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Accumulation of quinidine by human blood platelets: effects on platelet ultrastructure and 5-hydroxytryptamine

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Summary

1. We have studied the mechanism of quinidine uptake by normal human blood platelets.

2. Platelets in plasma or Krebs solution took up quinidine extremely rapidly, the maximal drug concentration ratio of platelet to medium being 24 to 1 after 1 minute.

3. The effects of metabolic inhibitors on uptake were equivocal. Ouabain had no effect but iodoacetate and dinitrophenol were effective. However, as accumulation was not inhibited by low temperature, it was probably not energy dependent.

4. Interactions between quinidine and 5-HT were also investigated.

5. Quinidine blocked 5-HT uptake, but conversely, neither 5-HT itself nor 5-HT uptake inhibitors, such as cocaine, dexamphetamine, and desipramine interfered with quinidine accumulation.

6. Electronmicrographs of platelets incubated with 10^{-5}, 10^{-4} or 2×10^{-3}M quinidine in Krebs solution for 2 h showed no changes at the lower concentrations, but gross ultastructural damage, including disintegration of the plasma membrane, at 2×10^{-3}M.

7. Further evidence for intracellular penetration was obtained because quinidine released endogenous 5-HT and ATP, and also ^{14}C-5-HT from loaded cells.

8. We conclude that quinidine is taken up by the platelet by a passive process, unrelated to the 5-HT transport mechanism. It is probably accumulated largely in the plasma membrane and outer protein layer, but intracellular penetration and ultrastructural damage may occur.

9. Although the effects on 5-HT and ATP were not due to platelet damage, this may occur *in vivo* and be the cause of quinidine induced thrombocytopenic purpura.

Introduction

Since quinidine is of clinical importance, and exerts potent cardiovascular effects, it would be useful to obtain information on its distribution in blood cells. Solomon & Zieve (1967) first showed that quinidine is taken up by human blood platelets *in vitro*, although they did not study the mechanism of uptake in any detail. We

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thought it worthwhile to make more extensive investigations using normal human platelets suspended in plasma or physiological saline.

The first experiments showed that quinidine binds very rapidly to the cell, probably to the outer plasma membrane. In addition some intracellular penetration took place. We therefore examined the effects of quinidine on endogenous 5-HT and ATP, on exogenous radioactive 5-HT in loaded platelets, and finally its actions on cellular ultrastructure by means of the electronmicroscope.

Methods

Isolation and separation of platelets

Blood was obtained from normal human volunteers of either sex. Approximately 20 ml of blood were mixed with 2·0 ml of 0·3 M disodium edetate in polycarbonate centrifuge tubes. The platelets were separated and the packed platelet volume measured as described by Hardisty & Stacey (1955). The mean platelet volume in these experiments was $4{\cdot}76 \pm 0{\cdot}4$ μl packed platelets/ml of platelet rich plasma ($n=40$).

Uptake and efflux experiments

The uptake and efflux of quinidine, 5-HT and ^{14}C-5-HT were measured by the procedures given earlier (Boullin & O'Brien, 1969) except that before assay the platelets were disrupted by sonification with a 'Biosonik 2' sonifier (Bronwill Scientific, Rochester, N.Y.), at probe setting 50 in either distilled water or 0·1 N H_2SO_4. The volume of fluid trapped between the packed platelets after centrifugation was determined by estimation of the radioactivity recovered from the platelet pellet after incubation with ^{14}C-inulin for 10–90 minutes.

The Krebs solution used was that of Umbreit, Burris & Stauffer (1964): NaCl, 121 mM; KCl, 4·9 mM; KH_2PO_4, 1·2 mM; $MgSO_4$, 1·2 mM and $NaHCO_3$, 25·5 mM. $CaCl_2$ was omitted and disodium edetate, 30 mM, and glucose, 11 mM, were added.

Quinidine estimation

After incubation the platelets were sonified in 500 μl of 0·1 N H_2SO_4, and the samples centrifuged at 20,000 *g* for 5 min to precipitate the platelet debris. The supernatant was removed and the fluorescence of quinidine read on an Aminco-Bowman spectrophotofluorimeter at excitation and emission wavelengths of 350 and 450 nm respectively (uncorrected values) (Udenfriend, Duggan, Vasta & Brodie, 1957).

Estimation of 5-HT

In several experiments the endogenous 5-HT or total 5-HT (^{14}C-5-HT+ endogenous) were estimated from 3–6 ml of platelet rich plasma by the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956).

Estimation of ATP

Platelets were lysed by sonification, centrifuged as described above and ATP was measured in the supernatant by the technique of Holmsen, Holmsen & Bernhardsen (1966).

Binding of quinidine to plasma proteins

Procedure A—protein precipitation

One millilitre samples of platelet free plasma were incubated at 37° C for 30 min with 10^{-5} to 10^{-3}M quinidine. At the end of this time proteins were precipitated with 1·0 ml of 2·88 M $ZnSO_4$ and 0·5 ml of 1·0 N NaOH. The quinidine remaining in the supernatant was then measured spectrophotofluorimetrically as described above.

