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Histamine release by sodium chloroplatinate

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1. The chloroplatinate ion can release histamine from the tissues in various species of animals.

2. This property is not shared by other complex ions with co-ordination number 6, or other complex platinum ions.

3. The release of histamine may form the basis of the known disease platinosis which affects people who work in platinum refineries, and which is characterized by symptoms of an allergic nature.

4. The mechanism of the liberation of histamine by chloroplatinate resembles that which occurs during anaphylactic shock.

During some research on heavy metals we have found that a complex platinum salt, sodium chloroplatinate, releases histamine from tissues (Parrot, Saindelle & Tazi, 1963).

In following up this work we have compared this release with that which occurs in anaphylaxis.

Methods

Recording of bronchomotility

We recorded the motility of the bronchi in the guinea-pig using a modification (Parrot, Nicot, Laborde & Canu, 1962) of the technique described by Konzett & Rössler (1940).

Male guinea-pigs weighing 300–900 g are anaesthetized with diallylmalonylurea (30 mg/kg subcutaneously). A tracheal cannula is inserted and connected to a respiratory pump. The animals are ventilated with a flow of 1 ml./100 g weight at a rate of 50–70 cycles/min (inspiration is active and expiration passive).

The inspiratory pressure is recorded by means of a Marey capsula which is inserted in the tracheal tube. Each bronchospasm appears as a sharp rise in the inspiratory pressure.

Estimation of the blood histamine content

We estimated the plasma histamine levels in the rat following the administration of the substances under investigation. The determination was carried out on the isolated guinea-pig ileum after extraction by the method of Barsoum & Gaddum

(1935) as modified by Code (1937). In the guinea-pig and the dog, we determined the whole blood histamine content fluorimetrically using a new automatic method (Ruff, Saindelle, Dutripon & Parrot, 1967).

Morphological study of the mast cells

We used our modification (Flavian, Saindelle & Parrot, 1967) of Norton's (1954) method. Small pieces of the mesentery of the rat were placed for 1 hr in an aqueous solution containing, in 100 ml., 4 parts formaldehyde and 4 parts toluidine blue. They were then immersed in acetone which was changed twice, and finally left for 12 hr in xylene which was changed after 6 hr. The pieces were then pinned to a cork board to enable us to choose a part of the mesentery which did not contain any large vessels. These latter are surrounded by a dense layer of fat which prevents accurate mounting. The chosen piece was mounted in a mixture of Canada balsam (2/3) and xylene (1/3), then examined microscopically.

Examination of the preparation showed that normally the mast cells are distributed abundantly around the vessels. The cells have a diameter of about 20 μ. The nucleus is not visible. The granulations, which are all intracellular, are strongly stained and cannot be distinguished individually.

Results

Action in vivo

Guinea-pig (Parrot, Saindelle & Tazi, 1963)

Intravenous administration. The intravenous administration of sodium chloroplatinate (10–20 mg/kg) into guinea-pigs produces, after a latent period of 1 min, a dyspnoea which is followed by increased intestinal motility. Death due to asphyxiation follows within 5 min as in the case of anaphylactic shock. At necropsy, the lungs are pale and distended.

At death, the histamine level of the whole blood, determined fluorimetrically as dihydrochloride, is greatly raised. It is greater than 1,000 μg/l., whereas in the normal animal the level determined by the same method does not exceed 500 μg/l.

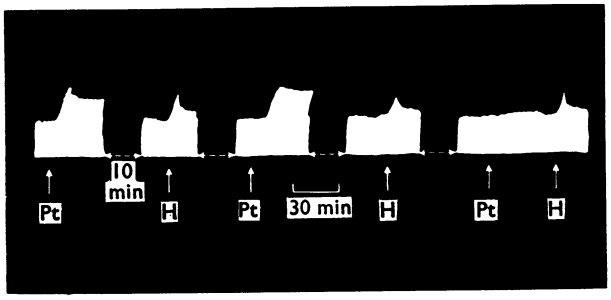

FIG. 1. Recording of broncho-motility in the guinea-pig using a technique based on that described by Konzett & Rössler (1940), and modified by Parrot, Nicot, Laborde & Canu (1962). Pt, sodium chloroplatinate (1 mg/kg intravenously); H, histamine dihydrochloride (5 μg/kg intravenously). At the third injection, the chloroplatinate has become almost inactive.

