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The accumulation of guanethidine by human blood platelets

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1. When human blood platelets were incubated aerobically in plasma containing 2×10^{-7} to 10^{-3}M radioactive guanethidine for 10 min to 6 hr, the drug was accumulated against a concentration gradient until concentration ratios (platelet/plasma) of up to 80 : 1 were obtained.

2. The decline in rate of uptake after 3 hr appeared to result from a decrease in platelet viability, because accumulation was reduced by prolonged incubation before addition of guanethidine.

3. Uptake was energy-dependent because it was inhibited by cold and ouabain.

4. Sodium ions were essential for guanethidine uptake and retention of 5-hydroxytryptamine (5-HT).

5. Accumulation was inhibited by 5-HT, desipramine, cocaine, dexamphetamine, bretylium, tyramine and noradrenaline ; bethanidine, *p*-chlorophenylalanine and (−)-α-methyldopa were inactive.

6. Guanethidine was tightly bound to platelets, only 10% being lost from labelled cells during 60 min incubation in drug-free plasma ; but efflux was increased by addition of amphetamine.

7. The binding sites for guanethidine seemed to be different from those for 5-HT since guanethidine accumulation was independent of 5-HT levels, and neither guanethidine uptake or release were affected by reserpine.

8. Guanethidine was not metabolized by platelets or plasma *in vitro*.

9. We consider that, if our results regarding uptake, binding and release of guanethidine are confirmed *in vivo*, and also found to apply to other pharmacologically active agents, then the eventual loss of a platelet-bound substance may increase pharmacological action by raising plasma levels.

Although there is considerable information concerning the mechanisms of uptake and sites of storage of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) by blood platelets, the fact that platelets can also accumulate a wide variety of other substances such as procaine, histamine, reserpine, quinidine, amino-acids, sugars

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and diuretic drugs has only recently been demonstrated (Solomon & Zieve, 1967; Zieve & Solomon, 1967, 1968a, b).

Recent work indicates that in several respects the blood platelet resembles the sympathetic nerve ending. Although 5-HT is the predominant amine in platelets and sympathetic nerves contain only NA, both structures preferentially accumulate the respective amine by an energy-dependent mechanism and store it in sub-cellular vesicles which show a dense core under the electron microscope (for references see Potter, 1967; Pletscher, 1968). As the uptake of guanethidine by sympathetic nerves is well established (for references see Boullin, 1968), it was thought that blood platelets might also take up guanethidine. This paper shows that this is the case: guanethidine is concentrated to a considerable degree by an energy-dependent process. As the ratio of the concentration of guanethidine in platelets to that in the incubation medium may exceed 50 : 1, the possibility that platelet-bound guanethidine may be of clinical significance is discussed.

Some of these experiments have been reported in preliminary communications (Boullin, 1967; Boullin & O'Brien, 1968b).

Methods

Blood was obtained by venipuncture from normal volunteers. The fluid was collected in either siliconed glass or plastic centrifuge tubes so that approximately 15 ml. blood was rapidly mixed with 1.5 ml. of a solution of disodium edetate (1 g/100 ml. 0.7% w/v sodium chloride solution). The separation of platelet-rich plasma was carried out according to the technique described by Hardisty & Stacey (1955) and Stacey (1961).

Incubation experiments with radioactive guanethidine

The procedure used was based on that described by Stacey (1961), with the following modifications: 1.2 ml. platelet-rich plasma, mixed with 0.3 ml. 0.7% saline, was incubated with radioactive guanethidine and various other drugs under an atmosphere of 95% oxygen and 5% carbon dioxide at 37° C for up to 6 hr in a Dubnoff metabolic incubator. In most experiments the incubations were made as follows: samples were first incubated for 10 min at 37° C. Radioactive guanethidine was then added in a volume of 10–50 μl. of distilled water and incubation continued for up to 6 hr. In experiments on the inhibition of guanethidine uptake by drugs, the preincubation period was extended to 30 min, the drugs being added after 10 min and guanethidine 20 min later. An exception was made with reserpine. After the initial incubation of 10 min, reserpine was added and incubation continued for another 2 hr; then radioactive guanethidine was added, and incubation continued.

Experiments with resuspended platelets

After preliminary incubations with guanethidine for 1.5 hr at 37° C the platelets were separated from the plasma/saline mixture by centrifugation at 3400 *g* for 5 min. This method of separation produced a platelet pellet that was not too highly packed for easy disruption by agitation but was sufficient to precipitate all the platelets. The solution was decanted, the interior of the centrifuge tubes were carefully dried with paper tissue, and then 1.5 ml. of fresh plasma/saline mixture was added. The platelets were resuspended by agitation of the tubes on a vortex mixer.

