HISTAMINE AND HEPARIN ANTAGONISM IN VIVO AND IN VITRO

BY

M. GERENDÁS and I. CSEFKÓ

From the Hungarian Biological Research Institute, Tihany, Lake Balaton

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In case rats or mice are administered intravenously large quantities of colloid solutions, — and this colloid solution is sufficiently labile — the animals show simptoms similar to anaphylactic shocks. This condition was called by *Lumière* an anaphylactoid shock. *Jancsó* has dealt with this question more intensively later (1928), in connection with his chemotherapeutic investigations on salvarsan derivates, and demostrated that also in the case of salvarsan an anaphylactoid shock was caused by the precipitation of the drug in the inner bloodstream, a supposition, which he was able to prove subsequently through in-vitro experiments. He observed that arsenobenzol derivates causing a very strong shock and being very toxic in therapy precipitate in the moment of their mixing with blood-plasma, or eventually shortly after it. It has however been established at the same time that salvarsan derivates are accumulated in the reticuloendothel *(RES)* cells according to their degree of intravasal precipitation. Lately *Jancsó* (1947) has established the connection between *RES* accumulation and histamine effect. The characteristic of the Jancsó phenomenon consists in the fact that on the effect of histamine the vesselwall of the endothel is transformed into *RES* and becomes capable of adsorbing and retaining Indian ink whether histamine is rubbed into the skin or administered subcutaneously, intraperitoneally or intravenously. Jancsó concludes from this fact that the hormone of the *RES* which introduces the accumulation is: histamine.

We found in our own, earlier investigations concerning this subject (*Csefkó, Gerendás, Udvardy,* 1948) that as the effect of histamine administered intravenously, the thrombin inactivating capacity of blood is temporarily shocklike, diminished, which means, that the inner substrate of the blood is tending towards clotting. Whereas the latest investigations of Gerendás, Pálos and Csefkó (1948) have established, that the heparin contained in the organism plays an important part in the raising of thrombin inactivation.

Based on these results we began the investigation as to the nature of anaphylactiod shock and as to the connection between the effects of histamine and heparin. We chose Indian ink as model-colloid.

Jancsó in his paper calls our attention to the fact, which has been also observed by us in our previous investigations, that in the production of the Jancsó phenomenon only defectless Indian ink protected by gelatine, and causing no anaphylactoid shock may be

used. There are, very great differences between Indian inks: some are of very labile construction, forming immediately thrombosis in the blood thus killing the animal, and therefore unutilizable for our purposes. We found, however, after elaborate researches certain preparations which precipitate in the small lungvessels only in form of small particles. This precipitation is so small that it does'nt cause any grave symptoms on the animal: difficult breathing is to be perceived for some minutes only. This Indian ink is at a critical point between stability and lability, and has been used in our subsequent experiments.

Our experiments were carried out on rats. The injection was administered — for the purpose of indisturbed operation — under a light aether anaesthesis which lasted no longer than the injection itself.

a. In case the rat is administred per 100 g of its bodyweight 1 ml of Indian ink of 10%, the animal wakes easily from its torpor only its respiration being slightly increased. From the Indian ink, and which after 15 minutes may only be observed in the eyes, and disappears entirely in 45 minutes. The animal moves lightly, reacts vigopously to pain, and defends itself. After 45 minutes it is bled to death by throat cutting. At the dissection spleen and liver are blackcoloured, saturated wiht Indian ink, the lungs are of a lightgrey colour, and there are no inky thrombuses to be perceived anywhere else in the vessels. Other deformation are not observed.

b. In case the animals are administered three minutes before the above injection 2 mg of histamine per 100 g of body-weight intravenously, and thereafter given the Indian ink injection, they show at their awakening from the ether-torpor heavy, difficult respiration, they collapse, stay leaning to one side, and sometimes small spasms occur in their limbs. They do not move about actively, and do not defend themselves when pinched or seized. The initial grey colouring disappears only with great difficulty from the skin, and is still visible in the eyes after 45 minutes. At their bleeding to death — after 45 minutes — only very little blood is let out. At the dissection, the lungs are of a slate grey colour, the liver and spleen brownishblack, there is a great dilatation and stagnation in the big vessels of abdomen and intestines, and the Indian ink is to be found in the large bloodvessels in thrombotic clots. Consequently, the Indian ink causing no anaphylactoid shock is precipitated in the large vessels by histamine administered intravenously. There this precipitation effect thrombosis and general stagnation and in the moment of the administration of Indian ink the animals show symptoms of an anaphylactoid shock, whereas, the Jancsó phenomenon is characterized bq the non-thrombuslike intracellular accumulation of Indian ink in the post-and praecapillary vessels. The difference is based on the different stability of the administered Indian ink. The Jancsó phenomenon is only to be observed in the perithoneum, in the soft connecting tissue under the skin in some parts of the small venes of the skin and in the mesentherium.

