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New Aspects on the Storage of 5-Hydroxytryptamine in Blood Platelets

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Blood platelets of many species contain 5-hydroxytryptamine (5HT), which they probably take up mainly from the blood. In fact, in platelets of man and guinea-pigs an active transport of the amine at the level of the cytoplasmatic membrane has been described. Furthermore, the platelets possess an efficient intracellular storage mechanism for 5HT, by which the amine is protected from metabolizing enzymes (like monoamine oxidase). Thus, in isolated platelets incubated for several hours in plasma or artificial media the 5HT content does not markedly decrease and only small amounts of 5HT metabolites such as 5-hydroxyindolacetic acid and 5-hydroxyindolylethanol (5-hydroxytryptophol) are formed[1,2]. The storage of 5HT in platelets resembles in many respects that of other biogenic amines in various cells. For instance the 5HT is stored like the catecholamines of the adrenal medulla in specific subcellular organelles together with nucleotides, e.g. adenosine-5'-triphosphate (ATP) and bivalent cations[3–5]. Furthermore, drugs like reserpine and tyramine interfere with the storage of these biogenic amines[6–8]. The platelets may therefore be considered as relatively simple and well accessible models for studying the storage process of monoamines.

The following presentation summarizes some new aspects of the 5HT storage in platelets and deals with the mechanism of the storage process.

1. *5HT storage sites*

Electron microscopy of isolated rabbit platelets after double fixation with glutaraldehyde and osmium tetroxide reveals the presence of very dense osmiophilic organelles which are clearly distinguishable from the classical ultrastructural elements, such as mitochondria, α-granules, glycogen granules, etc.[9–13]. Up to 10 osmiophilic organelles may be present per thin equatorial section of a platelet. They are spherical, 1000–2000 Å in diameter, surrounded by a single membrane, and contain a very dense osmiophilic core of 500–2000 Å diameter (Figure 1). Platelets of other species, e.g. man, guinea-pigs, cats, rats, show similar organelles, but these are in general fewer than in rabbit platelets. The following findings strongly indicate that the osmiophily of the dense core of the organelles is due to the presence of large amounts of 5HT:

1.1 5HT markedly reduces OsO_4 in vitro under the experimental conditions of the present experiments[9].

1.2 Reserpine and tyramine, which diminish the 5HT content of the platelets by more than 90%, cause a virtual disappearance of the organelles, although the number of the α-granules does not decrease (Figure 1)[9,11].

1.3 Incubation of platelets from reserpinized rabbits with large amounts of 5HT (1000 $\mu g/cm^3$) causes the reappearance of the osmiophilic organelles (Figure 1)[9].

1.4 Fixation of rabbit platelets with a technique specific for the visualization of monoamines by the electron microscope (glutaraldehyde followed by dichromate in the absence of OsO_4)[14] also reveals the presence of organelles. These are strongly electron dense and of the same number and shape as those found after fixation with glutaraldehyde and OsO_4. The other subcellular organelles including the α-granules are not or only poorly contrasted by the glutaraldehyde dichromate technique[10,15].

1.5 Comparison of various species shows that the concentration of 5HT in the platelets closely parallels the number of their osmiophilic organelles[9].

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1.6 ATP and histamine, which also occur in the platelets, do not reduce OsO_4 in vitro under the present experimental conditions. Furthermore, reserpine does not markedly diminish the ATP content of the organelles, but nevertheless these disappear after administration of the drug[9,16].

1.7 Catecholamines, which also reduce OsO_4, are not present in relevant amounts in the platelets[9].

It may therefore be concluded that the dense osmiophilic organelles represent specific storage sites of 5HT in the platelets which are distinct from other subcellular constituents, such as the α-granules.

2. Isolated storage organelles

The storage organelles described above have first been isolated from rabbit platelets[17]. By centrifugation of platelet homogenates in a continuous Urografin® gradient, a bottom fraction (bottom layer) is obtained which on electron microscopy consists of a homogeneous population of vesicle-like structures, the majority of which has a very dense osmiophilic core. They strongly resemble the 5HT organelles described in intact platelets. Virtually no other subcellular particles, such as α-granules and mitochondria, are present in this fraction (Figure 2). Homogenates of guinea-pig platelets centrifuged in the same way as those of rabbit platelets also yield a bottom layer consisting of a pure population of vesicles[18]. These resemble the organelles isolated from rabbit platelets with the exception that only very few of the guinea-pig organelles have an osmiophilic core, whereas the majority looks empty (Figure 2). Organelles of human platelets have not yet been isolated in highly purified form, but according to preliminary findings with partially purified preparations they behave like those of guinea-pigs.

Distribution studies in the various particulate fractions obtained by density gradient centrifugation of the platelet homogenates of rabbits[17] and guinea-pigs[18] as well as preliminary results with human platelets indicate that by far the highest concentration (in μg/μg protein) of 5HT occurs in the bottom fraction consisting of the organelles. The 5HT concentration in the other fractions, including those containing the α-granules and mitochondria, is less than 10% of that found in the bottom fraction (Figure 3). Furthermore, ^{14}C-5HT (Figure 3) as well as ^{14}C-dopamine and ^{14}C-norepinephrine injected i.p. also show a preferential localization in the particulate fraction containing the 5HT organelles[19].

These findings confirm the presence in the platelets of specific organelles storing 5HT. On electron microscopy the highly osmiophilic organelles isolated from rabbit platelets are identical with those detected in the platelets in situ. The isolated organelles of guinea-pigs closely resemble those of rabbits with regard to shape, size and physical density. Furthermore, the few organelles exhibiting a dense osmiophilic core are identical in appearance with the osmiophilic organelles found in intact guinea-pig platelets. The absence of osmiophily

