is not in accord with Udenfriend's hypothesis[12] that a particle contains all of the enzymes necessary for catecholamine synthesis.

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The influence of aerobic and anaerobic incubations on the uptake of serotonin by blood platelets*

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THE ABILITY of blood platelets to concentrate serotonin against large gradients is well established,[1] and it appears that this is due to an energy-requiring transport mechanism.[2, 3] Various lines of evidence lead to this conclusion: for example, uptake is inhibited by low temperature,[4, 5] by certain metabolic inhibitors,[6] and by drugs such as reserpine.[5] On the other hand, there are contradictory data in the literature with respect to the effect of anaerobiosis on the uptake of serotonin. Weissbach and Redfield[2] showed, for example, that a nitrogen atmosphere clearly suppressed the uptake of serotonin by platelets. Stacy[7] and Hughes and Brodie[8] claimed that the uptake of serotonin was equal in aerobic and anaerobic conditions.

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In this paper, we present evidence that shows serotonin uptake by blood platelets is suppressed by anaerobiosis, but that the suppression is dependent on both the length of incubation and on the concentration of serotonin used. That is, the uptake of serotonin by platelets incubated aerobically and anaerobically is different only when the gradient across the platelet membrane is of the appropriate order and when the platelets are incubated for sufficiently long periods. Moreover, data are presented which also suggest that the suppression of serotonin uptake in anaerobic incubations is not due to a simple depletion of ATP.

METHODS

Platelet-rich plasma was prepared from blood collected from volunteers into 1/24 vol. of chilled 5% disodium EDTA by centrifuging at 130 *g* for 20 min at 0°. The platelets were kept in melting ice until used and were exposed only to siliconized glass or cellulose nitrate throughout. Platelets were counted with a Coulter model B electronic particle counter according to Bull *et al.*[9] Platelets were counted before and after each experiment to rule out cell destruction.

Prior to the addition of serotonin, aliquots of platelet-rich plasma (from a single donor) were placed in a 37° bath and gassed with oxygen or nitrogen containing 5% CO_2 until the temperature rose to 37° (10–15 min) and for an additional 30 min. Serotonin dissolved in saline was added, and aliquots for analysis were taken at intervals through a separate piece of tubing without changing the atmosphere in the tubes.

The platelets were sedimented by centrifuging at 4500 *g* for 10 min. During early experiments, the serotonin content of the pellets was determined by fluorometry after isolating the amine on carboxymethylcellulose according to Contractor.[10] In subsequent experiments, the uptake of serotonin was followed by using ^{14}C-serotonin. The sedimented pellet was transferred to a tube containing 0·2 ml of 5 N KOH and digested at 60° for 20 min. One-tenth ml was then transferred to a vial containing 15 ml scintillation fluid.[11] Radioactivity was estimated at ambient temperature in a Nuclear Chicago model 6850 counter. Samples were corrected for quenching by use of an internal standard (0·005 μc ^{14}C-serotonin). Plasma radioactivity was determined by the same technique. Preliminary experiments in which serotonin uptake was measured both by fluorometric and radioactive methods showed that the two methods agreed within 5 per cent.

ATP was measured by a firefly luciferin-luciferase technique as previously described.[12]

RESULTS

The net uptake of serotonin as a function of time, atmosphere and concentration of serotonin in the medium is shown in Fig. 1. It is apparent from the data that suppression of the net uptake is manifest only at the higher serotonin concentrations and after prolonged incubation. At the lowest concentration employed, i.e. 30·8 n-mole/ml, no differences were observed in uptake between the platelets incubated aerobically and anaerobically. Moreover, even at the higher concentrations, statistically significant differences were not observed until the platelets were incubated for 90 min. It should be noted that in each experiment performed with the two higher serotonin concentrations, the values obatined in aerobically incubated platelets were higher than those obtained with the platelets incubated anaerobically when measurements were made at 90, 120 and 150 min. When the concentration was 123·3 n-moles/ml, the oxygenated platelets reached an average maximum of 23·5 n-mole/10^9 cells. The cells incubated anaerobically, but with the same concentration of serotonin accumulated only 18·0 n-mole/10^9 platelets. Moreover, there appeared to be a decrease in the serotonin content of the latter between 60 and 120 min. The magnitude of the decrease was not significant, but occurred in 11 of 11 determinations, showing the direction of the change to be significant.

Similar differences were noted in the experiments using 61·6 n-mole/ml: the cells incubated anaerobically averaged 32 per cent less serotonin at 120 min and 34 per cent less at 150 min.

