debris was usually formed in the absence of substrate than when glucose was present. In one experiment an exceptionally large amount of debris was formed without substrate; this was much more marked than with glucose and even more marked than with glucose-fluoride. However, the leakage of potassium was no greater without substrate than with glucose-fluoride. This indicates that potassium leakage is probably not mainly occasioned by mere tissue disintegration, but the two phenomena may well progress together. In the later experiments of this series the spun debris was included in the dry weight determinations.

DISCUSSION

We may conclude from the results recorded in Tables 1–3 that when both the supply of glucose and oxygen to the brain cells is cut off there is considerable leakage of potassium into the extracellular fluid. This loss of potassium may be one of the basic factors responsible for ischaemic injury to cells, as potassium is doubtless an essential intracellular constituent on whose presence in high concentration many enzymic processes may depend. Furthermore, in the case of brain, it is possible that the loss of potassium from damaged cells may have other consequences since in brain potassium ions have such remarkable effects on metabolism. The irritation and paralysis associated with vascular accidents may thus depend in part on these metabolic effects produced by potassium ions which have emerged from the primary focus of ischaemic damage out into adjacent regions, as well as on the escape of essential intracellular constituents (such as potassium) out of the initially injured tissue.

SUMMARY

1. During anaerobic metabolism in the presence of glucose there was no increase in the concentration of potassium in the fluid bathing slices of cerebral cortex. In some cases there was a decrease.

2. Under the same conditions, but in the absence of glucose, there was always a significant rise in the potassium concentration of the fluid surrounding the brain slices.

3. The action of glucose in preventing this loss of potassium is dynamic: it depends on the active utilization of glucose by the glycolyzing cell. Where glycolysis was inhibited by fluoride potassium leaked into the environmental fluid just as in the absence of glucose. Brain thus resembles red blood cells for which Danowski (1941) has described a similar effect.

4. The significance of these findings on the nature of ischaemic damage is briefly considered.

I am very grateful to Mr N. T. J. Bailey, Statistician to the Department of Medicine, Cambridge, for checking the figures in Tables 1 and 2, and for useful advice.

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The Activation of Phosphoglucomutasé by Metal Ions

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(*Received* 12 *July* 1948)

The existence of glucose-1-phosphate and its conversion to glucose-6-phosphate in muscle were first described by Cori & Cori (1936). Kendal & Stickland (1938) claimed that Mg^{++} and fructofuranose-1:6-diphosphate were both essential for this conversion, but Cori, Colowick & Cori (1938) stated that 'the contention of Kendal & Stickland that hexosediphosphate acts as a coenzyme and is essential for the reaction is not substantiated by our findings'. They found, on the other hand, that Mn^{++} and Co^{++} are more efficient activators than Mg^{++}, and that the enzyme (named by them phosphoglucomutase) shows a large residual activity in the absence of any added metal. This has led to the view, expressed typically by Sumner & Somers (1947), that 'phosphoglucomutase requires no coenzyme'.

The present paper deals with some of the effects of metallic ions on the activity of phosphoglucomutase. In a later publication the author hopes to

describe the role of fructofuranose-1:6-diphosphate (*HDP*), and to reconcile the conflicting statements of Cori *et al.* (1938) and Kendal & Stickland (1938). For the present it is enough to reassert that *HDP* is essential for maximal activity of phosphoglucomutase, as will be seen later in Table 1.

METHODS

Phosphoglucomutase. In most of the experiments the enzyme was partially purified by the method of Colowick & Sutherland (1942), but similar results can be obtained equally well in a simple dialyzed muscle extract.

Glucose-1-phosphate. This was prepared by a method substantially the same as that of Sumner & Somers (1943), and was used as the K salt.

Salts of metals. The salts used were A.R., where this quality was available (e.g. potash alum, chrome alum and lead acetate); in the other cases ordinary commercial samples were used without special purification.

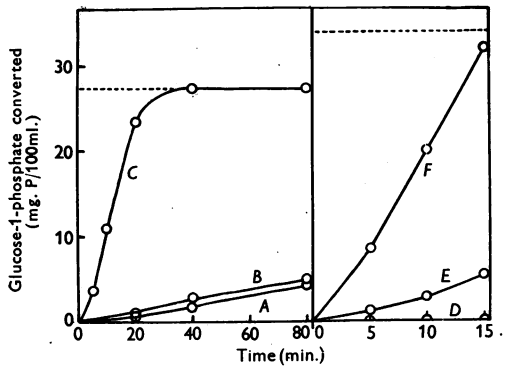

Fig. 1. The course of the conversion of glucose-1-phosphate to glucose-6-phosphate. Curve *A*, $Al^{+++}+HDP$; curve *B*, $Mg^{++}+HDP$; curve *C*, $Al^{+++}+Mg^{++}+HDP$; curve *D*, $Mg^{++}+HDP$; curve *E*, $Mn^{++}+HDP$ or $Cr^{+++}+Mn^{++}+HDP$; curve *F*, $Cr^{+++}+Mg^{++}+HDP$. The dotted lines indicate the amount of conversion at equilibrium. The concentration of *HDP* was 0·0003 M, and that of the metals was optimal (see text).