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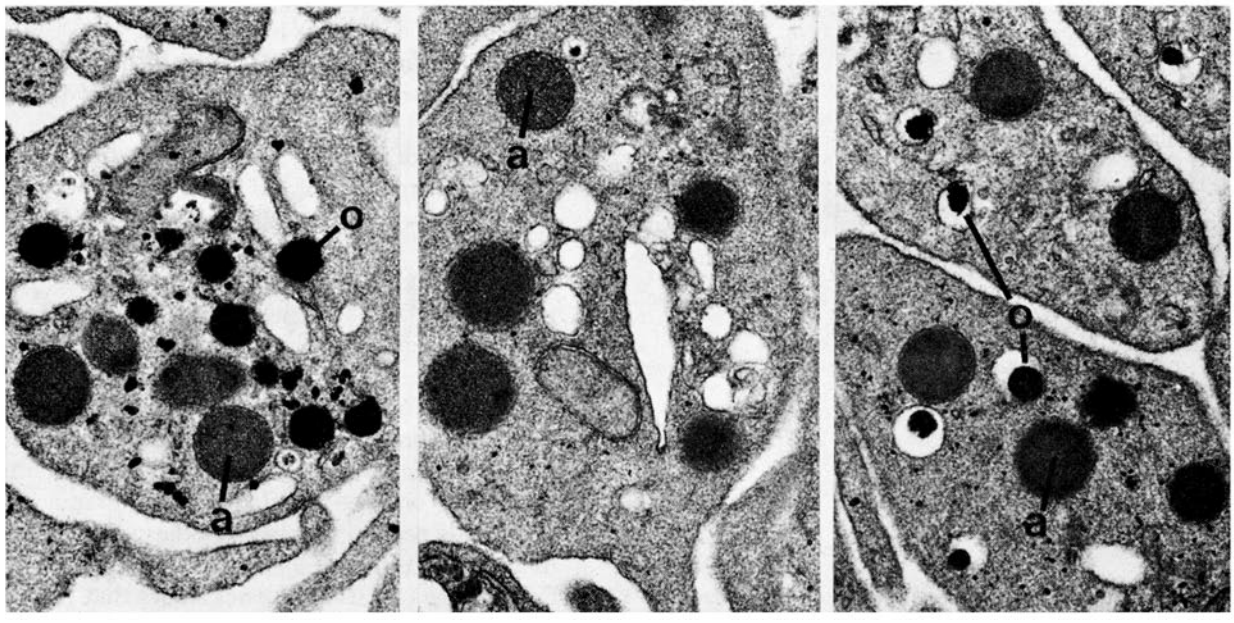

Fig. 1. Electron micrographs of rabbit platelets. Left: platelets of normal animals; middle: 5 mg/kg reserpine i.p. 16 h prior to experiment, right: platelets of reserpinized animals incubated at 37 °C for 2 h with 1000 μg/ml 5HT. a, α-granule; o, osmiophilic 5 HT organelle. ×30,000[36].

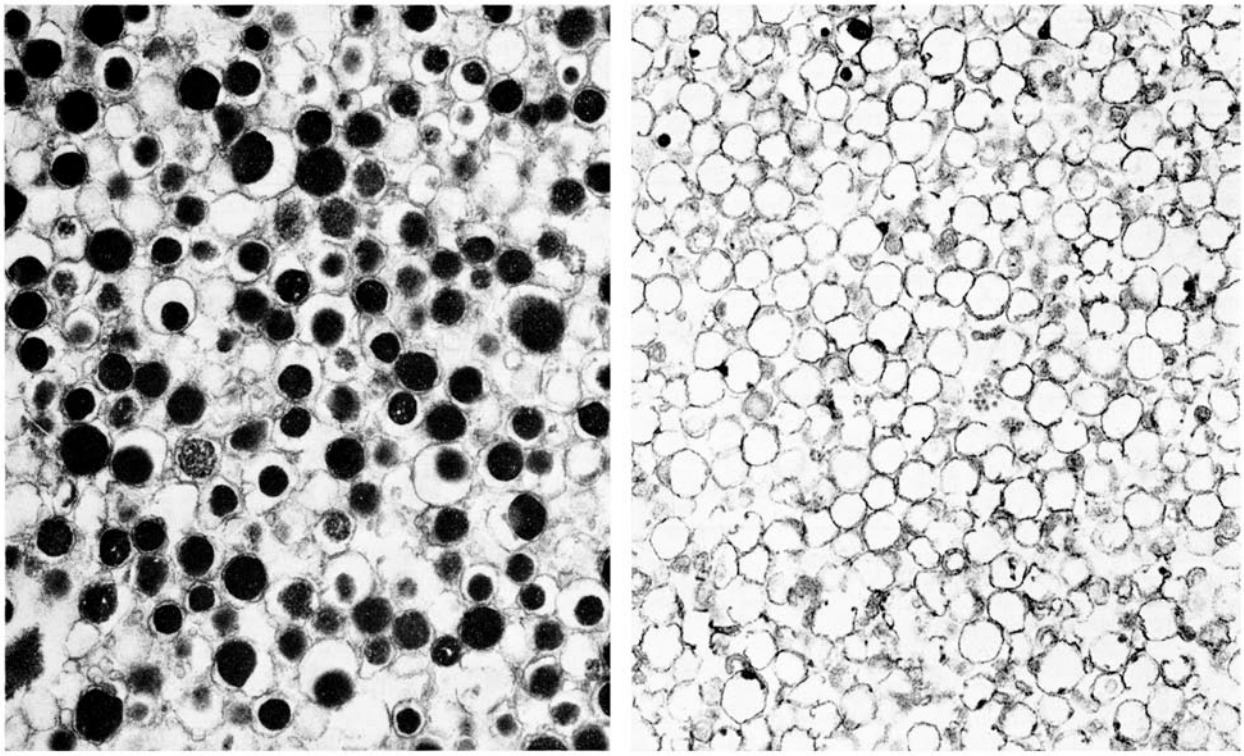

Fig. 2. 5-Hydroxytryptamine organelles from platelets of rabbits (left) and guinea-pigs (right)[18] isolated by density gradient centrifugation. Double fixation with glutaraldehyde and OsO_4. Double stain with uranyl acetate and lead citrate. × 28,000.

in the majority of the organelles obtained from platelets of guinea-pigs is probably not due to technical artefacts[18], but rather to the absence in the organelles of high amounts of 5HT (see below). Therefore, in the platelets in situ, these organelles (although present) cannot be recognized on electron microscopy with the fixation technique presently used. In fact, in platelets of guinea-pigs, in contrast to those of rabbits, dense osmiophilic storage organelles are only rarely seen [9,18]. However, if the platelets of guinea-pigs are exposed to 5HT, the number and size of the osmiophilic cores of the isolated organelles as well as the number of osmiophilic organelles in the intact platelets markedly increase[18].