Procedure B—dialysis

One millilitre of platelet free plasma was incubated with 10^{-5} to 10^{-3}M quinidine for 30 minutes. Samples were then dialysed against 0·2 M phosphate buffer for 18 h at 25° C and the quinidine was extracted and assayed by the method of Udenfriend (1962).

Drugs

The following substances were used: ^{14}C-5-HT creatinine sulphate monohydrate (specific activity 56 mCi/mmol, Radiochemical Centre, Amersham); ^{14}C-carboxylic acid-inulin (specific activity 3·08 μCi/mg, New England Nuclear Corp., Boston, Mass.); cocaine hydrochloride; desipramine hydrochloride; dexamphetamine sulphate; dinitrophenol (DNP); iodoacetic acid (IAC); ouabain; and quinidine sulphate.

Where indicated, experimental values are the mean $\pm$ standard error of the mean (m $\pm$ S.E.).

Electron microscopy

In some experiments the effect of quinidine on platelet ultrastructure was studied using the method of Roth, Stjarne, Bloom & Giarman (1968). After incubation with drug (see **Results**) platelets were sedimented by centrifugation at 20,000 *g* for 5 min and 5 ml of 5% glutaraldehyde in 0·13 M potassium phosphate, pH 7·0 were added. After exposure to glutaraldehyde for at least 1 h, the pellets which formed well defined discs measuring 2·0–5·0 mm in width and usually 0·5 mm in depth, could be teased off the wall of the tube and processed intact. The discs were exposed to 1% osmium tetroxide for 1 h, then dehydrated in graded ethanols and embedded in the flat end of a polyethylene capsule. The entire disc pellet was then removed, transected and turned on its side in a special vice-grip specimen holder (Ivan-Sorvall Co., Norwalk, Conn.), enabling thin sections to be cut which included the entire depth of the pellet (from tube wall to supernatant surface) along a full radius of the original disc. Thin sections with interference colours of pale gold to silver were cut on a Sorvall Mt-2 microtome and examined in a Zeiss EM-9 electron microscope. Thin sections were stained on the grids with uranyl acetate and lead citrate (or with lead citrate only) to provide contrast enhancement.

Results

Uptake of quinidine

When platelets suspended in plasma or Krebs solution were incubated with 10^{-5}M quinidine, uptake reached a steady state within 1 min (Fig. 1). This rapid uptake suggested that most of the drug binds directly to the plasma membrane of the

platelet or to the layer of plasma protein surrounding this membrane (Nakao & Angrist, 1968; Behnke, 1968). The relationship between the ratio C_i/C_f, where C_i is the concentration of drug in the platelet at the steady state, and C_f the corresponding concentration of free drug in the medium and the total concentration of drug added C_t (free + protein bound), is given in Fig. 2.

To estimate C_f in plasma it was necessary to determine the amount of quinidine which was bound to plasma proteins. This was done by assay of quinidine in the

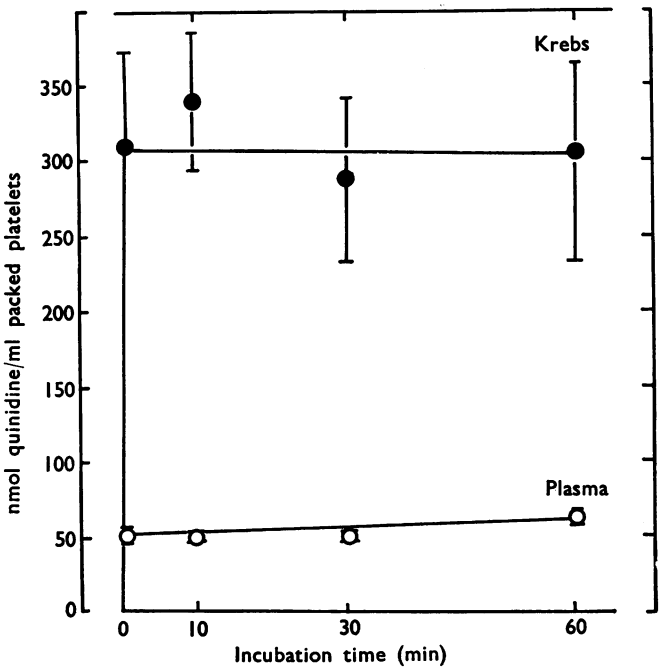

FIG. 1. Time course of quinidine uptake in plasma (○) and Krebs solution (●). Results are given as nmol/ml packed platelets (mean ± S.E.) from four to five experiments. Cells suspended in either plasma or Krebs solution were incubated with 10^{-5}M quinidine for 1–60 minutes.

