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Abstract

Compound 48/80, sinomenine, tween 20 and polyvinylpyrrolidone (PVP) were injected intravenously to dogs, in doses producing similar degree of profound hypotension, and changes in the plasma histamine content and coagulation time were followed on the blood from the femoral artery. After the injection of 48/80 or sinomenine plasma histamine rose rapidly and markedly, attaining its maximum within 2 minutes, but the increase was rather of a short duration. In contrast, after the injection of tween 20 or PVP a less marked increase in plasma histamine developed more slowly, but lasted longer. The blood coagulation time was prolonged in all the cases injected with 48/80, and occasionally with sinomenine. Both beginning and recovery of the prolongation of blood coagulation time were sluggish as compared with the changes of plasma histamine. Tween 20 and PVP did not induce any detectable change of the blood coagulation time. These data were discussed with reference to the sites of action of different histamine releasers.

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PLASMA HISTAMINE AND COAGULATION TIME OF THE BLOOD IN DOGS AFTER ADMINISTRATION OF DIFFERENT HISTAMINE RELEASERS

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Both heparin and histamine are now known to be concentrated in the granules of mast cells. Joint release of these two substances has been shown in the dog during anaphylactic or peptone shock (1—3) and also after injection of a histamine liberator, compound 48/80 (4). But in other animals, such as rats, guinea pigs and rabbits, it has not been possible to detect liberation of heparin coincident with the release of histamine in anaphylaxis or after injection of histamine releasers including compound 48/80 (5—7). RILEY (7, 8) explained these discrepancies as follows : In dogs these histamine releasers affect mainly the liver mast cells and heparin is released therefrom into the hepatic lymphatics, entering by way of the thoracic duct into the general circulation, while in other species heparin is hardly released in the liver but solely liberated from mast cells in other tissues, where the metachromatic material (heparin) is disposed of locally by macrophages and fibroblasts and/or adheres to adjacent connective tissue elements, while some may be bound by the basic histamine liberator itself.

It has previously been demonstrated in this laboratory that in dogs under anaphylactic shock (9) as well as under the effect of peptone (10) or compound 48/80 (11), histamine was released predominantly from the liver, while after injections of polyvinylpyrrolidone (PVP), tween 20 (11) and sinomenine (10, 11), it was released in a larger proportion from the skin rather than the liver. If RILEY's hypothesis is reliable, in this animal those histamine releasers which deplete histamine predominantly in the tissues other than the liver, should hardly increase heparin in the plasma even though plasma histamine might increase.

The present paper deals with the effects of different histamine releasers mentioned above upon the blood coagulation time in relation to the plasma histamine level.

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MATERIALS AND METHODS

Injection of histamine releasers: Dogs, weighing 7—16 kg in either sex, were anesthetized by intravenous injection of 35 mg/kg of pentobarbital sodium. The blood pressure from a carotid artery was recorded on a smoked drum. Into the femoral vein, 0.7—1 mg/kg of compound 48/80, 3 mg/kg of sinomenine hydrochloride, 30—50 mg/kg of tween 20, and 500 mg/kg of polyvinylpyrrolidone (PVP) were injected. These dose levels caused 70—80 per cent fall of the arterial blood pressure. The histamine releasers were dissolved in 0.9 % saline solution to make the concentrations of 0.1, 0.3, 5, and 25 per cent respectively in mentioned order, and injected in 30 seconds, except PVP which was given in 5 minutes.

Determination of plasma histamine: Blood samples for determinations of plasma histamine and coagulation time were obtained in a same syringe from the right femoral artery in which a blunt 16-gauge needle was inserted and fixed. Syringes and needles had been silicon-treated. Four and a half milliliters of the blood were mixed gently with 0.5 ml of 3.8 per cent sodium citrate and centrifuged to separate plasma. Plasma histamine was extracted by CODE's method (12) and assessed on atropinized guinea-pig ileum.

Determination of blood coagulation time: Blood coagulation time was determined on the arterial blood. The method was practically the same as that of LEE and WHITE (13) except we used the arterial blood. The time at which the blood was withdrawn was noted as accurately as possible. One milliliter of the blood was transferred from syringe to a small glass tube 9 mm in diameter, which had previously been rinsed out in normal saline solution. The tubes were placed in a constant temperature bath at 25°C, and then rotated endwise every thirty seconds. That point at which the blood no longer flowed from its position but maintained its surface contour when inverted was taken as the end-point. When the coagulation time was over 10 minutes, the intervals of observation were lengthened according to the degree of the prolongation. This test was usually duplicated with another one ml of the same blood sample and mean of the two determinations was adopted. Care was taken to exclude air bubble.