2.1 *Chemical composition*

The organelles contain mainly 5HT and 5'-phosphonucleotides, the concentrations of which are much higher than in the whole platelets (Table I). Appreciable amounts of bivalent metals, such as Ca and Mg, are also found[3,4,15,18,20]. Their concentration (in μmoles/μg proteins) is at least 50 times higher in the isolated organelles than in the intact platelets[18,21]. Soluble proteins, however, occur only to a minor extent in the organelles (about 4% of the ATP, calculated in μg/μg protein)[21]. The content of 5HT, related to that of the proteins, is about 35 times higher in organelles of rabbits than in those of guinea-pigs[18]. An attempt has been made to measure the volume of the isolated fresh rabbit organelles and to calculate the 5HT concentration in relation to the volume. A value as high as 20% was obtained[22].

The content of nucleotides is very high in organelles of both rabbit and guinea pig platelets (Table I). The majority of nucleotides consists of ATP, but besides other 5-phosphonucleotides are present, such as adeno-

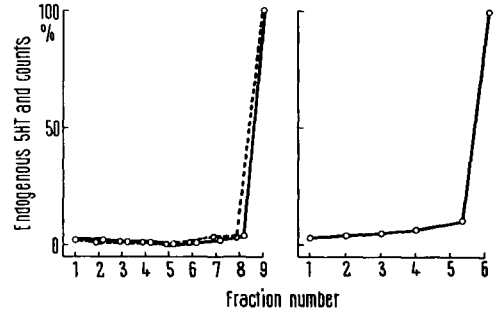

Fig. 3. Distribution of ^{14}C-5-hydroxytryptamine (5HT) and/or endogenous 5HT in platelets of rabbits (left)[19] and guinea-pigs (right) submitted to ultrasonication and subsequent density gradient centrifugation. ^{14}C-5HT (72 μg/kg) was injected i.p. 3 h before isolation of the platelets. The values (calculated as μmoles/μg proteins and counts per μg proteins respectively) found in the organelles (fraction 9 rabbits, fraction 6 guinea-pigs) were taken as 100%. Average of 2 experiments (rabbits) and typical experiment (guinea-pigs). ——, endogenous 5HT; – – –, ^{14}C-5HT.

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Table I. Content of 5-hydroxytryptamine (5HT) and adenosine-5'-triphosphate (ATP) of platelets and organelles of rabbits and guinea-pigs

	Rabbits		Guinea-pigs	
	Platelets	Organelles	Platelets	Organelles
5HT	$9.7 \pm 1.0 \times 10^{-5}$	$210 \pm 17 \times 10^{-4}$	$1.0 \pm 0.0 \times 10^{-6}$	$6.3 \pm 1.9 \times 10^{-4}$
ATP	$5.5 \pm 0.7 \times 10^{-5}$	$85 \pm 9 \times 10^{-4}$	$2.4 \pm 0.3 \times 10^{-5}$	$108 \pm 14.9 \times 10^{-4}$

The values are indicated in μmoles/μg proteins and represent averages with S.E. of 3–7 experiments.

Table II. Percentage amount of 5'-phosphonucleotides in isolated organelles of rabbit and guinea-pig platelets

	Rabbits	Guinea-pigs
ATP	57.0 ± 0.0	66.3 ± 0.8
ADP	13.8 ± 0.6	30.2 ± 1.2
GTP	15.0 ± 1.1	9.7 ± 2.5
UTP	5.0 ± 0.9	4.8 ± 0.1

The total value of the 5'-phosphonucleotides was taken as 100%. Averages with S.E. of 3–4 experiments. ATP, adenosine-5'-triphosphate; ADP, adenosine-5'-diphosphate; GTP, guanosine-5'-triphosphate; UTP, uridine-5'-triphosphate.

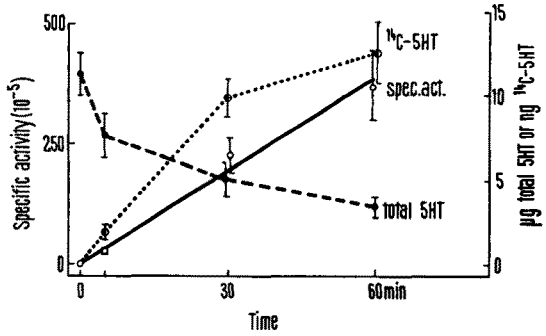
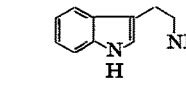
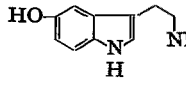
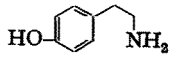
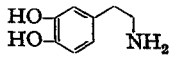
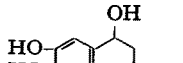
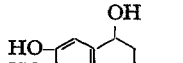
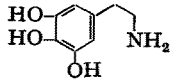


Fig. 4. Spontaneous liberation of endogenous 5-hydroxytryptamine (5HT) and accumulation of ^{14}C-5HT by isolated storage organelles of rabbit platelets incubated in plasma at 37 °C with 0.2 μg/ml ^{14}C-5HT (creatine sulfate monohydrate). Each point represents an average with S.E. of 4–5 experiments[8].

Table III. Accumulation of radioactive amines in isolated 5-hydroxytryptamine (5HT) organelles of rabbit platelets, incubated in plasma at 37 °C for 30 min

Amine		No. of expts.	Accumulation
	Tryptamine	3	10 ± 2
	5-Hydroxytryptamine	14	100 ± 9
	Tyramine	2	13 ± 3
	Dopamine	3	68 ± 2
	Epinephrine	4	36 ± 4
	Norepinephrine	6	29 ± 3
	5-Hydroxydopamine	3	13 ± 1

Concentration of the amines in the incubation fluid 0.57 μ*M*. The values represent averages with S.E. and are expressed in per cent of the ^{14}C-5HT accumulated by the organelles in the same experiment. Absolute accumulation of ^{14}C-5HT in ng/μg endogenous 5HT = 0.91 ± 0.08 (14 experiments)[25].

sine-5'-diphosphate (ADP), guanosine-5'-triphosphate (GTP) and uridine-5'-triphosphate (UTP) (Table II). No monophosphonucleotides have been found; they seem to be localized in other platelet compartments. The nucleotide pattern differs somewhat in organelles of rabbits and guinea-pigs, especially since the latter contain more ADP in relation to the nucleotides (Table II)[20,23].

The molar ratio 5HT to ATP shows marked differences in the two types of organelles. It amounts to 2.5 in those of rabbits, but only to 0.06 in guinea-pigs[18]. The findings with organelles of guinea-pigs therefore indicate that the ATP can be stored without major amounts of 5HT being present.

