one having a posture characteristic of sleep, with its eyes closed, crouching or lying with the other dormant mice. In contrast to our report on hibernating mollusks (*4*), the term "dormant" does not imply any hibernating mechanism, and the term "active" is used here to describe an animal that is moving in the cage, eating, and drinking.

Within 5 seconds after being removed from the cage, the animals were killed by cervical dislocation and decapitated. The whole brain was removed, blotted, weighed, and homogenized in ice-cold 0.2*M* perchloric acid. The steps from decapitation through homogenization were performed in about 1 minute for each brain. The determination of piperidine has been described (*4*). The whole homogenate was submitted to dansylation (dimethylaminonaphthalenesulfonyl chloride) (*6*). The dansyl derivatives were extracted into an organic solvent and separated by one-dimensional chromatography on silica gel with the solvent system cyclohexane–butyl acetate (2 : 1). The fraction which cochromatographed with standards of dansylated piperidine was scraped off and its piperidine content was measured by the mass spectrometry (*7*). A known quantity of dansylated pyrrolidine was added as an internal standard to the sample, and the mixture was introduced into the mass spectrometer (AEI model MS-902) and "flash" evaporated. The ion currents corresponding to dansylated pyrrolidine (*m/e* 304) and dansylated piperidine (*m/e* 318) were recorded with the use of the peak matching circuit of the spectrometer at a resolving power of about 2000 (Fig. 1). The actual quantity of piperidine in each sample was calculated from the area ratio of recorded currents between the known quantity of pyrrolidine and the unknown quantity of piperidine.

The piperidine concentration in the brain rose during dormancy to 36.6 ± 6.35 pmole/mg ($N = 10$), compared to 2.00 ± 0.55 pmole/mg ($N = 10$) found in active animals ($P < .001$). The weight of the brain was unchanged during the same interval (dormant: 405 ± 11 mg; active: 395 ± 8.3 mg). This unexpected increase in apparent piperidine concentration in the dormant mouse brain exceeded by several times any previously reported concentration of piperidine in the brain, or in any of its assayed components (*2–4*). The piperidine content found in the brain of active mice, however, concurs well with the measurements of others. It should be noted that our values have not been corrected for losses during extraction and thin-layer chromatography.

It is premature to discuss this finding in view of the scarcity of data on the direct effect of piperidine on mammalian nerve cells (*8*). The known pharmacological effects are mostly only peripheral or peripherally induced (*9*), and there is no reliable information as to the passage of administered piperidine either from the blood or from the cerebrospinal fluid into the brain tissue (*10*). However, in view of the similarity between piperidine action (*1*), its accumulation in dormant mollusks (*4*), and our data, one might expect that piperidine will be somehow connected with the sleep-wakefulness mechanisms in the mouse.

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Serotonin Storage in Platelets: Estimation of Storage-Packet Size

Abstract. *Storage-body diameter and volume, and the number of molecules of serotonin contained in a storage body, were estimated for blood platelets. In the human, 5.23 × 10^5 molecules of serotonin are contained in a storage body 198 nanometers in diameter, while in the cat, 31.2 × 10^5 molecules of this amine are contained in a storage body 298 nanometers in diameter.*

In platelets, serotonin is believed to be stored in discrete, membrane-bound organelles ("dense bodies"), probably complexed with adenosine triphosphate (ATP), divalent ions, and possibly a small amount of protein (*1*). Platelet serotonin, ATP, and divalent ions are released by thrombin in what appears to be an all-or-none process with a rapid time course (*2*). This release process may occur by exocytosis of the contents of individual storage bodies, in a fashion analogous to amine release from vesicles in the adrenal medulla and noradrenergic nerves (*3*).

Table 1. Serotonin storage in human and cat platelets (mean ± standard error of the mean).

Item	Human	Cat
Platelet		
Serotonin (10^6 molecules)	3.65 ± 0.28	35.4 ± 4.1
Storage bodies (No.)	6.4 ± 0.3	12.1 ± 0.6
Storage body		
Serotonin (10^5 molecules)	5.23 ± 0.69	31.2 ± 6.4
Diameter (nm)	198 ± 6	298 ± 7
Volume (10^6 nm^3)	6.69 ± 0.77	16.3 ± 1.1

In chemically transmitting nerve endings, it has proved useful to define a "storage packet" as the amount of transmitter released from a single membrane-limited vesicle. Adopting this terminology, we sought to estimate storage-packet size in serotonin-containing storage bodies from isolated platelets, in order to assist in the pharmacological and physiological evaluation of platelet function. In view of the many similarities between platelets and other amine-storing systems (*4*), the results may contribute to our understanding of secretory processes in general.