Drugs: Compound 48/80 was kindly supplied by Dr. J. J. BURNS, Burrough Wellcome Co., Tuckahoe, New York. Sinomenine hydrochloride (Shionogi Pharmaceutical Co., Osaka), tween 20 (Meito-sangyo Co., Nagoya), and polyvinylpyrrolidone (PVP, average mol. wt. 30,000; Ono Pharmaceutical Co., Kobe) were also donated by the courtesy of respective company. Other chemicals used were obtained from standard commercial sources.

RESULTS

Compound 48/80. The representative effects of this compound are graphically shown in Fig. 1. In this dog administered 1 mg/kg of this compound, the carotid pressure showed a rapid and profound fall beginning 15 seconds after the start of injection and reached the maximal fall of 82 per cent within about two minutes. Thereafter, it rose only slightly,

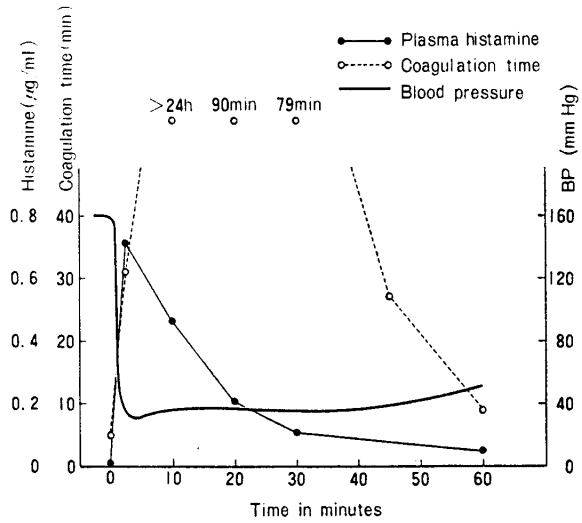

Fig. 1. Effects of compound 48/80 (1 mg/kg) on the arterial blood pressure, and the plasma histamine level and blood coagulation time of arterial blood. Dog, 9.0 kg male

lasting this state for about 40 minutes, then tended to recover. The plasma histamine level which was 0.005 μg/ml before injection markedly increased. As measured two minutes after the beginning of injection it showed the maximum of 0.72 μg/ml, and decreased rather rapidly until 20 minutes and thereafter fell down gradually. The increase of histamine was halved within 15 minutes after the injection, and 60 minutes later it was almost within the normal range. In this case the plasma histamine was not measured earlier than two minutes after the start of injection but in another case the maximum of 1.2 μg/ml was reached one minute after the beginning of the injection (Table 1).

Prolongation of the blood coagulation time was already noticed in the sample taken at two minutes after the injection, clotting by 32 minutes, and the blood obtained 10 minutes after was incoagulable for 24 hours. Since the sample 20 minutes after the injection clotted by 90 minutes, occurrence of the maximum prolongation was considered to be around 10 minutes after the injection. This time was much slower than appearance of the peak of plasma histamine. The recovery of coagulability was also slow, attaining not ealier than 60 minutes.

Tween 20. In one of the representative cases shown in Fig. 2, the injection of 50 mg/kg of this compound caused a steep fall of the arterial blood pressure after a slight transient descent immediately after the injec-

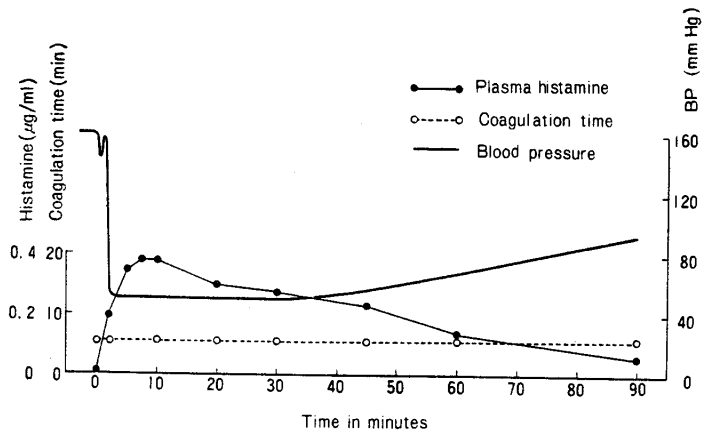

Fig. 2. Effects of tween 20 (50 mg/kg) on the arterial blood presure, and the plasma histamine level and blood coagulation time of arterial blood. Dog, 15.6 kg male

