Br. J. Pharmac. (1973), **49**, 279–287.

On the pharmacology and biochemistry of the amine-uptake mechanism in human blood platelets

I. C. CAMPBELL* AND A. TODRICK

Department of Clinical Research, Crichton Royal Hospital, Dumfries DG1 4TG, Scotland

Summary

1. The uptake of 5-hydroxytryptamine (5-HT) by human blood platelets *in vitro* has been studied with the object of identifying the biochemical mechanisms involved.

2. Drugs active in adrenergic systems are only moderate inhibitors of uptake, although prenylamine is as active as the less potent tricyclic anti-depressive drugs; phenoxybenzamine is almost inactive as a competitive inhibitor but is effective if pre-incubated with the platelets beforehand. This parallels its pharmacological pattern of action.

3. Inhibitors of oxidative phosphorylation do not inhibit 5-HT uptake, but iodoacetate inhibits, if pre-incubated with the platelets; *p*-chloromercuribenzoate does also, when the platelets are suspended in synthetic medium, but not in plasma.

4. Ouabain causes significant inhibition at 10^{-7}M; by 10^{-6}M it achieves its maximal effect, namely 40% inhibition; in K^+-deficient medium, uptake falls to 30% of normal; the K^+-dependent fraction of the uptake includes the ouabain-sensitive component. Mg^{++} has no effect.

5. A drug not possessing the imipramine structure, which has been tried in the treatment of depressive illness, 4-phenyl bicyclo (2,2,2) octan-1-amine, is a highly potent inhibitor of 5-HT uptake.

Introduction

The active uptake of 5-hydroxytryptamine (5-HT) into blood platelets and the effect of pharmacological agents thereon have been studied intensively, (Pletscher, 1968; Paasonen, 1972), because it can be more closely controlled experimentally than most other active transport systems for amines and because of the possible significance of blood platelets as a model of the nerve ending with respect to its amine re-uptake system (Maynert & Isaac, 1968; Murphy, Colburn, Davis & Bunney, 1970; Abrams & Solomon, 1970). This uptake is blocked by low concentrations of tricyclic anti-depressive drugs (Todrick & Tait, 1969) which, however, have negligible actions on many enzyme systems (Løvtrup, 1963, 1964). Since the pharmacological action of such drugs cannot be understood without a knowledge of the underlying biochemical processes, identification of the enzymes or other macromolecular components involved in the uptake process has been attempted.

* Present address: Department of Psychological Medicine, The University of Newcastle upon Tyne NE1 4LP, England.

Contradictory findings have been reported, particularly in respect of the action of ouabain (Weissbach, Redfield & Titus, 1960; Stacey, 1961; Pletscher, Burkhard, Tranzer & Gey, 1967).

Methods

Platelet preparations used in this study were obtained by withdrawing 20 ml of blood from the antecubital vein of healthy subjects and mixing immediately with 2 ml of an anticoagulant solution (0·7% sodium chloride, 1·0% disodium ethylenediamine tetraacetate). The medium is not physiologically normal due to the absence of Ca^{++}, but the platelet preparation is stable and uptake of 5-HT readily occurs. The mixture was centrifuged under refrigeration for 20 min at 110 *g* to separate the platelet-rich plasma. All glassware used was siliconed.

Except where specifically stated, the techniques used in these experiments were those described previously (Marshall, Stirling, Tait & Todrick, 1960; Yates, Todrick & Tait, 1963; Todrick & Tait, 1969). Platelet-rich plasma, 1·5 ml, was added to 0·5 ml total volume of solutions of 5-HT, inhibitors and other compounds where required and incubated at 37° C for 20 minutes. Ice-cold saline (10 ml) was then added and the platelets were centrifuged for 10 min at 1400 *g*, resuspended in saline and frozen to release 5-HT. Following zinc hydroxide precipitation and centrifugation, the 5-HT was estimated in an aliquot of the supernatant, acidified to 3 N HCl, using an Aminco-Bowman Spectrophotofluorometer set optimally (295–300/540–550 nm).

