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THE DISTRIBUTION OF 5-HYDROXYTRYPTAMINE AND ADENOSINETRIPHOSPHATE IN CYTOPLASMIC PARTICLES OF THE DOG'S SMALL INTESTINE

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The distribution of 5-hydroxytryptamine, adenosinetriphosphate, and succinic dehydrogenase in sucrose homogenates of the dog's small intestine has been studied. The adenosinetriphosphate was present in two different layers which could be separated by density gradient centrifugation. The upper layer contained also much succinic dehydrogenase, but no amine; it is probably composed of mitochondria. The lower layer contained not only adenosinetriphosphate but also the major portion of the particle-held 5-hydroxytryptamine. The mean molar ratio, amine : adenosinetriphosphate, in the lower layer was 2.6. The experiments suggest that adenosinetriphosphate in the intestine is of importance in the storage of 5-hydroxytryptamine, resembling the function of adenosinetriphosphate in the storage of the catechol amines of the adrenal medulla.

Many observations suggest that the pharmacologically active amines are stored in specific cell organelles which can be sedimented by centrifugation of tissue homogenates prepared in isotonic sucrose. Earlier work from this laboratory has shown that some of the 5-hydroxytryptamine present in homogenates of the dog duodenal mucosa can be sedimented by the use of a sucrose density gradient; by this method a considerable separation of mitochondria and 5-hydroxytryptamine-storing elements has been achieved (Baker, 1958, 1959).

In their storage location, amines seem to be associated with acidic compounds. For instance, in the mast cells histamine is found together with heparin, and in the chromaffin granules of the adrenal medulla the catechol amines occur together with adenosinetriphosphate. The work of Born (1956a; 1956b) and of Born and Gillson (1957) suggests that adenosinetriphosphate is also associated with 5-hydroxytryptamine in the platelets.

In most mammalian species the bulk of the 5-hydroxytryptamine is found in the epithelial lining of the gastro-intestinal tract. Information on the intracellular localization of the amine in this tissue is still far from complete. This is because the cells which carry the 5-hydroxytryptamine represent only a small fraction of those present in the mucous membrane. It seems likely, therefore, that the granular material rich in 5-hydroxytryptamine as prepared by Baker (1959) is still heterogeneous, with the elements carrying the amine contaminated by other structures. In the present work an attempt has been made to achieve a further purification and to find out if the tissue contains adenosinetriphosphate, and how it is distributed among the fractions isolated.

METHODS

Isolation and Purification of Cytoplasmic Granular Fractions

The procedure adopted followed that of Baker (1958; 1959), except that pentobarbitone sodium was used instead of ether as anaesthetic. Dogs were bled, the small intestine was taken out and the mucous membrane was removed and homogenized in 0.3 M sucrose. The portion used always included the duodenum and extended in different experiments to a varying degree to the lower parts of the small intestine. Unbroken cells, nuclei and coarse cell debris were removed by low-speed centrifugation at 900 *g* for 20 min. The supernatant from the low-speed centrifugation was spun at high speed, 11,000 *g* for 30 min., in order to sediment the amine-carrying material. This sediment was resuspended in 0.3 M sucrose and layered over a sucrose density gradient prepared the previous day and spun at 100,000 *g* for 1 hr. in the swinging bucket rotor SW 39L of the Spinco

ultracentrifuge. The fractions obtained were separated by the help of the Schuster cutter and those found rich in 5-hydroxytryptamine and adenosinetriphosphate were diluted with 0.3 M sucrose, to obtain a final molarity of about 1.0 M. They were then again layered over a sucrose density gradient. After a second centrifugation at 100,000 *g* for 1 hr. the fractions were separated and analysed for 5-hydroxytryptamine and adenosinetriphosphate and also for succinic dehydrogenase activity.

