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SEROTONIN TRANSPORT AND STORAGE IN RABBIT BLOOD PLATELETS—THE EFFECTS OF RESERPINE AND IMIPRAMINE*

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Abstract—Serotonin transport and storage in suspensions of washed rabbit platelets were investigated by following the exchange of platelet-bound [^{3}H]serotonin and [^{14}C]serotonin added to the suspending medium. Assuming a three-compartment system (suspending medium, platelet cytoplasm and platelet storage organelles), the transfer rates between the different compartments were calculated from the exchange data by statistical analysis. Reserpine reduced the storage organelle serotonin content by inhibiting the transfer of serotonin from the cytoplasm into the amine storage organelles. It also reduced the fraction of serotonin in the cytoplasm transferred per unit of time into the suspending medium. Imipramine (20 μM) inhibited the uptake of serotonin across the platelet plasma membrane into the cytoplasm and reduced the fraction of cytoplasmic serotonin transferred per unit of time into the suspending medium. At this concentration it had no effect on serotonin transport across the amine storage organelle membrane in either direction. The method used allows the serotonin transfer rates across the platelet plasma membrane to be distinguished from those across the amine storage organelle membrane in intact cells, and permits these transfer rates to be estimated simultaneously. The method may be used for determining the effects of drugs that interfere with transport and storage of biogenic amines and in defining the defects in diseases with abnormal transport or storage of biogenic amines.

We have recently developed a method of analysis of serotonin transport and storage in intact platelets [1, 2]. With this method it is possible to estimate the rates of serotonin transfer across the platelet plasma membrane and across the membrane of the storage organelles without introducing artifacts due to homogenization. Using this approach, we have now examined the effects of reserpine and imipramine, which are known to interfere with the transport and storage of biogenic amines such as adrenaline, noradrenaline and serotonin in nervous tissue [3], the adrenal medulla [4], and blood platelets [5]. In addition to confirming previously recognized effects of imipramine and reserpine on the transport and storage of biogenic amines, the present studies provide quantitative data on the effect of these drugs on serotonin transport across the amine storage organelle membrane in intact blood platelets.

MATERIALS AND METHODS

Non-radioactive compounds

Bovine thrombin was obtained from Parke, Davis & Co., Detroit, MI. Serotonin [5-hydroxytryptamine creatinine sulfate complex (5-HT)] was purchased from Sigma Chemical Co. (St. Louis, MO). Apyrase (EC 3.6.1.5) was prepared according to Molnar and Lorand [6]. This preparation hydrolyzed 18 nmoles ATP/min/μg of protein and 300 nmoles ADP/min/μg of protein (3 mg protein/ml of apyrase). Reserpine (Serpasil) was obtained from Ciba Co. Ltd., Dorval, Quebec, and was used for the experiments *in vivo*. Reserpine powder dissolved in absolute ethanol at a concentration of 0.33 mM or Serpasil was used for the experiments *in vitro*. Both preparations gave the same results compared with the appropriate control. Imipramine (Tofranil) was obtained from Geigy Canada Ltd., and dissolved in 0.85% saline. All concentrations given are the final concentrations in the platelet suspensions after all additions.

Radioactive compounds

5-Hydroxytryptamine [^{3}H] (G) creatinine sulfate ([^{3}H]serotonin; [^{3}H]-5-HT) in aqueous solution containing 2% ethanol (sp. act. between 11 and 17.3 Ci/m-mole) and 5-hydroxytryptamine-3'-[^{14}C]creatinine sulfate ([^{14}C]serotonin; [^{14}C]-5-HT) (sp. act. approximately 50 mCi/m-mole in different batches) were obtained from Amersham/Searle Corp., Arlington Heights, IL. The radioactive purity of the [^{14}C]-5-HT was determined by paper chromatography [5] and found to be between 96 and 98 per cent. Unlabeled serotonin was added to the radioactive serotonin when the final serotonin concentrations in the platelet suspensions were above 1 μM.

Platelet suspensions

Suspensions of washed platelets in Tyrode solution containing 0.35% albumin and apyrase (2 μl/ml of suspending medium) were prepared from rabbit blood by the method described by Ardlie *et al.* [7].

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Platelet release reaction

Suspensions of washed platelets were prepared from the blood of rabbits that had been injected intraperitoneally with 5 mg reserpine/kg 18 hr prior to the collection of the blood [8]. Control suspensions were prepared from rabbits that had not been treated with reserpine.

The washed platelets were equilibrated with [^{14}C]-5-HT at a concentration of 123 μM for 60 min. It had been established previously [2] in experiments in which serotonin uptake was measured fluorometrically that no further accumulation of serotonin in the platelets was achieved under these conditions after 20 min of incubation. The suspensions from reserpine-treated animals were equilibrated with serotonin in the presence of 2 μM reserpine. The control suspensions were equilibrated with serotonin in the absence of reserpine. After incubation with serotonin the platelets were resuspended in fresh Tyrode solution containing 0.35% albumin and unlabeled serotonin at the same concentration at which platelets had been previously equilibrated. The platelet release reaction was studied immediately after the platelets were resuspended. One ml of platelet suspension (10^6 platelets/mm^3) was warmed to 37° for 5 min and then incubated with Tyrode solution (0.1 ml) or with thrombin (in 0.1 ml of Tyrode solution) to give a final concentration of 0.45 units/ml for 3 min at 37° in a shaking device. The suspension was transferred rapidly into an Eppendorf centrifugation tube and centrifuged for 45 sec at 12,000 *g* in an Eppendorf microcentrifuge 3200. Samples for radioactivity determination were taken from the supernatant fluid. The radioactivity released by thrombin was corrected for the radioactivity found in the supernatant fluid of the aliquot treated with Tyrode solution. Released radioactivity was expressed as a percentage of the platelet-bound radioactivity in the control sample.

The effect of imipramine (20 μM) on the release of [^{14}C]-5-HT from washed rabbit platelets was studied in a similar manner. The methods used differed in two ways: (1) the rabbits from which blood was obtained were not treated with imipramine, and (2) the washed rabbit platelets were equilibrated with [^{14}C]-5-HT at a concentration of 100 μM in the presence or absence of 20 μM imipramine for 2 hr with one change of the suspending medium after 1 hr. The prolonged incubation time (as compared to the experiments in which the effect of reserpine was studied) was chosen since preliminary experiments had shown that, in the presence of 20 μM imipramine, the rate of serotonin uptake was reduced by more than 50 per cent.