tion. This fall in the pressure reached its maximum of 69 per cent two minutes after the commencement of injection. This level persisted for about 30 minutes and tended to recover slowly. The rise in plasma histamine level was less marked and slower than that of compound 48/80, reaching its maximal level of 0.38 μg/ml 7.5 minutes after the injection. Its recovery was very slow, maintaining one half of the maximum level even at 55 minutes after the injection. There could be recognized no prolongation of blood coagulation time in any sample taken in period of 1.5 hours after the injection.

PVP. Fig. 3 illustrates the representative case of this compound. The arterial blood pressure began to fall 1.5 minutes after the start of injection of 500 mg/kg of PVP and it fell down 48 per cent at the termination of 5-minute injection, reaching the maximal fall of 69 per cent one minute later. In this case the blood pressure once appeared to recover but it began to fall again around 60 minutes and the animal died 77 minutes after the injection. The effects on the plasma histamine and on the blood coagulation time resembled those of tween 20. The plasma histamine reached its peak of 0.36 μg/ml 5 minutes after the start of injection, and it declined to the preinjection level very slowly, recovering to one half of the maximal level at 45 minutes after the injection. There was no prolongation of the blood coagulation time at least for the period of 60-minute observation.

Sinomenine. In one case shown in Fig. 4, the intravenous injection of 3 mg/kg of sinomenine caused a rapid and profound fall of arterial pressure beginning 30 seconds after the start of injection. Such a delayed depressor

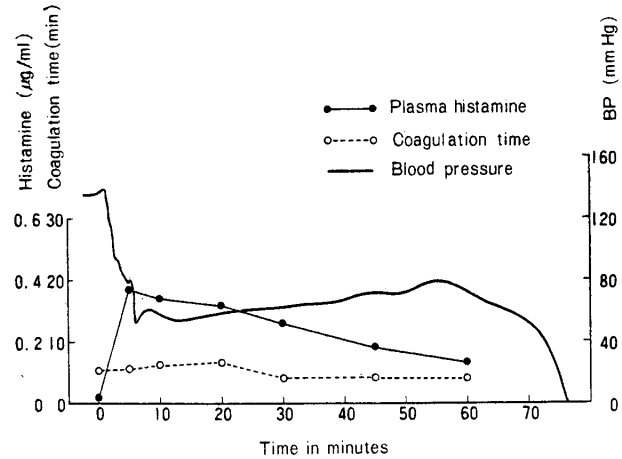

Fig. 3. Effects of PVP (500 mg/kg) on the arterial blood pressure, and the plasma histamine level and blood coagulation time of arterial blood. Dog, 8.8 kg male

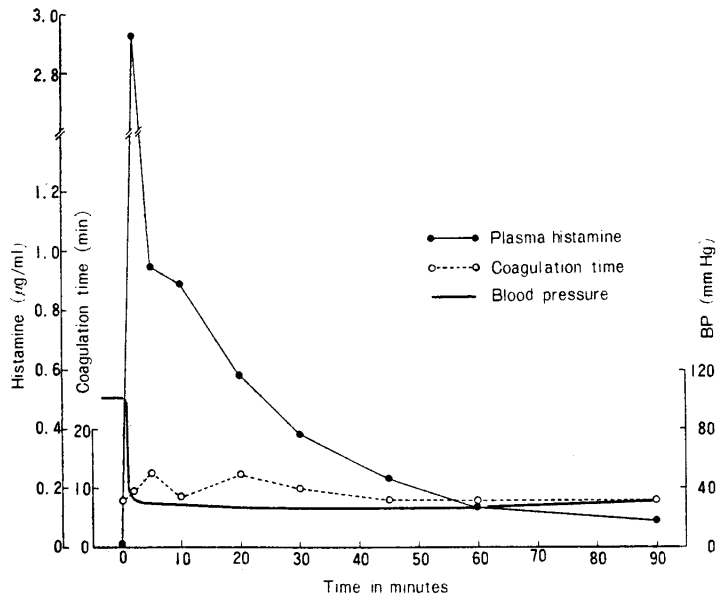

Fig. 4. Effects of sinomenine hydrochloride (3 mg/kg) on the arterial blood pressure, and the plasma histamine level and blood coagulation time of arterial blood. Dog, 9.6 kg female