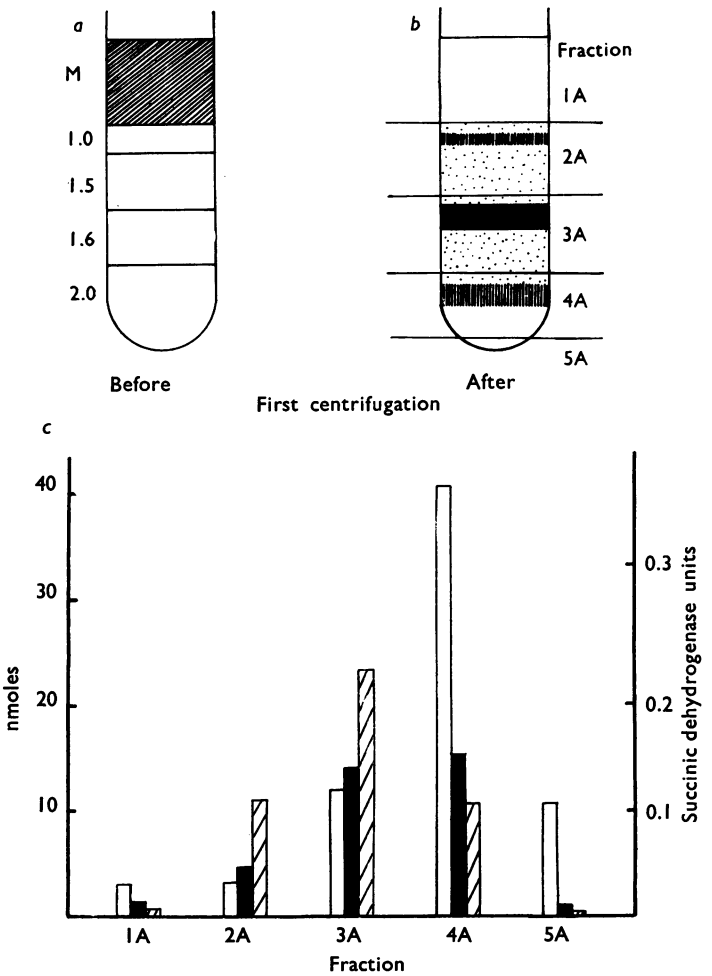

FIG. 1.—First density gradient centrifugation of cytoplasmic particles from the dog's small intestine. (1*a*) Density gradient before centrifugation, with the resuspended large cytoplasmic particles in 0.3 M sucrose at the top of the gradient (shaded area). (1*b*) Diagrammatic representation of the appearance after centrifugation for 60 min. at 100,000 *g*. The numbers at the right refer to the fractions obtained by cutting the tube. (1*c*) Distribution of 5-hydroxytryptamine (white columns), adenosinetriphosphate (black columns), and succinic dehydrogenase (shaded columns) in the particulate fractions.

The unit of succinic dehydrogenase activity is the amount of enzyme that will give an increase in optical density of 1.0 in 10 min.

Methods of Analysis

5-Hydroxytryptamine was extracted with *n*-butanol by the method of Udenfriend, Weissbach, and Clark (1955), as modified by Cargill-Thompson, Hardwick, and Wiseman (1958) and Baker (1959), and assayed on the rat gastric fundus preparation (Vane, 1957). Adenosinetriphosphate was estimated by the firefly luminescence method, as used by Holton (1959). Succinic dehydrogenase activity was determined by measuring the rate of reduction of cytochrome C, as described by Kuff and Schneider (1954).

RESULTS

The separation of the resuspended cytoplasmic particles in a sucrose density gradient is shown in Fig. 1a and 1b. The gradient was adapted from that described by Baker (1959), but the intermediate steps were chosen so as to make the distance between the different particulate fractions convenient for separation. There were three main particulate fractions, one a few mm. below the top of the gradient (2A), a second near the boundary of the 1.5 M and the 1.6 M sucrose (3A), and a third layer below the 1.6 M sucrose (4A).

The distribution of the 5-hydroxytryptamine, adenosinetriphosphate and succinic dehydrogenase in the various fractions is shown in Fig. 1c. The distribution of succinic dehydrogenase and of 5-hydroxytryptamine was essentially as found by Baker (1959); the mitochondrial enzyme had a maximum in fraction 3A, whereas the 5-hydroxytryptamine had a maximum in fraction 4A. The uppermost granular layer (2A) was poor in both enzyme and 5-hydroxytryptamine.