Organelles of rabbits also contain appreciable amounts of histamine ($90 \pm 11 \times 10^{-4}$ μmoles/μg protein)[3], whereas the organelles of guinea-pigs and probably those of man are very poor in this amine. The histamine will not be discussed in the following chapters.

2.2 *Incubation experiments*

Isolated 5HT organelles of rabbit platelets incubated in plasma or artificial media show a progressive diminution of their endogenous 5HT (Figure 4) and ATP which, between 5 and 90 min, is almost linear with time. The 5HT seems to decrease somewhat more markedly than the ATP. The reason for this rapid decline, which does not occur in isolated intact platelets, is not clear. It might be connected with the fact that the organelles have been separated from their physiological medium, i.e. the cytoplasm of the platelets. On the other hand, the isolated organelles are still capable of accumulating radioactive 5HT from the incubation medium (Figure 4), whereas labelled mono-, di- and triphosphonucleotides do not enter the organelles in appreciable

[23] M. DA PRADA and A. PLETSCHER, Biochem. J. *119*, 117 (1970).

amounts[3, 24]. Therefore, the membrane of the organelles seems to be permeable for exogenous 5HT, but not for nucleotides.

The amount of radioactive amine penetrating from the incubation medium into the isolated organelles markedly decreases with diminishing temperature[3]. It is also highly dependent on the chemical structure of the amine. Of all the amines tested, 5HT enters best into the organelles (Table III)[25]. Up to now, no evidence exists that this dependence on temperature and on chemical structure is the consequence of an active, carrier mediated transport at the level of the membrane of the organelles. Thus, absence from the incubation medium of metabolic substrates like glucose, presence of metabolic inhibitors like fluoride and iodoacetate or ouabain do not decrease the penetration of labelled 5HT into the isolated organelles[25]. The temperature sensitivity of the amine penetration as well as its dependence on the chemical structure of the amines may therefore be rather due to physico-chemical properties of the membrane of the organelles or to the different capacity of the various amines to form high molecular weight aggregates with nucleotides (see below).

Drugs which interfere with the transport and storage of 5HT in the platelets affect the organelles in different ways. For instance, imipramine, which considerably inhibits the uptake of 5HT by isolated platelets, does not markedly interfere with the penetration of the amine into the isolated organelles. In contrast, reserpine, which liberates 5HT from the platelets, inhibits the accumulation of ^{14}C-5HT in the isolated organelles[3]. In rabbit platelets, this drug shows a preferential localization at the level of the storage organelles. This is also the case for tyramine, another monoamine-liberating substance (Figure 5). However, reserpine mainly accumulates in the membranes of the organelles, whereas tyramine is preferentially found in their interior[26] (Table IV).

The mode of action of reserpine in liberating 5HT from the platelets is not clear. According to the present results, the drug does not cause disruption of the 5HT organelles. Thus, after pretreatment of rabbits with reserpine, density gradient centrifugation of the platelet homogenates (carried out as indicated above) yields a bottom layer consisting of a pure population of vacuole-like organelles. These contain dense osmiophilic cores only in rare instances, but have the same shape, size and physical density as the organelles isolated from untreated controls. Furthermore, the reserpinized organelles still contain a large amount of ATP (about 65% of controls), but a negligeable concentration of 5HT[16]. Therefore, reserpine seems to liberate 5HT rather selectively without causing other ultrastructural changes in the organelles. The preferential localization of reserpine

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[25] M. Da Prada and A. Pletscher, Life Sci. *8*, 65 (1969).
[26] M. Da Prada and A. Pletscher, Experientia *25*, 923 (1969).

Table IV. Distribution of ^{3}H-reserpine and ^{14}C-tyramine in isolated 5-hydroxytryptamine organelles of rabbit platelets

	Drugs	Membrane	Supernatant
in vitro	Reserpine	76.0 ± 4.8	24.0 ± 4.8
	Tyramine	3.0 ± 0.8	97.0 ± 0.8
in vivo	Reserpine	91.5 ± 1.1	8.5 ± 1.1
	Tyramine	1.8 ± 0.2	98.2 ± 0.2

In the in vitro experiments, isolated platelets were incubated with 350 µg/cm^3 ^{14}C-tyramine or 2 µg/cm^3 ^{3}H-reserpine for 1 h in plasma at 37 °C. In the in vivo experiment, ^{14}C-tyramine (0.4 mg/kg i.v.) and ^{3}H-reserpine (1 mg/kg i.p.) were injected 1 and 16 h resp. before exsanguination. The figures represent averages with S.E. of 3–4 experiments and are indicated in percentage of the radioactivity contained in the whole organelles[26].

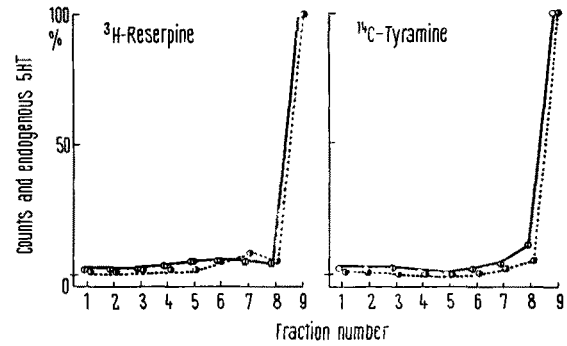

Fig. 5. Subcellular distribution of ^{3}H-reserpine and ^{14}C-tyramine in blood platelets of rabbits in comparison with the endogenous 5-hydroxytryptamine. ^{14}C-tyramine and ^{3}H-reserpine were injected 1 and 16 h respectively before exsanguination. The endogenous 5-hydroxytryptamine (5HT) as well as the radioactivity were calculated in µg/µg proteins and are indicated in per cent of the respective values found in the organelles (= 100%). Fraction 9 represents the pure 5HT organelles. Each point of each curve represents an average with S.E. of 2–4 experimental values[26]. ——, radioactive substances; ..., endogenous 5HT.