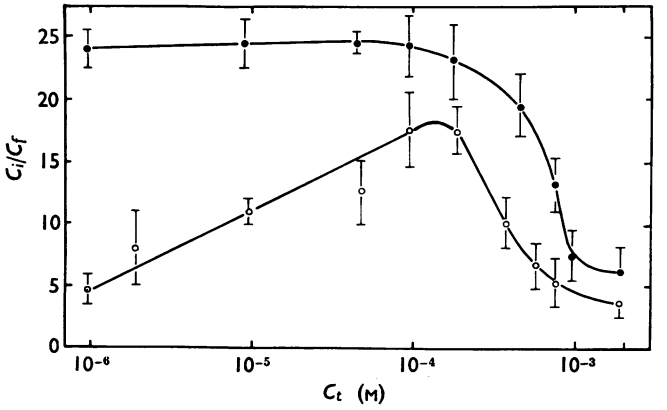

FIG. 2. Relationship between the concentration of quinidine in platelets and incubation media at equilibrium. The ratio C_i/C_f (platelet concentration to free medium concentration) is plotted against C_t (total medium concentration). Platelets were suspended in plasma (○) or Krebs solution (●) and incubated with 10^{-6} to 2×10^{-3}M quinidine for 30 minutes. Values are the mean ± S.E. from five experiments.

supernatant after precipitation of plasma proteins and by dialysis (see **Methods** and Table 1). Maximal binding of quinidine was 53% when the total quinidine concentration was 10^{-5}M. The values for 10^{-5} and 10^{-4}M given in Table 1 (obtained by Procedure A—protein precipitation) were used to calculate the ratios of C_t to C_f given in Fig. 2. At 10^{-3}M, a value of 9% quinidine binding was used (dialysis procedure) since this was in closer agreement with the results obtained by Conn & Luchi (1961).

Maximal uptake ratios in plasma occurred when C_t was between 10^{-4} and 2×10^{-4}M. The pattern of uptake of quinidine in Krebs solution was different from that in plasma. When C_t was less than 10^{-4}M, the ratio of C_i to C_f was constant and up to five times greater than comparable ratios in plasma. Removal of the plasma protein layer might have increased accumulation by allowing access of quinidine to a specific transport process for its intracellular binding. To determine whether this was the case, we studied the effects of low temperature, metabolic inhibitors, and substances which are specific inhibitors of the energy dependent mechanism for transporting 5-HT into platelets. In four experiments the following substances in concentrations of 10^{-5} and 10^{-4}M produced no significant inhibition of quinidine uptake by platelets: cocaine, desipramine, dexamphetamine and 5-HT. Most important, ouabain (10^{-4}M) and incubation at 4° or 22° C did not affect accumulation; this may suggest that an energy dependent process is not involved. It is surprising therefore that a combination of DNP and iodoacetate, 10^{-3}M, produced significant inhibition in plasma ($41{\cdot}0\pm 3{\cdot}9\%$) and in Krebs solution ($78{\cdot}9\pm 5{\cdot}4\%$).

TABLE 1. *Plasma protein binding of quinidine*

Concentration of quinidine in plasma (total drug added, C_t)	% Quinidine bound to protein Method	
	A Protein precipitation	B Dialysis
10^{-5}M	$52{\cdot}6\pm 0{\cdot}6$	$48{\cdot}9\pm 5{\cdot}4$
10^{-4}M	$43{\cdot}8\pm 1{\cdot}2$	$24{\cdot}3\pm 3{\cdot}6$
10^{-3}M	$34{\cdot}4\pm 1{\cdot}2$	$9{\cdot}3\pm 0{\cdot}5$

10^{-5}M to 10^{-3}M quinidine was incubated with plasma for 30 min and the values given (mean±S.E.) were calculated from the amount of quinidine determined by dialysis or protein precipitation in three experiments. At each concentration, less than 5% of the quinidine in control samples (protein-free solution) was co-precipitated.

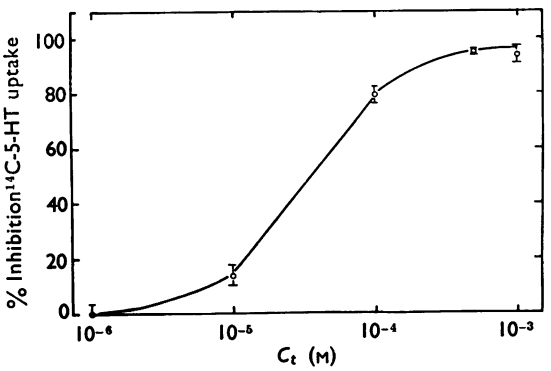

FIG. 3. Relationship between plasma quinidine concentration (C_t) and inhibition of ^{14}C-5-HT uptake. Results are % uptake inhibition (mean±S.E.) in three experiments. Platelets were suspended in plasma and incubated with 10^{-6} to 10^{-3}M quinidine for 60 min and then 10^{-5}M ^{14}C-5-HT was added and incubation continued for a further 90 minutes.

To obtain further information on the site of quinidine binding we studied its effects on 5-HT accumulation. As shown in Fig. 3, quinidine is a potent inhibitor of the uptake of ^{14}C-5-HT, the ID50 being $3{\cdot}6 \times 10^{-5}$M. This value is considerably greater than that for imipramine ($0{\cdot}05 \times 10^{-5}$M) yet is in very close agreement with those for cocaine ($2{\cdot}5 \times 10^{-5}$M) and chlorpromazine ($3{\cdot}5 \times 10^{-5}$M) (Stacey, 1961). Since this effect was observed when quinidine was added to platelet rich plasma only 1 min before 5-HT, it probably indicated that quinidine was blocking access to the 5-HT transport mechanism in the outer membrane (see **Discussion**).