In a modification of the procedure, the platelet-rich plasma was incubated with a drug or inhibitor at 37° C for a fixed time, usually 30 min, before addition of 5-HT; the control tubes were treated similarly. For the investigation of 5-HT uptake in synthetic media, the platelet-rich plasma was centrifuged for 20 min at 1400 *g*. The plasma was decanted and the tube walls dried with strips of filter paper. The plasma button was then resuspended in a phosphate buffer medium (Cooley & Cohen, 1967) or a variant of it lacking one or more cations (Table 1).

Platelets resuspended in this phosphate buffer medium by the technique of Dillard, Brecher & Cronkite (1951) took up 99·5 ± 15·9% (S.D.) (12 experiments) of the amount of 5-HT taken up by platelets resuspended in their own platelet-poor plasma.

Laboratory chemicals employed were: 5-hydroxytryptamine creatinine sulphate, *p*-chloromercuribenzoic acid, iodoacetic acid and 2,4 dinitrophenol (all B.D.H.). Acids were neutralized with 0·1 M NaOH during the preparation of the stock solutions.

TABLE 1. *Synthetic buffer media*

Medium	Cation concentration (mM) Na^+	K^+	Mg^{++}	Other components
Cooley & Cohen's (1967) standard phosphate buffer medium	143	5	0·29	Phosphate buffer 67 mM pH 7·6 Glucose 5·5 mM
K^+-deficient medium	148	0	0·29	
Na^+-deficient medium	0	148	0·29	
Mg^{++}-deficient medium	143	5	0	
K^+ & Mg^{++}-deficient medium	148	0	0	

Drugs

Amylobarbitone sodium (Amytal, Lilly); dichloroisoprenaline hydrochloride (Aldrich); ergotamine tartrate (Sigma); hydergine (Sandoz); iprindole hydrochloride (Prondol, John Wyeth); ouabain octahydrate (Sigma); phenoxybenzamine hydrochloride (Dibenyline, Smith Kline & French); phentolamine hydrochloride (Rogitine, Ciba); 4-phenyl bicyclo (2,2,2) octan-1-amine (EXP 561, du Pont de Nemours); prenylamine lactate (Segontin, Hoechst); propranalol (Inderal, I.C.I.); (−)-thyroxine sodium (B.D.H.). Fresh solutions of drug were prepared in saline for each experiment. Phenoxybenzamine was dissolved in ethanol and diluted 100-fold with saline (0·9% w/v NaCl solution) to make the stock solution, and working dilutions were prepared immediately; saline solutions containing ethanol were employed in the controls; the maximum ethanol concentration (0·1%) had no significant effect on the system.

Results

Action of pharmacological agents known to operate in adrenergic systems

The compounds investigated in a preliminary survey were the α-adrenoceptor blocking drugs, hydergine, phenoxybenzamine and phentolamine, the β-adrenoceptor blocking drugs, dichloroisoprenaline and propranalol and ergotamine and prenylamine. The inhibition by these drugs of 5-HT uptake into platelets suspended in plasma was measured at drug concentrations of 10^{-4}, 10^{-5} and 10^{-6}M, with and without 30 min pre-incubation. The results are given in Table 2. The experiments were also designed to show whether the drugs possessed any 5-HT releasing activity. No significant release was noted.

TABLE 2. *Inhibition of 5-hydroxytryptamine (5-HT) uptake into human platelets by drugs active in adrenergic systems*

Compound	Concentration (M)	Percentage inhibition* of 5-HT uptake at 37°C by platelets: Without pre-incubation of platelet-rich plasma and drug	Following pre-incubation of platelet-rich plasma with drug for 30 min
Hydergine	10^{-4}	50	55
	10^{-5}	15	25
Phentolamine	10^{-4}	55	45
	10^{-5}	5	− 5
	10^{-6}	− 5	− 5
Phenoxybenzamine	10^{-4}	20	80
	10^{-5}	0	25
Dichloroisoprenaline	10^{-4}	80	80
	10^{-5}	35	30
	10^{-6}	10	10
Propranalol	10^{-4}	90	95
	10^{-5}	25	20
	10^{-6}	20	− 5
Ergotamine	10^{-4}	35	55
	10^{-5}	5	0
	10^{-6}	10	0
Prenylamine	10^{-4}	80	85
	10^{-5}	50	80
	10^{-6}	20	25

* Figures are means from two incubation experiments each estimated in duplicate, rounded off to the nearest 5%.

