## Effect of Antigen-Antibody Reaction and of Some Drugs on <sup>14</sup>C-Serotonin (5HT) of Platelets. (On "<sup>14</sup>C-5HT Release Test")

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It was demonstrated by the following method that platelets took up 14C-5HT and that it reached the maximum by their incubation at at 37°C for 60 minutes. Normal human or rabbit platelet rich plasma and 14C-5 HT was incubated for various intervals at 37°C. Platelet buttons were obtained by centrifugation, washed once in normal saline and resuspended in distilled water. The platelets were then disrupted by repeated freezing and thawing and the debris deposited by centrifugation. One tenth ml of the supernatants were dried on steel planchets and their radioactivity measured in a gasflow counter. Both the effect of platelet counts on their uptake in the several constant concentrations of 14C-5 HT and that of <sup>14</sup>C-5 HT concentrations on the uptake in the several constant counts of platelets were examined.

Platelets taken up <sup>14</sup>C-5HT were prepared by the previous incubation of <sup>14</sup>C-5HT and normal platelets at 37°C for 60 minutes (<sup>14</sup>C-5HT-platelets).

Incubation of heterologous anti-platelet serum and 14C-5HT-platelet at 37°C for 60 minutes caused significant release of 14C-HT. Reactions of ovalbumin with rabbit antiovalbumin caused remarkable release of 14C-5HT from <sup>14</sup>C-5HT-platelets. This "<sup>14</sup>C-5HT Release Test" was performed as follows. <sup>14</sup>C-5HT-platelets, anti-ovalbumin serum and ovalbumin (or saline) were incubated at 37°C for 60 minutes and centrifuged at 4°C. Aliquots of 0.1 ml of the supernatants were dried on steel planchets and their radioactivity measured in a gas-flow counter (R, R). <sup>14</sup>C-HT taken up by the platelets in the presence of saline as a control (instead of ovalbumin) was measured as described above (U). The amount of 5HT released was expressed as a percentage of that taken up during incubation by the controls i.e. Release Index (R. I.) =  $\frac{R_{Ag}-R_S}{U_S} \times 100$ , where  $R_{Ag}$  and  $R_S$  were radioactivities of released  $^{14}\text{C-5HT}$  in the presence of ovalbumin and of saline, respectively, and U is uptaken of 5HT in the presence of saline. Examination over a wide range of antigen dilutions showed that there is an optimum concentration beyond or below which R.I. decreased. This concentration of ovalbum  $(10^{-2}\%)$  was different from the optimum concentration of antigen  $(10^{-4}\%)$  in the complement fixation reaction by ovalbumin and this anti-ovalbumin, and complement had no effect on R.I. Therefore 5HT release was proved to be independent of complement.

In some patients with a history of urticaria, anaphylaxis, purpura, etc. which seemed to be due to drug hypersensitivity, "14C-5HT Release Test" was positive. This test herein was performed by using patients' sera and causative drugs according to the method as described above for anti-ovalbumin and ovalbumin. In this test which is useful in the diagnosis of drug sensitive patients, drug concentrations without non-specific effect upon platelet 5HT must be used.

Drugs such as serpasil, quinidine sulfate, quinine hydrochloride, Periactin, Glycylon, etc. caused remarkable release of 5HT from platelets even in their concentrations lower than those usually administered; Wintermin, Hirnamin, Tofranil, etc. caused the significant release in the concentrations higher than those observed in the usual dosis; and most of antipyretics and sulfonamides has almost no effect on the release in the concentrations usually observed. On the other hand, Predonine and Tathion (glutathion) were observed to increase 5HT uptake of the platelet in their high concentrations. The mechanism of these effects remains to be elucidated.