Initial rate of [^{3}H]serotonin uptake by rabbit platelet suspensions

Platelets were suspended at a concentration of 10^4/mm^3 or 10^6/mm^3 in Tyrode solution containing 5 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethane sulfonic acid) buffer and apyrase (pH 7.35). To ensure accurate platelet concentrations, platelets diluted in 1% ammonium oxalate were counted by light microscopy in triplicate in Neubaur chambers. Platelets were kept at 37° in 1-ml aliquots in conical plastic Eppendorf tubes. Modified Tyrode solution (0.1 ml) or reserpine (2 μM) or imipramine (20 μM) was added to the aliquots of platelet suspension and incubated for 30 sec. At this time 1, 100 or 123 nmoles [^{3}H]-5-HT was added and mixed. Samples (0.1 ml) were removed 10, 20, 30, 40, 50 and 60 sec later and transferred immediately into 2 ml of suspending medium containing 123 μM non-radioactive serotonin and 0.4% formaldehyde [9], kept at 0° to stop immediately further [^{3}H]-5-HT uptake. This 2.1-ml sample was then filtered and washed with 8 ml of ice-cold (0°) modified Tyrode solution (no calcium, no magnesium) on a Millipore filter HAWP, diameter 25 mm, pore size 0.45 μm (Millipore Corp., Bedford MA) plated on a Millipore filter holder (Cat. No. 10.025.02) under vacuum. Filtration and washing required about 15 sec. The filters were dried overnight in scintillation counting vials. Then 10 ml of Triton-X-100–toluene scintillation fluid [10] was added to each vial. The vials were mixed with a vortex mixer for 30 sec, cooled to 4° and counted in a β-scintillation counter. The counts per min obtained from the filtered samples were corrected for non-specific binding to the filter and converted into nmoles [^{3}H]-5-HT taken up/10^9 platelets. In the absence of inhibitors the rate of [^{3}H]-5-HT uptake was linear with time for at least 1 min when a low concentration of serotonin (1 μM) was used, and less than 5 per cent of the total [^{3}H]-5-HT added was taken up during this time. The slope of the curve was calculated by linear regression analysis, expressed as nmoles of [^{3}H]-5-HT taken up/min/10^9 platelets and considered to represent the initial rate of serotonin uptake by the platelets. In the presence of reserpine or high concentrations of 5-HT (100 or 123 μM), the initial rate of uptake was obtained graphically by drawing the tangent to the [^{3}H]-5-HT uptake curve.

Serotonin exchange experiments

Experiments were performed in which the exchange of radioactive serotonin tracers between platelets and surrounding medium was determined. Under equilibrium conditions for the total serotonin, there is no net flux of serotonin but a net movement of tracer occurs when the tracer is not distributed according to the equilibrium amounts among the pools, e.g. if one label is outside the platelets and the other is inside. These experiments provide the data by which serotonin fluxes may be determined.

Suspensions of washed platelets were prepared from the blood of reserpine-treated and untreated rabbits. The platelets were equilibrated with [^{3}H]-5-HT (123 μM) for 60 min in the presence or absence of reserpine (2 μM) as described for the studies of the platelet release reaction. After incubation, the platelets were resuspended in fresh Tyrode solution containing albumin (0.35%) and apyrase (2 μl/ml of suspending medium). The platelet count was adjusted to 10^9/ml. In experiments in which the effect of imipramine was investigated, washed platelets were prepared from the blood of untreated rabbits. The platelets were then equilibrated with [^{3}H]-5-HT (100 μM) in the presence of imipramine (20 μM) for 120 min with a change of suspending medium after 60 min. After incubation, the platelets were resuspended in a medium containing either 123 μM (reserpine experiments) or 100 μM unlabeled

serotonin (imipramine experiments) and the drug which had been present during the previous incubation. All incubations were performed at 37°. After the platelets were resuspended in these media, [^{14}C]-5-HT was added to the suspending medium. (The change in serotonin concentration resulting from the addition of the radioactive serotonin was less than 0.3 μM.) Since the final concentrations of serotonin and drug were those of the previous incubation, no further net loss or uptake of serotonin could occur. The exchange of tracers between surrounding medium and platelets was determined; [^{14}C]-5-HT is taken up by the platelets and disappears from the surrounding medium. At the same time [^{3}H]-5-HT accumulates in the surrounding medium. Samples (approximately 0.5 ml) of platelet suspension were taken every 2 min. The platelets were removed by centrifugation in an Eppendorf microcentrifuge 3200 and the radioactivity in the supernatant fluid was determined. The exchange of tracers and thereby the exchange of serotonin can be observed until complete equilibrium of the tracers has taken place. Usually samples were taken for 60–80 min.

The high serotonin concentrations (100 or 123 μM) in the exchange experiments were chosen to ensure that there would be no appreciable net movement of serotonin across the platelet plasma membrane and to minimize the fraction of serotonin converted to its metabolites during the exchange experiments (see below and Ref. 2). Although part of the serotonin transfer across the platelet plasma membrane may be due to diffusion rather than due to active transport at the high serotonin concentrations used, these conditions were essential for later analysis. The high concentration of serotonin used in these experiments allowed us to estimate the maximum transport rates of serotonin between the different compartments which would otherwise be impossible with presently available techniques.

Analysis of serotonin exchange experiments

The exchange of serotonin between platelets and the suspending medium was followed with two radioactive tracers, ^{14}C and ^{3}H. At the beginning of the experiments the [^{3}H]-5-HT is largely within the platelet, and the [^{14}C]-5-HT is in the suspending medium. The serotonin exchange data are obtained as counts of ^{14}C and ^{3}H radioactivity in the suspending medium. These data may be converted to the fraction of total ^{14}C and ^{3}H in the platelet suspension. It is assumed that the platelet suspension contains three compartments: suspending medium (compartment 1); "cytoplasm" (compartment 2); and storage granules (compartment 3). Serotonin is exchanged between the granule compartment and the suspending medium via the "cytoplasm" compartment. The proportion of the serotonin in any compartment that is transferred to another compartment in 1 min is the fractional turnover rate between these compartments. Thus, for example, if a value of 1 is given to the amount of serotonin in any compartment and 10 per cent of this amount is transferred per min to a neighboring compartment, the fractional turnover rate from one to the other is 0.1 (min^{-1}). [For easier reading we have used the term "percentage of serotonin transferred between compartments" (unit: min^{-1}) in Results and Discussion. "Percentage of serotonin transferred between compartments" is obtained by multiplying the fractional turnover rate by 100.] For the transfer of serotonin from compartment 1 to compartment 2, the fractional turnover rate is represented by the symbol a_{21} and for the transfer from compartment 2 to 1 by the symbol a_{12}, etc. Under the steady state conditions of the exchange experiments, these fractional turnover rates for serotonin are constant. The fractional turnover rates are assumed to be equal for radioactive and non-radioactive serotonin. The fraction of tracer in compartment 1 (the suspending medium) is referred to by m_1 and may be described by the following equations for ^{14}C and ^{3}H tracers if a compartmental model is assumed [1].

$$[^{14}\text{C}]\ m_1 = A + Be^{-\lambda_1 t} + Ce^{-\lambda_2 t} \quad (1)$$

$$[^{3}\text{H}]\ m_1 = A + De^{-\lambda_1 t} + Ee^{-\lambda_2 t} \quad (2)$$

In these equations, the pre-exponential constants A, B, C, D and E are functions of the initial tracer distribution among the compartments and the fractional turnover rates (a_{12}, a_{21}, a_{23} and a_{32}), whereas the exponential constants, λ_1 and λ_2, are functions of the fractional turnover rates alone. These functions are given in the Appendix.

As the initial distribution of ^{14}C is known, all this tracer lying within the suspending medium, the fractional turnover rates describing the exchange of serotonin can be estimated from the ^{14}C data set: A, B, C, λ_1 and λ_2 that give the best fit of Eqn. 1 to the data being determined.

Although the ^{3}H data set cannot be used to calculate the fractional turnover rates, the initial distribution of ^{3}H within the platelet being unknown, this data set is valuable in that the equation describing it, Eqn. 2, has constants in common with Eqn. 1 (A, λ_1 and λ_2). The simultaneous fitting of both data sets to Eqns. 1 and 2 thus provides more precise estimates of these constants. The fitting employs a Bayesian multivariate technique of analysis which takes into account the correlation of errors in both data sets. Details of this technique have been described previously [1]. (The expanding simplex method of Nelder and Mead [11] was used to search for the estimates of A, B, C, D, E, λ_1 and λ_2 with highest posterior probability. The criterion of maximum likelihood was that of Box and Draper [12].) From the estimates of A, B, C, λ_1 and λ_2, the fractional turnover rates a_{12}, a_{21}, a_{23} and a_{32} may be calculated from the work of Robertson *et al.* [13] (see Appendix).

