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GLUCOSE UPTAKE BY THE CORN SCUTELLUM.*

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Abstract—The rate of uptake of glucose into corn scutellum slices was dependent both on glucose concentration and the time the slices were incubated in water prior to the addition of glucose. As the length of the incubation period was increased, so was the rate of glucose uptake. This effect was nullified by high glucose concentrations (e.g. $4{\cdot}9 \times 10^{-2}$ M) during the uptake period. Incubation in the absence of glucose caused the total reducing sugars and glucose to decrease in the tissue during the first hour and increase slightly thereafter. The sucrose and glucose-6-phosphate contents decreased throughout the 4-hr period of measurement. During incubation in the presence of different concentrations of glucose, the contents of reducing sugars, glucose, sucrose and glucose-6-phosphate decreased more slowly than during incubation in water or were maintained at levels equal to or greater than initial levels. Mannose inhibited glucose uptake. During incubation with mannose the slices accumulated mannose and mannose-6-phosphate, and evidence is presented that both mannose and mannose-6-phosphate are responsible for the inhibition of glucose uptake. It is suggested that glucose-6-phosphate competitively inhibits an enzymatic step (hexokinase-catalyzed reaction?) associated with net glucose uptake.

INTRODUCTION

ALTHOUGH there is extensive literature on sugar absorption by animal and bacterial cells, comparatively little work has been done on sugar uptake by higher plant tissues. Bieleski[1,2] and Glasziou[3,4] reported detailed studies on the uptake and transformation of sugars by slices of sugar cane internodes.

During germination of the cereal grain the starch of the endosperm is degraded to maltose and glucose primarily through the action of alpha and beta amylase. The transport of the soluble sugar from the endosperm to the developing seedlings is facilitated by the scutellum, the single cotyledon of the cereals, which is positioned between the endosperm and the root-shoot axis. Glucose from the endosperm is absorbed by the scutellum and converted to sucrose in which form it is transported.[5] The scutellum thus appears to be an excellent tissue for the study of sugar uptake.

RESULTS

Rate of Glucose Uptake

The scutellum slices were usually incubated in water at 30° for various periods of time prior to the addition of glucose. In some instances however glucose (or mannose) was present in

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[1] R. L. BIELESKI, *Australian J. Biol. Sci.* **13**, 203 (1960).
[2] R. L. BIELESKI, *Australain J. Biol. Sci.* **13**, 221 (1960).
[3] K. T. GLASZIOU, *Plant Physiol.* **35**, 895 (1960).
[4] K. T. GLASZIOU, *Plant Physiol.* **36**, 175 (1961).
[5] J. EDELMAN, S. I. SHIBKO and A. J. KEYS, *J. Exp. Bot.* **10**, 178 (1959).

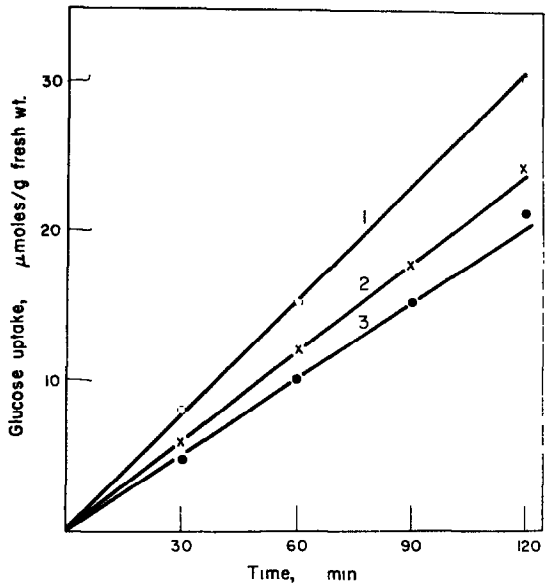

FIG. 1. EFFECT OF PRIOR INCUBATION IN THE PRESENCE OR ABSENCE OF GLUCOSE ON THE SUBSEQUENT RATE OF GLUCOSE UPTAKE.

Length of prior incubation period: 90 min in water (curve 1), 30 min in water (curve 3), and 90 min in $2{\cdot}45 \times 10^{-2}$ M glucose (curve 2). At the end of the incubation periods, the scutellum slices were washed in running distilled water for 2 min and replaced in flasks containing $4{\cdot}9 \times 10^{-3}$ M glucose. The glucose uptake measurements (shown above) were begun at this time.

