STORAGE OF 5-HYDROXYTRYPTAMINE IN MEGAKARYOCYTES

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INTRODUCTION

The blood platelets of various species, including man, contain specific, highly osmiophilic subcellular organelles storing 5-hydroxytryptamine (5HT). Strong evidence exists that the osmiophilia of the organelles is directly due to the presence of large concentrations of 5HT (1, 2, 3). It has not yet been established whether the 5HT organelles are formed in the circulating blood platelets or whether the megakaryocytes are already capable of storing the amine at specific sites. In fact, except in very rare instances, the megakaryocytes, the stem cells of the platelets, do not contain typical 5HT organelles, even in species, e.g. the rabbit, whose platelets are rich in these ultrastructural elements (2, 3). This paper deals with the problem of whether megakaryocytes are capable of storing exogenous 5HT at specific subcellular sites.

MATERIAL AND METHODS

Treatment of Animals

Guinea pigs weighing 500–700 g and rabbits weighing 2000–2500 g, of mixed sex, were used for the experiments. Part of the animals received 3 single ntraperitoneal doses, each of 230 mg/kg 5HT-creatinine sulfate (corresponding to 100 mg/kg 5HT base) at intervals of 8 hr. Some of the 5HT-treated rabbits were given intraperitoneal injections of reserpine (5 mg/kg), 4 hr after the last 5HT injection. Other rabbits received 4 intraperitoneal doses, each of 100 mg/kg 5-hydroxydopamine[1]-hydrobromide (corresponding to 66 mg/kg base) at intervals of 8 hr. All animals were bled, under light ether anesthesia, through a polyethylene cannula in the carotid artery before sacrifice. The bone marrow of femur, tibia, and humerus was blown out of the bones by a jet of nitrogen immediately after their removal.

Electron Microscopy

Blocks of bone marrow, about 1 mm in diameter, were fixed in a solution containing 3% glutaraldehyde and phosphate buffer 0.1 M, pH 7.0–7.4 at 4°C for 2–4 hr. After fixation the tissue samples were stored overnight in a solution containing 7% sucrose and 0.1 M phosphate buffer, pH 7.0–7.4, at 4°C. Some of the blocks were then fixed with 2% OsO_4 in phosphate buffer 0.1 M, pH 7.0–7.4 for 1 hr, whereas others were treated with a solution of 5% dichromate, pH 4.0, at 4°C for 18 hr without OsO_4 fixation. All the blocks were dehydrated in alcohol and propylene oxide and embedded into Epon. The thin sections of about 500 A were either not contrasted or contrasted with lead citrate or double contrasted with uranium acetate and lead citrate. A Philips EM 300 was used for the examination of the sections.

5HT Analysis

The 5HT of the bone marrow was measured by a spectrophotofluorimetric method (4), and the values were related to the fresh tissue weight. In rabbits the marrow was taken from the femora whereas in guinea pigs a pool of marrow from various bones (femora, tibiae, humeri) was used.

RESULTS

Normal Megakaryocytes

The mature megakaryocytes, i.e. the granular and thrombocytogenic cells, which were the object of the present study showed the characteristic ultrastructural picture described earlier after fixation with glutaraldehyde and OsO_4 (3, 5). No typical 5HT-storing organelles as seen in the circulating platelets (1, 2, 3) could be detected. In both rabbits (Fig. 1) and guinea pigs (Fig. 2), however, the cytoplasm of the megakaryocytes contained a large number of empty-looking vesicles of various sizes and shapes. Vesicles about 1000–2000 A in diameter which contain a tiny, dense osmiophilic core (up to about 200 A in diameter) and are reminiscent of the 5HT storage organelles of the platelets were seen very exceptionally in megakaryocytes of rabbits but were never seen in those of guinea pigs.

5HT Treatment

Megakaryocytes of rabbits (Fig. 3) and guinea pigs (Fig. 4) treated with 5HT were similar in

[1] 3,4,5-trihydroxyphenylethylamine, synthesized by Dr. A. Brossi, Chemical Research Department, F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland.

ultrastructure to those of controls. However, in addition, numerous osmiophilic elements (up to a few hundred per equatorial section of mature megakaryocytes) appeared, which were strikingly similar to the 5HT storage organelles of the blood platelets of the same species (compare Fig. 1 with Fig. 3, and Fig. 2 with Fig. 4). Thus, the organelles of the megakaryocytes had a diameter of 1000–2000 A; they were surrounded by a single membrane and contained a very dense osmiophilic core with a diameter of about 1000–1500 A (Fig. 3). In addition to these dense organelles, numerous empty-looking vesicles of a similar size persisted in the cytoplasm of the megakaryocytes. The vesicles of the Golgi apparatus also remained empty. The α-granules showed no modification in their ultrastructure and, especially, no increase in their osmiophilia when compared to those of the control megakaryocytes. Furthermore, no dense osmiophilic deposits were found in the demarcation membrane system.

