addition of these compounds to a perfusion of artificial sea water does not affect the electrical activity of isolated giant axons. In the presence of reduced calcium and magnesium spontaneous firing of the fiber occurs. This may be reversed by returning the nerve to sea water with normal concentrations of calcium and magnesium. A decrease in hyperirritability may also be obtained by adding dilantin or mesantoin to water deficient in calcium and magnesium. Continued exposure to the latter solution reversibly abolishes signs of electrical activity. Dilantin labelled with N^{15} was found to penetrate into the interior of the giant axon of squid. The concentration in the axoplasm of the fiber was close to equilibrium within one hour.

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Effect of Histamine and Antihistaminics on Coagulation of Normal and Heparinized Rabbit Plasma. (18470)

THOMAS J. HALEY AND FRANCES STOLARSKY.*

From the School of Medicine, University of California at Los Angeles.

Haley and Harris(1) recently reported that several antihistaminic compounds significantly decreased the coagulation time in both normal and roentgen ray irradiated guinea pigs. This indicated that the antihistaminic compounds were able to inactivate the circulating anticoagulant (heparin?) found in the blood of animals subjected to whole body ionizing radiation(2). However, when one considers the chemical structure of the antihistaminics in relation to other compounds shown to be heparin inactivators both chemically and in a coagulation system(3,4), it becomes difficult to explain the antiheparin activity of the antihistaminics. The structure of these drugs, with the exception of the phenothiazine derivatives, bears no resemblance to the nuclear structure (phenazine, thiazine or oxazine) previously shown to be essential for heparin inactivation(3). Furthermore, the essential primary amine group is absent and the antihistaminics have a tertiary or cyclized amine group in its place. It was these considerations which prompted our *in vitro* investigation of the effect of both histamine and the antihistaminics upon the coagulation of normal and heparinized rabbit plasma.

Experimental. Blood was obtained from 5 rabbits by cardiac puncture every 2 weeks. The pooled blood was mixed with 3.8% citrate at a ratio of 1:4, then centrifuged and the supernatant plasma removed and stored at 4°C until used. All the plasma was filtered through glass wool before use and no plasma was used which was more than 4 days old. The method and chemicals used for studying the effects of histamine and the antihistamines on coagulation were the same as previously described for the dyes(4). Histamine and the antihistamines were dissolved in physiological saline to give final concentrations of 10, 25, 50, 100, 250, 500, 750, and 1000 μg per 0.1 cc. Six separate determinations were made with each concentration of drug, with and without heparin 0.1 μg (0.12 Toronto Units) in the system. The following antihistaminic drugs were investigated: diphenhydramine, tripelennamine, phenazoline, pyranisamine, thonzylamine, phenindamine, Tagathen, metaphenilene,

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TABLE I. Typical Effects of Antihistamines on Rabbit Plasma Coagulation.

Drug	Plasma	Control value	Conc. in μg/0.1 cc 10	25	50	100	250	500	750	1000
Tripelennamine	Normal	66*	65	65	67	65	70	67	70	67
	Heparinized	221	213	220	205	216	201	158	131	108
Phenindamine	Normal	60	60	60	64	69	71	106	212	276
	Heparinized	227	218	208	204	165	86	109	213	274
Thonzylamine	Normal	59	60	59	59	57	57	57	56	57
	Heparinized	174	170	163	162	167	170	158	147	150

* Avg values of coagulation times in seconds.

Doxylamine, Phenergan, methapyrilene, Thenfadil, Chloroprophenpyridamine, Di-Paralene, chlorocyclizine, pyrathiazine, Anthallan, p-Fluorobenzyl D.P.E., P.D. Co. AH-853, 194-B, Ambodryl, Bristol C-5581H, No. 204 and Foralamin. The Beckman pH-meter was used to determine the pH values of both histamine and the antihistamines at their highest concentration (1000 μg/0.1 cc). Control evaluations using hydrochloric, fumaric, maleic and succinic acids were made at the following pH's: 2, 3, 4, 5, and 6.

Results. The protocol of typical experiments showing the effects of the various antihistamines on normal and heparinized rabbit plasma is given in Table I. Fig. 1 illustrates the typical patterns of response obtained with the various antihistaminics when they are grouped according to the effect produced. The heparin inactivation observed at the lower doses is probably due to the alkaline nature of the antihistaminics and their combination with the acidic heparin molecule. Control evaluations with the various acids showed that the coagulation system, with or without heparin, was unaffected except at pH 2. At this value the systems became incoagulable except in the case of hydrochloric acid which caused the plasma proteins to precipitate.

