HISTAMINE EFFECT AND TROMBIN INACTIVATION

BY

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Pharmacological investigations in connection with histamine have been carried out for a long time in order to establish, whether histamine had any influence on blood-clotting. The investigations, however, undertaken on experimental animals have led to very contradictory results.

Some authors, as for instance *Dale* and *Laidlaw*, (1910) did not observe any changes, whereas *Barger* and *Dale* (1922) found later, that histamine was able to produce intravasal blood-clotting. In experiments on dogs *Biedl* and *Kraus* (1912) found a clotting inhibitory effect, whereas Modrakowsky 1912) found an accelerating one. In the more recent literature on this subject there are not te be found either uniform- or satisfactory dates. (f. i. Guggenheim, 1940). On investigations on humans *Dzsinich* and *Pély* (1934) made the observation, that blood-clotting at the highest degree of the histamine effect is very much protracted, but that it turns normal again after discharge of the reaction. According to *Howell* (1924) the quantity of heparin — the physiological substance of the organism preventing blood-clotting — increases to a great extent at the end of the peptone-shock-effect in dogs.

According to him this effect has to be attributed to the protecting mechanism of the organism trying to eliminate the shock-producing substance from the body. It follows from the above-said that the difference between histamine effect and pepton-shock can be defined as follows: the clotting of the blood is uncertain at the effect of histamine, whereas it becomes definitely retarded as the effect of a peptone-shock.

Jancsó (1931) established that the primary condition and introduction of the accumulation of the reticuloendothel-system (RES) consits in the precipitation of the colloids circulating in the blood or brought into it in the course of the experiments — because there is fine fibrinnetwork formed around them, and accumulation is already brought about through the fibrin-colloid complex.

The further elucidation of the question has been considerably promoted by Jancsó's observation, (1947) according to wich histamine is to be considered as the activator and the directing hormone of the RES.

Thus, according to Jancsó, the preliminary condition of the accumulation of RES by the cells is that a colloid-fibrin precipitation has to be effected in the blood and on the other hand, that the stimulant of this accumulation in the organism is histamine.

In consequence we had to accept the data of the literature establishang the accelerating effect of histamine in blood-clotting which

permits to assume that appearance of histamine in the blood leads to the precipitation of fibrin. We tried to clear up in our researches the coming about and the mechanism of this process.

The investigations on thrombin inactivation served as a further help in our experiments. According to recent investigations the thrombin inactivating capacity of blood is a very significant factor from the point of view of coagulability, *(Lenggenhager* 1941, *Gerendás,* 1946 and 1948.) and the shift in the formation or disappearance of thrombin as related to one-another promotes or inhibits the process of fibrinprecipitation. It was observed, that in case of a thrombin injection into the bloodstream, the organism protected itself against intravasal clotting by increased inactivation. *(Gerendás* and *Csapó* 1948). In opposition to this theory, it could be supposed, that histamine effects just a contradictory influence on inactivator decreasing its activity and promoting the coagulability of the blood, as well as the appearance of intravasal fibrin traces.

It has to be emphasized, that we have already established in our previos experiments that decreased inactivation is generally followed by increased coagulability (a shorter clotting time) and increased inactivation by decreased coagulability (a longer clotting time). This can be easily understood, as by diminution of inactivation the formation of thrombin is facilitated, and it remains longer in an active condition which means that its efficacy is increased.

EXPERIMENTS

In the first part of our investigations we studied in invivo experiments the effects of histamine on thrombin inactivation of the circulating blood. In the second part of our experiments our observations were supported by experiments in vitro.

EXPERIMENTS IN VIVO

We used rabbits for our in vivo experiments. In the thrombin inactivation fests 1 ccm of blood was extracted from the vene of the ear, or of the opened thigh vene of the anaesthetised animal.

The blood was left to clot and centrifuged 20 minutes subsequently. We examined thereafter the thrombin inactivating capacity of the serum separated from the coagulum. The principle of the thrombin inactivating test consists in our method to add thrombin of an exactly known activity to the serum, and to observe the decrease of its activity during 10 minutes.