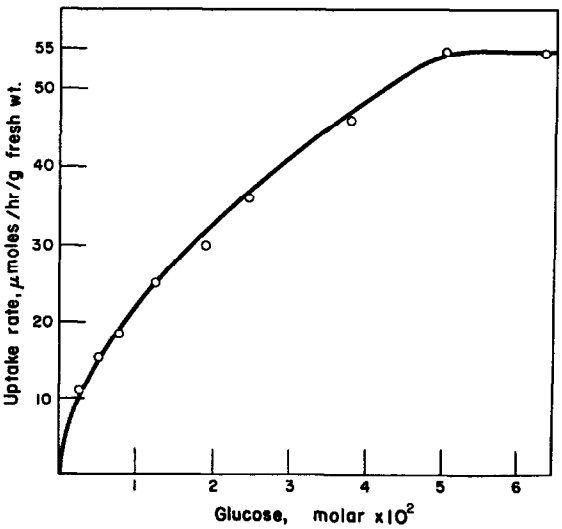

FIG. 2. RATE OF GLUCOSE UPTAKE INTO SCUTELLUM SLICES AS A FUNCTION OF GLUCOSE CONCENTRATION.

The slices were incubated at 30° for 90 min prior to the addition of glucose. Rates were obtained from a series of measurements over a 2 hr period.

the medium during prior incubation, but in these instances the slices were washed in water before the rate of uptake was determined.

The results of kinetic studies on uptake as a function of both time and glucose concentration show the complexity of the uptake process. At low glucose concentrations (e.g. $4{\cdot}9 \times 10^{-3}$ M) the rate of glucose uptake remained constant with time even after the external solution was largely depleted of glucose (Fig. 1, curve 1). After 120 min, 72 per cent of the glucose initially added had entered the slices. During this period the average glucose concentration decreased from $4{\cdot}4 \times 10^{-3}$ M during the first 30 min period of measurement to $1{\cdot}8 \times 10^{-3}$ M during the last 30 min period without a decrease in uptake rate. However, from the data of Fig. 2, which show the uptake rate as a function of glucose concentration, it would be expected that such a decrease in glucose contentration would have resulted in a 50 per cent reduction in uptake rate. In some experiments the rate of uptake decreased after 90–120 min,

TABLE 1. EFFECT OF LENGTH OF THE INCUBATION PERIOD, PRIOR TO THE ADDITION OF GLUCOSE, ON THE SUBSEQUENT RATE OF GLUCOSE UPTAKE

Prior incubation period (min)	Uptake rate* (μmole per hr per g fresh wt.)			
	$2{\cdot}45 \times 10^{-3}$ M Glucose†	$4{\cdot}9 \times 10^{-3}$ M Glucose	$2{\cdot}45 \times 10^{-2}$ M Glucose	$4{\cdot}9 \times 10^{-2}$ M Glucose
30	5·9	10·0	31·1	49·4
60	8·8	12·2	32·8	47·2
90	11·9	14·4	35·0	55·0
120	14·9	16·1	36·7	58·0
180	17·6	18·9	42·2	58·0

* The numbers in the columns are averages of three rate determinations.

† These values refer to the initial glucose concentrations.

but in all cases the initial rate of uptake was maintained for periods longer than would be expected from the data shown in Fig. 2.

Another phenomenon perhaps related to the above was observed. An increase in the prior incubation period in water from 30–90 min was followed by a 50 per cent increase in rate of glucose uptake (Fig. 1). This effect of prior incubation was nullified wholly or in part by adding glucose to the incubation medium (Fig. 1) or by increasing the glucose concentration in the uptake vessel. The effect of increasing the length of the prior incubation in water on the subsequent rate of uptake from four concentrations of glucose is shown in Table 1. The length of the prior incubation period had very little effect when a $4{\cdot}9 \times 10^{-2}$ M glucose concentration was used, but a threefold increase in rate of uptake from $2{\cdot}45 \times 10^{-3}$ M glucose solution was obtained with an increase in the incubation period from 30–180 min.

The effect of prior incubation on glucose uptake was not due to a metabolic change caused by slicing the scutellum, since the effect was also obtained with whole scutella. Nor was the effect due to temperature, since it was also observed at 25°, the temperature at which germination took place. The observed phenomena were not associated with changes in oxygen consumption; the slices consumed oxygen at a rate of 800–900 μl per hr per g at 30°. The rate was constant over a 4-hr period, and was unchanged by the addition of glucose to a concentration of $4{\cdot}9 \times 10^{-2}$ M.