As the platelets are at equilibrium with respect to serotonin and as the amount of serotonin within the suspending medium is known, the amounts of serotonin in the two platelet compartments may be calculated as follows: let S_1 be the total amount of serotonin in compartment 1, S_2 the total serotonin in compartment 2, and S_3 the total serotonin in compartment 3. Then (amount of serotonin in compartment 1) × (fraction of serotonin in compartment 1 transferred to compartment 2 every min)

= flux from compartment 1 to 2 (amount/unit time)

$= a_{21}S_1$

Similarly:

flux from compartment 2 to 1 (amount/unit time)

$= a_{12}S_2$

At equilibrium:

$$a_{21}S_1 = a_{12}S_2$$

or:

$$S_2 = S_1 \frac{a_{21}}{a_{12}}$$

Similarly:

$$S_3 = S_2 \frac{a_{32}}{a_{23}}$$

The analysis of the exchange experiments as described above is based on the assumption that the [^{14}C]-5-HT added to the platelet suspension is not converted to other compounds to an appreciable extent during the experiment. We have previously reported [2] that this assumption is justified for washed rabbit platelets in the absence of drugs that influence serotonin transport or storage. However, Pletscher [5] found that part of the endogenous platelet serotonin as well as part of exogenously added serotonin is metabolized in the presence of reserpine. We confirmed the observation of Pletscher [5] and found that under the conditions of our experiments approximately 2 per cent of the [^{14}C]-5-HT (123 μM) added to the platelet suspension (10^9 platelets/ml) was metabolized within 1 hr in the presence of 2 μM reserpine. Further experiments were done to demonstrate that the small amounts of 5-HT metabolites formed will not influence the results obtained in the serotonin exchange experiments greatly.

Experiment 1. In this experiment, we examined whether or not the amount of 5-HT metabolites in platelets in the presence of reserpine is large or small compared to the platelet content of 5-HT. Platelets from reserpine-treated rabbits were equilibrated with [^{14}C]-5-HT at a concentration of 123 μM in the presence of reserpine (2 μM) for 90 min with a change of the suspending medium after 60 min. They were then resuspended in fresh medium containing 123 μM unlabeled serotonin (platelet count, 10^9/ml). Immediately upon resuspension, [^{14}C]-5-HT and its radioactive metabolites were determined by paper chromatography [5] in aliquots of the total platelet suspension and the supernatant fluid from the platelet suspension. Platelet-bound [^{14}C]-5-HT and radioactive metabolites were calculated; 2.9 per cent of the total platelet-bound radioactivity was found in 5-hydroxytryptophol (5-HT'ol), the only 5-HT metabolite formed within the platelets to any extent [14]. This indicates that the amount of metabolites present in reserpine-treated platelets is very small under the exchange conditions of the present experiments. Absolute values may be calculated from Table 2. Thus, if all the 5-HT'ol (1.28 nmoles/10^9 platelets) were in the cytoplasmic compartment, it would contribute 7.9 per cent of the total 5-HT + metabolite content in this compartment. If it were exclusively in the granule compartment, it would contribute 4.6 per cent of the total 5-HT + metabolite content in this compartment.

Experiment 2. In this experiment, we examined whether newly formed 5-HT'ol would accumulate in the suspending medium or be retained in the platelets. Platelets from reserpine-treated rabbits were equilibrated with 123 μM [^{14}C]-5-HT and then resuspended at a concentration of 10^9/ml in a medium containing [^{14}C]-5-HT of the same concentration (123 μM). The accumulation of [^{14}C]-5-HT'ol in the platelet suspension and its distribution between platelets and the suspending medium were determined. At all times (up to 60 min), between 80 and 95 per cent of the total [^{14}C]-5-HT'-ol formed was found in the suspending medium. (Control experiments showed that the formation of radioactive 5-HT'ol by platelets in the absence of reserpine was negligible.) This indicates that the majority of 5-HT'ol formed in blood platelets in the presence of reserpine accumulates in the suspending medium.

Experiment 3. In this experiment, the absolute amounts of 5-HT'ol formed within 60 min in a platelet suspension containing reserpine were estimated. Platelets from reserpine-treated rabbits were equilibrated with unlabeled 5-HT at a concentration of 123 μM and resuspended in a medium containing [^{14}C]-5-HT at a concentration of 123 μM (platelet count 10^9/ml). The formation of [^{14}C]-5-HT'ol was studied over a period of 60 min. In this experiment, 1.5 per cent of the total [^{14}C]-5-HT was metabolized to [^{14}C]-5-HT'ol. Since the [^{14}C]-5-HT'ol accumulates practically exclusively in the suspending medium (see Exp. 2) and since the concentration of [^{14}C]-5-HT in the suspending medium is known (123 μM at the beginning of the experiment and only slightly less after 60 min because of the small amount metabolized), one can calculate that about 0.015 × 123 nmoles [^{14}C]-5-HT'ol is formed by 10^9 platelets during the 60 min of the exchange experiment. Dividing this number by 60, we obtain the amount of 5-HT'ol formed/min (0.031 nmole/min/10^9 platelets). Table 2 of Results indicates that the exchange of serotonin across the platelet plasma membrane in the presence of reserpine is 8.6 ± 1.3 nmoles/min/10^9 platelets. Thus, the formation and flux of 5-HT'ol from the platelets into the suspending medium contribute probably less than 0.4 per cent to the total flux of ^{14}C across the platelet plasma membrane. The experiments in which the initial uptake of serotonin was determined (Fig. 1) support further the conclusion that the small amount of 5-HT'ol formed during the exchange experiments does not influence the numerical results significantly. Practically the same values were obtained when uptake of serotonin into the platelets was determined by either calculation from the exchange experiments or by direct determination in short-term incubation experiments (see Results).

Consequently, a correction was made to the radioactivity as measured in the supernatant fluid to reflect the true amount of radioactive serotonin present when analyzing the exchange of serotonin in the presence of reserpine. Only this correction needed to be made since the main metabolite, 5-HT'ol, collected in the surrounding medium. Since it was found that approximately 2 per cent of the radioactivity in the supernatant fluid after 1 hr was not serotonin, the appropriate percentage was subtracted from each supernatant fluid radioactivity measurement, taking into account the time of measurement, e.g. 1 per cent was subtracted at 30 min. The subsequent mathematical analysis for fractional turnover rates showed little sensitivity to this correction.

Imipramine almost completely prevents the formation of serotonin metabolites in the suspension of washed rabbit platelets [5]. Therefore, no correction was applied in experiments in which the serotonin exchange in the presence of imipramine was investigated.

RESULTS

Effects of reserpine on serotonin storage and transport

Liberation of serotonin from platelets. Washed rabbit platelets were labeled with [^{14}C]-5-HT (1 μM), washed and resuspended in Tyrode solution containing albumin (0.35%) and a small amount of apyrase. Upon addition of reserpine (2 μM), about 20 per cent of the initially platelet-bound ^{14}C accumulated in the suspending medium within 60 min.

Inhibition of serotonin accumulation by platelets. Washed rabbit platelets rapidly accumulated [^{14}C]-5-HT added to the suspending medium. Reserpine (2 μM) inhibited this accumulation of [^{14}C]-5-HT. However, reserpine (2 μM) did not inhibit the initial rate of [^{14}C]-5-HT uptake at either low (1 μM) or high (123 μM) 5-HT concentrations (Fig. 1). The initial rate of 5-HT uptake at 123 μM was about three times greater than that at 1 μM 5-HT.