Measurement of the course of reaction. The reaction was followed by determining the residual glucose-1-phosphate by 5 min. hydrolysis in N-HCl at 100°. The reaction mixtures contained the substrate, etc., at the following final concentrations, unless otherwise stated: glucose-1-phosphate, 0·01 M; Na phosphate buffer, pH 7·5, 0·005 M (approx.); *HDP*, 0·0003 M. The various metals and their concentration will be given in the text and tables. For the sake of uniformity, concentrations are expressed in terms of the 'molarity' of cation (e.g. Cr^{+++}) and not of the actual salt used (e.g. $Cr_2(SO_4)_3$). The reaction mixtures were made up in lots of 1·0 or 2·0 ml. in 6 × 0·5 in. test tubes, and were placed in the water bath at 38° for some 5 min. before the addition of the enzyme solution (0·1 or 0·2 ml. at a predetermined dilution). The reaction was stopped, usually after 15 min., by the addition of 3 vol. of 4% trichloroacetic acid, and the inorganic PO_4 determined in samples of the filtrate, before and after 5 min. acid hydrolysis at 100° (Fiske & Subbarow, 1925). When the time course of the reaction was to be followed, larger volumes of mixture were made, and 1·5 or 2·0 ml. samples withdrawn from each into trichloroacetic acid at zero time and after the required intervals.

The use of a single point to measure relative reaction velocities was justified by the observation that the reaction is almost linear until the equilibrium point is nearly reached. A slight lag in the first quarter of the reaction was sometimes noticed (Fig. 1). To obtain the greatest possible accuracy, the concentration of the enzyme was always adjusted so that, in the fastest reaction of any series, some 75% of the conversion had taken place in the time allowed (15 min.). Finally, the most important points were checked by following the course of the reaction to the equilibrium point.

RESULTS

The basic observation is that maximal activation of phosphoglucomutase is achieved only with a combination of three components, viz. two metals and *HDP* (Table 1). Of these three components, the present work is concerned only with the two metals, and, in all the experiments that follow, it may be assumed that *HDP* is present at a concentration of 0·0003 M unless the contrary is stated. A wide range of metals has been tested for their power to take the place of either Mg^{++} or Al^{+++} in the system shown in Table 1, and these will be dealt with in turn.

Table 1. *The indispensability of three components for full phosphoglucomutase activity*

(Reaction mixture as described in text. Concentrations of additions: Mg^{++}, 0·003 M; Al^{+++}, 0·0005 M; *HDP*, 0·0003 M.)

Additions	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.) Exp. 1	Exp. 2	Exp. 3
$Mg^{++}+HDP$	1·1	0·0	0·0
$Al^{+++}+HDP$	3·1	1·3	0·0
$Mg^{++}+Al^{+++}$	1·6	1·7	0·7
$Mg^{++}+Al^{+++}+HDP$	27·7	22·6	11·0

Magnesium. The indispensability of Mg^{++} was demonstrated by the use of a test system containing substrate, enzyme, Al^{+++} and *HDP*. The addition of other metals to this system showed that only Mg^{++} led to the appearance of any considerable activity (Table 2). Cr^{+++} is not included in this table, as it is a special case which will be considered later. The concentrations used, except in the case of Mg^{++}, were 0·002 and 0·0002 M, and it is most unlikely that any activity should exist which would not be detected at one or other of these concentrations. Mg^{++} was tested at 0·004 M, the concentration shown to be optimal for glycolysis by Lohmann (1931); Cori *et al.* (1938) observed an ill-defined optimum for phosphoglucomutase at *c.* 0·005–0·01 M.

Of special interest are the negative results with Mn^{++} and Co^{++}; these will call for further comment later.

