MORPHOLOGICAL AND PHARMACOLOGICAL EFFECTS OF RESERPINE, GIVEN ALONE OR AFTER IPRONIAZID, ON THE CATECHOL AMINES OF THE ADRENAL GLANDS OF THE RAT

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ABSTRACT

Adrenomedullary cells, after fixation with OsO_4, are filled with well formed granules which are considered to represent their catechol amine content. The submicroscopic appearance of these cells was studied in reserpine-treated rats during the late phase of catechol amine depletion and during the period of its restoration. At 3 days after the beginning of reserpine treatment, the granules appeared to be emptied of their content and small vesicles containing scattered, dense deposits of, presumably, catechol amines began to be seen. At 9 days after the beginning of treatment, these deposits had already become granules and the cells had attained a completely normal appearance. The submicroscopic structure of the adrenomedullary cells of rats pretreated with iproniazid (before reserpine), in which a complete inhibition of monoamine oxidase activity had thus been obtained, was similar to that seen in non-treated animals. In numerous cases, however, some characteristic features were noted: the sacs which usually contained a dense granule of catechol amines appeared swollen and many fine granules could be seen around them; the latter were dispersed in a way suggesting that they may represent a partial breakdown of the large granules which, under the inhibitory action of iproniazid, do not release the catechol amines contained within them.

The action of reserpine in releasing tissue amines (serotonin, catechol amines, and histamine) has been recognized only relatively recently. The adrenomedullary stores of epinephrine and norepinephrine have been shown to be depleted almost completely by reserpine, although this effect is delayed when compared to that seen in other tissues (5). The same time interval is needed when other methods of releasing adrenal catechol amines are used (acetylcholine, cold, splanchnic stimulation, insulin, etc.) (2–5, 21).

It has been noted also that the restoration curves of epinephrine and norepinephrine are quite different. Norepinephrine stores reach their maximal concentration on the 7th day following depletion, while epinephrine stores only begin to build up from the 7th day onwards (4). This is one of the facts which support the hypothesis that epinephrine is derived from norepinephrine by methylation within the cell, and it conflicts with the views of those workers who hold (9, 12), on the basis of histochemical coloration, that there are two types of cell involved in adrenalin and noradrenalin secretion. Other workers, using the electron microscope, have not been able to demonstrate two types of cell (6, 8, 20).

The electron microscope has shed new light on the synthesis and storage of adrenal catechol amines (7, 10, 14, 19). As early as 1902, it was observed that the reaction between catechol amines and OsO_4 is rapid and that the product is stable; hence, this treatment has become one of the most common methods for the study of chromaffin cells. By means of this reaction, catechol amines can be indirectly revealed in granules possessing a typical density and appearance when adrenomedullary tissue sections are examined under the electron microscope. The presence of catechol amines in the granules of the adrenal medullary cell can also be confirmed by various control tests based on the method of cell fractionation by ultracentrifugation (2, 14, 19).

The granules of epinephrine and norepinephrine are not identifiable with mitochondria. The erroneous presumption that they are was made initially because of the difficulty of separating the granules of catechol amines from the mitochondria by ultracentrifugation, their sedimentation coefficients being similar, and because of their equal content of ATP and proteins (2).[1]

A membrane appears to envelop the granules containing catechol amines. This membrane, according to some workers (16), seems to be present even after catechol amine depletion. Its presence might account for the interval of time that elapses between the venous injection of isolated granules and their pharmacological effect.

The objectives of this investigation were: (*a*) to study the appearance of the granules, supposedly containing catechol amines, at different times after reserpine treatment; (*b*) to follow the electron microscopic modifications seen during the progressive reappearance of granules after depletion of the depots; (*c*) to see whether there is some correlation between the morphological appearance and the catechol amine content of the tissue as shown by pharmacological methods; and (*d*) to see whether iproniazid, a well known antimonoamine oxidase capable of inhibiting the catechol amine depletion in rat adrenals after treatment with reserpine, would modify the morphological appearance of depletion and, if so, to what extent.

Experiments were carried out in which the adrenal glands were examined at various intervals of time after treatment with reserpine: in one gland the submicroscopic appearance of the medulla was analyzed, whereas in the contralateral gland the catechol amine content was biologically assayed. Iproniazid was injected into some of the rats before reserpine treatment in order to inhibit monoamine oxidases. It is well known that reserpine does not release catechol amines from the adrenals of rats in which monoamine oxidase activity has previously been inhibited (13, 15). The ultrastructural modifications under these conditions, however, are not known. It might be supposed that, after iproniazid, reserpine is unable to release catechol amines from the granules, or that, if they are released, the catechol amines are protected from oxidative enzymes by iproniazid.

[1] The function of ATP is still not clear, and one only knows that ATP varies in proportion to the content of catechol amines (4) in such a way that one is tempted to think that it plays some important role in the process of synthesis and storage of the amines.