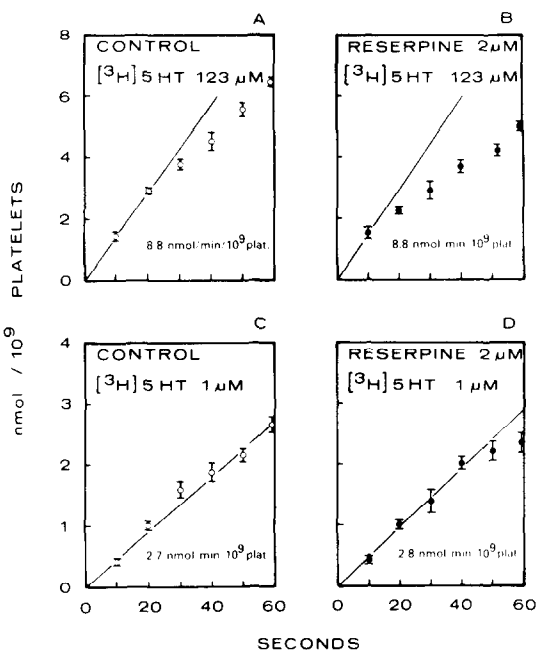

Fig. 1. Effect of reserpine on the initial rate of uptake of [^{3}H]-5-HT. Panels A and B: Washed rabbit platelets (10^6/mm^3) were incubated for 30 sec with saline (A) or 2 μM reserpine (B), before the addition of 123 μM [^{3}H]-5-HT. Mean and standard error (S. E. M.) of triplicate determinations. (This was typical of five experiments. Mean $\pm$ S. E. M. in these five experiments was 7.9 $\pm$ 0.4 nmoles/min/10^9 platelets for the control platelets and 7.8 $\pm$ 0.4 nmoles/min/10^9 platelets for the reserpine-treated platelets; $P < 0.8$.) Panels C and D: Washed rabbit platelets (10^4/mm^3) were incubated for 30 sec with saline (C) or 2 μM reserpine (D) before addition of 1 μM [^{3}H]-5-HT. Mean $\pm$ S. E. M. of triplicate determinations. (This was typical of eleven experiments. Mean $\pm$ S. E. M. in these eleven experiments was: 3.0 $\pm$ 0.2 nmoles/min/10^9 platelets for the control platelets and 2.8 $\pm$ 0.2 nmoles/min/10^9 platelets for the reserpine-treated platelets; $P < 0.5$.) In these experiments less than 8 per cent of the serotonin was taken up by the platelets during the observation period; therefore the concentration of serotonin was not appreciably depleted during the time of study.

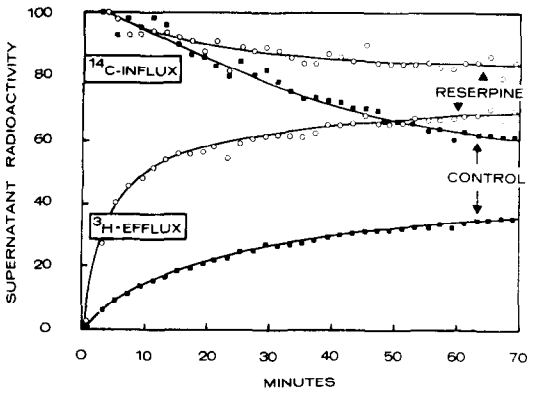

Fig. 2. Effect of reserpine on 5-HT exchange. Platelets from rabbits that had received 5 mg/kg of reserpine intraperitoneally 18 hr prior to the collection of blood, were equilibrated with [^{3}H]-5-HT at a concentration of 123 μM in the presence of 2 μM reserpine and then resuspended in [^{14}C]-5-HT and reserpine at the same concentrations. Exchange of ^{3}H and ^{14}C between platelets and suspending medium was followed in supernatant fluid samples of aliquots of the platelet suspension taken at 2-min intervals. Details are described in Materials and Methods. Control platelets were prepared without reserpine in the same way from blood of animals that had not been given any reserpine. The platelet count was 10^6/mm^3. This was one of five similar experiments. Key: (O——O) exchange of ^{3}H and ^{14}C in the presence of reserpine; (■——■) exchange of ^{3}H and ^{14}C in the control platelet suspension.

Effect on exchange of serotonin between platelets and suspending medium. Washed rabbit platelets from either reserpine-treated animals or untreated control animals were incubated with [^{3}H]-5-HT at a concentration of 123 μM in either the presence or absence of 2 μM reserpine. The platelets were resuspended in [^{14}C]-5-HT and reserpine at the same concentration at which they had been equilibrated. Supernatant fluids from samples of the platelet suspension were prepared at 2-min intervals. The concentration of ^{3}H and ^{14}C in each supernatant fluid was determined (Fig. 2). Analysis of the data showed that transfer from the cytoplasmic compartment into the granule compartment (amine storage granules) was significantly diminished in the presence of reserpine. However, the percentage of serotonin in the amine storage organelles that was transferred into the cytoplasm per unit of time was not significantly altered (Table 1). As a consequence of these changes in transport, the amount of serotonin in the amine storage organelles was greatly reduced (by about 85 per cent) compared to the control platelets (Table 2).

Reserpine (2 μM) did not affect the rate of serotonin exchange between the suspending medium and the cytoplasm across the platelet plasma membrane (Table 2). In agreement with this, the percentage of serotonin transferred from the suspending medium into the cytoplasm per min was unaltered by reserpine. However, the percentage of serotonin in the

Table 1. Effect of reserpine on transfer of serotonin between suspending medium and different compartments of a suspension of washed rabbit platelets*

Compartments	Percentage of serotonin transferred between compartments/min		
	Control platelets	Reserpine-treated platelets	Significance of difference between means†
Suspending medium to cytoplasm	7.1 ± 1.2	6.9 ± 1.0	Not significant
Cytoplasm to suspending medium	73.4 ± 8.9	48.1 ± 1.6	Significant
Cytoplasm to storage granules	29.6 ± 6.6	4.5 ± 0.7	Significant
Storage granules to cytoplasm	1.7 ± 0.3	2.4 ± 0.3	Not significant

* Results are expressed as means ± S. E. M. of five experiments. In these studies serotonin transport has been described by non-linear equations containing several constants. Significance of the change of the values of these constants was assessed through (a) Student's *t*-test and (b) direct determination of 95 per cent joint confidence contours for the estimated values in each experiment. Details of these techniques have been described [1]. The reserpine treatment and experimental details are described in Materials and Methods. The serotonin concentration in the suspending medium was 123 μM. The platelet count was 10^6/mm^3.

† At the 95 per cent level of confidence.

cytoplasm transferred into the suspending medium per unit of time was reduced by about one-third (Table 1), and consequently the cytoplasmic 5-HT pool was slightly increased in the presence of reserpine (Table 2).

Effect of reserpine on thrombin-induced release of serotonin. In the next set of experiments, we examined whether the values for the size of the cytoplasmic compartment and the granule compartment obtained from the exchange experiments are comparable to the values for releasable and non-releasable serotonin of platelets. Washed platelets from reserpine-treated rabbits were equilibrated with [^{14}C]-5-HT (123 μM) in the presence of reserpine (2 μM) and resuspended in Tyrode solution containing albumin (0.35%) and unlabeled serotonin at the same concentration at which the platelets had previously been equilibrated. Upon addition of thrombin (0.45 units/ml), reserpine-treated platelets released 72 ± 3 (mean ± S. E. M.) per cent of their radioactivity whereas the control platelets released 91 ± 3 per cent of their radioactivity. This difference was significant ($P < 0.05$; $N = 4$).