Table 2. *The irreplaceability of* Mg^{++}

(Reaction mixture and conditions of experiment as described in the text. Concentrations: Al^{+++} (0·005 M) and *HDP* (0·0003 M) present throughout; Mg^{++}, 0·003 M, other metals, 0·002 M. The other metals were also tested at 0·0002 M, with similar negative results. Other metals tested, at 0·002 and 0·0002 M, with completely negative results, were Be^{++}, Ca^{++}, Zn^{++}, Pb^{++}, Hg^{++}, UO_2^{++}, La^{+++}, Bi^{+++}, Zr^{++++}, Ce^{+++}.)

Addition	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.) 1st series	2nd series	3rd series
None	2·7	0·1	0·2
Mg^{++}	26·4	21·7	12·3
Fe^{+++}	3·3	—	—
Mn^{++}	3·2	—	—
Sr^{++}	—	2·3	—
Ba^{++}	—	1·7	—
Cu^{++}	—	2·0	—
VO^{++}	—	—	1·1
Ni^{++}	—	—	2·5
Co^{++}	—	—	1·0
Ti^{++++}	—	—	1·1

The relationship between concentration of Mg^{++} and degree of activity is shown in Fig. 2. The optimum, 0·003 M, agreed closely with that found

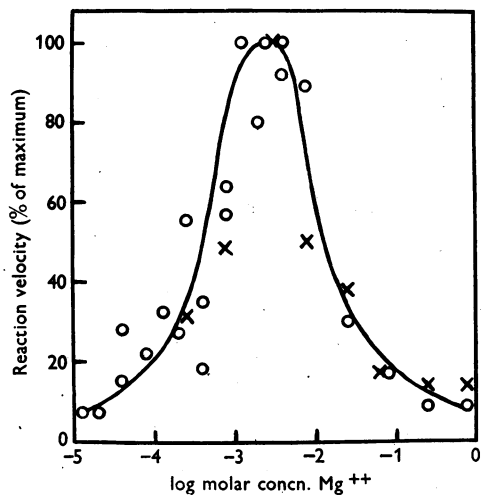

Fig. 2. The relationship between concentration of Mg^{++} and activity of phosphoglucomutase. *HDP* (0·0003 M) present throughout. Circles: in the presence of Al^{+++} (0·0005 M); crosses: in the presence of Cr^{+++} (0·00002 M).

by Lohmann (1931) for the whole glycolytic process, but not so closely with that found by Cori *et al.* (1938) for partially activated phosphoglucomutase. It was the same whether the second metal were Al^{+++} or Cr^{+++}; the optimum for Mg^{++} has not been specially determined for any other metals, and Mg^{++} has been used at 0·003 M in all other experiments.

The second metal. In a test system containing substrate, enzyme, Mg^{++} and *HDP*, the inclusion of Al^{+++} led to the appearance of a high enzymic activity (Table 1), and this power was shared by a number of other metals, notably Cr^{+++}, Pb^{++}, Fe^{+++}, UO_2^{++}, Ce^{+++}, Ti^{++++}, Be^{++}, etc. (Table 3).

Table 3. *Metals which can supplement* Mg^{++}

(Reaction mixture and conditions of experiment as usual. Concentrations: Mg^{++} (0·003 or 0·004 M) and *HDP* (0·0003 M) present throughout; Al^{+++} and other metals, 0·0002 M. The other metals were also tested at 0·00002 M, without revealing any further activity. Other metals tested, at 0·0002 and 0·00002 M, with completely negative results, were Zn^{++}, Ca^{++}, Sr^{++}, Cu^{++}, Hg^{++}, Ni^{++}, VO^{++}, Bi^{+++}, Ag^{+}, and Cd^{++}. The salts used were the sulphates, except those of Be, Ca, Sr, Ba, Hg, Zr, Cs and Sn (chlorides), UO_2, La and Ag (nitrates), and Pb (acetate).)

Addition	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.) 1st series	2nd series	3rd series	4th series
None	0·7	3·5	2·8	2·3
Al^{+++}	16·2	21·7	12·3	25·6
Mn^{++}	1·8	—	—	—
Be^{++}	13·7	—	—	—
Cr^{+++}	37·6	—	—	—
Fe^{+++}	24·4	—	—	—
Ba^{++}	—	8·4	—	—
Pb^{++}	—	19·0	—	—
UO_2^{++}	—	16·9	—	—
La^{+++}	—	—	5·8	—
Zr^{++++}	—	—	5·3	—
Ti^{++++}	—	—	9·8	—
Ce^{+++}	—	—	10·6	—
Cs^{+}	—	—	—	4·0
Tl^{+}	—	—	—	2·9
Sn^{++}	—	—	—	5·8

With all these metals the activity was found only if Mg^{++} was present at the same time (Table 4). A partial exception to this rule was found in Cr^{+++}, which at relatively high concentrations (of the order of 10^{-4} M) showed some activity in the absence of Mg^{++} (see bottom of Table 4). This point will be considered in rather more detail later.