response with this histamine liberator has already been reported by MAYEDA (14). The maximal fall of 76 per cent was reached 1.5 minutes later. The blood pressure, thereafter, maintained this low level and re-

covery was extremely slight even at 90 minutes. The plasma histamine level showed a rapid and marked rise, attaining to the maximal level of 2.9 μg/ml two minutes after the beginning of injection. This rise was transient, falling to 0.93 μg/ml at 5 minutes, and thereafter the recovery ensued rather slowly. In this case there could not be recognized any appreciable delay in the coagulation time.

Fig. 5 shows another case which was administered the same dose of sinomenine but responded with a much severe shock. The blood pressure fell abruptly, reaching its maximal descent of 91 per cent 10 minutes after

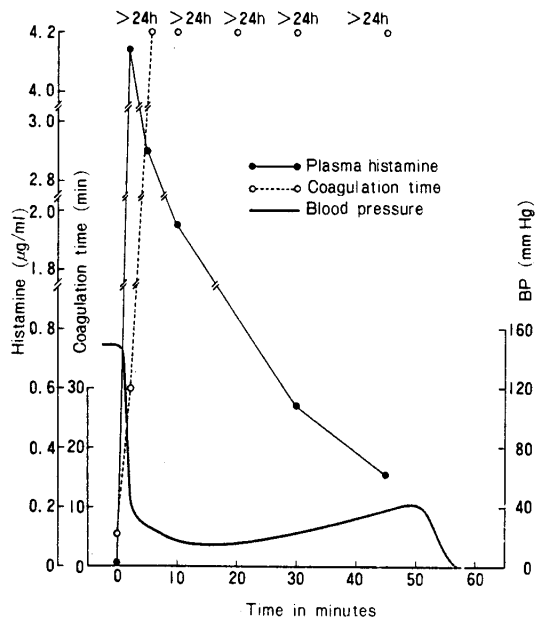

Fig. 5. The same as Fig. 4, but a case showing unusually severe reactions to sinomenine hydrochloride 3 mg/kg. Dog, 11.2 kg male

the injection. Although once it turned to recover around 50 minutes, it fell again and finally the animal died other 5 minutes later. The rise in the plasma histamine was extremely marked, coming up to the maximal level of as high as 4.15 μg/ml two minutes after starting injection. This is the highest histamine level attained throughout the present experiments. This high level turned down rapidly at first and then slowly, still showing a value of 0.32 μg/ml 45 minutes later.

In this case the blood coagulation time was markedly prolonged. The blood sample obtained two minutes after the injection clotted by 30 minutes,

but the samples obtained 6, 10, 20, 30 and 45 minutes after the injection all remained incoagulable for over 24 hours.

Comparison of the effect of each histamine releaser. Each of these four histamine releasers was repeatedly tried on 3—4 dogs and the results are summarized in Table 1. The table clearly indicates the characteristics of each histamine releasers. Despite the doses injected were such as to induce profound hypotension of comparable depth, the degree of change in the plasma histamine or prolongation of blood coagulation time was not the same in each histamine releaser. In doses used, sinomenine raised plasma histamine level most remarkably (max. 2.8 μg/ml, av.). This effect was followed by compound 48/80 (max. 0.93 μg/ml, av.), and the effects of PVP and tween 20 were much weaker (max. both 0.24 μg/ml, av.). The rises of plasma histamine induced by compound 48/80 and sinomenine were very rapid, reaching the maximal height within two minutes ; and it took only 7.3 and 5 minutes in average, respectively, to fall down to their half levels. In contrast, the rises of plasma histamine induced by PVP and tween were much slower, reaching the maximum by 5—10 minutes after the

Table 1. Flasma histamine content and coagulation time of the arterial blood after intravenous injection of different histamine releasers