Experiments with washed platelets

This procedure was similar to that outlined for resuspension of platelets except that artificial media were used, as described in results, and the centrifugation and resuspension were repeated three times.

Estimation of guanethidine uptake

At the end of incubation, a tightly packed platelet pellet was obtained by high speed centrifugation at 20,000 *g* for 5 min. The incubation medium was then decanted, the inside surfaces of the centrifuge tubes were dried and then the tubes were immersed in solid carbon dioxide at −78° C to freeze the pellet. After thawing, the lysed platelets were dissolved in 1 ml. of distilled water. This process was repeated and another 1 ml. of distilled water added. A 1 ml. aliquot of this platelet extract and 0.2 ml. of the incubation medium were put in counting vials and 10 ml. of Bray's solution (Bray, 1960) was added. Radioactivity was counted in a Packard Tricarb liquid scintillation spectrometer. In some experiments radioactive guanethidine was separated from metabolites by chloroform extraction (Furst, 1968). 0.25 ml. of 60% KOH was added to 1 ml. aliquots of the pellet extract and the incubation medium. The alkaline extracts were shaken for 10 min with 10 vol. of chloroform. 1 ml. of the chloroform layer (containing unmetabolized guanethidine) was put in a counting vial and the solvent evaporated on a water bath before addition of scintillator. 1 ml. of the aqueous layer (containing guanethidine metabolites) was neutralized with HCl and counted in 10 ml. Bray's solution. Samples were corrected for quenching by addition of internal standards, and in all experiments the extracellular distribution of guanethidine in the platelet pellet was determined by addition of radioactive substance to the incubation medium immediately before removal of platelets by high-speed centrifugation. Uptake was calculated after subtraction of the extracellular guanethidine and is expressed as moles guanethidine/ml. packed platelets. The packed platelet volume in 0.5 ml. platelet-rich plasma was determined with a thrombocytocrit (Hardisty & Stacey, 1955).

5-HT estimation

5-HT in an aliquot of the platelet pellet extract was estimated spectrophotofluorimetrically (Stacey, 1961).

Drugs

The weights of all drugs refer to the base. The following compounds were used:

Bethanidine hydrochloride, bretylium tosylate, *p*-chlorophenylalanine, cocaine hydrochloride, desipramine hydrochloride, dexamphetamine sulphate, α-methyldopa, 5-hydroxytryptamine creatinine sulphate, (−)-noradrenaline hydrochloride, ouabain, reserpine phosphate, tyramine hydrochloride.

^{14}C-guanethidine sulphate (specific activity 3.14 μc/mg) or ^{3}H-guanethidine sulphate (specific activity 132 μc/mg) were used as described by Boullin (1968).

Results

Kinetics of guanethidine uptake

Guanethidine was accumulated by human blood platelets incubated in plasma containing a wide range of concentrations (2×10^{-7} to 10^{-3}M). Uptake was linear

for at least 1.5 hr ; the rate of uptake began to decline after 3 hr but steady state conditions were not reached even after 6 hr incubation (Fig. 1). The uptake and pattern of accumulation of guanethidine was completely different from the uptake of 5-HT. Figure 2 shows results of experiments where platelets taken from the same volunteers were incubated with 10^{-5}M 5-HT or guanethidine for 90 min. Whereas the accumulation of 5-HT had almost ceased after 90 min, guanethidine was still being taken up at an undiminished rate ; the possible explanation of the linear uptake of guanethidine will be discussed later in this paper.

Although guanethidine uptake was comparatively slow, the concentration of drug in the platelets (C_i) exceeded the plasma concentration (C_o) shortly after starting incubation. In most experiments incubation was continued for no longer than 90 min to avoid undue reduction in platelet viability. At the end of this time there was a linear relationship between the ratio C_i/C_o and the final concentration of drug in the plasma, C_o (Fig. 3). Although guanethidine uptake did not satisfy Michaelis-Menten kinetics, Fig. 3 suggests that accumulation did involve a process that was progressively saturated as the plasma drug concentration was increased. It may be estimated from Fig. 3 that by extrapolation to 10^{-2}M C_i/C_o approaches zero. The minimum possible value could be unity if all active processes were eliminated. A higher constant value, up to 2.6 : 1, however, might be obtained depending on the pK_a values of guanethidine (Boullin, 1966) and the electrochemical gradient of the platelet (Solomon & Zieve, 1967).

