cortisone is known to depress IF production (14,15), IF may be the factor limiting this infection and preventing spread of virus to the central nervous system, thus determining the outcome of the infection. Also, the observations of Larke(16) on dose responses to viruses, together with these findings of rabies induced IF, suggest that IF production may be involved in the classical post-exposure antirabies prophylaxis (Pasteur treatment).

Summary. The induction of interferon by rabies virus was studied in order to establish the status of this virus as an inducer of interferon in the cells it infects. Previous investigators, using *in vitro* systems, have been unable to demonstrate interferon production from cells infected with rabies virus, and this has caused some confusion among researchers working on rabies. However, using an *in vivo* system, we were able to isolate and characterize rabies induced interferon from several tissues. The brain, in which the virus reached its highest titer, also contained the largest amount of interferon, with the other levels of interferon corresponding to the centrifugal spread of rabies virus from the brain to the organs.

We are greatly indebted to Dr. Royce Z. Lockart, Jr. for his criticisms in reviewing this manuscript and for his many helpful discussions thoughout the course of these studies. The valuable assistance of Mrs. Marilyn Mays Stewart is gratefully acknowledged.

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Received June 9, 1966. P.S.E.B.M., 1966, v123.

Effect of Adenosine Derivatives and Antihistaminics on Platelet Aggregation. (31569)

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It is well documented that ADP is a powerful platelet aggregator, both *in vivo* and *in vitro,* and that some adenosine analogues inhibit the ADP effect(1-6). Several adenosine derivates, prepared in our laboratories by Dr. Gerzon, were tested for inhibitory activity using rabbit platelet rich plasma.

O'Brien(7) reported that the antihistaminic, dephenylhydramine hydrochloride, had an inhibitory effect on platelet aggregation. Several antihistaminics were tested for their inhibitory activity on ADP induced aggregation to ascertain whether this was correlated to antihistaminic activity.

Materials and methods. Rabbits were used exclusively for this study and, unless otherwise stated, no anticoagulant was employed at any time. All surfaces coming in contact

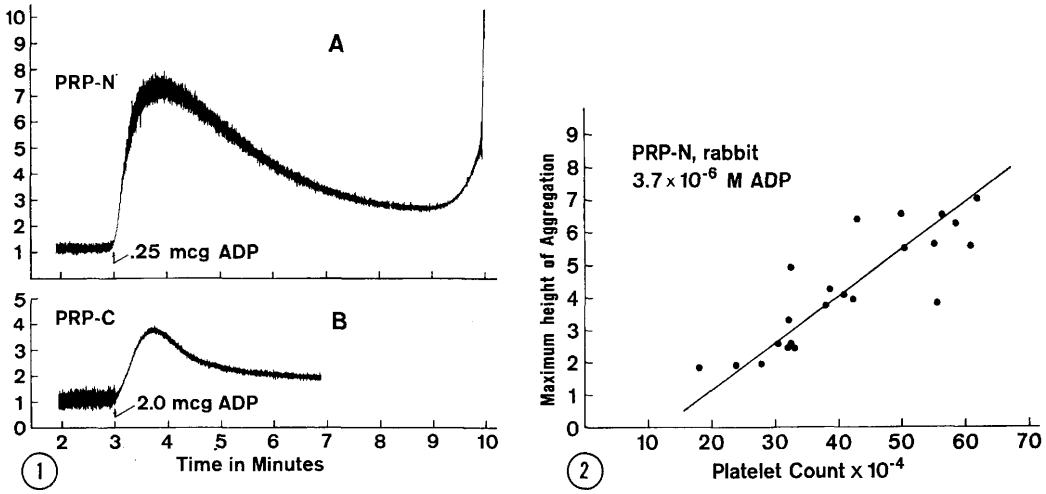


FIG. 1. ADP induced platelet aggregation in native V.S. citrated blood of the same rabbit. The platelet aggregation response is recorded on the ordinate.

FIG. 2. Effect of platelet concentration on ADP induced aggregation.

with the blood or plasma were freshly siliconized, siliconized tubing being used only once and the silicone removed from glassware with a NaOH-acetone mixture before being re-siliconized. A 10% solution of General Electric SC-87 silicone in low boiling petroleum ether was used for siliconizing. We found these precautions necessary for our studies with native blood and plasma to obtain a good platelet rich plasma (PRP) preparation which remained stable for several hours.

