of control animals. × 45,000.

FIGURE 15  Portions of several follicular cells and a capillary are shown. No change from control can be seen in the endoplasmic reticulum, mitochondria, lysosomal structures, or microvilli of the follicular cells (F). C, Colloid. The capillary endothelium (E) also appears normal. × 20,000.

likely that 5-HT may also be bound in some way within parafollicular cell granules.

Reserpine blocks uptake of amines by storage granules in many systems, including adrenergic nerve granules (Euler, 1969), adrenomedullary granules (Lishajko, 1971), and 5-HT organelles of blood platelets (DaPrada and Pletscher, 1969 a). The mechanism has not been worked out, but in platelets reserpine becomes bound to the 5-HT organelles (DaPrada and Pletscher, 1969 a) and, specifically, to the membrane of these organelles (DaPrada and Pletscher, 1969 b). The suggestion, therefore, has been made that reserpine acts at the level of the membrane of the 5-HT organelles in platelets (DaPrada and Pletscher, 1969 b). However, no evidence has been found indicating that the storage of 5-HT in platelets is linked to a process of energy-dependent transport through the membrane of the subcellular storage organelles. Consequently, it has been postulated that a change in the properties of the membrane of these organelles might affect the stability of the 5-HT nucleotide aggregates within them (DaPrada and Pletscher, 1969 b). In the present study, changes induced by reserpine were limited to the interior of parafollicular cell granules, and these changes were correlated with evidence indicating that the granules had lost their ability to bind 5-HT. However, it is possible that reserpine could have changed the internal stability of the parafollicular
cell granules by acting on the granular membrane in a way that was not morphologically apparent.

The submicroscopic changes we found in parafollicular cell granules are different from the changes that other investigators have ascribed to reserpine in other amine-storing cells. These studies of adrenal medulla (Clementi and Zocche, 1963; Yates, 1963), abdominal sympathetic paraganglia (Mascorro and Yates, 1971), and platelets (DaPrada et al., 1968) have described either a loss of electron opacity of the matrices of the various granules or the replacement of the granules with empty-appearing cytoplasmic vesicles. On the other hand, even massive doses of reserpine failed to produce morphologic changes in cells of the carotid body (Duncan and Yates, 1967). Differences in these studies may be due to varying susceptibility of different tissues to reserpine, the different doses of reserpine used in each study, the different methods of administration of the drug, and the different times after injection of reserpine at which tissues were obtained for examination. The different results might also be due to modification of the interactions with reserpine by differences in the nonamine content of the granules. For instance, in addition to amines, adrenomedullary granules also contain chromogranins and parafollicular cell granules contain thyrocalcitonin.

In conclusion, the present study has confirmed the association of 5-HT with the mature small secretory granules of rat parafollicular cells. These granules share with other amine-storage organelles a susceptibility to reserpine treatment which leads to changes in the shape and internal substructure of the parafollicular cell granules, along with a loss of their ability to bind 5-HT. The effect of reserpine on the storage of thyrocalcitonin and the function of parafollicular cells is unknown and should be determined in the future.

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REFERENCES

Ábogg, G. H., and B. Uvnäs. 1971. A mucopolysaccharide-protein complex with amine binding properties in rat thrombocytes. Acta Physiol. Scand. 81:568.

Bernes, K. H., M. DaPrada, and A. Fletscher. 1969. Physico-chemical properties of 5-hydroxytryptamine organelles of blood platelets. Agents Actions. 1:35.

Carlsson, A. 1966. Drugs which block the storage of 5-hydroxytryptamine and related amines. In Handbook of Experimental Pharmacology. 5-Hydroxytryptamine and Related Indole Alkyl Amines. Springer-Verlag New York Inc., New York. XIX:529.

Clayton, J. A., and C. M. Szego. 1967. Depletion of rat thyroid serotonin accompanied by increased blood flow as an acute response to thyroid-stimulating hormone. Endocrinology. 80:689.

Clayton, J. A., and D. T. Masuoka. 1968. TSH-induced mobilization of serotonin from perivascular mast cells in the rat thyroid. Endocrinology. 83:263.

Clementi, F., and G. P. Zocche. 1963. Morphological and pharmacological effects of reserpine given alone or after iproniazid on the catecholamines of the adrenal glands of the rat. J. Cell Biol. 25:587.

DaPrada, M., A. Fletscher, J. P. Tranzer, and H. Knochel. 1968. Action of reserpine on subcellular 5-hydroxytryptamine organelles of blood platelets. Life Sci. 7 (Part 1):477.

DaPrada, M., and A. Fletscher. 1969 a. Storage of exogenous monoamines and reserpine in 5-hydroxytryptamine organelles of blood platelets. Eur. J. Pharmacol. 7:45.

DaPrada, M., and A. Fletscher. 1969 b. Different localizations of reserpine and tyramine within the 5-hydroxytryptamine organelles of blood platelets. Experientia (Basel). 25:923.

DaPrada, M., K. H. Bernes, and A. Fletscher. 1971. Storage of catecholamines in adrenal medullary granules: formation of aggregates with nucleotides. Life Sci. 10 (Part 1):639.

Duncan, D., and R. D. Yates. 1967. Ultrastructure of the carotid body of the cat as revealed by various fixatives and the use of reserpine. Anat. Rec. 157:567.

Ellis, I., A. Hennig, and D. E. Schwartz. 1971. Stereology: Application to biomedical research. Physiol. Rev. 51:138.