Platelet-rich plasma (PRP) and platelet pellets were prepared from human and cat blood (*5*). Portions of each sample were taken for platelet counts (*6*) and serotonin assay (*7*). In order to

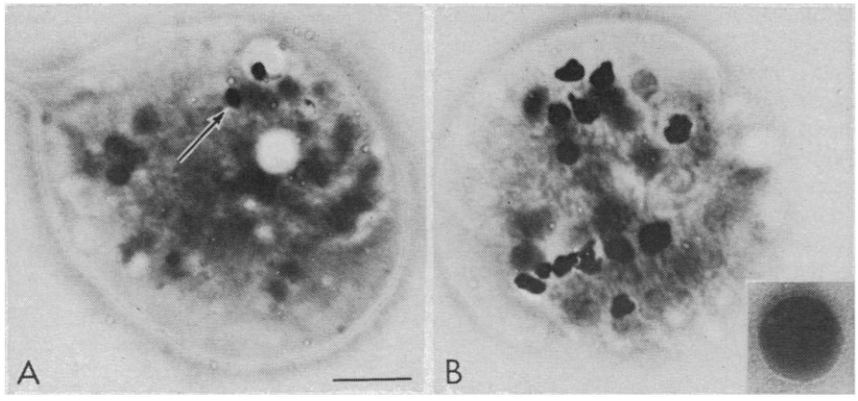

Fig. 1. Unfixed, unstained platelets, photographed at 30 kv (marker, 1 μm). (A) Human platelet. A dense body is indicated by the arrow. (B) Cat platelet. Several dense bodies are apparent. (Inset) A single dense body from a human platelet. The edge of the dense body is sharply delimited by an electron-lucent border (seen best at the lower portion of the body), which may represent the negative image of a membrane.

calculate the number of storage bodies per platelet, PRP aliquots from each sample were dried on coated grids and examined unstained (*8*). When viewed at an accelerating voltage of 30 kv, platelets from both species contained electron-opaque structures believed to represent serotonin storage bodies (*9*) (see Fig. 1, A and B). Counts were made of dense bodies in 100 platelets from each sample.

Assuming that almost all of the platelet serotonin is stored in dense bodies, and that it is equally distributed between storage sites (*1*), we calculated average values of 5.23×10^5 molecules of serotonin per storage body in the human platelet, and 31.2×10^5 molecules in the cat platelet (Table 1). The discrepancy between storage-packet values for human and cat platelets led us to investigate the relative volumes of the storage bodies in the two species, in order to determine whether serotonin packet size corresponds to actual storage-body volume. Since storage-body profiles were often irregular in both types of platelets, we cut out profiles from 20 randomly selected platelet micrographs. The profiles were weighed and compared to the weights of circles of known diameter cut from the same paper (*10*). It was then possible to calculate that human storage bodies have a mean diameter of 198 nm and are significantly smaller than cat storage bodies, which have a mean diameter of 298 nm (*11*). That dense bodies were not flattened when dried on the grids was determined by tilting them in the electron microscope.

The accuracy of these figures was checked by measuring the diameters of the membranes and cores of storage bodies in serial sections of conventionally fixed and embedded platelets (*8*). The membrane profiles in the cat averaged 330 nm, whereas the size of the cores averaged 116 nm. Thus, we are probably describing a membrane-bound vesicle in our characterization of the platelet storage body (Fig. 1, inset).

Using the diameters obtained, we calculated the average volume of the human platelet storage body to be 6.69×10^6 nm^3, and that of the cat to be 16.3×10^6 nm^3 (Table 1). Thus the greater amount of serotonin stored in the cat storage body corresponds to its larger volume. Furthermore, because the difference in the amount of serotonin stored appears somewhat larger than the difference in storage-body volume, it is possible that differences exist in the molar ratios of serotonin, ATP, and divalent ions contained in the storage bodies of the two species (*11*). It is known, for example, that the molar ratio of serotonin to ATP in isolated storage bodies of guinea pig platelets is 0.06, while the ratio in storage bodies of rabbit platelets is 2.5 (*12*).

Because of methodological limitations, it has not yet been possible to calculate storage-packet size in cholinergic or adrenergic nerve endings with techniques as accurate as those used here (*13*). Nevertheless, in view of the relation between packet size and storage-body volume in platelets, the estimate of 1.2×10^4 molecules of noradrenaline (*14*) in a storage body 50 nm in diameter (*15*) seems reasonable. As more accurate measurements become available for other systems, the nature of the relation between storage-body volume, storage-packet size, and the postulated quantal nature of the neurotransmitter release process can be determined.

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