An indication of intracellular penetration could be obtained by seeing if quinidine released 5-HT and ATP, since it is known from the work of Da Prada & Pletscher (1968) that the indolealkylamine is bound within the platelet in intracellular organelles in a complex with ATP in the molar ratio 5-HT to ATP=2 to 1.

Effects of quinidine on 5-HT and ATP

We found that quinidine released 5-HT from platelets in plasma (Table 2). Incubation for 2 h with 10^{-4}M quinidine caused the loss of 63% of the 5-HT; 10^{-5}M was without effect. We could not detect a significant change in the platelet ATP content in these experiments. During the 2 h incubation the cell counts determined with the Coulter counter did not change, suggesting that quinidine was not releasing 5-HT by causing cell lysis. An increase in particle count would have been expected if cell lysis had occurred. As can be seen from Table 2, there is no simple relationship between the platelet quinidine content and the amount of 5-HT released.

The results of similar experiments in which we measured the release of ^{14}C-5-HT from loaded platelets in plasma are given in Fig. 4. There was a linear relationship between the log of plasma quinidine concentration (C_t) and the percentage loss of ^{14}C-5-HT over a considerable concentration range. The concentration required to release 50% of the bound ^{14}C-5-HT was $1{\cdot}2 \times 10^{-4}$M. The time course of ^{14}C-5-HT release produced by 10^{-4}M quinidine is shown in Fig. 5. Assuming linearity for the first 10 min of incubation, an initial rate of loss of 8·8 (nmol/min)/ml packed platelets was calculated.

Quinidine also released exogenous 5-HT from platelets in Krebs solution and the pattern of efflux was similar to that seen in plasma (Fig. 5). An initial rate of loss of 7·3 (nmol/min)/ml packed platelets was calculated from the graph. These findings on the release of ^{14}C-5-HT are further evidence in favour of the intracellular

TABLE 2. *Effect of quinidine on endogenous 5-HT and ATP in human blood platelets*

Platelet quinidine concentration	Total quinidine in medium	5-HT content before incubation	5-HT lost	ATP lost
		(nmol/ml packed platelets)		
225±28	10^{-5}M	208±37	N.D.	N.D.
2002±213	10^{-4}M	208±37	131±20 (63%)	70±44*
		Ratio of quinidine content to 5-HT lost		
	10^{-4}M	15·3		

N.D.=none detectable; *=not significant. Results are the mean±S.E. from three experiments. Platelets were obtained from 6 ml platelet rich plasma, resuspended in Krebs solution as described in **Methods** and incubated with 10^{-5} or 10^{-4}M quinidine for 2 hours. Value in parenthesis is % 5-HT lost from total endogenous content. The lower portion of the Table gives the ratio found after 10^{-4}M quinidine only. Quinidine content in the platelets was 2,002 nmol/ml packed platelets at the end of incubation. Endogenous ATP concentration was 1612±221 nmol/ml packed platelets. There was no detectable loss of endogenous 5-HT in the absence of quinidine.

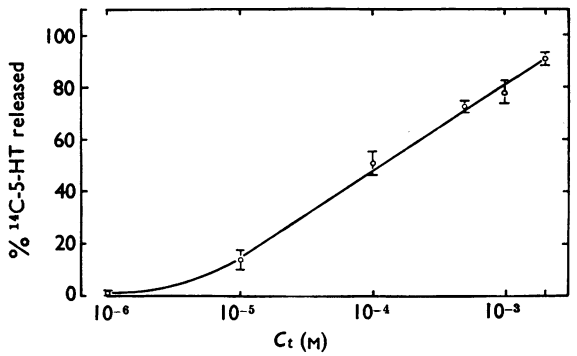

FIG. 4. Relationship between plasma quinidine concentration (C_t) and release of ^{14}C-5-HT from loaded platelets. Cells were suspended in plasma, incubated with 10^{-5}M ^{14}C-5-HT for 90 min, and then resuspended in 5-HT free plasma containing 10^{-6} to 2×10^{-3}M quinidine and incubated for 2 h as described in **Methods**. The ^{14}C-5-HT content before resuspension was 921 ± 106 nmol/ml packed platelets. Results are the mean $\pm$ S.E. from four experiments.