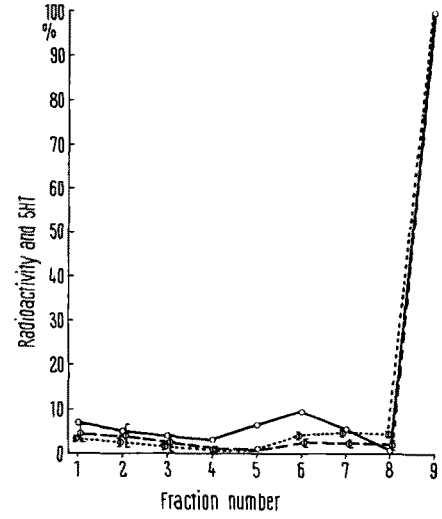

Fig. 6. Subcellular distribution of ^{14}C-adenosine- or ^{14}C-guanosine-5′-triphosphate (ATP) in storage organelles of rabbit platelets. The platelets were incubated in modified Tyrode solution at 37 °C for 2 h with 0.5 µg/ml ^{14}C-adenosine or 0.5 µg/ml ^{14}C-guanosine. The radioactivity as well as the endogenous 5-hydroxytryptamine (5HT) were related to the proteins and indicated in per cent of the respective values found in the organelles (fraction 9)[24]. – – –, ^{14}C-ATP (averages with S.E. of 3 experiments); ——, guanosine-5′-triphosphate (1 experiment); ..., endogenous 5HT (averages with S.E. of 3 experiments).

in the membranes of the organelles might indicate an action at this level. In contrast, tyramine seems to interfere with the 5HT-ATP metal aggregates present within the organelles, as will be discussed below.

The 5HT organelles of platelets also store newly synthesized nucleotides. Thus, after incubation of rabbit platelets with ^{3}H-adenine or ^{14}C-adenosine, small but significant amounts (about 1% of the total ATP) of labelled ATP are found in the interior of the isolated organelles. Similarly, incubation of the platelets with ^{14}C-guanine or ^{14}C-guanosine induces the appearance of small amounts of ^{14}C-GTP in the organelles. Of all the particulate fractions of the platelets, the concentration of labelled triphosphonucleotides is by far the highest in the 5HT organelles (Figure 6) which do not, however, contain labelled mono- and diphosphonucleotides[24]. The radioactive triphosphonucleotides seem to be formed outside, but stored inside the organelles, since the isolated organelles are not able to synthesize triphosphonucleotides from precursors like adenine or guanine and their nucleosides.

The mode of transport of the labelled triphosphonucleotides into the storage organelles has not yet been clarified. The appearance of newly synthesized nucleotides in the organelles might indicate that under normal conditions their storage capacity for nucleotides is not fully saturated. Furthermore, the possibility has to be considered that the triphosphonucleotides of the organelles of rabbit platelets have a certain turnover, which would be in contrast to findings with human platelets[27].

3. *Storage mechanism for 5HT and nucleotides*

According to the findings indicated above, the content of low molecular weight substances, e.g. 5HT, nucleotides and bivalent cations, is very much higher in the storage organelles than in their surrounding medium, i.e. the cytoplasm of the platelets. The concentration in the organelles is such that these could not be osmotically stable if 5HT and ATP were present in a monomolecular form or even as mixed salts or complexes of low molecular weight. Experiments with the analytical ultracentrifuge have shed some light on the possible mode of storage of the low molecular weight constituents of 5HT organelles. Thus, the contents of isolated 5HT organelles (after removal of their membrane) show very high apparent average molecular weights which markedly increase with diminishing temperature and rising concentration of the solutes (Figure 7)[28]. This indicates the formation of aggregates. Since the 5HT organelles contain very little protein, but large amounts of 5HT, ATP and bivalent cations, the aggregates probably consist mainly of these low molecular weight compounds.

Experiments with artificial solutions confirm this view. In aqueous solutions of ATP or 5HT alone, aggregation occurs to a small extent only. However, if bivalent metals like Ca and Mg are added to the ATP solution, the apparent molecular weights markedly increase (Figure 8). This increase is further enhanced with rising concentration of the solutes, with diminishing temperature, and it also depends on the molar ratio of ATP to bivalent cations. On cooling, a second bottom phase separates which probably consists of aggregates of very large size. It is transparent, highly viscous and contains about 4–5 times as much ATP as the upper phase[29]. Bivalent cations added to a solution of 5HT do not cause an appreciable increase of the average apparent molecular weight. However, on addition of 5HT to a solution of ATP + small amounts of bivalent cations,

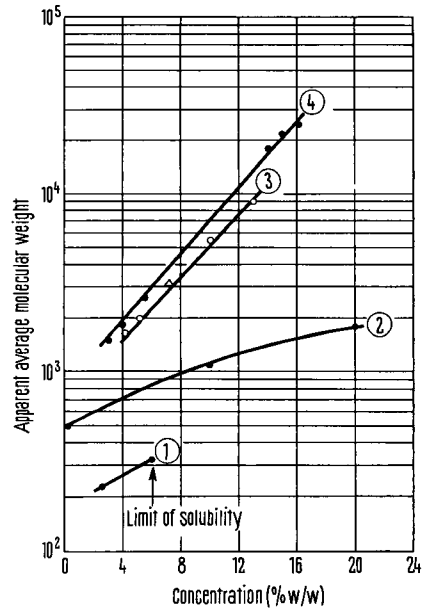

Fig. 7. Dependence of the apparent average molecular weight on the concentration[28].
1. 5-hydroxytryptamine (5HT) (as oxalate or hydrochloride); 2. adenosine-5′-triphosphate (ATP); 3. 5HT + ATP, molar ratio 2:1; 4. content of isolated storage organelles of rabbit platelets.

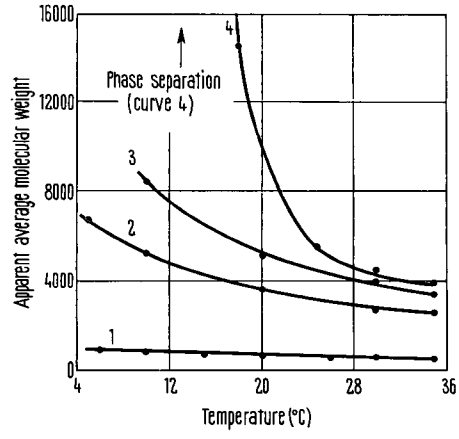

Fig. 8. Apparent average molecular weights of a 12% w/v aqueous solution of adenosine-5′-triphosphate (ATP)[30]. Curve 1: without additives. Curve 2: addition of $MgCl_2$ and $CaCl_2$, molar ratios to ATP: 0.8 and 0.2 respectively. Curve 3: addition of 5-hydroxytryptamine (5HT), $MgCl_2$ and $CaCl_2$, molar ratios to ATP: 1, 0.8 and 0.2 respectively. Curve 4: addition of 5HT, $MgCl_2$ and $CaCl_2$, molar ratios to ATP: 2, 0.8 and 0.2 respectively. The pH was adjusted to 6.

the apparent average molecular weight further increases. This increase is enhanced with rising concentration and diminishing temperature (Figure 8) and reaches its maximum at a molar ratio of 5HT to ATP of about 2. Again, a second, highly viscous and transparent bottom phase separates on cooling (Figure 9) which probably consists of aggregates of very large size and which contains 5HT, ATP and bivalent cations in high concentrations[28, 30, 33]. It can therefore be assumed that ATP-metal aggregates are capable of incorporating 5HT, forming 5HT-ATP-metal aggregates.