Effects of imipramine on serotonin storage and transport

Liberation of serotonin from platelets. Imipramine (20 μM), added to a suspension of washed rabbit platelets prelabeled with [^{14}C]-5-HT at a low concentration (1 μM), caused the accumulation of only a very small amount of ^{14}C in the suspending medium upon prolonged incubation (Fig. 3). When platelets were prelabeled with [^{14}C]-5-HT at a high concentration (100 μM) and resuspended in a medium containing no added serotonin, imipramine (20 μM) caused the accumulation (net efflux) of a larger amount of ^{14}C in the suspending medium (Fig. 3),

Table 2. Effect of reserpine on serotonin content in different compartments of a rabbit platelet suspension and its exchange between these compartments*

Compartment	Control platelets	Reserpine-treated platelets	Significance of difference between means
	Serotonin (nmoles/10^9 platelets)		
Cytoplasm	10.8 ± 0.8	16.3 ± 3.3	$P < 0.2$
Storage granules	177 ± 19	28.1 ± 3.3	$P < 0.001$
	Serotonin (nmoles/min/10^9 platelets)		
Exchange of serotonin between suspending medium and platelet cytoplasm	8.9 ± 1.5	8.6 ± 1.3	$P < 0.9$
Exchange of serotonin between platelet cytoplasm and platelet storage organelles	3.1 ± 0.8	0.67 ± 0.11	$P < 0.02$

* Means ± S. E. M. of five experiments. The significance of the differences was calculated by Student's *t*-test.

The reserpine treatment and experimental details are described in Materials and Methods. The serotonin concentration in the suspending medium was 123 μM. The platelet count was 10^6/mm^3.

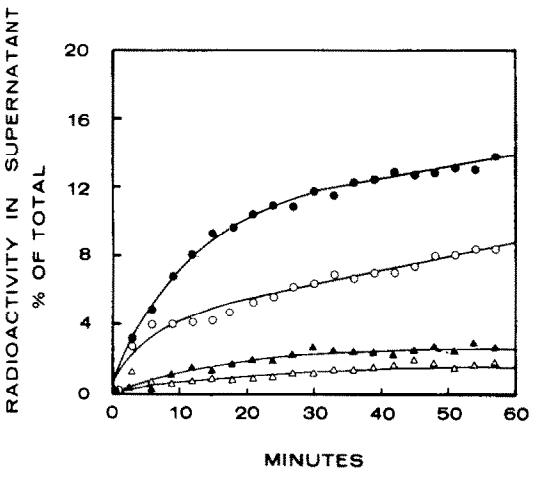

Fig. 3. Effect of imipramine on the liberation of ^{14}C from platelets prelabeled with [^{14}C]-5-HT and the accumulation of radioactivity in supernatant fluid. Washed platelets incubated with either 1 μM [^{14}C]-5-HT (△, ▲) or 100 μM [^{14}C]-5-HT (○, ●) were resuspended in the presence (▲, ●) or absence (△, ○) of imipramine (20 μM). Radioactivity was determined in the supernatant fluid from aliquots of the two suspensions taken at 3-min intervals. The platelet count was 10^6/mm^3. This was one of three similar experiments.

indicating that imipramine reduced the amount of serotonin in the platelets.

Inhibition of serotonin accumulation by platelets. Imipramine (20 μM) powerfully inhibited the initial uptake of [^{3}H]-5-HT at low (1.5 μM) and high (100 μM) serotonin concentrations (Fig. 4).

Effect on exchange of serotonin between platelets and suspending medium. The reduced amount of serotonin in blood platelets in the presence of imipramine could be due to the diminished uptake of serotonin across the platelet plasma membrane demonstrated above or due to diminished uptake of serotonin into the amine storage organelles from the cytoplasm, or both. This was examined in experiments in which the exchange of serotonin between washed rabbit platelets and the suspending medium was followed in the presence or absence of 20 μM imipramine as described in Materials and Methods. Analysis of these experiments showed that 20 μM imipramine inhibited the exchange of serotonin across the platelet plasma membrane (Table 3). Imipramine reduced the fraction of serotonin transferred per unit of time from the suspending medium into the platelet cytoplasm and

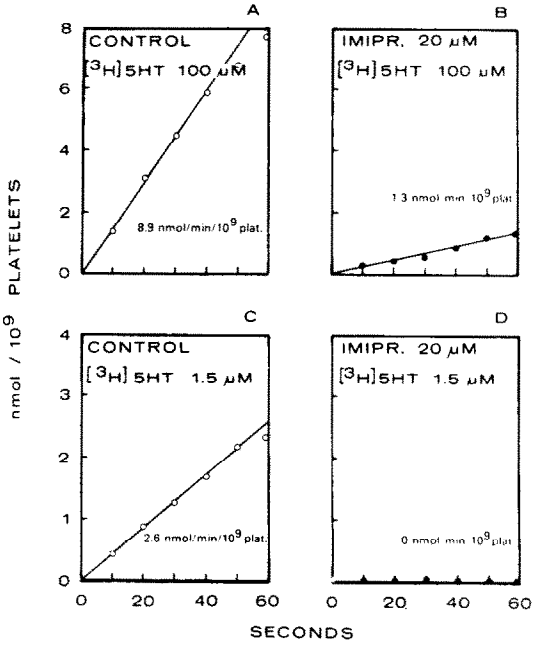

Fig. 4. Effect of imipramine on the initial rate of uptake of [^{3}H]-5-HT by washed rabbit platelets. Panels A and B: Washed rabbit platelets (10^6/mm^3) were incubated for 10 min with saline (A) or 20 μM imipramine (B) before the addition of 100 μM [^{3}H]-5-HT. Mean of duplicate determinations. (This was typical of two experiments.) Panels C and D: Washed rabbit platelets (10^4/mm^3) were incubated for 30 sec with saline (C) or 20 μM imipramine (D), before the addition of 1.5 μM [^{3}H]-5-HT. Mean of duplicate determinations. (This was typical of five experiments.)

Table 3. Effect of imipramine on serotonin content in different compartments of a rabbit platelet suspension and its exchange between these compartments*

Compartment	Control platelets	Imipramine-treated platelets	Significance of difference between means
	Serotonin (nmoles/10^9 platelets)		
Cytoplasm	11.3 ± 1.8	8.5 ± 2.1	$P < 0.4$
Storage granules	179 ± 25	155 ± 7	$P < 0.4$
	Serotonin (nmoles/min/10^9 platelets)		
Exchange of serotonin between suspending medium and platelet cytoplasm	8.4 ± 1.6	3.2 ± 1.4	$P < 0.05$
Exchange of serotonin between platelet cytoplasm and platelet storage organelles	2.5 ± 0.3	1.8 ± 0.3	$P < 0.2$

* Means ± S. E. M. of three experiments. The significance of the differences was calculated by Student's *t*-test.

The imipramine concentration was 20 μM; the serotonin concentration in the suspending medium was 100 μM. The platelet count was 10^6/mm^3.