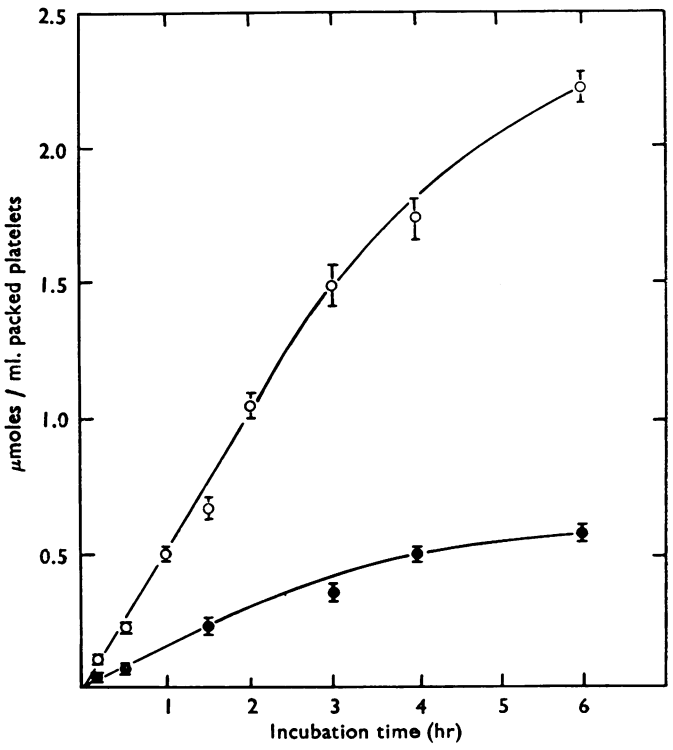

FIG. 1. Uptake of guanethidine by human blood platelets incubated in plasma/saline (4/1, see text) containing 10^{-4} (○) or 10^{-5}M (●) ^{3}H-guanethidine. Results are mean ± S.E. of five to nine determinations.

The C_i/C_o ratios shown in Fig. 3 obtained after 90 min incubation ranged between 7:1 when C_o was 0.96×10^{-3}M, and 30:1 at the lowest concentration, when C_o was 1.7×10^{-7}M. The C_i/C_o ratios obtained after 6 hr incubation were at least 2.5 times greater than the 90 min values; at the lowest plasma concentration of 2×10^{-7}M the C_i/C_o ratio was between 70 and 80:1. At such high ratios the accumulation of drug by the platelets had the effect of reducing plasma levels considerably—by

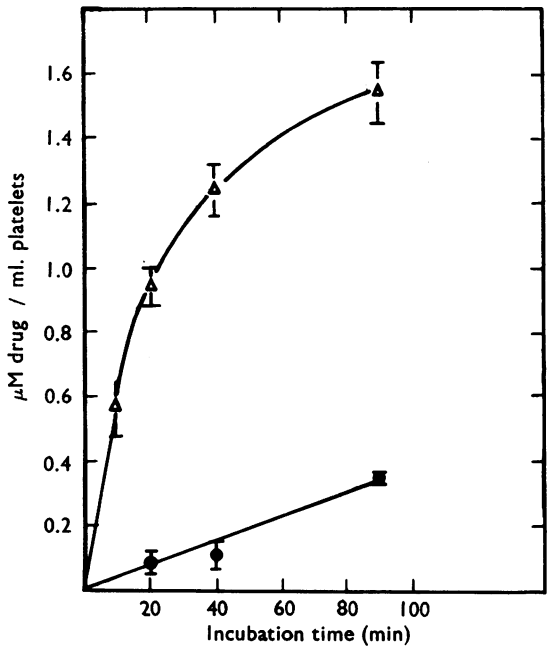

FIG. 2. Uptake of guanethidine and 5-HT compared. Samples of plasma from the same volunteers were incubated separately for 10 to 90 min with 10^{-5}M 5-HT (△) and 10^{-5}M ^{14}C-guanethidine (●). Experimental conditions as described for Fig. 1. The uptake of 5-HT was approaching the steady state after 90 min, whereas the uptake of guanethidine was linear. Mean values ± S.E. obtained in four volunteers are shown.

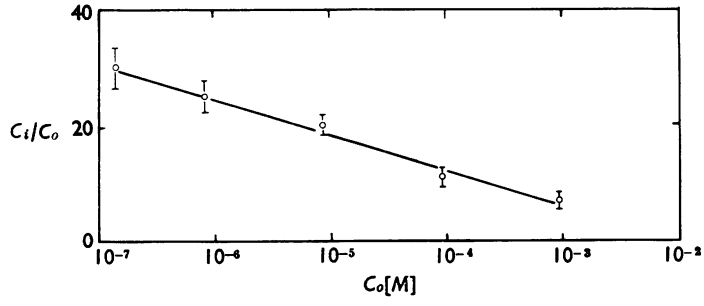

FIG. 3. Relationship between concentration of guanethidine in platelets and final plasma concentration after 90 min incubation. The final plasma concentration of guanethidine (C_o) is plotted logarithmically as abscissa against the ratio of the concentration of substance in platelets (C_i): C_o plotted arithmetically as ordinate. The linear relationship between C_i/C_o and C_o is evidence for a saturable component in the uptake process for guanethidine by platelets. Mean values ± S.E. for four to nine determinations are given.

about 35%. Thus a substantial proportion of the total drug in each incubation tube entered the platelets.