We used for our investigations mixtures of the following composition:

Incubating mixture at 20 C°

Serum of the experimental animal	0.4 ml
Distilled water	0.1 „
Standard thrombin solution	0.4 „

After stirring the incubating mixture, samples were taken from it in the subsequent 1, 2, 3, 5, and 10 minutes, and their activity was examined on the following clotting-test:

at 20 C°

Clotting test

Oxalat plasma of ox	0.1 ml
Distilled water	0.1 „
Sample from incubating mixture	0.1 „

We put the mixture serving for the determination of the clotting time into the cavities of a china dish. The occurrence of the clotting was established by the help of a small glass loop. We measured the time elapsing from the adding of the thrombin till the formation of the first massive fibrin clot with a stop-watch. As a result of inactivation we obtained gradually increasing clotting times. From the clotting times we defined by the help of an empiric relation, their corresponding thrombin-activity and calculated, base on the dates thus obtained, the factor of the velocity of reaction, according to the following formula:

$$k = \frac{1}{t} \cdot 2.3 \cdot \log \frac{a}{a-x}.$$

For further details as to this method we refer to our previous publications. (Gerendás, 1949, 1950.).

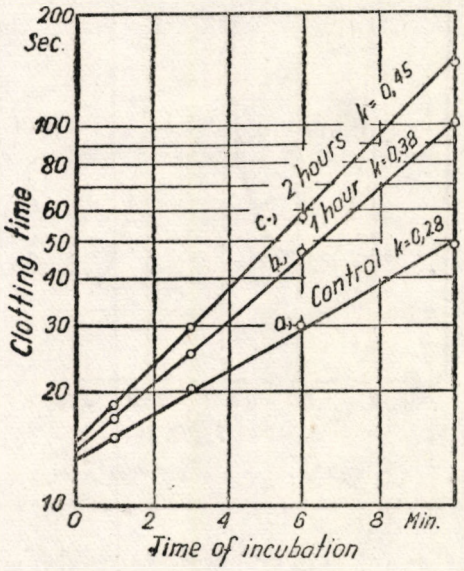

Figure No. 1. Change in the thrombin inactivating capacity of rabbit serum after intravenous infusion of histamine.

In one part of the experiments histamine was administered intravenously in infusions, in other cases by simple intravenous injections.

In case of infusions, 12mg of histamine dissolve in 12 ml. of distilled water were infused in a space of time of 20 minutes. Preseding the experiment, the thrombin inactivating capacity of the blood-serum of the rabbit has been established. The curve thus obtained (Figure No. 1, curve a) represent the control curve before the administration of

histamine. Then also measured the inactivation after the first and the second hour following to the administration of the histamine (Figure No. 1, curves *b* and *c*.)

The figure shows that the clotting times were prolonged i. e. the velocity of inactivation was increased. This phenomenon seemed to be contrary to the effect expected as it showed that histamine applied intravenously increased the effect of inactivation.

This phenomenon seems to be similar to that, described by *Gerendás* and *Csapó* (l. c.) occuring after the intravenous administration of thrombin. These authors found, that as the effect of the administra-

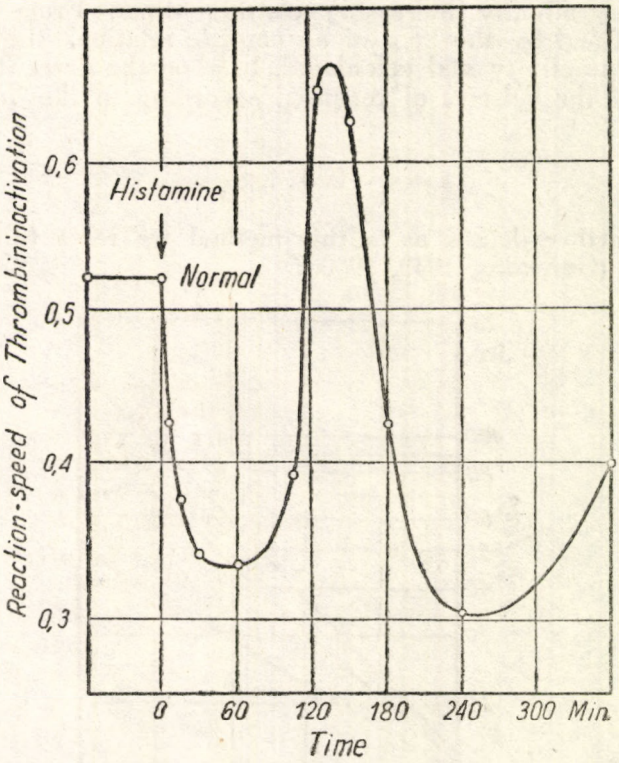

Figure No. 2. Change in the thrombin inactivating capacity of rabbit serum after an intravenous injection of 1.5 g of histamine.

