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INHIBITION BY GUANIDINO COMPOUNDS OF PLATELET AGGREGATION INDUCED BY ADENOSINE DIPHOSPHATE*

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Abstract—Various guanidino and related compounds were tested for their ability to inhibit platelet aggregation induced by adenosine diphosphate. The effect of the more active compounds on several platelet functions and on thrombic activity was also determined. Some amino alkylguanidines, alkylenediguanidines, and substituted alkylenediguanidines proved to be quite potent inhibitors, with no antithrombic activity or damaging effect on platelets. The most active compound tested was 1,4-diguanidino diphenylsulfone, of which 0·038 mM caused 50 per cent inhibition.

THE FUNDAMENTAL role of platelet aggregation in the formation of a hemostatic plug or thrombus is well established.[1] After adenosine diphosphate (ADP) was shown to be the constituent of red blood cells causing platelet aggregation,[2, 3] evidence of this nucleotide's key role in physiologic and pathologic platelet function began to accumulate. Thrombin-induced platelet aggregation was mediated by ADP which originated from within the platelets,[4, 5] and platelet ADP was also responsible for aggregation caused by connective tissue particles,[6, 7] serotonin (5-hydroxytyptamine), and epinephrine.[8, 9] Honour and Mitchell[10] suggested that ADP or a similar aggregating agent, released from a site of injury in a vascular wall, probably initiates the formation of a hemostatic platelet plug. Marr *et al.*[11] confirmed the presence of ADP at sites of hemorrhage. ADP-induced aggregation requires the presence of an optimal concentration of calcium ions[12] and one[12] or more[13] components of plasma, but the entire mechanism is not yet clear.

The importance of ADP in platelet function led investigators to test various substances for their inhibitory capacity toward ADP-induced platelet aggregation. Active agents reviewed elsewhere[1, 14] include compounds structurally similar to ADP, e.g. adenosine, AMP, and 2-chloroadenosine; metabolic inhibitors such as iodoacetate; sulfhydryl inhibitors; and calcium chelating agents.

Salzman and Chambers[15] and our group[16] found that tosylarginine methylester (TAMe) inhibits ADP-induced platelet aggregation. Certain related aminoacid derivatives also proved active.[15, 17, 18] We have extended these studies to include additional guanidino compounds as well as compounds related in other ways, in an attempt to find more potent inhibitors and to relate inhibitory potency to chemical structure. A

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preliminary report has been made of this work.[18] The compounds most active against ADP-induced platelet aggregation were also tested for possible interference with other platelet functions such as clot retraction and ability to retain serotonin against a concentration gradient; and for antithrombic effect as measured by prolongation of thrombin clotting time or inhibition of thrombin-induced release of serotonin from platelets.

MATERIAL AND METHODS

Materials

Platelet-rich plasma (PRP), anticoagulated with acid-citrate-dextrose (ACD), isotonic Tris buffer pH 7·5, and ADP (Sigma Chemical Co.) were prepared by methods previously described.[19] Highly purified human thrombin was prepared by Dr. Kent D. Miller, New York State Department of Health, Albany, in conjunction with the American National Red Cross. It was used in a concentration of 30 units/ml unless otherwise stated. Serotonin creatinine $SO_4 \cdot H_2O$ (Mann Research Laboratories), in a 0·01 M stock solution, and epinephrine (Adrenaline; Vitarine Co.), 1 mg/ml, were further diluted in Tris buffer immediately before use.

We are grateful to the following individuals (indicated by letters in the tables) who supplied compounds to be tested for inhibitory activity: Dr. B. C. Pressman (P), Department of Biophysics and Physical Chemistry, Johnson Foundation, University of Pennsylvania—the alkylguanidines and some of their derivatives; Dr. W. Troll (T), Department of Industrial Medicine, New York University School of Medicine—ϵ-aminocaproic acid ethylester (EACA); and Dr. E. Shaw (S), Department of Biochemistry, Tulane University School of Medicine—*p*-aminobenzamidine. Burroughs Wellcome (BW), CIBA Pharmaceutical (C), and Merck Sharp & Dohme (M) kindly furnished compounds of their own manufacture. Biguanide, guanylurea, aminoguanidine, and other compounds were purchased from K & K Laboratories (K); the ω-guanidino acids, their esters, and other compounds from Cyclo Chemical Corp. (Cy); several compounds from Mann Research Laboratories (MR), Calbiochem (CB), and Eastman Organic Chemicals (E).