Complete protection against asphyxiation can be obtained by a subcutaneous injection of mepyramine (20 mg/kg) 30 min previously. In a guinea-pig which has been pre-treated with this drug, the LD50 for histamine injected intravenously is increased eighty-fold (48 mg/kg instead of 0.6 mg/kg in the normal guinea-pig) (Bovet & Walthert, 1944 ; Halpern, 1947 ; Bovet & Bovet-Nitti, 1948).

The intravenous administration of 1–2 mg/kg of sodium chloroplatinate produces a bronchospasm after 45 sec which is as severe as that produced by histamine dihydrochloride (5 μg/kg) injected by the same route (see Fig. 1).

The bronchospasm due to chloroplatinate is longer lasting, and whereas the same dose of histamine always produces the same response, after several doses of the chloroplatinate the response disappears. Soon the animal does not respond to the salt, and it can withstand a fatal dose of this compound. These results suggest that the chloroplatinate is not toxic by itself, but that the early injections release one or several toxic substances from the animal.

Local administration. We have also studied the local action of sodium chloroplatinate. The injection of 0.2 ml. of a 10^{-4} g/ml. solution into the abdominal skin is followed by an increased capillary permeability ; we have demonstrated this phenomenon in the guinea-pig by showing the local accumulation of Evans blue (1 ml. of a 0.5% solution) which has been intravenously injected 10 min before killing the animal.

Protection against the action of the chloroplatinate. We have established that the prior administration of some general anaesthetics protects the guinea-pig from the shock produced by chloroplatinate. This has been shown with urethane (1 g/kg intraperitoneally) and with ethyl alcohol (1.3 g/kg orally or 1 g/kg intravenously).

Rat

Sodium chloroplatinate has been shown to be much less active in the rat than in the guinea-pig ; it is known that the same fact applies to histamine (Rocha e Silva, 1955).

Intravenous administration. Histamine release can be demonstrated in this species: 10 min after the intravenous injection of sodium chloroplatinate (40 mg/kg) there seems to occur a pruritus of the muzzle and the feet, and a cooling of the extremities. We have established that the plasma histamine level, estimated by a biological method (expressed as dihydrochloride), is greater than 1,000 μg/l. whereas the normal value is on average 157 ± 79 μg/l. (Saindelle, 1964).

Action on mesenteric mast cells. We injected 20 ml. of a Tyrode solution containing sodium chloroplatinate (10^{-3} g/ml.) intraperitoneally into rats. Control animals were given 20 ml. Tyrode solution by the same route. We found that in the treated animals the mast cells of the mesentery had undergone some important changes: granulations had appeared around the cells and the cell size increased (see Fig. 2).

Dog

The intravenous injection of sodium chloroplatinate (30 mg/kg) into a dog which had been anaesthetized either with chloralose (100 mg/kg intravenously) or with diallylmalonylurea (30 mg/kg intravenously) led to death after some minutes. The histamine content of the whole blood (expressed as dihydrochloride) rose considerably, from 20 to 1,000 μg/l. in 2–5 min.

When we gave a lower dose (10 mg/kg) of chloroplatinate to another animal we did not observe any rise in the histamine content of whole blood.

Action in vitro

Isolated guinea-pig ileum

Demonstration of histamine release. When the isolated guinea-pig ileum is placed in Tyrode solution at 37° C it contracts strongly 10–15 sec after the addition