tion of thrombin the organism in defence increased inactivation and retarded clotting. If increased clotting is effected by histamine, it can be just as well expected that the organism will eliminate it after a certain time, by some compensatory mechanism, and will even probably, overcompensate it. It seemed therefore probable that the primary histamine effect occurred during the hour following the puncture, and that have only observed a compensatory phenomenon instead. We therefore, repeated the experiment but instead of infusion we administred histamine intravenously (1mg per 1000 gr body weight), in 30 seconds.

Blood was drawn in the 3rd, 15th, 30th, 60th, 90th, 180th, 240th, and 360th minute after the injection and the thrombin inactivating ca-

pacity was determined by the above-described method. The reaction-velocity constants, calculated from these data, are shown on Figure 2. It can be observed that the administration of histamine is followed by a prompt decrease of the inactivation. This decrease of the inactivation continues for 60 minutes subsequently to the administration of histamine, then the speed of inactivation accelerates rapidly, and in the 100th minute it already exceeds the normal inactivation velocity. A maximum speed value is reached in about 130 minutes, which is followed again by a decrease of speed. The curve reaches, after several fluctuations, its normal value.

Also in case the animal was given a large dose of histamine, so that it perished in a histamine shock, thrombin inactivation of the blood-serum has been determined before the injection and after the death of the animal. We observed that the inaction of the later blood-sample was considerably weaker and that the coagulation of the blood tended decidedly towards the thrombotic direction.

We got similar results at the repetition of the experiments.

From the results of our investigations it becomes comprehensible why literary data about the effect of histamine on the blood-clotting are so contradictory. The authors were not aware of the fact, that they have been faced with phenomenon taking place periodically where it depended on the time sample-taking whether they obtained inactivation or increasing of the clotting.

It can be established as a consequence of the results of our experiments, that as the effect of histamine, the succession of two phenomena is determining the events taking place in the blood-stream. *The primary effect, — which lasts 0.5—1 hour — after the administration of histamine, is a decided thrombin inactivation, and a tendency of the coagulability of the blood towards increased clotting.*

The secondary effect, on the other hand, manifests itself in an increased thrombin inactivation, This can be considered as a compensatory activity of the organism, and leads to prevention of coagulation.

Our opinion concerning the observed phenomenon is resumed, as follows: The decrease of thrombin inactivation occurs in each case, when histamine gets suddenly, shock-like into the blood. In consequence of diminished inactivation the equilibrium of the coagulating system is altered, the quantity of thrombin increases, and fibrin is formed. Our investigations are thus explaining the observations of *Jancsó* (loc. cit.) as the fibrin generating in consequence of the above described process draws the alien colloids to be found in the circulation to itself, just as Jancsó has observed it in his experiments as the effect of histamine.

In consequence of this process however, the blood is getting into an over-coagulated condition and therefore the defensive phase is instantly setting in. Which increases inactivation and prevents thrombosis.

We however consider it possible that while the histamine takes such part in the introduction of accumulation, the coagulability of the blood is in certain cases directed in a too large degree into a thrombotic direction. This may lead not only to the precipitation of the alien colloids, but also to that of the thrombocytes, and may thus effect thrombosis.

The organism, tending constantly towards the maintenance of the inner equilibrium, is compensating the shift of the coagulation when it tends in a thrombotic direction. It is very probable that a greater quantity of heparin — the clotting inhibiting substance of the organism — is produced in these cases, and carried into the circulation. Histamine effect gets thus compensated by the heparine effect.

The above-said present a new aspect of RES accumulation, and thrombin is given also a part in its mechanism.

EXPERIMENTS IN VITRO

We carried out experiments in order to determine, wether the influence of histamine in increasing coagulation and inhibiting inactivation could also be demonstrated by experiments in vitro.

These experiments may be summarized in four points.