The rabbits were anesthetized with secobarbital sodium and the carotid artery was cannulated with PE240 tubing and bled into a centrifuge tube in an ice-bath. The blood was centrifuged at 4°C and 100 $\times$ *g* for 20 minutes. The supernatant PRP was transferred into a new tube and stored in an ice-bath until used.

For the study of platelet aggregation the procedure and instrumentation described by Mustard *et al*(8) was employed. A constant final volume of 1.40 ml was used in all instances, consisting of 1.0 ml of PRP and 0.4 ml of imidazole buffer, pH 7.4, containing the substances to be added. Unless otherwise stated, the drug tested was incubated in the aggregometer with stirring (1100 rpm) and at 37°C for 3 minutes prior to addition of the aggregation inducer. Platelet aggregation was induced by 4.2×10^{-7} M ADP.

The antihistaminic drugs were obtained from the following sources: methapyriline hydrochloride, Eli Lilly & Co.; methdilazine hydrochloride, Mead Johnson & Co.; promethazine hydrochloride, Wyeth Laboratories, Inc.; diphenylhydramine hydrochloride, Parke Davis & Co.; trimeprazine tartrate, Smith Kline & French Laboratories; chlorpheniramine maleate, Schering Corp.

Collagen from tendon (Sigma Chemical Corp.), 0.3 g in 100 ml tyrodes buffer, was fragmented in a Waring blender for 10 minutes. The suspension was centrifuged at 30 $\times$ *g* for 10 minutes and the supernatant was used to produce platelet aggregation. The thrombin was Bovine, Topical obtained from Parke Davis & Co.

Results. A typical record obtained using ADP to induce platelet aggregation is presented in Fig. 1, curve A. The upward slope of the curve represents a decrease in optical density due to aggregation of the platelets. The decline of the curve after maximal aggregation indicates de-aggregation illustrating the reversibility of this reaction apparently due to ADP degradation by the plasma enzymes (9-12). Following this, aggregation again occurs just prior to fibrin formation presumably due to thrombin. Hence, by using native PRP (PRP-N) both

platelet aggregation and fibrin formation can be observed.

Using citrated PRP (PRP-C), one part 3.8% sodium citrate in saline to 9 parts of blood, a decreased ADP response was obtained(8), and fibrin formation was prevented as shown in curve B, Fig. 1. Since Ca^{++} is required for the aggregation reaction (13), the citrate effect is not unexpected. Because the citrate concentration would be critical for studies with inhibitors of platelet aggregation, we used PRP-N for all our studies. Furthermore, citrate is a metabolically active substance which may influence platelet metabolism.

Since PRP-N is less stable than PRP-C it was important to test the aggregating response to ADP over a period of time. It was found that with time there was an acceleration of fibrin formation, but the ADP response remained unchanged as long as there was no visible evidence of platelet settling in the stock PRP-N stored in the ice bath. Similarly, the inhibitory response to a compound remained constant.

The maximum height of the curve corresponding to platelet aggregation varied from one PRP-N preparation to the next due to platelet responsiveness to ADP, rate of ADP degradation and also it depended on the platelet concentration as shown in Fig. 2. Dilution of the PRP-N with platelet poor plasma from the same rabbit to a standard platelet count sometimes resulted in a poor PRP-N preparation. Therefore, to standardize the inhibition studies the assumption was made that in any one PRP-N preparation the percent inhibition of the standard ADP aggregation response, calculated from the point on the curve of maximum aggregation, is proportional to that obtained with an arbitrarily chosen standard compound. By plotting percent inhibition against molar concentration on semilog graph paper a straight line was obtained. From the intercept of this line at 50% inhibition the molar concentration can be obtained from the graph. The molar concentration producing 50% inhibition was used to calculate relative potency (Rp). Two or more points between 20 and 80% inhibition were used for each dose response curve.

The effect of several adenosine derivatives on ADP induced aggregation is presented in Table 1. Some of these compounds were quite insoluble and it was not possible to add a high enough concentration to obtain a dose response curve. In those instances the percent inhibition at the highest dose tested is reported.

The adenosine derivatives show a relatively high specificity for inhibition of platelet aggregation. Apparently the amino function at R_1 is required, Nos. 1, 14, and 15. With the exception of No. 4, 5'-adamantoyladenosine, any change from adenosine in positions R_2, R_3, or R_4 resulted in a decrease in activity. Changing the ribose moity from the 5 position (adenosine No. 1) to the 3 position (isoadenosine No. 2) resulted in a 100-fold decrease in activity.