Ericson, L. E. 1970. Subcellular localization of 5-hydroxytryptamine in the parafollicular cells of the mouse thyroid gland. An autoradiographic study. J. Ultrastruct. Res. 31:162.

Euler, U. S. Von. 1969. Adrenergic neuroeffector transmission. In The Structure and Function of Nervous Tissue. Vol. II. G. H. Bourne, editor. Academic Press Inc., New York. II:424.
Falck, B. 1962. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta Physiol. Stand. Suppl.* 186:451.

Falck, B., B. Larsson, C. Von Mecklenburg, E. Roqvist, and K. Svenaeus. 1964. On the presence of a second specific cell system in mammalian thyroid gland. *Acta Physiol. Stand.* 62:5491.

Falck, B., and Ch. Owman. 1968. 5-Hydroxytryptamine and related amines in endocrine cell systems. In Advances in Pharmacology. S. Garattini and P. A. Shore, editors. Academic Press Inc., New York. 211.

Gershon, M. D., and L. L. Ross. 1966 a. Radioisotopic studies of the binding, exchange and distributions of 5-hydroxytryptamine synthesized from its radioactive precursor. *J. Physiol. (Lond.)*. 186:577.

Gershon, M. D., and L. L. Ross. 1966 b. Location of sites of 5-hydroxytryptamine storage and metabolism by radioautography. *J. Physiol. (Lond.)*. 186:477.

Gershon, M. D., and E. A. Nunez. 1970. Histochemical and radioautographic studies of serotonin and parafollicular cells in the thyroid gland of the prehibernating bat. *Endocrinology*. 86:160.

Gershon, M. D., B. E. Belshaw, and E. A. Nunez. 1971. Biochemical, histochemical and ultrastructural studies of thyroid serotonin, parafollicular and follicular cells during development in the dog. *Am. J. Anat.* 132:5.

Gershon, M. D., and R. F. Altman. 1971. An analysis of the uptake of 5-hydroxytryptamine by the myenteric plexus of the small intestine of the guinea pig. *J. Pharmacol. Exp. Thr.* 179:29.

Hagopian, M., M. D. Gershon, and E. A. Nunez. 1972. An ultrastructural study of the effect of reserpine on ventricular cardiac muscle of active and hibernating bats (Myotis lucifugus) *Lab. Invest.* 27:59.

Hirsch, P. F., and P. L. Munson. 1969. Thyrocalcitonin. *Physiol. Rev.* 49:548.

Jaim-Etcheverry, G., and L. M. Zieher. 1968. Cytochemical localization of monoamine stores in sheep thyroid gland at the electron microscope level. *Experientia (Basel)*. 24:593.

Kirshner, A. G., and N. Kirshner. 1969. A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla. II. Physical characterization. *Biochem. Biophys. Acta* 181:219.

Koe, B. K., and A. Weissman. 1966. p-Chlorophenylalanine: a specific depleter of brain serotonin. *J. Pharmacol. Exp. Thr.* 154:499.

Lishajko, F. 1971. Studies on catecholamine release and uptake in adrenomedullary storage granules. *Acta Physiol. Stand. Suppl.* 362:1.

Lovenberg, W., E. Jeger, and A. Sjoersma. 1967. Tryptophan hydroxylation: measurements in pineal gland, brainstem, and carcinoi tumors. *Science (Wash. D. C.)*. 155:217.

Mascorro, J. A., and R. D. Yates. 1971. Ultrastructural studies of the effects of reserpine on mouse adrenal sympathetic paraganglia. *Anat. Rec.* 170:269.

Melander, A., and F. Sundler. 1972. Significance of thyroid mast cells in thyroid hormone secretion. *Endocrinology*. 90:302.

Muncholl, E. 1960. Die hemmung der noradrena
it aufnahme des herzens durch reserpin und die wirkung von tyramin. *Arch. Exp. Pathol. Pharmakol.* 240:234.

Nunez, E. A., R. P. Gould, and S. J. Holt. 1969. A study of granule formation in the bat parafollicular cell. *J. Cell Sci.* 5:12.

Nunez, E. A., R. P. Gould, and S. J. Holt. 1970. Seasonal changes in secretory granules and crystalloid inclusions of bat thyroid parafollicular cells. *J. Cell Sci.* 6:821.

Nunez, E. A., and M. D. Gershon. 1972. Synthesis and storage of serotonin by parafollicular (C) cells of the thyroid gland of active, prehibernating and hibernating bats. *Endocrinology*. 90:1008.

Paasonen, M. K. 1938. 5-Hydroxytryptamine in mammalian thyroid. *Experientia (Basel)*. 4:95.

Reynolds, F. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208.

Rogers, A. W. 1971. Recent development in the use of autoradiographic techniques with electron microscopy. *Philos. Trans. R. Soc. Lond. Ser. B. Biol. Sci.* 261:159.

Salpeter, M. M., J. Bachmann, and E. E. Salpeter. 1969. Resolution in electron microscopic radioautography. *J. Cell Biol.* 41:1.

Shore, P. A. 1962. Release of serotonin and catecholamines by drugs. *Pharmacol. Rev.* 14:531.

Snyder, S. H., J. Axelrod, and M. Zweig. 1965. A sensitive and specific fluorescence assay for tissue serotonin. *Biochem. Pharmacol.* 14:831.

Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4:475.

Williams, M. W. 1969. The assessment of electron microscopic autoradiographs. *Adv. Opt. Electron Microsc.* 3:219.

Yates, R. D. 1963. An electron microscopic study of the effects of reserpine on adrenomedullary cells of the Syrian hamster. *Anat. Rec.* 146:29.