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EFFECT OF ATP ON THE INTERMEDIARY STEPS OF THE REACTION OF THE ($Na^+ + K^+$)-DEPENDENT ENZYME SYSTEM

III. EFFECT ON THE *p*-NITROPHENYLPHOSPHATASE ACTIVITY OF THE SYSTEM

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SUMMARY

1. It has been investigated how K^+, Mg^{2+}, ATP, and Na^+ influence the *p*-nitrophenylphosphatase activity of the ($Na^+ + K^+$)-dependent enzyme system.

2. K^+ activates the phosphatase activity in the presence of Mg^{2+}. The activity increases to a maximum and again decreases when the K^+ concentration is increased; this is under conditions where the ionic strength increases with the K^+ concentration. When the ionic strength is kept constant by choline, (a) the activity does not go through a maximum when the K^+ concentration is increased, but increases to a certain level which is lower than the maximum obtained with K^+ without choline, i.e. with a lower ionic strength; (b) the apparent affinity for K^+ for activation decreases. Tris has the same effect as choline.

3. The effect of Na^+ in the presence of K^+ depends on the K^+:Na^+ concentration ratio. At a low ratio, Na^+ increases the activity while with a higher ratio, Na^+ decreases the phosphatase activity.

4. The effect of ATP depends on the cations in the medium. With K^+, ATP decreases the apparent affinity for K^+ and decreases V. The inhibition by ATP can partly be overcome by an increase in the K^+ concentration.

With K^+ plus Na^+ ATP has two effects, (a) at a low K^+:Na^+ concentration ratio, ATP in low concentrations increases the catalytic activity; with higher concentrations of ATP, the activity again decreases. (b) At a high K^+:Na^+ concentration ratio, ATP decreases the activity.

5. *p*-Nitrophenylphosphate competes for the effect of ATP just as ATP competes for the effect of *p*-nitrophenylphosphate.

6. CTP and ITP, but not GTP, have an activating effect in the presence of a low K^+:Na^+ ratio just as ATP, but the concentration necessary to obtain a given effect is much higher. All the triphosphates inhibit the activity in the presence of K^+ and in the following order: ATP > GTP > CTP > ITP.

7. The experiments suggest that there are two phosphatase activities—one which is due to a ${}^{o}K_m^+/{}^{i}Na_n^+$ form of the system (i for inside, o for outside, m and n

are numbers); ATP in low concentrations increases the catalytic activity of this form. Another which is due to a $^{o}K_m^+/^{i}K_n^+$ form of the system, and which has a higher activity than the $^{o}K_m^+/^{i}Na_n^+$ form; ATP decreases the activity of this form by decreasing the affinity for K^+ on the i-site of the system.

INTRODUCTION

In two previous papers it has been shown that the reaction of the $(Na^+ + K^+)$-dependent enzyme system with ATP [1] as well as with MgATP [2] leads to an increase in the apparent affinity for Na^+ relative to K^+ for the site where Na^+ activates the ATPase activity, the i-site of the system (i for inside). This was seen under conditions where the site where K^+ activates the ATPase activity, the o-site (o for outside), is saturated with K^+, i.e ATP as well as MgATP seems to shift the equilibrium between a $^{o}K_m^+/^{i}K_n^+$ and a $^{o}K_m^+/^{i}Na_n^+$ form of the system towards a $^{o}K_m^+/^{i}Na_n^+$ form. It was furthermore found that a decrease in the free ATP/free Mg^{2+} ratio at a given MgATP concentration leads to a decrease in activity suggesting either that free ATP is necessary for activity, or that free Mg^{2+} inhibits [2].

Preparations of the $(Na^+ + K^+)$-dependent enzyme system have a K^+-activated phosphatase activity which is influenced by K^+, Na^+, ATP and g-strophanthin in such a way that it is reasonable to assume that it is due to the $(Na^+ + K^+)$-dependent enzyme system [3–20]. This is further supported by the observation that the $(Na^+ + K^+)$-dependent ATPase activity of preparations from different tissues correlates to the K^+-dependent phosphatase activity [11] and that the ratio between the two activities stays constant during the purification of the $(Na^+ + K^+)$-activated enzyme system [21].

As ATP influences the phosphatase activity in the presence of Na^+ and K^+, it has been investigated whether the effect of ATP on the phosphatase activity can further elucidate the effect of ATP on the ATPase activity.

METHODS

The enzyme was prepared as previously described [1]; it was stored at −20 °C.

The Tris salt of *p*-nitrophenylphosphate was used as substrate. The phosphatase activity was tested in 1 ml of a solution buffered with 30 mM Tris–HCl, pH 7.4, 37 °C. The reaction was started by addition of enzyme which was preheated to 37 °C for 2 min before use. In the experiments where ATP (the Tris salt) was added, the test tube also contained 1 mM phosphoenolpyruvate and pyruvate kinase (glycerol preparation from Boehringer). The reaction was stopped after 5 min by addition of 0.1 vol. 50% trichloroacetic acid. The colour of the released *p*-nitrophenol was developed by the addition of 2 ml 0.5 M Tris base and the extinction read at 410 nm. The activity was a rectilinear function of time under the conditions used. The activity was corrected for the small activity found with Mg^{2+} without added K^+.

The figures show typical results, which have all been reproduced 3–5 or more times.

RESULTS

In Fig. 1 is shown how K^+ influences the hydrolysis of *p*-nitrophenylphosphate 4 mM in the presence of 4 mM Mg^{2+}. The sum of K^+ plus choline, and of K^+ plus Na^+ has been kept constant at 100 mM.

Without Na^+ or choline, the activity goes through a maximum when the K^+ concentration is increased. The concentration of K^+ to give maximum activity increases with the Mg^{2+} concentration (not shown) and is 10 mM with 4 mM Mg^{2+} (Fig. 1).