c. In case the animals are given three minutes before the administration of histamine at least the threefold quantity of heparin, (at

least 6 mg of heparin per 100 g of bodyweight, dissolved in distilled water) and histamine in the above dosis is injected subsequently, we may observe, that after the receiving of Indian ink the animals wake easily from the ether-torpor, the grey colouring disappears very quickly (in 10 minutes) from their skins, and the eyes too become colourless in 20 minutes. There is no sign of an anaphylactoid shock on the animals, — as contrasted with the animals of group b. When bled, to death 45 minutes later, bleeding is abundant. At the dissection liver and spleen are of a coal-black colour, the lungs are of a normal rose colour and conditions of the abdomen are entirely normal. Indian ink thrombuses are not to be found in the vessels, and there appears not even the Jancsó phenomenon, which is observed at group *b)*. (With the exception of the duodenum, where it appears even when histamine is not administered).

d. In case histamine is given 3 minutes before the aministration of heparin both in the above doses and lastly Indian ink, there is no external difference to be seen, as compared with the animals of groupe *c)*. At the dissection, however, a difference between the two groups can be observed in as much as there is a very slight greyish tint in the colouring of the lungs which proves that Indian ink was precipitated in the small vessels.

The following conclusions may be drawn from our investigations: Histamine, or histaminelike substances are causing precipitation of the colloids (in the present case of Indian ink) in the blood. In the case of using Indian ink which is on the critical point between stability and lability it happens therofore, that the colloid, effecting no precipitation in normal circumstances, is precipitated nevertheless in small particles in the lungs. It is a well known fact that the lungs are, under normal condition, the organs with the highest histamine content, consequently histamine added to the organism is adsorbed most rapidly by the lungs. This is probably also the reason why the Indian ink in the critical condition between stability and lability, precipitates in small particles in the lungs even when it does not precipitate in the other organs. The action of histamine in promoting thrombosis or, as in our present experiments, in expressly producing it, appears, after the experimental results, to be proved. Thus it seems natural that, at the effect of heparin, this thrombotic process did not occur.

The suspending effect of heparin on thrombosis, and on the accumulation of RES, is therefore, beyond doubt, based on the antagonism between histamine and heparin.

It seemed therefore, probable that on basis of the above-said there occurs some chemical association between heparin and histamine, which could be eventually proved optically. It is known, that heparin gets a violet colouring with toluidine-blue. (*Jorpes*, 1936.). We therefore investigated, whether this dye-reaction is influenced by histamine. We added a solution of toluidine blue to heparin which effected the characteristic violet colouring of the solution. If we repeated the same experiment — with the only change of giving previously histamine to the solution — the violet colouring did not take place, the solution kept its toluidine-blue colour unchanged; this means, that in

this case heparine was already associated to histamine and gave, consequently, no reaction with toluidine-blue. On farther investigations of the question it has also been proved, that the linkage between heparin and histamine was stronger than that between heparin and toluidine-blue. This was established by adding first toluidine-blue to heparin and obtaining thus the violet colour-reaction. In adding subsequently histamine to this solution the colour reaction was undone, and toluidine-blue colouring reappeared.

In order to investigate the proportions of the bond thorouhly, we have undertaken the following experiment:

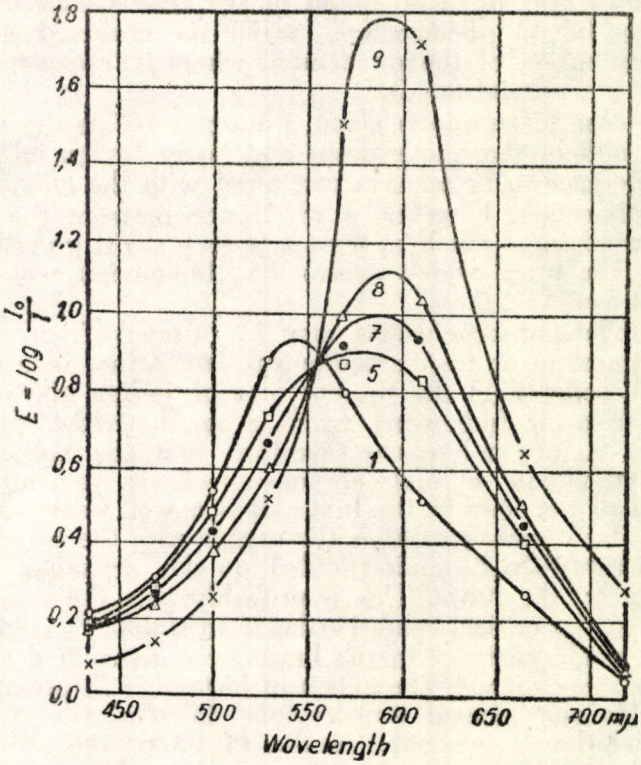

Figure No. 1. Changes of the spectrum of the heparin-toluidine-blue solution at the effect of histamine. Dates of the curves shown on Table No. 1.

