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A NEW VIEW OF ADRENERGIC NERVE FIBRES, EXPLAINING THE ACTION OF RESERPINE, BRETYLIUM, AND GUANETHIDINE*

BY

J. H. BURN, M.D., Hon.Sc.D., F.R.S.

Oxford

It is scarcely too much to say that our ideas of the sympathetic control of vascular tone are undergoing a revolution at the present time. At the beginning of this century Elliott (1904) put forward the suggestion that a post-ganglionic sympathetic fibre might transmit its impulse by liberating adrenaline. That suggestion has been generally accepted, with the reservation that the transmitter might not be adrenaline itself but something closely related to it. During the years 1920 to 1937 Cannon and his colleagues established this mode of sympathetic action, and Euler (1948) and Peart (1949) finally showed that the transmitter was for the most part noradrenaline. The picture seemed to be complete, and the matter seemed to be settled.

The Perfused Vessels of the Dog Leg

Almost by accident I made some observations 30 years ago which were not explained by this simple scheme. I had been introduced to perfusion experiments by Sir Henry Dale and had seen their usefulness in analysing the action of histamine. In 1928 the Dale–Schuster pump became available, and I set out to study the effect of stimulating the sympathetic fibres on the vessels of the dog hindleg. The blood was pumped into the femoral artery, and was collected from the femoral vein. A second pump then drove it through the lungs, and then it went back to the leg. To set up the perfusion with the use of only one dog rather than two, it was necessary to leave the hindleg without a circulation for about 45 minutes. When the perfusion began, the tone in the hindleg vessels was very low, and therefore adrenaline was added drop by drop to the blood in the venous reservoir to bring the tone up to its normal level. I then observed the effect of stimulating the post-ganglionic fibres in the lumbar sympathetic chain, and saw that it caused, not vasoconstriction, but vasodilatation.

Vasodilatation was often the only response obtained throughout an experiment, but, in some experiments, after an hour or two the response changed to vasoconstriction. It was usually true that brief stimulation, for three seconds only, caused vasodilatation, while in the later stages of an experiment a longer stimulation for 30 seconds caused vasoconstriction. It appeared that the longer the time during which adrenaline was added to the blood the more likely was stimulation to cause vasoconstriction, and I came to the conclusion that "if sympathetic stimulation releases adrenaline, then a store of adrenaline is required at the sympathetic nerve endings ready for release." I thought that in my experiments this store must have been destroyed during the period in which the hindleg vessels were without a circulation, and that with the addition of adrenaline to the blood the store was gradually replenished (Burn, 1932).

Later work showed that the vasodilatation caused by sympathetic stimulation was due to the release of acetylcholine, and indicated that there were cholinergic fibres in the lumbar sympathetic chain (Bülbring and Burn, 1935). This work did not, however, explain why cholinergic fibres were unaffected by a period of anoxia which put the adrenergic fibres out of action.

Noradrenaline in Organs

After Euler had found that noradrenaline was present in sympathetic nerves, and had suggested that it was the transmitter, Schmiterlöw (1948) demonstrated that noradrenaline was present in the walls of the blood-vessels, and Euler and Purkhold (1951) demonstrated that noradrenaline was present in the spleen, the liver, the kidney, and the salivary glands. Goodall (1951) also demonstrated that it was present in the heart. Moreover, this noradrenaline disappeared from the organs when the sympathetic nerves degenerated, and Euler therefore concluded that the noradrenaline in the organs must be located in the terminations of the sympathetic nerves themselves.

Action of Reserpine

The beginning of recent developments can be traced to the observation of Bertler, Carlsson, and Rosengren (1956) that when a large dose of reserpine was given by intravenous injection to a rabbit, and the rabbit was killed 16 hours later, the heart no longer contained any noradrenaline. Whereas normal rabbit hearts contained 1.57 μg./g., hearts from rabbits injected with reserpine contained only 0.03 μg./g. Later Burn and Rand (1957) showed that the injection of reserpine had a similar effect on the noradrenaline in the wall of the thoracic aorta, both in the rabbit and in the dog. They also examined the effect of stimulating the lumbar sympathetic on the perfused vessels of the dog's hindleg. In these experiments the perfusion began at the moment of stopping the natural circulation, so that in a normal dog stimulation always caused the expected vasoconstriction.

