

THE MECHANISM OF ANAPHYLAXIS

By

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Mr. President.

I am very proud and grateful to have been invited to take part in this historic occasion. It commemorates one of the great events in the history of Physiology and Pathology. It has been to me a matter of very great regret that I have been prevented, by a medical embargo, from making the journey to Paris, to take part in this commemorative ceremony; but I am fortunate in that Dr. Halpern has most kindly undertaken to read, on my behalf, the communication which I had hoped to be able to present here in person.

The interest of an occasion which would, in any case, have been a most notable one, is greatly enhanced by the fact that Professor Portier, who took part in the dramatic discovery of the anaphylactic reaction, and in the immediate recognition of its great and novel importance, is able to take a personal and prominent part in this celebration. You will have heard from him, I am sure, an account of the peculiar circumstances in which that discovery was made. The late Professor Charles Richet and he were experimenting with a naturally poisonous protein, "actinocongestine", which they had extracted from the tentacles of a sea-anemone. Having observed its action, when they injected it intravenously into dogs in different doses, they preserved the animals which had survived the smaller initial injections, and repeated the injections into these dogs after an interval of some weeks, expecting to find some degree of specific immunity to the poison

already established, and to be able to enhance this in the familiar manner, by a series of increasing injections. Contrary to this expectation, the second injections, even of doses which at the first had produced but slight effects, evoked symptoms which, in the rapidity of their onset, their great severity and their frequently fatal termination, appeared to be similar to those which had followed first injections of the larger, lethal doses. The effect of the first injection, then, had apparently been, not the production of immunity, prophylaxis, but of the opposite condition, which they termed "anaphylaxis".

Nobody who had first seen this phenomenon, in the circumstances in which Portier and Richet thus discovered it, would have adopted a different interpretation of its nature. Yet it was not long before it became clear that such an explanation of its meaning was not compatible with other aspects of the phenomenon. In the years immediately following the initial discovery, the observations of Arthus on the rabbit, of Theobald Smith and of Rosenau and Anderson on the guinea-pig showed that a comparable, rigidly specific sensitiveness, or anaphylaxis, appeared in these species, as the result of a preliminary injection, a few weeks earlier, of normally innocuous foreign proteins, such as those in the blood serum of a horse or in the albumin of a fowl's egg. The reactions by which this sensitiveness was manifested in these different animal types were, indeed, rather remarkably different from those described by Portier and Richet in their experiments on the dog; but it was soon made clear that this difference had no connexion with the natural toxicity of the substances with which the first observations had been made. For, in the dog also, which had received, a few weeks earlier, a preparatory injection of a naturally non-poisonous foreign protein, such as that of horse serum or egg-albumin, a reinjection of the same substance would elicit a reaction entirely comparable, in its nature and its violence, to that which Portier and Richet had first seen with a second injection of their actinocongestine.

Anaphylaxis, then, was not, as had seemed at first to be

so obvious, a condition of acquired sensitiveness, or diminished resistance to a natural poison; it was a condition in which the animal responded to a previously injected foreign protein, even to a normally quite inactive one, as though it were, indeed, a powerful poison. And, in the years which followed, it often seemed to me that a great deal of fruitless effort might have been saved, and a great deal of confusion and needless controversy avoided, if all of the many investigators, who became engaged in the study of anaphylaxis and the mechanism of its production, had kept clearly in view this outcome of the observations made at these early stages of the investigation. Portier and Richet's initial observation was, in fact, not only sound in itself, but highly significant, when it was correctly interpreted in the light of further evidence. For it showed that the reaction of an animal, sensitized by a prior injection, to a further injection, even of a normally inactive protein, had a close and even a deceptive resemblance to the reaction of a normal animal of the same species to a naturally poisonous protein — to any, indeed, of a large class of such poisons. Later we shall see that a similar syndrome may be produced by an even much wider range of poisonous substances, many of them not even of a protein nature. It ought, accordingly, to have been clear, even from a consideration of Portier and Richet's earliest experiments and of those which so quickly followed them, that it is not the syndrome of the shock which is characteristic of the anaphylactic condition; it is the sensitizing effect of a previous injection, which causes that syndrome to appear in response to the injection of a protein which would not normally have any such effect.