The compounds were usually freshly prepared. Occasionally it was necessary to add concentrated HCl for completed issolution. The solutions were diluted to the desired concentration with Tris buffer and, if necessary, neutralized with 1 or 5 N NaOH immediately before use.

Experimental procedure

To 0·24 ml PRP was added 0·03 ml of compound or, in the control sample, Tris buffer. To test platelet aggregation, 0·03 ml of 2·5 or 25 μM ADP, 3 units/ml thrombin, 55 μM epinephrine, or 100 μM serotonin was added. With the last two compounds, 0·015 ml of 0·4 M $CaCl_2$ was also added. Aggregation, observed grossly after shaking the tubes for 1 min, was graded from 0 to 4+. Aggregation was 4+ in the control tubes. The concentration of inhibitor that reduced aggregation to 2+ was regarded as the 50 per cent inhibitory concentration, and the lowest concentration causing total inhibition was designated as the 100 per cent inhibitory concentration. It was not necessary to preincubate the inhibitors with PRP. We found, as did Salzman and Chambers,[15] that maximal inhibition occurred immediately. Platelet aggregation and its inhibition were occasionally measured by changes in optical density (O.D.):[20] the concentration

found to cause half the maximal change in O.D. agreed well with the 50 per cent inhibitory concentration determined by gross inspection of test tubes.

In many experiments, additional tests were carried out with the 100 per cent inhibitory concentration. Platelets in PRP were allowed to take up ^{14}C-labeled serotonin as described elsewhere.[19] To 2·7 ml labeled PRP was added 0·3 ml of compound or Tris buffer and the mixture distributed into several tubes to test spontaneous serotonin leakage, thrombin-induced serotonin release, thrombin clotting time, and clot retraction, as described previously.[19] Normal values for these tests were labeled N, and the criteria for grading abnormalities were as follows. In control tubes, thrombin caused clotting in 10–15 sec, a delay of up to 60 sec was designated as partially abnormal or PA, and higher values were designated as markedly abnormal or MA. In the clot retraction test, PA denoted partial inhibition, whereas MA denoted total absence of retraction. In supernatants of control samples without thrombin, 5–15 per cent of ^{14}C was detected, hence 85–95 per cent of added ^{14}C-labeled serotonin had been taken up by the platelets. Values in the supernatant between 15 and 50 per cent were considered partial leakage (PA) and higher values, marked leakage (MA). Thrombin caused release of 75–90 per cent of the serotonin in control samples free of inhibitory compounds. Lower values of release were considered as partial inhibition (PA). None of the compounds completely inhibited thrombin-induced release of serotonin.

RESULTS

The final concentration of compounds that caused 50 and 100 per cent inhibition of platelet aggregation induced by 2·5 μM ADP appear in Table 1, which also shows the effect of the 100 per cent inhibitory concentrations of numerous compounds on some physiologic characteristics of platelets and thrombin. The compounds were graded according to their 50 per cent inhibitory concentration as follows: potent, 1·5 mM or less; moderate, 2–10 mM; weak, 10–50 mM; negligible, over 50 mM.

Group A included arginine and its derivatives. Arginine itself proved a weak inhibitor, irrespective of its stereochemical configuration. A similar compound but with an oxygen atom (A XVIII) substituted for one methylene group was also a weak inhibitor —only twice as active as arginine. De-α-amination of arginine had a negligible effect since δ-guanidinovaleric acid (A XVI) was a weak inhibitor, and even the esterified form (A XVII) was only four times as effective as arginine. The effect of γ-guanidinobutyric acid was negligible and its methylester was a weak inhibitor. Group A compounds in which the amino group was blocked but the carboxyl group free (A III, IV, and V) were only slightly more effective than arginine.

Compounds in which the α-amino group was free and the carboxyl group either blocked (A VI and VII) or absent (A VIII) were 9–16 times as effective as arginine. Similar activity was observed when the α-amino group was blocked and the carboxyl group either blocked (A IX to XIV) or absent (A XV). In the most potent inhibitor in this group, tosyl arginine methylester (A XIII), the L-configuration was somewhat more effective than a mixture of the D- and L-forms. All the arginine esters and amides prolonged thrombin clotting time, and the majority partially inhibited the release of serotonin from platelets by thrombin.