MATERIALS AND METHODS

10 Sprague-Dawley rats, with a mean weight of 200 gm, were given reserpine (Serpasil, Ciba Pharmaceutical Products, Inc., Summit, New Jersey), 2.5 mg/kg subcutaneously, for 3 consecutive days. The rats were kept at a constant temperature of 25°C

Abbreviations for Legends

N, nucleus
m, mitochondrion
mc, cell membrane
mn, nuclear membrane
er, endoplasmic reticulum
G, Golgi complex

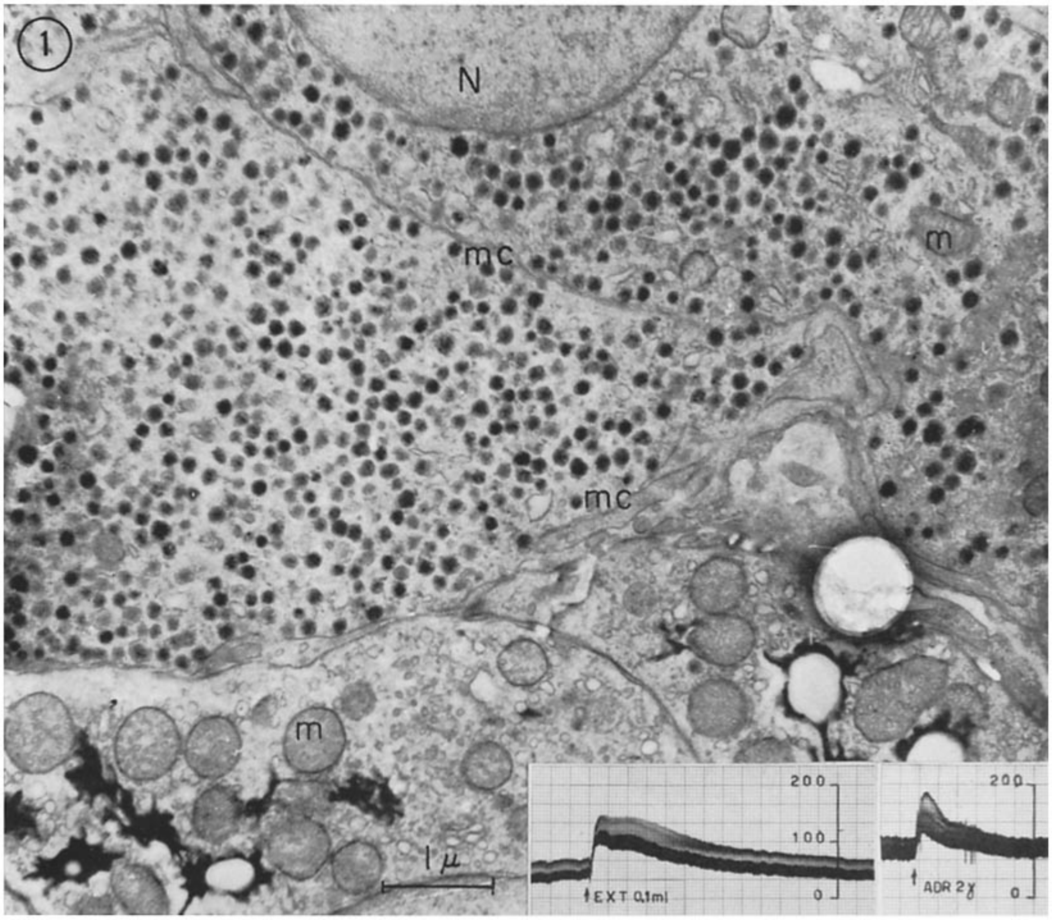
FIGURE 1

Electron microscopic view of one adrenal gland of a normal rat. The medulla is filled with granules containing catechol amines. × 14,000.

Lower right: Arterial pressure tracings after injection of an extract obtained from the contralateral gland (left) and after injection of 2 μg epinephrine (right).