The type of reaction itself had, indeed, been known for many years as that given by the animal body, and by that of the dog in particular, to a whole group of naturally poisonous proteins, or to poisonous products obtained artificially, from naturally inert proteins, by partial hydrolytic cleavage. As long ago as 1880, Schmidt-Mülheim had described the reaction of the dog to the intravenous injection of a sufficient dose (about 0.3 gramme per kilogramme) of the so-called

"Peptone" of Witte—a mixture of albumoses, and peptones obtained by the peptic digestion of blood-fibrin. He had observed the collapse of the arterial pressure, accompanied by a great congestion of the liver and the gastrointestinal mucous membrane and, especially, the loss of the coagulability of the blood, which followed such an intravenous injection of peptone into a dog. And, quite early in the study of anaphylaxis, the effects produced by such injections of "peptone" in the dog and in other species, and particularly in the guinea-pig, had become a centre of attention. For the reaction of the anaphylactic guinea-pig, to reinjection of the sensitizing antigen, was rather strikingly different from that which was first seen in the dog. The dog had shown the syndrome long familiar as the effect of injecting peptone—circulatory collapse, intense and obstructive congestion of the liver, retrograde congestion of the gastro-intestinal canal with capillary haemorrhages, and loss by the blood of its normal power of clotting. The anaphylactic guinea-pig, on the other hand, responded to a reinjection of the antigen with a suffocating, valve-like constriction of the bronchioles, so that the animal characteristically died in a few minutes from asphyxia, with the lungs in a state of permanent distension—the so-called Auer-Lewis syndrome. It accordingly appeared to be highly suggestive, when Biedl and Kraus found that this previously unfamiliar reaction, shown by the anaphylactic guinea-pig in response to its antigen, was also reproduced in the normal guinea-pig by an intravenous injection of peptone. And it must be admitted that it was very natural that further investigations should proceed, for a time, on the assumption that the anaphylactic condition was due to the appearance in the blood, as the result of the first injection, of something which caused the sensitizing protein, when reinjected, to undergo a rapid, enzymatic hydrolysis, with a resulting production of a poisonous cleavage product of the "peptone" type, a non-specific "Apotoxine" (Richet) or "Anaphylatoxine". And then, when evidence had been obtained to show that the anaphylactic condition was due to the formation of an immune substance

or antibody of some kind, and probably of a precipitin, the theory of the formation of an anaphylatoxin underwent a number of variations in detail. Some proponents of this theory supposed that a specific precipitate, formed in the blood when the sensitizing antigen was reinjected, was attacked by a natural protease of the blood, with a resulting formation of poisonous cleavage products; others, who distrusted this form of the theory from quantitative considerations, supposed that the formation of the precipitate would remove an antitryptic factor present in the normal blood, and thereby initiate an autolytic cleavage of the blood proteins in general, with the production from them, again, of poisonous cleavage products. Others, again, regarded the formation of a specific precipitate in the circulating blood as disturbing the delicate equilibrium between its plasma proteins and its formed elements, in a manner comparable to the effect produced by contact with a foreign surface; so that the blood was supposed to have become involved in the earlier phases of the coagulation process, and therewith to have acquired a toxicity responsible for the symptoms. Yet others supposed that the blood acquired toxicity from a more vaguely conceived disturbance of the colloidal solution of its plasma constituents.

It should be noted that, at this juncture, the guinea-pig appears to have become the experimental animal used in a very large proportion of the experiments on anaphylaxis. The dramatic and rapidly fatal reaction, the "Auer-Lewis syndrome", with which it responded to a reinjection of the sensitizing antigen, was a comparatively novel discovery; and it was peculiarly easy to overlook the true significance of Biedl and Kraus's experiments with peptone, which might properly have been taken to indicate that there was nothing in the syndrome itself which was characteristic of anaphylaxis. It was found that the fresh blood serum of a guinea-pig would readily acquire toxic properties under a variety of manipulations *in vitro*, enabling it to produce a reaction more or less closely reproducing the Auer-Lewis syndrome, when it was injected into the circulation of a normal guinea-pig. It was