The fact that agmatine (A VIII), an amino alkylguanidine, was half as active as TAMe (A XIII), in which the α-amino group is blocked, led us to test other amino alkylguanidines (group B). Their effect was variable: B III and IV were quite active and

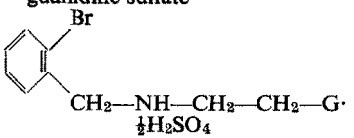
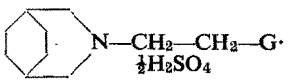
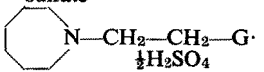
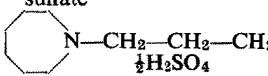
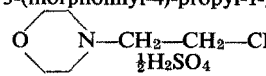
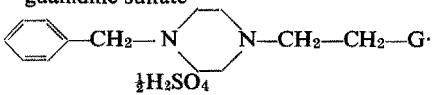
TABLE 1. EFFECT OF VARIOUS COMPOUNDS ON ADP-INDUCED PLATELET AGGREGATION AND OTHER PLATELET AND THROMBIN FUNCTIONS

Compound		Source[a]	Inhibition of ADP-induced platelet aggregation		Effect of 100% inhibitory concentration on:			
			50% Inhibitory concentration (mM/l.)	100% Inhibitory concentration (mM/l.)	Thrombin clotting time	Clot retraction	Leakage of serotonin	Thrombin-induced serotonin release
A. Arginine and its derivatives								
I	D-arginine HCl	Cy	50	100				
II	L-arginine HCl	E	50	100				
III	*p*-tosyl-L-arginine	MR	15					
IV	benzoyl-L-arginine	MR	15					
V	hippuryl-L-arginine	MR	>10					
VI	L-arginine amide 2HCl	Cy	5·5	25	PA[b]	N[b]	N	N
VII	L-arginine methylester 2HCl	Cy	4·5	15	PA	N	N	PA
VIII	agmatine sulfate	CB	3	10	PA	N	N	PA
IX	N-α-tosyl-L-arginine amide HCl	MR	3·3	10	PA	N	N	N
X	benzoyl-L-arginine amide HCl · H_2O	MR	8	33	PA	N	N	N
XI	benzoyl-L-arginine ethylester HCl	MR	4	12·5	MA[b]	N	N	PA
XII	benzoyl-L-arginine methylester HCl	MR	6·2	12·5	MA	N	N	PA
XIII	*p*-tosyl-L-arginine methylester HCl	MR	1·5		MA	N	N	PA
XIV	*p*-tosyl-DL-arginine methylester HCl	MR	2·5	5				
XV	N-α-*p*-tosylagmatine HCl	Cy	4	12·5	PA	PA	N	PA
XVI	δ-guanidinovaleric acid	Cy	>50					
XVII	δ-guardinovaleric acid methylester HCl	Cy	12·5	>45			N	
XVIII	L-canavanine H_2SO_4	MR	25	50				
B. Substituted aminoalkylguanidines								
I	3(N,N-diethyl)amino-2 hydroxypropyl-1-guanidine sulfate	BW 62-232	10					

[a], [b] See text.

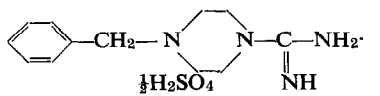
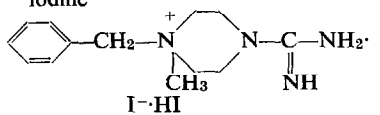
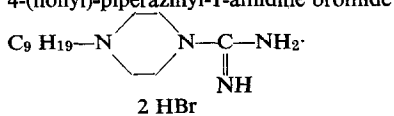
[c] Guanidino group.