Isoadenosine diphosphate at a concentration as high as 2.6×10^{-4} M did not produce platelet aggregation itself but decreased the rate of ADP induced aggregation without having any effect on the extent of aggregation as determined by the maximum response obtained.

The effect of several antihistaminics on ADP induced platelet aggregation is presented in Table 2. The results show that there was no direct correlation between antihistaminic and platelet aggregation inhibitory activity. Compounds 1, 3, and 4 are of the same range of antihistaminic potency, yet there was a 4-fold difference in aggregation inhibitory activity. Compounds No. 3 and 6 were of equal potency with respect to aggregation inhibition, yet No. 6 is a more potent antihistaminic. All of the compounds have an amino function in common, but further structure activity relationship was not apparent. Histamine at a concentration as high as 7×10^{-4} M did not produce platelet aggregation but at this dose ADP induced aggregation was inhibited 23%. Constantine (14) also observed inhibition of ADP aggregation with histamine.

To determine whether preincubation of drug with the PRP-N prior to ADP addition was essential, adenosine derivative No. 4 and methdilazine were added after ADP aggre-

gation had just started. It was found, Fig. 3, that both compounds still had an inhibitory effect; however, the adenosine derivative, curves B and C, produced less inhibition when added after ADP, whereas methdilazine, curves D and E, produced equal inhibition

TABLE I. Inhibitory Effect of Adenosine Derivatives on Platelet Aggregation.

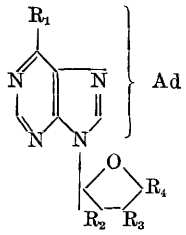
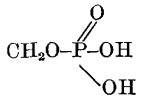
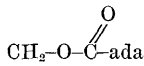
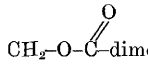
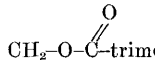
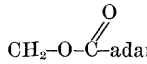
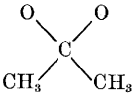
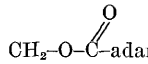
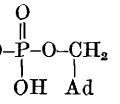
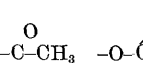
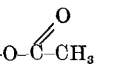
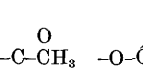
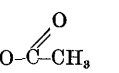
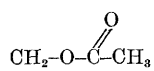
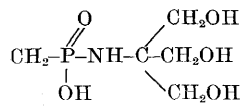
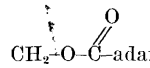
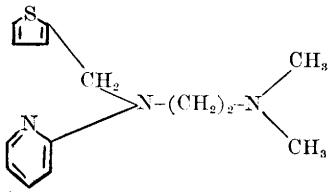
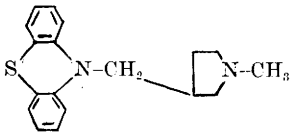
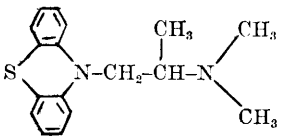
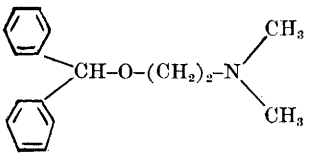
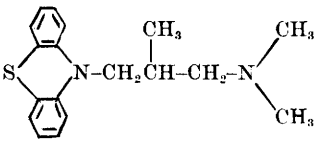
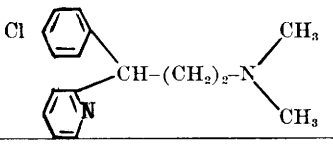
		R_1	R_2	R_3	R_4	Relative potency (4.2×10^{-7}M ADP)
						
1.	Adenosine	NH_2	OH	OH	CH_2OH, 50% inhibition at 2.7×10^{-7}M	1
2.	Isoadenosine	NH_2	OH	OH	CH_2OH, ribose p- to R_1	.015
3.	AMP	NH_2	OH	OH		.135
4.	5'-adamantoyl adenosine	NH_2	OH	OH		1.2
5.		NH_2	OH	OH		.023
6.		NH_2	OH	OH		31% inhibition 1.3×10^{-5}M
7.		NH_2	H	OH		18% inhibition 9.9×10^{-5}M
8.		NH_2				0% inhibition 7.2×10^{-6}M
9.		NH_2	OH	$-O-PO_3H_2$	CH_2OH	.006
10.		NH_2	OH		CH OH	20% inhibition 2.9×10^{-5}M
11.		NH_2			CH_2OH	.08
12.		NH_2				.004
13.		NH_2	OH	OH		.009
14.		OH	OH	OH		30% inhibition 8.3×10^{-5}M
15.		SH	OH	OH	CH_2OH	0% inhibition 2.5×10^{-4}M