1. We investigated, whether histamine is influencing the clotting time of recalcinated rabbit-plasma containing oxalate. We used for the experiments rabbitblood, containing 2‰ of Natriumoxalate and from it obtained the plasma by centrifugating. The clotting times after the recalcination were obtained with the following combinations:

	I.	II.	
Rabbit plasma containing oxalate	0.1 ml	0.1 ml	
Distilled water	0.1 „	0.0 „	
Histamine of various cencentrations.	0.0 „	0.1 „	Water bath
m/40 CaCl solution	0.1 „	0.1 „	37 C°

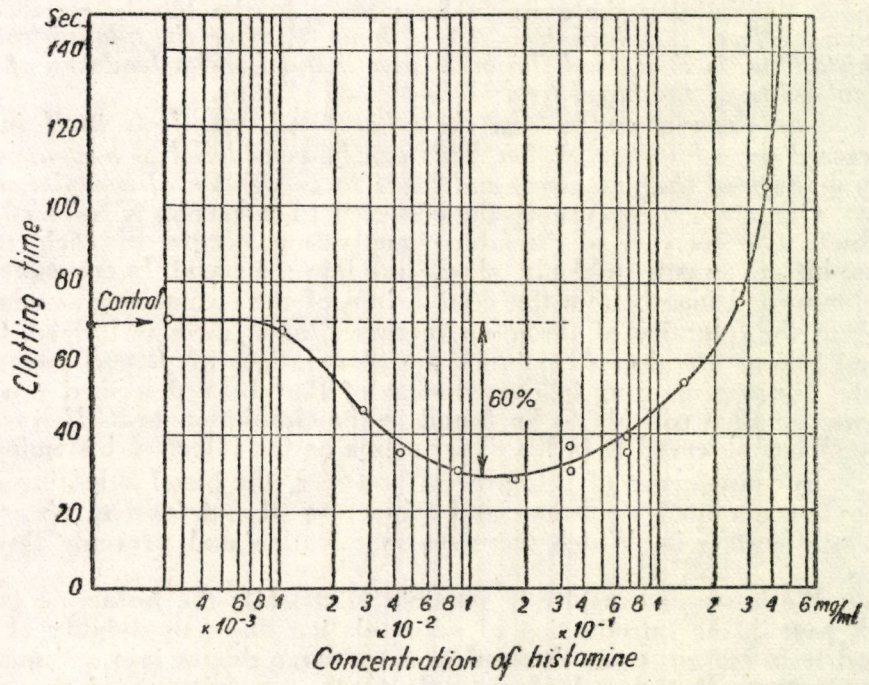

Figure No. 3. Changes in the clotting times of recalcinated oxalate plasma under the action of histamine.

By system No. 1. (control test, without histamine) the starting point of the curve has been established, whereas by system No. II-in using histamine solutions of various concentrations the dependence of the clotting time from the histamine concentration has been determined. (Fig. No. 3.). The histamine concentrations of the above systems are given in mg/ml.

No histamine effect has been observed at concentrations between 0,003—0,01 mg/ml. Beginning at a concentration of 0.01 mg/ml till 0,1 mg/l an increasing clotting-effect has been tested. (On the figure the clotting times get shorter). At a further increase of the concentration its accelerating effect on the clotting begins to decrease. From a concentration of 2,66 mg/ml of histamine a considerable increase of the clotting time could be observed, which means, a coagulation inhibiting effect.

It can be established from the results of these experiments that *histamine is effecting an accelarting influence on the clotting of recalcinated rabbitplasma at concentrations of 0,01—2,66 mg/ml* and there is to be observed a shortening of the clotting time of about 60% at the optimum concentration (0.1 mg/ml).

2. We examined subsequently, whether histamine is influencing the clotting time of the oxalated rabbit plasma, when coagulation is effected with thrombin.

The following combination was used to determine the clotting times:

	I.	II.
Rabbit plasma oxalate containing	0.1 ml	0.1 ml
Distilled water	0.1 „	0.0 „
Histamine sol. of various concentrations	0.0 „	0.1 „
Thrombin sol. of various concentrations 0.1	„	0.1 „

This series of experiments, as well as the further experiments were carried out at a room temperature (20 C°). Thrombin solutions in six different concentrations were used in the experiments. These solutions produced coagulation in the first series of experiments (control experiments, without histamine) in 15, 22, 30, 60, 100 and 150 secs, respectively. Subsequently the clotting times were determined in varying the concentration of the histamine solutions, and the curves of the coagulation were constructed thereafter based on these results. (Fig. No. 4.). The concentrations of histamine in the used conbinations are given in mg/ml.