Histamine releaser	No. exp.		Plasma histamine (μg/ml) and coagulation time (min)		Time (min)* of max. plasma histamine and coagulation	Half life (min)	Max. fall of blood pressure in 30 min (%)
			Before shock	After shock			
Compound 48/80 0.7—1 mg/kg	3	Histamine	0.018 (0.005—0.04)	0.93 (0.72—1.2)	1, 2†, 2†	7.3 (3—15)	79 (72—84)
		Coagulation time	6 (1—7)	> 24 hr	8.3 (5—10)	—	
Tween 20 30—50 mg/kg	4	Histamine	0.01 (0.01—0.01)	0.24 (0.17—0.38)	7.0 (5—10)	50 (40—60)	75 (69—83)
		Coagulation time	4.2 (3.5—5)	no increase	—	—	
PVP 500 mg/kg	3	Histamine	0.027 (0.005—0.02)	0.24 (0.18—0.36)	6.7 (5—10)	50 (45—60)	70 (65—80)
		Coagulation time	4.5 (3.5—5)	no increase	—	—	
Sinomenine HCl 3 mg/kg	3	Histamine	0.018 (0.01—0.02)	2.8 (1.3—4.2)	2†, 2†, 2†	5 (4—9)	82 (77—90)
		Coagulation time	5.7 (5.5—7.5)	>24 hr in one case‡, no increase in other two	>5 in one case‡	—	

* Time from the beginning of injection.
† Not determined earlier than 2 min.
‡ Dead 55 min after the injection.

beginning of injections and also required a longer period for the recovery, taking about 50 minutes to drop to the half level of the maximum.

Of these four compounds it was only compound 48/80 that invariably induced the prolongation of blood coagulation time. With sinomenine, tried on three dogs, the prolongation was observed only in one case, which suffered a fatal shock and attended with an exceptionally marked increase in plasma histamine level, and in other two cases, which survived shock, no prolongation could be detected while they still showed considerable elevations of plasma histamine more markedly than in the cases of compound 48/80. In all the cases treated with PVP and tween 20 there could not be recognized any prolongation of the blood coagulation time. As is obvious from Table 1 and also from Figs. 1 and 5, either with compound 48/80 or sinomenine, the prolongation of coagulation time was slower in onset but longer in lasting as compared with the elevation of plasma histamine level.

DISCUSSION

NISHIYAMA, TASAKA and IRINO (11) reported that compound 48/80 liberated histamine from the liver and skeletal muscle of the dog in a higher proportion than the skin while tween 20 and PVP depleted predominantly the skin of histamine. As to sinomenine, these authors confirmed the earlier finding of MAYEDA (10) that histamine was released at higher percentage from the skin, but still a considerable amount of histamine was also lost from the liver. Later, KAWAMOTO (15) substantiated these findings by histological observations of mast cells in tissue sections, which revealed that under the effects of these compounds, the relative intensities of the disruption of mast cells in the liver and skin paralleled well with the rate of histamine release as reported by NISHIYAMA *et al.* (11).

In the present experiments, when these four histamine releasers were injected intravenously into dogs in doses sufficient to induce a severe hypotension, it was only compound 48/80 that we could always observe a prolongation of the blood coagulation time. In one case out of three of sinomenine such a prolongation was encountered, but this may be rather unusual since in this case the animal was highly sensitive, responding with a fatal shock and an extremely high increase in plasma histamine. Neither tween 20 nor PVP did cause any prolongation of coagulation time in all three or four cases. These observations suggest that it is the release of histamine from the liver but not from the skin or other tissue that is causally related to occurrence of the prolongation of blood coagulation

time.

From his histological observations, RILEY (16) inferred that, when histamine is liberated from mast cells into the connective tissue, the tissue becomes flooded by protein-rich lymph which seeps through the walls of vessels rendered more permeable by histamine and thus possibly results in the activations of the fixed connective tissue cells to put their phagocytic potentialities in motion so that heparin or heparin-containing granules discharged from the mast cells are taken up by these cells. This may be conceivable for the most connective tissues other than the liver since in those tissues a majority of mast cells are not distributed so closely to the vascular endothelium as in the liver. It is only in the dog that we can observe a diminution of blood coagulability or an increase in plasma heparin content during anaphylactic shock or after the injection of histamine liberators. It is known that the main site of histamine release in peptone shock (10, 17) and anaphylactic shock (9, 18) in dogs is the liver, and also that in the hepatectomized dogs peptone shock (19) and anaphylactic shock (20) do not liberate heparin into the circulating blood. All these evidences collectively indicate that the mast cells in the liver are the only source of the plasma heparin of this animal responsible for the prolongation of blood coagulation time.