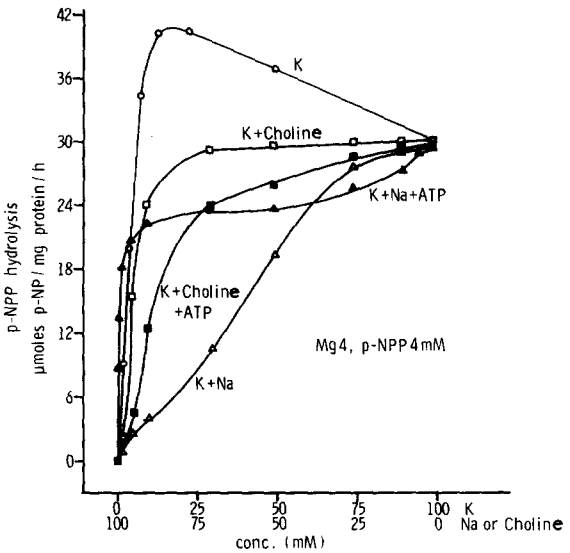

Fig. 1. The effect of choline, Na^+ and ATP on the activation by K^+ of the *p*-nitrophenylphosphatase activity of the ($Na^+ + K^+$)-dependent enzyme system. The concentration of Mg^{2+} was 4 mM and of *p*-nitrophenylphosphate 4 mM. The sum of the concentrations of K^+ and choline and of K^+ and Na^+, respectively, was kept constant at 100 mM. The ATP concentration was 0.1 mM. In this and the following figure the test was made in 30 mM Tris–HCl, pH 7.4, 37 °C.

With choline or Na^+, the top of the curve disappears and the concentration of K^+ necessary to give a certain effect increases, more so with Na^+ than with choline. Tris has the same effect as choline, which may suggest that the effect of choline is an effect on the ionic strength, cf. ref. 19 (see discussion). In the following, choline chloride has been used to keep the ionic strength constant.

With K^+ plus choline, ATP apparently decreases the affinity for K^+ (Fig. 1), and the S-shape of the curve, which was difficult to see without ATP, becomes more pronounced. ATP also gives a slight decrease in the activity with 100 mM K^+; this can hardly be seen with the low concentrations of ATP used in Fig. 1, but see below.

With K^+ plus Na^+ and ATP, the activation curve consists of two parts, a first steeper part where the activity is considerably higher than without ATP, and where K^+ activates in lower concentrations than with choline both with and without ATP (Fig. 1). This part of the curve levels off at a low K^+:Na^+ concentration ratio and is followed by a second S-shaped part which has a much lower slope. And for this part

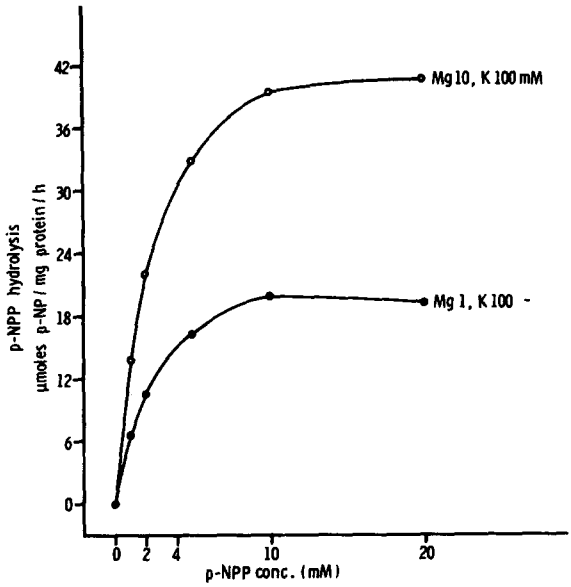

Fig. 2. The effect of varying concentrations of *p*-nitrophenylphosphate on the *p*-nitrophenylphosphatase activity in the presence of 10 mM Mg^{2+}, 100 mM K^+, and of 1 mM Mg^{2+}, and 100 mM K^+, respectively.

of the curve ATP decreases the activity when the K^+:Na^+ ratio becomes higher than 60:40; it shows that ATP increases the inhibitory effect of small concentrations of Na^+ in the presence of high concentration of K^+.

The 4 mM *p*-nitrophenylphosphate used in the experiments in Fig. 1 is not the optimum concentration. As seen from Fig. 2, the activity increases with the *p*-nitrophenylphosphate concentration up to 10 mM or higher. The concentration for half maximum effect is about 2 mM, and it seems to be independent of the Mg^{2+}

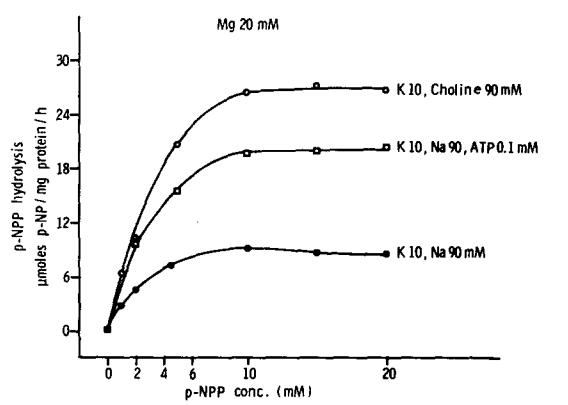

Fig. 3. The effect of varying concentrations of *p*-nitrophenylphosphate on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+} and 10 mM K^+ with 90 mM choline, with 90 mM Na^+ and with 90 mM Na^+ plus 0.1 mM ATP, respectively.

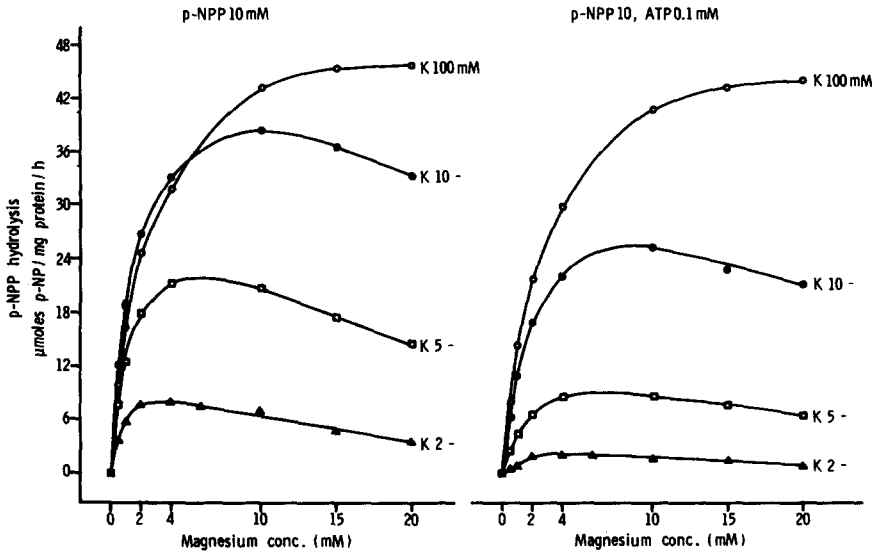

Fig. 4. The effect of varying concentrations of Mg^{2+} on the *p*-nitrophenylphosphatase activity in the presence of 10 mM *p*-nitrophenylphosphate (p-NPP) and varying concentrations of K^+ with and without 0.1 mM ATP. The sum of the concentrations of K^+, choline, and Mg^{2+} was kept constant at 140 mequiv/l. K^+ plus choline = 100 mM.