The relationship between the concentration of the individual metals and the degree of activity produced in the presence of Mg^{++} and *HDP* showed in every case (except that of Cr^{+++}) a fairly sharp optimum. Although each single enzyme preparation gave a smooth and reproducible relationship between activity and concentration of cation, different preparations gave slightly different curves, so it is impossible to be very precise about the affinities of the enzyme for the metals. These points are illus-

Table 4. *The indispensability of* Mg^{++}

(Reaction mixture and conditions of experiment as before. *HDP* (0·0003 M) was present throughout; Mg^{++}, 0·003 M.)

'Second metal'	Concentration of 'second metal' (M × 10^5)	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.)		
		Mg^{++} alone	'Second metal' alone	Both together
Al^{+++}	60	1·1	3·1	27·7
Al^{+++}	40	0·7	0·4	23·4
Be^{++}	23	0·8	0·0	9·8
UO_2^{++}	67	0·0	0·0	9·6
UO_2^{++}	20	1·5	1·1	9·5
Pb^{++}	50	0·0	0·4	20·1
Fe^{+++}	67	0·8	0·0	12·7
Ce^{+++}	40	0·0	0·0	15·8
Cr^{+++}	4	0·0	0·0	24·8
Cr^{+++}	2	1·5	2·0	20·1
Cr^{+++}	1·2	0·5	0·0	19·7
Cr^{+++}	45	0·8	9·0	25·9
Cr^{+++}	12	0·5	15·9	22·3

trated in the case of Al^{+++} in Fig. 3; the other metals (except Cr^{+++}) gave curves of similar shape, whose characteristics are shown numerically in Table 5.

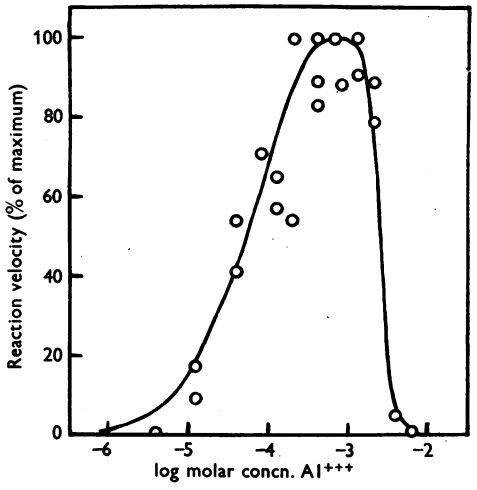

Fig. 3. The relationship between concentration of Al^{+++} and activity of phosphoglucomutase. *HDP* (0·0003 M) and Mg^{++} (0·003 M) present throughout.

The maximal activity reached with $Al^{+++} + Mg^{++}$, $Cr^{+++} + Mg^{++}$, and $Pb^{++} + Mg^{++}$, each at their optimal concentrations, was the same. With Fe^{+++}, Fe^{++}, and Ce^{+++} the maximal activity in the presence of Mg^{++} was only slightly lower, being from 70 to 90% of that with $Al^{+++} + Mg^{++}$. The other metals gave results which varied widely from one phosphoglucomutase preparation to another (Table 5). In view of this variability it must be admitted that some of the metals, which in Table 3 showed a small activity (e.g. Ba^{++}, La^{+++}, Zr^{++++}, and Sn^{++}) and which were there tested only with a single phosphoglucomutase preparation, might show greater activity if tests were carried out on a wider scale.

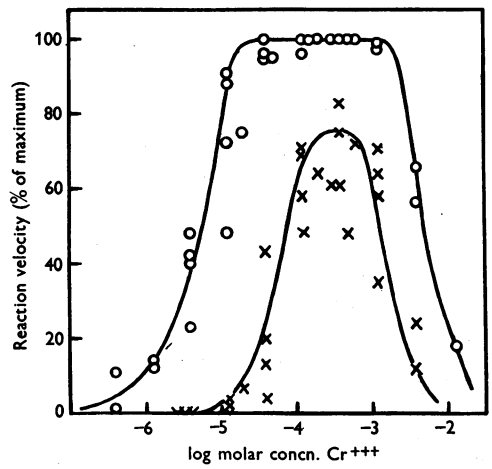

Fig. 4. The relationship between concentration of Cr^{+++} and activity of phosphoglucomutase. Circles: in the presence of *HDP* (0·0003 M) and Mg^{++} (0·003 M); crosses: in the presence of *HDP* (0·0003 M) only.