We added to heparin solutions of equal quantities, histamine solution in gradually increasing quantities, and added to each sample an identical quantity, i. e. 0.05 °/00 °/ toluidine-blue solution. (Table No. 1.) We obtained thus a colour-series which showed according to the quantity of the histamine employed, beginning with the violet colour of heparin-toluidine-blue, gradually the transition from violet into toluidine-blue colour. The absorption spectra of the solutions were then determined by a Pulfrich-photometer, (Fig. No. 1.) which showed distinctly the transition of the two basic spectra into each other (On the Figure the absorption spectra of samples 1, 5, 7, 8, and 9 are shown.) The biggest change between the two spectra was observed at 610 m μ. The extinction variations obtained at this wavelength are

given on figure No. 2. It may be established from this curve, that the transition between the two modifications occurs at a histamine concentration of 10 mg/ml, which is approximately equivalent to molecule proportion of 2 : 1 between histamine and heparin; it seems, consequently, that for the neutralisation of one molecule of heparin two molecules of histamine were needed in this experiment. We are very well aware of the fact that this reaction is not specific in in vitro conditions, and a good many compounds are turning the violet colour of heparin-toluidine-blue into toluidine-blue again, and that a heat-effect, or the change of the pH decomposes also the heparin-toluidine-blue bond. Nevertheless the colour-reaction made the calculation of the molecule proportions possible — in the case of histamine. — The chemical investigation of the details of reaction are pendent.

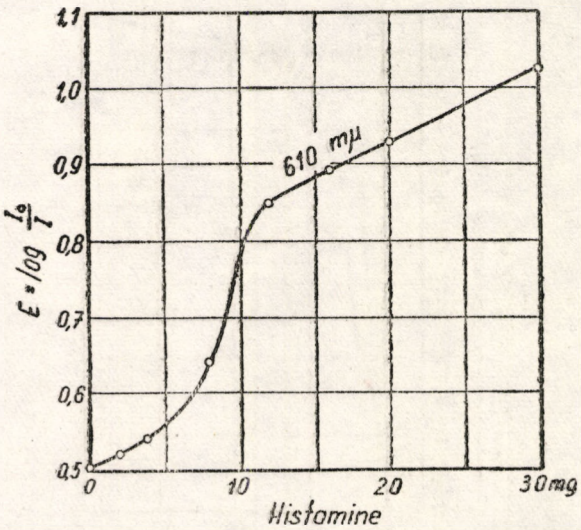

Figure No. 2. The variations of the extinction of the heparin-toluidine-blue solution at the effect of histamine at 610 mμ.

All the sings indicate consequently that the precipitation of the labile colloids in the blood is caused by the histamine circulating in the blood, or is at least in a great measure facilitated by its associating with the heparin present in the blood, and by increasing thus the coagulability of the blood. This is also proved by the observation that Indian ink on the critical point between stability and lability causes severe thrombosis in rats at the effect of histamine administered intravenously. Our opinion was already at the animal experiments, that the antagonistic effect of heparin is actually directed against histamine, and was not the consequence of the increase of stability of the colloids. This was also proved by the in vitro experiments described above. In this connection we also succeeded in clearing up the approximate quantitative proportions between histamine and heparin.

We established by our previously described experiments, that Indian ink of a sufficiently labile construction precipitates in the whole circulatory system, as the effect of histamine administered

intravenously and that thrombin, containing Indian ink may be observed in the big bloodvessels. This precipitation can be prevented by heparin.

We further investigated whether if animals were administered Indian ink, which subsequetly accumulated by the RES, there were to be observed any signs in thrombin-inactivation and coagulation of the blood indicating the liberation of heparin. We wanted to elucidate by this, whether the organism defends itself on such occasions by mobilisation of heparin against precipitation and against thrombus formation.

In these experiments rabbits were given 5 ml of Indian ink of 15% per 1000 g bodyweight, protected by gelatine, (Indian ink Weber). Changs of the inactivation and coagulation of the blood were observed immediately after the administration.

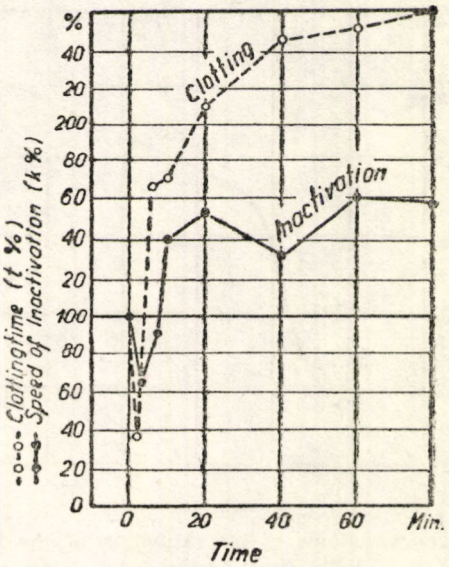

Figure No. 3.