It has been suggested that the ATP content of platelets is limiting in their ability to concentrate serotonin.[3, 13] Therefore, simultaneous measurements of serotonin and ATP were made under conditions in which serotonin was accumulated by the platelets. Six time course studies in which such measurements were made are summarized in Fig. 2. In each instance, serotonin (61·6 n-mole/ml)

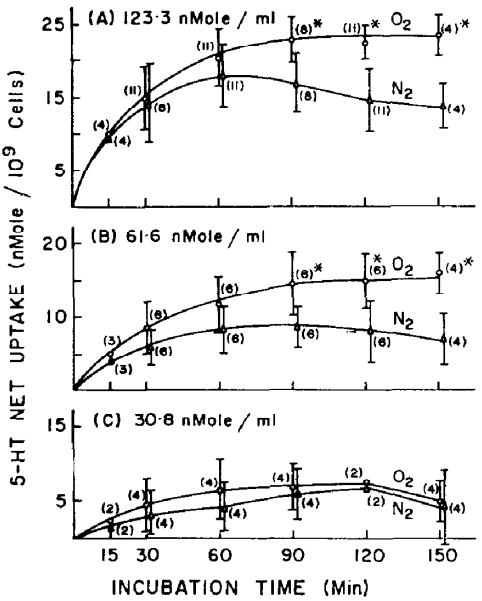

FIG. 1. The net uptake of serotonin (5-HT) is shown as a function of time, incubation condition and concentration of serotonin in the medium. The points marked with an asterisk are statistically significant (Student's t test for paired samples), $P < 0{\cdot}01$, with the exception of point B (90 min) for which $P < 0{\cdot}05$.

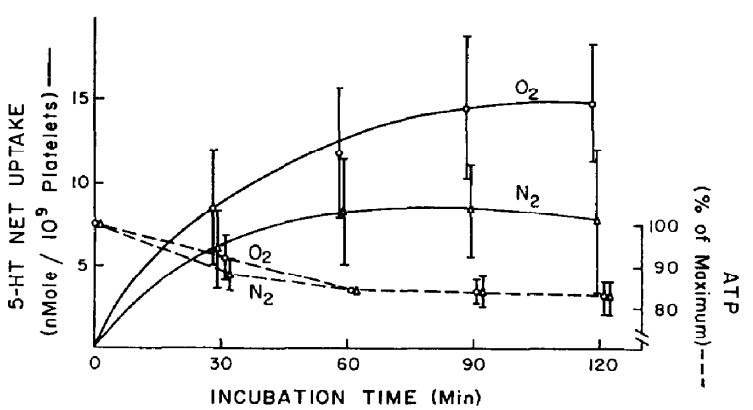

FIG. 2. Six experiments were performed in which simultaneous measurements of serotonin uptake and ATP content were made. The data for serotonin uptake are the same as those shown in Fig. 1 B. It is apparent that although anaerobic incubation suppresses the uptake of serotonin, ATP is not depressed. ATP is expressed as per cent of maximum (zero time) because of the variability of this compound from donor to donor (mean = 53 n-mole/10^9 platelets with a range of 32–73 n-mole).

was added to paired aliquots of platelet-rich plasma and aerobic and anaerobic incubations were performed. It is clear from the data that the net ATP content of the cells was not limiting in the uptake of serotonin, since the ATP levels were identical irrespective of the incubation conditions, but, as mentioned above, the amount of serotonin concentrated was significantly higher in the oxygenated cells.

DISCUSSION

The disagreement in the literature about the effect of anaerobiosis on the uptake of serotonin by platelets is explained by the data presented. Weissbach and Redfield[2, 6] demonstrated 10–30 per cent suppression of serotonin uptake by anaerobic conditions. Their experimental conditions were similar to the higher concentrations used in the present study (Fig. 1, A and B). Moreover, they incubated their platelets for 60–90 min.

Stacey[7] and Hughes and Brodie,[8] on the other hand, reported no suppression of serotonin uptake by anaerobiosis. The amount of serotonin employed by these investigators approximated the lowest concentration in the present study (Fig. 1, C). In addition, the incubation of platelets with serotonin was continued for only 25–60 min.

From the data in this paper it is clear that the uptake of serotonin is limited by anaerobiosis only when the incubations are prolonged and when an appropriate concentration of the amine is employed. The reason for this suppression is not clear, but membrane damage by prolonged anaerobic incubation could account for the observed decrease in net uptake by either inhibiting the influx or increasing the efflux of serotonin. This possibility cannot be rejected, but the fact that the platelets can maintain equal concentrations under both conditions (at a concentration of 30·1 n-moles, Fig. 1) make this seem unlikely.

Platelets are known to have an active pathway of oxidative phosphorylation, and inhibition of this pathway by anaerobiosis could impair serotonin uptake by exhaustion of cellular ATP. The ATP content of platelets, however, was not depressed by anaerobiosis (Fig. 2). From these data, it is clear that exhaustion of total cellular ATP is not the explanation for these findings. It is understood, however, that platelet ATP exists in at least two compartments[14] and the depletion of one of these, if it were very small with respect to total ATP, could inhibit the transport of serotonin and not be reflected in changes in total cellular ATP.

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