Chromium behaved exceptionally in several ways. In the presence of Mg^{++}, full activity equal to that with $Al^{+++} + Mg^{++}$ was found over the whole range of Cr^{++} concentrations from 10^{-5} to 2×10^{-3} M, and half the maximum was reached at 5×10^{-6} M (Fig. 4). This high affinity of the enzyme for Cr^{+++} distinguishes this element from Al^{+++} and all the other metals. A second point of difference was that Cr^{+++} showed a considerable degree of activity in the absence of Mg^{++}. This activity was only some 75% of that observed when Mg^{++} was present too, and was found only at the higher part of the range

Table 5. *The relationships between the concentrations of various metals and the phosphoglucomutase activity produced by them in the presence of* $Mg^{++}+HDP$

Metal	Salt used	Relative activity ($Al^{+++}=100$)	Concentration giving half the maximal activity (M)	Optimal concentration (M)
Al^{+++}	$KAl(SO_4)_2.12H_2O$	100	6×10^{-5}	5×10^{-4}
Cr^{+++}	$KCr(SO_4)_2.12H_2O$	100	5×10^{-6}	10^{-5} to 2×10^{-3}
Pb^{++}	$Pb(OOC.CH_3)_2$	100	3×10^{-5}	4×10^{-4}
Fe^{+++}	$KFe(SO_4)_2.12H_2O$	70–80	1×10^{-4}	4×10^{-4}
	$Fe(NO_3)_3.9H_2O$			
Fe^{++}	$Fe(NH_4)_2(SO_4)_2.6H_2O$	70	5×10^{-5}	4×10^{-4}
Ce^{+++}	$Ce_2(SO_4)_3$	80–90	6×10^{-5}	3×10^{-4}
Ti^{++++}	$Ti(SO_4)_2$	30–80 (5 enzyme samples)	4×10^{-5}	3×10^{-4}
		0 (1 enzyme sample)		
UO_2^{++}	$UO_2(NO_3)_2.6H_2O$	35–80 (6 enzyme samples)	4×10^{-4}	3×10^{-4}
		0 (2 enzyme samples)		
Be^{++}	$BeCl_2.4H_2O$	15–70	1×10^{-4}	5×10^{-4}

of concentrations of Cr^{+++}. There was consequently an appreciable range of Cr^{+++} concentrations over which no activity was seen unless both metals were present together (from 10^{-5} to 4×10^{-5} M). The activity with Cr^{+++} alone showed a sharp maximum at 3×10^{-4} M, half this maximum being reached at about 8×10^{-5} M.

Manganese and cobalt. Cori *et al.* (1938) showed that the activity of phosphoglucomutase is increased by Mg^{++}, Mn^{++} and Co^{++}, the degree of activity produced being roughly the same for all three, and the concentration required for full activation being greater for Mg^{++} than for the other two. They also observed that at lower enzyme concentrations the efficacy of Mg^{++} became less than that of Mn^{++} or Co^{++}. However, at the still lower enzyme concentrations necessitated by the greater activity of the systems dealt with in the present work, the activity with Mg^{++} was negligibly small, or zero (see Tables 4 and 6–8). Under the same conditions the activity with Mn^{++} added alone was considerably greater than that with Mg^{++} alone, but still small compared with that given by $Mg^{++}+Cr^{+++}+HDP$. The ratio

$$\frac{\text{Activity with } Mg^{++}+Cr^{+++}+HDP}{\text{Activity with } Mn^{++}}$$

for eight different enzyme preparations is shown in Table 6; it varied from 5 to 12, with an average of 7·5. Co^{++}, used alone, showed about the same activity as Mn^{++} (Table 8).

In an earlier section it was stated that neither Mn^{++} nor Co^{++} could replace Mg^{++} in the complete system (Table 2). In the experiments to which that table refers, these metals were tested at concentrations of 0·002 and 0·0002 M. The difference in behaviour was so curious that it seemed advisable to test these two metals more thoroughly, and in particular at the optimal concentrations already established by Cori *et al.* (1938). The results appear in Tables 6–8. It is plain that (*a*) neither Mn^{++} nor Co^{++} can act in the same way as Mg^{++} in supplementing the action of $Cr^{+++}+HDP$, and (*b*) they will not supplement the action of any of the other metals which, in co-operation with $Mg^{++}+HDP$, can produce full activity of the enzyme. In fact, many of the metals which, when present with Mg^{++}, stimulate the enzyme, had an inhibitory effect on that activity which is produced by Mn^{++} alone. Another point of interest is that the effect of Mn^{++} is independent of the presence of *HDP* (see Table 9).