claimed, for example, that the serum acquired a toxicity of this supposedly significant kind, when it was incubated *in vitro* with suspensions of living or dead bacteria, of kaolin or Kieselguhr, or after emulsification with chloroform, or with sols of starch, inulin or agar-agar. Only when it had been emulsified with chloroform was there clear evidence of proteolytic cleavage in the serum; and in that case the effect of its injection was to produce a massive intravascular clotting of the blood, with symptoms which only superficially recalled those seen in the anaphylactic shock. It must be admitted that it is not possible to offer complete explanations of the toxicity of all these preparations. Some of them did produce symptoms more definitely suggestive of, though in no case completely identical with, those of the anaphylactic reaction. In any case, however, those who devoted so much time and labour to the investigation of their properties seem to have wandered a long way from their initial objective. There was curiously little connexion, indeed, between the effects of these artificial "anaphylatoxins" on normal guinea-pigs, and the specific sensitiveness to a protein caused by an earlier small injection, or passively conferred, after a shorter interval, by injection of a specific precipitin for that protein, which was the true anaphylaxis. The only link, indeed, was in the resemblance, varying in degree, between the syndromes produced by the anaphylatoxins in normal guinea-pigs, and that seen in the anaphylactic reaction of the sensitized guinea-pig to the sensitizing antigen. And it cannot be too strongly insisted that there is nothing characteristic in this syndrome, which can be produced in the guinea-pig by a whole range of poisons, the actions of which have no proper connexion with anaphylaxis; just as we saw earlier that the very different syndrome, seen in the anaphylactic reaction of the dog, can be produced in normal dogs by a similarly wide variety of poisons. *It is not the syndrome which is anaphylactic; anaphylaxis is the production of a common syndrome by a special kind of immunological reaction.*

I have been obliged, by consideration of the time avail-

able on an occasion of this kind, to dismiss rather dogmatically this large volume of evidence on the artificial production of the so-called anaphylatoxins. And the need for brevity will oblige me to be at least as dogmatic, in presenting the evidence which seems to me to have a direct and positive significance, for an understanding of the nature, on the one hand, of the anaphylactic condition and, on the other hand, of the mechanism of the reaction of the anaphylactic animal to the protein to which it has been sensitized. The former of these problems is one of immunology, while the latter involves considerations proper to physiology and pharmacology as well.

THE NATURE OF THE ANAPHYLACTIC CONDITION

(a) *In the guinea-pig.* In my opinion, the nature of the true anaphylaxis in the guinea-pig was made clear by the immunological experiments of the late Richard Weil in 1913, in which he followed the development of passive anaphylaxis after an intravenous injection of a precipitin. Weil showed that the specific sensitiveness appeared in parallel with the disappearance of the precipitin from the blood. He found, further, that this sensitiveness was immediately masked, replaced by an immunity, when a further injection of precipitin was made into the circulation. Concurrently it was shown by Schultz, in Washington, and by myself in London, that the isolated plain muscle of an anaphylactic guinea-pig, freed in my own experiments from all traces of blood, reacts specifically to the sensitizing antigen. In experiments made with plain muscle of the uterus from young females, I was able to demonstrate *in vitro* all the main features of the anaphylactic condition in the guinea-pig—its exquisite specificity, its disappearance (desensitization) after reaction to an effective dose of the antigen, and its passive transfer by antibody. Later, with C. H. Kellaway, I was able, with the same preparation, to demonstrate *in vitro* the immunity which Weil

had demonstrated *in vivo*. We showed that the plain muscle, sensitized by an antibody fixed to its cells, could be completely protected from the effect of the antigen, when an excess of the same antibody was added to the fluid in which the preparation was suspended.

More recently my former colleague, Sir Percival Hartley, has shown how readily plain muscle from a normal guinea-pig can be made anaphylactically sensitive, by simply soaking it for 24-48 hours, in the cold, in a dilution of the appropriate antibody. It is then washed and suspended in a warm saline solution, to test the effect upon it of the corresponding antigen.

It seems to me that evidence such as this has proved beyond reasonable doubt, that anaphylaxis in the guinea-pig, causing it to die from a suffocating contraction of the plain muscle of the bronchioles when the antigen is reinjected, is due to the presence of an ordinary, precipitating antibody, abnormal only in its distribution, in that it is predominantly attached to the fixed cells of the tissues, and especially to those of the plain muscle, and present only in minor quantities in the circulating blood and body fluids.

