PENTOSE METABOLISM IN EXTRACTS OF YEAST AND MAMMALIAN TISSUES*

by

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In an earlier publication[1] an enzyme was described which catalyzes the transfer of phosphate from adenosine triphosphate (ATP) to ribose. This enzyme, designated *ribokinase*, is present in extracts of bakers' and brewers' yeast. The reaction products could not be isolated, since it was found that interfering enzymes were not eliminated by partial purification of the enzyme; a 15-fold purified enzyme solution contained phosphatases and the enzyme complex which degrades ribose-5-phosphate (R-5-P) anaerobically. A study of degradation of pentose compounds by yeast was undertaken in order to establish a criterion to be used in determining the products of the ribokinase reaction. The work of DICKENS[2], DISCHE[3], SCHLENK AND WALDVOGEL[4], RACKER[5] AND LAMPEN*** was a useful guide in this connection. The metabolism of R-5-P in extracts of brain, skeletal muscle and heart muscle will also be reported.

MATERIALS

Pentose degradation in yeast was studied with a protein fraction of brewers' yeast maceration juice, precipitated at pH 7.6 between 0.45 and 0.65 saturation with ammonium sulfate, as described in the earlier publication. The preparation of the mammalian tissue extracts is described in the EXPERIMENTAL section.

Ribose was a gift from Hoffman-LaRoche, Inc. *Glycolaldehyde* was prepared either by hydrolysis of glycolaldehyde diethyl acetal (Eastman Kodak Co.) or by decarboxylation of dihydroxymaleic acid§ by heating an aqueous solution to 60° for 30 minutes, by which time all effervescence had ceased. The concentration of glycolaldehyde in the resulting solutions was determined by titration with periodate.

Ribose-5-phosphate was prepared by hydrolysis of ATP[6]. *Arabinose-5-phosphate* (A-5-P) (as the brucine salt) and *xylose-5-phosphate* (X-5-P) (as the barium salt) were obtained from the P. A. Levene Collection of the Rockefeller Institute, through the courtesy of Dr LYMAN C. CRAIG. *Ribose-1-phosphate* (R-1-P) was the crystalline dipotassium salt† kindly supplied by Dr GRAHAM T. WEBSTER. *Fructose-1,6-diphosphate* was purified by a modification of the method of NEUBERG *et al.*[7,8]. DL-*glyceraldehyde-3-phosphate*[9] was a gift from Drs H. O. L. FISCHER AND E. BAER.

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*** J. O. LAMPEN: Unpublished work, Washington University, 1947.

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ATP was purchased from Rohm and Haas, Inc. *Diphosphopyridine nucleotide* (DPN) of 48% purity was prepared by Dr S. F. VELICK by the method of WILLIAMSON AND GREEN[10]. *Reduced diphosphopyridine nucleotide* (DPNH) was prepared by Dr F. E. HUNTER, Jr., by the method of OHLMEYER[11]. *Triphosphopyridine nucleotide* (TPN) was prepared from beef liver by the method of WARBURG *et al.*[12]

Crystalline *aldolase*[13], crystalline D-*glyceraldehyde-3-phosphate dehydrogenase*[14] and partially purified *zwischenferment*[15] were prepared by standard procedures.

Tris(hydroxymethyl)aminomethane was purified as described elsewhere[16].

METHODS

Reducing sugar was determined by the method of NELSON[17]. *Pentose* was determined by the method of MEJBAUM[18] slightly modified as to $FeCl_3$ concentration and heating time. *Fructose* was determined by the ROE[19] procedure. *Glycolaldehyde* was measured either as reducing sugar or by the diphenylamine reaction of DISCHE AND BORENFREUND[20], both methods being first standardized by the periodate titration. *Formaldehyde* was distilled from the periodate oxidation mixtures after titration with sodium arsenite. It was found that the amount of formaldehyde distilled was proportional to the amount of water distilled, owing to the high degree of hydration of formaldehyde in dilute solution[21]. Formaldehyde in the distillate was determined by the chromotropic acid method[22] as modified by MACFADYEN[23] and BRICKER AND JOHNSON[24]. The standard of reference was freshly resublimed hexamethylene tetramine, hydrolyzed by heating in a sealed tube with H_2SO_4. Good recovery of known amounts of formaldehyde was obtained by this method.