FIGURE 2

Higher power view of the normal adrenal medulla showing granules containing catechol amines surrounded by a membrane (arrows). × 25,000.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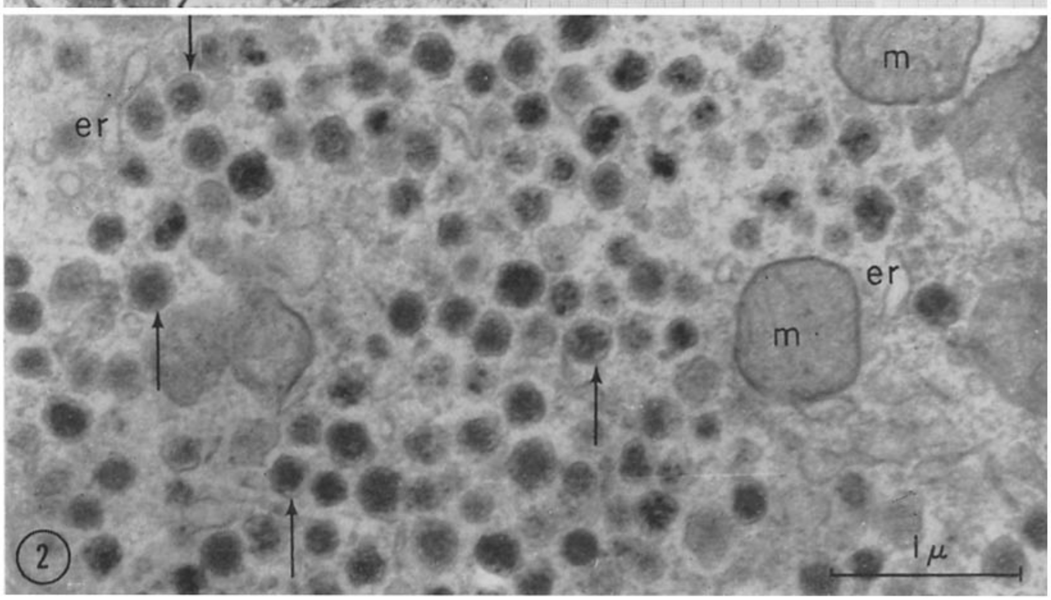
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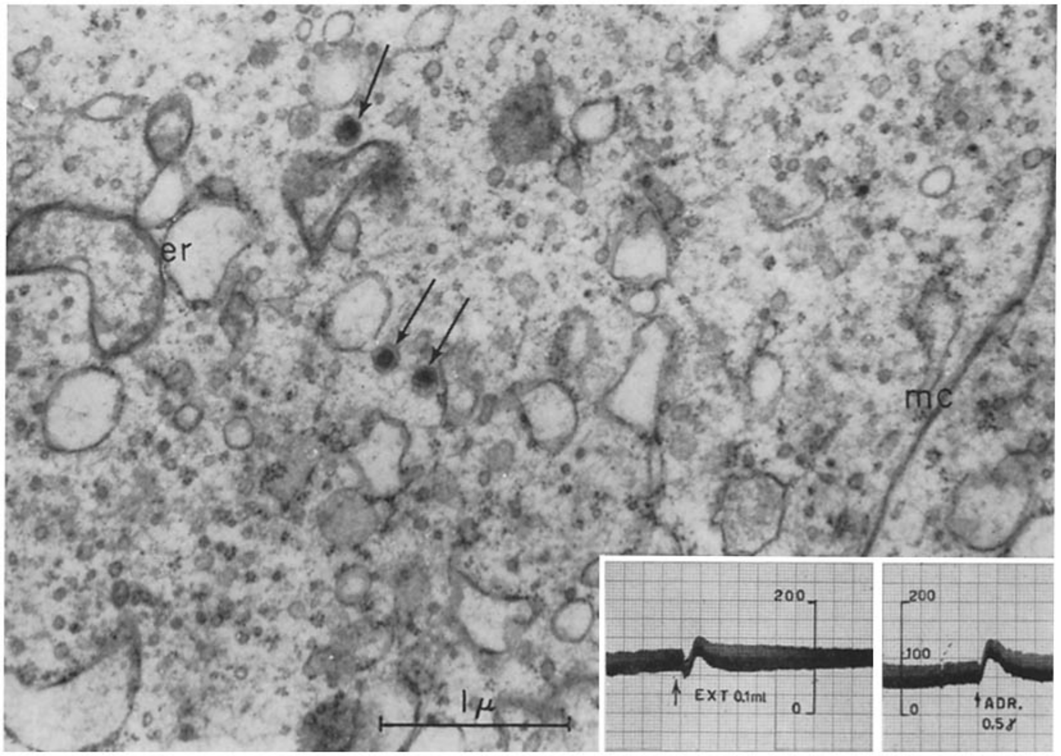


FIGURE 3

Electron microscopic view of the adrenal medulla 3 days after the beginning of the treatment with reserpine. Only very few granules are still present (arrows). There is slight swelling of the endoplasmic reticulum. × 25,000.

Lower right: arterial pressure tracings after injection of an extract obtained from the contralateral gland (left) and 0.5 μg epinephrine (right).

to prevent the hypothermic and toxic effects of reserpine. Two animals were killed 4 hours after the last injection of reserpine, two after 24 hours, two after 3 days, two after 6 days, and two after 9 days.

In order to block monoamine oxidase activity, four rats received iproniazid, 100 mg/kg intraperitoneally, 4 hours before each treatment with reserpine.