The distribution of adenosinetriphosphate differed from that of the two other constituents. Little was recovered in fraction 2A; the adenosinetriphosphate present was approximately equally divided between the other two particulate

fractions, 3A and 4A. In other experiments the mitochondria (3A) contained up to 3 times the amount of adenosinetriphosphate found in the dense particulate fraction (4A).

In order to determine whether adenosinetriphosphate was associated with the 5-hydroxytryptamine-containing granules, experiments were performed in which the fraction rich in 5-hydroxytryptamine (fraction 4A of Fig. 1b) was diluted with 0.3 M sucrose and again centrifuged in a sucrose density gradient. The gradient used in this centrifugation is shown in Fig. 2a, and the appearance of the gradient tube at the end of the centrifugation in Fig. 2b. The gradient differed from that used in the first gradient centrifugation in that the layer of 1.0 M sucrose at the top of the gradient was omitted. This layer was unnecessary, since the molarity of the resuspended particulate material was adjusted to approximately 1.0 M. Equal volumes of 1.5 M, 1.6 M, and 2.0 M layers of sucrose were used (Fig. 2a).

The distribution of the particulate elements after a centrifugation for 1 hr. at 100,000 *g* is shown in Fig. 2b. There were two layers, of which the larger one, fraction 4B, was present in a level equal in density to that from which the material had been obtained in the first density gradient centrifugation (fraction 4A of Fig. 1b).

Fig. 2c shows the distribution of the components in the different layers. In this figure the % distribution of the material recovered is given. It can be seen that fraction 4B was again rich in both adenosinetriphosphate and 5-hydroxytryptamine, but succinic dehydrogenase activity was still associated with this fraction. Unlike fraction 1A of the first density gradient, the top fraction, 1B, contained about one-quarter of the 5-hydroxytryptamine and also of the succinic dehydrogenase activity; this indicates that some release had accompanied the dilution with 0.3 M sucrose of the particulate material from fraction 4A, which had been recovered in strongly hypertonic sucrose. This is analogous to observations on the chromaffin granules of the adrenal medulla (Hillarp and Nilson, 1954; Eade, 1958).

A second experiment was carried out in which use was made of the experience gained from the results just described. An initial high-speed