Table 4. Effect of imipramine on transfer of serotonin between different compartments of washed rabbit platelets*

Compartments	Percentage of serotonin transferred between compartments/min		
	Control platelets	Imipramine-treated platelets	Significance of difference between means†
Suspending medium to platelet cytoplasm	8.4 ± 1.6	3.2 ± 1.4	Significant
Platelet cytoplasm to suspending medium	70.6 ± 2.3	34.7 ± 7.6	Significant
Platelet cytoplasm to storage granules	23.4 ± 3.0	24.7 ± 7.9	Not significant
Storage granules to cytoplasm	1.5 ± 0.1	1.2 ± 0.2	Not significant

* Results are expressed as means ± S. E. M. of three independent experiments. The significance of the differences between the means was calculated as described in Table 1. The concentration of serotonin in the suspending medium was 100 μM. The imipramine concentration was 20 μM. The platelet count was 10^6/mm^3.

† At the 95 per cent level of confidence.

reduced the fraction of serotonin lost per unit of time from the cytoplasm into the suspending medium (Table 4). However, the exchange of serotonin across the amine storage organelle membrane was not significantly reduced at this concentration of imipramine (Table 4).

Effect of imipramine on thrombin-induced release of serotonin. Platelets were equilibrated with serotonin in the presence of imipramine as described in Materials and Methods. Upon addition of thrombin (0.4 units/ml), imipramine-treated platelets released 96 ± 3 (mean ± S. E. M.) per cent of their radioactivity whereas the control platelets released 88 ± 3 per cent of their radioactivity. This difference was not significant ($P < 0.2$; $N = 3$).

DISCUSSION

Reserpine [8, 15–23] and imipramine [24, 25] interfere with the storage and transport of biogenic amines in nervous tissue [3, 26–39], adrenal medulla [4, 40–42], blood platelets [43–52] and other cells such as mast cells [53]. Because they can be rapidly isolated in pure form, platelets are useful for the investigation of transport and storage of biogenic amines [54, 55]. The present studies show that it is possible to examine quantitatively the effects of imipramine and reserpine on serotonin transfer across the platelet plasma membrane and the amine storage organelle membrane in intact platelets.

Reserpine

Reserpine liberates serotonin from blood platelets [8, 43, 44, 48] and inhibits accumulation of serotonin by platelets [45, 56–58]. Reserpine binds to the amine storage organelle membrane [59, 60] but is also found outside the amine storage organelles [60], for example, in the platelet plasma membrane [55]. However, its precise location and distribution outside the amine storage organelles are presently unknown [60]. Although it had been suggested originally that reserpine exerts its effects by inhibiting the active uptake across the platelet plasma membrane [45, 56, 61, 62], more recent experiments did not show an effect of reserpine on the initial uptake of serotonin by platelets [57, 58]. However, reserpine reduced the diffusion of serotonin from the platelets into the surrounding medium [63] and inhibited the uptake of serotonin into (isolated) amine storage organelles [50]. In the experiments reported in this paper, with intact platelets it was possible to examine quantitatively the rates of exchange of serotonin across the plasma membrane and the granule membrane. Using this intact platelet system, we found that reserpine exerted a greater effect at the storage organelle membrane (transfer from the cytoplasm to the granules) than at the plasma membrane (transfer from cytoplasm to suspending fluid). Values for the serotonin transfer rates were calculated by statistical analysis from experiments in which the exchange of radioactive serotonin between platelets and the suspending medium was followed using methods developed previously [1, 2]. An attempt was made to examine the validity of the numerical values obtained by this method, by comparing the rate of exchange across the platelet plasma membrane with the initial rate of uptake of serotonin measured in independent experiments. For this purpose, a method was developed that made it possible to measure serotonin uptake at very short time intervals (10 sec) after its addition. This was necessary since it was found that the uptake of [^{3}H]-5-HT or [^{14}C]-5-HT in the presence of reserpine was linear for less than 1 min. The values obtained with this method for the initial rate of uptake of serotonin by washed rabbit platelets were similar to the values obtained for the transfer of serotonin from the suspending medium into the platelet cytoplasm by statistical analysis of the serotonin exchange experiments. At a serotonin concentration of 123 μM, the rate of transfer from the suspending medium into the platelets was 7.8 ± 0.4 nmoles/min/10^9 platelets when measured by the filtration method and 8.9 ± 1.5 nmoles/min/10^9 platelets when calculated from the exchange experiments.

Imipramine

In contrast to reserpine, imipramine causes no or only very little liberation of serotonin from the

platelets [49]. However, similar to reserpine, imipramine inhibits the accumulation of serotonin by blood platelets [24, 46, 51, 62, 64–66]. All investigators agree that this inhibition is due to an inhibition of the active serotonin uptake across the platelet plasma membrane [5, 52], although there is some dispute about whether this inhibition is competitive [64, 66] or non-competitive [67] in nature. The effect of imipramine on serotonin uptake across the platelet plasma membrane was confirmed in the initial uptake studies as well as in the serotonin exchange experiments. In the presence of imipramine, we also observed some accumulation of platelet serotonin in the suspending medium, indicating a small reduction of the serotonin storage capacity. This finding is in agreement with the hypothesis—put forward by Markwardt [68]—that any drug which inhibits the uptake causes a slow release of endogenous serotonin as well. This is so because the passive diffusion of serotonin out of the platelet is counteracted by active uptake across the platelet plasma membrane [68, 69]. In addition, it was found in these experiments that imipramine at a concentration of 20 μM reduced the fraction of serotonin transferred from the platelet cytoplasm into the suspending medium per unit of time. This is consistent with the suggestion of Gey and Pletscher [70] that tricyclic drugs such as chlorpromazine and chlorprothixene inhibit liberation of endogenous monoamine compounds in brain tissue by reducing the permeability of brain membranes to the amines. Similarly Lingjaerde [71] demonstrated recently that doxepin, another tricyclic drug, diminished the efflux of radioactive serotonin from preloaded platelets at a concentration of 10 μM. The diminished fraction of serotonin transferred from the platelets into the suspending medium per unit of time provides an explanation for the finding that platelets liberate only a small amount of their serotonin during incubation with imipramine.

In vitro, it has been shown that imipramine at high concentrations (>40 μM) [50] inhibits the uptake of serotonin into isolated amine storage organelles. Furthermore, small amounts of imipramine accumulate in the amine storage organelles [72] when intact platelets are incubated with this drug. Imipramine has been shown *in vitro* to displace serotonin from its adenosine triphosphate complex [73]. However, our data on the transfer rates of serotonin across the amine storage granule membrane in intact platelets indicate that imipramine at a lower concentration (20 μM) has no significant effect at this site.

Effect of reserpine and imipramine on intracellular serotonin distribution

It was possible to calculate from the percentages of serotonin transferred into the neighboring compartment(s) per unit of time under the equilibrium conditions of the present experiments the amounts of serotonin (+its metabolites; see Materials and Methods) in the intermediate (extragranular, cytoplasmic) pool and the storage compartment (amine storage organelles). This calculation showed that the intermediate compartment contained about 5–10 per cent of the platelet serotonin, the remaining 90–95 per cent being in the storage compartment. It is generally thought that only the serotonin bound to the amine storage organelles but not the extragranular material can be released from platelets during the release reaction [74]. Thus, it is possible to examine the validity of the numerical results obtained from analysis of the serotonin exchange by measuring the amount of releasable serotonin. Thrombin caused a maximal release of about 90 per cent of the platelet-bound serotonin in the absence of either imipramine or reserpine. This value is similar to those reported from other laboratories [75]. The value of 5–10 per cent for non-releasable, extragranular serotonin in normal washed rabbit platelets is similar to the estimate of extragranular noradrenaline in sympathetic nerve tissue [76] and the particle-free store of adrenaline in the adrenal medulla [77, 78].