A control animal was studied for each group of treated rats. The rats were decapitated and the adrenal glands of each animal were rapidly removed. For the purpose of electron microscopic examination, one adrenal gland was immersed at once in 1 per cent osmium tetroxide, buffered at pH 7.4 according to Palade (17), and then cut into small pieces and left for 2 hours at 4°C. After a quick washing, the small

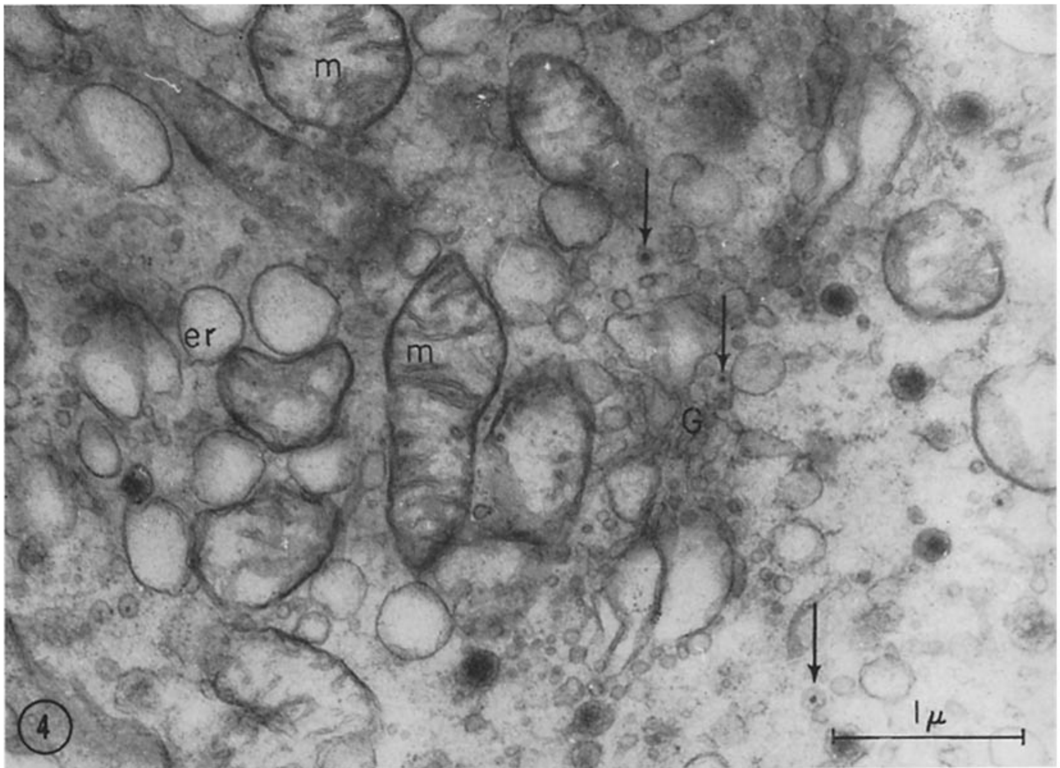
FIGURE 4

Adrenal medulla 4 days after the beginning of reserpine treatment. Newly formed granules can be seen near the Golgi complex (arrows). × 25,000.

FIGURE 5

Adrenal medulla 4 days after the beginning of reserpine treatment. Newly formed granules can be seen far from the Golgi complex and near the endoplasmic reticulum (arrows). × 25,000.

