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THE PART PLAYED BY CALCIUM IN DETERMINING THE RESPONSE TO STIMULATION OF SYMPATHETIC POSTGANGLIONIC FIBRES

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The inhibition of the pendular movements in a loop of rabbit ileum caused by stimulating the periarterial nerves in the mesentery depends on the calcium concentration in the bathing fluid. The inhibition is small when the concentration is low, and increases as the concentration rises. In the lower ranges of calcium concentration there is rarely any change in the response to noradrenaline, so that the increase in inhibition is due to an increase in the amount of noradrenaline released. The effect of calcium is antagonized by magnesium. In the presence of hyoscine, nicotine inhibits the ileum, and this inhibition also depends on the concentration of calcium. Acetylcholine can sometimes be shown to cause inhibition, and again this inhibition depends on the concentration of calcium. These and other experiments show a close similarity between the release of catechol amines from the adrenal medulla by acetylcholine and the release of noradrenaline from the postganglionic fibre by stimulation or by nicotine. In both instances the calcium concentration plays a decisive part.

The part played by calcium in the discharge of catechol amines from the adrenal medulla in response to acetylcholine has recently been demonstrated by Douglas & Rubin (1961, 1963). They perfused the adrenal gland in the cat with Locke solution, and measured the output of catechol amines in the adrenal vein, injecting acetylcholine to provoke a secretion. They observed that when calcium was omitted from the perfusion fluid, acetylcholine did not cause a secretion. They found that when calcium was added to the perfusion fluid, the amount of catechol amines released was related quantitatively to the amount of calcium added. They showed that calcium was the one ion which was necessary, for acetylcholine caused a release of catechol amines when the gland was perfused with a solution containing only sucrose, calcium and dextrose. On the basis of these and a great many more experiments, Douglas & Rubin reached the conclusion that the role of acetylcholine as a transmitter was to cause a brief change in the adrenal medullary cells which allowed calcium ions to penetrate them and to release the catechol amines.

Burn & Rand (1959) have suggested that the release of noradrenaline from the sympathetic postganglionic fibre is effected by acetylcholine acting as an intermediary. They have given reasons for thinking that, within the same fibre, acetylcholine is released first, and that this in its turn releases noradrenaline. If this hypothesis is true there should be a parallelism between the events in the adrenal

medulla and those in the postganglionic fibre. We therefore wished to see if the release of noradrenaline in response to postganglionic stimulation, and also in response to acetylcholine or nicotine, would be modified by calcium in the same way.

METHODS

We have used the rabbit isolated ileum preparation as described by Finkleman (1930), stimulating the periarterial nerves to produce inhibition of the pendular movements. The preparation was set up in an organ-bath of 50 ml. capacity, using Locke or Tyrode solution. The former contained (g/l.): NaCl 8.5, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5 and dextrose 2.0; the latter contained NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $NaHCO_3$ 1.0, NaH_2PO_4 0.05, $MgCl_2$ 0.1 and dextrose 2.0. The bath was bubbled with 5% carbon dioxide in oxygen and maintained at 33° C.

The electrodes were of the pattern described by Burn & Rand (1960); rectangular wave supramaximal pulses of 1 msec duration were used. Solutions of acetylcholine bromide, hyoscine hydrobromide and nicotine acid tartrate were prepared; doses of the first two were expressed in terms of the salts, and of the last in terms of the base.

RESULTS

Effect of calcium on the response to stimulation. The first experiments were made to determine the effect of raising the concentration of calcium on the inhibitory response to stimulation. The record in Fig. 1 shows the response to 300 shocks

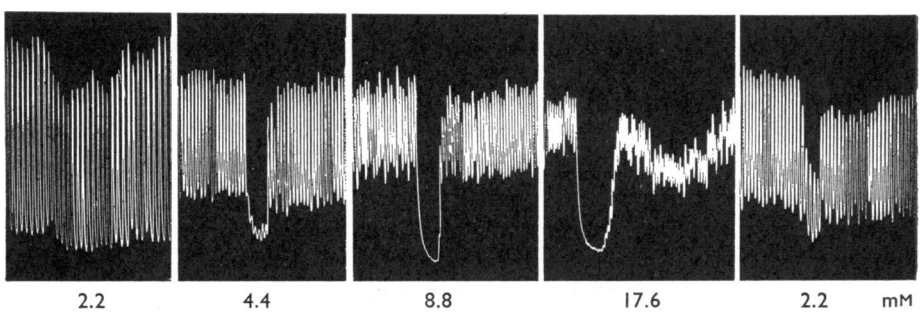

Fig. 1. Rabbit ileum preparation in Locke solution. Stimulation of periarterial nerves with 300 shocks at 10 shocks/sec. The concentration of calcium (mM) present in the bath is given below each panel. Note the increase in the inhibition as the concentration rose from 2.2 to 8.8 mM.