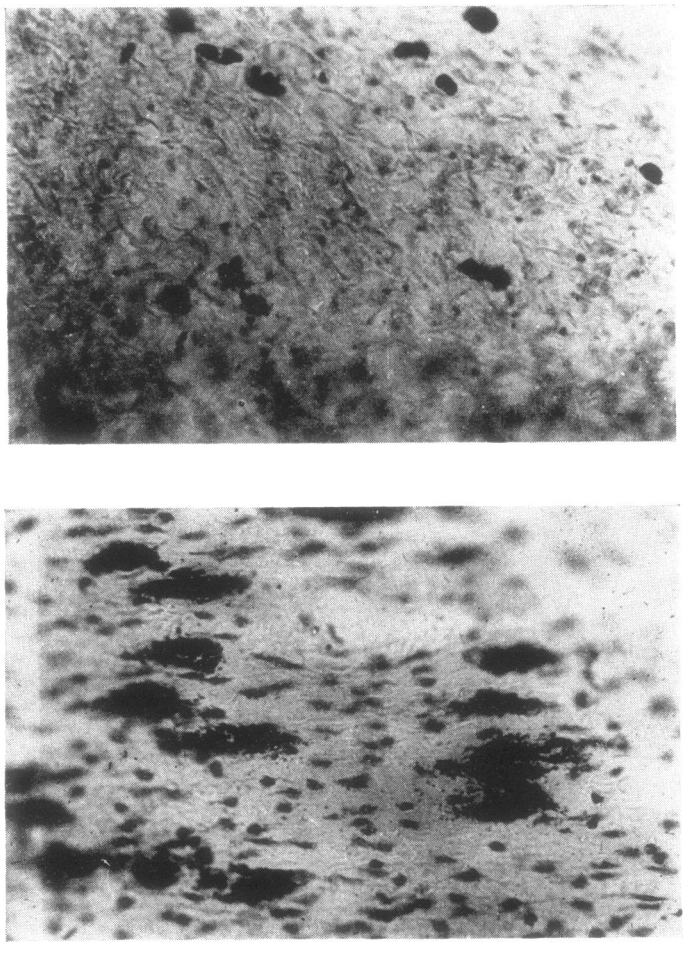

FIG. 2. Mesenteric mast cells, 20 min after an intraperitoneal injection of 20 ml. of (a) Tyrode solution and (b) Tyrode solution containing chloroplatinate (1 mg/ml.).

of sodium chloroplatinate (1 mg in an 8 ml. bath). When the same dose is added again, it is less effective, and the latent period increases to 30–40 sec. A third dose fails to produce any effect, whereas the sensitivity to histamine remains almost the same (see Fig. 3).

The action of the platinum salt disappears almost completely when the isolated guinea-pig ileum is bathed in Tyrode solution containing mepyramine (10^{-8} g/ml.) (see Fig. 4).

Attempt to obtain a transfer of the liberated substance. We have tried to carry out some experiments on the transfer of the substance liberated from the ileum, and to demonstrate this by using a second piece of ileum as a test object. We used two different techniques. First, we submitted a piece of ileum to the action of chloroplatinate, and then tested the Tyrode solution in which this piece had been bathed on a second piece of ileum which had previously been desensitized to this salt: the solution failed to cause a contraction of this second piece of ileum.

Second, we cut the whole of the small intestine of the guinea-pig into pieces 0.3 mm thick. After incubating these pieces for 15 min at 37° C in Tyrode solution containing chloroplatinate (10^{-3} g/ml.), we showed that, if histamine is present in the

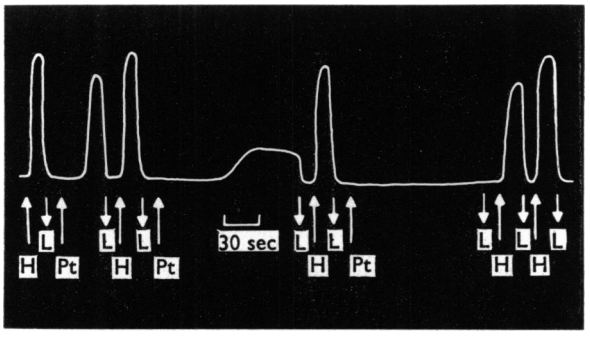

FIG. 3. Contractions of the isolated guinea-pig ileum. Bath volume, 8 ml. H, histamine dihydrochloride (0.2 μg); Pt, sodium chloroplatinate (1 mg); L, washing.

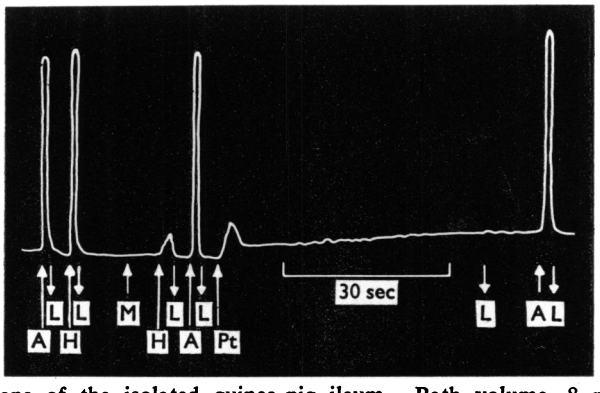

FIG. 4. Contractions of the isolated guinea-pig ileum. Bath volume, 8 ml. H, histamine dihydrochloride (0.2 μg); A, acetylcholine chloride (0.2 μg); Pt, sodium chloroplatinate (1 mg); L, washing; M, mepyramine (10^{-8} g/ml.) added to the Tyrode solution.

incubation solution, its level is below the threshold sensitivity of the test object to this substance (0.02 μg/ml.).

