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LIBERATION OF HISTAMINE FROM THE PERFUSED LUNG BY STAPHYLOCOCCAL TOXIN

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The theory of Lewis [1927] that cell injury is associated with the liberation of a histamine-like substance and that the similarity between the effects of many injurious agents is explained by this common mechanism has also been applied to the action of bacterial toxins. Some indirect evidence for this view has been put forward for the skin reactions produced by streptococcal and diphtheria toxins, which show their effects only after a latent period of hours or days. We have sought direct evidence of the liberation of histamine by staphylococcal toxin, using the methods employed by Feldberg & Kellaway [1937] in the previous communication. Staphylococcal toxin, if administered intravenously, causes symptoms after a latent period of a few minutes only [Kellaway *et al.* 1930]. The relative shortness of this latent period made this toxin peculiarly suitable for our purpose.

Methods

Guinea-pig's and cat's lungs were perfused with Tyrode solution through the pulmonary artery and the outflowing fluids were assayed for histamine, using the technique described in the previous communication. A slight alteration was introduced in the method of perfusion of the guinea-pig's lung. It was dissected from the thorax and placed on a specially moulded wax sheet. No cannula was tied into the left auricle, but the heart was cut off at its base and the venous outflow, together with the fluid which exuded from the lung surface, were collected together. In the perfusion of the cat's lung a cannula was tied into the left auricle and

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the venous perfusate and the fluid exuding from the lung surface ("leakage fluid") were collected and assayed separately. When toxin was injected into the arterial cannula it was quickly washed through the lungs. To permit of longer contact, in some experiments the perfusion was stopped for ½–2 min. while the injection was being made.

We used a single batch of staphylococcal toxin, which was prepared in nutrient broth containing 0·5 p.c. agar. To prevent bacterial growth during storage 0·01 p.c. merthiolate was added. The toxin was centrifuged before use to remove the bulk of the cocci. It completely haemolysed 1 p.c. suspension of rabbit red cells in a dilution of 1 : 640. Injected intravenously 0·5 c.c. killed a 3·1 kg. rabbit in 30 min. For neutralization, 1·0 c.c. required >10 and <20 I.U. of antitoxin. It was therefore less active than that used by Kellaway *et al.* [1930].

Results

In preliminary experiments it was found that the crude toxin had a stimulating action on the isolated guinea-pig's jejunum, which was used for the routine assay. This action was apparently not due to the toxin itself but to the presence in the broth of a histamine-like substance.[1] Kellaway *et al.* showed that such a substance was present in control sterile broth samples and accounted wholly for the immediate depressor effect they observed.

Following the addition of 0·2 c.c. of 5 p.c. solution of the toxin to the 3 c.c. bath, the gut contracted immediately and relaxed again quickly on changing the fluid in the bath. With repeated administration, no diminution of the stimulating action was observed. Repeated assays of the toxin against histamine on different days showed that the stimulating action of 1 c.c. of toxin corresponded to that of $4{\cdot}2\gamma \pm 10$ p.c. of histamine. The toxin had an immediate depressor action on the cat's blood pressure and yielded the same histamine equivalent when assayed by this test.

The presence in the crude toxin of histamine or of a histamine-like substance complicated our experiments. The effects of this substance on the perfused lung had to be distinguished from those of the toxin itself and its presence in the perfusate differentiated from the output of histamine caused by the toxin. Fortunately the toxin acted on the lung only after a latent period of 10–40 min., which made the distinction possible.

[1] With a later batch of toxin, we were able to show that the whole of the stimulant action on the guinea-pig intestine was present in the sterile broth and was not increased by the addition of agar or by the growth of staphylococci.

Perfusion of guinea-pig's lung

Effect of the lungs. A few seconds after the injection of 0·5 c.c. of the crude toxin into the pulmonary artery strong bronchoconstriction sets in; the respiratory movements diminish, stopping completely in the following 20–30 sec. No further changes are observed for 10–15 min.; the lung then swells gradually, fluid accumulates in it and parts of its substance become glassy and transparent. Similar, but more profound changes, have been described after large doses of snake venom [Feldberg & Kellaway, 1937]. The outflowing fluid becomes turbid and frothy; if boiled it becomes opalescent and coagulable protein may be precipitated. These changes become less evident if perfusion is continued for 1–1½ hours after the toxin injection.