Mechanism of guanethidine uptake

The concentration ratios C_i/C_o were at least an order of magnitude greater than those which might be predicted on the basis of guanethidine entering platelets by diffusion along the electrochemical gradient existing between plasma and the platelet interior (see Solomon & Zieve, 1967).

Additional experiments clearly showed that guanethidine uptake at 37° C involved an energy-requiring process. First, 10^{-5}M ouabain produced a 52% inhibition of the uptake of 10^{-5}M guanethidine over 90 min at 37° C, and second, incubation at 3° C almost completely inhibited uptake of 10^{-5}M guanethidine over 2 hr (Table 1). Table 1 also shows the effect of various drugs on guanethidine uptake, the potential inhibitors being added to platelet-rich plasma in a concentration of 10^{-5}M 20 min before incubation with 10^{-5}M guanethidine for 90 min. The order of potency shown is similar to that found by Stacey (1961) for a series of related compounds which were tested as inhibitors of the uptake of 5-HT. In particular, it was interesting that in the present work the most potent inhibitory drug was 5-HT itself.

Ionic requirement for uptake of guanethidine

Da Prada *et al.* (1967), and Iversen & Kravitz (1966), have shown that the uptake of 5-HT by platelets and the uptake of NA by sympathetic nerves is sodium-dependent. Accordingly the effect of Na^+ on the uptake of guanethidine was determined.

Platelets were suspended in an artificial medium containing (mM) NaCl 134; KCl 5.5; Na_2HPO_4 4.5, glucose 10. This was termed 100% sodium solution. During 90 min incubation in this medium, containing 10^{-4}M ^{14}C-guanethidine, drug was accumulated but 5-HT levels did not change (Fig. 4). When NaCl was replaced by choline Cl (134 mM), so that the Na^+ was reduced by 96.6%, similar responses were obtained. When platelets were incubated with 10^{-4}M guanethidine in a sodium-free medium consisting of (mM) sucrose 138.5, KCl 5.5, glucose 10, however, uptake of guanethidine was reduced by 54.1% during 90 min incubation and the endogenous platelet 5-HT content declined by 78.5% (Fig. 4). Although some accumulation of

TABLE 1. *Effect of potential inhibitors on the uptake of guanethidine by platelets*

Inhibitor	% Inhibition (mean ± S.E.)
Low temperature (3° C)	98±0·1
5-HT	86±2·1
Desipramine	79±3·3
Cocaine	68±4·1
Dexamphetamine	61±3·2
Bretylium	58±2·2
Ouabain	52±2·9
Tyramine	40±1·9
Noradrenaline	20±1·5

The following substances produced 0 to 18% inhibition: bethanidine; *p*-chlorophenylalanine; debrisoquin; α-methyldopa.
The potential inhibitory drugs were added to the plasma 20 min before incubating with guanethidine for a further 90 min. Equimolar concentrations (10^{-5}M) of inhibitor and guanethidine were used. Figures for percentage inhibition refer to mean values of four determinations in two experiments.

guanethidine did occur in these experiments the C_i/C_o ratio after 90 min was only 1.6. Uptake of this order of magnitude could be accounted for by diffusion along the electrochemical gradient existing between platelet water and plasma (Solomon & Zieve, 1967).

These results suggest that the guanethidine uptake process is not only energy-dependent, but also requires Na^+. Thus removal of the cation prevents active accumulation, with the result that guanethidine enters platelets only by diffusion.

The next experiments were designed to determine the site of action of the substances which inhibited guanethidine uptake.

Site of action of guanethidine uptake inhibitors

According to current views, inhibitors of 5-HT and NA uptake of the imipramine-type act on the outer membrane of the platelet (Pletscher, 1968) and sympathetic nerve (Carlsson, 1966). This certainly seems to be the case for 5-HT inhibition of