TABLE 1.—*continued.*

II	2[N(2 bromo-benzyl)-amino]-ethyl-1-guanidine sulfate  $CH_2—NH—CH_2—CH_2—G\cdot$ $\frac{1}{2}H_2SO_4$	BW 60-101	2·4				
III	[1-(1,4 cyclohexyl)amino 2-ethyl] guanidine sulfate  $N—CH_2—CH_2—G\cdot$ $\frac{1}{2}H_2SO_4$	C SU-10465	1·2	4	N	N	N	N
IV[a]	[(2-octahydro-1-azocinyl) ethyl] guanidine sulfate  $N—CH_2—CH_2—G\cdot$ $\frac{1}{2}H_2SO_4$	C SU-5864	2	4	N	N	N	N
V	[3-(octahydro-1-azocinyl) propyl] guanidine sulfate  $N—CH_2—CH_2—CH_2—G\cdot$ $\frac{1}{2}H_2SO_4$	C SU-6097	1·2	4	N	MA	MA	N
VI	3-(morpholinyl-4)-propyl-1-guanidine sulfate  $N—CH_2—CH_2—CH_2—G\cdot$ $\frac{1}{2}H_2SO_4$	BW 62-318	6·5	25				
VII	[2-(1 phenylmethyl-4-piperazinyl)-ethyl] guanidine sulfate  $CH_2—N\quad N—CH_2—CH_2—G\cdot$ $\frac{1}{2}H_2SO_4$	C SU-7639	0·71	3·3	N	N	N	N

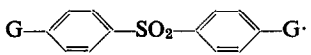
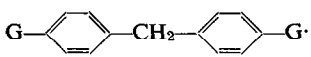
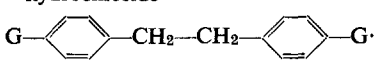
[a] Ismelin.

TABLE 1.—*continued.*

	Compound	Source[a]	Inhibition of ADP-induced platelet aggregation: 50% Inhibitory concentration (mM/l.)	100% Inhibitory concentration (mM/l.)	Effect of 100% inhibitory concentration on: Thrombin clotting time	Clot retraction	Leakage of serotonin	Thrombin-induced serotonin release
VIII	4(benzyl)piperazinyl-1-amidine sulfate 	BW 932	1·5	6	N	PA	N	N
IX	4,4′(benzyl-methyl)piperazinyl-1-amidine iodine 	BW 938	0·39	0·8	N	N	N	N
X	4-(nonyl)-piperazinyl-1-amidine bromide 	BW 52-66	12·3					
C. Alkylenediguanidines								
I	ethylenediguanidine H_2SO_4	M	2·5	10	N	N	N	N
II[e]	diguanidinobutane H_2SO_4	CB	0·3	1·25	N	N	N	PA
III	diguanidinopentane 2 HI	BW	0·3	3	N	N	N	N
IV	diguanidinohexane 2 HI	BW	0·3	3·5	N	N	N	N
V[f]	decamethylenediguanidine 2 HCl	BW	0·4	3·5	PA	MA	N	PA

[e] Arcaine. [f] Synthalin A.

TABLE 1.—*continued.*

D. Modified alkylenediguanidines								
I	(ethoxyethane)diguanidine hydrochloride G—$(CH_2)_2$—O—$(CH_2)_2$—G· 2 HCl	BW 51-283	1·25	5	PA	PA	N	N
II	(ethylene dioxyditrimethylene) diguanidine sulfate G—$(CH_2)_3$—O—$(CH_2)_2$—O—$(CH_2)_3$—G· H_2SO_4	M L284163-1-1	0·5	3·3	N	N	N	N
III	1,4-diguanidino-diphenyl sulfone hydrochloride  2 HCl	M L284154-1-2 BW 65-276	0·038	0·15	N	N	N	N
IV	1,4-diguanidino-diphenyl methyl hydrochloride hydrate  2 HCl H_2O	BW 65-332	0·06	0·3	N	N	N	N
V	1,4-diguanidino-diphenyl ethyl hydrochloride  2 HCl	BW 65-353	0·14	0·5	PA	N	N	N

did not affect platelet functions; B V and VIII were equally active but harmful. Two compounds (B VII and IX) were very potent and had no apparent deleterious effect on platelet or thrombic activity.

Alkylenediguanidines (group C) were also very potent inhibitors except for ethylenediguanidine (C I). The 2-, 5-, and 6-methylenediguanidines (C I, III, and IV) had no deleterious effects on platelets or thrombic activity. However, diguanidinobutane (C II) partially inhibited thrombin-induced release of serotonin; and decamethylenediguanidine C V) slightly prolonged clotting time, completely inhibited clot retraction, and partially interfered with thrombin-induced release of serotonin from platelets.