Pre-incubation did not, generally, enhance the degree of inhibition, but it did with phenoxybenzamine. Further experiments comparing the effects of phenoxybenzamine and hydergine confirmed this (Figure 1). Hydergine inhibition was not increased by pre-incubation; without pre-incubation, inhibition by phenoxybenzamine was hardly significant but, after 30 min pre-incubation, it increased significantly (to over 80% at 10^{-4}M).

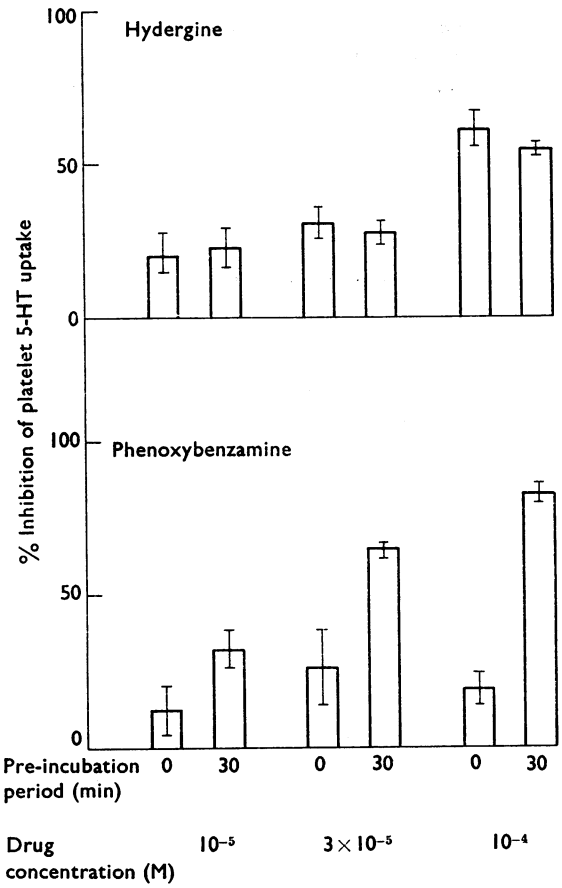

FIG. 1. Influence of pre-incubation with α-adrenoceptor blocking drugs at 37° C on the uptake of 5-hydroxytryptamine (5-HT) by platelets *in vitro*. Each bar is the mean ± S.E.M. of 6 experiments on platelets from different individuals.

Phenoxybenzamine apart, the β-adrenoceptor blocking drugs were more effective inhibitors than the α-adrenoceptor blocking drugs, though prenylamine was the most potent of the compounds examined.

Effect of compounds with known biochemical actions

Inhibition of 5-HT uptake into platelets was measured both with and without 30 min pre-incubation at 37° C; the experimental results are given in Table 3.

TABLE 3. *Inhibition of 5-hydroxytryptamine (5-HT) uptake into platelets by compounds possessing specific biochemical actions*

Compound	Concentration (M)	Percentage inhibition* of 5-HT uptake at 37°C: Without pre-incubation of platelet-rich plasma with drug	With 30 min pre-incubation of platelet-rich plasma with drug
Malonate	10^{-3}	5	—
Amylobarbitone	10^{-4}	15	10
	10^{-5}	5	20
	10^{-6}	25	15
2,4 Dinitrophenol	10^{-4}	− 5	—
	10^{-5}	0	—
	10^{-6}	− 5	—
(−)-Thyroxine	10^{-4}	5	0
	10^{-5}	0	0
	10^{-6}	20	0
p-Chloromercuribenzoate	10^{-4}	5	5
	10^{-5}	5	5
	10^{-6}	5	10
Iodoacetate	10^{-2}	0	65
	10^{-3}	− 5	−10
	10^{-4}	−15	+ 5
Ouabain	10^{-4}	50	40
	10^{-5}	25	35
	10^{-6}	35	40

* Mean of two incubations each estimated in duplicate, rounded off to nearest 5%.