Obviously this conception of anaphylaxis in the guinea-pig, as due to a predominantly cellular fixation of an ordinary antibody, is applicable only to soluble antigens. It clearly cannot be applied, for example, to what some observers have termed anaphylaxis to the blood corpuscles of another species. An intravascular haemolysis in the guinea-pig may, indeed, evoke symptoms resembling those of a true anaphylactic reaction in that animal. I feel obliged, however, again to insist that it is not the symptoms which are characteristic of anaphylaxis, but the conditions under which a common syndrome is evoked. And, from that point of view, it appears to me that we ought, in the interests of precision, to limit the application of the term anaphylaxis to the condition to which it was first applied and is still in most general use—the condition in which a previous injection of a soluble antigen has produced a specific sensitiveness to that substance.

(b) *In the dog.* In the dog, again, there is clear evidence, which was first obtained by Manwaring, to show that the condition of anaphylaxis is due to a predominant fixation of an antibody, presumably again a precipitin, to the fixed cells of a tissue. The cells in this case, however, are not those of plain muscle; on the contrary, the sensitization by antibody is concentrated in the dog on the cells of the liver. If the liver of an anaphylactic dog is excluded from the circulation, injection of the antigen into the bloodstream produces no shock; and if isolated preparations of plain muscle are made from an anaphylactic dog, they give no significant response to contact with the antigen. Nevertheless, when, with the liver in the circulation, the reinjected antigen has elicited the shock, the viscera and the blood vessels, and the blood itself, all become involved in the ensuing general syndrome. Some substances have been poured into the blood from the primarily affected liver cells, to produce these secondary effects on other organs; and we must consider a little later what these substances are.

(c) *In the rabbit.* In several ways the phenomenon of anaphylaxis in the rabbit appears to differ from that in the other species. It is far less regular in its occurrence than in the guinea-pig. The Arthus phenomenon, a local reaction to injections of a foreign protein into or beneath the rabbit's skin, seems clearly to involve a local sensitization of the tissues, presumably by the fixation of a precipitating antibody. On the other hand, the conditions for the production of a passive anaphylaxis in the rabbit, and for the elicitation of a shock in such an animal by intravenous injection of the antigen, appear to be quite different from those seen in the guinea-pig. The optimum conditions for the production of the shock in the rabbit seem to be created, when a serum giving a specific precipitate with the antigen is injected intravenously, and the antigen soon after it, or even simultaneously; so that no interval is required, as in the guinea-pig, for the removal of the precipitin from the circulation and its predominant fixation in the tissues. The shock in the rabbit then—the evoca-

tion of which is, in any case, a comparatively capricious phenomenon—would appear to be due, most probably, to the interaction of the antigen with the specific precipitin in the circulating blood, and therein to present a sharp contrast to the anaphylactic shocks in the guinea-pig and the dog.

If we enquire why such a reaction in the blood should cause a shock-like reaction in the rabbit, it seems relevant to observe that the rabbit's blood is peculiarly rich in platelets, and that it is apparently the platelets in this species which normally contain nearly the whole of the singularly high content of histamine to be found in the rabbit's blood. To the significance of this we must turn our attention almost immediately. We may note here that the central feature of the syndrome, which has been called the anaphylactic shock in the rabbit, is an obstruction of the circulation through the lungs, leading to a failure of the right side of the heart in a state of distension; and that this lethal reaction accompanies an almost complete disappearance of the platelets from the blood, and the agglutination of the few which remain.

THE MECHANISM OF THE ANAPHYLACTIC SHOCK

THE MECHANISM OF THE ANAPHYLACTIC REACTION

It was in 1910 that the late P. P. Laidlaw and I, making a first study of the organic diamine, histamine, observed the remarkable resemblance between the different symptoms which it produced in the different species and those of the anaphylactic shock in the same species—collapse of the systemic circulation in the dog, suffocating constriction of the bronchioles and permanent pulmonary distension in the guinea-pig, and congestive failure of the right side of the heart in the rabbit. Not many months earlier Biedl and Kraus had drawn attention to the even more complete resemblance between the different symptoms produced by the Peptone-