In rabbits given reserpine injections after administration of 5HT, the appearance of the strongly osmiophilic organelles was abolished in the megakaryocytes. This treatment did not induce other detectable ultrastructural changes.

The organelles that appeared after 5HT treatment also showed strong, specific electron opacity when fixed with glutaraldehyde and dichromate in the absence of OsO_4. Megakaryocytes of animals not exposed to 5HT did not reveal such structures when fixed by the same technique (compare Fig. 5 with Fig. 6). The electron-opaque organelles that appeared after 5HT exposure resembled in number and size the highly osmiophilic organelles described above. The other fine structural elements of the megakaryocytes were hardly recognizable because they lacked contrast. Only after additional staining with lead citrate did some ultrastructural elements, e.g. ribosomes and glycogen particles, appear. The α-granules were not (or only poorly) contrasted by the dichromate treatment. Apart from megakaryocytes and blood platelets, no other cells of the bone marrow showed an enhanced contrast of their subcellular organelles due to the dichromate treatment, in controls or in 5HT-treated animals.

5-Hydroxydopamine

Treatment of rabbits with 5-hydroxydopamine also induced the appearance in the megakaryocytes of highly osmiophilic organelles (Fig. 7). These organelles closely resembled the osmiophilic organelles that appeared in the megakaryocytes after 5HT treatment as described above. No other ultrastructural changes were seen.

5HT Analysis

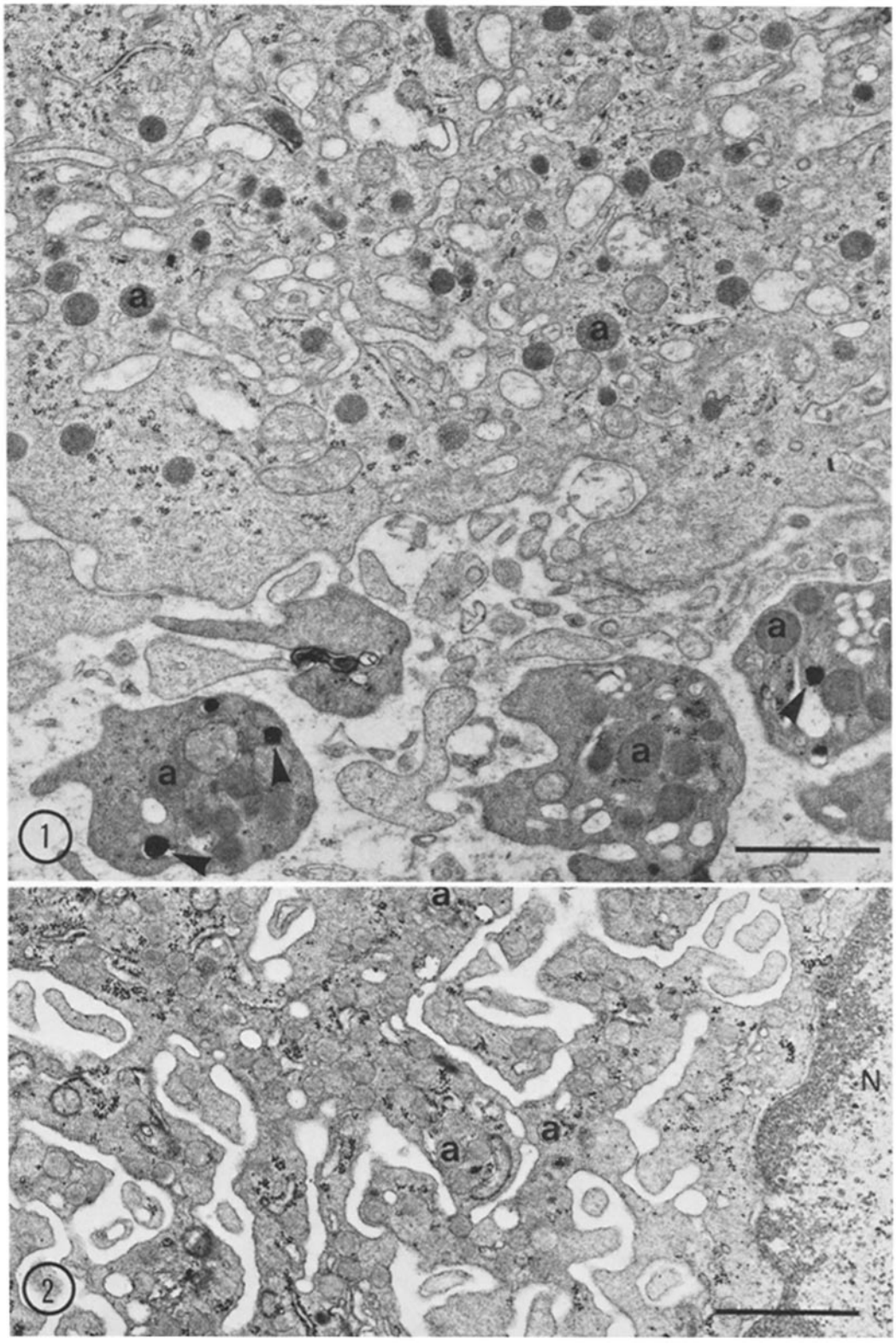
The quantitative analysis of the bone marrow revealed a considerable increase (up to 80-fold in guinea pigs) in its 5HT content in animals treated with this amine. This rise was markedly diminished by reserpine treatment (Table I).