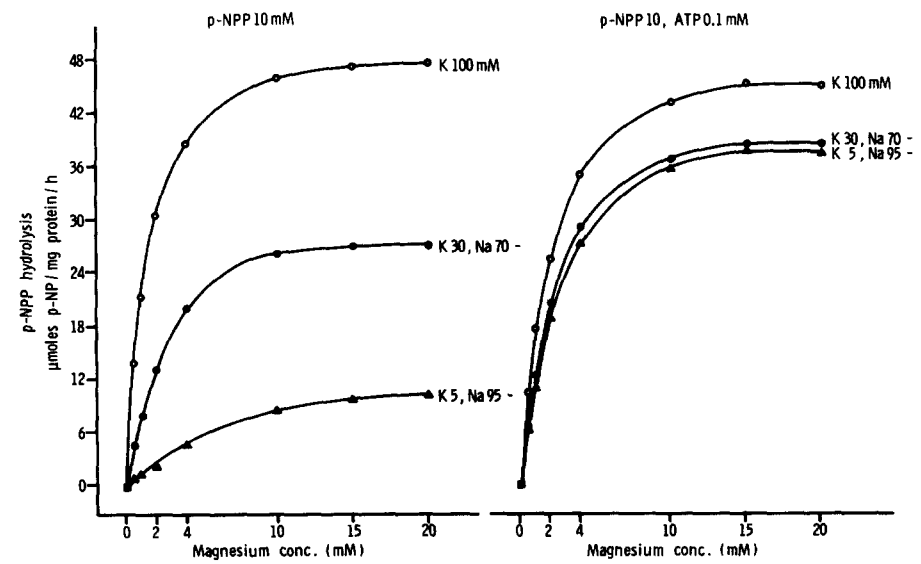

Fig. 5. The effect of varying concentrations of Mg^{2+} on the *p*-nitrophenylphosphatase activity with 10 mM *p*-nitrophenylphosphate (p-NPP) and varying concentrations of K^+ and $K^+ + Na^+$ with and without 0.1 mM ATP. The sum of the concentrations of K^+, Na^+, and Mg^{2+} was kept constant at 140 mequiv/l.

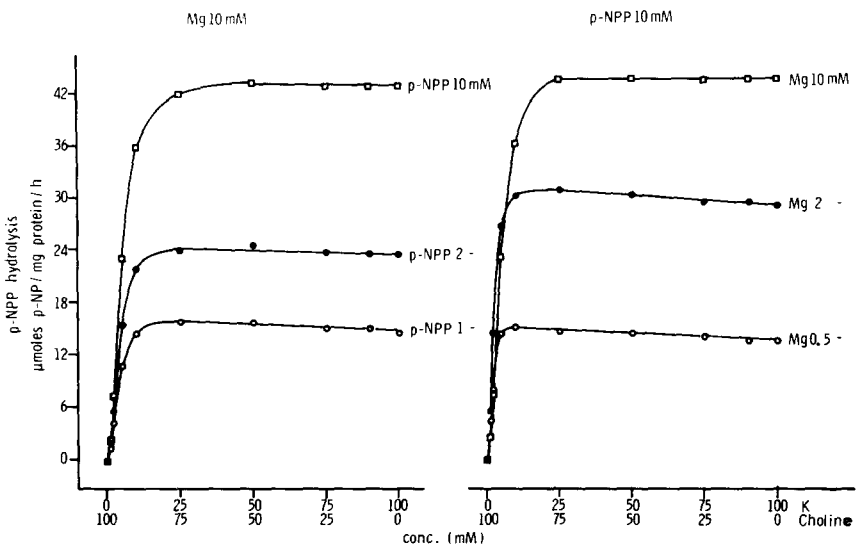

Fig. 6. The effect of K^+ in the presence of choline on the *p*-nitrophenylphosphatase activity with 10 mM Mg^{2+} and varying concentrations of *p*-nitrophenylphosphate (p-NPP) and with 10 mM *p*-nitrophenylphosphate and varying concentrations of Mg^{2+}, respectively. The sum of K^+ and choline was kept constant at 100 mM.

concentration, of the concentration of K^+, and whether or not there is Na^+ or Na^+ plus ATP besides Mg^{2+} in the medium (Figs 2 and 3).

The effect of Mg^{2+} on the activity seems to be more complex (Figs 4 and 5). With K^+ plus choline, the activity goes through a maximum when the Mg^{2+} concentration is increased, and the maximum moves towards higher concentrations of Mg^{2+} with an increase in the K^+ concentration (Fig. 4). This is seen both without and with ATP, but without ATP the slope of the ascending part of the curve with 100 mM K^+ is lower than with 10 mM K^+, suggesting that the activating effect of Mg^{2+} decreases with an increase in the K^+ concentration. With K^+ plus Na^+, the activity does not go through a maximum, but increases with the Mg^{2+} concentration up to 15–20 mM both without and with ATP (Fig. 5). Without ATP, the concentration of Mg^{2+} for half maximum activity increases when the K^+:Na^+ ratio is decreased; this effect is much less pronounced in the presence of ATP.

In agreement with the observations in Fig. 4 that K^+ in the presence of choline decreases the activating effect of Mg^{2+}, it is found that Mg^{2+} decreases the activating effect of K^+ in the presence of choline (Fig. 6). It is seen that with 10 mM *p*-nitrophenylphosphate the slope of the ascending part of the curve with 10 mM Mg^{2+} is lower than with 2 mM Mg^{2+}. The effect is not very pronounced, but it is consistently found both in the experiments where K^+ is varied at a fixed Mg^{2+} (Fig. 4), and where Mg^{2+} is varied at a fixed K^+ concentration (Fig. 6).