The most effective inhibitors were modified alkylenediguanidines (group D). Two of them (D III and IV) were more than five times as active as alkylenediguanidines. Neither these two compounds nor D II interfered with clot retraction or with the action of thrombin, in contrast to the other two in this group (D I and V).

Since most of the active compounds had a free guanidino group, we tested other compounds containing this group. Aminoguanidine, guanylurea, and biguanide had no noticeable inhibitory effect. The inhibitory potency of the alkylguanidines increased with length of the chain; there was a linear relationship between chain length and the logarithm of the 50 per cent inhibitory concentration (Fig. 1). Other platelet functions were also affected; butylguanidine partially inhibited clot retraction, and compounds with longer methylene chains completely inhibited clot retraction and caused either partial leakage of serotonin from the platelets (pentyl- and hexylguanidine) or complete leakage (heptylguanidine).

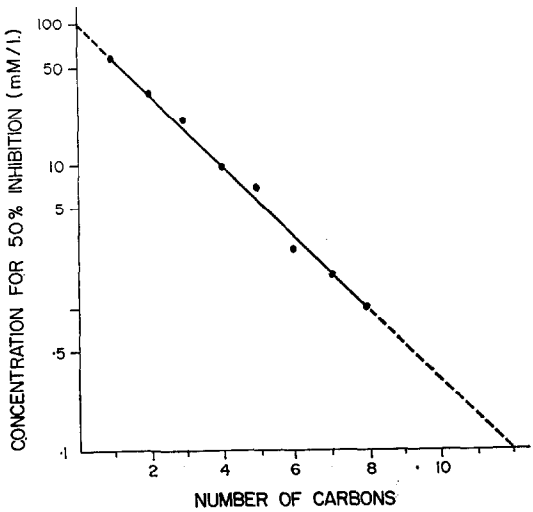
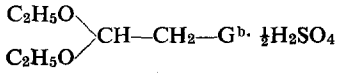
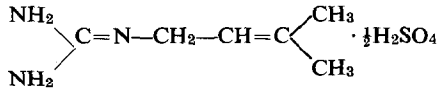
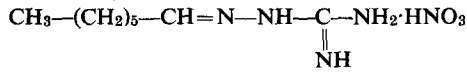
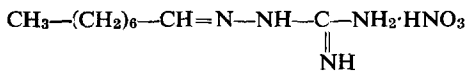
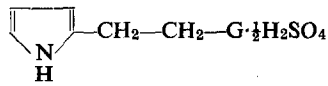
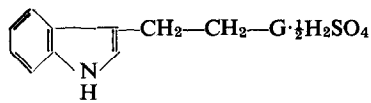
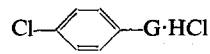
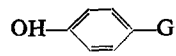
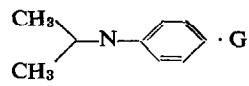
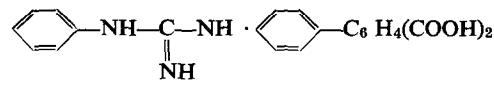
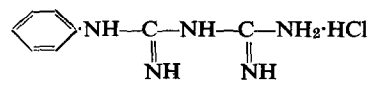


FIG. 1. Effect of number of methylene groups on the inhibitory activity of *n*-alkylguanidines

The 50 per cent inhibitory concentrations of other compounds with guanidino or amino groups are given in Table 2. Although two substituted alkylguanidines (E III and IV) as well as tryptylguanidine (F I) were potent inhibitors, they impaired clot retraction and caused spontaneous leakage of serotonin from the platelets. Tests involving phenylguanidine derivatives, and benzyl- and phenethylguanidine and their

TABLE 2. INHIBITION OF ADP-INDUCED PLATELET AGGREGATION BY VARIOUS COMPOUNDS

	Compound	Source[a]	50% Inhibitory concentration (mM/l.)
E. Substituted alkylguanidines			
I		BW 58-179	>50
II[c]		K	8·3
III[d]		P	0·66
IV[d]		P	0·4
V		C SU6026	6
F. Tryptyl guanidine			
I[e]		BW 63-178	1
G. Guanidinobenzimidazole 1[d] 2-guanidinobenzimidazole		K	5·5
H. Derivatives of phenylguanidine			
I		BW 65-100	3·5
II		BW 58-170	15
III		BW 58-171	5
IV		K	2
V		K	8·3

[a] See text. [b] Guanidino group. [c] Galegine. [d] The compound caused spontaneous leakage of serotonin and completely inhibited clot retraction.