Lower right: Injected extract produces slight hypotension.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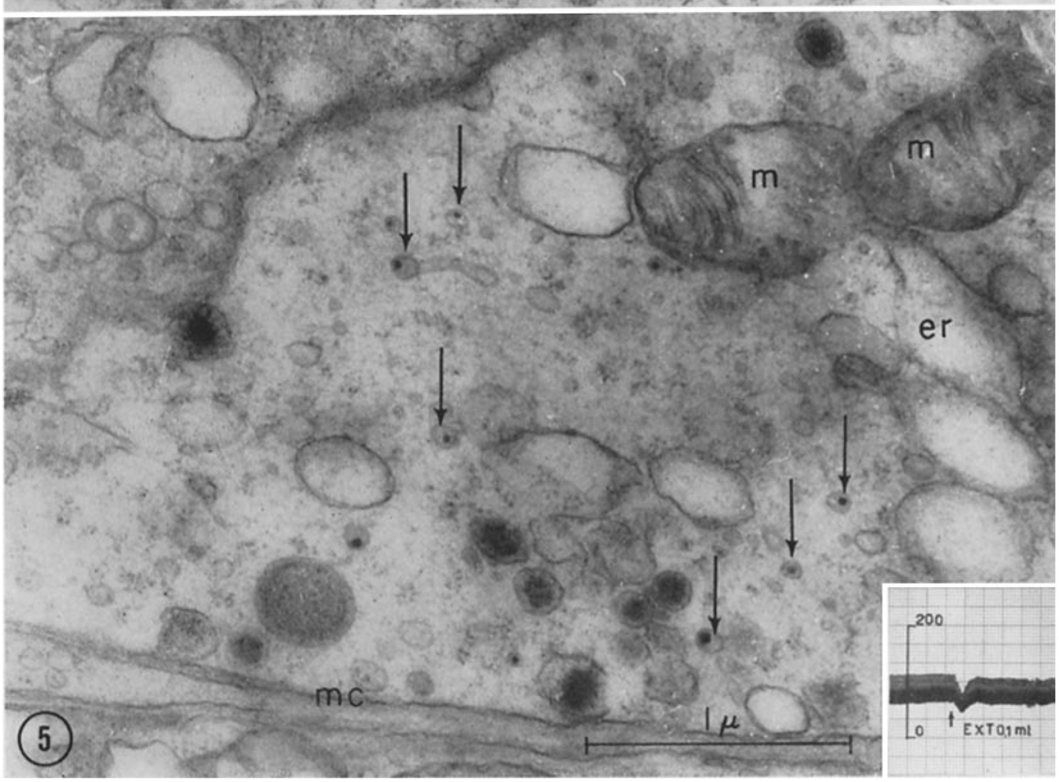
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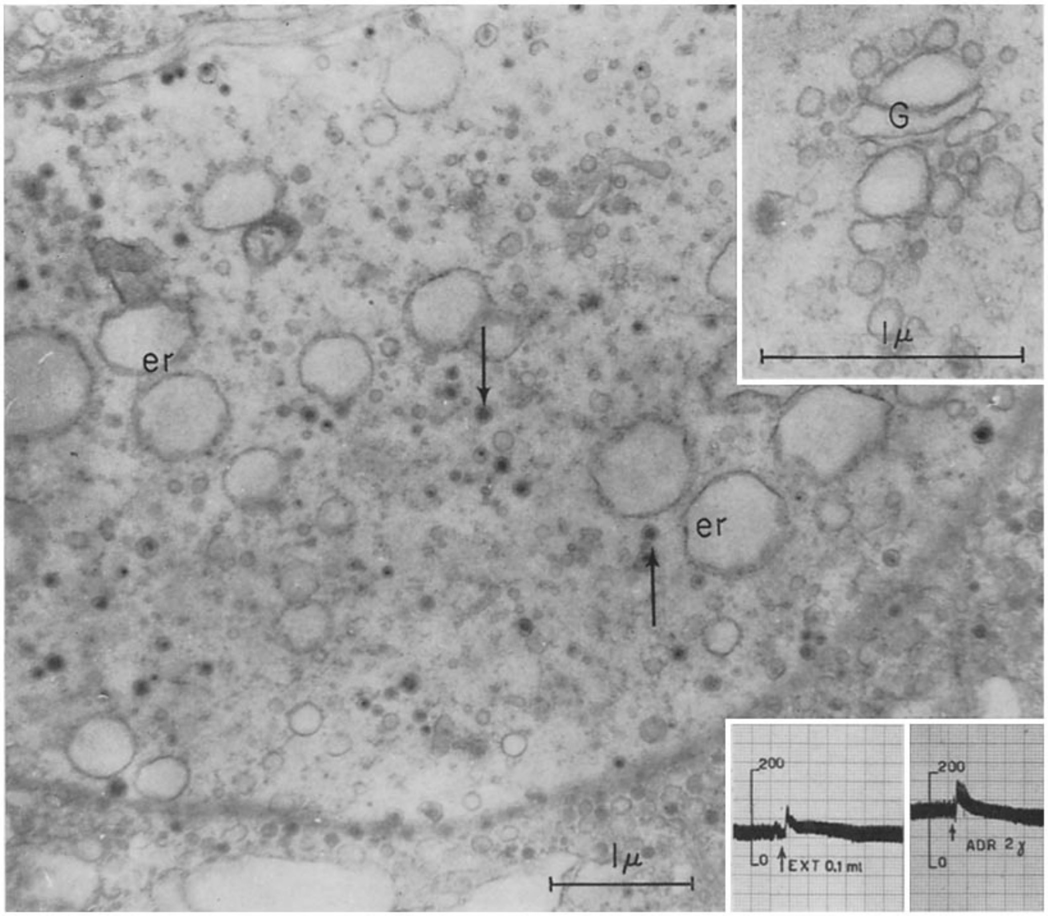


FIGURE 6

Adrenal medulla 6 days after the beginning of reserpine treatment. A fair amount of small granules is present. The endoplasmic reticulum is slightly swollen while the Golgi complex (inset, top right) now appears less conspicuous. × 18,000; inset, × 35,000.

Injected extract produces slight hypertensive response (lower right).

pieces were dehydrated in acetone and embedded in Vestopal (18). The sections were cut with a Porter-Blum microtome and examined under a Philips EM 100A electron microscope.

