Enzymic Formation of Serotonin in Mammalian Blood Platelets and Red Cells

Blood platelets, according to PAASONEN[1], are a useful model system in the studies of the uptake, storage and release mechanisms of 5-HT and other biogenic amines. Many studies have been carried out in this field. Thus, the presence of 5-HT was reported many years ago[2,3], also platelets have been shown to contain the suitable mechanisms for the uptake, storage and release of 5-HT[4,5]. On the other hand little work has been done on the metabolism[6] and none at all on its biosynthesis.

Up to the present it has been believed that blood platelets lack the serotonin producing enzyme and only the histamine could be synthesized[7,8]. Recently the tryptophan hydroxylation in mammalian systems was extensively studied by LOVENBERG et al.[9]. Studying the hydroxylating enzyme in various tissues, these workers found a rather significant activity in human platelets. From this last finding, one can expect the existence of 5-hydroxytryptophan (5-HTP)-decarboxylase (EC 4.1.1.28) in human platelets.

While much work has been done on platelet 5-HT, our knowledge about it in red cells is very poor. Thus, only the existence of 5-HT in red cells is known, in quantities in an order of magnitude of 0.01–0.02 µg/ml[10]. This paper presents data for the decarboxylation of 5-HTP and some properties of 5-HTP-decarboxylase in human and beef platelets and red cells.

Methods. 25 ml freshly collected venous blood were transferred into siliconized polypropylene centrifuge tubes containing 2.9 ml anticoagulant solution[11] and immediately blood platelets and red cells suspensions were separated by centrifugation[12]. The deliberation of enzyme was achieved by freezing the platelets and red cells suspensions 3 times at $-100\,°C$ for 3 min and thawing at 20 °C for 3 min. In this situation, blood platelets and red cells were used as enzyme preparation. A 0.3 ml aliquot was taken to measure the 5-HTP-decarboxylase activity, by a radiochemical test similar to that described by SEKERIS and KARLSON[13] and adapted by us for 5-HTP[14,15]. Proteins were determined by the method of LOWRY et al.[16].

Results. Under the experimental conditions used, the enzyme activity is higher in the case of beef blood platelets and red cells (Table I). It should be noted here that the variation in enzyme activities from one species (human) to the other (beef) do not indicate the actual difference in enzyme concentration, since the determinations were performed on crude preparations and therefore may have been influenced in different ways by the environmental factors, such as the presence of endogenous substrate, product, activators or inhibitors. We shall refer to this later. As was also indicated in the same table, the platelets are a richer enzyme source than are red cells in both cases. Apart from the above-mentioned enzyme activities for blood platelets and red cells, we attempted to find whether any activity exists in plasma and especially in human plasma. Indeed a small activity was found, but it is not sure whether this activity results from plasma 5-HTP-decarboxylase, or from ruptured platelets during isolation, although all the necessary precautions had been taken.

The enzymic nature of the produced serotonin was tested by measurement of the 5-HTP-decarboxylase in the presence of hydroxylamine, a potent inhibitor. 50% inhibition was obtained at $10^{-3} M$ concentration of the inhibitor. This finding also suggests the participation of pyridoxal phosphate in the reaction system. For a better study of the relation between coenzyme and apoenzyme, an attempt was made to liberate the apoenzyme. A portion of crude extract from beef platelets was allowed to pass through a sephadex G 25 column (2.5 × 10 cm), which was buffered at pH 7.2. The efluent was used as enzyme preparation. The results of these experiments are tabulated in Table II. The fact that the values of enzyme

Table I. 5-Hydroxytryptophan-decarboxylase activity of human and beef platelets and red cells

Subject	Enzyme source	Specific activity in mU/mg protein
Human	Platelets	1.95
	Red cells	0.69
Beef	Platelets	2.60
	Red cells	0.80

The enzymic assays were performed as previously described[13–15]. Each value represents the average of duplicated determinations.

Table II. 5-hydroxytryptophan-decarboxylase activity in relation to coenzyme and activator

Enzyme source	Incubation conditions	Specific activity in mU/mg protein
Beef platelets	Crude extract	2.6
	Sephadex fraction	3.1
	Sephadex fraction + pyridoxal phosphate (100 µg)	3.0
	Sephadex fraction + pyridoxal phosphate (100 µg) + $FeSO_4$ ($3.3 \times 10^{-3} M$)	3.2

The experimental conditions are the same as those in Table I.

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activity for sephadex fraction alone and sephadex fraction plus pyridoxal phosphate are nearly the same indicates that coenzyme is not separated from the apoenzyme. On the other hand, the fact that crude extract has less enzyme activity than sephadex fraction may be attributed to the presence of endogenous substrate, product or inhibitors. This assumption is more true in the case of inhibition caused by the presence of the product, since the platelets are known to contain serotonin[2,3].