The immediate immobilization of the lung is an effect of the histamine-like substance in the broth, whereas the late changes must be attributed to the toxin itself.

Assay of perfusate. The histamine-like principle present in the broth is recovered in the fluid flowing out from the lung during the first 10–20 min. after the injection. The sample collected in the first 2 or 3 min. contains the bulk of it, the concentration diminishing rapidly in the subsequent samples. Fluid collected about 15 min. after the injection may be completely inactive. The total activity of the samples collected within this period corresponds to the histamine equivalent of the injected crude toxin. During the next 10–20 min. a renewed activity is noted in the samples. The onset of this rise in activity occurs in some experiments (cf. Fig. 1) before the perfusate has lost all activity. The renewed activity reaches a maximum 40–60 min. after the injection and then slowly declines.

The results of a typical experiment are graphed in Fig. 1, in which the abscissae give the time of perfusion in hours after the injection of toxin, the ordinates the histamine equivalents of the outflowing fluid in γ per min. During, and for some 40 sec. after the injection of toxin (0·5 c.c.), perfusion was stopped. The rate of flow varied between 0·4 and 0·8 c.c. per min. The outflowing fluid before the injection had no detectable activity. The histamine equivalent of the first three samples collected during the first 15 min. corresponded to 2·06γ, over half of which was found in the first 2 min. sample. The fourth sample collected during the next 15 min. had an activity corresponding to 0·3γ of histamine. At the end of the experiment the toxin was assayed on the same preparation, and the dose used had a histamine equivalent of 2·2γ. Thus the 2·06γ of the first three samples and about half of the activity of the fourth sample

must be attributed to the histamine-like principle in the crude toxin. In Fig. 1, the area representing this activity is shaded. The remaining half of the activity of the fourth sample is part of the renewed activity, which increases in the following two samples. Thereafter it decreases again, but even the last sample of fluid collected $2\frac{3}{4}$ hours after the injection of toxin had not become completely inactive. In other experiments the renewed activity disappeared sooner.

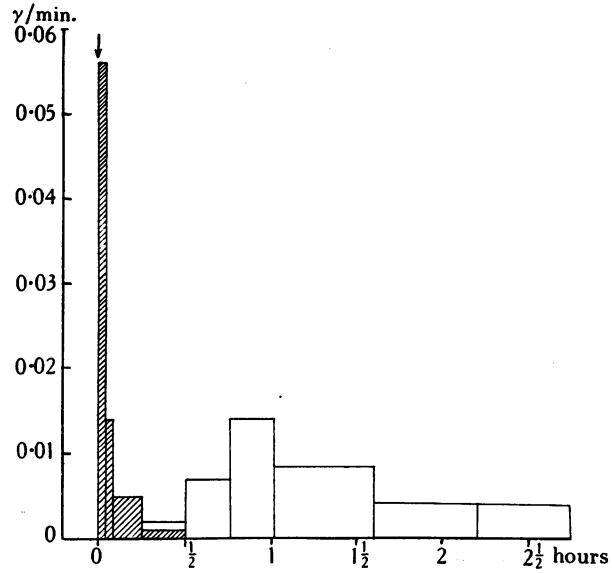

Fig. 1. Histamine output from perfused guinea-pig's lungs after injection of 0·5 c.c. of toxin at (↓) (for details see text).

Identification. This renewed activity of the perfusate is due to histamine liberated from the lungs. A sample, taken when the activity was strongest, was tested not only on the guinea-pig's jejunum but also on the arterial blood pressure and suprarenals of the cat. Such an assay is illustrated in the following paper of Feldberg & O'Connor [1937]. The sample caused a fall of arterial blood pressure which was not abolished by atropine and an output of adrenaline from the suprarenals. All three reactions matched with the same histamine concentration.

The late onset of the renewed activity in the perfusate excludes the possibility that it is due to the stimulating substance derived from the toxin, which is fully recovered in the early samples. The substance responsible for the renewed activity probably originates from the lungs.

In the previous paper on snake venoms it could actually be demonstrated that the lung had lost that amount of histamine, which was assayed in the perfusate. The amounts of histamine liberated by our toxin were too small for such a demonstration to be made with certainty. Nevertheless we must assume a similar mechanism and can therefore regard the active substance responsible for the renewed activity as being part of the lung histamine.