Biological assays of the amine content of the contralateral gland were carried out using a homogenate prepared in 0.1 N hydrochloric acid, the ratio being 20 mg of gland per 1 ml of HCl solution. This preparation was spun at 8,000 *g* in a refrigerated centrifuge to eliminate nuclei and cellular debris. The clear extract thus obtained was injected into a peripheral vein of a rat which had been adrenalectomized 24 hours previously. Blood pressure changes in the carotid artery were recorded by means of an electromanometer connected to a 150 Sanborn recorder and the pressure changes so obtained were compared with those induced in the same animal by graded doses of epinephrine, from 0.5 to 5 μg.

RESULTS AND DISCUSSION

The normal medullary cell of the rat contains a large number of osmiophilic bodies which look like granules surrounded by a membrane (Fig. 1). These granules, which were found in cells presumed to be fully mature and at rest, were all of almost equal size. The material within the granules appeared dense, dark, and uneven (Fig. 2). The cells contained few mitochondria and very little endoplasmic reticulum. Numerous vesicles (pinocytotic?) were noted at the level of the cell membranes (Fig. 2). The Golgi complex was seen in its usual form as large sacs and numerous vesicles. Some authors believe that the catechol amine granules are formed in the Golgi zone (8). When the extract made from the contralateral gland (see Fig. 1) was injected into an adrenalectomized rat, it induced a hypertensive response which was estimated to be similar to that induced by a solution containing 20 μg/ml epinephrine (a catechol amine content which, expressed as epinephrine, would correspond to 1 mg epinephrine per 1 gm adrenal tissue). The hypertensive response induced by injecting the adrenal homogenate lasted longer than that induced by the administration of epinephrine solution.

What impressed one most when one examined the adrenal medulla 3 days after the beginning of treatment with reserpine was the almost total disappearance of the catechol amine granules (Fig. 3). The mitochondria did not appear to have changed. The endoplasmic reticulum and the Golgi complex were perhaps slightly swollen. In only a few sections could one still observe some granules of reduced size full of the dense material. Biological assays of catechol amines confirmed the almost complete depletion of the adrenal medulla (Fig. 3).

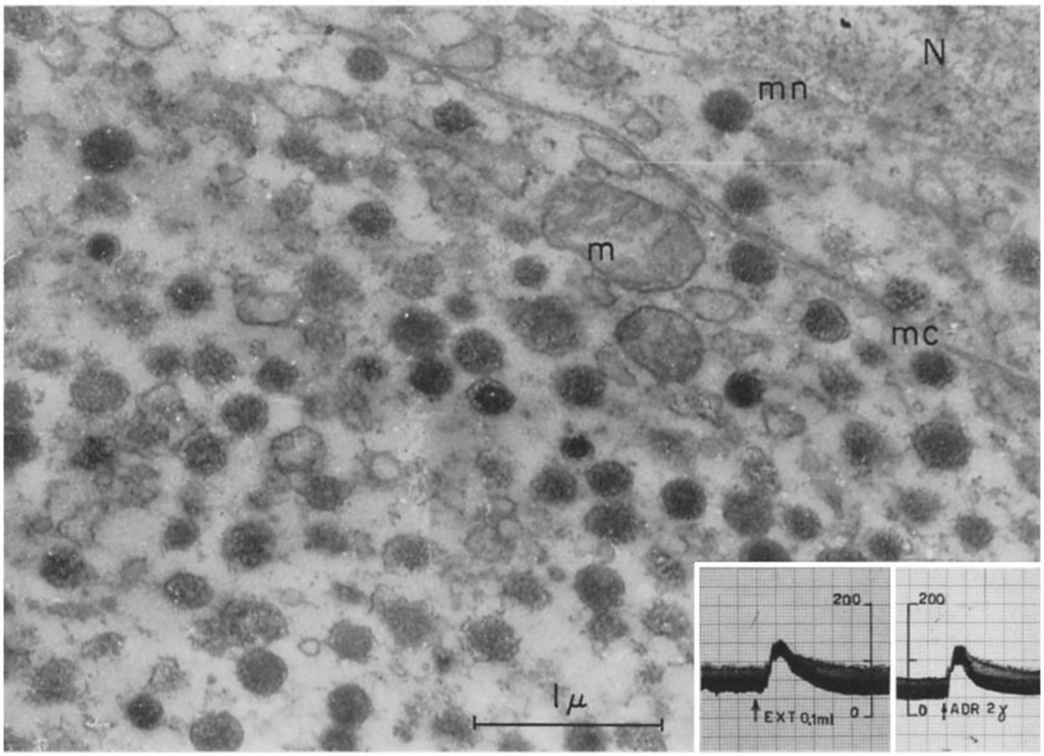


FIGURE 7

Adrenal medulla 9 days after the beginning of reserpine treatment. The submicroscopic appearance of the gland is normal. × 25,000.

Lower right: Injected extract produces a hypertensive response (left) which can be superimposed on that induced by 2 μg of epinephrine (right).