DISCUSSION

The following findings strongly suggest that the osmiophilic organelles that appear in megakaryocytes of rabbits and guinea pigs after exposure to 5HT are storage sites for biogenic amines: (*a*) The organelles appear after exposure of the animals to 5HT; (*b*) 5HT has been shown to reduce OsO_4, under the present experimental conditions, and can therefore be visualized by electron microscopy (1); (*c*) the organelles closely resemble those found in normal rabbit platelets (1, 2). Furthermore, megakaryocytes behave like platelets of guinea pigs, since in both cells similar osmiophilic or-

FIGURE 1 Part of a megakaryocyte and platelets in the bone marrow of a normal rabbit. Note the very dense osmiophilic organelles (►) that represent the 5HT storage sites in the blood platelets. The cytoplasm of the megakaryocyte is completely devoid of such structures. Numerous, typical α-granules (*a*) exist already in the cytoplasm of the megakaryocytes. Their electron opacity is always much lower than that of the 5HT-storing organelles found in the blood platelets. Double fixation with glutaraldehyde and OsO_4. Double staining with uranyl acetate and lead citrate. Bar, 1μ. × 22,000.

FIGURE 2 Part of a megakaryocyte of the bone marrow of a normal guinea pig. Note the absence of the very dense organelles that were seen in the rabbit platelets in the previous figure. α-granules (*a*). Nucleus of the megakaryocyte (*N*). Double fixation with glutaraldehyde and OsO_4. Double staining with uranyl acetate and lead citrate. Bar, 1μ. × 22,000.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ganelles appear after exposure to 5HT. These organelles in the platelets of rabbits as well as guinea pigs have been shown to be major storage sites for 5HT, by combined electron microscopy and biochemical investigations after their isolation from the platelets (6, 7). (*d*) osmiophilic organelles also appear in the megakaryocytes after the animals have been treated with 5-hydroxydopamine. This amine, like 5HT, has been shown to accumulate in the storage organelles of blood platelets and to be easily visualized by electron microscopy (8). (*e*) the organelles that appear in the megakaryocytes after treatment with 5HT can be selectively contrasted by double fixation with glutaraldehyde and dichromate in the absence of OsO_4. It is generally accepted that this technique is cytochemically specific for the detection of biogenic amines by electron microscopy (2, 9). (*f*) reserpine, which abolishes the storage of endogenous and exogenous 5HT in the blood platelets (10, 11), also abolishes the appearance of osmiophilic organelles in the megakaryocytes after their exposure to 5HT, but no other ultrastructural changes are detected. (*g*) the bone marrow of animals exposed to 5HT contains up to 80 times more of this amine than the marrow of controls. Reserpine markedly counteracts this increase of 5HT. However, these changes probably do not occur exclusively in the megakaryocytes. Thus, the exogenous 5HT may also be stored in other elements of the bone marrow, e.g. the platelets, which (especially those of guinea pigs) have been shown to take up considerable amounts of the amine (7, 12, 13).