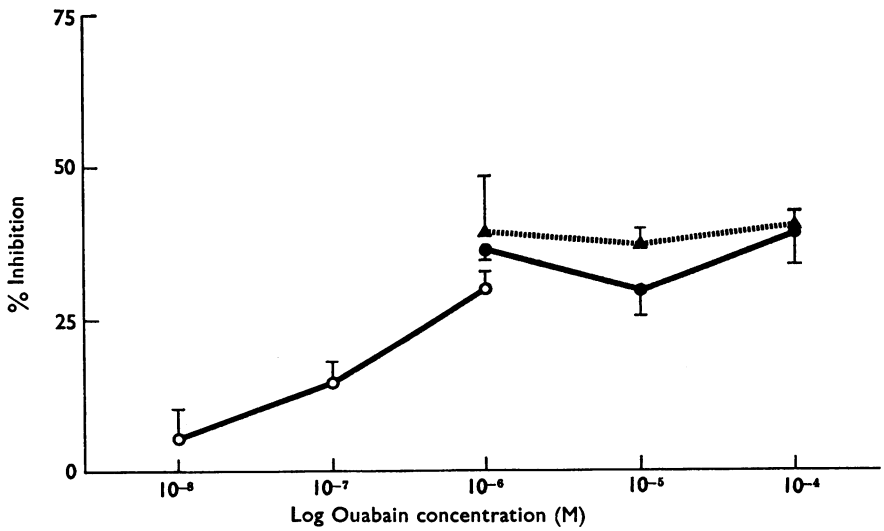

FIG. 2. Effect of ouabain on platelet uptake of 5-hydroxytryptamine (5-HT) at 37° C *in vitro*. ▲– – –▲, 30 min pre-incubation of ouabain with platelets at 37° C before addition of 5-HT, (1·0 μg of base/ml); ●——● and ○——○, no pre-incubation, Series I and II respectively. Points show mean ± S.E.M. of four or more estimates.

Previous extensive experience with this experimental procedure gave a standard deviation of 8·3% with slight but significant heteroscedasticity (Williams & Todrick, 1969). Therefore, inhibitions of up to 15% given in Table 3 and elsewhere are not significantly different from zero. Only two compounds inhibited. Iodoacetate was ineffective without pre-incubation, even at 10^{-2}M, but caused significant inhibition after pre-incubation, though only at this high concentration. The inhibition

produced by ouabain, although not more than 50% under any circumstances, was too large to be ignored and, within the limits of experimental error, was independent of drug concentration. It was, therefore, studied in greater detail. The results are given in Figure 2. In the range 10^{-8}–10^{-6}M, a normal concentration dependence was found but the maximal inhibition achieved was again only 40%. Pre-incubation for 30 min at 37° C did not affect the level of maximum inhibition at 10^{-4} and 10^{-6}M, though there was an inexplicable difference at 10^{-5}M.

For comparison with previous published work, some of these inhibitors were tested on platelets suspended in synthetic buffer medium. The results (Table 4) indicate that considerable binding of inhibitor by plasma protein occurs.

Effect of cations on uptake of 5-hydroxytryptamine by platelets

In the light of the specific action of ouabain on cation flux, uptake in cation-deficient solutions was investigated. The results are given in Table 5.