[e] The compound caused partial leakage of serotonin and partial inhibition of clot retraction.

TABLE 2.—*continued*

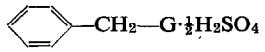
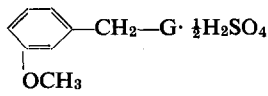
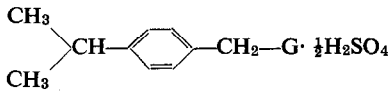
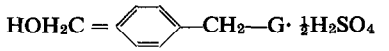
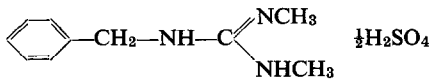
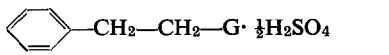
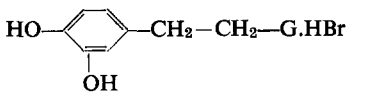
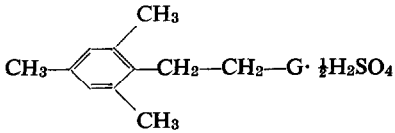
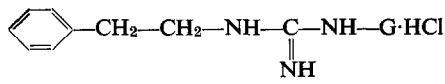
	Compound	Source[a]	50% Inhibitory concentration (mM/l.)
K. Benzylguanidine and its derivatives			
I		P	8
II		BW 60-137a	8·3
III		BW 62-393	3
IV		BW 62-388	3
V[t]		BW	12·5
[t] Bethanidine.			
L. Phenethylguanidine and its derivatives			
I		BW 247-C-58a	6
II		BW 62-127a	4·2
III		BW 59-426	2·5
IV		K	10
M. Lysine derivatives			
I	L-lysine ethylester 2 HCl	MR	10
II	N-benzoyl-L-lysine methylester HCl	Cy	10
III	ϵ-aminocaproic acid ethylester HCl	T	8
IV	ϵ-aminocaproic acid methylester HCl	Cy	15
N. Monoamines			
I	norvalamine HCl	CB	35
II	norleucamine HCl	CB	30
P. Diamines and their substitutes			
I	putrescine 2 HCl	MR	25
II	cadaverine 2 HCl	MR	6·25
III	1,6-hexanediamine 2 HCl	E	2·5
IV	pentamethonium bromide	K	10
V	hexamethonium bromide	K	5
VI	spermine 4 HCl	CB	3·5

TABLE 2.—*continued*

	Compound	Source[a]	50% Inhibitory concentration (mM/l.)
Q. Aromatic amino compounds			
I	cyclohexylamine HCl	K	20
II	β phenylethylamine HCl	K	13
III	tyramine	K	6·5
IV	2-aminomethyl benzimidazole HCl	K	10
R. Benzamidine and its derivatives			
I[g]	benzamidine HCl	K	3·1
II[h]	*p*-aminobenzamidine HCl	S	1·25
S. Benzimidazole			
I	benzimidazole	MR	25

[g] The compound caused partial leakage of serotonin.

[h] This compound caused partial prolongation of clotting time and partial inhibition of clot ratraction.

derivatives, failed to reveal any potent inhibitors, although some compounds were moderately active (H I, III, IV, and V; K I, II, III, and IV; L I, II, and III). Lysine derivatives (group M) were moderate or weak inhibitors; one (M II) was slightly less active than the corresponding arginine derivative. The monoamino compounds (N I and II) were weak inhibitors. Only four of the diamines and their substitutes (P II, III, V, and VI) had moderate inhibitory effects; two others (P I and IV) were weak. No potent inhibitor was found among the aromatic amino compounds (group Q). One related compound (R II) was potent but prolonged clotting time slightly and partially interfered with clot retraction, and a third (S I) was weak.