In Fig. 7, the experiments from Fig. 6 have been repeated, but with K^+ plus Na^+ instead of K^+ plus choline. The picture is quite different. With the higher concentrations of Mg^{2+} and of *p*-nitrophenylphosphate, the curves seem to consists of two parts like the curves for the effect of $K^+ + Na^+$ in the presence of ATP (cf. Fig. 1). There is a first steep part where K^+ activates insmall concentrations relative to the

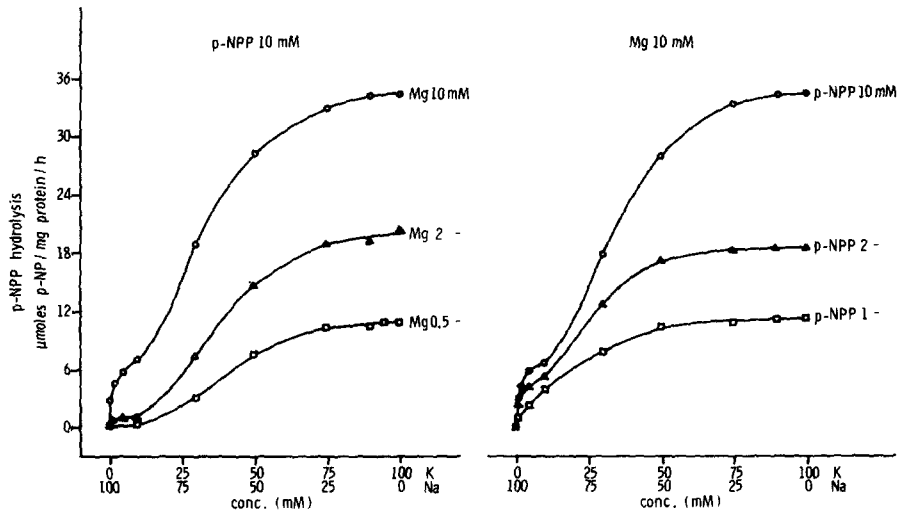

Fig. 7. The effect of $Na^+ + K^+$ on the *p*-nitrophenylphosphatase activity in the presence of 10 mM *p*-nitrophenylphosphate (p-NPP) and varying concentrations of Mg^{2+} and with 10 mM Mg^{2+} and varying concentrations of *p*-nitrophenylphosphate, respectively. The sum of the concentrations of Na^+ and K^+ was kept constant at 100 mM.

concentrations of Na^+, and this tends to level off and is followed by a second part which is S-shaped, and with a lower apparent affinity for K^+ relative to Na^+.

It requires apparently a high concentration of Mg^{2+} as well as of *p*-nitrophenylphosphate to see the steep part of the curve without ATP in the medium. Fig. 8 shows, however, that it is also necessary to have a certain concentration of Na^+. When the

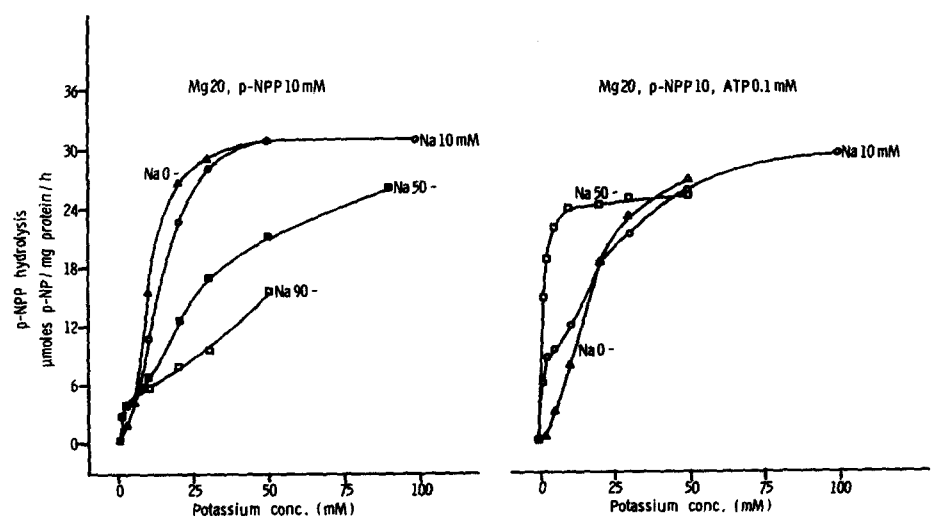

Fig. 8. The effect of K^+ on the *p*-nitrophenylphosphatase activity with 20 mM Mg^{2+}, 10 mM *p*-nitrophenylphosphate (p-NPP) and with varying concentrations of Na^+ and choline without and with 0.1 mM ATP. The sum of the concentrations of Na^+, K^+, and choline was kept constant at 150 mM.

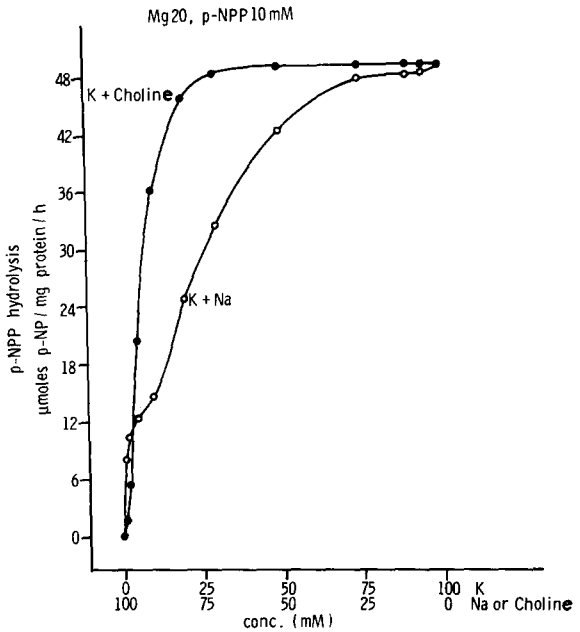

Fig. 9. The effect of K^+ in the presence of choline and of Na^+, respectively, on the *p*-nitrophenylphosphatase activity with 20 mM Mg^{2+}, 10 mM *p*-nitrophenylphosphate (p-NPP). The sum of the concentrations of K^+ and choline, and of K^+ and Na^+ was kept constant at 100 mM.