at 10 shocks/sec, in increasing concentrations of calcium. In 2.2 mM the inhibition was very small. In 4.4 mM the inhibition was greatly increased, and it was further increased in 8.8 mM. Beyond this, when the concentration rose to 17.6 mM the inhibition was prolonged but not increased. When the concentration was dropped to 2.2 mM, the inhibition was reduced to about its original size. Many observations of this kind were made, and the result was always the same.

Effect on the response to noradrenaline. Observations were also made in Tyrode solution, which is similar in composition to Krebs solution and is used by many workers. (Tyrode solution contains magnesium and when it was used the results were different in one particular, as will be seen.) The increase in the inhibition as

the calcium concentration rose was evident not only when the concentration was raised above normal, but also when it was raised from below normal to normal. The increase might have been due to an increase in the amount of noradrenaline liberated, or to an increase in the effect of noradrenaline. We therefore compared the effect of stimulation with that of noradrenaline in different calcium concentrations. A record of such a comparison, made when Tyrode solution was used, is shown in Fig. 2. In the left-hand panel are the effects of stimulating at 10 shocks/sec for

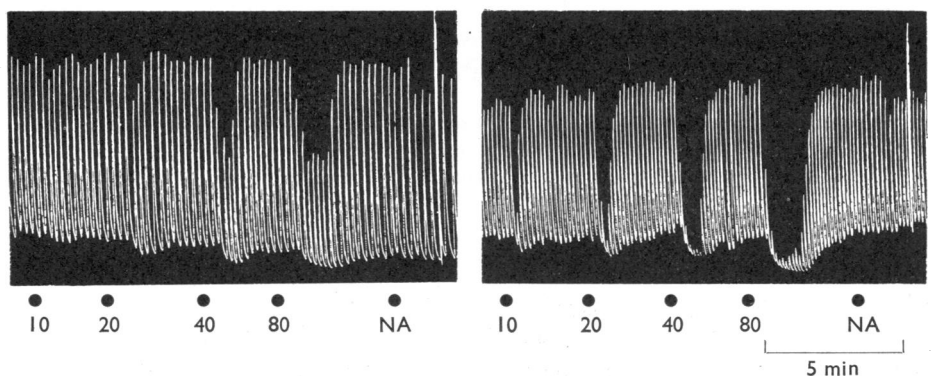

Fig. 2. Rabbit ileum preparation in Tyrode solution. Stimulation of periarterial nerves at 10 shocks/sec for 10, 20, 40 and 80 sec. The left-hand panel shows the responses in 0.6 mM-calcium, and also the response when noradrenaline (NA, 10^{-8} g/ml.) was added to the bath for 10 min. The right-hand panel shows the responses in 1.8 mM-calcium. Note that the response to noradrenaline remained the same.

10, 20, 40 and 80 sec. The effect of adding noradrenaline (10^{-8} g/ml.) is also shown. These observations were made in 0.6 mM-calcium. The right-hand panel shows the effects in 1.8 mM-calcium. The inhibition in response to stimulation was increased in every instance, but there was no change in the response to noradrenaline. The result shown in Fig. 2 was typical of the effect of raising the concentration from 0.6 to 1.8 mM-calcium ; there was no change in the response to noradrenaline in six out of seven experiments, and the increase seen in one experiment was slight. However, when the calcium concentration was raised above 1.8 mM the situation was different. In most experiments there was some increase in the response to noradrenaline, though sometimes there was none. Thus the effect of raising the calcium concentration on the response to stimulation was in no way parallel to the much smaller effect on the response to noradrenaline.