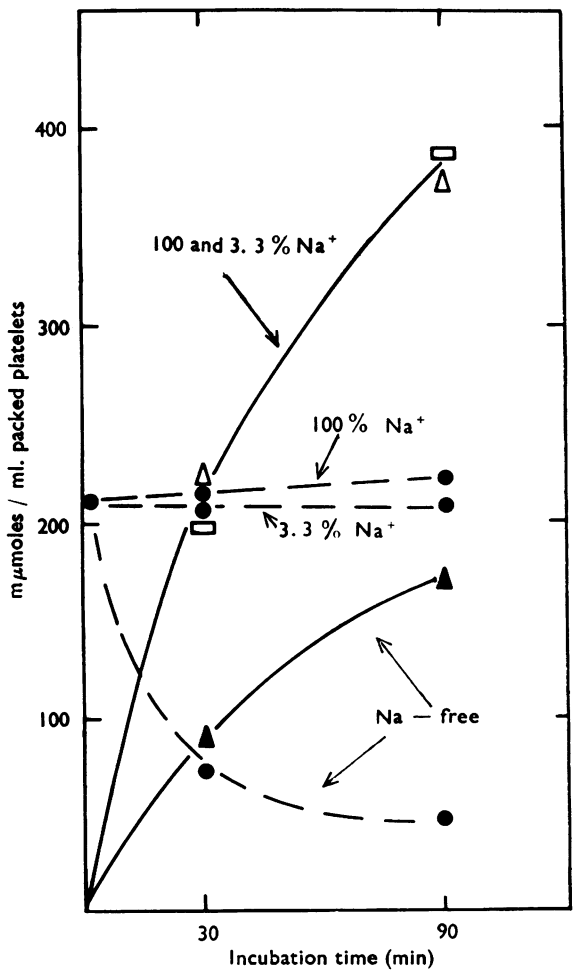

FIG. 4. Effect of Na+ on the uptake of guanethidine and 5-HT content of platelets incubated in artificial media. Platelets were incubated in modified Krebs-Ringer phosphate containing 0, 3.3 or 100% of the normal Na+ concentration (138.5 mM) as described in the text, for 30 and 90 min. Endogenous 5-HT levels (●, - - - -) were determined before incubation and after 30 and 90 min, and guanethidine levels (□, △, ▲, ——) after incubation with 10^{-4}M ^{14}C-guanethidine for 30 and 90 min. Levels of 5-HT and guanethidine in the presence of 3.3% Na+ (●, □) were not different from levels when 100% Na+ was present (5-HT, ●; guanethidine, △). Complete removal of Na+ reduced 5-HT content (●) and guanethidine uptake (▲). Results are mean values obtained in two experiments.

guanethidine uptake by platelets as indicated by the following experiments. When platelets were incubated with 10^{-5}M 5-HT for 10 min before the addition of 10^{-5} or 10^{-4}M guanethidine, uptake was reduced from 179 ± 7.5 mμmoles guanethidine/ml. packed platelets to 27 ± 3.1 mμmoles/ml. packed platelets (85% inhibition, see Table 1). The 5-HT content of the platelets was 580 mμmoles/ml. packed platelets. In similar experiments when the 5-HT content of platelets was increased from 580 to 1,575 mμmoles/ml. packed cells by extending the 5-HT incubation period from 10 to 90 min before addition of guanethidine, the inhibition of guanethidine uptake produced by 5-HT was still 85%. The 5-HT content of the platelets remained static during the 90 min that guanethidine was taken up; furthermore, the guanethidine accumulation did not involve displacement of 5-HT.

To summarize, these results indicate that the ability of 5-HT to inhibit uptake of guanethidine was not influenced by the level of 5-HT in the platelets. Thus 5-HT appears to exert its inhibitory action on the outer membrane of the platelets and not by saturating storage sites in the interior of the cell.

Site of guanethidine accumulation

It was of interest to attempt to identify the site of guanethidine accumulation in platelets. There were several possibilities: adsorption onto or penetration within the exterior cell membrane; accumulation of free guanethidine in platelet water or isolation in one of the several types of granules known to exist in platelets. Possibly the most obvious site was the dense-core granules (" vesicles ") which are considered to be the storage sites for platelet 5-HT (Pletscher, 1968).

Experiments were therefore carried out to try to find out if guanethidine was also stored in these vesicles. The results of such tests make this unlikely. First, platelets were incubated with 10^{-5}M guanethidine for 3 hr and 5-HT levels determined. In two experiments the 5-HT content of platelets was 199 and 209 mμmoles/ml. packed platelets before incubation with guanethidine and 192 and 206 mμmoles/ml. packed platelets after incubation; similar results were also obtained with platelets incubated in artificial media (see below). Second, the effect of reserpine on guanethidine uptake was examined: platelets were incubated with 10^{-5}M reserpine for 2 hr; then 10^{-5}M guanethidine was added and incubation continued for another 90 min. The results of these experiments are given in Table 2; reserpine treatment did not affect the uptake of guanethidine. The effect of reserpine on 5-HT levels in platelets was not examined in this series of experiments, but in two earlier experiments 10^{-5}M reserpine reduced the endogenous 5-HT content of platelets by approximately 40% in identical experimental conditions; 5-HT levels were (mμmole/ml.