Witte, and those of the anaphylactic shock in the different species. Peptone had produced, in the dog, the obstructive congestion of the liver, retrograde congestion of the alimentary viscera and incoagulability of the blood, seen also in the dog's anaphylactic shock, but absent from the actions of histamine. This defect in the otherwise remarkable resemblance made us hesitate to suggest any direct or causal relationships, between the effects of histamine and the symptoms of the anaphylactic reaction. And this hesitation continued, until, about 1926, definite evidence began to become available of the presence of histamine as a natural constituent of the animal body, widely distributed in the cells of its active organs and tissues, held, apparently, in some inactivating association with the protoplasm of the living, uninjured cell, but readily liberated in active form from a cell exposed to even a slight injury of any kind. An intelligible conception thus began to emerge, of the anaphylactic shock as due, primarily, to a reaction of the reinjected antigen with a precipitating antibody which had become incorporated into tissue cells—especially into cells of the plain muscle in the guinea-pig and cells of the liver in the dog. The consequent aggregating reaction, like any other kind of injury to the cell, would then immediately liberate physiologically active substances from their normally inactivating associations with the cell protoplasm; and since histamine would be by far the most active of these, the actions of histamine, with their special characters in the different species, would form the most conspicuous feature of the anaphylactic shock in each case. This conception soon received direct experimental confirmation. Feldberg and his co-workers, in Berlin, demonstrated the release of histamine, when the antigen was added to the saline fluid perfusing the lung of an anaphylactic guinea-pig; while Dragstedt and his co-workers, in Chicago, found that histamine appeared in the lymph flowing from the liver of an anaphylactic dog, when the shock was produced by injecting the antigen into the general circulation. And another feature, required to complete the picture of the anaphylactic shock but

missing from the histamine syndrome, was supplied a few years later, when it was discovered that the primary injury of the liver cells in an anaphylactic dog, by contact with the antigen, or in those of a normal dog by contact with "peptone", caused them to pour into the general circulation, not only histamine, with the consequent general vasodilatation and spasms of visceral plain muscle, but also heparin, with the resulting loss of the coagulability of the blood. It is, of course, possible that a cellular antibody might be attached, not only to cells of the solid tissues, but to the leucocytes and platelets in the blood, where its presence, with that of histamine, might play a significant part in the genesis of the shock in certain species. And, with that additional possibility in mind, we may briefly consider the separate applications of this general conception to the anaphylactic reactions of different species.

(a) *In the guinea-pig.* The resemblance of the histamine action to the anaphylactic shock is particularly close in this species; and the fact that the release of histamine could be demonstrated when the antigen was applied to the anaphylactic plain muscle, isolated as completely as possible from other tissues, appeared to leave little doubt of its significance. Rocha e Silva, however, has recently observed that the histamine released from an isolated guinea-pig's lung, when the so-called "agar-anaphylatoxin" is added to the saline perfusion, is much greater in amount than that which is released from a similar preparation of the anaphylactic lung, when the specific antigen is perfused. He appears to argue from this that histamine from other sources than the sensitized plain muscle itself takes part in the anaphylactic shock in this species. The observation is interesting in itself, as evidence that the agar-treated serum thus releases histamine from the lung in the absence of blood; and it is to be presumed that the relatively large output of histamine thus evoked is mainly from the endothelium of the blood capillaries and the walls of the alveoli, of which the lung tissue, with its remarkably high content of histamine, is predominantly composed. The

point of chief importance for our problem, however, is not the *quantity* of the histamine released, but its effectiveness in producing the constriction of the bronchioles which forms the central feature of the anaphylactic shock. And in the true anaphylactic shock, as Kellaway and I were able to demonstrate, the constriction of the bronchioles is much more forcible and complete than that produced by any of the anaphylatoxins, even though their effects may be accompanied by a much larger output of histamine, from other tissue cells, than that which is released, in the anaphylactic reaction, directly from the cells of the plain muscle itself. In the same connexion, it may be noted that the anaphylatoxins cause a practically complete disappearance of the platelets from the blood of the guinea-pig, while the specific antigen, in the true anaphylactic shock, has very little effect of that kind.