Effect of magnesium. Douglas & Rubin (1963) observed that, when Locke solution to which 10 mM-magnesium had been added was used to perfuse the adrenal gland, the response to acetylcholine was much less. In order to see if sympathetic stimulation would be similarly affected, we stimulated at two frequencies, using two different trains of shocks at each frequency. The control observations are shown in Fig. 3, *a*. Magnesium chloride (10 mM) was then added to the bath, and, as shown in Fig. 3, *b*, the tone, the amplitude of the rhythm, and the effects of the four stimulations were much reduced. The magnesium

was present in the bath for 15 min. On changing the bath fluid to Locke solution without magnesium, the tone and the amplitude were restored, but the effects of stimulation remained much smaller than in the control (Fig. 3, *c*). Even when

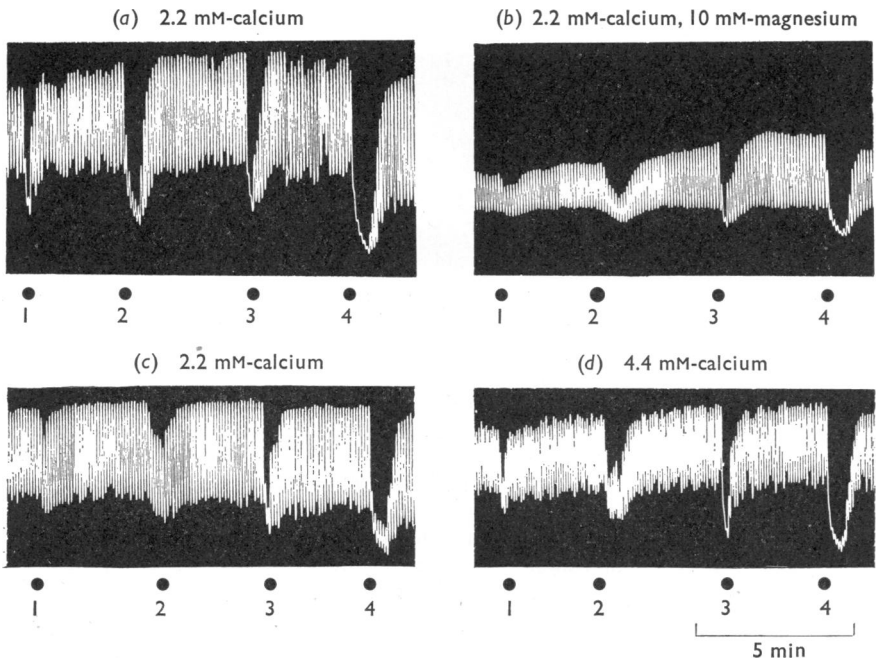

Fig. 3. Rabbit ileum preparation in Locke solution. Stimulation at 1 was at 10 shocks/sec for 10 sec, at 2 it was 10 shocks/sec for 30 sec, at 3 it was 30 shocks/sec for 10 sec and at 4 it was 30 shocks/sec for 30 sec. (*a*) Control in Locke solution; (*b*) the same stimuli applied after the addition of 10 mM-magnesium to the bath, which reduced the amplitude of contractions, the mean tone (the base line was the same in all panels) and the effect of stimulation; (*c*) after the magnesium had been removed, showing that the amplitude and tone were restored, but the inhibitions were much smaller than in (*a*); (*d*) when the calcium was raised to 4.4 mM the inhibitions were still less than in (*a*), though greater than in (*c*). There was an interval of 10 min between each panel.

the calcium concentration was raised to 4.4 mM, which increased the effects of stimulation, the responses at the low frequency of 10 shocks/sec were still less than in the control (Fig. 3, *d*). Thus magnesium had a similar inhibitory effect on the response to stimulation to that which Douglas & Rubin had observed in the adrenal gland in response to acetylcholine.