The curves are very similar to those obtained at the recalcination tests. The concentration of the thrombin solution employed was proportionate to the clotting increasing effect of histamine which means the weaker the concentration of the applied thrombin solution, the less quantity of histamine was need to produce a clotting increasing effect. The increasing effect of histamine was already observed at the weakest concentration (=0,003 mg/ml), in which coagulation was effected in 150 secs. (Curve No. I). The optimum effect of this curve appears at a histamine concentration of 0,66 mg/ml. whereas the inhibiting effect of histamine starts from concentrations of 2,5 mg/ml. onwards.

At the other curves the effect of histamine could only be observed at greater concentrations.

In comparing the curves it can be established that the clotting increasing effect of histamine is most developped in curve No. I. (smallest thrombin concentration) where the clotting time diminishes by 66% at the optimum concentration.

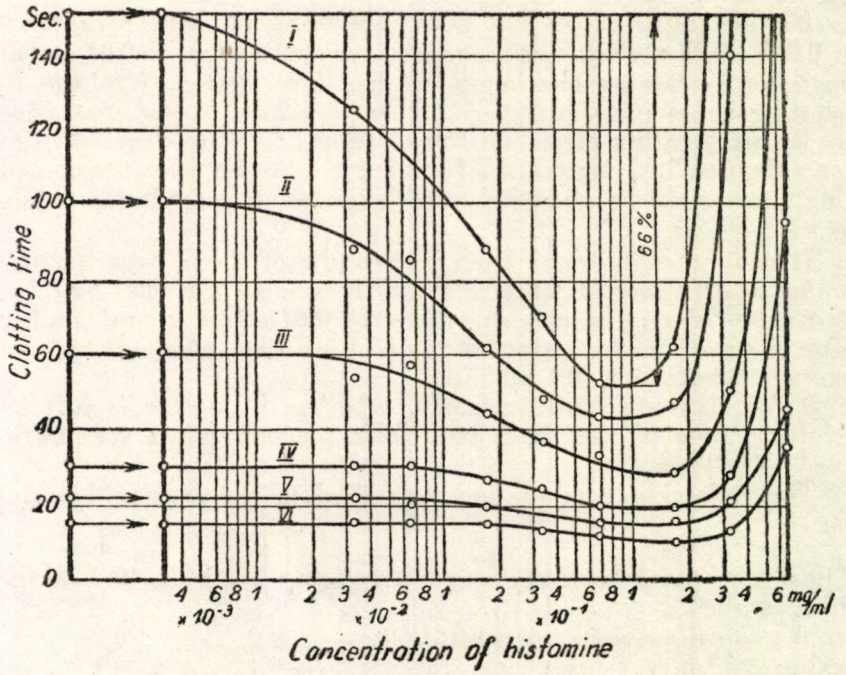

Figure No. 4. Changes in the clotting times at the effect of histamine on oxalate plasma clotted with thrombin.

3. After the accelerating effect of histamine on the coagulation of recalcinated plasma as well as at coagulation with thrombin was already established investigated subsquently, whether the accelerating effect of histamine appeared also in the clotting of pure fibrinogen by pure thrombin.

The following combination was used for the experiment:

	I.	II.
Fibrinogen (3 mg/ml	0.1 ml	0.1 ml
Histamine sol. in var. conc.	0.0 „	0.1 „
Distilled water	0.1 „	0.0 „
Thrombin sol. in var. conc.	0.1 „	0.1 „

The fibrinogen solution was prepared according to Laki's method. (Laki 1942). We prepared histamine solutions in concentrations of 0.0125, 0,05, 0,3125, 0,625, 1,25, 2,5 and 5,0 mg/ml. Thrombin solutions were chosen to clot system No. I in 13, 20, 30, 60, 120 seconds respectively. The data of the clotting are shown on Fig. No. 5.

The curves show that histamine has no clotting-accelerating effect in this system, but inhibiting effect at greater concentrations may be observed here too.