The origin of the 5HT-storing organelles of the megakaryocytes remains to be further investigated. It is possible that these organelles arise by pinocytosis from the canalicular system which is in continuity with the external cell membrane (5). This is unlikely, however, because a clear continuity of even a single dense organelle with elements of the canalicular system could never be observed. On the other hand, it is conceivable that organelles which contain no or little endogenous 5HT but which are capable of storing this amine if exposed to it preexist in the megakaryocytes. In fact, from the blood platelets of guinea pigs organelles have been isolated which are poor in endogenous 5HT and osmiophilic cores but rich in adenosine triphosphate (ATP). On exposure of the platelets to exogenous 5HT, both the amine content and the number of osmiophilic cores of the organelles markedly increase, probably due to the formation of high molecular weight aggregates between 5HT and ATP (14).

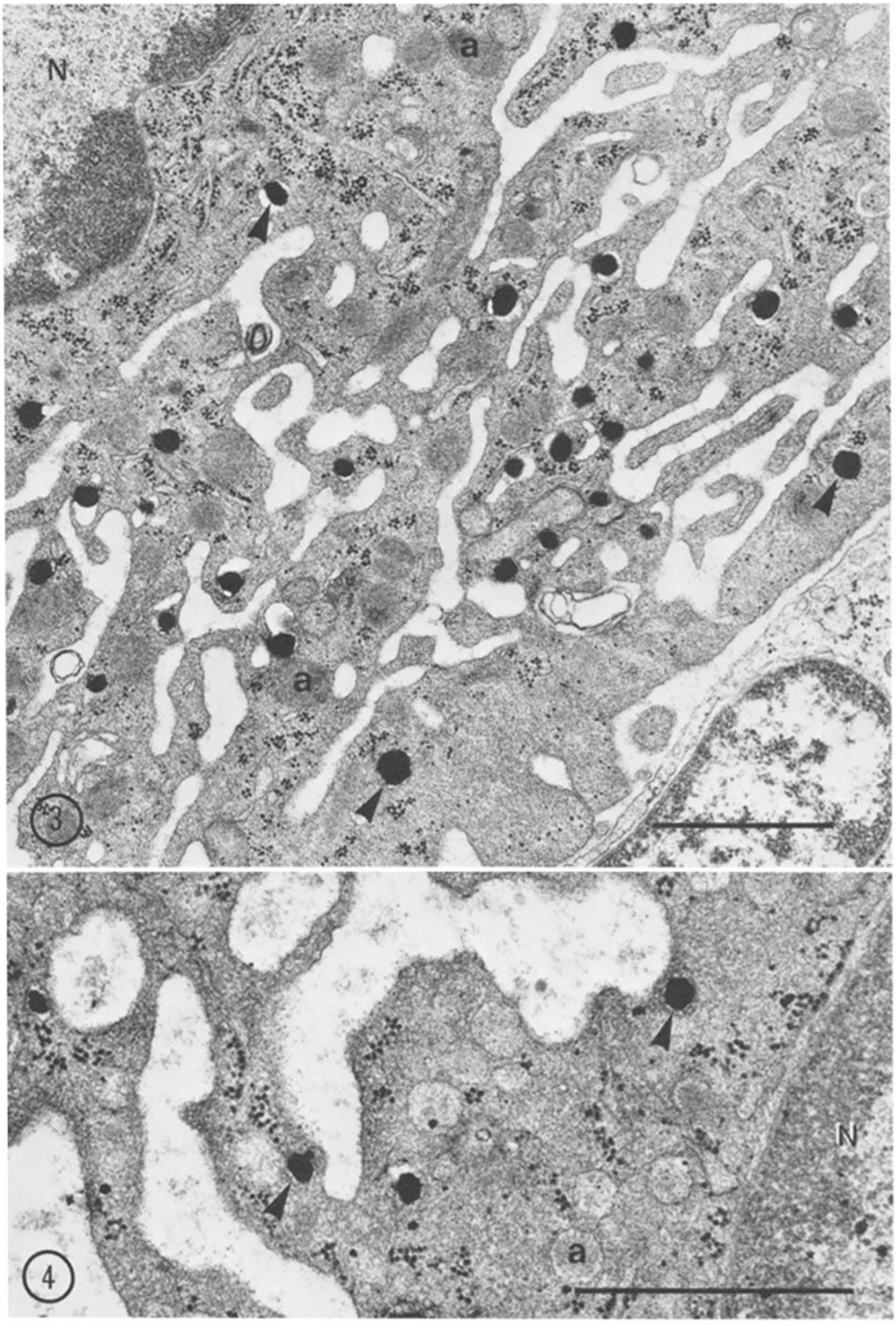
It might thus be speculated, by analogy with the platelets of guinea pigs, that the megakaryocytes contain organelles which store ATP but which are not detectable on electron microscopy. Once the platelets have been severed from the megakaryocytes, they probably take up 5HT in the circulating blood, especially in species like the rabbit. The 5HT might be stored in the preexisting organelles which, as a consequence, would become osmiophilic.