The effect of pH. The need for three activating components was observed equally at all pH values at which the enzyme was active (Table 10). These results were obtained with the use of a mixed phosphate (0·005 M) and veronal (0·005 M) buffer. Veronal at high concentrations inhibited the enzyme (50 % inhibition at 0·0125 M), but at 0·005 M the inhibition was only *c.* 10 %, and as the veronal was present in all the reaction mixtures the inhibition can be ignored. Borate is less suitable for buffering phosphoglucomutase, as it also inhibits it completely and at a rather lower concentration (50 % inhibition at 0·007 M). The substrate also contributes largely to the buffering of the reaction mixtures used in these experiments, so the appropriate mixtures of glucose-1-phosphate, phosphate and veronal were made up, and their pH's adjusted to the required values by a colorimetric method, before they were measured out into the tubes in which the reaction was to take place. The fact that no difference in behaviour was noted obviated the need to make more precise measurements of the pH values. The enzyme preparation (at pH 7·5) was used at such great dilution that its addition had no effect on the pH of the mixtures.

Table 6. *The failure of* Mn^{++} *to replace* Mg^{++} *in the system* $Mg^{++}+Cr^{+++}+HDP$

(Reaction mixture and conditions of experiment as given in the text. Concentrations: *HDP*, 0·0003 M (present throughout); Mg^{++}, 0·003 M; Mn^{++}, 0·00125 M; Cr^{+++}, 0·00002 or 0·00004 M.)

Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.) in the presence of					Ratio of activities $\frac{Cr^{+++}+Mg^{++}}{Mn^{++}}$
Mg^{++}	Mn^{++}	Cr^{+++}	$Cr^{+++}+Mg^{++}$	$Cr^{+++}+Mn^{++}$	
1·0	1·4	0·0	17·2	1·9	12·3
0·0	5·3	3·5	32·0	5·4	6·0
1·2	4·6	1·3	22·9	2·4	5·0
0·0	2·0	0·6	13·1	2·5	6·6
0·0	1·9	—	20·7	—	10·9
1·3	4·4	—	22·6	—	5·6
2·8	3·3	—	27·0	—	8·2
1·6	5·7	3·8	31·2	—	5·5

Table 7. *The failure of* Mn^{++} *to replace* Mg^{++} *in the system* $Mg^{++}+HDP+$ *other metals*

(Reaction mixtures and conditions of experiment as described in the text. Concentrations: *HDP*, 0·0003 M (present throughout); Mg^{++}, 0·003 M; Mn^{++}, 0·00125 M; Al^{+++}, 0·0004 M; Pb^{++}, 0·0005 M; Fe^{+++}, 0·00033 M; UO_2^{++}, 0·00067 M; Ce^{+++}, 0·0004 M.)

Second metal (X)	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.) in the presence of				
	Mg^{++}	Mn^{++}	X	$Mg^{++}+X$	$Mn^{++}+X$
Al^{+++}	0·8	3·3	2·3	23·2	1·3
Al^{+++}	1·2	4·6	4·6	18·8	0·7
Al^{+++}	0·0	2·0	0·0	12·8	1·5
Fe^{+++}	0·8	3·3	0·0	12·7	2·5
Fe^{+++}	0·0	2·0	1·4	10·1	1·5
Pb^{++}	0·0	2·3	0·4	10·1	0·0
UO_2^{++}	0·0	2·3	0·0	9·6	0·0
Ce^{+++}	0·0	2·3	0·0	15·8	1·6

Table 8. *The failure of* Co^{++} *to replace* Mg^{++} *in the system* $Mg^{++}+HDP+$ *various metals*

(Reaction mixture and conditions of experiment as before. Concentrations: *HDP*, 0·0003 M (present throughout); Cr^{+++}, 0·00002 or 0·00004 M; Al^{+++}, 0·0004 M; Fe^{+++}, 0·00033 M; Pb^{++}, 0·0005 M; Ce^{+++}, 0·0004 M; Mg^{++}, 0·003 M; Co^{++}, 0·0017 M.)