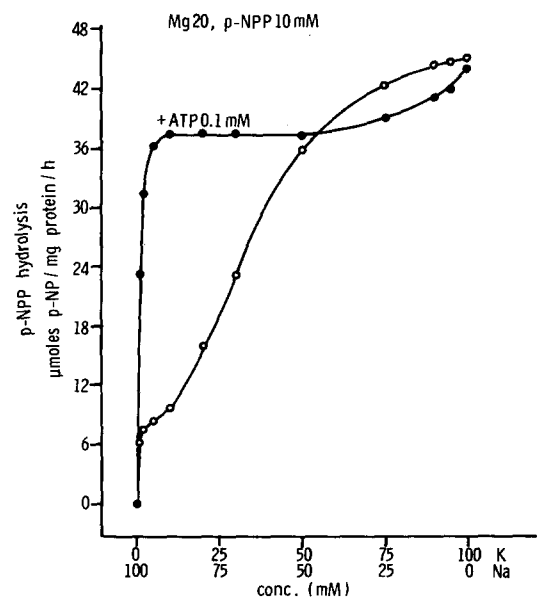

Fig. 10. The effect of $Na^+ + K^+$ on the *p*-nitrophenylphosphatase activity with 20 mM Mg^{2+}, 10 mM *p*-nitrophenylphosphate (p-NPP) without and with 0.1 mM of ATP. The sum of the concentrations of K^+ and Na^+ was kept constant at 100 mM.

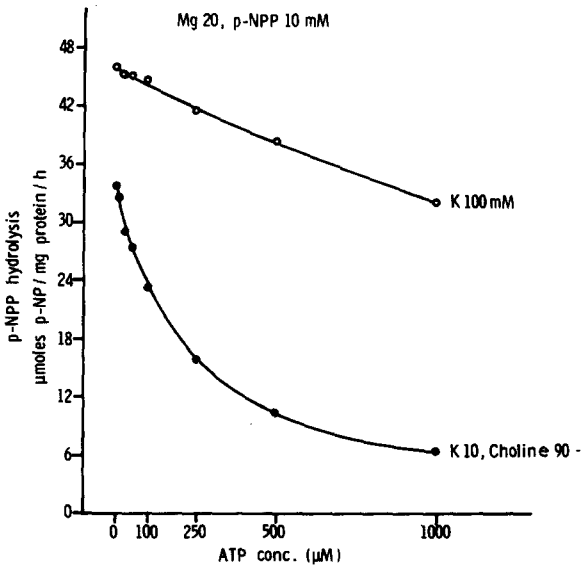

Fig. 11. The effect of ATP on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+}, 10 mM *p*-nitrophenylphosphate (p-NPP) with 100 mM K^+ and with 10 mM K^+ plus 90 mM choline, respectively.

K^+ concentration is increased the steep part of the curve is not seen without ATP with 10 mM Na^+, but with 50 and 90 mM. With ATP, on the other hand, it is seen with 10 mM Na^+, and the fraction of the activity which is given by this part of the curve increases with the Na^+ concentration up to at least 50 mM.

In Figs 9 and 10, part of the experiments in Fig. 1 have been repeated, but with saturating concentrations of Mg^{2+} and *p*-nitrophenylphosphate, 20 and 10 mM, respectively. Fig. 9 shows that the slope of the steep part of the curve is steeper with K^+ plus Na^+ than with K^+ plus choline; this is consistently found. And from Fig. 10,

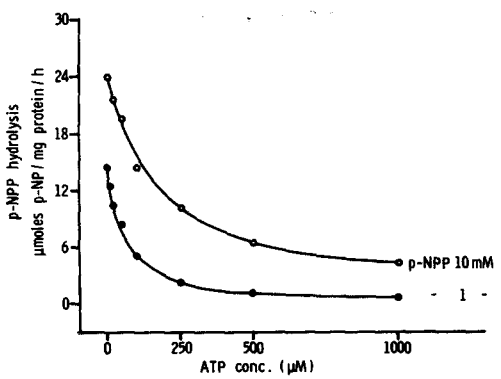

Fig. 12. The effect of ATP on the *p*-nitrophenylphosphatase activity in the presence of 10 mM Mg^{2+}, 10 mM K^+, 90 mM choline with 10 mM *p*-nitrophenylphosphate (p-NPP) and with 1 mM *p*-nitrophenylphosphate, respectively.

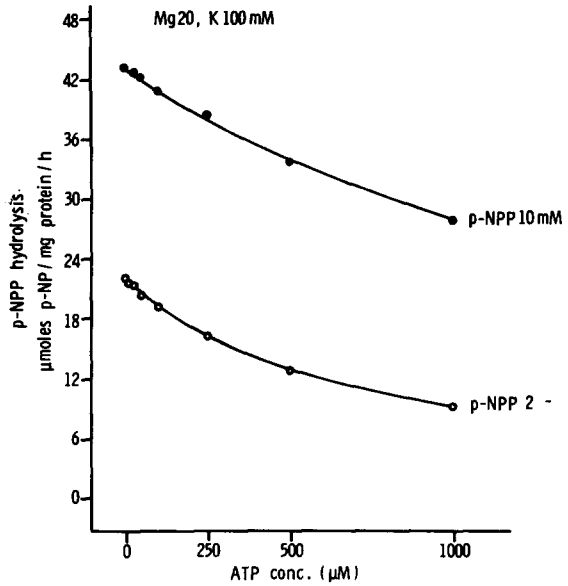

Fig. 13. The effect of ATP on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+}, 100 mM K^+ with 10 mM *p*-nitrophenylphosphate (p-NPP) and with 2 mM *p*-nitrophenylphosphate, respectively.

that ATP increases the fraction of the activity given by the steep part and apparently without influencing the apparent affinity for K^+ for this part of the curve. When the curves in Fig. 10 are read from right towards left, it is seen that small concentrations of Na^+ in the presence of high concentrations of K^+ decrease the activity more with