Effect on continuous stimulation. When the adrenal gland was perfused with Locke solution containing acetylcholine, the output of catechol amines was high at first, but became steadily less as time went on. If the calcium in the perfusing fluid was raised, the output of catechol amines also rose, becoming many times greater. To imitate this procedure we stimulated the periarterial nerves continuously when the calcium concentration was low, and obtained a steadily maintained inhibition which was however not complete. We then raised the calcium concentration in the

bath and observed that the inhibition became much more complete, remaining so until the stimulation stopped. Such an effect is seen in Figs. 4, *a* and *b*. In Fig. 4, *a*, the stimulation began in 0.6 mM-calcium, and was then continued when the concentration was raised to 1.8 mM. In Fig. 4, *b*, the stimulation began in a

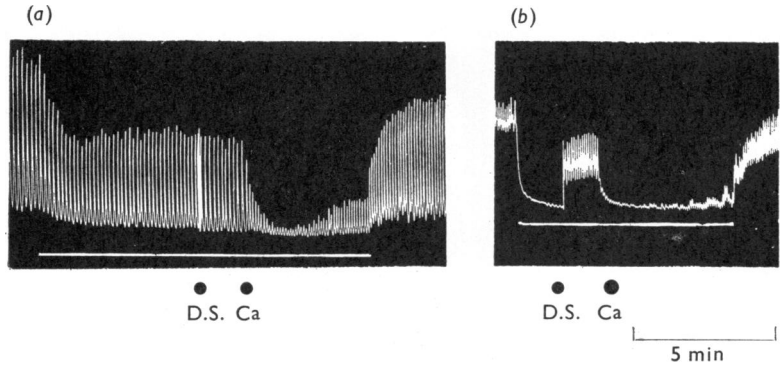

Fig. 4. (*a*) Rabbit ileum preparation in Tyrode solution; calcium concentration, 0.6 mM. Stimulation of sympathetic fibres for 15 min during the white line caused incomplete inhibition. The drum was stopped for about 5 min at D.S., while the stimulation continued. At Ca calcium was added to raise the concentration to 1.8 mM. The inhibition at once increased, and remained so until the stimulation was stopped. (*b*) Rabbit ileum preparation in Tyrode solution; calcium was added to make the concentration 6 mM. Stimulation during the white horizontal line was at 10 shocks/sec and continued for 15 min. At D.S. the drum was stopped for 8 min until the inhibition had declined and the rhythm had partly returned. At Ca the calcium concentration was raised to 18 mM. The inhibition at once became complete and remained almost complete until the stimulation stopped.

calcium concentration of 6 mM, and was continued until some of the tone and rhythm had returned. The calcium concentration was then raised to 18 mM and this produced prompt inhibition which continued until the stimulation stopped about 5 min later. The rhythm at once returned. These results appear to be the precise counterpart of the changes observed in the adrenal gland.

The action of nicotine and of acetylcholine. In the presence of hyoscine (10^{-7} g/ml.) nicotine when added to the bath in a concentration of about 2×10^{-6} g/ml. inhibits the pendular movements of the ileum. We compared this effect in Tyrode solution containing different amounts of calcium. In Fig. 5 the ileum was first placed in Tyrode solution containing 0.6 mM-calcium. The addition of nicotine caused a very brief inhibition which gave way to a motor response; at the end of 1 min the nicotine was washed out of the bath. The calcium concentration was then raised to 1.8 mM, in which nicotine produced a larger inhibition, not followed by a motor response. The concentration was then raised to 3.6 mM, in which nicotine produced a much larger inhibition. Thus the inhibition produced by nicotine in the presence of hyoscine was affected by changes in calcium concentration exactly as was the inhibition produced by sympathetic stimulation.

While nicotine produces inhibition in nearly all preparations provided that hyoscine is present, acetylcholine rarely does so in Tyrode solution, though it is effective in Locke solution, which does not contain magnesium. Unfortunately we