Histamine output. The histamine liberated by the toxin is best expressed as a percentage of the lung histamine, because this varies greatly in different animals. In the experiment of Fig. 1, the output of histamine in $2\frac{3}{4}$ hours amounted to $8{\cdot}96\gamma$. Another $0{\cdot}85\gamma$ was recovered in the 6·9 c.c. of fluid draining from the excised lung ("drainage fluid") during a period of 50 min. The lung was then extracted and yielded $54{\cdot}6\gamma$ of histamine. The lung therefore originally contained $64{\cdot}4\gamma$ of histamine, 15 p.c. of which had been recovered from the perfusate and drainage fluid. In different experiments the output varied between 4 and 15 p.c. of the lung histamine. Our experiments were spread over a month during which the toxin lost some of its activity, the low values for the output being obtained in the later experiments.

There was some parallelism between the histamine output and the effects observed on the lung. In the experiment of Fig. 1, for instance, the whole right lower lobe had become glassy, and transparent patches in the other parts were abundant and ran together. In those experiments in which the histamine output amounted only to 4–6 p.c. of the lung histamine, the effects on the lung were less pronounced.

The drainage fluid in the experiment of Fig. 1 was collected in three successive samples. 3 c.c. were collected in the first 5 min. and 1·9 c.c. in the following 10 min. Both samples had a histamine concentration of about 1 in 7·5 million which was somewhat stronger than the histamine concentration of 1 in 11 million assayed in the last sample of perfusate. The third sample of 2 c.c. of drainage fluid, collected in 35 min., had a histamine concentration of about 1 in 10 million. This decrease in the histamine concentration has been observed in all those experiments in which the lung was excised when the histamine liberation was coming to an end.

Perfusion of cat's lung

Two experiments have been done with results similar to those earlier obtained on the guinea-pig's lung. There was an immediate bronchoconstriction due to the action of the histamine-like principle present in the toxin, followed after some 20 min. by the effects of the toxin itself.

The immediate immobilization of the lung was not as complete as in the guinea-pig's lung and could be overcome by increased ventilation. Later, fluid accumulated in the lung causing swelling and the appearance of glassy patches, which increased in size and number during the first hour. The venous outflow decreased as the effects due to the toxin developed and in one experiment stopped completely some 30 min. after the injection. On the other hand, the leakage fluid became more abundant; it could be further increased for a short period by increasing the lung ventilation, thus pressing out some of the accumulated fluid. Fluid appeared also in the trachea.

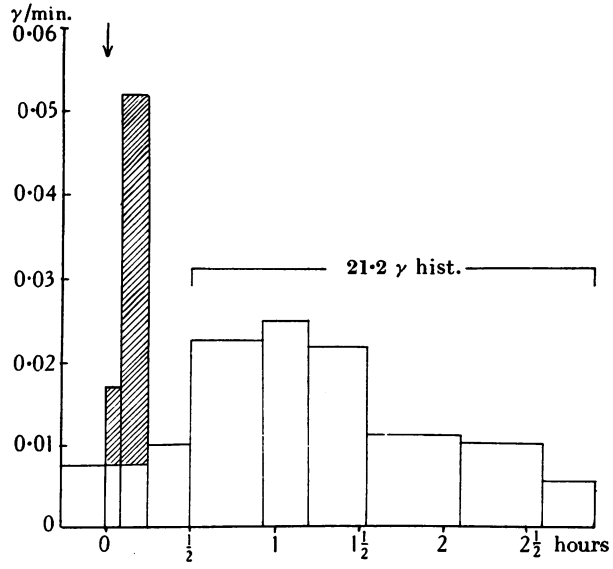

Fig. 2. Histamine output from perfused cat's lungs after injection of 1 c.c. of toxin at (↓) (for details see text).