TABLE 2. CHANGE IN THE GLUCOSE, GLUCOSE-6-PHOSPHATE, TOTAL REDUCING SUGAR AND SUCROSE CONTENT OF SCUTELLUM SLICES WITH LENGTH OF INCUBATION PERIOD IN THE PRESENCE OR ABSENCE OF GLUCOSE

Incubation period (hr)	No addition				$4{\cdot}9 \times 10^{-3}$ M Glucose				$4{\cdot}9 \times 10^{-2}$ M Glucose			
	Glucose*	G-6-P*	R.S.†	Sucrose†	Glucose	G-6-P	R.S.	Sucrose	Glucose	G-6-P	R.S.	Sucrose
0	2·3	0·83	8·3	51·8								
1	1·4	0·41	5·0	38·6	1·5	0·50	6·3	48·8	4·1	0·85	10·1	52·0
2	1·7	0·33	5·7	34·8	1·8	0·38	7·3	37·4	3·7	0·81	9·7	57·9
4	1·6	0·21	5·7	21·9	2·0	0·36	7·8	31·3	3·8	0·51	12·7	57·3

* Values are μmole per g fresh wt. and are each an average of 3 or 4 determinations.
† Values are μmole per g fresh wt. and are each an average of 4 or 5 determinations.

Changes in Tissue Sugars

The change which occurred in the slices during incubation in water resulting in a greater rate of glucose uptake was related, apparently, to the endogenous level of glucose or a product of glucose metabolism. This conclusion is based on the observation that glucose nullified, wholly or in part, the effect of prior incubation. Consequently, the slices were analyzed for their contents of glucose, total reducing sugars, sucrose, glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P). Analyses were performed on extracts prepared from scutellum slices immediately after weighing and after 1, 2 or 4 hr incubation at 30° either in the presence or absence of glucose. The results of these analyses are shown in Table 2.

In the absence of exogenous glucose, G-6-P decreased to one-fourth the initial amount after 4 hr, while the glucose content decreased during the first hour and increased slightly thereafter. In order to maintain the amount of G-6-P at the level initially present in the tissue, it was necessary to supply an exogenous glucose concentration of $4{\cdot}9 \times 10^{-2}$ M. Even at this concentration the amount of G-6-P decreased about 40 per cent below the initial level after 4 hr. This probably was a result of the decrease in exogenous glucose. (The amounts of F-6-P in the slices were found to be at levels expected from the equilibrium condition of the phosphoglucose isomerase reaction.)

At a glucose concentration of $4{\cdot}9 \times 10^{-2}$ M in the external solution, the endogenous glucose was maintained at a level about 2 μmole higher than that in the controls. However, considering that 50 μmole of glucose entered the slices per hour, it is apparent that glucose was not being accumulated as the free sugar but was utilized as it entered the cells. It was utilized, in part, to maintain the sucrose content and to increase the reducing sugar content of the tissue. However, a comparison of the amounts of reducing sugar and sucrose in the absence and presence of $4{\cdot}9 \times 10^{-2}$ M glucose shows that these sugars account for only 66 per cent (after 1 hr) or 38 per cent (after 4 hr) of the glucose taken up. The starch content of the slices was small (5–6 μmole, calculated as glucose) and did not increase when the slices were incubated with glucose. Inasmuch as the addition of glucose did not increase the oxygen consumption of the slices, the glucose taken up which did not appear as reducing sugar or sucrose must have been utilized in the synthesis of other compounds.

The carbohydrate analyses show that the amounts of both G-6-P and sucrose in the slices decreased during incubation in water but could be maintained approximately at the initial levels by incubation with glucose. The possibility exists, therefore, that the levels of these compounds are factors involved in the effect of prior incubation on glucose uptake. Evidence that hexose monophosphates can inhibit glucose uptake was obtained from experiments with mannose.

Inhibition by Mannose

Glucose uptake from a $4{\cdot}9 \times 10^{-2}$ M solution was inhibited 80 per cent by the presence of an equimolar concentration of mannose. Furthermore, incubation of slices in $4{\cdot}9 \times 10^{-2}$ M mannose for an hour followed by washing for 2–3 min in several changes of water resulted in an inhibition of the uptake of subsequently added glucose (Table 3). Inhibition by prior incubation with mannose decreased with time during glucose uptake and was less pronounced in the presence of high glucose concentrations.