SUMMARY

Administration of 5-hydroxytryptamine (5HT) to rabbits and guinea pigs causes the appearance in the megakaryocytes of numerous organelles, which are highly osmiophilic and also give a positive chromaffin reaction in the absence of OsO_4. The effect of 5-hydroxydopamine is similar to that of 5HT. Administration of reserpine after 5HT counteracts the appearance of osmiophilic organelles in the megakaryocytes and the 5HT increase seen without reserpine in the bone marrow.

FIGURE 3 Part of a megakaryocyte from a rabbit treated with 5HT. Numerous, very dense osmiophilic organelles (►) have now appeared, which closely resemble those found in the platelets of normal rabbits (compare with Fig. 1). All of the other subcellular elements, especially the α-granules (*a*), show no change in their appearance or electron opacity after treatment with 5HT. Nucleus of the megakaryocyte (*N*). Double fixation with glutaraldehyde and OsO_4. Double staining with uranyl acetate and lead citrate. Bar, 1μ. × 28,000.

FIGURE 4 Part of a megakaryocyte of guinea pig treated with 5HT. Note the appearance of similar, very dense osmiophilic organelles (►) which are absent in the megakaryocytes of nontreated animals (compare with Fig. 2). The α-granules (*a*) have a varying electron opacity, which is, however, always much lower than that of the highly osmiophilic organelles that appear after the animals have been treated with 5HT. Nucleus of the megakaryocyte (*N*). Double fixation with glutaraldehyde and OsO_4. Double staining with uranyl acetate and lead citrate. Bar, 1μ. × 44,000.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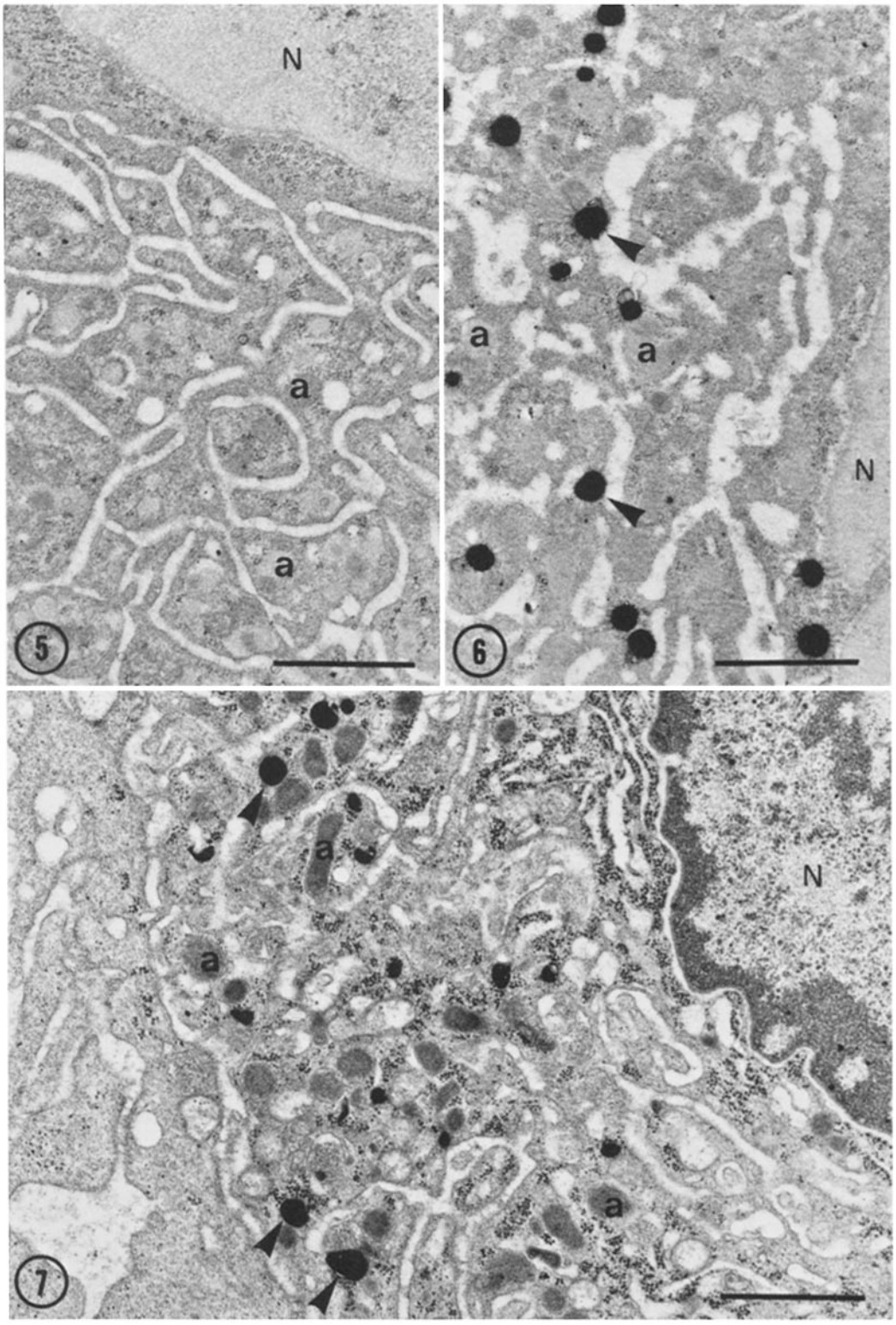
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TABLE I