Assay of perfusate and histamine output. The results of one experiment are shown in Fig. 2. The outflowing fluid had some activity before the injection of toxin, the significance of which has been discussed in the preceding paper. The fluid collected during 15 min. immediately following the injection contained all the histamine-like principle of the injected 1 c.c. of toxin. This part of the activity is represented by the shaded area. When the toxin had been washed out, the activity of the perfusate returned to about its original value. The renewed activity did not start until about 30 min. after the injection, the latent period being

somewhat longer than in the guinea-pig experiments. The identification of this activity as being due to histamine liberated from the lung was made in the same way as in the experiments on guinea-pigs. The histamine output reached its maximum about 1 hour after the injection of toxin, then decreased again and came to an end after $2\frac{1}{4}$ hours. The output during that period amounted to $21{\cdot}2\gamma$ of histamine. Another $3{\cdot}7\gamma$ was assayed in the drainage fluid, bringing the total output to $24{\cdot}9\gamma$. Although this value is greater than in experiments on guinea-pigs, the percentage loss of the lung histamine was of the same order. The lung yielded by extraction 348γ of histamine, the total output representing a loss of 6·7 p.c. In the other experiment the loss from the lung amounted to $96{\cdot}5\gamma$ or 6·9 p.c. of the lung histamine. The output started about 40 min. after the injection of the toxin (1 c.c.), reached its maximum in 3 hours and was not complete when the perfusion was stopped after $4\frac{1}{2}$ hours. Of the $96{\cdot}5\gamma$, 19γ were assayed in the drainage fluid.

In the experiment of Fig. 2 in which perfusion was stopped when the histamine output had come to an end, the drainage fluid was collected in two successive samples; the first 20 min. sample of 11 c.c. had a histamine concentration of 1 in 3·5 million in comparison with one of 1 in 15 million in the last sample of perfusate. The second 30 min. sample of 2·5 c.c. had a histamine concentration of 1 in 4 million. This result was similar to those in the experiments on guinea-pigs, which have already been discussed. In the other experiment perfusion was stopped before the histamine output had come to an end, and the histamine concentration of the second sample of drainage fluid had actually increased instead of being diminished. The first 5 min. sample of 19 c.c. had a concentration of 1 in 2·5 million, the second 10 min. sample of 6·6 c.c. a concentration of 1 in 800,000. The next 3·1 c.c. collected in 35 min. showed no further increase; the histamine concentration being 1 in 1 million. We may safely conclude that if perfusion had been stopped at a still earlier stage, the third sample of drainage fluid would have shown a further increase in its histamine concentration. The experiment represents a condition of the lung midway between two limits—one in which the liberation of histamine is at its height after the toxin injection and the other when it is returning to normal.

Discussion

The theory of Lewis that histamine is liberated from the tissues by bacterial toxin has, at least so far as staphylococcal toxin is concerned, been proven by the experiments here recorded. We were able to show that

this toxin, after a latent period characteristic of its action, causes an output of histamine from the perfused lungs of guinea-pigs and cats. Much of what has been said in the preceding paper concerning the part played by the liberation of histamine in the symptomatology of poisoning by snake venoms is also applicable here. Briefly we may say that those symptoms which resemble histamine effects may be attributed to the action of liberated histamine, whereas those symptoms which cannot be reproduced by histamine must be regarded as being due either to the direct effect of the cell injury caused by the toxin or to the liberation of substances other than histamine from the injured cells. Thus the acute fall of arterial blood pressure in cats with the concomitant rise in pulmonary pressure and the peripheral vaso-dilatation are manifestations which may be regarded as the effects of liberated histamine. On the other hand the hæmorrhagic lung œdema, which has been described by Russ [1916] and by Kellaway *et al.* [1930] can scarcely be explained by histamine. The effects of the liberated histamine may combine with those of the cell injury. For instance the vascular reaction produced by intradermal puncture of bacterial toxins into the human skin differs from the triple response caused by puncturing in histamine. This difference may be due to modification of the triple response by additional cell injury.

The evidence here presented of the liberation of histamine by staphylococcal toxin makes it likely that this mechanism is involved in the action of other bacterial toxins.

Summary

Staphylococcal toxin, after a latent period of between 10 and 40 min., causes an output of histamine from the perfused lungs. With the toxin used, of which 0·5 c.c. was injected into the guinea-pig's lung and 1 c.c. into cat's lung, the histamine output amounted to between 4 and 15 p.c. of the lung histamine. The role of this mechanism in the symptomatology of poisoning by staphylococcal toxin is discussed.

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