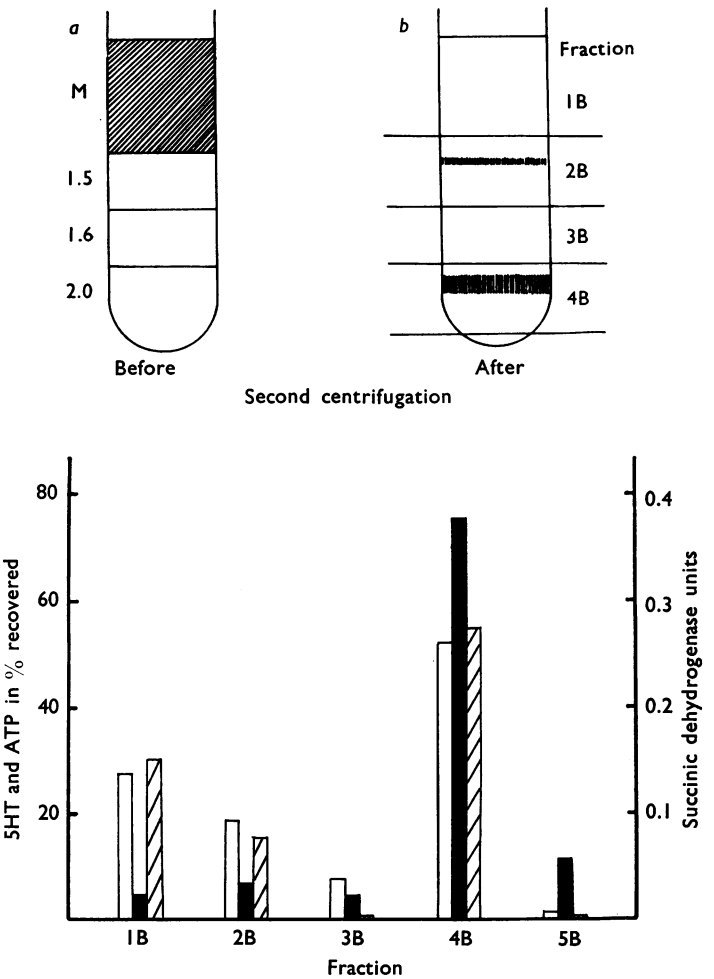

FIG. 2.—Second density gradient centrifugation of the granular fraction (4A) rich in 5-hydroxytryptamine and adenosinetriphosphate obtained in the gradient shown in Fig. 1*b*. (2*a*) Gradient before centrifugation. (2*b*) Appearance of the gradient after centrifugation for 60 min. at 100,000 *g* and position of the fractions collected. (2*c*) Determination of 5-hydroxytryptamine (white columns), adenosinetriphosphate (black columns), and succinic dehydrogenase (hatched columns) in the different fractions. Note that in this diagram 5-hydroxytryptamine and adenosinetriphosphate are expressed in terms of the total material recovered.

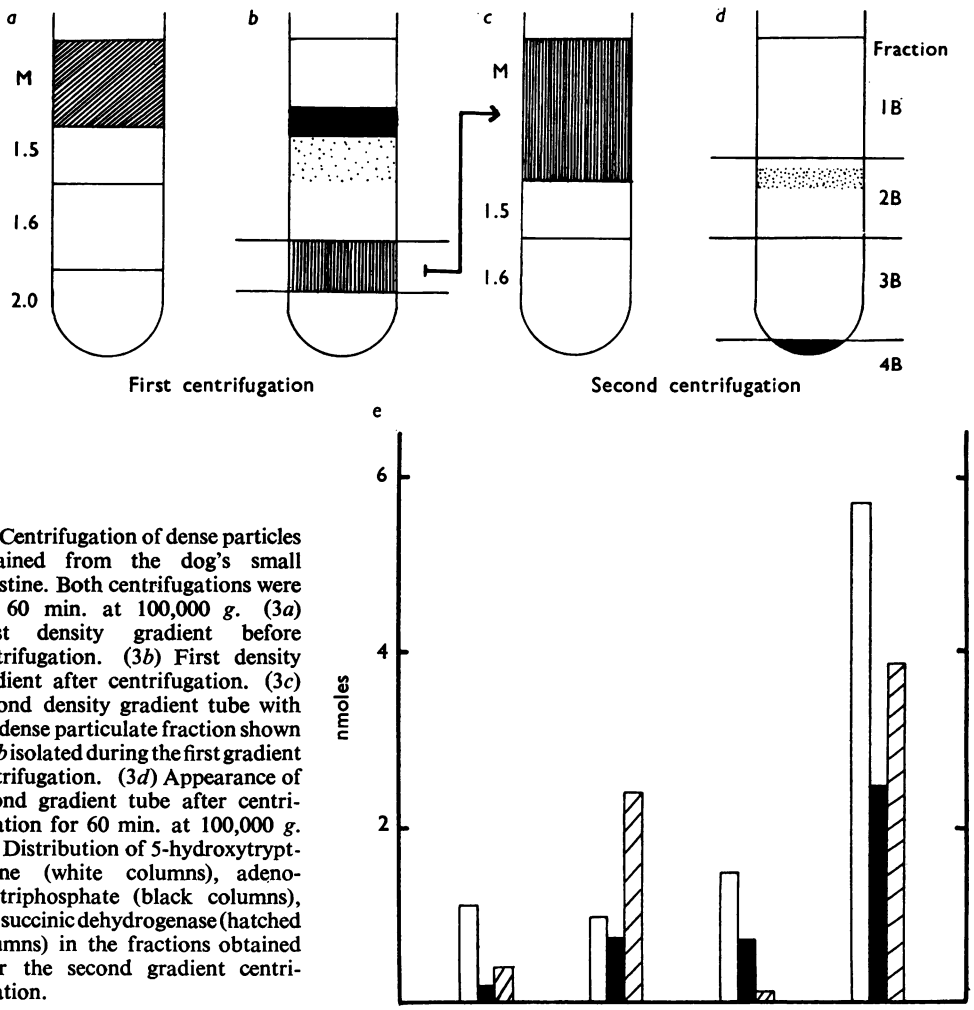

FIG. 3.—Centrifugation of dense particles obtained from the dog's small intestine. Both centrifugations were for 60 min. at 100,000 *g*. (3*a*) First density gradient before centrifugation. (3*b*) First density gradient after centrifugation. (3*c*) Second density gradient tube with the dense particulate fraction shown in 3*b* isolated during the first gradient centrifugation. (3*d*) Appearance of second gradient tube after centrifugation for 60 min. at 100,000 *g*. (3*e*) Distribution of 5-hydroxytryptamine (white columns), adenosinetriphosphate (black columns), and succinic dehydrogenase (hatched columns) in the fractions obtained after the second gradient centrifugation.