*Triose phosphate**, *fructose-1,6-diphosphate* and *hexose-6-phosphate** were determined by their ability to react with DPN or TPN in the presence of the specific enzymes[13,14,15]. Oxidation or reduction of pyridine nucleotides was measured by observing the absorption band at 340 mμ with a Beckman quartz spectrophotometer, model DU. The extinction coefficients used in computing the results were those reported by HORECKER AND KORNBERG[25].

Inorganic orthophosphate was determined by KING's[26] modification of the method of FISKE AND SUBBAROW[27].

Periodate was determined by titration with sodium arsenite[28].

Chromatography of sugars on filter paper strips was carried out as described previously[1].

EXPERIMENTAL

Pentose metabolism in yeast extracts. When the yeast protein fraction was incubated with R-5-P there was rapid disappearance of material giving the pentose colour in the orcinol test, while A-5-P and X-5-P were unaffected. Fig. 1 represents a typical exper-

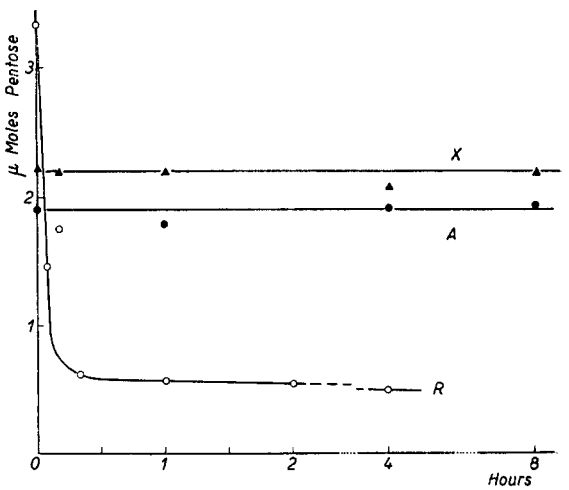

Fig. 1. Degradation of pentose phosphate by extracts of brewers' yeast. The substrates were incubated at 30° with 0.40 ml of the fraction of yeast extract precipitated between 0.45 and 0.65 saturation with $(NH_4)_2SO_4$; tris(hydroxymethyl) aminomethane 0.04 *M* pH 7.6; NaF 0.1 *M*, final volume 1.4 ml. The "zero time" values were obtained by having the fixing agent present before addition of the protein solution. Pentose was determined in the protein-free filtrates. A-arabinose-5-phosphate; R-ribose-5-phosphate; X-xylose-5-phosphate.

* The D-glyceraldehyde phosphate dehydrogenase preparation used contained triose phosphate isomerase, so both triose phosphates were measured together. The zwischenferment contained hexose-6-phosphate isomerase, so that fructose-6-phosphate and glucose-6-phosphate were measured together.

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iment. The slow rate of disappearance of pentose following the initial rapid phase may mean that an equilibrium point is being approached, or may be a reflection of the effect on the enzyme of diminished substrate concentration. Some of the remaining pentose is free ribose, which is not attacked by the degrading system. Ribose-3-phosphate has been tested recently, and it is not degraded by this protein fraction*.

It was observed that as pentose disappeared there was a concomitant increase in material reacting as fructose in the ROE test (Table I). This observation, together with the results of paper chromatography, made it seem likely that fructose phosphates were being formed, by the mechanism proposed by SCHLENK AND WALDVOGEL[4]. In another experiment (Table II) the incubation mixtures were fixed in 0.15 *N* HCl and then

TABLE I

FORMATION OF KETOSE DURING DEGRADATION OF RIBOSE-5-PHOSPHATE BY BREWERS' YEAST EXTRACT

Incubation of R-5-P at 30° with a protein fraction precipitated between 0.51 and 0.65 saturation with $(NH_4)_2SO_4$; 0.07 *M* tris(hydroxymethyl)aminomethane, pH 7.8.