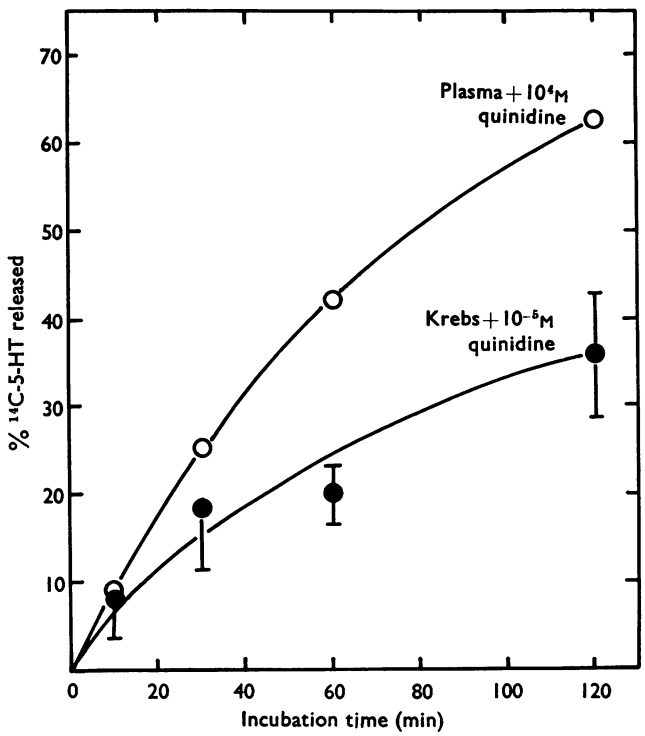

FIG. 5. Release of ^{14}C-5-HT by quinidine. Platelets were loaded by incubation in plasma containing 10^{-5}M ^{14}C-5-HT for 90 minutes. The cells were then resuspended in either plasma (○) or Krebs solution (●) as described in **Methods** and incubated for up to 120 min in the presence of 10^{-5} or 10^{-4}M quinidine. The % loss values are the net losses due to quinidine alone in all instances. The loss due to the resuspension procedure was $1{\cdot}6\pm0{\cdot}5\%$. The ^{14}C-5-HT content of the loaded cells was $1{,}036\pm88$ nmol/ml packed platelets. Results are given as the % released (mean $\pm$ S.E. from four experiments, Krebs solution, or the mean of two experiments, plasma).

penetration of quinidine. Additional experiments in which we measured the change in the total 5-HT (endogenous + ^{14}C-5-HT) and ATP caused by quinidine from ^{14}C-5-HT loaded platelets are summarized in Table 3. The molar ratio of ATP to 5-HT of 2·38 to 1 in these loaded cells is between the value of 1·79 to 1 found by Boullin & O'Brien (1970) in children, and 3·23 to 1 found by Born, Ingram & Stacey (1958) for adults. The quantity of 5-HT released represented 61·4% endogenous and 57% exogenous amine, there being no change in the ratio of exogenous to endogenous indolealkylamine. Additionally there was a decrease in ATP concentration of 400 ± 26 nmol/ml cells (Table 3).

These changes could well have been due to an effect of quinidine on platelet structure, and therefore we thought it was important at this point to study the effects of quinidine on structure by electronmicroscopy.

Effects of quinidine on platelet ultrastructure

Platelets were incubated in Krebs solution containing 10^{-5}, 10^{-4} or 2×10^{-3}M quinidine for 2 h and then processed for electronmicroscopy as described in **Methods**. We found no clearcut ultrastructural changes in platelets incubated with 10^{-5} or 10^{-4}M quinidine (compare Fig. 6 with Fig. 7), but very gross changes were evident with $2{\cdot}10^{-3}$M (compare Figs. 6–8). The principal changes were as follows: first, the shape of the cells was abnormal in that all were spherical with almost no spicules or pseudopodia; second, the integrity of the plasma membrane was lost, and glycogen granules appeared in the extracellular space. There seemed to be a redistribution of glycogen granules from clusters to a fairly random dispersal; possibly there was also an increase in the actual number of granules/platelet. Other changes included a generalized loss of discrete platelet ultrastructure, with disappearance of α-granules, mitochondria, microtubules and normal vacuoles. Additionally some platelets appeared to be in process of disintegration, but were not completely broken up after 2 h when they were fixed for electronmicroscopy.

Quinidine efflux

An estimate of the tightness of quinidine binding can be made by measuring efflux from quinidine loaded cells as shown in Fig. 9. In both plasma and Krebs solution, most drug (60–80%) was lost during the resuspension alone, but the subsequent losses were significantly greater in plasma than Krebs solution ($P<0{\cdot}05$). In the

TABLE 3. *Release of 5-HT and ATP by quinidine*

	Platelet content of 5-HT (nmol/ml packed cells)		
	Before incubation	After incubation	Amount released
5-HT: Total	1216±119	503±48	713±103
Endogenous	280±29	108±9	172±18
^{14}C-5-HT	936±79	395±29	541±49
^{14}C-5-HT	3·3	3·7	3·12
Endogenous 5-HT	Platelet content of ATP		
	2900±130	2500±157	*400±26

Values are the mean ± S.E. obtained in three experiments. Platelets suspended in plasma were first loaded by incubation in 10^{-5}M ^{14}C-5-HT for 90 min and then reincubated in 5-HT free Krebs solution for 2 h with 10^{-4}M quinidine. Quinidine content was 2,156±229 nmol/ml packed cells. Loss of endogenous 5-HT in absence of quinidine was not detectable; ^{14}C-5-HT was lost to the extent of 6·2±2·4%. There was no detectable loss of ATP in the absence of quinidine. *$P<0{\cdot}001$.