However, when the dog had been given reserpine on the two preceding days, stimulation caused dilatation as in my 1932 experiments; the dilator effect was abolished in the presence of atropine, and was due to acetylcholine. Since the sympathetic transmitter is known to be mainly noradrenaline and not adrenaline,

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noradrenaline was added drop by drop to the venous reservoir, 0.5 mg. being added to 1 litre of blood. The noradrenaline caused vasoconstriction, but when no more was added the constriction gradually disappeared, and the tone in the vessels returned to the initial level. When sympathetic stimulation was applied at this point vasoconstriction was produced. Thus it was learnt that sympathetic stimulation caused vasodilatation, and was unable to cause vasoconstriction when there was no store of noradrenaline in the vessel walls, and it was also learnt that an infusion of noradrenaline restored the power to cause vasoconstriction (Burn and Rand, 1958a). This evidence confirmed the view put forward in 1932, which has already been quoted.

From the physiological point of view it was important to know whether the effect of noradrenaline in restoring the constrictor effect of sympathetic stimulation applied only when the animal had been given reserpine. Was the effect of an infusion of noradrenaline one which could be produced in the perfusion of normal vessels ? We found that the effect was readily seen in normal vessels, though the increase was of short duration. Longer-lasting effects were obtained when we made plethysmograph records of the leg volume of dogs anaesthetized with chloralose. Having recorded the effect of stimulating the lumbar sympathetic chain, using stimuli of varying strengths, we gave an intravenous infusion of noradrenaline, and when the infusion was complete (the amount infused varying from 0.25 to 0.5 mg.) we waited until the direct effect of the noradrenaline had passed off. Stimuli of a strength which previously had no effect now produced vasoconstriction, and the smallest effective stimulus was in some experiments reduced to one-sixth of its previous value (Burn and Rand, 1960a).

Effect of Variation in the Store

The results indicated that the effect of an impulse passing along a post-ganglionic sympathetic fibre varied greatly according to the size of the store of noradrenaline present at the nerve ending. It is therefore evident that the action of reserpine in lowering the blood-pressure is due to the reduction of the store of noradrenaline in the vessel walls and in other organs such as the heart and spleen. When the patient is given reserpine the amount of noradrenaline in the sympathetic nerve endings is reduced, and therefore the impulses passing down the sympathetic nerves cause less vasoconstriction.

Direct evidence that the infusion of noradrenaline increased the amount of extractable noradrenaline was obtained by Pennefather and Rand (1960) for the kidney and for the horn of the uterus. They used these organs because they are paired. Evidence was also obtained by Whitby, Hertting, and Axelrod (1960) for the spleen and for the heart, using radioactive noradrenaline.

It is likely that in those who are subject to emotional crises there will be a larger output of noradrenaline from the adrenal medulla than in others. This larger output will fill the stores at sympathetic nerve endings and may thus be responsible for a higher level of blood-pressure.

Bretylium

A second substance, which was introduced as a hypotensive agent in 1959, is bretylium. The forerunner of bretylium was choline 2,6-xylyl ether bromide, which was synthesized by Hey (1952) in Leeds, and was shown by Exley (1957) to prevent the release of noradrenaline when post-ganglionic sympathetic fibres were stimulated. The blocking action of bretylium has been thought to be due to a local anaesthetic action on the nerve fibres, and Boura and Green and their colleagues (1960) have found that radioactive bretylium was taken up in greater amount by sympathetic ganglia than by other ganglia, and in greater amount by adrenergic nerves than by cholinergic nerves. Thus Boura and Green supposed that bretylium acted by preventing the conduction of impulses along the sympathetic post-ganglionic fibres. The action has, however, been located by Exley and Fleming (1960) on the terminal portion of these fibres rather than along their course.

The work of Burn and Rand (1960b) led them to support the conclusion of Exley and Fleming and to give it a more precise form. They were interested in the curious observation that many sympathetic effects were produced in the body by nicotine and by acetylcholine in the presence of atropine, in circumstances in which nicotine and acetylcholine were acting beyond the location of sympathetic ganglia. An example of this is given by the vessels of the rabbit ear. When the fibres from the superior cervical ganglion are stimulated the vessels constrict. They also constrict when a small quantity of nicotine is injected into the fluid perfusing the vessels, or when, in the presence of atropine, a small quantity of acetylcholine is injected. If the ear is taken from a rabbit previously given reserpine, then stimulation of the sympathetic fibres does not cause constriction, and likewise the injection of nicotine or of acetylcholine does not cause constriction (Burn and Rand, 1958b). From this it was evident that the constriction caused in the normal ear by nicotine or by acetylcholine was due to the release of noradrenaline, just as was the constriction caused by sympathetic stimulation.