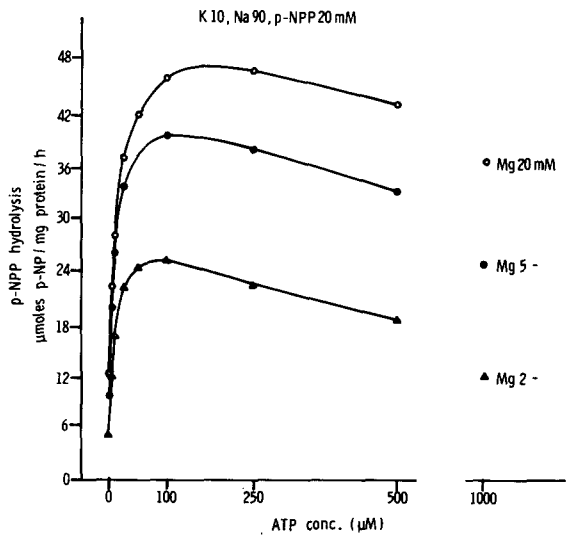

Fig. 14. The effect of ATP on the *p*-nitrophenylphosphatase activity in the presence of 10 mM K^+, 90 mM Na^+, 20 mM *p*-nitrophenylphosphate (p-NPP) and with different concentrations of Mg^{2+}, 2, 5, and 20 mM respectively.

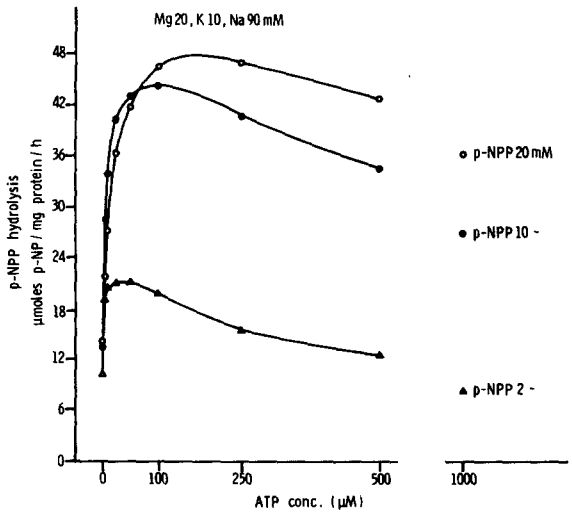

Fig. 15. The effect of ATP on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+}, 10 mM K^+, 90 mM Na^+ and with different concentrations of *p*-nitrophenylphosphate (p-NPP), 2, 10 and 20 mM respectively.

ATP than without (cf. Fig. 1), suggesting that ATP increases an affinity for Na^+ relative to K^+ for the low slope part of the curve.

The effect of ATP on the activity thus depends on the cations in the medium. With K^+ plus choline, ATP decreases the activity, and as seen from Fig. 11, the inhibitory effect of ATP increases with a decrease in the K^+ concentration. But the effect of ATP also depends on the *p*-nitrophenylphosphate concentration in such a way that the lower the *p*-nitrophenylphosphate concentration the more pronounced is the

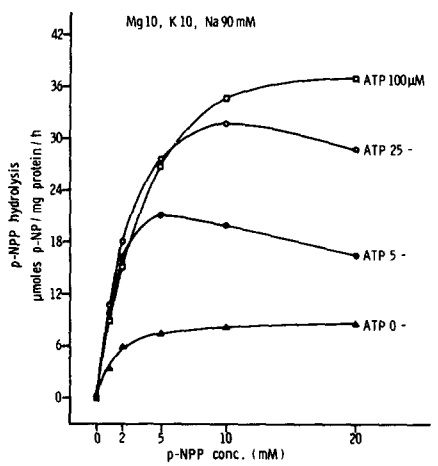

Fig. 16. The effect of *p*-nitrophenylphosphate (p-NPP) on the *p*-nitrophenylphosphatese activity in the presence of 10 mM Mg^{2+}, 10 mM K^+, 90 mM Na^+ without and with ATP, 5, 25 and 100 μM respectively.

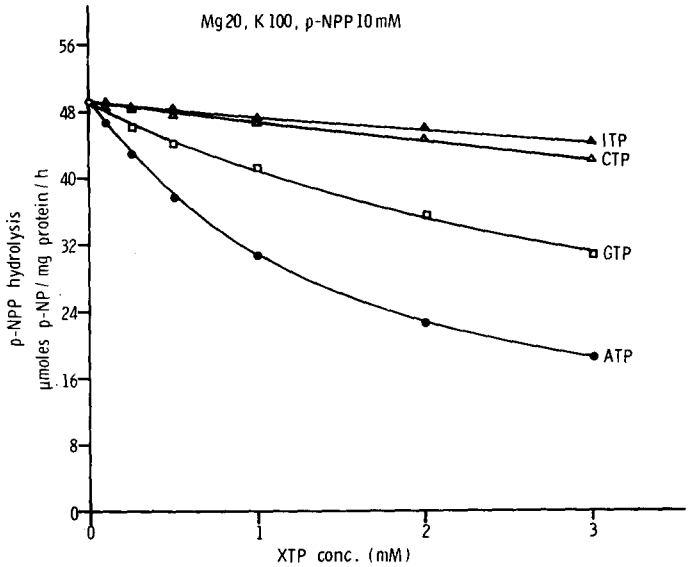

Fig. 17. The effect of varying concentrations of ATP, ITP, CTP, and GTP on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+}, 100 mM K^+, and 10 mM *p*-nitrophenylphosphate (p-NPP).

inhibitory effect of ATP both when the K^+ concentration is high and when it is low (Figs 12 and 13).

With K^+ plus Na^+, the effect of ATP depends on the K^+:Na^+ ratio. When this ratio is high, ATP decreases the activity as discussed in relation to Figs 1 and 10. When the ratio is low, ATP in low concentrations gives a considerable increase in

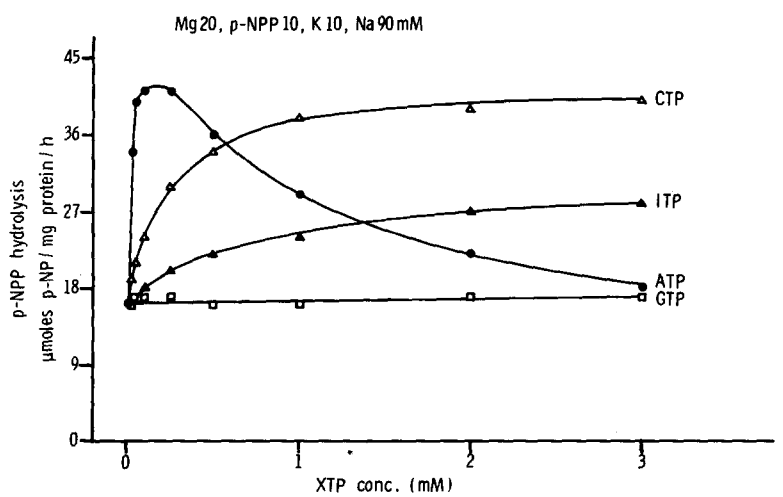

Fig. 18. The effect of varying concentrations of ATP, CTP, GTP, and ITP on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+}, 10 mM *p*-nitrophenylphosphate (p-NPP) 10 mM K^+ and 90 mM Na^+.