Absence of Mg^{++} did not reduce 5-HT uptake. In a K^+-deficient medium the

TABLE 4. *Inhibition of 5-hydroxytryptamine (5-HT) uptake into platelets in synthetic buffer medium*

Compound	Concentration (M)	Conditions	Percentage inhibition* of 5-HT uptake at 37°C: In plasma	In synthetic buffer medium
Iodoacetate	10^{-2}	Without pre-incubation	0	50
		With 30 min pre-incubation	65	100
p-Chloromercuribenzoate	10^{-4}	Without pre-incubation	5	90
	2×10^{-5}	,, ,,	−10	60

* Mean of two incubations each estimated in duplicate, rounded off to nearest 5%.

TABLE 5. *Reduction of 5-hydroxytryptamine (5-HT) uptake into platelets in cation deficient media*

A. Alterations in cations only

Medium	% Loss of activity* ±S.E.M. (n=4)	Significance	Significance of difference between deficient media
K^+-deficient	76·7±3·5	$P<0.001$	$P<0.1>0.05$
K^+ & Mg^{++}-deficient	65·2±1·9	$P<0.001$	
Mg^{++}-deficient	−0·6±4·3	$P=0.90$	
Na^+-deficient	No net uptake at all. Irregular loss of endogenous 5-HT		

B. Inhibition by ouabain 10^{-5}M in cation-deficient media

Medium	Mean % inhibition† ±S.E.M. (n=4)	Significance	Significance of difference in the effect of ouabain between standard and Mg^{++}-deficient media
Standard buffer	41·6±2·3	$P<0.001$	$P<0.1>0.05$
Mg^{++}-deficient	51·2±3·2	$P<0.001$	
K^+-deficient	6·9±2·4	$P<0.1>0.05$	

* By comparison with standard buffer medium suspensions of same platelets (n=4). † By comparison with uptake in same medium in absence of ouabain (n=4).

TABLE 6. *Inhibition of 5-hydroxytryptamine (5-HT) uptake into platelets by potential anti-depressive drugs*

Iprindole		EXP 561	
Concentration (M)	% Inhibition	Concentration (M)	% Inhibition
3×10^{-4}	81 (4)	10^{-6}	87 (4)
10^{-4}	52 (8)	3×10^{-7}	62 (4)
3×10^{-5}	23 (4)	10^{-7}	55 (6)
10^{-5}	8 (4)	3×10^{-8}	18 (4)
Concentration giving 50% inhibition*	9×10^{-5}		$1{\cdot}5\times10^{-7}$
Slope of line† (% inhibition/unit log molar concentration)	57		43

* Determined graphically. † Mean slope for 13 tricyclic anti-depressive drugs=46±1·6 (S.E.M.) % inhibition/unit log molar concentration. (Todrick & Tait, 1969.)

uptake fell to about 30% and this was not significantly altered if Mg^{++} was also absent. In a Na^+-deficient medium there was no measurable nett uptake; there was in fact an irregular but sometimes complete loss of endogenous 5-HT.

In the complete synthetic medium, 10^{-5}M ouabain caused 42% inhibition of 5-HT uptake, which was significantly greater than that observed in platelet rich plasma ($P<0{\cdot}05$). In K^+-deficient medium, the already reduced uptake of 5-HT was not further inhibited by ouabain but, in Mg^{++}-deficient medium, ouabain had its full effect.

Action of drugs tested clinically for anti-depressive activity

The tricyclic anti-depressive drugs are among the most potent inhibitors of 5-HT uptake by platelets (Stacey, 1961). Two compounds which differ considerably from the tricyclics in chemical structure but have also undergone trial for the treatment of depressive illness have been tested as inhibitors (Table 6). Iprindole (Prondol, John Wyeth) appears to be only a weak inhibitor of 5-HT uptake but 4-phenyl bicyclo (2,2,2) octan-1-amine (EXP 561, du Pont de Nemours) is more potent than any compound hitherto examined in this laboratory.