The striking similarity in the action of acetylcholine and in the action of sympathetic stimulation both in the effect they produce and in the way of producing it is not confined to the rabbit-ear vessels. Thus it is seen in the isolated atria of the rabbit heart, where, in the presence of atropine, acetylcholine causes acceleration of the rate, but not when the atria are taken from a rabbit which has been treated with reserpine. It is also seen in the pilomotor response of the cat's tail. This can be studied by removing most of the hair of the cat's tail with clippers, leaving a series of tufts. Stimulation of the lumbar sympathetic chain causes the tufts to erect. The injection of acetylcholine into the skin at the base of a tuft also causes that tuft to erect. We found that noradrenaline was present in the skin of the cat's tail ; when a cat was injected with reserpine the noradrenaline in the skin of the tail was greatly reduced. When a cat was injected with reserpine the injection of acetylcholine into the skin at the base of a tuft had almost no effect in causing erection of the tuft (Burn, Leach, Rand, and Thompson, 1959).

Another situation where similar observations were made was in the colon of the rabbit, after a portion of the colon was removed from the freshly killed animal, together with the extrinsic nerves, and was suspended in a bath. Sympathetic stimulation caused inhibition of the pendular movements, and the addition of a low concentration of nicotine to the bath had the same effect. If the rabbit had been injected with reserpine, however, both sympathetic stimulation and nicotine lost their inhibitory action (Gillespie and Mackenna, 1959).

Thompson (1958) has described a preparation of the cat's nictitating membrane which can be set up in a bath with its sympathetic supply. Stimulation of the sympathetic fibres causes contraction of the smooth muscle of the membrane, and so also does addition of nicotine to the bath. When a cat has been injected with reserpine the isolated nictitating membrane no longer contracts on sympathetic stimulation or on the addition of nicotine to the bath.

The noradrenaline which can be extracted from an organ with a sympathetic supply disappears not only when the animal is injected with reserpine but also when the nerves degenerate (Euler and Purkhold, 1951). Stimulation of the sympathetic fibres causes contraction of the spleen, and so also does injection of acetylcholine into the splenic artery. However, when the sympathetic fibres degenerate, injection of acetylcholine into the splenic artery no longer causes contraction of the spleen (Daly and Scott, 1961). When a cat was treated with reserpine, stimulation of the sympathetic fibres no longer caused contraction of the spleen and the amount of noradrenaline in the spleen was found to be greatly reduced.

In the six examples which have been given the same curious parallelism was found. Sympathetic stimulation caused effects which were mimicked by acetylcholine (in the presence of atropine) or by nicotine. These effects were no longer seen in animals treated with reserpine or in organs to which the sympathetic fibres had degenerated. Thus it appeared that sympathetic stimulation acted by liberating noradrenaline from the store (probably in the nerve endings), and acetylcholine or nicotine also acted by liberating noradrenaline from the same store. What was the meaning of this common action? Did it mean that the sympathetic nerves liberated acetylcholine?

Cholinergic Fibres in the Sympathetic Nerves

The work of Brücke (1935) suggested that this might be so. He had shown that acetylcholine caused erection of the hairs of the tail as already described, the amount required being small, about 5 μg. But in addition he made the observation that when a larger amount was injected, such as 0.2 mg., 40 times greater, there was again a brief erection of the tuft, after which sympathetic stimulation was no more effective. The larger amount of acetylcholine had blocked sympathetic stimulation, though stimulation still erected adjacent tufts where acetylcholine had not been injected. Burn and Rand (1960b) confirmed these observations, which suggested that the sympathetic fibres to the cat's tail were cholinergic, and that the acetylcholine released by impulses passing down them caused a discharge of noradrenaline.