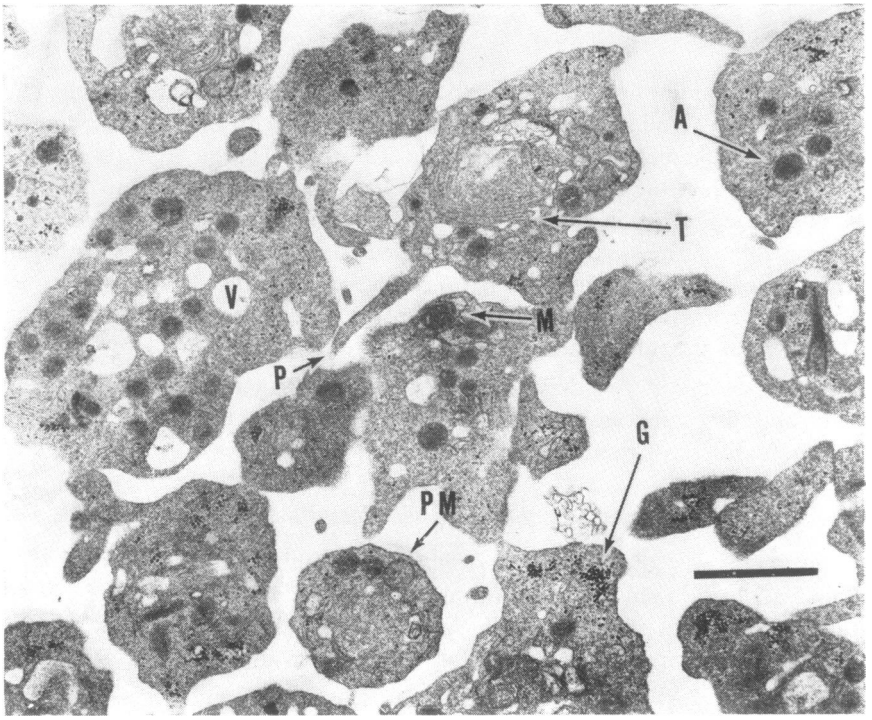

FIG. 6. Electronmicrograph of normal human platelets incubated in Krebs solution for 2 h; no quinidine added. Notice abundance of vacuoles (V) and numerous pseudopodia (P). The plasma membrane (PM) surrounding the platelet can be seen, and glycogen granules (G) are usually in clusters. Several platelets show the characteristic system of microtubules (T), and all cells contain mitochondria (M) and α granules (A). Magnification $\times 16{,}800$, scale 1 μm.

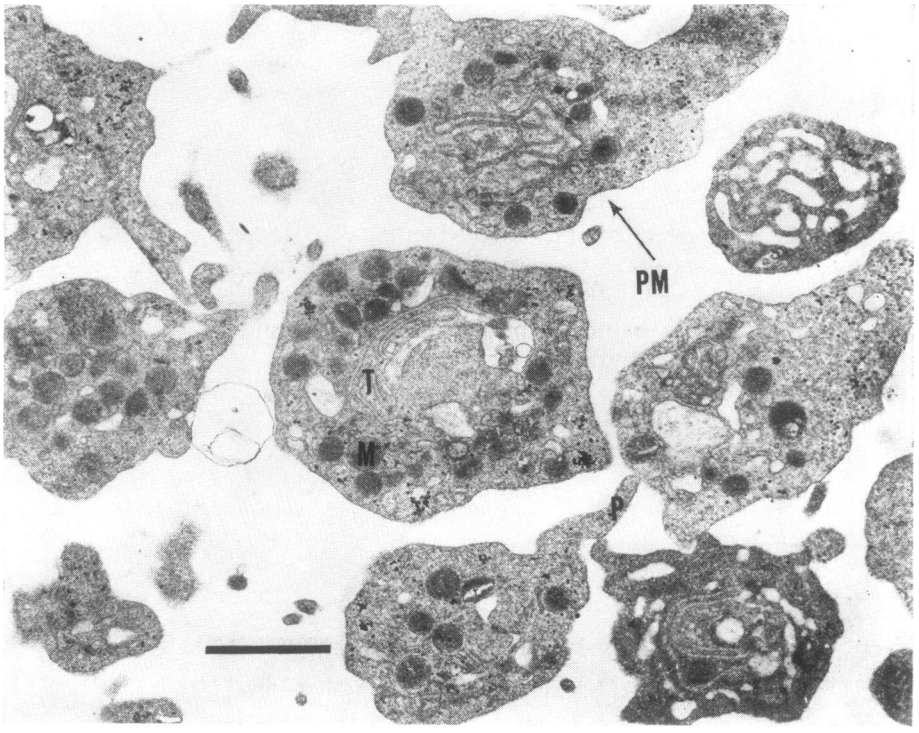

FIG. 7. Electronmicrograph of normal human platelets incubated with 10^{-4}M quinidine sulphate for 2 h in Krebs solution. Magnification, scale and legend as in Fig. 6. There are no overt ultrastructural changes.