In reserpine-treated platelets from reserpine-treated animals, the absolute amount of serotonin in the extragranular compartment did not change significantly, whereas the storage granule pool was greatly diminished (calculated from the exchange experiments). Consequently the proportion of the total platelet serotonin (+metabolites) within the intermediate (extragranular) compartment rose to 25–40 per cent under the equilibrium conditions of these experiments whereas 60–75 per cent remained in the granules. Upon stimulation with thrombin, about 70 per cent of the platelet serotonin was released.

In contrast, imipramine, which diminished the platelet serotonin content only slightly, did not change the relative distribution of serotonin between the extragranular and the granular compartment significantly as calculated from the exchange experiments. This is consistent with the finding that imipramine did not change the releasable proportion of the total platelet serotonin.

The method of studying serotonin exchange in intact platelets used in these experiments may be applicable in investigating the sites at which other drugs interfere with the storage and transport of biogenic amines. It may also prove helpful in defining more precisely the nature of the defect in some diseases that are thought to be related to disorders of transport or storage of biogenic amines [55, 79–81].

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REFERENCES

1. D. J. Allen, H.-J. Reimers, I. A. Feuerstein and J. F. Mustard, *Biometrics* **31**, 921 (1975).
2. H.-J. Reimers, D. J. Allen, I. A. Feuerstein and J. F. Mustard, *J. Cell Biol.* **65**, 359 (1975).
3. U. S. v. Euler, in *Catecholamines* (Eds. H. Blaschko and E. Muscholl), p. 186. Handbuch der experimentellen Pharmakologie, Heffter Heubner, New Series XXXIII, Springer, Berlin (1972).

4. L. Stjärne, in *Catecholamines* (Eds. H. Blaschko and E. Muscholl), p. 231. Handbuch der experimentellen Pharmakologie, Heffter Heubner, New Series XXXIII, Springer, Berlin (1972).
5. A. Pletscher, *Br. J. Pharmac. Chemother.* **32**, 1 (1968).
6. J. Molnar and L. Lorand, *Archs Biochem. Biophys.* **93**, 353 (1961).
7. N. G. Ardlie, D. W. Perry, M. A. Packham and J. F. Mustard, *Proc. Soc. exp. Biol. Med.* **136**, 1021 (1971).
8. P. A. Shore, A. Pletscher, E. G. Tomich, R. Kuntzmann and B. B. Brodie, *J. Pharmac. exp. Ther.* **117**, 232 (1956).
9. J. L. Costa and D. L. Murphy, *Nature, Lond.* **255**, 407 (1975).
10. G. W. Pasternak and S. H. Snyder, *Molec. Pharmac.* **10**, 183 (1974).
11. J. A. Nelder and R. Mead, *Compt. J.* **7**, 308 (1964).
12. G. E. P. Box and D. R. Draper, *Biometrika* **52**, 355 (1965).
13. J. S. Robertson, D. C. Tosteson and J. L. Gamble, *J. Lab. clin. Med.* **49**, 497 (1957).
14. M. DaPrada, G. Bartholini and A. Pletscher, *Experientia* **21**, 135 (1965).
15. M. Holzbauer and M. Vogt, *J. Neurochem.* **1**, 8 (1956).
16. A. Berther, A. Carlsson and E. Rosengren, *Naturwissenschaften* **43**, 521 (1956).
17. V. Erspamer, *Experientia* **12**, 63 (1956).
18. R. M. Hardisty, G. I. C. Ingram and R. S. Stacey, *Experientia* **12**, 424 (1956).
19. P. A. Shore, A. Pletscher, E. G. Tomich, A. Carlsson, R. Kuntzmann and B. B. Brodie, *Ann. N.Y. Acad. Sci* **66**, 609 (1957).
20. B. B. Brodie, E. G. Tomich, R. Kuntzmann and P. A. Shore, *J. Pharmac. exp. Ther.* **119**, 461 (1957).
21. A. Dahlström, K. Fuxe and N. Å. Hillarp, *Acta Pharmac. tox.* **22**, 277 (1965).
22. P. A. Shore, *Pharmac. Rev.* **18**, 561 (1966).
23. G. Taugner, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **274**, 299 (1972).
24. E. F. Marshall, G. S. Sterling, A. C. Tait and A. Todrick, *Br. J. Pharmac. Chemother.* **15**, 35 (1960).
25. H. S. Alpers and H. E. Himwich, *J. Pharmac. exp. Ther.* **180**, 531 (1972).
26. G. Hertting, J. Axelrod and L. G. Whitby, *J. Pharmac. exp. Ther.* **134**, 146 (1961).
27. H. J. Dengler, H. E. Spiegel and E. O. Titus, *Nature, Lond.* **191**, 816 (1961).
28. H. J. Dengler, H. E. Spiegel and E. O. Titus, *Science, N.Y.* **133**, 1072 (1961).
29. J. Axelrod, G. Hertting and L. Potter, *Nature, Lond.* **194**, 297 (1962).
30. H. J. Dengler, I. A. Michaelson, H. E. Spiegel and E. O. Titus, *Int. J. Neuropharmac.* **1**, 23 (1962).
31. E. O. Titus and H. E. Spiegel, *Fedn Proc.* **21**, 179 (1962).
32. L. L. Iversen, J. Glowinski and J. Axelrod, *J. Pharmac. exp. Ther.* **150**, 173 (1965).
33. A. Carlsson, K. Fuxe, B. Hamberger and M. Lundquist, *Acta physiol. scand.* **67**, 481 (1966).
34. S. B. Ross and A. L. Renyi, *Life Sci.* **6**, 1407 (1967).
35. A. Carlsson, K. Fuxe and U. Ungerstedt, *J. Pharm. Pharmac.* **20**, 150 (1968).
36. A. Carlsson, H. Corrodi, H. Fuxe and T. Hökfelt, *Eur. J. Pharmacol.* **5**, 367 (1969).
37. E. G. Shaskan and S. H. Snyder, *J. Pharmac. exp. Ther.* **175**, 404 (1970).
38. P. Lidbrink, G. Jånsson and K. Fuxe, *Neuropharmacology*, **10**, 54 (1971).
39. B. Hamberger and J. R. Tuck, *Eur. J. clin. Pharmac.* **5**, 229 (1973).
40. A. Carlsson, N. Å. Hillarp and B. Hökfelt, *J. biol. Chem.* **227**, 243 (1957).
41. T. A. Slotkin, *Life Sci.* **13**, 675 (1973).
42. N. Kirshner, *Life Sci.* **14**, 1153 (1974).
43. A. Carlsson, P. A. Shore and B. B. Brodie, *J. Pharmac. exp. Ther.* **120**, 334 (1957).
44. F. B. Hughes, P. A. Shore and B. B. Brodie, *Experientia* **14**, 178 (1958).
45. F. B. Hughes and B. B. Brodie, *J. Pharmac. exp. Ther.* **127**, 96 (1959).
46. C. M. Yates, A. Todrick and A. C. Tait, *J. Pharm. Pharmac.* **16**, 460 (1964).
47. A. Pletscher, W. P. Burkard, J. P. Tranzer and K. F. Gey, *Life Sci.* **6**, 273 (1967).
48. J. P. Tranzer, M. Da Prada and A. Pletscher, *Nature, Lond.* **212**, 1574 (1966).
49. A. Pletscher and J. P. Tranzer, *Experientia* **23**, 289 (1967).
50. M. Da Prada and A. Pletscher, *Br. J. Pharmac. Chemother.* **34**, 591 (1968).
51. A. Todrick and G. C. Tait, *J. Pharm. Pharmac.* **21**, 751 (1969).
52. A. Pletscher, M. Da Prada, K. H. Berneis and J. P. Tranzer, *Experientia* **27**, 993 (1971).
53. S. E. Jansson, *Acta physiol. scand.* **82**, 35 (1970).
54. M. K. Paasonen, in *Pharmacology and the Future of Man* (Eds. F. E. Bloom and G. H. Acheson), Vol. 4, p. 328. S. Karger, Basel (1973).
55. J. M. Sneddon, in *Progress in Neurobiology* (Eds. G. A. Kerkut and J. W. Phillis), Vol. 1, Part 2, p. 151. Pergamon Press, Oxford (1973).
56. I. Sano, Y. Yakimoto and K. Taniguchi, *Am. J. Physiol.* **195**, 495 (1959).
57. H. J. Weiss, T. B. Tschopp, J. Rogers and H. Brand, *J. clin. Invest.* **54**, 421 (1974).
58. J. L. Costa, S. A. Silver and J. L. Murphy, *Fedn Proc.* **34**, 275 (1975).
59. M. Da Prada and A. Pletscher, *Experientia* **25**, 923 (1969).
60. S. J. Enna, M. Da Prada and A. Pletscher, *J. Pharmac. exp. Ther.* **191**, 164 (1974).
61. B. F. Minter and N. Crawford, *Biochem. Pharmac.* **23**, 351 (1974).
62. Z. Fuks, R. C. Lanman and L. S. Schanker, *Int. J. Neuropharmac.* **3**, 623 (1964).
63. G. Bartholini, M. Da Prada and A. Pletscher, *Nature Lond.* **205**, 400 (1965).
64. R. S. Stacey, *Br. J. Pharmac. Chemother.* **16**, 284 (1961).
65. L. Ahtee and L. Saarnivaara, *J. Pharm. Pharmac.* **23**, 495 (1971).
66. J. Tuomisto, *J. Pharm. Pharmac.* **26**, 92 (1974).
67. G. V. R. Born, K. Juengjaroen and F. Michal, *Br. J. Pharmac.* **44**, 117 (1972).
68. F. Markwardt, *Annls Med. exp. Biol. Fenn.* **46**, 407 (1968).
69. M. Okuda and Y. Nemerson, *Am. J. Physiol.* **220**, 283 (1971).
70. K. F. Gey and A. Pletscher, *Nature, Lond.* **194**, 387 (1962).
71. O. Lingjaerde, *Psychopharmacologia* **47**, 183 (1976).
72. M. Da Prada and A. Pletscher, *Eur. J. Pharmac.* **32**, 179 (1975).
73. T. Nogrady, H. J. Nogrady, P. D. Hradina and G. M. Ling, *Molec. Pharmac.* **11**, 795 (1975).
74. H. Holmsen, H. J. Day and H. Stormorken, *Scand. J. Haemat.* **1**, (suppl.) (1969).
75. F. Markwardt and W. Barthel, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **249**, 176 (1964).
76. U. S. V. Euler, *Acta physiol. scand.* **67**, 430 (1966).
77. N. Å. Hillarp, *Acta. physiol. scand.* **50**, 8 (1960).
78. N. Kirshner, H. J. Sage and W. J. Smith, *Molec. Pharmac.* **3**, 254 (1967).
79. J. G. White, J. R. Edson, S. J. Desnick and C. J. Witkop, Jr., *Am. J. Path.* **63**, 319 (1971).
80. F. T. Lott, T. N. Chase and D. L. Murphy, *Pediat. Res.* **6**, 730 (1972).