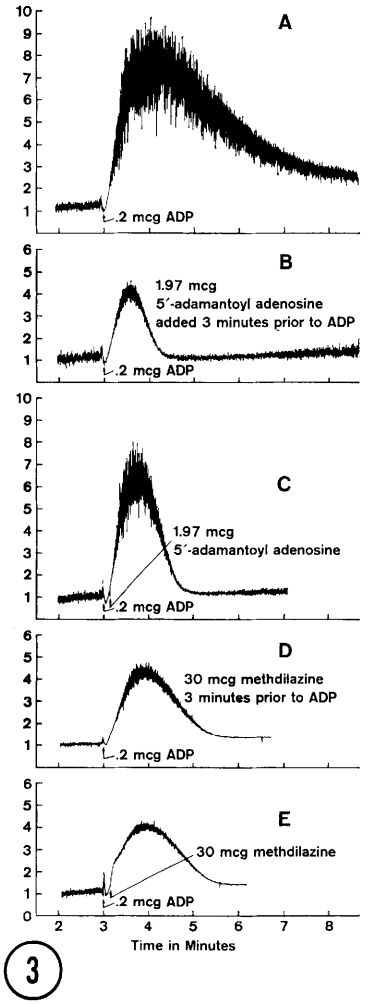
TABLE II. Inhibitory Effect of Antihistaminics on Platelet Aggregation.

	Compound		Single dose (adult), mg	Relative potency (4.2×10^{-7}M ADP)
1.		methapyrilene hydrochloride	50–100*	1 (50% inhibition at 4.6×10^{-4}M)
2.		methdilazine hydrochloride	8†	5.4
3.		promethazine hydrochloride	25–50*	2.0
4.		diphenylhydramine hydrochloride	50*	0.5
5.		trimeprazine tartrate	2.5†	2.9
6.		chlorpheniramine maleate	2–4*	1.9

* Goodman, L. S., and Gilman, A., The Pharmacological Basis of Therapeutics, 1965, 3rd ed.
† Goth, A., Medical Pharmacology, 1964, 2nd ed.

when added either prior to or after ADP. It is also interesting to note that the antihitaminics decreased the amplitude of pen deflection as shown on the record, Fig. 3, which indicates sphering of the platelets(20). Similarly, the size of the aggregates as smaller after antihistaminic addition than after the adenosine derivatives.

Both compounds also inhibited collagen induced aggregation, but slightly higher doses of drug were required than with ADP induced aggregation. It was interesting to find that heparin, which has no effect on ADP induced aggregation, curves A and B, Fig. 4, partially inhibited the collagen response curves C and D, Fig. 4. Increasing the dose of heparin up to 400 μg had no further inhibitory effect. With PRP-N there is a spontaneous production of thrombin as seen in curve A where platelet aggregation occurs just prior to fibrin formation (confirmed by examination under the microscope). Hence, the collagen response, curve C, may possibly be exaggerated due to spontaneous thrombin for-