Four days after the start of reserpine treatment, the medullary cells began to recover. The Golgi complex was filled with sacs and vesicles (Fig. 4). Near the Golgi complex were noted granules, probably newly formed, which were characterized by their small size and different content. There were also mature granules which were very rich in osmiophilic material. In addition, the cell was full of small vesicles.

The injection of medullary extract 4 days after the beginning of reserpine treatment did not produce hypertension but hypotension (Fig. 5). It is not easy to explain this finding, although it should be kept in mind that total gland extracts were used and hence adrenocortical hormones were also present.

Six days after the beginning of treatment, the cell contained a fair number of chromaffin granules, but they were still of very small size (Fig. 6). The endoplasmic reticulum was swollen, while the Golgi complex now appeared to be less conspicuous (Fig. 6).

Blood pressure recordings confirmed that catechol amines were beginning to be resynthesized: the injected extracts produced clear hypertensive responses similar to those obtained by injecting adrenal extracts from untreated animals, although the responses were of a much lesser degree than those induced by a solution containing 20 μg of epinephrine (see Fig. 1).

Nine days after the beginning of treatment, the normal submicroscopic appearance of the granules had been restored and they seemed to be filled with dense, granular material (Fig. 7). The Golgi complex and the endoplasmic reticulum were normal. The number of pinocytotic vesicles at the level of the cell membrane appeared to be reduced. The amount of amines present appeared to be normal on biological assay (Fig. 7). The blood pressure response to injections of extracts could be superimposed on that induced by epinephrine, as far as form and duration were concerned.

The data obtained from animals pretreated with iproniazid were also interesting. After treatment with iproniazid and reserpine combined, the medullary cells appeared to be similar to those of the controls. It was generally observed that the granules maintained their dense content, and their number did not seem to be diminished (Fig. 8). But in many sections a very interesting feature was observed: smaller granules were present all around the dense granules. In some cases the dense granules disappeared and it seemed as though they were replaced by a diffuse network of the smaller and more dispersed granules. It was possible to see all the stages from the swelling of the small sacs containing the dense granules to the progressive dissolution of these granules inside the sacs (Fig. 9). Some empty sacs, which probably represented the last stage of the process, were also seen. The inhibition by iproniazid of a discharge mechanism accounts for the development of the dispersion. Biological assays of the adrenal medulla of rats treated with reserpine and iproniazid demonstrated the persistence of a high catechol amine activity, at least equal to that of non-treated animals.

The striking correlation, during different periods of the secretory cycle, between the granular content of the adrenomedullary cells, as estimated by electron microscopy, and the content of catechol amines, as assayed biologically, provides a direct demonstration that the catechol amines are contained in the dense granules, a fact for which histochemical and biochemical evidence is available.

Our observations would seem to indicate that, after treatment with reserpine, other cellular organelles, in addition to the Golgi complex, were modified during the period of reappearance of the secretory granules, *i.e.* the endoplasmic reticulum was swollen, and numerous vesicles were dispersed in the hyaloplasm. It is possible that while the Golgi complex is concerned with one phase of the secretory cycle, other organelles may have previously provided some of the material packed into the granules within the Golgi apparatus.

Pretreatment with iproniazid was found to prevent the release of the dense material contained in the granules. The morphological patterns which