Discussion

Reviews (Pletscher, 1968; Murphy *et al.*, 1970) quote reports that inhibitors of oxidative phosphorylation block the uptake of 5-HT by platelets. The present results do not confirm this and reference to the original papers (Sano, Kakimoto & Taniguchi, 1958; Born & Gillson, 1959; Weissbach & Redfield, 1960) reveals that inhibition was slight and inhibitor concentration high. It is, moreover, difficult to assess the statistical significance of the data. There is more evidence to support the view that the glycolytic cycle provides the energy for the active uptake of 5-HT by platelets (Hughes & Brodie, 1959; Weissbach & Redfield, 1960; Waller, Lohr, Grignani & Gross, 1959).

The cation-transport inhibitor, ouabain, was originally found not to inhibit 5-HT uptake by platelets at 10^{-4}M in a plasma medium (Weissbach *et al.*, 1960; Stacey, 1961), though the former workers noted inhibition in a buffer medium at an unphysiological pH (5·7). Pletscher *et al.* (1967) found inhibition of 5-HT uptake by guinea-pig platelets from a modified Tyrode medium was 55% at 10^{-5}M and 60% at 10^{-4}M ouabain. Qualitatively similar data are given in Fig. 2 of this paper, a maximum, but partial, inhibition of 40% being reached with 10^{-6}M ouabain. However, Sneddon (1971) found that the inhibition by ouabain of 5-HT uptake into rat platelets was concentration-dependent up to 10^{-3}M.

Two-thirds of the 5-HT uptake was found to be K^+-dependent; approximately half of this was the ouabain-sensitive component. Sneddon (1971) found that 5-HT uptake into rat platelets was also reduced by two-thirds in the absence of K^+ but the results are not wholly concordant since in the rat platelets the ouabain inhibition was approximately equal to the whole of the K^+-dependent fraction.

The discrepancy between the results reported here and those of Weissbach *et al.* (1960) may be related to the higher 5-HT concentration which they used, 15 μg/ml, compared with 1·0 μg/ml used in the present work and 0·17 μg/ml of Pletscher *et al.* (1967).

Blood platelets from different species differ widely in their normal 5-HT content (Garattini & Valzelli, 1965) and their capacity for taking up exogenous 5-HT *in vitro*. Human and guinea-pig platelets resemble one another closely in having low (0·15—0·2 μg/ml) endogenous levels of 5-HT and a high uptake potential (Stacey, 1961; Pletscher *et al.*, 1967) in contrast to other species, including rats and rabbits, with much higher endogenous levels and low uptake potential (Sneddon, 1969; Campbell, unpublished results).

The agreement between the present results and those of Pletscher *et al.* (1967) and the discrepancy with Sneddon's findings may reflect the similarity between human and guinea-pig platelets and their differences from those of other species.

EXP 561 is one of the most potent inhibitors of human platelet 5-HT uptake yet tested. The complete inhibition produced by it and tricyclic anti-depressive compounds (Todrick & Tait, 1969) suggests that they do not inhibit by acting on the Na^+/K^+-dependent ATPase transport mechanism.

This research was supported by a grant from Geigy (U.K.) Ltd. We are also indebted to the Scottish Hospital Endowments Research Trust for the continued loan of a spectrophotofluorometer and are grateful to Miss V. A. Fawcett and Mr. A. V. Sherry for valuable technical assistance.

REFERENCES

ABRAMS, W. B. & SOLOMON, H. M. (1970). The human platelet as a pharmacologic model for the adrenergic neurone. *Clin. Pharmac. Therap.*, **10**, 702–709.

BORN, G. V. R. & GILLSON, R. E. (1959). Studies on the uptake of 5-hydroxytryptamine by blood platelets. *J. Physiol., Lond.*, **146**, 472–491.

COOLEY, M. H. & COHEN, P. (1967). Potassium transport in human blood platelets. *J. lab. clin. Med.*, **70**, 69–79.

DILLARD, G. H. L., BRECHER, G. & CRONKITE, E. P. (1951). Separation, concentration and transfusion of platelets. *Proc. Soc. exp. Biol. Med.*, **78**, 796–799.

GARATTINI, S. & VALZELLI, L. (1965). *Serotonin*, pp. 242–276. Amsterdam: Elsevier.