5HT and ATP together also aggregate in the absence of bivalent cations. The aggregation is markedly enhanced by small and moderate amounts of bivalent cations, and the second phase already separates at higher temperatures than without the metal ions. Ca is more effective than Mg. Very large amounts of bivalent cations, however, cause disaggregation and disappearance of the bottom phase so that the solution becomes homogeneous again (Figure 9)[4, 21, 28, 33]. It thus seems possible to change the concentration of 5HT between the two aqueous phases by proper adjustment of the concentration of the alkaline-earth metal ions.

Various amines, e.g. dopamine and norepinephrine, also markedly aggregate with ATP, especially in the presence of Ca and Mg. However, other amines like tyramine and amphetamine show very little tendency for aggregation[4, 31, 34] and cause disruption of aggregates formed in vitro between monoamines (e.g. 5HT or noradrenaline), ATP and bivalent metals. Furthermore, tyramine and amphetamine induce the dissolution of the bottom phase obtained on cooling of a solution containing monoamines, ATP and bivalent metals. Dopamine has no disaggregating effect[34]. It is of interest that tyramine and amphetamine cause liberation of 5HT from intact platelets in vitro[7]. Whether these drugs act by disruption of the 5HT-ATP aggregates within the 5HT organelles remains to be elucidated.

Nucleotides like ADP and GTP also form aggregates with monoamines and/or bivalent cations, whereas UTP exhibits this property only to a minor extent[23, 29, 30].

The *molecular structure* of the ATP aggregates in the presence of bivalent cations has been investigated on the basis of nuclear magnetic resonance spectra. Accordingly, ATP seems to aggregate by vertical stacking of

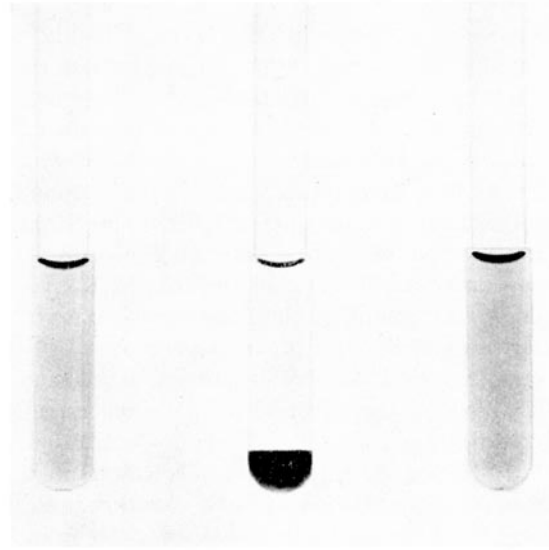

Fig. 9. Effect of bivalent cations on the formation of a second phase in aqueous solution containing 15% w/w adenosine-5′-triphosphate (ATP) and 5-hydroxytryptamine (5HT). Molar ratio 1:2.3. Left: no addition of bivalent metals, middle: addition of 0.25 mol $CaCl_2$ (per mol amine), right: addition of 0.25 mol $CaCl_2$ and 2 mol $MgCl_2$ (per mol amine)[33].

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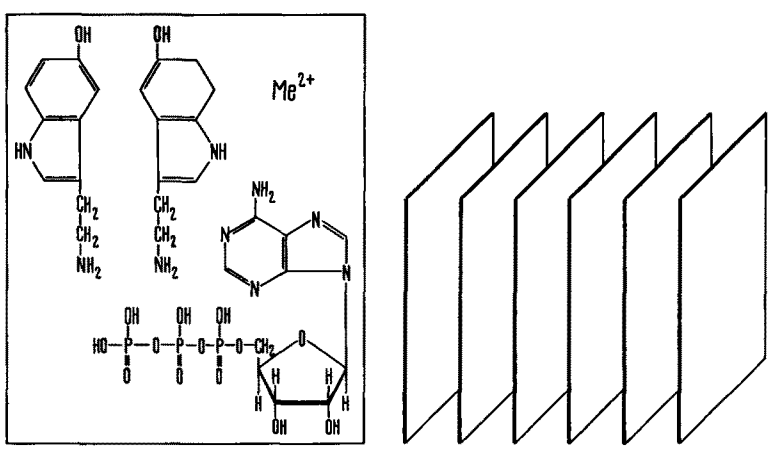

Fig. 10. Hypothetical structure of aggregates formed by 5-hydroxytryptamine, adenosine-5′-triphosphate and bivalent cations.

the aromatic rings, probably due to van der Waals forces[29]. It may be anticipated that in the presence of 5HT plus ATP subunits (consisting of 1 mol ATP and 2–3 moles 5HT) are formed which also aggregate by vertical stacking (Figure 10).

4. *Megakaryocytes*

Megakaryocytes do not show typical highly dense osmiophilic 5HT storage organelles, not even in species, e.g. the rabbit, whose platelets are rich in these subcellular structures[10]. The megakaryocytes contain a large number of empty-looking vesicles, however, which in rabbits exhibit, exceptionally, a tiny, dense osmiophilic core of about 100 Å in diameter[15] (Figure 11). Repeated i.p. injections of 5HT to rabbits and guinea-pigs cause the appearance in the cytoplasm of the megakaryocytes of numerous osmiophilic elements which show striking similarities with the 5HT organelles of blood platelets of the same species. Thus, these organelles have a diameter of 1000–2000 Å, are surrounded by a single membrane and contain very dense osmiophilic cores with a diameter of about 1000–1500 Å (Figure 11)[15]. Reserpine, which interferes with the 5HT storage, abolishes the appearance of osmiophilic organelles in megakaryocytes in rabbits pretreated with 5HT and also diminishes the 5HT rise of the bone marrow. These observations and other findings discussed elsewhere[15] indicate that the presence of dense osmiophilic cores in megakaryocytes after 5HT treatment is due to the storage of the amine in preexisting organelles. The capacity of the preformed organelles of the megakaryocytes to store exogenous 5HT may be explained by the presence in the organelles of nucleotides and bivalent cations with which the amine forms aggregates of high molecular weight. Since nucleotides and bivalent metals cannot be visualized by the presently used electron microsopic techniques, the preformed storage organelles of normal megakaryocytes are only recognizable on electron microscopy when they have accumulated exogenous 5HT.