TABLE 2. *Effect of reserpine on the uptake of guanethidine by platelets*

Reserpine concentration (M)	Guanethidine uptake (mμmoles/ml. packed platelets)
0	$145 \pm 11{\cdot}2$
10^{-5}	$152 \pm 17{\cdot}9$

Platelets were incubated in plasma/saline (4/1, see text) for 2 hr with or without 10^{-5}M reserpine. 10^{-5}M ^{3}H-guanethidine was then added and incubation continued for another 1·5 hr. Results are the mean ± S.E. of four determinations. (Uptake of guanethidine by platelets incubated for only 10 min before addition of drug was $179 \pm 7{\cdot}5$ mμmoles/ml. packed platelets, nine determinations.)

packed platelets): before incubation 178, 212; after incubation 103 and 136 (42% and 36% reduction respectively). In two other experiments in which the plasma concentration of reserpine was increased to 10^{-4}M there was a 23% inhibition of uptake of 10^{-5}M guanethidine over 90 min using identical experimental conditions.

These results strongly suggest that guanethidine uptake did not involve displacement of 5-HT from storage sites and that the drug was not bound in 5-HT storage granules in the platelets. We have no further information on the binding sites of guanethidine in platelets, although the high C_i/C_o ratios obtained suggest guanethidine retention involved either a " pump " mechanism or intracellular binding.

The next experiments were designed to assess the degree of guanethidine binding to the cells.

Guanethidine binding by platelets

Platelets were first incubated with 10^{-5}M guanethidine for 90 min when they contained 245 ± 12.1 mμmoles/ml. packed cells (mean ±S.E. of four experiments). Then the platelets were separated from the incubation medium by centrifugation and resuspended in drug-free plasma and reincubated. The resuspension procedure itself caused the loss of 10.3 ± 1.8% of the platelet-bound drug (four determinations). During the next 30 min a further 10% was lost but efflux then ceased for at least the next 30 min (Fig. 5). Clearly, guanethidine was tightly bound to platelets.

Metabolism of guanethidine by platelets

Platelets were incubated for 1.5, 3, 4, and 6 hr with 10^{-4}M guanethidine. Then the platelet pellet and plasma were extracted with chloroform as described in **Methods**.

There was no difference in the proportion of radioactivity represented by metabolites in either platelets or plasma at any of the incubation times studied. In

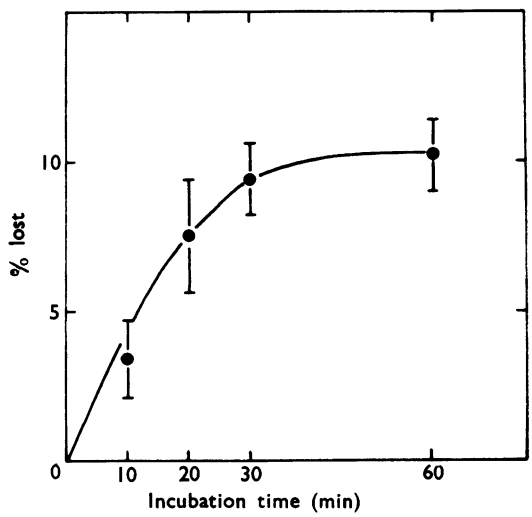

FIG. 5. Efflux of guanethidine from platelets. The figure shows the % loss of guanethidine from labelled cells after resuspension in drug-free medium for 10 to 60 min. The platelets were first labelled with ^{3}H-guanethidine by incubation in plasma/saline (4/1) for 90 min. They were then separated from this incubation medium by centrifugation and resuspended. Before resuspension the platelets contained 245 ± 12.1 mμmoles guanethidine/ml. packed platelets. The resuspension procedure alone caused the loss of 10.3 ± 1.8% in addition to that shown here. Each result is the mean ±S.E. of five determinations.

eighteen determinations $1.48 \pm 0.11\%$ of radioactivity in platelets and $7.67 \pm 0.21\%$ of radioactivity in plasma represented guanethidine metabolites. As the recovery of added guanethidine from platelets and plasma varied between 87 and 104% (mean 95.1%) we conclude that neither platelets nor plasma metabolize guanethidine *in vitro*.