Crossed tachyphylaxis between chloroplatinate and the antigen. We wondered whether the actions of chloroplatinate and the antigen on the sensitized animal would affect each other.

The animals were sensitized by the injection of three doses of a mixture containing 5 mg of pure crystalline egg albumen, 0.25 ml. of Freund's adjuvant and 0.25 ml. of saline. The injections were given at 48 hr intervals, the first subcutaneously and the other two intramuscularly.

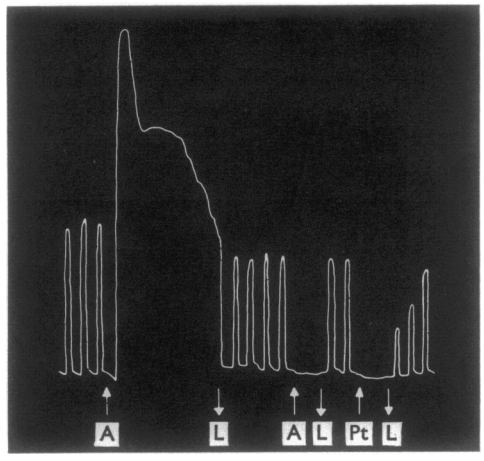

FIG. 5. Contractions of a piece of guinea-pig ileum taken from an animal which had been sensitized to egg albumen 3 weeks previously. Bath volume, 8 ml. A, Egg albumen (10 mg); Pt, sodium chloroplatinate (1 mg); L, washing. The other contractions were produced by the addition of 0.5 μg histamine dihydrochloride to the bath.

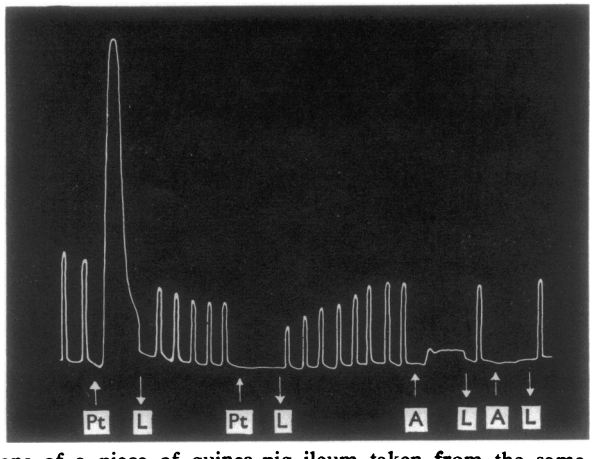

FIG. 6. Contractions of a piece of guinea-pig ileum taken from the same animal as that in Fig. 5. Bath volume, 8 ml. A, Egg albumen (10 mg); Pt, sodium chloroplatinate (1 mg); L, washing. The other contractions were produced by the addition of histamine dihydrochloride (0.5 μg) to the bath.

Three to six weeks later, the addition of the antigen (10 mg) to the isolated ileum (in an 8 ml. bath) resulted in a marked contraction after a latent period of 10 sec. A second dose of the antigen did not produce any effect. This sequence of events constitutes the Schultz-Dale phenomenon. Figure 5 shows that the ileum which has been desensitized to the antigen failed to contract in response to sodium chloroplatinate (1 mg), whereas the sensitivity to histamine remained the same.

A second piece of ileum taken from the same animal responded at first to the chloroplatinate (1 mg in an 8 ml. bath) (Fig. 6). It will be seen that when the ileum has been desensitized to the salt, the antigen can still produce just a weak contraction of the ileum.

Inhibition of histamine release. (a) Effect of temperature: After incubation of the isolated guinea-pig ileum for 15 min at 45° C, followed by restoration of the temperature to 37° C, the ileum no longer releases histamine in response to sodium chloroplatinate, although its sensitivity to histamine is unaltered. We have observed the same phenomenon with egg albumen (1 mg in an 8 ml. bath) using a piece of ileum taken from a sensitized animal.