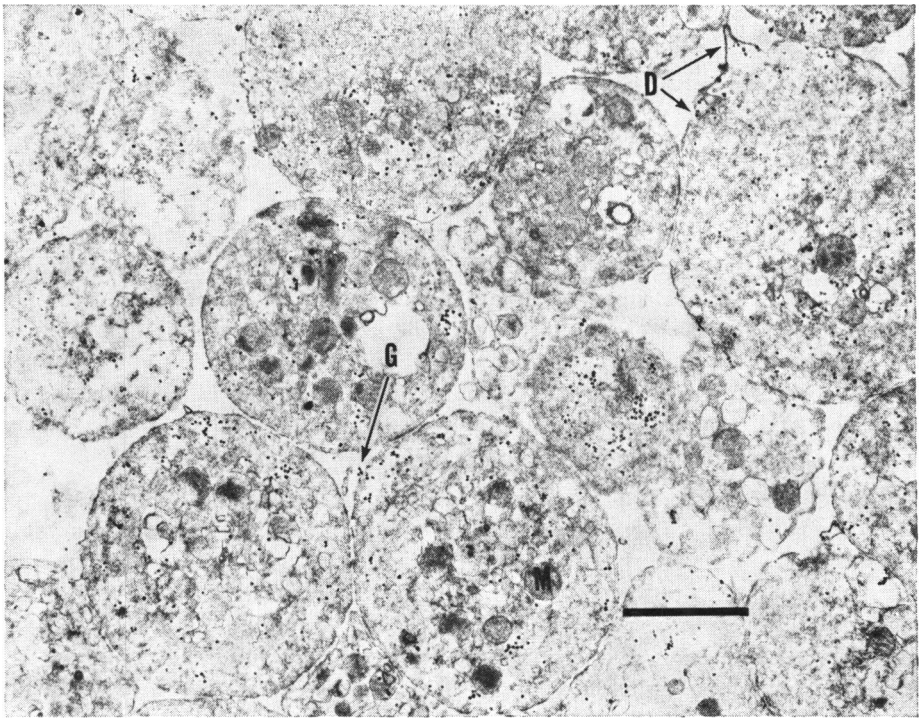

FIG. 8. Electronmicrograph of normal human platelets incubated with 2×10^{-3}M quinidine sulphate for 2 h in Krebs solution. Magnification, scale and legend as in Fig. 6. Notice the gross ultrastructural changes in all cells. Platelets are rounded and there are no pseudopodia. Mitochondria and α granules are sparsely distributed, and are absent from some cells. The plasma membrane is displaced and breached in many places (that is 'D'), and glycogen granules have passed into the extracellular space; those granules remaining within the cells are not in clusters, but randomly dispersed.

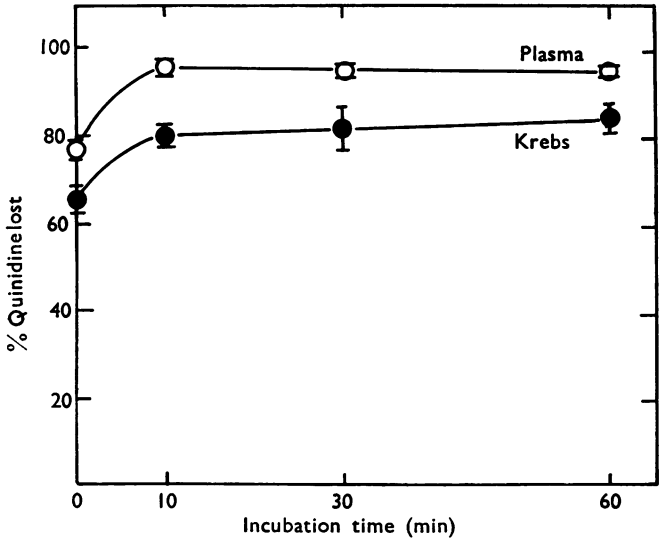

FIG. 9. Efflux of quinidine from platelets in plasma or Krebs solution. Platelets were suspended in plasma or Krebs solution and incubated with 10^{-5}M quinidine for 30 minutes. They were then resuspended in either fresh plasma (○) or Krebs solution (●) as described in **Methods** and reincubated for 60 minutes. The values given at zero time refer to losses due to the resuspension procedure alone. Results are the % lost (mean ±S.E.) in four experiments.

latter, almost 20% of the bound drug was retained by the platelets after 60 min incubation when C_i to C_f was approximately 40 to 1.

For platelets suspended in plasma 95% of the quinidine was lost in 10 min with no further loss thereafter, and C_i to C_f was 22 to 1.

Discussion

In 1963 Shulman studied the binding of quinidine-antibody complexes to human blood platelets. From his findings that washed platelets accumulated less quinidine that those suspended in their own plasma, and that platelet ghosts do not bind immunologically detectable amounts of drug he concluded that quinidine does not attach directly to the platelet membrane. Solomon & Zieve (1967) found, however, by measuring quinidine directly, that it bound avidly to human blood platelets. We confirm this and show that uptake occurs in Krebs solution, indicating that formation of an antibody complex is not a prerequisite for binding.