In other words, we had a new conception of an adrenergic mechanism. Hitherto an adrenergic nerve had been conceived as a nerve in which the impulse released noradrenaline directly. Now it appeared that a sympathetic nerve might be cholinergic, liberating acetylcholine, and that the acetylcholine might in turn release noradrenaline from a store. Such a mechanism might be blocked by a large dose of acetylcholine which would paralyse the effect of small amounts of acetylcholine released from the nerve.

The conception that post-ganglionic sympathetic fibres might in some cases be cholinergic was not new. Thus Dale and Feldberg (1934) showed that all the fibres to the sweat glands were cholinergic. Earlier than this, Euler and Gaddum (1931) had found that some fibres from the superior cervical ganglion to the vessels of the tongue were cholinergic; Bülbring and Burn (1935) showed that some fibres to the vessels of the dog's hindleg were cholinergic. Other examples were given by the nictitating membrane (Bacq and Fredericq, 1935), by the dog uterus (Sherif, 1935), and by the cat heart (Folkow, Frost, Haeger, and Uvnäs, 1948). But, except for the fibres to the sweat glands, cholinergic fibres in the sympathetic nerves were regarded as being mixed together with adrenergic fibres which were assumed to constitute the principal innervation.

Hence the idea put forward by Burn and Rand (1959), that cholinergic fibres might be part of an adrenergic mechanism, was a new idea which had not been previously considered. If adrenergic effects were obtained as a result of the release of noradrenaline by cholinergic fibres, the question was raised whether there were, in addition, adrenergic fibres in the hitherto accepted sense which liberated noradrenaline without the intervention of acetylcholine.

The Splenic Nerves

The first step towards deciding this was to see if cholinergic fibres could be demonstrated in the long post-ganglionic fibres running to the spleen and in those running from the superior cervical ganglion to the vessels of the rabbit ear.

When acetylcholine was injected intravenously into a cat which had been treated with reserpine the spleen dilated. When the splenic nerves were stimulated in such a cat the spleen did not constrict as it usually does, but dilated. This dilatation was evidently due to the liberation of acetylcholine, because it was greater in the presence of eserine (which is an anticholinesterase) and it was abolished by atropine. Brandon and Rand (1961) examined the perfused spleen in a cat which had been treated with reserpine, allowing the effluent from the vein to run over a strip of muscle from the guinea-pig ileum. Stimulation of the splenic nerves had no effect on the contractions of this muscle. However, when an anticholinesterase (neostigmine) was added to the perfusion fluid, stimulation of the splenic nerves was followed by contraction of the ileum. In the presence of atropine this contraction did not occur. The evidence clearly showed the presence of cholinergic fibres in the splenic nerves.

By similar methods the presence of cholinergic fibres was demonstrated in the post-ganglionic supply to the vessels of the rabbit ear, in the hypogastric nerves to the cat uterus, and in the post-ganglionic supply to the nictitating membrane of the cat.

Action of Bretylium

The evidence thus indicated that in all post-ganglionic sympathetic fibres some cholinergic fibres were present. How could it be determined whether these were only an admixture or whether they constituted the whole supply? At this point bretylium provided a guide. In the first place the chemical structure of bretylium suggested that it would be likely to act more in relation to acetylcholine than to noradrenaline. Then bretylium was found to block the action of acetylcholine on the isolated atria of the rabbit heart. As already described, in the presence of atropine, acetylcholine quickens the

rate and force of the atrial beat. Bretylium blocked this action in the same concentration as it blocked the action of sympathetic stimulation (Huković, 1960). Then bretylium was found to block the action of acetylcholine (in the presence of atropine) in causing constriction in the vessels of the perfused rabbit ear, and it blocked the constriction caused by nerve stimulation in the same concentration.

Thus the evidence suggested that the action of bretylium was indeed exerted on the nerve terminations as Exley and Fleming had found, and that specifically it blocked the action of the acetylcholine liberated by the cholinergic fibres, so that it could not release noradrenaline. Now, since bretylium blocked the whole of the post-ganglionic sympathetic supply to the atria and to the rabbit-ear vessels, it followed that not merely some but all of these fibres were cholinergic. The earlier view was that bretylium prevented the release of noradrenaline. But tyramine is an agent which releases noradrenaline from the store at the sympathetic nerve ending by a direct action on the granules containing noradrenaline (Schümann, 1961). It was observed that, when bretylium blocked the release of noradrenaline by sympathetic stimulation, the effect of tyramine was not only undiminished but was increased (Burn and Rand, 1960b). Thus bretylium appeared to be an agent which prevented the acetylcholine coming from cholinergic fibres from liberating noradrenaline from the store, without interfering with the action of tyramine on the store.