Figure No. 3. shows the graphic demonstration of our experiments. (The medium values of the data of 8 experiments on animals.) The inactivation curve shows the percentual changes of the *k* values, expressing the velocity of the reaction, compared as to the initial values. The percentual changes of the clotting times expressed in seconds are demonstrated on the figure.

These experiments prove that inactivation is suddenly decreasing immediately after the injection of Indian ink, when Indian ink gets precipitated in the bloodstream on the surface of the RES cellules. Shortly afterwards, however, the inactivation is powerfully accelerated, and remains for a long time on a high level. The clotting times are undergoing a correspoding change, the coagulation is consequently accelerated at first, but later falls back beyond the initial value.

We succeeded, therefore, to demonstrate through these experiments that in the first phase of the accumulation when the precipi-

tation of the Indian ink is dominating, — and according to literary data — histamine gets liberated, inactivation suddenly decreases and clotting times are shortened. Immediately aftedwards, however the action of a compensating phase may be observed which appears as well in the coagulation as in the inactivation. This compensation is evidently brought about by the liberation of heparin.

If we observe also the duration of the circulation of Indian ink in the blood, we can see that in the 25-th minute after the injection there is no more Indian ink to be proved in the circulation. These tests were undertaken with the blood-drop method, described by *Jancsó*. During this time, the Indian ink got partly stored by the RES, and partly precipited on the walls of the cells. According to our opinion, the originating small thrombuses of Indian ink are causing the prolonged mobilisation of heparin as a defence action of the organism.

TABLE I.

Composition of the histamine- heparin- toluidine-blue solutions for the examination of the absorption spectra. Dates are given in ml.

No. of the samples	1	2	3	4	5	6	7	8	9
10 mg/ml heparin	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	—
10 mg/ml histamine	—	0.2	0.4	0.8	1.2	1.6	2.0	3.0	3.0
Distilled water	3.0	2.8	2.6	2.2	1.8	1.4	1.0	—	2.0
0.05 mg/ml toluidine blue	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Volumen	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0

REFERENCES

CSEFKÓ I. and GERENDÁS M.: Ann. Inst. Biol. Pervest. Hung. **1**. (1949—50) 206.

DRAGSTEDT C. S., WELLS J. A. and ROCH de SILVA M.: Proc. Soc. Exp. Biol. Med. **51**. (1942) 191.

GERENDÁS M., CSEFKÓ I. and UDVARDY M. D. F.: Nature, **162**. (1948) 257.

GERENDÁS M, PÁLOS Á. L. and CSEFKÓ I.: Ann. Inst. Biol. Pervest. Hung. **1**. (1949—50) 190.

JANCSÓ M.: Acta Univ. Segediensis Sect. Med. **2**. (1928) 246.
Klin. Wschr. **10**. (1931) 537.
Nature, **160**. (1947) 227.

JORPES E.: Acta Med. Scand. **88**. (1936) 437.

АНТАГОНИЗМ ГИСТАМИНА И ГЕПАРИНА IN VIVO И IN VITRO

Автор: МИХАЙЛ ГЕРЕНДАШ

РЕЗЮМЕ

Делая выводы из наших опытов, можно установить, что под действием гистамина осаждение коллойдов, добавленных к крови ускоряется. Этот процесс задерживает гепарин. Из этого следует, что гепарин и гистамин добавленные наружно, компенсируют свое действие с точки зрения инактивации и коагуляции крови.

Во второй части наших опытов мы изучали вопрос о том, что случается, если гистамин освобождается в ток крови в начале накопления. В этом случае инактивация уменьшается и коагуляция ускоряется. Но после этого организм немедленно мобилизирует гепарин, а действие этого гепарина проявляется и в инактивации, и в коагуляции.

На основе наших опытов мы пришли к выводу, что гепарин и гистамин компенсируют свое действие в организме отчасти путем своего влияния на коагуляцию крови, а отчасти потому, что организм отвечает на всякое действие гистамина мобилизацией гепарина, а — с другой стороны — на всякую мобилизацию гепарина организм отвечает действием гистамина.

Антагонизм между гистамином и гепарином можно доказать и действием гистамина на цветную реакцию толуидиновой синьки с гепарином. На основе оптического исследования цветной реакции мы с приблизительной точностью установили, что в этом процессе 2 молекула гистамина соединяются с одним молекулом гепарина.

По нашему мнению гистамин и гепарин оказывают значительное и антагонистическое влияние на формацию и на предотвращение тромбоза и на процесс накопления (В ретикуло-эндотелияльной системе.)