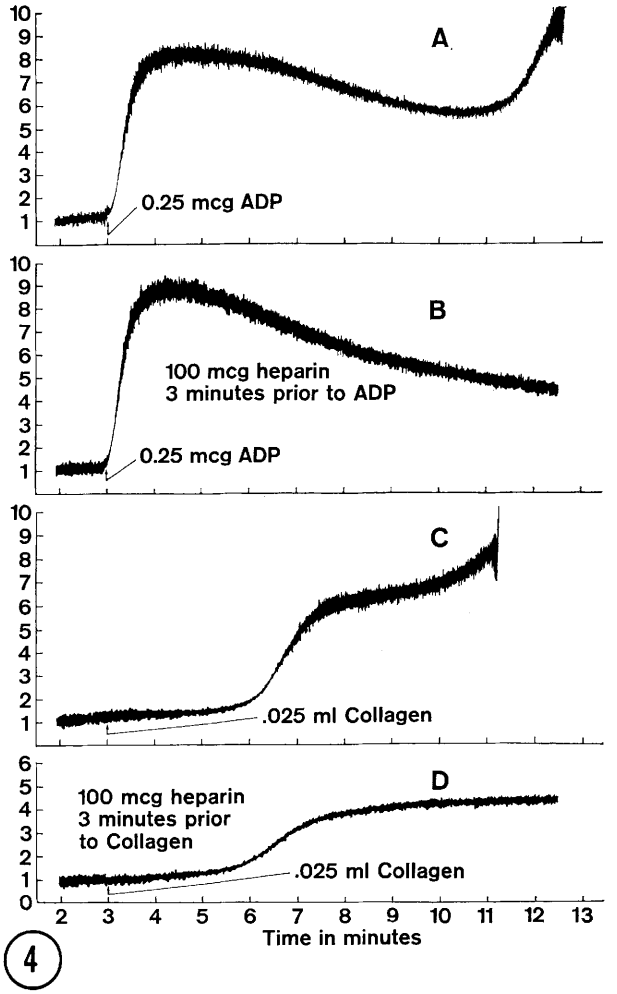


FIG. 3. Inhibitory effect of 5'-adamantoyl adenosine and methdilazine added prior to and after ADP. The platelet aggregation response is recorded on the ordinate.

FIG. 4. Effect of heparin on ADP and collagen induced aggregation. The platelet aggregation response is recorded on the ordinate.

mation. Since we have been able to completely inhibit the thrombin response with heparin, curve B and also after thrombin added to the PRP-N, the inhibition of the collagen induced aggregation seen in curve D may at least partly be due to a block of the thrombin effect. Therefore, it appears that heparin has minimal inhibitory activity on collagen induced aggregation.

Discussion. The high degree of specificity illustrated by the adenosine derivatives for inhibition of ADP induced aggregation and also for production of aggregation(15) is interesting in contrast to the broad spectrum of inhibitors reported, antihistaminics (8 and this report), substituted amino acids(16), sulfhydryl inhibitors(17), thrombin inhibitors (18), anti-inflammatory compounds(19), and others. Furthermore, the potency of adenosine as compared to the antihistaminics is much greater, about 1000 times as great. Spaet *et al*(21) found that inhibition of ADP induced platelet aggregation by AMP was associated with inhibition of platelet ADPase whereas the inhibition produced by diphenylhydramine was not. They therefore postulate two different pathways for the inhibition platelet aggregation. Our results also indi-

cate two possible mechanisms for inhibition; one where a specific block of the ADP reaction is involved and the other possibly a general surface action on the platelet membrane.

The PRP-N of one rabbit was found to be about 100 times less sensitive to adenosine inhibition of ADP induced aggregation than that normally observed. On the other hand, the inhibitory effect of methapyrilene hydrochloride was in the normal range. A possible explanation for this appears to be that the plasma of this particular rabbit had a very high adenosine desaminase activity.

Summary. The inhibitory activity of adenosine derivatives on ADP induced platelet aggregation appears to be quite specific. Only one derivative, 5'-adamantoyl adenosine, retained the potency of adenosine. Isoadenosine was 1/100 as active as adenosine. Isoadenosine diphosphate did not induce platelet aggregation at a dose as high as 2.6×10^{-4} M as compared to ADP which produces extensive aggregation at 4.2×10^{-7} M. All of the 6 antihistaminics tested inhibited ADP induced aggregation, but there was no correlation between antihistaminic and aggregation inhibitory potency. Both classes of compounds also inhibited collagen induced aggregation.

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Received July 1, 1966. P.S.E.B.M., 1966, v123.

Phagocytic and Bactericidal Capacity of Polymorphonuclear Leucocytes Recovered from Venous Blood of Human Beings.* (31570)

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The phagocytic efficiency of polymorphonuclear granulocytic leucocytes (PMNG) derived from diabetic but non-ketotic animals and man has been found equal to that of cells derived from control subjects(1,2,3). Metabolic acidosis due to the ketosis of poorly regulated diabetes mellitus, however, has been found to depress phagocytic vigor(3).

Since phagocytosis results in death or destruction of many types of ingested micro-

* This investigation was supported by USPHS Grants AI-02406-06, HE-02792-09 and T1-Ai-69.