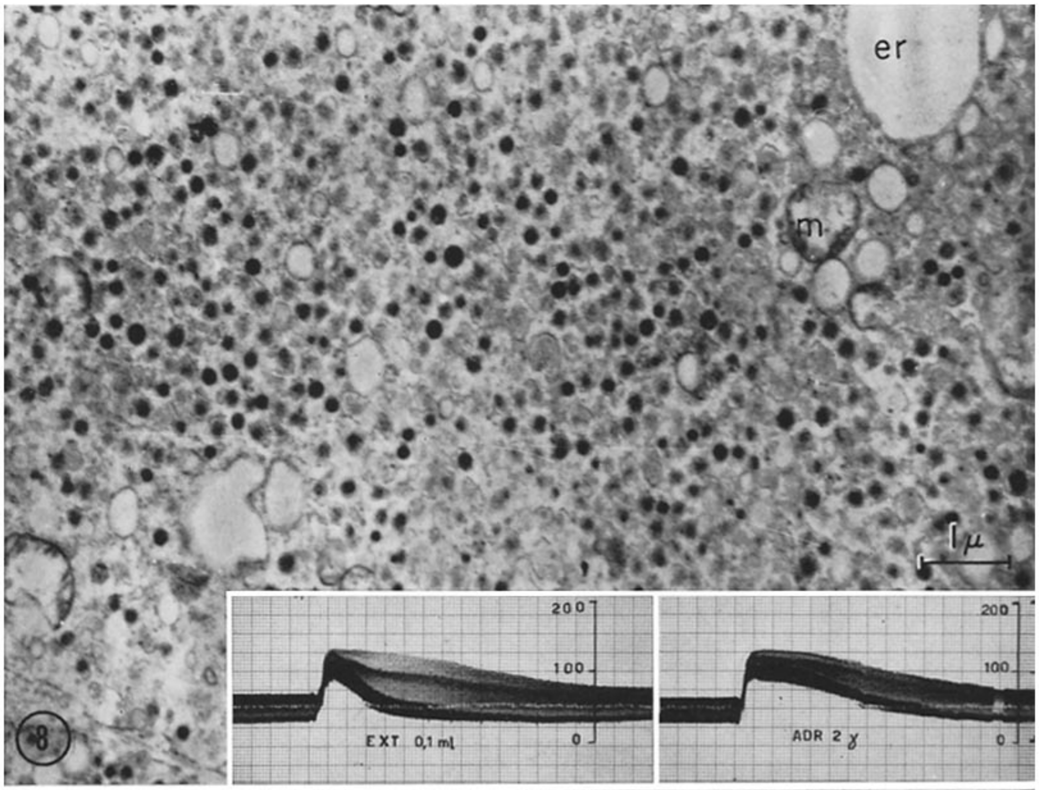
FIGURE 8

Adrenal medulla of a rat treated with iproniazid and reserpine. The granules containing catechol amines generally maintain their normal appearance and their number is unchanged. × 12,000.

Lower right: Injected extract producess a hypertensive response (left) which can be superimposed on that induced by 2 μg of epinephrine (right).

FIGURE 9

Adrenal medulla of a rat treated with iproniazid and reserpine. In some sections, smaller granules are developing from the dense granules containing catechol amines. The small granules seem to be replacing the large ones by forming a diffuse network. Various stages of the process can be seen. 9*a*, × 25,000; 9*b*, × 30,000.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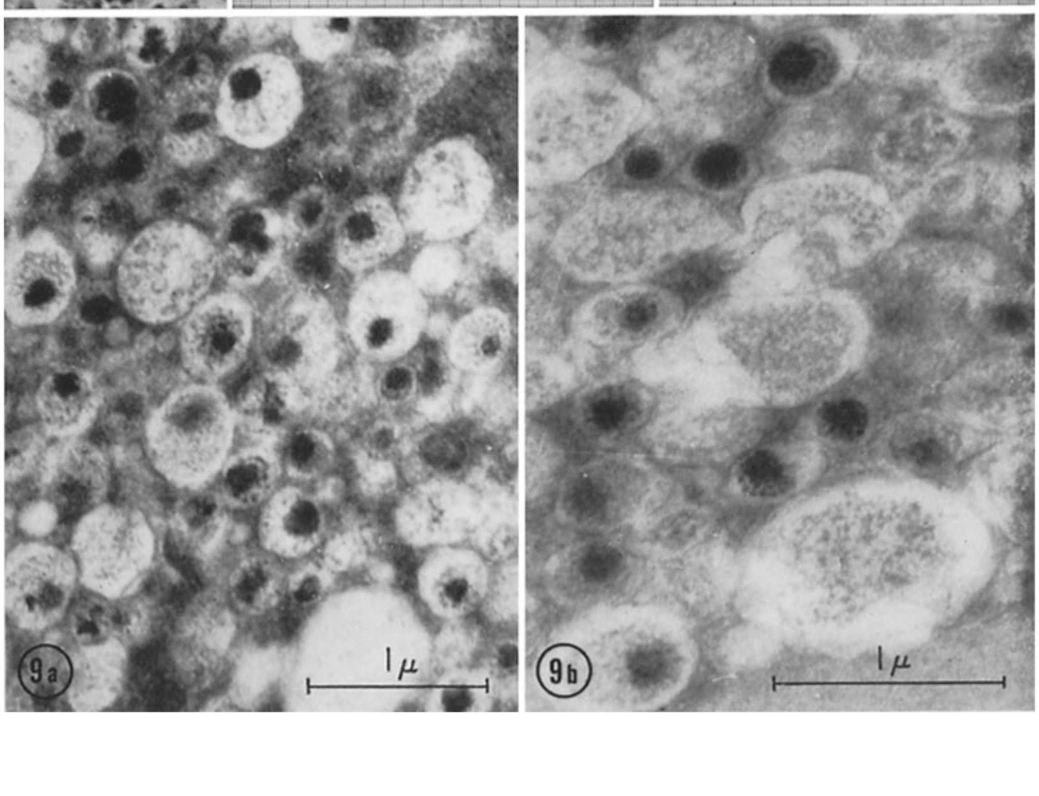
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we observed provide support for the views advanced by Axelrod and his coworkers (1, 11), according to which the monoamine oxidase blocking agents would act by inhibiting the liberation of catechol amines from their binding sites in the tissues.

The occurrence of the osmiophilic catechol amine-containing granules fragmented into smaller, finely scattered granules, often observed in animals treated with iproniazid and reserpine, may represent an early stage of the reserpine effect.

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