Two diguanidino compounds active against ADP-induced aggregation—arcaine (C II) and diguanidino diphenyl sulfone (DGPS) (D III)—were also tested for their capacity to inhibit platelet aggregation induced by thrombin, serotonin, and epinephrine. Thrombin-induced aggregation was inhibited by the same concentration of DGPS as that shown in Table 1, whereas a fourfold concentration of arcaine was needed to inhibit this aggregation. With either serotonin or epinephrine, it was necessary to add $CaCl_2$ to bring about 4+ aggregation. A concentration of 0·25 μM ADP was used as a control for these studies, since it caused 4+ aggregation only in the presence of added $CaCl_2$. Since arcaine and DGPS inhibition is antagonised by calcium,[21] higher concentrations of the inhibitors were required than those shown in Table 1. A final concentration of 0·5 mM DGPS totally inhibited aggregation induced by ADP, serotonin, or epinephrine. Because of arcaine's limited solubility the highest concentration tested was 5 mM, which completely inhibited aggregation induced by serotonin and markedly inhibited ADP- or epinephrine-induced aggregation.

DISCUSSION

In examining the relationship between chemical configuration and inhibitory effect on ADP-induced platelet aggregation, it was clear that arginine and its derivatives with a free carboxyl group had no appreciable inhibitory effect. Similar results were obtained by Salzman and Chambers.[15] In contrast, arginine derivatives with a blocked or missing carboxyl group had a more pronounced effect, perhaps due to a higher overall positive

charge. Among arginine derivatives with both the carboxyl and α-amino groups blocked, those with a tosyl group proved stronger inhibitors than similar compounds with a benzoyl group, and TAMe was the most effective. According to Salzman and Chambers[15] and McLean *et al.*,[17] the benzoylarginine methyl- and ethylesters were more active than TAMe. This discrepancy is thus far unexplained. Since benzoylarginine methylester was somewhat more active than the corresponding lysine derivative attention was focused on the guanidino group.

Guanidine itself[15] and substituted guanidines, e.g. amino guanidine, guanylurea, and biguanide had no noticeable inhibitory activity. Certain guanidino compounds (B V and VIII; C V; D I; E III and IV; and F I) were potent inhibitors but caused leakage of serotonin, partial or complete inhibition of clot retraction, or both. Their inhibitory effect on ADP-induced platelet aggregation was thus probably due to platelet damage rather than to a more specific mechanism.

Comparison of arginine methylester with δ-guanidinovaleric acid methylester showed that the presence of an α-amino group enhanced potency, and comparison of butylguanidine with agmatine revealed that it prevented deleterious effects on platelets. Substitutions on the amino group of the aminoalkylguanidines had variable effects; some compounds were less and others more active than agmatine. The most potent inhibitor in this group (B IX) was about as active as the alkylenediguanidines. Interestingly enough, a similar compound one-fourth as active (B VIII) differed by having a trivalent nitrogen rather than a quaternary nitrogen with a positive charge. This suggests the importance of a strong charge on the second basic group.

Comparison of diguanidinopentane and -hexane with pentyl- and hexylguanidine revealed that the presence of a second guanidino group, rather than an amino group, in the terminal position of the molecule also abolished the deleterious effects and further enhanced inhibitory potency. Moreover, diguanidinobutane was approximately ten times more inhibitory than agmatine. Similar enhancement of inhibitory potency was noted in studies of enzymatic activity. Blaschko *et al.*[22] found that compounds with two amidino or guanidino groups were stronger inhibitors of histaminase (diamine oxidase) than compounds with one amidino or guanidino group. In studies of mitochondrial respiration, Guillory and Slater[23] found that decamethylenediguanidine (Synthalin) had a more pronounced inhibitory effect than the alkylguanidines or phenethylbiguanide. In our studies, Synthalin was not only a potent inhibitor of ADP-induced aggregation but also interfered with thrombin clotting time, clot retraction, and thrombin-induced release of serotonin. Therefore, it seems that alkylenediguanidines with a longer carbon chain resemble alkylguanidines in their harmful effect on platelets. This may result from a detergent-like activity.

Diamine and dimethonium compounds were less active inhibitors than diguanidino compounds. Diguanidinopentane was about 30 times as active as pentamethonium, and diguanidinohexane was about 16 times as potent as hexamethonium. These results suggest that the charge of the basic group is not the sole consideration in determining potency. The unsubstituted diamines (P II and III) were slightly more active than the corresponding methonium compounds.