81. A. Barbeau, G. Campanella, R. Butterworth and K. Yamada, *Neurology* **25**, 1 (1975).
82. N. R. Amundson, *Mathematical Methods in Chemical Engineering*, p. 121. Prentice-Hall, Englewood Cliffs, N.J. (1966).

APPENDIX

The platelet suspension is assumed to be comprised of three compartments: suspending medium (compartment 1), "cytoplasm" (compartment 2), and storage organelles (compartment 3). Symbols: a_{21} is the fraction of serotonin in compartment 1 (suspending medium) that transfers to compartment 2 ("cytoplasm") in unit time; a_{12} is the fraction of serotonin in compartment 2 that transfers to compartment 1 in unit time; a_{32} is the fraction of serotonin in compartment 2 that transfers to compartment 3 (storage organelles) in unit time; and a_{23} is the fraction of serotonin in compartment 3 that transfers to compartment 2 in unit time. If there is neither creation nor destruction of the serotonin tracer within a compartment, then, for that compartment:

rate of accumulation of tracer in the compartment = (rate at which serotonin tracer is transferred from the other compartments into this compartment) − (rate at which serotonin tracer is lost to the other compartments).

Thus, for compartment 1

$$\frac{dm_1}{dt} = a_{12}m_2 - a_{21}m_1$$

Similarly, for compartment 2

$$\frac{dm_2}{dt} = a_{21}m_1 + a_{23}m_3 - a_{12}m_2 - a_{32}m_2$$

and for compartment 3

$$\frac{dm_3}{dt} = a_{32}m_2 - a_{23}m_3$$

This set of simultaneous differential equations may be rewritten in vector notation and solved through matrix diagonalization [82].

As described in Materials and Methods, the solution for m_1 takes the form of

$$m_1(t) = A + Be^{-\lambda_1 t} + Ce^{-\lambda_2 t}$$

in which A, B, C, λ_1 and λ_2 are expressed in terms of the fractional turnover rates (a_{12}, a_{21}, a_{23} and a_{32}) and the initial distribution of tracer [$m_1(0)$, $m_2(0)$, $m_3(0)$]. These relationships then follow:

$$A = \frac{a_{12}a_{23}}{\lambda_1\lambda_2}$$

$$B = \frac{a_{12}}{(\lambda_1 - \lambda_2)}\left[\frac{(a_{23} - \lambda_1)}{\lambda_1}\left(\frac{(a_{21} - \lambda_1)a_{21}m_1(0)}{a_{12}(a_{23} - a_{21})} + m_2(0) - \frac{a_{23}(a_{23} - \lambda_2)m_3(0)}{a_{32}(a_{23} - a_{21})}\right)\right]$$

$$C = \frac{(a_{23} - \lambda_2)}{-\lambda_2}\left[\frac{(a_{21} - \lambda_1)a_{21}m_1(0)}{a_{12}(a_{23} - a_{21})} - m_2(0) + \frac{a_{23}(a_{23} - \lambda_1)m_3(0)}{a_{32}(a_{23} - a_{21})}\right]$$

$$\lambda_1 = (x - y)/2$$

$$\lambda_2 = (x + y)/2$$

where

$$x = (a_{21} + a_{12} + a_{23} + a_{32})$$

$$y = [x^2 - 4(a_{21}a_{32} + a_{21}a_{23} + a_{12}a_{23})]^{1/2}$$

The initial distribution of the ^{14}C tracer is known [$m_1(0) = 1$, $m_2(0) = m_3(0) = 0$] and thus the fractional turnover rates may be calculated from the estimated values of A, B, C, λ_1 and λ_2. The relationships permitting direct calculation of fractional turnover rates were obtained from the work of Robertson *et al.* [13], and are:

$$a_{21} = B\lambda_1 + C\lambda_2$$

$$a_{12} = (\lambda_1 + \lambda_2 - a_{21}) - \frac{\lambda_1\lambda_2(1 - A)}{a_{21}}$$

$$a_{23} = \frac{A\lambda_1\lambda_2}{a_{12}}$$

$$a_{32} = \frac{\lambda_1\lambda_2(1 - A)}{a_{21}} - a_{23}$$