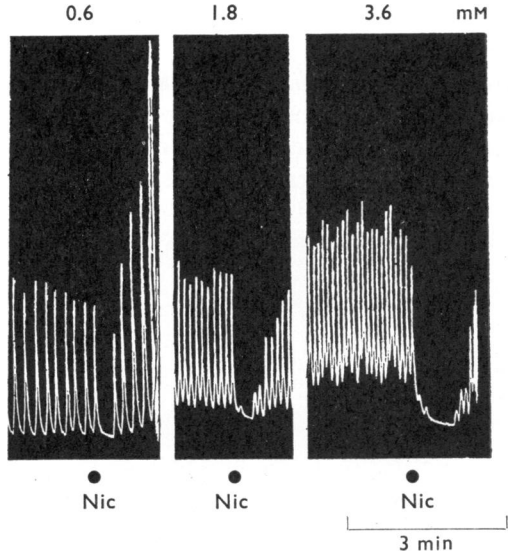

Fig. 5. Rabbit ileum preparation in Tyrode solution. Hyoscine (10^{-7} g/ ml.) was present in the bath. In the left-hand panel the calcium concentration was 0.6 mM. At Nic nicotine (2×10^{-6} g/ml.) was added to the bath; it caused a small inhibition followed by a motor response. In the middle panel the calcium concentration was 1.8 mM; nicotine caused a greater inhibition. In the right-hand panel the calcium concentration was 3.6 mM; nicotine caused a still greater inhibition.

did not test the effect of changing the calcium concentration in Locke solution, and this remains to be done. In Tyrode solution the main effect of acetylcholine in the presence of hyoscine is a contraction of the loop of ileum. However, in some preparations this contraction is preceded by a short period of inhibition, and in one experiment we were able to observe that this period of inhibition was longer when the calcium concentration was higher. Before acetylcholine was added to the bath the drum was driven at a faster rate, so that the interval between each wave of the pendular movement could be measured. The measurements were made under magnification when the record was varnished. The results are shown in Table 1. The distance between two waves before acetylcholine was added was almost constant. This distance diminished as the calcium concentration rose, the pendular movements occurring at a faster rate. Table 1 shows that when acetylcholine was

TABLE 1

THE EFFECT OF ACETYLCHOLINE IN INHIBITING PENDULAR MOVEMENTS OF THE RABBIT ISOLATED ILEUM PREPARATION AT DIFFERENT CALCIUM CONCENTRATIONS IN TYRODE SOLUTION

This result was exceptional (see text). The acetylcholine concentration was 40 μg/ml.

Calcium concentration (mM)	Distance between two pendular waves: Before acetylcholine (mm)	With acetylcholine (mm)	Increase (mm)	%
1·8	2·9	4·0	1·1	38
5·4	1·7	4·0	2·3	134
10·8	1·2	5·8	4·6	383

added the distance to the next wave increased, and that the increase was proportional to the amount of calcium present.

Release of noradrenaline by calcium. Douglas & Rubin (1961) observed that, when the adrenal gland had been perfused for a time with calcium-free Locke solution, the addition of calcium by itself caused an output of catechol amines. We carried out the experiment illustrated in Fig. 6 in which the ileum preparation

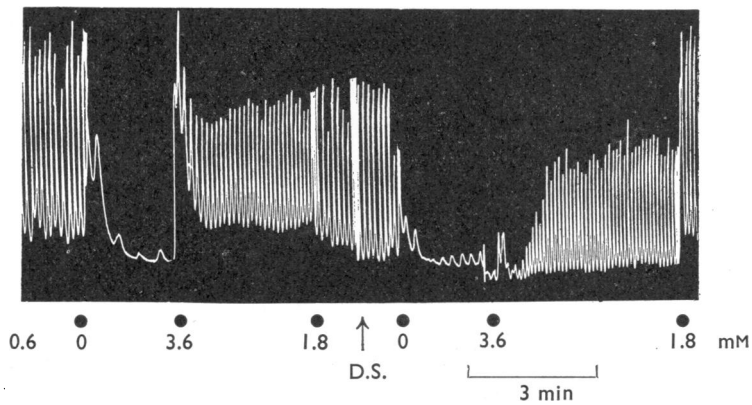

Fig. 6. Rabbit ileum preparation in Tyrode solution, calcium concentration 0.6 mM. At the first dot, the fluid in the bath was changed to calcium-free Tyrode solution. At the second dot, calcium was added to make 3.6 mM; note the immediate contraction and restoration of rhythm. The bath fluid was then changed to Tyrode solution with 1.8 mM-calcium. At D.S. the drum was stopped for 10 min. The bath fluid was next changed to calcium-free Tyrode solution, and this was left for 20 min, the drum being stopped. At the next dot calcium was again added to make 3.6 mM. There was no prompt contraction and the rhythm which developed slowly had a low mean tone. On changing the bath fluid to Tyrode solution with 1.8 mM calcium the mean tone promptly rose.

was first suspended in Tyrode solution with 1.8 mM-calcium. The bath fluid was then changed to calcium-free Tyrode solution. Within 2 min the rhythm was arrested and without delay calcium was added to 3.6 mM. There was a prompt contraction and resumption of the rhythm. The ileum was then left for about 10 min in Tyrode solution with 1.8 mM-calcium.