At each of the sites where acetylcholine acts the blocking agents differ. Thus at the termination of the parasympathetic nerves the blocking agent is atropine. At the neuromuscular junction the blocking agent is tubocurarine. At sympathetic and parasympathetic ganglia the blocking agent is hexamethonium. Now we have a fourth site, at the terminations of the post-ganglionic sympathetic fibres, where the blocking agent is bretylium.

Action of Hemicholinium

This was the picture in July, 1959, when Rand and I parted company, he going to Sydney and I into retirement. When in Sydney, Rand became interested in the action of hemicholinium, a substance introduced by Long and Schueler (1954). The essential feature of the action of hemicholinium was discovered by MacIntosh, Birks, and Sastry (1956) in Montreal. They showed that it interfered with the formation of acetylcholine. They perfused the superior cervical ganglion, and collected the venous effluent, estimating the acetylcholine present in it. The addition of hemicholinium to the perfusion fluid did not alter the amount of acetylcholine in the venous effluent so long as the preganglionic fibres were stimulated at a slow rate; but, when the rate was faster, hemicholinium reduced the amount of acetylcholine leaving the ganglion and reduced the transmission of impulses through the ganglion. Both the defect of transmission and the fall in the outflow of acetylcholine were made good by the addition of choline to the perfusion fluid. MacIntosh and his colleagues demonstrated that the synthesis of acetylcholine by brain tissue was diminished by hemicholinium, but that the synthesis returned to its normal rate when choline was added.

Chang and Rand (1960) tested the effect of hemicholinium in a series of isolated preparations in which the sympathetic nerves could be stimulated. They used the hypogastric nerve to the vas deferens of the guinea-pig; the hypogastric nerve to the rabbit uterus; the sympathetic supply to the rabbit colon; the sympathetic supply to the vessels of the rabbit ear; and the sympathetic supply to the cat atria. With each of these preparations they obtained evidence that the effect of stimulation of the sympathetic fibres was blocked in the presence of hemicholinium, but was restored when the hemicholinium was removed or when choline was added. With the preparation of the vas deferens they were able to show that the inhibitory effect of hemicholinium was evident only when the rate of stimulation was fast. In addition they tested the effect of hemicholinium on the pilomotor response in the cat's tail to sympathetic stimulation. They found that when hemicholinium was injected into the skin at the base of the tufts, then during stimulation those tufts were slower in erecting and quicker in falling back.

Thus Chang and Rand found that in six preparations with a sympathetic innervation hemicholinium blocked the effect of stimulation in the same way as it blocked the effect of stimulating the phrenic nerve on the rat diaphragm. This indicated the existence of a cholinergic transmission at the terminations of the sympathetic post-ganglionic fibres. Since the block of stimulation was usually complete, and always nearly complete, the observations indicated that the innervation was not only cholinergic in part but was entirely cholinergic.

Location of the Store

The conception of the adrenergic nerves has hitherto been founded on the evidence that noradrenaline is present in the nerves (Euler, 1948) and is released on stimulation (Peart, 1949). Schümann (1956) discovered that dopamine, the precursor of noradrenaline, is present in the nerves, and Holtz and Westermann (1956) found that the enzyme dopa-decarboxylase, which converts the amino-acid dopa into dopamine, is present in the nerves. In view of these observations, how is the suggestion of a cholinergic mechanism to release the noradrenaline to be envisaged?