(b) Effect of some metabolic inhibitors: The release of histamine by chloroplatinate is completely abolished after incubation of the isolated guinea-pig ileum for 15 min in the presence of ethyl alcohol (1%) or urethane (4%), although the sensitivity to histamine is unaltered.

Isolated mesentery of the rat

We have investigated the action of sodium chloroplatinate (10^{-3} g/ml.) on the isolated rat mesentery incubated in Tyrode solution at 37° C, and we did not observe any cytological changes in the mast cells.

Relationship between the spatial configuration of the complex platinum salts and histamine release

We have established that among all the complex platinum salts, only the chloroplatinate ($PtCl_6^{2-}$), with co-ordination number 6, causes the liberation of histamine from guinea-pig tissues.

The chloroplatinite ($PtCl_4^{2-}$), with co-ordination number 4, is devoid of this property both when tested *in vivo* (20 mg/kg) and on the isolated ileum (1 mg in an 8 ml. bath).

We wondered whether other complex ions with co-ordination number 6 were able to release histamine. So we studied the actions of ammonium chloro-iridate, chlororuthenate, chloropalladate and chlororhodate: none of these salts caused the release of histamine either *in vivo* (20 mg/kg) or on the isolated guinea-pig ileum (1 mg in an 8 ml. bath).

Discussion

Analogies between the release of histamine by chloroplatinate and by antigen

Several experiments suggested that the mechanism of histamine release by chloroplatinate resembles that which occurs during anaphylaxis.

Crossed tachyphylaxis between specific antigen and chloroplatinate on the guinea-pig ileum. Among the various histamine liberators which we studied, only the chloroplatinate caused the guinea-pig ileum to contract, and exhibited crossed tachyphylaxis with Schultz-Dale phenomenon. Thus, Cobra venoms, octylamine and 48/80 were without any activity on the ileum.

Effect of temperature. The inhibition of histamine release by chloroplatinate from the guinea-pig ileum seen following incubation of the ileum at 45° C resembles the results obtained by Mongar & Schild (1957). These workers found that the release of histamine from the isolated guinea-pig ileum during anaphylaxis is considerably reduced after preliminary incubation of the tissue at 45° C. It is thus probable that a thermolabile factor is involved in these two types of histamine release. This factor seems to be different from complement as it was described by Mongar & Schild (1957), because this latter factor is destroyed only after heating to 52°–54° C for 25 minutes. We have further shown that when the thermolabile factor has been inactivated, the addition of fresh guinea-pig serum to the isolated ileum does not restore the ability of the chloroplatinate to release histamine from this organ. This is exactly what Mongar (1958) found with specific antigen. The nature of this factor, which is probably enzymatic, remains unknown.

Failure of transfer. We were not able to demonstrate a transfer of the substance liberated by chloroplatinate from the isolated guinea-pig ileum.

These negative results resemble those which Dworetzky (1959) obtained in the case of anaphylactic shock. This author put two pieces of ileum in the same organ bath ; one was sensitized to egg albumen, and the other came from a normal animal. The addition of specific antigen produced the Schultz–Dale phenomenon in the sensitized piece, but elicited a contraction of the normal piece on only two out of seven occasions. This phenomenon can be explained by the fact that the quantity of histamine released from the sensitized ileum is high enough to raise the local concentration around the receptors of the sensitized ileum, but not enough to diffuse out and reach the receptors on the second piece of ileum.

Protection by general anaesthetics. The protection which urethane affords to the guinea-pig ileum against platinic shock is similar to the protection against anaphylactic shock afforded by anaesthetics as described by Besredka (1907).

It does not seem that the protection against the action of chloroplatinate can be linked with general anaesthesia: we also have demonstrated it using the isolated guinea-pig ileum just as Geiger *et al.* (1956) showed it with reference to the Schultz–Dale phenomenon.

Analogies to and difference from a known disease : platinosis

The liberation of histamine by chloroplatinate resembles a disease which affects people who work in platinum refineries, and which is characterized by skin eruptions and respiratory symptoms of an allergic nature (Roberts, 1951 ; Hebert, 1966). Human platinosis appears only after a period of exposure to the hazard, whereas the liberation of histamine occurs in the guinea-pig after the first injection of the chloroplatinate. The delay in the appearance of platinosis suggests that the platinum salts could combine with a protein *in vivo* to form an antigen which is responsible

for the sensitization. According to this concept, the same substance would be capable of causing a sensitization on the one hand, and of immediately liberating histamine on the other.

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