The experiment was then repeated but the ileum remained in calcium-free Tyrode solution for 20 min instead of 2 min. On raising the calcium to 3.6 mM at the end of this time there was no abrupt contraction, but there was a slow development of a rhythm with a low mean tone. On washing out the bath with Tyrode solution containing 1.8 mM, the mean tone rose at once.

In further trials using Tyrode solution, we were unable to repeat this result. However, when we used Locke solution, which does not contain magnesium, we obtained the result with regularity.

DISCUSSION

The observations which have been described show that the inhibitory effect of stimulating the periarterial nerves on the pendular movements of a loop of rabbit

ileum depends on the concentration of calcium present. When the ileum was suspended in Tyrode solution containing one-third of the normal calcium concentration, the inhibition due to stimulation was slight, and was greatly increased on raising the concentration to normal. In this range there was rarely any change in the response to noradrenaline, and therefore the increase in the inhibition seemed to be due to an increase in the amount of noradrenaline liberated. We have in fact observed that changes in calcium concentration modify the effect of stimulating the sympathetic postganglionic fibres to the ileum in the same way as Douglas & Rubin (1961, 1963) found that they modify the amount of catechol amines released from the perfused adrenal medulla by acetylcholine.

In the presence of hyoscine, nicotine and sometimes also acetylcholine inhibit the ileum when added to the bath, and this inhibition like that due to sympathetic stimulation was also increased as the calcium concentration was raised. In the adrenal medulla the action of calcium was antagonized by magnesium, and we observed a similar antagonism when stimulating the sympathetic fibres to the ileum. When the adrenal medulla was stimulated continuously by including acetylcholine in the perfusion fluid, the output of catechol amines gradually fell to a low level, and at that point a rise in the calcium concentration produced a large rise in the output of catechol amines. This occurred over a wide range of calcium concentrations, up to an eightfold increase. We have similarly observed that, when the sympathetic nerves were stimulated continuously, a rise in the calcium concentration in the bath increased the inhibition, this increase being seen even when the rise was from the already high figure of 6mM to 18 mM, which is ten-times the normal concentration in Tyrode solution.

Douglas & Rubin (1961, 1963) observed that, after a period of perfusion with a calcium-free solution, calcium itself increased the output of catechol amines. They attributed this to an increase in the permeability of the chromaffin cells which occurred when calcium was absent. The readmission of calcium was followed by unimpeded entry of the calcium into the chromaffin cells with release of catechol amines. We observed changes which were consistent with a similar release of noradrenaline by calcium after a period in which calcium was absent. These changes were only once seen when Tyrode solution was used, but were seen in all experiments in which Locke solution was used. Tyrode solution contains magnesium. Douglas & Rubin (1963) observed that magnesium inhibited the effect of calcium in releasing catechol amines after a period of calcium deprivation.

The similarities between the postganglionic fibre and the adrenal medulla which we have observed indicate that the release of noradrenaline from the postganglionic fibre is due to the action of calcium. Just as acetylcholine in the adrenal medulla, in the view of Douglas & Rubin (1961, 1963), depolarizes the membrane of the chromaffin cells, or else produces a change in the permeability of the membrane to calcium, and thus allows calcium to enter and release catechol amines, so in the postganglionic fibre the sympathetic impulse produces a change which enables calcium to release noradrenaline from the granules within the fibre. Nicotine can certainly do this as well as the sympathetic impulse, and we have a little evidence that acetylcholine also has this power.

According to the view that the nerve impulse itself directly releases noradrenaline, it would seem that the impulse may itself effect a change in the permeability of the terminal part of the fibre membrane to calcium, allowing calcium to enter and to release noradrenaline. According to the view that the impulse first releases acetylcholine, then this acetylcholine may have the function of changing the permeability of the terminal part of the fibre membrane to calcium, allowing calcium to enter and to release noradrenaline. Of course the " entry " of calcium into the fibre may actually be a release of calcium from a binding site in the fibre membrane.

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