This question can only be settled with the help of the histochemists and the electron microscopists; thus work has recently been done by Abrahams, Koelle, and Smart (1957) on the nerve fibres running from the supraoptic nucleus to the posterior lobe of the pituitary body. Staining showed that these fibres contained acetylcholinesterase, and that therefore they were probably cholinergic. Now this tract of nerves has been examined by Gerschenfeld, Tramezzani, and De Robertis (1960), using the electron microscope, and they observed the presence in the fibres of secretory granules, which increased in size towards the terminations of the fibres, from a diameter of 600 Å in the middle of the fibres to a diameter of 1,500 Å near the endings. These granules probably contained vasopressin, for when examined in a dehydrated animal those at the endings had disappeared. At the nerve endings, however, there were in addition synaptic vesicles of a diameter of 400 Å, similar to those found in sympathetic ganglia. Both Koelle and his colleagues and De Robertis and his colleagues have put forward the suggestion that, within a single fibre, a nerve impulse might release a transmitter (acetylcholine) from synaptic vesicles, and that this transmitter might in turn release vasopressin from the secretory granules. If this suggestion were to be proved correct, then a similar situation might be found in the

sympathetic post-ganglionic fibres, where acetylcholine might release noradrenaline from the same fibre.

Action of Guanethidine

It remains to describe the action of guanethidine. From what has been said in explaining the action of reserpine and of bretylium, it is easy to explain the action of guanethidine. This substance appears from present evidence to be the best of the three substances for the control of hypertension. Guanethidine, like reserpine, has the property of releasing the stores of noradrenaline in the walls of the arteries, in the heart, and in other organs possessing a sympathetic innervation. The disadvantage of reserpine, however, is that it enters the brain, and produces effects there by reducing the stores of noradrenaline and of hydroxytryptamine. The patient becomes depressed and may even suffer from melancholia. With guanethidine these effects on the brain are not seen. It is a strongly basic substance which does not pass the blood–brain barrier. The main value of guanethidine therefore is that it acts like reserpine in the periphery but has no effect on the brain.

This, however, is not the only action of guanethidine, though it is the most important one. In addition, guanethidine has an action like that of bretylium on sympathetic post-ganglionic nerve endings. Thus, when tested on the isolated preparation of the vas deferens, guanethidine, like bretylium, blocks the effect of sympathetic stimulation, and this is seen long before there is a decline of the amount of noradrenaline present in the vas deferens. Whether this blocking action is of clinical importance is not certain. Guanethidine finally has some action which resembles, superficially at least, the peripheral action of cocaine. When injected into a spinal cat, guanethidine causes a moderate though prolonged rise of blood-pressure and a sustained contraction of the nictitating membrane. The effect of noradrenaline is then increased and the effect of tyramine is depressed. Thus in the spinal cat the immediate effect of guanethidine is very like that of cocaine (Burn and Robinson, 1952).

Conclusion

The evidence that all sympathetic post-ganglionic fibres release acetylcholine, which in turn releases noradrenaline, is weighty, but for a conception of such importance cannot be regarded as complete; that is to say, it provides a working hypothesis which must be tested by further observations. The hypothesis affords a very satisfactory explanation of the observations in the perfused hindleg which were made in 1932 and again recently. However, further work is required on the mechanism of vasoconstriction following sympathetic stimulation in skin vessels, in particular those of the perfused rabbit ear. For it may be that there is here and in other places a direct release of noradrenaline without the intervention of acetylcholine. Dilatation of skin vessels has not yet been obtained experimentally, though it is easy to produce in muscle vessels. It seems likely, however, that such a dilatation is seen in the skin when a young lady blushes, and that this blush is due to the direct effect of acetylcholine.

Summary

The current conception of the adrenergic nerve fibres is that the nerve impulse releases mainly noradrenaline from the nerve ending. Recently evidence has accumulated that the nerve fibre may first release acetylcholine, and that this acetylcholine then releases noradrenaline. This action of acetylcholine is nicotine-like, and not muscarine-like, and therefore is not abolished by atropine.

The noradrenaline present in the sympathetic post-ganglionic fibres is reduced in amount when the animal is treated with reserpine or with guanethidine. When the noradrenaline is reduced, the effect of sympathetic impulses is diminished. This reduction appears to account for the hypotensive action of reserpine and of guanethidine. Guanethidine has the advantage over reserpine in that it does not affect the brain.

The amount of noradrenaline which is released by a sympathetic impulse is increased when a slow intravenous infusion of noradrenaline is given. It thus appears that the noradrenaline present in sympathetic fibres is not only synthesized there but also accumulates as a result of uptake from the blood.

Bretylium probably acts by blocking the action of the acetylcholine liberated by the nerve impulse, so that it cannot liberate noradrenaline. Guanethidine also has this blocking property.

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