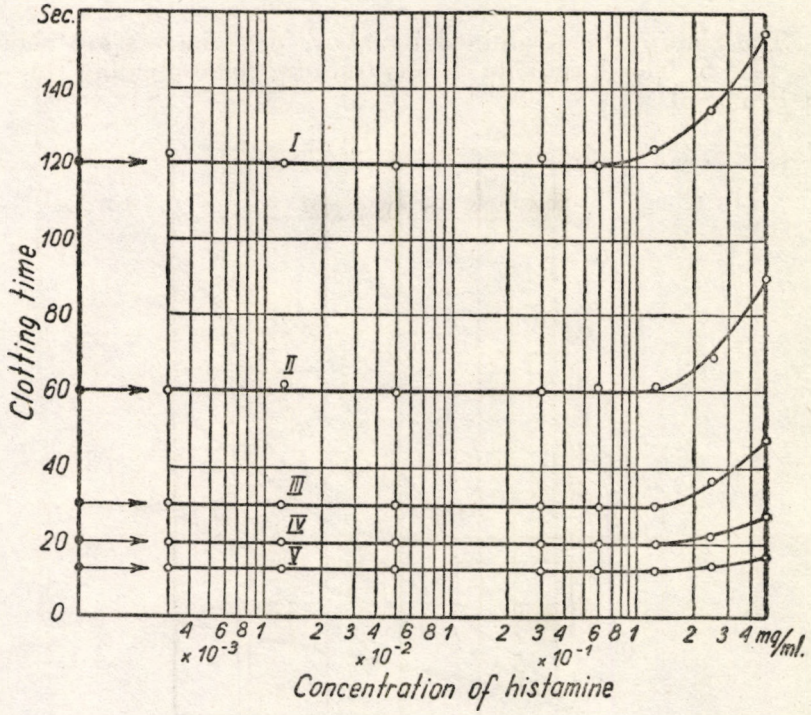

Figure No. 5. Changes in the clotting times effected by histamine on fibrinogen coaguluted with thrombin. Histamine has no accelating effect.

Thus we see that histamine in itself has no accelerating effect on the action of thrombin, and it may be concluded from the results of the experiments that for producing the accelerating effect of histamine some other factor or factors too are need in the plasma. There seem to be two possibilities: histamine has either an accelerating effect on the thrombin producing system, or it inhibits the action of the thrombin in activating system, Our subsequent experiments prove the later supposition.

4. We worked with the following combinations:

Inactivation of the serum without histamine (Control)

Serum. (ox)	0.4 ml
Distilled water	0.1 „
Stanard thrombin solution	04 „

Inactivation of histamine serum

Serum. (ox)	0.4 ml
Histamine sol. var. conc.....	0.1 „
Standard thrombin sol.	0.4 „

The activity of the samples was determined by the following test:

Fibrinogen solution ..... 0.1 ml.
Sample taken from mixture.. 0.1 „

The clotting times obtined by above experiments are shown on figure No. 6. The histamine concentrations corresponding to system given in mg/ml.

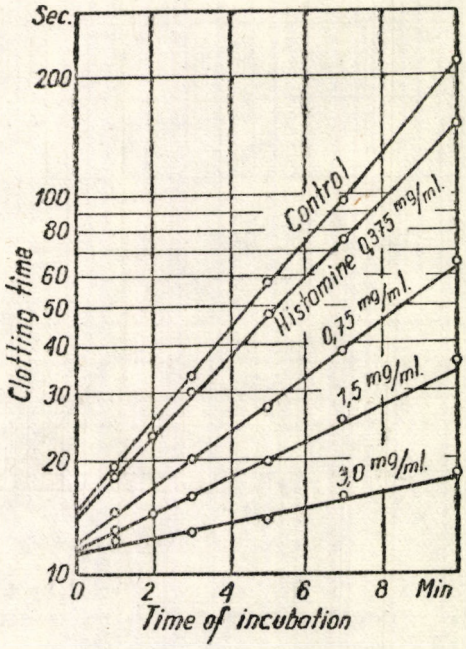

Figure No. 6. Decrease in the thrombin inactivating capacity of serum at the effect of increasing concentrations of histamine.

The curves are showing a diminishing inactivation in proportion to the increase of histamine concentration.