Plasma proteins form an outer layer on the platelet membrane (Nakao & Angrist, 1968 ; Behnke, 1968). These molecules might therefore bind considerable amounts of quinidine, and it has been suggested that quinidine acts on cells by virtue of avid binding to membrane proteins, thereby altering cell permeability (Conn & Luchi, 1961). One possible explanation for the enhanced quinidine uptake seen in Krebs solution concerns the role of this outer protein layer. Possibly, the binding of drug to this layer prevents further accumulation by the platelet itself. In other words, this serves as a physical barrier reducing the amount of drug coming into contact with the platelet membrane. If this is the case, this layer of molecules acts as a binding compartment for quinidine so that there is a relationship between three compartments as follows:

$$\underset{\text{Pool}}{\text{free quinidine}}\ \underset{(1)}{} \rightleftharpoons \underset{(2)}{\text{outer protein layer}} \rightleftharpoons \underset{(3)}{\text{platelet membrane}}$$

The relative importance of the compartments is unknown.

It seems probable that uptake is a passive process, as it is not inhibited by low temperature or ouabain. It is certainly dissimilar from the 5-HT transport mechanism, as 5-HT and 5-HT uptake inhibitors do not block uptake. The inhibition of quinidine uptake by dinitrophenol and iodoacetate only occurs at high concentrations and may be due to actions unrelated to inhibition of intermediary metabolism.

The next point to be considered is the subcellular distribution of quinidine. From our work it appears that a small proportion of the quinidine may be located intracellularly. We think most is simply attached to the outer membrane, because 60–80% is lost during resuspension. Only the small fraction which is retained after efflux do we consider bound inside the cell. Thus although the quinidine concentration in the medium increased considerably during efflux, the high concentration gradient, C_i to C_f still favoured further efflux; the fact that drug remained in the platelet during reincubation is evidence for intracellular binding, and the loss of endogenous 5-HT and ATP and exogenous 5-HT from loaded cells further strengthens this view. Several groups have shown, using both biochemical and electronmicroscopic techniques, that 5-HT is bound intracellularly in platelets in electron dense organelles (Tranzer, Da Prada & Pletscher, 1966, 1968 ; Bak, Hassler, May & Westerman, 1967 ; Davis & White, 1968).

Regarding the sites of distribution of quinidine in platelets suspended in plasma or Krebs solution, the fact that quinidine causes comparable rates of loss of 5-HT from platelets in either medium suggests that the same 5-HT store is being depleted in each instance. Therefore, some quinidine is penetrating to the same site in each case. Furthermore, quinidine depletes 5-HT and ATP in the molar ratio 1·78 to 1 ; this is close to the ratio of 2 to 1 found by Berneis, Da Prada & Pletscher (1969) to be the ideal proportion of 5-HT and ATP for micelle formation *in vitro*.

The foregoing remarks apply to the results obtained with 10^{-5} and 10^{-4}M quinidine. There is no doubt that very high concentrations (2×10^{-3}M) produced severe platelet damage (Figs. 6–8). At this concentration quinidine certainly entered the platelet, because the outer plasma membrane was broken and there was partial to complete disappearance of mitochondria, α-granules, microtubules and normal vacuoles. The redistribution and possible increase in glycogen granules is in favour of an effect of quinidine on intermediary metabolism.

This information obtained with the electronmicroscope is very important in relation to the pharmacological effects of quinidine. These all occurred with 10^{-5} or 10^{-4}M, when no ultrastructural changes were noted (Fig. 7). In particular, 10^{-4}M quinidine released approximately 60% of endogenous and exogenous ^{14}C-labelled 5-HT, without altering the exogenous to endogenous 5-HT ratio (Table 3). Consequently the combined pharmacological and electronmicroscopic results not only indicate that exogenous and endogenous indolealkylamine are sequestered in a single pool, but that quinidine affects this pool without causing overt changes in platelet ultrastructure.

The relationship of these comments to earlier work is that they agree with the findings of Davis & Wilson (1969) that the ED50 for ^{14}C-5-HT release was 3×10^{-5}M, and indicate that their effects also did not involve platelet damage. On the other hand, the complete inhibition of radioactive 5-HT uptake noted by Bridges & Baldini (1966) was undoubtedly due to an action of quinidine causing severe platelet damage.

One limitation of our experiments was that the uptake of quinidine and its effects on platelet ultrastructure were measured in calcium free media. Therefore we cannot dismiss the possibility that different results might be obtained if calcium were present. However, this does not seem likely because the extensive accumulation of many other substances by platelets, including chlorpromazine, imipramine and reserpine (Ahtee & Paasonen, 1966 ; Boullin & O'Brien, 1968 ; Solomon & Zieve, 1967) has been studied in calcium free conditions ; also the uptake of catecholamines is dependent on sodium, but not calcium (Iversen & Kravitz, 1966 ; Tissari, Schönhöfer, Bogdanski & Brodie, 1969).

The uptake of quinidine by platelets and its effects on 5-HT, ATP and ultrastructure are of interest both pharmacologically and clinically. As a potent and rapid releaser of platelet 5-HT, its actions should be studied on 5-HT and other amines in other tissues. Furthermore, as we have shown that quinidine can actually cause platelet damage in high concentrations *in vitro*, it seems highly probable that similar effects may also occur *in vivo* at lower plasma concentrations, because the cells are in contact with the drug for days rather than hours. Thus the well known thrombocytopenic purpura observed in some quinidine treated patients is likely to result from platelet destruction.

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