Time *Minutes*	*Pentose disappeared* *Micromoles**	*Ketose accumulated* *Micromoles**	*Pentose equivalent***
5	1.54	0.25	1.00
15	2.55	0.82	3.28
45	3.69	1.24	4.96
90	5.17	1.07	4.28
150	5.23	1.01	4.04
720	5.34	1.15	4.60

* The values are relative to amounts found in an aliquot taken at 0.5 minutes.

** Fructose-6-phosphate and fructose-1,6-diphosphate give between half and two-thirds as intense a color as an equimolar amount of fructose. Each mole of fructose is derived from two moles of pentose. Thus, as an approximation, the last column is derived from the previous one by applying the arbitrary factor 4.

TABLE II

PRODUCTS OF ANAEROBIC DEGRADATION OF RIBOSE-5-PHOSPHATE BY BREWERS' YEAST

R-5-P* incubated at 30° with the fraction of yeast extract precipitated between 0.50 and 0.65 saturation with $(NH_4)_2SO_4$; NaF 0.1 *M*, tris(hydroxymethyl)aminomethane 0.04 *M*, pH 7.6.

Time *Minutes*	*Pentose disappeared* *Micromoles*	*Recovery of 3-carbon fragment* *Micromoles*				
		As triose	*As fructose diphosphate*	*As hexose-6-phosphate*	*Total*	%**
10	1.17	0.21	0.10	0.55	0.86	71
30	1.25	0.20	0.09	0.78	1.07	89
75	1.17	0.15	0.05	0.68	0.88	73
	Average 1.20					

* Initial amount of R-5-P = 2.44 micromoles.

** Calculated on the basis of 1.20 micromoles of pentose disappearing.

* A. J. GUARINO, AIDA T. DE CORI, AND H. Z. SABLE: Unpublished experiments 1951.

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neutralized, upon which the protein precipitated*. The protein-free filtrates were analyzed enzymatically. Up to 89% of the phosphorylated moiety of R-5-P was recovered as triose phosphates and hexose mono- and di-phosphates. This evidence indicates that R-5-P was cleaved to triose phosphate and an unphosphorylated two-carbon fragment. Since the extracts are rich in most of the enzymes of anaerobic glycolysis, both triose phosphates would be present regardless of which one is the primary product. Triose phosphate condensation would then give fructose-1,6-diphosphate, and dephosphorylation would yield fructose-6-phosphate.

Glycolaldehyde. The fate of carbon atoms 1 and 2 of R-5-P has not been determined. Glycolaldehyde has been suggested as a possible product[2,3] but has never been found as a product of pentose degradation by cell free extracts of animal tissues or of micro-organisms**. Experiments have been carried out with the yeast extract which appear to exclude glycolaldehyde from consideration as a product of R-5-P degradation in this system. In the paper chromatograms[1], glycolaldehyde was found to move almost as rapidly as the solvent front. R-5-P was incubated with a degrading system of known potency, and the mixture fixed with $Ba(OH)_2$–$Zn(SO_4)$***. This reagent does not remove glycolaldehyde from solution. The filtrate was subjected to paper chromatography, and no rapidly moving component was found, nor was there any material reacting as glycolaldehyde in the diphenylamine test. In another experiment tests were carried out to see whether or not glycolaldehyde would remain if it were formed. Glycolaldehyde was incubated with the protein fraction with catalytic amounts of DPN or TPN, and also in the absence of coenzyme (Fig. 2). In the latter case there was no disappearance of glycolaldehyde over a period of 140 minutes§. It is clear therefore that if any glycolaldehyde had been formed during R-5-P degradation, it should have been detected.