centrifugation in 0.3 M sucrose was again followed by two density gradient centrifugations of the resuspended high-speed sediment. The first sucrose gradient (Fig. 3a) was set up with 1.5 M, 1.6 M, and 2.0 M sucrose. The volumes were chosen so as to obtain a separation of the dense layer rich in 5-hydroxytryptamine from the other particulate layers of lower density. Fig. 3b shows that a satisfactory separation of this layer in the lower part of the tube was achieved.

The dense layer obtained in the first density gradient centrifugation was isolated and again diluted with 0.3 M sucrose to a molarity of 1.0 M. This suspension was layered over a second density gradient as shown in Fig. 3c. The gradient did not contain a layer of 2.0 M sucrose at the bottom of the tube; this layer was omitted in order to obtain a sedimentation of particulate material of highest density.

Fig. 3d shows that a sediment was obtained in the second density gradient centrifugation. As in the preceding experiment some opaque material was also present at the top of the gradient.

Fig. 3e shows the composition of tube at the end of the second gradient centrifugation. The sediment, which probably contributed only 1% of the total volume of the tube, contained 63% of the total 5-hydroxytryptamine and also 63% of the total adenosinetriphosphate. The sedimentation of both 5-hydroxytryptamine and adenosinetriphosphate clearly shows that both substances were associated with a particulate fraction. It may be noted that the sediment also contained some succinic dehydrogenase activity.

Molar Ratio, 5-Hydroxytryptamine : Adenosinetriphosphate

Three experiments were carried out in which 5-hydroxytryptamine and adenosinetriphosphate were determined in the fractions obtained after the first density gradient centrifugation. The mean figure for total 5-hydroxytryptamine, as recovered in all these fractions, was 8.7 ± 2.8 nmoles/g. of fresh tissue; the corresponding figure for adenosinetriphosphate was 6.0 ± 2.1 nmoles: this gives a molar ratio of 1.5. The mean 5-hydroxytryptamine content of the dense particle fraction was 4.1 ± 0.9 nmoles, the mean adenosinetriphosphate content 1.6 ± 0.4 nmoles; thus the mean molar ratio was 2.6. This increase in the molar ratio is accounted for by the fact that some adenosinetriphosphate was removed without a concomitant loss of 5-hydroxytryptamine. It seems likely that the adenosinetriphosphate removed was present in mitochondrial elements.

Discussion

The work reported in this paper confirms and extends previous observations, according to which a substantial part of the intestinal 5-hydroxytryptamine is present in a structural element that can be sedimented by high-speed centrifugation of a sucrose homogenate. Density gradient centrifugation was successful in achieving a partial separation of the particle-held 5-hydroxytryptamine from succinic dehydrogenase, but even with the higher degree of purification now achieved some enzymic activity was still found in the fraction that was rich in 5-hydroxytryptamine. Preliminary studies by electron microscopy of the fractions obtained revealed the presence of fragmented mitochondria; it is probable, therefore, that the succinic dehydrogenase is associated with these fragments.

The main interest of the present experiments lies in the fact that the fractions rich in 5-hydroxytryptamine invariably also contained similar amounts of adenosinetriphosphate. In the adrenal medulla, molar ratios of amine:adenosinetriphosphate of about 4 have been reported, and it is of interest that the figure of approximately 3 found in the present experiments is not very different from the ratio in the adrenal medulla. At the present stage of our knowledge the mechanism by which the 5-hydroxytryptamine is held at its site of storage is unknown, but the present experiments show that enough adenosinetriphosphate is present to assign to it a role similar to that which it plays in the storage of the catechol amines in the chromaffin tissue and in the adrenergic neurone.

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