

# Down's Syndrome: Transport, Storage, and Metabolism of Serotonin in Blood Platelets

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## *Extract*

Serotonin (5HT) transport and metabolism were studied in platelets from 14 trisomy-21 patients who were matched with 12 sibling and 9 non-sibling control subjects with regard to age, sex, home environment, diet and weight. Endogenous 5HT content, in nanograms per milligram platelet protein, was reduced from  $446 \pm 66$  in the sibling controls and from  $492 \pm 74$  in the non-sibling controls to  $158 \pm 16$  in the Down's syndrome group, a difference of 61% and 68% respectively ( $P < 0.001$ ). Initial uptake of  $^{14}\text{C}$ -5HT measured after a 3-min incubation period was reduced 35% ( $P < 0.05$ ) in the platelets from Down's syndrome patients, while the efflux of radiolabeled amine from platelets was not significantly altered in these patients. Since platelets do not synthesize 5HT, the decreased transport found may explain the lower 5HT levels in these patients. Mongols did not differ from control subjects in the activity of platelet monoamine oxidase.

## *Speculation*

Similarities in the uptake, storage, and release of 5HT between the human blood platelet and the serotonergic neuron suggest that the 5HT abnormality observed in mongol platelets may reflect disordered metabolism of the monoamine within the central nervous system.

## *Introduction*

Low levels of 5HT have been reported in whole blood [25, 27] and platelets [12, 18] from patients with Down's syndrome. Explanations for this biochemical abnormality have included alterations in the metabolism of 5HT [11, 14] or in the uptake, storage, or release [4, 13] of the amine by blood platelets, the cellular structures which contain virtually all the 5HT present in whole blood [22, 28]. The possibility that low levels of 5HT in platelets relate to the central

nervous system defect in Down's syndrome is suggested by recent evidence that the human platelet has certain structural and functional similarities to amine-containing neurons [1, 19-21]. On the assumption that the whole blood abnormality of 5HT in Down's syndrome may reflect defective metabolism of this putative neurotransmitter amine in the central nervous system, L-5-hydroxytryptophan, the immediate precursor of 5HT, has been administered to neonatal mongols and has been shown to produce some alteration in neuromuscular function [2].

The patient populations in previous metabolic studies, when defined, have consisted of institutionalized mongols, a source which may distort biochemical observations through the influence of diet and intercurrent disease [29]. The present study was designed to evaluate aspects of 5HT metabolism in the platelets of Down's syndrome patients who were matched with control subjects in regard to age, sex, home environment, diet and weight.

#### Patients and Methods

Fourteen individuals [33], 9 males and 5 females, 8–25 years old, with the clinical characteristics of Down's syndrome were found to have trisomy-21 by karyotype analysis. All patients lived at home and attended day care centers for retarded children. Each was found to be free of intercurrent disease by medical evaluation.

The first control group consisted of 12 non-mentally retarded siblings [33] (7 males and 5 females, 7–24 years old) of the Down's syndrome patients who lived in the same household. The second control group was composed of 9 non-sibling controls [33] (5 males, 4 females) who were aged matched with the Down's syndrome patients. Laboratory studies of the Down's syndrome patients and their siblings were performed in batches with patient and control samples equally distributed on each study day. All blood samples were coded before analysis. Medications, if any, were discontinued at least 2 wk prior to the study and a dietary history was used to exclude ingestion of unusual foods. Foods containing high concentrations of 5HT were excluded for a period beginning 48 hr prior to collection of blood.

All blood was drawn between 9 and 11 AM. Venous blood containing 10 units heparin/ml was centrifuged at  $125 \times g$  for 20 min to yield platelet-rich plasma with an average of one leukocyte per  $10^5$  platelets. Uptake of 5HT was measured by incubation of platelet-rich plasma with  $^{14}\text{C}$ -5-hydroxytryptamine creatinine sulfate (40 mCi/mmol) [32] and harvesting of aliquots of the suspended platelets by centrifugation ( $12,000 \times g$ ) after 3- and 30-min periods of incubation. Labeled amine was extracted from the platelet pellet into 0.4 N perchloric acid and counted by liquid scintillation spectrometry. The radiolabeled compound was identified by thin layer chromatography with authentic indoleamines used as carriers [15]. Platelet protein content was determined by the method of Lowry *et al.* [16] on aliquots from the  $12,000 \times g$  platelet pellet washed with isotonic saline.