(b) *In the dog.* Although the anaphylactic reaction in the dog was the first to be observed—the historic event, indeed, which is commemorated on this occasion—it appears to me that there are details of the mechanism of its production which are yet incompletely understood. A primary reaction in the liver, with secondary effects due to portal obstruction, on the one hand, and to the liberation of products from the poisoned liver, histamine and heparin in particular—these are on evidence which seems to be convincing. Recent observations by Rocha e Silva and others, however, appear to me to show quite definitely, that the full reaction of the anaphylactic liver with the antigen involves the cooperation, not merely of the blood, but of the blood in its natural, coagulable condition. Only when both are present, the sensitized liver and the coagulable blood, can the specific antigen produce the full, intensely congestive reaction, with the resulting abundant output of histamine and heparin; but the evidence yet available appears to be inadequate to explain the nature of the contribution made by the blood to the full reaction.

(c) *In the rabbit.* As I have already suggested, the available evidence, concerning the phenomenon which has been described as the acute anaphylactic shock in the rabbit, sug-

gests that the primary reaction may well take place in the blood. The rabbit's blood contains an unusual abundance of platelets, and these appear to be of a special kind and to contain a large proportion, at least, of the histamine in which, moreover, the rabbit's blood is peculiarly rich. There is direct evidence, indeed, that the addition, *in vitro*, of a specific antigen to the blood of a rabbit containing the corresponding antibody, causes a release of histamine from the cellular elements of the blood into the plasma. The distinction, indeed, between the anaphylactic and the immune conditions seems to be much less clearly defined in the rabbit than in the dog and, particularly, in the guinea-pig. The reaction which has been recognized as an anaphylactic shock in the rabbit may involve, therefore, a primary antigen-antibody reaction in the blood, with a release of histamine into its plasma and a resulting constriction of the pulmonary vessels, to the obstructive effect of which clumps of agglutinated platelets may also contribute—apart from any possibility of a direct sensitization of the pulmonary arterioles to the effect of the antigen.

CONCLUSION

Anaphylaxis, then, is not the opposite of immunity, as its discoverers naturally supposed when they observed a special manifestation of it. It is, on the contrary, a phase of immunity, in which a precipitating antibody is located in such a relation to living cells of the fixed tissues or, in certain cases, of the circulating blood, that its union with the reinjected antigen subjects the cells to a sudden injury. To this injury, as to injury of any kind, mechanical or chemical, these cells respond by the liberation of substances normally held in their protoplasm. Some of these substances have powerful physiological or pharmacodynamic actions, which appear when they are thus liberated; and conspicuous among such active substances are histamine and heparin, to the actions of which, as we have seen, we can attribute different phases of the anaphylactic syndrome, as this is observed in different species.

On such a conception of anaphylaxis, it is clear that there is nothing specific or characteristic about the syndrome, the association of symptoms seen in the anaphylactic shock. It is the production of that syndrome by an immunological reaction that identifies the shock as anaphylactic. A shock-reaction of the same general type can be produced in a variety of ways, and by a growing range of chemical substances; some of these are thrown out of solution, so as to form precipitates, when injected into the blood stream, and others so directly injure fixed tissue cells, as to provoke a sudden liberation from them of histamine; and thus have been produced reactions which those who observed them have described as "anaphylactoid reactions", "crises nitritoides", or "crises hémoclasiques".

Then there is the group of naturally acquired specific idiopathies, commonly termed "allergies", and most familiar in man. It seems to be beyond doubt that these are also due to specific antibodies, predominantly fixed to the cells of the sensitive tissues—to those of the epidermis in the case of an urticaria, to those of the naso-pharyngeal epithelium in the case of a hay-fever or other specific rhinitis, and to those of the epithelium and the plain muscle coats of the bronchioles, in the case of a specific allergic asthma. Obviously such a naturally acquired condition, is closely related to, if not identical with, some kinds, at least, of artificially produced anaphylaxis. And the part played, in the production of the characteristic symptoms, by the release of histamine when the antigen makes contact with the allergic cells, is made clear, not only by the nature of the symptoms themselves, but by the successful suppression of these symptoms, in many cases, by remedies of the growing class of the "antihistamines", the development of which had its beginning with the researches made during the war and the Occupation, here, in Paris, by Dr. Halpern and Dr. Bovet, and now being so eagerly pursued all over the world.

And we may feel certain that all such advances of knowledge, with regard to related conditions, will promote a more

complete understanding of the condition of anaphylaxis, as produced under the now classical, experimental conditions. And we may be glad, I think, to recognize that progress is so well advanced towards a complete understanding of the phenomenon of anaphylaxis, as we celebrate this fiftieth anniversary of its discovery.

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