Explanation of the linear uptake of guanethidine

The efflux experiments described show that although a small amount of guanethidine was lost from platelets suspended in drug-free medium during the first 30 min, efflux ceased for the next 30 min. After that time, however, efflux recommences (Boullin & O'Brien, unpublished observations; see also Fig. 6). This efflux may be due to death of some cells with subsequent loss of drug. We now give direct evidence that platelet viability is decreased by prolonged incubations. Platelets preincubated for 10 min before addition of 10^{-5}M guanethidine to plasma took up 179 ± 7.5 mμmoles/ml. packed cells (nine experiments). When the preincubation period was extended to 2 hr the uptake was reduced by 19% to 145 ± 11.2 mμmoles/ml. packed cells (five experiments). This difference was significant ($P<0.01$). On the basis of available experimental evidence, therefore, we consider that linear uptake was due to failure to saturate binding sites, and the decline in the rate of guanethidine uptake with time was probably due to a decrease in cell viability *in vitro*.

Release of guanethidine by platelets

The possible significance of release of platelet-bound drug will be considered in the discussion, but experimental evidence that guanethidine can be released from platelets will now be presented:

Platelets were first incubated in plasma containing 10^{-5}M guanethidine for 90 min. They were then resuspended in either drug-free plasma or in plasma containing 10^{-4}M dexamphetamine. The results are shown in Fig. 6; amphetamine caused an approximately 50% increase in guanethidine efflux over 90 min.

In two other experiments with 10^{-6} and 10^{-5}M reserpine, the efflux of guanethidine over 90 min was not affected.

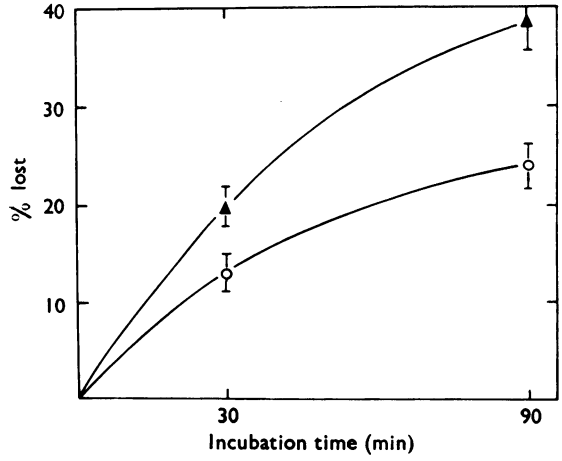

FIG. 6. Effect of amphetamine on efflux of guanethidine from platelets. Platelets were first labelled with drug by incubation with 10^{-5}M ^{3}H-guanethidine for 90 min. The cells were then resuspended in either drug-free medium (○) or in medium containing 10^{-4}M dexamphetamine (▲), when the efflux shown was obtained. Each point is the mean ± S.E. of four determinations.

Discussion

In 1967, Solomon & Zieve described the uptake of procaine, histamine, quinidine and reserpine by human blood platelets incubated in plasma and in a variety of artificial media. Although the uptake of procaine and histamine could be adequately explained by diffusion, the accumulation of reserpine and quinidine was too great to be accounted for on this basis ; the authors considered that reserpine uptake was largely a result of intracellular binding, while quinidine was accumulated by active transport and/or binding.

The present results indicate that the mechanism of accumulation of guanethidine by blood platelets resembles that of quinidine, 5-HT and NA. Thus desipramine and cocaine are extremely potent inhibitors of the uptake of all three compounds, while dexamphetamine is less potent and alpha-methyldopa is inactive. Furthermore, low temperature and ouabain are effective inhibitors of the uptake of guanethidine, 5-HT and noradrenaline, and the retention of all three drugs requires extracellular Na^+ (this communication ; Pletscher, 1968 ; Iversen & Kravitz, 1966). It is reasonable to conclude therefore that the mechanisms of accumulation of guanethidine and 5-HT by blood platelets, and guanethidine and NA by sympathetic nerves, are similar. Once inside the nerve-ending or platelet, however, the three substances may be stored in different sites. Efflux experiments showed that guanethidine was tightly bound by platelets and unlike 5-HT is probably not accumulated in vesicles in platelets (Pletscher, 1968).

There are several pieces of evidence to indicate that the storage sites for guanethidine in platelets are different from those for 5-HT. Probably the most convincing are those obtained in experiments with reserpine. Recent work (da Prada *et al.*, 1967 ; Pletscher, 1968) has shown that the classical effect of reserpine in releasing platelet 5-HT is associated with a concomitant disappearance of the postulated binding sites—the dense-core granules. Furthermore, the inhibitory effect of reserpine on 5-HT uptake seems to be an indirect effect resulting from damage to and/or disappearance of these 5-HT containing granules. Yet in our experiments with guanethidine we found that reserpine affected neither the uptake of the drug into platelets nor the efflux of guanethidine from platelets. On the other hand, reserpine was quite effective in releasing 5-HT.