Mast cells are abundant in the dog liver, which are located preferentially along the hepatic venous tributaries, adhering to the endothelial lining of the vessels (15, 21). Another characteristic feature of these veins is the presence of periodically arranged constrictor muscles (21, 22, 23) which are sensitive to histamine (24). Therefore, when these mast cells are disrupted, histamine released may rapidly diffuse out into the hepatic venous blood and constricts these sluice musclatures, leading to an elevation of the intrasinusoidal pressure, which facilitates plasma transudation, through the capillary walls rendered more permeable by histamine, into the perivascular lymphatics connecting to the thoracic duct. This is evidenced by the facts that in dogs the lymph flow from the thoracic duct is markedly accelerated after the intravenous injection of histamine (25) or histamine liberators (14, 26), and this acceleration is largely prevented by the previous ligation of the periportal lymphatics (25, 26). Since histamine is easily diffusible, and its release by basic liberators is known to be of very rapid process, as will be discussed later, it is easily conceivable that a major quantity of histamine liberated from mast cells of the wall of suprahepatic veins enters into blood therein, while a small portion is washed away into the thoracic duct lymph as demonstrated by OHKURA (27) in dogs subjected to peptone- and anaphylactic shock. But, heparin released may be taken away to the

lymphatic drainage with an aid of an increased formation of lymph because of a rather slow rate of release and difficulty of diffusing out owing to its large molecular size.

The prolongation of the blood coagulation time observed with 48/80 as well as with sinomenine began more slowly but lasted longer than the rise in the plasma histamine. This may easily be accounted for by the difference in the main paths through which they enter into the blood circulation. WHITE and WOODARD (19) reported that in dogs during anaphylaxis or peptone shock the heparin concentration in the thoracic duct lymph was higher than in the arterial or hepatic venous blood. These authors explained the slow elevation of plasma heparin is due to a relatively gentle flowing stream in the thoracic duct rather than the lengthening of circulation time of the liver as conjectured by JAQUES and WATER (20). This is a confirmation of GLEY and PACHON (28) and CHITTENDEN, MENDEL and HENDERSON (29) who pointed out the important role of the thoracic duct for the pathway of liver heparin to enter into blood circulation.

Tween 20 and PVP raised plasma histamine but this effect was not so drastic as with 48/80 or sinomenine and its time course was much slower. A very rapid increase in the plasma histamine after the administration of compound 48/80 may be due to the anatomical situation of mast cells peculiar to the canine liver. But, this does not fully explain a sharp rise of plasma histamine observable after sinomenine which affects predominantly the mast cells in the skin rather than in the liver. There are many indications that the histamine release by 48/80 and also by sinomenine is a very rapid process in the whole animal of the dog (4, 14) and rat (30) as well as in the isolated mast cells of rat (31, 32), while the release by tween 20 has been demonstrated to occur rather slowly and to continue for a longer duration in the rat (30). Therefore, differences between these releasers in the rapidity and duration of the increase in plasma histamine level may have a greater bearing on the release mechanism. The questions as to why the effect of a single histamine-releasing agent on mast cells differs with the sites, even in the same animal, and also why such a pattern of histamine release varies between different releasers, still remain to be unravelled.

SUMMARY

Compound 48/80, sinomenine, tween 20 and polyvinylpyrrolidone (PVP) were injected intravenously to dogs, in doses producing similar

degree of profound hypotension, and changes in the plasma histamine content and coagulation time were followed on the blood from the femoral artery.

After the injection of 48/80 or sinomenine plasma histamine rose rapidly and markedly, attaining its maximum within 2 minutes, but the increase was rather of a short duration. In contrast, after the injection of tween 20 or PVP a less marked increase in plasma histamine developed more slowly, but lasted longer. The blood coagulation time was prolonged in all the cases injected with 48/80, and occasionally with sinomenine. Both beginning and recovery of the prolongation of blood coagulation time were sluggish as compared with the changes of plasma histamine. Tween 20 and PVP did not induce any detectable change of the blood coagulation time. These data were discussed with reference to the sites of action of different histamine releasers.

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