In the same Table the data of sephadex fraction plus pyridoxal phosphate plus ferric ions, a known activator of 'aromatic amino acid decarboxylase'[17] are shown. The fact that the value of enzyme activity in this case is about the same as those of the other fractions mentioned above, denotes either that ferric ions are not required for the decarboxylation in this enzymic reaction, or are attached to the apoenzyme. For a further explanation of this problem, an attempt was made to check the existence or not of ferric ions in the protein fraction. Thus a portion of sephadex fraction was tested for ferric ion concentration, which was found to be half that of crude extract. The instrument used was the Perkin-Elmer mod. 303 atomic absorption spectrophotometer.

Discussion. The activity of 5-HTP-decarboxylase in human and beef blood platelets and red cells was measured by radiochemical techniques. The relatively large amount of 5-HTP-decarboxylase found suggests the existence of a metabolic pathway for the production of serotonin inside the blood platelets and red cells. Up to now, it was believed that no 5-HTP-decarboxylase exists in the platelets[7] and that these blood constituents were only a storage site for serotonin[4,5]. On the contrary, the existence of serotonin producing enzyme was expected at least for human platelets, since recently LOVENBERG et al.[9] have shown significant activity of tryptophan hydroxylase in human platelets.

The properties of 5-HTP-decarboxylase studied in beef platelets are in accordance with the findings of earlier investigators. Thus the finding that coenzyme is not separated from the apoenzyme, after treatment with sephadex, which suggests that the coenzyme is tightly bound on the apoenzyme, has also been reported by CLARK et al.[7]. From our data, one could not explain whether the ferric ions found in the protein fraction are attached to the apoenzyme or belong to another protein of sephadex fraction. Consequently the main problem, of whether 5-HTP-decarboxylase has any requirement or not for ferric ions, will remain unexplained until extensive purification of the enzyme is obtained. Previous work on this subject has led to contradictory results. Thus LOVENBERG et al.[17] have shown no metal requirement for the decarboxylation of 'aromatic amino acid decarboxylase', while SEKERIS[18], working on the blowfly *Calliphora erythrocephala*, has found a full dependence of a sephadex fraction containing DOPA decarboxylase on ferric ions, while other metallic ions such as Zn, Mg, Mn cannot substitute them.

From the physiological point of view, platelets' serotonin may be involved in the coagulation of blood[19], or may interfere in certain blood diseases, such as thrombocytopenia. The fact that platelets, except their function in known mechanisms, namely uptake storage and release, also contain the biosynthetic mechanism of serotonin, may denote that all these mechanisms help one another to perform the above-mentioned physiological roles, or any others yet unknown. Referring now to the red cells, we cannot express any opinion about the physiological role of serotonin from the present data. Perhaps it may be related to the platelets' serotonin, or may have any other(s) special role(s). This subject apparently needs a further investigation.

Zusammenfassung. Throbozyten aus Menschen- und Rinderblut enthalten eine hohe Aktivität an 5-Hydroxytryptophan-Decarboxylase und für diese enzymatische Reaktion scheint Pyridoxalphosphat als Cofaktor eine Rolle zu spielen.

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The Allergic Macrophage of Graft Versus Host Disease

Graft versus host disease (GVH) is a progressive and often fatal disorder following the injection of incompatible lymphoid cells into hosts which are unable to reject them. Failure of rejection is due either to immaturity of the host, immunosuppression or genetic factors. When parental lymphoid cells of an inbred mouse strain are injected into first generation hybrids, the cells colonise the lymph nodes and spleen which become enlarged. Subsequently the animals lose weight and develop diarrhoea and dermatitis. Autopsy shows lymphoid atrophy, ulceration of the gut and round celled infiltrations of the liver. The pathogenesis of this disease has not yet been elucidated although it is clear that interaction of the injected lymphoid cells with host tissue must play an important part. This report draws attention to an altered reactivity of macrophages in this disease. Macrophages from affected animals are autoaggressive towards lymphocytes and cell death occurs on contact.

Materials and methods. General plan of experiments: GVH was produced by injecting lymphoid cells from donor mice into F×1 hybrid recipient mice. The treated animals are subsequently referred to as runts. Cell culture monolayers of peritoneal macrophages were prepared from donor, recipient and runt mice. Lymphoid cells from donor, recipient and runt animals were added to these monolayers. The degree of interaction was judged by microscopic examination of stained preparations.

Animals. Donor and recipient strains were C57Bl/6J and B6AF1/J (offspring of C57Bl/6J males and A/J females) obtained from Jackson Laboratories, Bar Harbour, Maine.

Preparation of lymphoid cells. Lymph nodes and spleens were removed aseptically, cut in small fragments and very gently dissociated in tissue culture medium 199 (Glaxo) using a loose fitting hand operated glass homogeniser. Tissue fragments were allowed to sediment and the cells in the supernatant were washed once and