Histamine, consequently, reduces the velocity of the thrombin inactivating process. In order to elucidate this connection, we have calculated the velocity constant of the inactivating-reaction of the curves as obtained with each hisamine concentration. The changes of the k values thus obtained are shown on Fig. No. 7. It is evident from the curve that the inactivation inhibiting effect of histamine begins at a concentration of 0,1 mg/ml. and that greater quantities of histamine are inhibiting inactivation almost entirely.

From these experiments in vitro it may also be seen that histamine is producing its clotting-accelerating effect by inhibiting the action of the thrombin-inactivating system of the blood. Thus thrombin generating in blood, or added to it, remains in greater quantities and for longer periods active and may consequently better produce its effect.

We cannot however explain the fact that according to our experimental results, a larger concentration of histamine is needed in vitro, than in vivo. We have injected at the intravenous histamine administration. l/mg histamine per kg. of body weight, which corresponds approximately to a concentration of 0,01 mg/ml in the blood. This quantity of histamine reduces the thrombin-inactivation in the blood of the animal from $k=0.52$ to $k=0.33$. (Fig. No. 2.). The diminishing effect of histamine inactivation at in vitro experiments begins at a concentration of 0.1 mg/ml. only i. e. at an approximately tenfold concentration.

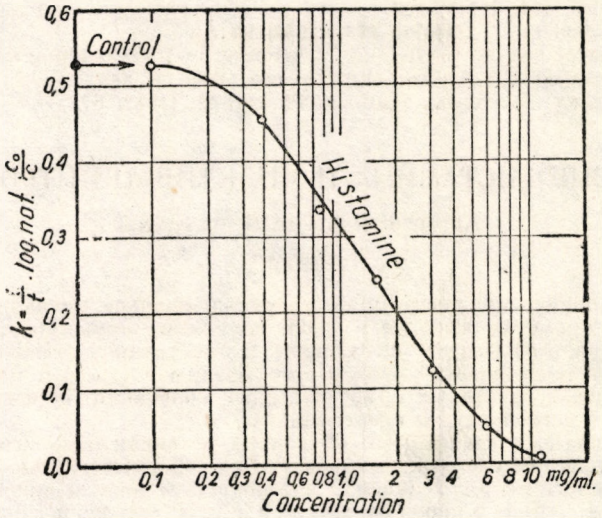

Figure No. 7. Diminution of the velocity constant of the thrombin inactivating reaction at the effect of histamine.

As an explanation of this difference it might be supposed, that activity of histamine is greater in circulating blood, than in serum, in which the humoral and cellular factors have already undergone a big change during the process of coagulation.

It might be further assumed as a consequence of our experimental results that histamine in itself is not responsible for the influence on the inactivation, but it liberates probably some substance or substances in the organism, producing a histamine like effect, or increasing the effect of histamine. This our opinion is based on the fact that the maximum effect of histamine is observed an hour after its administration, whereas the well known pharmacological effects of histamine manifest themselves already some minutes after injection. It might be consequently assumed that the effect of histamine on blood-coagulation is to a certain degree an indirect effect, and this explains also the big differences between the in vivo and in vitro experiments. In spite of these facts it seems beyond doubt that histamine exercises a significant effect on the thrombin inactivating capacity of the blood.

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ДЕЙСТВИЕ ГИСТАМИНА И ИНАКТИВАЦИЯ ТРОМБИНА

Автор: ГЕРЕНДАШ МАХАЙЛ

РЕЗЮМЕ

Мы установили, что гистамин внутривенно вначале уменьшает способность к инактивации тромбина сыворотки проб, взятых из крови. (способность крови к свертыванию растет.) Позднее способность к инактивации становится выше нормального уровня (способность крови к свертыванию опадает) и только несколько часов спустя, (после некоторых компенсаторных колебаний) кровь снова приобретает нормальную способность к свертыванию.

На основе вышеуказанных мы объясняем механизм накопления в ретикулоэндотелияльной системе следующим образом: Под действием гистамина освобождающегося в физиологических условиях, способность крови к инактивации крови падает, уровень тромбина в крови возрастет, а внутри сосудов возникает преципитат фибрина. Этот преципитат тянет к себе инородные коллоиды.

Мы установили также, с помощью опытов in citro что гистамин тормозит свертывание плазмы путем рекальцинации или же с помощью тромбина, а причиной этого действия является ослабление инактивации тромбина.