Table 1. Endogenous serotonin content of platelets

Subjects	Serotonin, ng/mg protein
Down's syndrome ( $n = 14$ )	$158 \pm 16^1$
Down's syndrome siblings ( $n = 12$ )	$446 \pm 66$
Non-sibling control subjects ( $n = 9$ )	$492 \pm 74$

<sup>1</sup>  $P < 0.001$ ; mean  $\pm$  SE.

In other experiments,  $^{14}\text{C}$ -5HT was added directly to fresh platelet-rich plasma to yield a final concentration of  $1 \times 10^{-6}$  M. After 30 min, platelets were sedimented by centrifugation at  $1100 \times g$ , then washed with isotonic saline and resuspended in either autologous plasma or Tris-phosphate buffer (pH 7.2) that contained glucose (5.9 mM) and 3.2% sodium citrate. These preparations were reincubated at  $37^\circ$  and aliquots were harvested after 30 and 60 min. The  $^{14}\text{C}$ -5HT which was retained in the platelets was extracted and quantitated by the same methods as were used in the uptake studies.

Levels of 5HT in platelets were measured fluorometrically [26] and platelet monoamine oxidase activity was determined by the deamination of 2- $^{14}\text{C}$ -tryptamine,  $8 \times 10^{-5}$  M [30].

#### Results

Endogenous 5HT content was reduced by 61% in the Down's syndrome group as compared with sibling controls ( $P < 0.001$ ) and by 68% in comparison with non-sibling controls ( $P < 0.001$ ) (Table I). Concentration of protein in platelets, in micrograms per milliliter platelet-rich plasma, was  $450 \pm 97$  for sibling controls,  $565 \pm 80$  for non-sibling controls, and  $564 \pm 67$  for the Down's syndrome group. The differences were not statistically significant. There were no differences in hematocrits, white blood cell counts or platelet counts between mongol and control groups.

#### Uptake of 5HT by Mongol and Control Platelets

The  $^{14}\text{C}$ -5HT content of platelets which had been incubated for 3 min was determined in order to measure the initial rate of amine uptake (*i.e.*, at a time when vesicular binding and storage processes exert relatively little influence on the concentration of radioactivity) [19]. The use of radiolabeled 5HT permitted measurement of amine uptake at early time periods. The 3-min time point with a final  $^{14}\text{C}$ -5HT concentration of  $10^{-6}$  M provides a measure of the initial rate of amine uptake at the cellular membrane which was found to be linear for only 5 min [11]. A greater con-

Table II. Uptake of  $^{14}\text{C}$ -serotonin by platelets

Subjects	$^{14}\text{C}$ -serotonin uptake, pmoles/mg protein	
	3-min incubation, $1 \times 10^{-6}$ M	20-min incubation, $1 \times 10^{-6}$ M
Down's syndrome ( $n = 14$ )	$227 \pm 19^1$	$1240 \pm 97^2$
Down's syndrome siblings ( $n = 12$ )	$339 \pm 45$	$1740 \pm 330$
Non-sibling control subjects ( $n = 9$ )	$392 \pm 55$	$1850 \pm 320$

<sup>1</sup>  $P < 0.05$  for differences from either control group.

<sup>2</sup>  $P < 0.05$  for differences between patient and combined control groups.

Table III. Efflux of  $^{14}\text{C}$ -serotonin from platelets

Subjects	Minutes				
	0	30		60	
	pmoles/mg protein	pmoles/mg protein	% change from 0 time	pmoles/mg protein	% change from 0 time
Down's syndrome ( $n = 14$ )	$520 \pm 38$	$445 \pm 39$	(-14.1%)	$443 \pm 39$	(-14.5%)
Down's syndrome siblings ( $n = 12$ )	$736 \pm 83$	$688 \pm 92$	(-6.1%)	$673 \pm 72$	(-8.2%)

Table IV. Monoamine oxidase (MAO) activity in platelets

Subjects	MAO activity, nanomoles/mg protein/hr
Down's syndrome ( $n = 14$ )	$4.48 \pm 0.37$
Down's syndrome siblings ( $n = 12$ )	$5.11 \pm 0.76$

centration of  $^{14}\text{C}$ -5HT ( $1 \times 10^{-5}$  M) and a longer incubation period (20 min) was used in order to saturate the transport mechanism and to determine intracellular (including intravesicular) binding capacity. In this experiment, platelets from Down's syndrome patients incorporated significantly less ( $P < 0.05$ ) radioactivity than did platelets from either of the control groups (Table II). When platelets were incubated for 20 min with a 10-fold greater concentration of  $^{14}\text{C}$ -5HT, a similar difference in the accumulation of 5HT in platelets was observed, although greater variance in individual values resulted in borderline statistical significance ( $P = 0.10$ ). When the two control groups were combined and compared with the Down's syndrome group, the differences achieved statistical significance ( $P < 0.05$ ). Assay of radioactivity indicated that over 95% was in 5HT.