Effect of 5-Hydroxytryptamine (5HT), Alone or in Combination with Reserpine, on the 5HT Content of the Bone Marrow

Treatment	Rabbits			Guinea pigs
	Femur	Tibia	Humerus	
Controls	0.77 ± 0.06	0.80 ± 0.19	1.40 ± 0.46	0.19 ± 0.05
5HT	8.39 ± 2.51	6.94 ± 1.85	8.37 ± 1.98	16.91 ± 2.56
5HT + reserpine	1.14 ± 0.21	0.80 ± 0.22	0.84 ± 0.34	1.31 ± 0.26

230 mg/kg 5HT-creatinine sulfate (corresponding to 100 mg/kg 5HT base) were injected intraperitoneally at intervals of 8 hr. Reserpine (5 mg/kg) was given intraperitoneally 4 hr after the last 5HT administration. In guinea pigs, a pool of marrow from femora, tibiae, and humeri was used. The figures represent averages (with SE) of 4–9 experiments. The results are expressed in μg 5HT/g fresh bone marrow.

From these findings it is concluded that megakaryocytes store exogenous 5HT in specific subcellular organelles, which may be the precursors of the 5HT-storage organelles in the blood platelets.

This work is dedicated to the memory of Nicole Granboulan.

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FIGURE 5 Part of a megakaryocyte of an untreated rabbit after glutaraldehyde fixation followed by dichromate treatment without OsO_4 fixation. Various ultrastructural elements of the megakaryocyte, such as α-granules (*a*), ribosomes, empty-looking vesicles, the demarcation membrane system, and the nucleus (*N*), are readily recognizable. Single lead citrate stain. Bar, 1μ. × 22,000.

FIGURE 6 Part of a megakaryocyte from a rabbit treated with 5HT, after glutaraldehyde fixation followed by dichromate treatment without OsO_4 fixation. The ultrastructural elements of the cell appear to be like those of Fig. 5, except that numerous, dense chromaffin organelles (►) are present in the cytoplasm. Single lead citrate stain. Bar, 1μ. × 22,000.

FIGURE 7 Part of a megakaryocyte of a rabbit treated with 5-hydroxydopamine. Note the appearance of numerous, highly osmiophilic organelles (►). The very dense organelles closely resemble, in shape, size, and contrast, those found in megakaryocytes after exposure of the animals to 5HT (compare with Fig. 3). The other fine structural elements, e.g. the α-granules (*a*), are not changed by this treatment when compared with megakaryocytes of control animals (compare with Fig. 1). Double fixation with glutaraldehyde and OsO_4. Double staining with uranyl acetate and lead citrate. Bar, 1μ. × 22,000.