5. *Concluding remarks*

The results presented above strongly indicate that in the platelets the 5HT is stored in specific subcellular organelles which are distinctly different from other ultrastructural elements, e.g. the α-granules. These specific organelles probably preexist already in the megakaryocytes, but do not yet contain relevant amounts of 5HT. Under normal conditions, megakaryocytes may have very little contact with circulating 5HT, since the great majority of the amine which reaches the blood (e.g. from the enterochromaffin cells) is taken up and stored in the circulating platelets which have an efficient uptake mechanism for 5HT at the level of the cytoplasmatic membrane. Only if the quantity of 5HT to be taken up by the platelets exceeds their storage capacity (e.g. after repeated i.p. administration of 5HT) do relevant amounts of the amine reach the megakaryocytes, where they are stored in the preexisting organelles.

Good evidence also exists that the 5HT of the platelet organelles is stored, as high molecular weight aggregates with nucleotides and bivalent cations. This seems to be the reason why the organelles are osmotically stable despite their high content of low molecular weight substances (5HT, nucleotides, bivalent metals). In addition, the physico-chemical properties of the 5HT-nucleotide aggregates probably explain some biological findings at the level of the storage organelles, e.g. the uptake of monoamines without an active transport process being involved, and possibly the liberation of 5HT by some drugs (e.g. tyramine). Furthermore, the biphasic effect of bivalent cations on the amine-nucleotide aggregates may also be of biological significance. The role of the small amounts of proteins found in the organelles remains to be elucidated. Simple adsorption of the 5HT and nucleotides to the proteins without prior aggregation of these low molecular weight compounds can be excluded on thermodynamic grounds. Intragranular proteins might, however, stabilize the 5HT-nucleotide-metal aggregates[35].

Based on the above-mentioned findings, it may be speculated that the process of 5HT storage in the blood platelets proceeds in the following way: The storage organelles are probably formed already in the megakaryocytes. Originally these organelles do not contain relevant amounts of 5HT, but may have a relatively large content of nucleotides, such as ATP which occur in the form of high molecular weight aggregates with bivalent cations. Once the platelets have been severed from the stem cell, they become exposed to 5HT in the circulating blood. The amine, taken up into the platelets by an active transport mechanism at the level of the cytoplasmatic membrane, penetrates into the storage organelles whose membrane is freely permeable for 5HT. Inside the organelles, the amine is probably incorporated into the preexisting nucleotide-metal aggregates, whereby amine-metal-nucleotide aggregates are formed. As a result of this incorporation, the concentration gradient of the free amine between the external medium (cytoplasm) and the interior of the organelles would be maintained. In consequence, more of the 5HT might enter the organelles and be incorporated into the nucleotide-metal aggregates leading to an accumulation of 5HT. Since amines seem to be reversibly bound to the nucleotides[30,31], a state of equilibrium between aggregated and non-aggregated 5HT molecules may exist in the organelles, whereby the majority of the 5HT is probably present in the aggregated form and only very little in monomolecular solution. This might be the physico-

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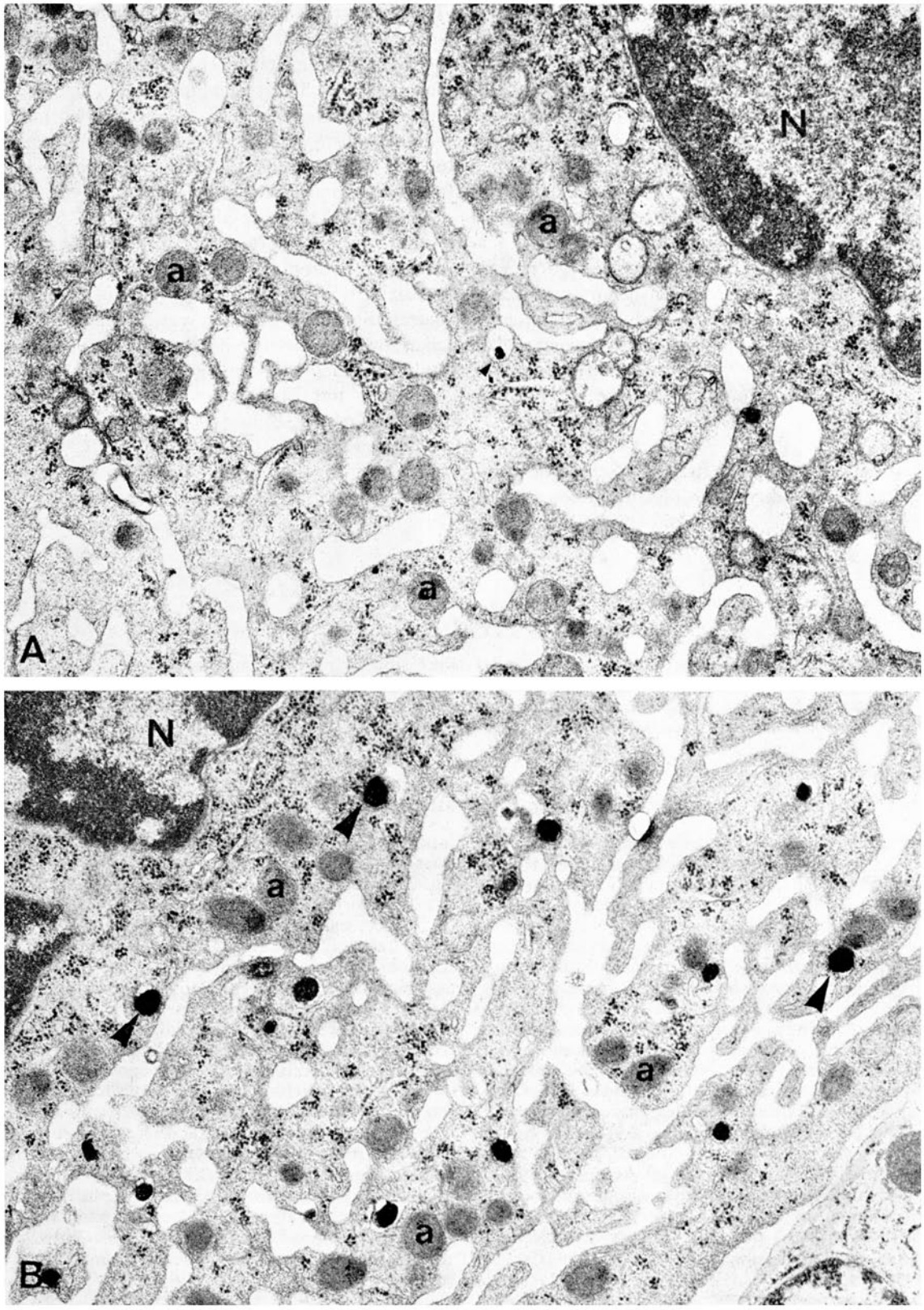