Because inhibition by mannose persisted after washing, extracts of the slices were analyzed for mannose, mannose-6-phosphate (M-6-P) and other sugars (Table 4). Both mannose and M-6-P were present in the tissue at relatively high levels immediately after washing the slices. However, the mannose content declined rapidly while the M-6-P content

remained constant. The decrease in mannose was not due to metabolic utilization, but to leakage of mannose into the bathing solution (Table 5). Since it was possible to wash out the mannose, the amount of mannose found at time-zero (6–8 μmole) may have been that which was left due to insufficient washing following the mannose incubation. Whether the mannose was inside or outside the cells remains to be determined.

TABLE 3. INHIBITION OF GLUCOSE UPTAKE BY PRIOR INCUBATION OF THE SCUTELLUM SLICES IN MANNOSE*

Uptake period (hr)	Inhibition (%)	
	$4{\cdot}9 \times 10^{-3}$ M Glucose	$4{\cdot}9 \times 10^{-2}$ M Glucose
0·5	68	—
1·0	61	51
1·5	61	—
2·0	58	42
3·0	—	33
4·0	—	27

* The slices were incubated in $4{\cdot}9 \times 10^{-2}$ M mannose for 1 hr and then washed for 2 min in running distilled water. The washed slices were placed in a glucose solution and uptake measurements begun immediately.

After prior incubation with mannose, slices took up glucose from a $4{\cdot}9 \times 10^{-3}$ M solution at a rate only 32 per cent as great as that of the control slices (68 per cent inhibition, Table 3). In a similar experiment two groups of slices were incubated in mannose ($4{\cdot}9 \times 10^{-2}$ M) for 1 hr. One group was washed in water for 2–3 min as before, but the other group was washed for 1 hr with several changes of water to remove most of the mannose. The rates of glucose uptake into these two groups of slices were compared to the rate obtained with slices which had been incubated with water for 1 hr and washed for 2–3 min. Mannose treatment followed by a short washing period caused a 60 per cent inhibition of uptake from a $4{\cdot}9 \times 10^{-3}$ M glucose solution while the longer washing period decreased the inhibition to 38 per cent.

TABLE 4. CHANGES IN THE SUGAR CONTENT OF SCUTELLUM SLICES WITH TIME AFTER INCUBATION IN MANNOSE*

Sugar	Time period following mannose treatment (hr)			
	0	1	2	4
	(μmole per g fresh wt.)			
Glucose	4·0	2·9	2·3	3·6
Fructose	1·7	1·4	1·4	1·8
Mannose	6·2	2·8	0·8	0·9
G-6-P	<0·05	<0·05	0·09	0·08
F-6-P	<0·05	<0·05	<0·05	<0·05
M-6-P	7·9	8·4	7·4	8·8

* The slices were incubated with $4{\cdot}9 \times 10^{-2}$ M mannose for 1 hr and then washed for 2 min in running distilled water. The washed slices were incubated in water at 30° for the period shown, and then were killed and extracted (see Experiment section).

TABLE 5. LEAKAGE OF MANNOSE FROM SCUTELLUM SLICES INTO THE BATHING SOLUTION*

Sugar	Time period following mannose treatment (hr)		
	0	1	2
	(μmole per g fresh wt.)		
M-6-P (in slices)	8·0	8·3	8·3
Mannose (in slices)	8·1	0·7	0·5
Mannose (in bathing solution)	—	6·2	5·7

* The slices were incubated with $4{\cdot}9 \times 10^{-2}$ M mannose for 1 hr and then washed for 2 min in running distilled water. The washed slices were incubated in water at 30° for the periods shown and then were removed, washed, killed and extracted (see Experimental section). A portion of the bathing solution was removed for mannose determination just prior to killing the slices.

The above data suggest that free mannose was partially responsible for the inhibition of glucose uptake and that the inhibition remaining after extended periods of washing was due to M-6-P. Furthermore, it appears that both mannose and M-6-P exert their effect by inhibiting the utilization of glucose after it has entered the cells. The very low amounts of G-6-P and F-6-P in the tissues after mannose treatment indicate an inhibition of glucose phosphorylation (Table 4). To determine the effect of prior incubation in mannose on the accumulation of glucose and G-6-P, mannose-treated slices were placed in $4{\cdot}9 \times 10^{-2}$ M glucose for 1 hr, after which the slices were analyzed for these two sugars. The analysis showed that the G-6-P content increased to only 0·26 μmole per g while the glucose increased to 6·7 μmole per g. Slices which received no mannose treatment contained 0·71 μmole per g and 3·0 μmole per g respectively of G-6-P and glucose after a 1 hr incubation in the same concentration of glucose.