#### 5HT Storage and Efflux from Platelets

The amount of 5HT retained by platelets during prolonged incubation was measured by observing the 5HT content of  $^{14}\text{C}$ -5HT loaded cells which were re-

suspended in platelet-poor plasma obtained from each subject. Platelets from the Down's syndrome patients had a significantly lower concentration of the amine at the time of resuspension, which reflected the above-described differences in 5HT uptake and binding. However, loss of  $^{14}\text{C}$ -5HT from platelets resuspended for 30 and 60 min increased only moderately (6-8%) in the mongol group and this did not represent a significant difference when compared with the control subjects (Table III). When prelabeled platelets were suspended in 5HT-free buffer instead of in autologous plasma, uptake and release results were virtually identical to those cited above.

#### Monoamine Oxidase Activity

In order to determine whether the depressed content of 5HT in mongol platelets could be related to an increased rate of enzymatic degradation, monoamine oxidase activity was measured using  $^{14}\text{C}$ -tryptamine as substrate. Mongols did not differ from control subjects with respect to monoamine oxidase activity (Table IV).

#### Effects of Imipramine on the Efflux of 5HT from Platelets

The 5HT which is released from platelets can be taken up again by a specific membrane transport mechanism [8]. Thus the low content of 5HT in mongol platelets, or the reportedly increased rate of 5HT release from prelabeled platelets resuspended in 5HT-free plasma [4], may be due to a continuing defect in the reuptake of the amine as well as to defective binding. Imipramine competitively inhibits accumulation of 5HT by platelets [10, 17, 31] and was used to examine whether a loss of the amine similar to that observed in the Down's syndrome patients might be observed when reuptake was blocked to a similar degree (30-40%). Imipramine ( $10^{-7}$  M) added *in vitro* produced a 10-20% greater loss of  $^{14}\text{C}$ -5HT from the platelets of both mongol and control subjects ( $P < 0.05$ , Fig. 1).

## Discussion

Alterations in the 5HT content of platelets might be explained by differences in systemic synthesis, or by changes in the release, uptake, or binding of the amine. Our results indicate that the endogenous 5HT content of platelets from Down's syndrome patients is markedly reduced and that the initial rate of 5HT uptake by these platelets is depressed. Since platelet 5HT is thought to derive predominantly from the uptake of circulating 5HT rather than from synthesis in the platelet, the altered transport of 5HT observed provides a mechanism for explaining the change in endogenous 5HT levels. The low endogenous content is in agreement with that reported in several other studies [1-4], and a defect in the total accumulation of 5HT by mongol platelets was previously reported by Jerome and Kamoun using high concentrations of nonradiolabeled 5HT [13].

In contrast, storage of 5HT as measured by the rate of release of  $^{14}\text{C}$ -5HT during prolonged incubation is not significantly altered in these patients. Measurement of storage of 5HT in platelets is complex because of the reuptake of the slowly released amine which occurs when prelabeled platelets are resuspended in 5HT-free plasma or buffer solutions. We have shown that an increased loss of radioactivity occurs when pre-labeled platelets are incubated with imipramine, which suggests that the reuptake of released 5HT is at least one factor that is necessary in order to maintain concentration of amine in platelets. The concentration of imipramine used in this study, which is comparable to levels in plasma achieved clinically in man, probably does not directly cause the release of significant amounts of endogenous 5HT which, however, can occur with much higher drug concentrations ( $10^{-4}$  M) [24]. The effect of imipramine is small at  $10^{-7}$  M concentrations, but a definite loss of platelet 5HT was observed which is of the same order of magnitude as that seen in the release experiments with Down's syndrome patients. This indicates that the degree of diminished 5HT uptake observed in Down's syndrome platelets is sufficient to account for the small increase in amine "release" seen in these patients. In this regard, it is interesting to note a previous study [4] on the efflux of 5HT from mongol platelets in which the concentration of platelet 5HT was decreased approximately 50% in the Down's syndrome group at the end of the preincubation period, a finding that is in agreement with our own (Table III). It seems entirely possible that the low content of 5HT at the time of resuspension is related to defective uptake of the amine as well as to the observed increased rate of "release".