Second metal (X)	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.) in the presence of				
	Mg^{++}	Co^{++}	X	$Mg^{++}+X$	$Co^{++}+X$
Cr^{+++}	1·2	5·7	1·3	22·9	7·1
Cr^{+++}	0·0	1·9	0·6	13·1	2·5
Al^{+++}	1·2	5·7	4·6	18·8	1·3
Al^{+++}	0·0	1·9	0·0	12·8	1·1
Fe^{+++}	0·0	1·9	1·4	10·1	0·3
Pb^{++}	1·2	5·5	0·4	23·0	6·7
Ce^{+++}	1·2	5·5	0·0	23·6	4·6

Table 9. *The absence of any effect of HDP on the activity of phosphoglucomutase activated by* Mn^{++} *alone*

(Reaction mixture and conditions of experiment as before. Concentrations: Mn^{++}, 0·00125 M; *HDP*, 0·0003 M.)

Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.)	
HDP absent	*HDP* present
24·0	24·4
12·0	11·0
6·0	6·5
3·1	2·6

The effect of progressive dilution of the enzyme. Since the activity of phosphoglucomutase with Mg^{++}, Cr^{+++} and *HDP* is some ten times that previously observed with Mg^{++} or Mn^{++}, it is clear that in order to measure this activity either the time scale of the experiments must be shortened or the enzyme concentration must be reduced. In the present work the method chosen was that of decreasing the enzyme concentration, keeping the time scale constant.

With the object of determining the proper enzyme concentration, a large number of routine tests have been carried out, using serial twofold dilutions of the enzyme, and the results of these show some

features of interest. Fig. 5 gives a diagrammatic summary of many such experiments. At the highest concentrations no activator was needed; with decreasing concentrations this activity rapidly fell off (Fig. 5, curve *A*), but full activity could still be achieved by addition of Mg^{++} or Mn^{++}. With a small further dilution of the enzyme, the Mg^{++} activation rapidly disappeared (curve *B*), while the activation with Mn^{++} fell off more slowly (curve *C*). At a point where the activity with Mg^{++} had vanished, and that with Mn^{++} had become very small, full activity could still be produced by the addition of Cr^{+++} and Mg^{++}. Eventually this activity also abruptly disappeared with further dilution (curve *D*).

Table 10. *The indispensability of two metals at various* pH *values*

(The preparation of the reaction mixtures and the conditions of experiment are described in the text. Concentrations: *HDP*, 0·0003 M (present throughout); Cr^{+++}, 0·00002 M; Mg^{++}, 0·003 M.)

	Glucose-1-phosphate converted in 15 min. (mg. P/100 ml.)		
	pH 7·5	pH 8·0	pH 8·5
Cr^{+++} alone	1·2	2·8	1·4
Mg^{++} alone	1·9	2·8	0·9
$Cr^{+++} + Mg^{++}$	27·2	25·7	18·7

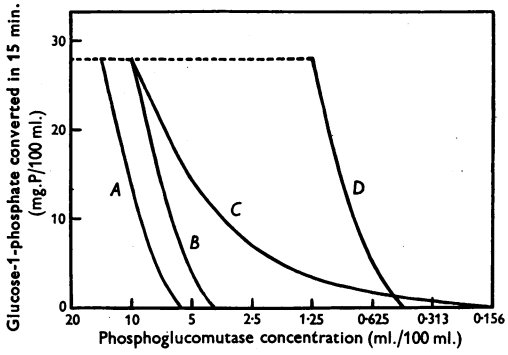

Fig. 5. Diagrammatic representation of the effect of dilution of phosphoglucomutase on its activity in the presence of various activators. Curve *A*, no activator; curve *B*, Mg^{++}; curve *C*, Mn^{++}; curve *D*, $Mg^{++} + Cr^{+++}$. *HDP* (0·0003 M) present throughout.

A curious point is that the velocity of reaction was proportional to the enzyme concentration only in the experiments where Mn^{++} was the sole activator (Fig. 5, curve *C*; see also Table 9, where the four results were obtained with successive twofold dilutions of the enzyme). That the reaction velocity should diminish more rapidly than the enzyme concentration might be expected in experiments with no added activator, or in those with Mg^{++} added alone (curves *A* and *B*), but in fact precisely the same relationship was observed when the enzyme was fully activated with Mg^{++} and Cr^{+++} (curve *D*).

Owing chiefly to variable losses in activity during the heat treatment in the purification of the enzyme, the absolute volume of enzyme required varied considerably from one preparation to another. On the other hand, the relative volumes required for measuring the activity in the presence of different combinations of activators were fairly constant. The scale of enzyme concentrations shown in Fig. 5 is the rough average of those found with a large number of phosphoglucomutase preparations, in which the final volume of the enzyme solution was one fifth of that of the original muscle extract.