DISCUSSION

The data suggest that the rate of glucose phosphorylation was the limiting factor in net glucose uptake. Mannose treatment inhibited both net glucose uptake and glucose phosphorylation. However, it did not inhibit glucose penetration into the cells since mannose-treated slices maintained a higher glucose content in the presence of external glucose than did untreated slices. Both mannose and M-6-P appear to inhibit glucose uptake. Mannose, as a free sugar, was responsible for that fraction of the inhibition which was relieved when mannose was washed from the cells. The remaining inhibition is assumed to be due to M-6-P although this assumption could not be tested because the tissue could not be freed of M-6-P.

In the non-inhibited tissue the rate of glucose uptake was influenced by both the length of the prior incubation period and the concentration of glucose in the external medium. These observations can be explained if it is assumed that G-6-P competitively inhibits glucose phosphorylation. By this reasoning the reduction in the amount of G-6-P during incubation in the absence of glucose (Table 2) would account for the effect of the prior incubation on glucose uptake. The further assumption that the inhibition is competitive is necessary since the glucose concentration ($4{\cdot}9 \times 10^{-2}$ M) which supported the highest rate of uptake also maintained the G-6-P content of the slices at its initial level ($\sim$ 0·8 μmole per g). Calculation of the glucose/G-6-P ratios from the data of Table 2 shows that during incubation in the absence of glucose

the ratio increases from 2·8 initially to 7·6 after 4 hr while during incubation with $4·9 \times 10^{-2}$ M glucose the ratio remains at about 4·7 for the first 2 hr. However, the concentration of G-6-P and glucose in the immediate vicinity of the enzymatic phosphorylation step are of interest here, and the data obtained for the whole tissue do not provide this information.

According to the postulates outlined above, the rate of glucose uptake is controlled by a hexokinase-type reaction. Glucose, mannose, G-6-P and M-6-P compete for the same site on the enzyme, and glucose phosphorylation is inhibited to varying degrees depending on the concentrations of the sugar and sugar phosphates. If this is the correct interpretation of these results, then the association constant of G-6-P for the enzyme must be much greater than that of M-6-P since M-6-P inhibited glucose uptake from a $4·9 \times 10^{-3}$ M glucose solution by only 35 per cent when it was present at a level 10 times as great as the maximum G-6-P level found.

Hexokinases from brain and other animal tissues have been studied in some detail,[6–8] and have been found to be non-competitively inhibited by G-6-P. Crystalline hexokinase preparations from yeast are not inhibited by G-6-P.[7] Very little information is available on hexokinase from higher plants. A particulate hexokinase from wheatgerm[9] was inhibited only 17 per cent by 5×10^{-3} M G-6-P. A concentration 1·5 times as great as that of the glucose in the reaction mixture. However, the presence, in crude extracts, of phosphohexose isomerase and phosphofructokinase would remove the G-6-P. An attempt to prepare a highly purified hexokinase from corn scutella is presently under way in this laboratory.

EXPERIMENTAL

Plant Materials

Corn grains (*Zea mays* L., var. Funks G-50 or Funks G-76) were soaked in aerated tap-water for 24 hr and then placed on moist filter paper in the dark at 24° to 25° for 72 hr. The scutella were excised from the germinated grains and cut transversely into slices 0·5 mm or less in thickness. The slices were washed in distilled water until the wash water remained clear, and then were blotted on filter paper and weighed in groups of 1 g. Excising, slicing and weighing the scutella required 75–85 min.

Measurement of Glucose Uptake

Each group of slices (1 g fresh wt.) was placed in a 25 ml Erlenmeyer flask containing 6·5–8·9 ml of distilled water. The flasks were placed in a "Gyrotory" water bath at 30° (New Brunswick Scientific Company, New Brunswick, N.J.) and rotated at approximately 180 rev/min. After a period of incubation in the water bath (the length of the incubation period prior to the addition of glucose to the flasks was a parameter in these experiments), the flasks were removed and a glucose solution (0·22 M) was added to bring the fluid volume to 9·0 ml. One min after the addition of glucose, an 0·1 ml portion of the bathing solution was removed from each flask and the flasks were returned to the water bath. At predetermined intervals, following the addition of glucose, additional 0·1 ml portions were removed. The glucose content of each 0·1 ml sample was determined with the use of glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N.J.). The decrease in the glucose content of the bathing solution was assumed to be equal to the glucose taken up by the scutellum slices.