Although at the concentrations of reserpine used up to 80% was probably bound to plasma protein (Solomon & Zieve, 1967), the amount of substance remaining free in the plasma was at least three times greater than the quantity (0.3 μg/ml.) which was found by Carlsson, Shore & Brodie (1957) to cause maximal release of 5-HT from platelets in plasma in similar experimental conditions. Thus guanethidine appears to be present either in a free form in platelet water, or in a different storage compartment. Additional evidence that 5-HT and guanethidine are bound in different sites in platelets was obtained in experiments where the uptake of both drugs was measured. Apart from cold, 5-HT was the most potent inhibitor of the uptake of guanethidine, yet this inhibitory effect appeared to be exerted on the outer membrane of the cells and to be independent of their 5-HT content. Thus, 5-HT produced 85% inhibition of guanethidine uptake whether the platelets contained merely the naturally occurring (endogenous) amount (580 mμmoles/ml. packed platelets) or were saturated by prolonged incubation with 5-HT (1,575 mμmoles/ml. packed cells). Moreover, the quantity of guanethidine accumulated

over 90 min in these circumstances was quite substantial—164 mμmoles/ml. packed platelets—when the plasma contained 10^{-4}M guanethidine. Finally we found that amphetamine—a pharmacological antagonist of guanethidine—was effective in releasing guanethidine. The concentrations of amphetamine required to bring about release were at least ten times lower than the threshold concentration required to release platelet 5-HT (da Prada *et al.*, 1967).

Previous attempts to demonstrate the release of guanethidine by amphetamine in the isolated perfused rat heart were unsuccessful (Boullin, Furst & McMartin, unpublished observations) possibly because the ratio of specific to non-specific binding sites is very low (Boullin, 1968). It is tempting to speculate that the clear-cut effect of amphetamine on guanethidine efflux described here may be due to the fact that platelets may be compared with the specific binding sites in other tissues—namely the adrenergic nerve endings—and that enhanced release was demonstrable because all drug was bound in specific sites.

Another question to be considered is the cause of the linear uptake of guanethidine over a prolonged period. Previously it has been reported that guanethidine uptake is slow in heart (Schanker & Morrison, 1965 ; Boullin, 1966), but steady-state conditions were attained after 3 hr. In the platelet experiments uptake was linear for this time. Possible causes of such an effect were rapid metabolism of guanethidine by platelets and subsequent loss into plasma, or a rate of guanethidine efflux from platelets approximating the rate of uptake. Neither of these explanations is acceptable on the basis of the experimental results given here. Indeed, we have evidence that the cause of the decline in the rate of uptake of guanethidine during incubation for 3 to 6 hr may have been due to a decrease in platelet viability. This is because in experiments on 5-HT uptake, accumulation over 90 min was reduced by 19% from 179 to 145 mμmoles/ml. packed cells, when the preincubation period was extended from 10 to 120 min ; Born & Gillson (1959) have reported similar results. The observed decline in uptake may therefore be an artefact of the *in vitro* situation and may not occur in man, where the C_i/C_o ratios may be considerably higher than reported here.

We wish now to discuss the possibility that platelets may take up guanethidine and other drugs in sufficient quantity for the subsequent release of the drug to modulate pharmacological action by increasing plasma levels.

Table 3 gives some concentration ratios for drugs in platelets which have been found experimentally. Thus ratios of 25 to 50 : 1 have been reported with guanethidine and debrisoquin (Boullin & O'Brien, 1968a), 100 to 200 : 1 for reserpine and up

TABLE 3. *Changes in distribution of drugs between platelets and plasma resulting from decline in plasma levels*

Substance	Platelet/plasma conc. ratio	Ref.	% in platelets at peak plasma level	% in platelets when plasma level has declined by	
				50%	90%
Guanethidine	25:1	1	9·0	16·6	50
Guanethidine	50:1	2	16·6	28·6	66·6
Quinidine	100:1	3	28·6	44·4	80
Reserpine	200:1	3	44·4	61·5	88·8
Quinidine	800:1	3	76·1	86·4	96·9

1. Boullin & O'Brien (1968a).
2. This paper.
3. Solomon & Zieve (1967).

to 800:1 for quinidine (Solomon & Zieve, 1967). The remainder of the table gives theoretical calculations of the percentage of substance present in the platelets compared to the plasma, the calculations being based on a figure of 4 μl. platelets/ml. whole blood (Hardisty & Stacey, 1955). It is clear that the proportion of drug retained by the platelets will increase in relation to the total blood content as plasma levels decline due to metabolism. The significance of this platelet-bound fraction is not known, but Table 3 shows that its release may increase plasma levels. It does not seem likely that the quantity of substance lost from platelets would be sufficient to produce a general increase in plasma levels or pharmacological action, but such changes may occur in a local situation where platelets accumulate in quantity and, in fact, release of substances from platelets *in vitro* has been observed by Mills, Robb & Roberts (1968).

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