The absolute activity of phosphoglucomutase preparations. Schlamowit & Greenberg (1948) say that the method of Colowick & Sutherland (1942) gives activities of phosphoglucomutase up to 3300 units/mg. of protein, these activities being measured in the presence of 0·00125 M Mn^{++}. Similar measurements on four samples of phosphoglucomutase in the presence of optimal concentrations of Mg^{++}, Cr^{+++}, and *HDP* gave values of 20,000, 37,000, 26,000 and 11,000 units/mg. dry weight, figures which confirm the value for the ratio:

$$\frac{\text{Activity with } Mg^{++} + Cr^{+++} + HDP}{\text{Activity with } Mn^{++}}$$

shown in Table 6.

DISCUSSION

The chief facts presented in this communication do not call for further discussion at the moment. It might, however, be profitable to inquire into the possible physiological implications. Previously the most active form of phosphoglucomutase had been that obtained by the addition of Co^{++}, Mn^{++} or Mg^{++}. The concentrations of Co^{++} or Mn^{++} required were enormously in excess of those which could occur in animal tissues, so Mg^{++} was accepted as the only physiological activator. The present results show that, at a great dilution of the enzyme, Mg^{++}, either alone or in co-operation with *HDP*, imparts no activity to the enzyme, and that another metal is required in addition. This phenomenon is capable of more than one interpretation, but assuming for the moment that the activation by two metals is of importance *in vivo*, then only Cr^{+++} appears to be worth considering as an actual physiological component of the system, because of the higher concentrations required with all the others. Cr^{+++} shows maximal activity at a concentration of 10^{-5} M, or 50 μg./100 g. It remains to be seen whether a concentration of this order is to be found in animal tissues; the only report in the literature is that of Dutoit & Zbinden (1930), who by spectro-

graphic analysis detected traces of Cr^{+++} in all organs, with most in thyroid and spleen.

The relationship between the activation by Mn^{++} and that by $Mg^{++}+Cr^{+++}+HDP$ is curious. The variability in the ratio of these two activities (see Table 6) at first indicated that two different enzymes might be concerned, but the absence of any marked change in the ratio during the purification of the enzyme, and the variability in some other properties of the enzyme between one preparation and the next, suggest that this is not so. Further work on the interrelations between the activations by various metals and combinations is proceeding.

SUMMARY

1. The greatest activity of phosphoglucomutase is found to occur only in the simultaneous presence of three activators, hexosediphosphate, Mg^{++} and a second metal.

2. The second metal may be Al^{+++}, Cr^{+++}, Pb^{++}, Fe^{+++}, or Ce^{+++}; some other metals also show smaller activity.

3. In this system Mg^{++} cannot be replaced by Mn^{++} or Co^{++}. The maximal activity is some ten times that previously observed with Mn^{++} as the only activator.

4. Of all the 'second metals' studied, only Cr^{+++} shows any activity in the absence of Mg^{++}, and that only at relatively high concentrations.

5. Consideration of affinities suggests that if this 'two-metal' activation has any physiological importance, then Cr^{+++} and Mg^{++} are the metals concerned.

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The Effect of the Peroxide Concentration and other Factors on the Decomposition of Hydrogen Peroxide by Catalase

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(*Received* 6 *May* 1948)

When catalase is added to hydrogen peroxide there is an initial rapid evolution of oxygen which lasts for about 2 min. After this oxygen is given off at a steady rate which slowly decreases in the course of about an hour. This is not necessarily due to a decrease in the peroxide concentration, since it is quite marked in experiments where there is a large excess of peroxide (Morgulis, Beber & Rabkin, 1926; George, 1947). The first problem in studying the kinetics of the reaction is to determine to what extent destruction of the enzyme is responsible for these changes in the rate as the reaction proceeds.

The results of some of the early investigations are difficult to interpret because only the total amount of oxygen evolved from a given amount of catalase and peroxide is recorded, so that the initial rapid reaction and the steady evolution cannot be distinguished. Provided the catalase is not present in excess the initial rapid reaction represents a small proportion of the total reaction possible, and as this condition obtained in most of the early investigations the results refer mainly to the subsequent steady rate. This is found to be directly proportional to the enzyme concentration, whereas the variation with peroxide concentration is more complicated. Above an optimum concentration as the peroxide is increased the reaction proceeds more slowly (Evans, 1907; Morgulis *et al.* 1926).

There is no doubt that the gradual decrease in the rate, after the initial rapid reaction is over, is due to enzyme destruction, and several kinetic equations have been developed to account for it in the papers of Yamasaki (1920), Morgulis (1921), Northrop (1924–5) and Williams (1927–8). There

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