The results of preliminary experiments showed that pH in the range 3·1–7·2 had no effect

[6] R. K. CRANE and A. SOLS, *J. Biol. Chem.* **203**, 273 (1953).
[7] R. K. CRANE and A. SOLS, *J. Biol. Chem.* **210**, 597 (1954).
[8] H. WEIL-MALHERBE and R. BONE, *Biochem. J.* **49**, 339 (1951).
[9] P. SALTMAN, *J. Biol. Chem.* **200**, 145 (1952).

on glucose uptake. Accordingly, buffer was not added to the bathing solutions in the experiments reported in this paper. The pH of the bathing solution in the absence of added buffer was 4·6–4·8.

Preparation of Scutellum Extracts

One g of scutellum slices was placed in a 50 ml beaker and killed by the addition of 20 ml of boiling 80% ethanol, boiling was continued for 30 sec, and after 1 hr at room temperature the ethanol solution decanted into a 150 ml beaker. This was repeated and the slices were then washed with three 5-ml portions of 80% ethanol. The combined extracts were evaporated on a steam bath until the volume was reduced to approximately 2 ml. The remaining aqueous solution was transferred to a graduated 12 ml centrifuge tube, the pH adjusted to 7·0–7·2, and water added to bring the volume to 6·0 ml. The solution was centrifuged at 1000 *g* for 10 min and the supernatant fluid was decanted into a plastic tube and frozen. This solution was analyzed for its content of sucrose, glucose, fructose, mannose, reducing sugars and hexose monophosphates.

To test for the completeness of the extraction of G-6-P, the residue of the slices was re-extracted again with 20 ml of boiling 80% ethanol. The aqueous solution remaining after removal of the ethanol contained no G-6-P.

Known amounts of authentic hexose monophosphates, added to the scutellum slices just prior to killing them with boiling 80% ethanol, were recovered to the extent of 90 to 100%.

Carbohydrate Analysis

For the analysis of sucrose, glucose and reducing sugars, a portion of the scutellum extract was cleared with $ZnSO_4$ and $Ba(OH)_2$ according to the method of Nelson.[10] Glucose was determined by the glucose oxidase method. Sucrose was determined by analyzing the cleared extract for glucose before and after invertase treatment. Reducing sugars were determined by the procedure of Nelson[10] using the modified copper reagent of Somogyi.[11]

G-6-P, F-6-P, M-6-P, glucose, fructose and mannose were assayed by measuring the amount of $NADPH_2$ formed in the presence of the scutellum extract, G-6-P dehydrogenase and the proper combination of the enzymes; phosphoglucose isomerase, phosphomannose isomerase and hexokinase. In addition to the scutellum extract and the appropriate enzymes, the cuvettes contained 40 μmole glycylglycine buffer (pH 7·5), 0·8 μmole NADP, 5 μmole $MgCl_2$, 5 μmole ATP (when hexokinase was used) and water to 3·0 ml. The stock enzyme suspensions were diluted with glycylglycine buffer (0·04 M, pH 7·5) just prior to use. In the blank cuvette buffer was substituted for G-6-P dehydrogenase. The reduction of NADP was followed in the Beckman DU spectrophotometer at 340 mμ. The reaction was considered to be complete when there was no change in absorbancy for 2–4 min. When M-6-P or mannose was being assayed, however, the reaction was considered complete when there was a change in absorbancy of 3 per cent or less in 10 min.

Each enzyme except phosphomannose isomerase was added in the amount of 0·5–2·0 E.U. per cuvette. Phosphomannose isomerase was obtained as a lyophilized powder from rabbit muscle, and 0·5 mg of this crude preparation was added to the cuvette. The rabbit-muscle powder also contained phosphoglucose isomerase, but no hexokinase or G-6-P dehydrogenase activity was observed. When known amounts of authentic hexoses and hexose

[10] N. Nelson, *J. Biol. Chem.* **153**, 375 (1944).
[11] M. Somogyi, *J. Biol. Chem.* **195**, 19 (1952).

monophosphates were added to the cuvettes in place of the scutellum extract the amount of $NADPH_2$ formed was within 10 per cent of the calculated amount.

Reagents

G-6-P dehydrogenase, hexokinase, and phosphoglucose isomerase were obtained from the California Corporation for Biochemical Research (prepared by C. F. Boehringer and Son). Phosphohexose isomerase (phosphomannose isomerase), NADP, ATP and mannose were obtained from The Sigma Chemical Company.

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