Comparison of the effects of the various compounds upon normal plasma reveals that histamine, Anthallan, chlorocyclizine, Di-Paralene, Foralamin, Ambodryl, Phenergan, Tagathen, C-5581H and phenindamine have critical levels above which they act as anticoagulants. However, such is not the case with pyrathiazine, 204, PD-AH-853, p-Fluorobenzyl D.P.E., 194-B, methaphenilene, pyranisamine, tripelannamine, phenazoline, diphenhydramine, chloroprophenpyridamine, Doxylamine, Thenfadil, methapyrilene, and thonzylamine. The latter 3 compounds are unique among the compounds investigated in that they have no effect on either normal or heparinized plasma. Pyrathiazine, phenazoline, 204, diphenyhydramine, methaphenilene, P.D. AH-853, 194-B, p-Fluorobenzyl D.P.E., tripelennamine and pyranisamine have critical levels for exerting their maximum antiheparin action and above those concentrations no further inactivation results. Critical heparin inactivation concentrations, above which an anticoagulant effect is observed, occur with histamine, Anthallan, Ambodryl, chlorocyclizine, Di-Paralene, chloroprophenpyridamine, Phenergan, doxylamine, C-5581H, Tagathen, Foralamin and phenindamine.

Discussion. Our results indicate that both histamine and the antihistaminic drugs, with the exception of Thenfadil, methapyrilene and thonzylamine, are capable of inactivating heparin but the quantities required for complete inactivation can only be attained at toxic levels insofar as human dosage is concerned. The inactivation of heparin may be due to the chemical combination of the basic antihistaminic molecule and the acidic heparin molecule. However, one should not overlook the possibility that both histamine and the antihistamines could increase platelet disintegrative activity producing a more rapid release of the platelet thromboplastin, such as has been postulated by Butler *et al.*(5). His results with platelet-free and platelet-rich dog plasma of animals receiving increasing doses of histamine show a similarity to the results

5. Butler, S., Hall, F. R., and Sanford, H. N., *J. Lab. Clin. Med.*, 1950, v36, 710.

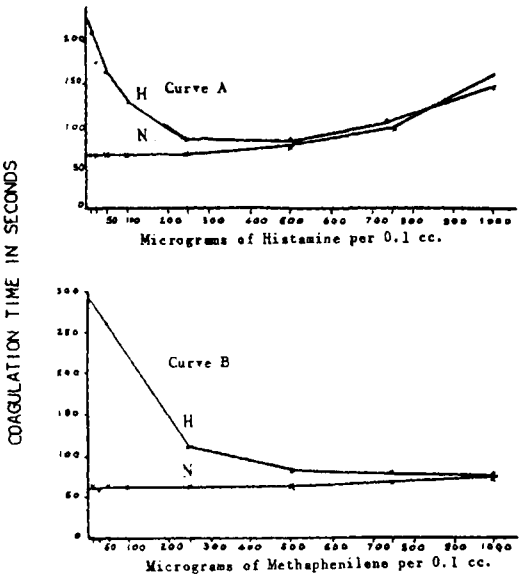

FIG. 1.

Effect of drugs on normal and heparinized rabbit plasma.

H = heparinized plasma
N = Normal plasma

Curve A—Histamine, Phenergan, Tagathen, Ambodryl, Anthallan, chlorocyclizine, Foralamin, Chloroprophenpyridamine, Doxylamine, phenindamine, C-5581-H.

Curve B—Methaphenilene, pyranisamine, tripelennamine, phenazoline, diphenhydramine, PD-AH-853, 194-B, p-fluorobenzyl D.P.E., Pyrathia zine No. 204.

obtained by us using rabbit plasma. However, the concentrations of added thromboplastin and platelets in our experiments were the same in the normal and the heparinized plasma so that any increase in thromboplastin due to platelet disintegration should be reflected in a decreased coagulation time in the normal plasma. Inasmuch as this did not occur, it is probable that the mode of action of both histamine and the antihistaminics as heparin inactivators is by direct chemical combination similar to that observed with the dyes(3,4).

Summary. The effects of histamine and a large number of antihistaminic drugs upon the coagulation of normal and heparinized rabbit plasma have been investigated. It has been established that the amount of each compound required for an antiheparin effect is critical. At concentrations below the critical level the drugs have little or no effect upon the coagulation system studied. At concentrations above this level several of the antihistaminics as well as histamine produced a progressively increased incoagulability of the system. This anticoagulant effect is related to concentration and the action is on some substance other than heparin. Thenfadil, methapyrilene and thonzylamine had no effect upon normal or heparinized plasma. Of all the compounds tested, 194-B, methaphenilene, pyranisamine, 204, phenazoline, tripelennamine, diphenhydramine, pyrathiazine, P.D. AH-853 and p-Fluorobenzyl D.P.E. gave the best results in that, upon reaching the critical concentration for heparin inactivation, they did not become anticoagulants themselves. It has been postulated that the antiheparin activity of these compounds is due to their direct combination with heparin.

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