Fig. 11. Parts of megakaryocytes of the bone marrow of rabbits: normal animal (A), animal pretreated with 5-hydroxytryptamine (5HT) (B). In the cytoplasm of the megakaryocyte of the 5HT-pretreated animal, numerous very dense osmiophilic organelles (▶) have appeared which closely resemble those found in the platelets of normal rabbits (compare with Figure 1). Such organelles are absent in the megakaryocyte of the normal rabbit (A); note, however, the single tiny, highly osmiophilic core in one vacuole (▶) which is an exceptional finding. All the other subcellular elements, especially the α-granules (a), did not not change their aspect by the treatment with 5HT. N, nucleus of the megakaryocyte. Double fixation with glutaraldehyde and OsO_4. Double stain with uranyl acetate and lead citrate. ×28,000.

chemical explanation for the finding that the 5HT stored in platelets is freely exchangeable with exogenous 5HT[32].

The platelets of rabbits probably take up more 5HT during their life span than those of guinea-pigs. Thus, in mature rabbit platelets, most of the organelles seem to be virtually saturated with 5HT, whereas in guinea-pigs and possibly in man this is not the case, because the majority of these organelles do not show osmiophily on electron microscopical examination.

Formation of high molecular weight aggregates with nucleotides and bivalent metals in specific subcellular organelles seems to be a general principle for the storage of biogenic amines. Thus, evidence for an aggregation of catecholamines and ATP has also recently been obtained in chromaffin granules of bovine adrenal medulla[35].

Zusammenfassung. Blutplättchen verschiedener Spezies speichern 5-Hydroxytryptamin zusammen mit Nukleotiden (hauptsächlich Adenosin- und Guanosin-5'-Triphosphat) und bivalenten Kationen (Ca und Mg) in spezifischen, subzellulären Organellen, welche elektronenoptisch eine starke Osmiophilie aufweisen. Speicherstellen für 5HT scheinen bereits in den Megakaryozyten vorhanden zu sein, da in diesen Zellen nach Zufuhr von exogenem 5HT osmiophile Organellen erscheinen, die denjenigen der Plättchen entsprechen. Die Aufnahme von biogenen Monoaminen durch isolierte Speicherorganellen hängt von der chemischen Struktur der Amine und der Temperarur ab; es bestehen keine Anhaltspunkte für einen aktiven 5HT-Transport.

Analytische Ultrazentrifugation des Inhalts von isolierten Speicherorganellen von Kaninchen-Blutplättchen sowie von artifiziellen Lösungen ergibt, dass 5HT wahrscheinlich in Form hochmolekularer, gemischter Aggregate mit Nukleotiden und bivalenten Kationen akkumuliert. Die Bildung solcher Aggregate erklärt die osmotische Stabilität sowie möglicherweise andere biologische Eigenschaften der Speicherorganellen vor allem bezüglich Aufnahme von 5HT und dessen Freisetzung durch gewisse Substanzen, z. B. Tyramin.

SPECIALIA

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Oriented Overgrowth (Epitaxy) of a Polyamide Model Biopolymer on Non-Identical Polyamides

With the epitaxy of polyethylene on polyoxymethylene as well as on Nylon[6] (poly-ε-caprolactam) the first epitaxial association of non-identical macromolecular compounds was described[1,2]. These previous results suggested the idea that epitaxy of the natural polyamides, the proteins, and other natural macromolecular compounds might be of potential significance in biological ultrastructures and in the molecular mechanisms of biological processes[1,2]. In view of these thoughts epitaxy of non-identical polyamides seems to be a point of particular interest.

As non-identical polyamides the polyamide model biopolymer poly-γ-benzyl-L-glutamate (PBLG) as a deposit and a drawn double oriented film of Nylon 6 as a substrate were chosen. Both polyamides proved to be especially suitable for studying epitaxy, since PBLG is soluble in solvents in which Nylon 6 is insoluble. Under biological aspects it is remarkable that the molecules of PBLG-crystals oriented on alkali halides are reported to be in α-helix conformation[3].

The PBLG obtained from Dr. SCHUCHARDT, Munich, had an indicated molecular weight of 200,000–400,000. In the Nylon film the hydrogen-bonded (001) sheets were oriented approximately parallel to the plane of the film.

Initial attempts to obtain oriented crystals of PBLG on the surface of N 6-films using xylene or tetrachloroethylene as solvents were unsuccessful. In contrast thereto and in accordance with previous experience[3] crystallization from mesitylene gave the desired result. Following the experimental method by which epitaxy of PBLG on alkali halides had been obtained[3], 6 cm³ of a highly concentrated solution (1.7×10^{-3} g PBLG/ml) were placed in a test tube immersed in a thermal oil bath heated to 110 °C for crystallization. A film of Nylon 6 was suspended in the crystallizing medium for 10 min. Upon removal, the substrate film carrying the overgrowth of PBLG was dried at ambient temperature.

The PBLG was oriented on the film surface in the form of long-needle-like objects, often bent in a characteristic manner, the long needle axis being preferably inclined about $60 \pm 2°$ to the drawing direction of the film, i.e. to the axis of the Nylon 6 macromolecules. These needles were observed only in one position from 'bottom left to top right' in accordance with the symmetry of the hydrogen-bonded (001) sheets of the surface of the substrate film (Figures a and b). Beside these needles others were formed with the long axis parallel or perpendicular to the drawing direction of the substrate film. The average thickness of the needles was about 2000 to 3000 Å, in contrast to PBLG crystals oriented on alkali halides, which have a thickness of about 600 Å at the utmost[3].

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