Of two substituted diguanidino compounds, D IV was five times as active as the active alkylenediguanidines, while D III (DGPS), slightly more potent than D IV, was the most active compound tested. The mechanism by which they inhibit ADP-induced platelet aggregation is specific, since they had neither an antithrombic effect

nor a deleterious effect on platelets. Kinetic studies of ADP-induced aggregation and its inhibition[21] revealed that DGPS (D 111) as well as arcaine (C 11) are noncompetitive inhibitors with respect to ADP but are antagonized by calcium, which is essential for ADP-induced aggregation.[12] However, DGPS does not inhibit other calcium-dependent reactions such as clot retraction and serotonin release.[14] These findings suggest that DGPS may compete with calcium in combining with ADP and/or plasma protein[12, 13] during ADP-induced aggregation. We have obtained evidence that a complex forms between DGPS and ADP (M. B. Zucker, L. Skoza, Z. Jerushalmy and R. Grant, unpublished data). Pressman and Park[24] have indicated that competition between guanidine and magnesium may explain the inhibitory effect of guanidine on energy transfer in oxidative phosphorylation. It is doubtful that arcaine or DGPS will be useful *in vivo* since concentrations that would inhibit platelet aggregation were lethal to rabbits (J. Peterson and M. B. Zucker, unpublished observations).

REFERENCES

1. A. J. Marcus and M. B. Zucker, *The Physiology of Blood Platelets. Recent Biochemical, Morphologic and Clinical Research.* Grune and Stratton, New York and London (1965).
2. A. J. Hellem, *Scand. J. clin. Lab. Invest.* **12**, Suppl. 51 (1960).
3. A. Gaarder, J. Jonsen, S. Laland, A. Hellem and P. A. Owren, *Nature, Lond.* **192**, 531 (1961).
4. R. Käser-Glanzmann and E. F. Lüscher, *Thromb. Diath. haemorrh.* **7**, 480 (1962).
5. R. J. Haslam, *Nature, Lond.* **202**, 765 (1964).
6. T. Hovig, *Thromb. Diath. haemorrh.* **9**, 264 (1963).
7. T. H. Spaet and M. B. Zucker, *Am. J. Physiol.* **206**, 1267 (1964).
8. J. R. O'Brien, *J. clin. Path.* **17**, 275 (1964).
9. R. J. Haslam, *Physiology of Hemostasis and Thrombosis* (Ed., S. A. Johnson),Thomas, Springfield, Ill. (in press).
10. A. J. Honour and J. R. A. Mitchell, *Brit. J. exp. Path.* **45**, 75 (1964).
11. J. Marr, J. J. Barboriak and S. A. Johnson, *Nature, Lond.* **205**, 259 (1965).
12. G. V. R. Born and M. J. Cross, *J. Physiol., Lond.* **170**, 397 (1964).
13. D. Deykin, C. R. Pritzker and E. M. Scolnick, *Nature, Lond.* **208**, 296 (1965).
14. M. B. Zucker and Z. Jerushalmy, *The Physiology of Hemostasis and Thrombosis*, (Ed. S. A. Johnson), Thomas, Springfield, cf. f.9 Ill. (in press).
15. E. W. Salzman and D. A. Chambers, *Nature, Lond.*, **204**, 698 (1964).
16. M. B. Zucker and Z. Jerushalmy, *Blood* **25**, 603 (1965).
17. J. R. McLean, R. E. Maxwell and D. Hertler, *Life Sci.* **3**, 1313 (1964).
18. Z. Jerushalmy, L. Skoza and M. B. Zucker, *Fedn Proc.* **24**, (No. 2, Part 1), 402 (1965).
19. Z. Jerushalmy and M. B. Zucker, *Thromb. Diath. haemorrh.* **15**, 413 (1966).
20. J. R. O'Brien, *J. clin. Path.* **15**, 446 (1962).
21. M. B. Zucker, L. Skoza and Z. Jerushalmy, *Fed. Proc.* **25**, 554 (1966).
22. H. Blaschko, F. N. Fastier and I. Wajda, *Biochem. J.* **49**, 250 (1951).
23. R. J. Guillory and E. C. Slater, *Biochim. biophys. Acta* **105**, 221 (1965).
24. B. C. Pressman and J. K. Park, *Biochem. biophys. Res. Commun.* **11**, 182 (1963).