HUGHES, F. B. & BRODIE, B. B. (1959). The mechanism of serotonin and catecholamine uptake by platelets. *J. Pharmac. exp. Ther.*, **127**, 96–102.

LØVTRUP, S. (1963). A comparative study of the influence of chlorpromazine and imipramine on mitochondrial activity: (i) oxidation and phosphorylation. *J. Neurochem.*, **10**, 471–477.

LØVTRUP, S. (1964). A comparative study of the influence of chlorpromazine and imipramine on mitochondrial activity: (ii) cytochrome oxidase and $NADH_2$ cytochrome C reductase. *J. Neurochem.*, **11**, 377–386.

MARSHALL, E. F., STIRLING, G. S., TAIT, A. C. & TODRICK, A. (1960). The effect of iproniazid and imipramine on the blood platelet 5-hydroxytryptamine level in man. *Br. J. Pharmac. Chemother.*, **15**, 35–41.

MAYNERT, E. W. & ISAAC, L. (1968). Uptake and binding of serotonin by the platelet and its granules. *Adv. Pharmac.*, **6A**, 113–122.

MURPHY, D. L., COLBURN, R. W., DAVIS, J. M. & BUNNEY, W. E. (1970). Imipramine and lithium effects on depressed and manic-depressive patients. *Am. J. Psychiat.*, **127**, 339–344.

PAASONEN, M. K. (1972). Blood platelets as a model for aminergic neurones. In: *Proc. 5th Int. Cong. on Pharmacology*, San Francisco, July 1972. pp. 18–19.

PLETSCHER, A. (1968). Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. *Br. J. Pharmac. Chemother.*, **32**, 1–16.

PLETSCHER, A., BURKHARD, W. P., TRANZER, J. P. & GEY, K. F. (1967). Two sites of 5-hydroxytryptamine uptake in blood platelets. *Life Sci.*, **6**, 273–280.

SANO, I., KAKIMOTO, Y. & TANIGUCHI, K. (1958). Binding and transport of serotonin in rabbit blood platelets and action of reserpine. *Am. J. Physiol.*, **195**, 495–498.

SNEDDON, J. M. (1969). Sodium dependent accumulation of 5-hydroxytryptamine by rat blood platelets. *Br. J. Pharmac.*, **37**, 680–688.

SNEDDON, J. M. (1971). Relationship between internal Na^+/K^+ and the accumulation of ^{14}C-5-hydroxytryptamine by rat platelets. *Br. J. Pharmac.*, **43**, 834–844.

STACEY, R. S. (1961). Uptake of 5-hydroxytryptamine by platelets. *Br. J. Pharmac. Chemother.*, **16**, 284–295.

TODRICK, A. & TAIT, A. C. (1969). The inhibition of human platelet 5-hydroxytryptamine uptake by tricyclic anti-depressive drugs. The relation between structure and potency. *J. Pharm. Pharmac.*, **21**, 751–762.

WALLER, H. D., LOHR, G. W., GRIGNANI, F. & GROSS, R. (1959). Uber der Energiestoffwechsel normaler menschlicher Thrombozyten. *Thromb. Diäth. Haemorrh.*, **3**, 520–547.

WEISSBACH, H. & REDFIELD, B. G. (1960). Factors affecting the uptake of 5-hydroxytryptamine by human platelets in an inorganic medium. *J. Biol. Chem.*, **235**, 3287–3291.

WEISSBACH, H., REDFIELD, B. G. & TITUS, E. O. (1960). The effect of cardiac glycosides and inorganic ions on binding of serotonin by platelets. *Nature, Lond.*, **185**, 99–100.

WILLIAMS, D. A. & TODRICK, A. (1969). Appendix—Statistical evaluation of relative potencies of selected anti-depressive compounds. *J. Pharm. Pharmac.*, **21**, 761–762.

YATES, C. M., TODRICK, A. & TAIT, A. C. (1963). Aspects of the clinical chemistry of desmethylimipramine in man. *J. Pharm. Pharmac.*, **15**, 432–439.

(*Received December* 2, 1972)