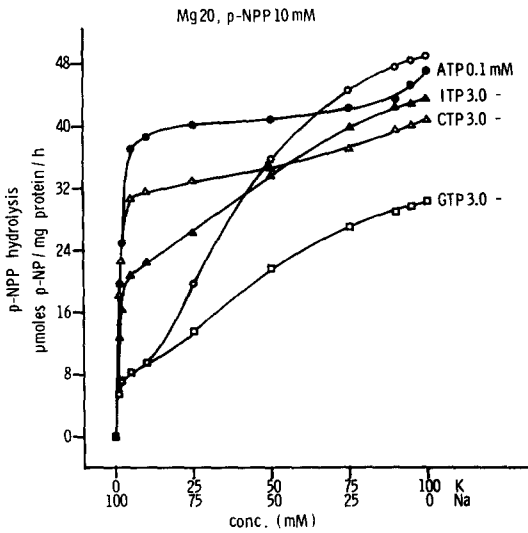

Fig. 19. The effect of $Na^+ + K^+$ on the *p*-nitrophenylphosphatase activity in the presence of 20 mM Mg^{2+}, 10 mM *p*-nitrophenylphosphate (p-NPP) and with 0.1 mM ATP, 3 mM ITP, 3 mM CTP, 3 mM GTP and without a triphosphate respectively.

activity as it was seen from Figs 1 and 10. But when the ATP concentration is further increased, the activity again decreases (Fig. 14), and the maximum tends to move towards higher ATP concentrations when the Mg^{2+} concentration is increased.

The effect of ATP in the presence of a low K^+:Na^+ concentration ratio is also influenced by *p*-nitrophenylphosphate and in such a way that there seems to be a double competition between ATP and *p*-nitrophenylphosphate (Figs 15 and 16). The slope of the ascending part of the curve for the effect of ATP decreases with an increase in the *p*-nitrophenylphosphate concentration and vice versa; the concentration of ATP necessary to give maximum effect increases with an increase in the *p*-nitrophenylphosphate concentration just as the concentration of *p*-nitrophenylphosphate necessary to give maximum effect increases with the ATP concentration. Finally, the inhibitory effect of ATP decreases with an increase in the *p*-nitrophenylphosphate concentration just as the inhibitory effect of *p*-nitrophenylphosphate decreases with an increase in the ATP concentration. Without ATP there is no or only a slight inhibitory effect of *p*-nitrophenylphosphate in the concentration used (Fig. 16, cf. also Figs 2 and 3).

CTP, ITP and GTP inhibit the activity in the presence of K^+ plus choline just as ATP, but the concentrations necessary to give a certain inhibition are much higher than for ATP (Fig. 17, cf. ref. 11).

CTP and ITP, but not GTP, activate the phosphatase in the presence of a low K^+:Na^+ concentration ratio just as ATP, but the concentrations necessary to give an effect are much higher, and with ITP the maximum effect which can be obtained is lower than with ATP and CTP (Figs 18 and 19, cf. refs 11 and 17).

A comparison between Figs 17, 18 and 19 shows that there is no correlation between the activating effect of the triphosphates with K^+ plus Na^+ and the inhibitory effect with K^+ plus choline.

DISCUSSION

Choline which was added to keep the ionic strength constant decreases the apparent affinity for K^+. It suggests that choline influences the activity by competing for K^+ for activation just as Na^+ (see below), but with an affinity for choline (and for Tris which has the same effect) which is much lower than for Na^+.

With K^+ plus choline, ATP decreases the activity, and the effect of ATP decreases with an increase in the K^+ concentration; ATP also decreases the apparent affinity for K^+ (cf. refs 5, 13, 17). This agrees with the observation that ATP decreases the apparent affinity of the $(Na^+ + K^+)$-activated enzyme system for K^+ and vice versa [1, 22, 23]. ATP decreases the affinity for K^+ on the "Na^+-site" of the system, the i-site [1]. It suggests that K^+ on this site is necessary for the phosphatase activity and as K^+ from the outside also seems to be necessary [24] it suggests that it is the ${}^{o}K_m^+/{}^{i}K_n^+$ form of the system which has the phosphatase activity (o for outside, m and n are numbers) (see however ref. 24).

With K^+ plus Na^+, ATP increases the activity when the K^+:Na^+ ratio is low. This has been taken to indicate that ATP in the presence of Na^+ increases the affinity for K^+ for activation [11, 13, 15]. The results given in Figs 7–10 suggest, however, another explanation.

Without ATP, a high concentration of Na^+ increases the activity in the presence of a low concentration of K^+; this is seen from the first steep part of the curves in Figs 8 and 9, and it agrees with the observations by Albers and Koval [20]. As Na^+ alone has no effect, it must be due to a combined effect of the two cations on the system. The effect is seen in the same low K^+:Na^+ concentration range which activates the $(Na^+ + K^+)$-ATPase. For the ATPase activity the activation is due to a combined effect of Na^+ on the "Na^+-site", the i-site of the system and of K^+ on the "K^+-site", the o-site of the system. There is a "Na^+-site" and a "K^+-site" also for the phosphatase activity and with the same cation characteristics as for the ATPase activity [17]. This and the similarities in the effect of a low K^+ and a high Na^+ concentration on the two activities suggest that it is also a combined effect of Na^+ on the "Na^+-site" and of K^+ on the "K^+-site" which gives the increase in phosphatase activity with a low K^+:Na^+ concentration ratio.

p-Nitrophenylphosphate cannot give a transport of cations in the intact cell [24], at least not with a high concentration of Na^+ in contact with the outside of the membrane and without a nucleotide [25]. It seems therefore unlikely that the reaction with *p*-nitrophenylphosphate with the low K^+ and high Na^+ concentration can give a cycling of a carrier site between inside and outside. A combined effect of K^+ and Na^+ then suggests that the "Na^+-site" and the "K^+-site" exist simultaneously, and that the activity with a low K^+:Na^+ ratio is due to a ${}^{o}K_m^+/{}^{i}Na_n^+$ form of the system. It suggests that the first steep part of the curve is due to the conversion of an inactive Na^+ form ${}^{o}Na_m^+/{}^{i}Na_n^+$ into a low active ${}^{o}K_m^+/{}^{i}Na_n^+$ form (m and n are numbers).