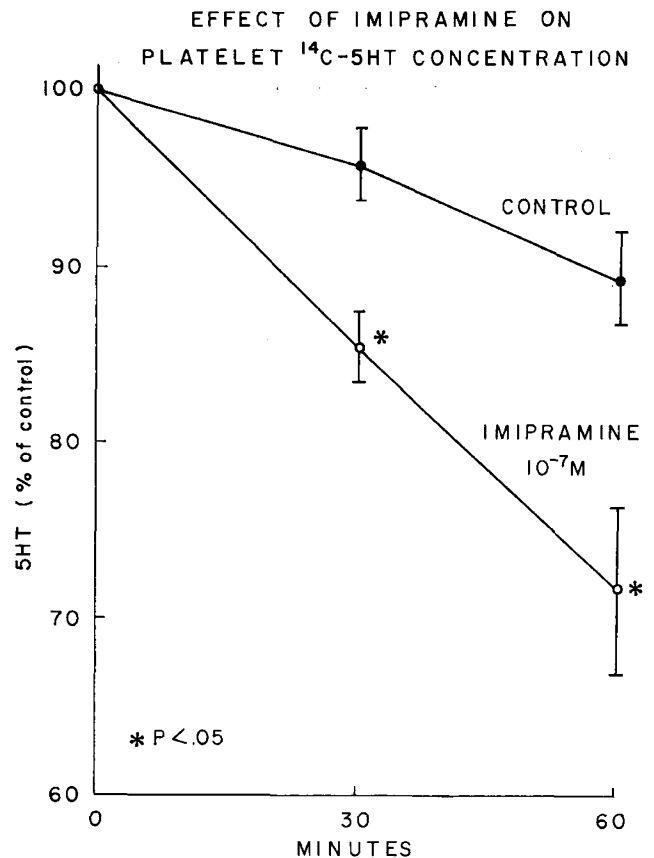


Fig. 1. Effect of imipramine on concentration of  $^{14}\text{C}$ -serotonin (5HT) in platelets.

Platelets contain high concentrations of ATP [7] and the suggestion by Born *et al.* [9] that 5HT was bound to ATP in human platelets has been supported by recent evidence [3]. ATP has also been implicated in an active transport system for 5HT at the cellular membrane [23]. Consequently, decreased uptake of 5HT by mongol platelets might be explained by a defect in platelet ATP content or function. A decrease in total ATP concentration has recently been suggested for Down's syndrome patients [5], but this has not been confirmed in other studies [13].

Since the catabolic route for 5HT is oxidative deamination, the finding of normal monoamine oxidase activity in Down's syndrome platelets makes it unlikely that the low endogenous content of 5HT is a direct result of increased degradation of 5HT. The possibility remains that defective synthesis of the amine by enterochromaffin cells located in the gastrointestinal tract contributes to the low 5HT content of mongol platelets.

Although the low content of 5HT in Down's syndrome platelets has no known clinical significance, the

similarities between human blood platelets and amine-containing neurons make imperative a comparative study of 5HT metabolism in human blood platelets and brain.

#### Summary

Endogenous 5HT content of blood platelets was reduced by 60–70% in Down's syndrome patients as compared with a group of sibling and non-sibling control subjects. The initial uptake of radiolabeled 5HT was reduced by 35% in the Down's syndrome group, although efflux of the amine and activity of monoamine oxidase were not altered in these patients. The decreased transport of 5HT into the blood platelet may explain the low level of this amine in the blood of Down's syndrome patients.

#### Addendum

As this paper was being prepared for publication, Boullin and O'Brien [6] reported results that are in agreement with our findings of low content of 5HT in platelets, and defective uptake of the amine by mongol platelets. In addition, they observed an increased rate of <sup>14</sup>C-5HT efflux from mongol platelets which was associated with a low ATP concentration. The difference from our finding of no significant increase in <sup>14</sup>C-5HT efflux may possibly be related to methodological differences, including their use of ethylenediaminetetraacetic acid as anticoagulant. They also stated the need for a study of 5HT in brain of Down's syndrome patients.

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  32. New England Nuclear Corporation, Boston, Mass.
  33. Informed consent for participating in the study was obtained from parents of patients and control subjects in accordance with the provisions set forth in the Declaration of Helsinki.
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