A further increase in the K^+:Na^+ concentration ratio gives a further increase in the phosphatase activity along an S-shaped curve with a much lower slope. The K^+:Na^+ concentration for half maximum increase in activity read from this part of the curve (Fig. 10), is about 30:70 suggesting an apparent affinity for K^+ which is about 2.3 times higher than for Na^+. This is close to the K^+:Na^+ affinity ratio of 2.5:1 for the "Na^+-site" of the system found when there is no ATP present [1]. It

suggests that the increase in phosphatase activity given by this part of the curve is due to a replacement of Na^+ on the "Na^+-site" by K^+. As K^+ is necessary on the "K^+-site" for activity [24], it means that the increase in activity when the K^+:Na^+ ratio is increased is due to a conversion of the low active ${}^0K_m^+/{}^iNa_n^+$ form into a high active ${}^0K_m^+/{}^iK_n^+$ form of the system. The low slope part of the curve thus shows the sum of two activities, one due to the ${}^0K_m^+/{}^iNa_n^+$ form is decreasing and the other due to the ${}^0K_m^+/{}^iK_n^+$ form is increasing when more and more of the system is transformed from the ${}^0K_m^+/{}^iNa_n^+$ form into the ${}^0K_m^+/{}^iK_n^+$ form by an increase in the K^+:Na^+ ratio. But as the ${}^0K_m^+/{}^iK_n^+$ form has a higher activity than the ${}^0K_m^+/{}^iNa_n^+$ form, this transformation leads to a further increase in activity.

ATP increases the fraction of the activity given by the first steep part of the curve, and it seems not to influence the apparent affinity for K^+ for this part of the curve. It suggests that ATP increases the catalytic activity of the ${}^0K_m^+/{}^iNa_n^+$ form of the system against *p*-nitrophenylphosphate.

ATP has also an effect on the low slope part of the curve. When this curve is read from right towards left, it is seen that the inhibitory effect of low concentrations of Na^+ in the presence of high concentrations of K^+ is more pronounced with ATP than without. It suggests that ATP increases the apparent affinity for Na^+ relative to K^+. And as ATP has this effect on the "Na^+-site" of the system [1, 2], it gives support to the view that this part of the curve reflects the competition between Na^+ and K^+ for the "Na^+-site" of the sytem.

It seems thus as if the phosphatase activity requires a combined effect of Na^+ and K^+ as does the ATPase activity. But for the phosphatase it is the ${}^0K_m^+/{}^iK_n^+$ form which has the highest activity, while the ${}^0Na_m^+/{}^iNa_n^+$ form has no activity and the ${}^0K_m^+/{}^iNa_n^+$ has an intermediary activity. For the ATPase it is the ${}^0K_m^+/{}^iNa_n^+$ form which has the highest activity while the ${}^0K_m^+/{}^iK_n^+$ form has no activity, and the ${}^0Na_m^+/{}^iNa_n^+$ form has a low activity.

In a previous paper [2] it was found that a high concentration of Mg^{2+} relative to the concentration of ATP inhibits the ATPase activity in the presence of optimum concentrations of Na^+ and K^+, i.e. of the ${}^0K_m^+/{}^iNa_n^+$ form of the system. The effect was independent of the MgATP concentration, suggesting that it could not be due to an inhibitory effect of MgATP. It must then be due either to an inhibitory effect of free Mg^{2+} or to a lack of free ATP meaning that a decrease in the free Mg^{2+} concentration leads to an increase in activity of the ${}^0K_m^+/{}^iNa_n^+$ form, and/or that free ATP is necessary, and that therefore an increase in the concentration of free ATP leads to an increase in activity. In the present paper it is found that ATP in the presence of Mg^{2+} increases the catalytic activity of the ${}^0K_m^+/{}^iNa_n^+$ form of the system towards *p*-nitrophenylphosphate. Considering the similarities between the phosphatase and the ATPase activity of the system, it seems likely that the increase in catalytic activity of the ${}^0K_m^+/{}^iNa_n^+$ form towards the two substrates must have a common cause.

In the ATPase experiments the decrease in the Mg^{2+}:ATP ratio which leads to an increase in activity leads to a decrease in MgATP; in the phosphatase experiments the increase in activity is seen under conditions where the MgATP concentration is increased; this excludes MgATP as a common factor. Mg^{2+} in the concentrations used has no inhibitory effect on the phosphatase activity in the presence of Na^+ plus K^+ (cf. Fig. 5), which means that the small decrease in the concentration of free Mg^{2+}

seen when ATP is added (20 mM Mg^{2+}, 0.1 mM ATP) cannot lead to an increase in the activity, and thereby not explain the effect of ATP. This leaves free ATP as a common factor; it increases in concentration with the increase in activity of the ${}^{0}K_{m}^{+}/{}^{i}Na_{n}^{+}$ form both in the ATPase and the phosphatase experiments. This would mean that free ATP activates the catalytic activity of the ${}^{0}K_{m}^{+}/{}^{i}Na_{n}^{+}$ form of the system both towards *p*-nitrophenylphosphate and ATP.

About 25 μM ATP gives half maximum activation in the presence of 20 mM Mg^{2+} and 20 mM *p*-nitrophenylphosphate. Considering the complexing of ATP with Mg^{2+} and the apparent competition between *p*-nitrophenylphosphate and ATP for the activation, the affinity for free ATP for activation must be high.

As discussed in a previous paper [2] it is not possible from our present knowledge about the system to tell whether an activating effect of ATP_f means that there is an activator site on the system besides a substrate site, or that ATP_f is the substrate and therefore is necessary for the ATPase activity.

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