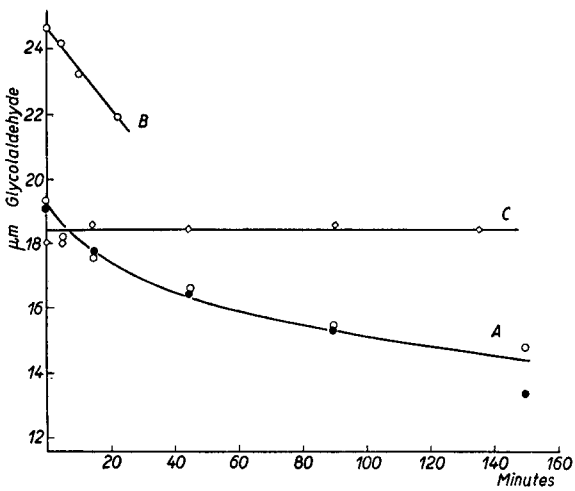

Fig. 2. Coenzyme-linked dismutation of glycolaldehyde in an extract of brewers' yeast. Glycolaldehyde was incubated with catalytic amounts of DPN or TPN in the presence of same protein fraction used for the experiment in Fig. 1; tris(hydroxymethyl)aminomethane 0.04 *M*, pH 7.6. Aliquots were fixed in $Ba(OH)_2$–$ZnSO_4$ and glycolaldehyde determined as reducing sugar.

Curve A ○-----○ Glycolaldehyde/DPN = 180/1
Curve A ●-----● Glycolaldehyde/TPN = 67/1
Curve B Glycolaldehyde/DPN = 42/1
Curve C No coenzyme

* Perchloric acid which was used for deproteinization in other experiments cannot be used here because the perchlorate ion interferes with the enzymatic tests.

** Recently KAUSHAL *et al.*[29] have isolated glycolaldehyde from culture media in which *Acetobacter acetigenum* was grown in the presence of D-xylose and L-arabinose.

*** M. SOMOGYI, quoted by NELSON[17].

§ In another experiment not shown in this figure, the effect of inorganic arsenate was tested. In the presence of catalytic amounts of the coenzymes, the presence or absence of arsenate had no observable effect on the rate or extent of disappearance of glycolaldehyde.

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The disappearance of glycolaldehyde in the presence of catalytic amounts of coenzyme probably occurs by a mechanism similar to that proposed by RACKER[30], a dismutation catalyzed by alcohol dehydrogenase and aldehyde dehydrogenase. That the former activity is present in this protein fraction is shown by the fact that reduced DPN and TPN are rapidly reoxidized when glycolaldehyde is added (Fig. 3). Yeast alcohol dehydrogenase has been reported to be specific for DPN[31], while yeast aldehyde dehydrogenase reacts with either DPN or TPN[32]. Work is now in progress to determine whether yeast also contains a TPN-linked alcohol dehydrogenase.

The product of the ribokinase reaction. Evidence regarding the nature of the phosphorylated product was obtained as follows: the usual reaction mixtures[1] were prepared. In a typical experiment 5.55 micromoles of ribose were phosphorylated in 75 minutes, as determined by the reducing power of aliquots fixed with $Ba(OH)_2$–$Zn(SO_4)$*. The remainder of the incubated mixture was fixed in 0.2 *N* HCl and neutralized with NaOH.

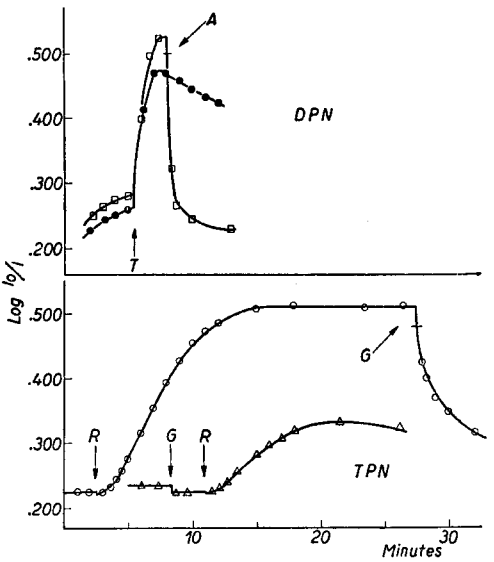

Fig. 3. Oxidation of reduced coenzymes by glycolaldehyde. Experiments were carried out in spectrophotometer cuvettes, with the same protein fraction as that used for the experiment of Fig. 1. The upper portion of the Figure represents an experiment in which 0.26 micromoles of DPN were present, and the extract fortified with crystalline triose phosphate dehydrogenase and the other components of the triose phosphate test[14]. At T 0.4 omicromoles of glyceraldehyde-3-phosphate were added, in order to form reduced DPN.

● ----- ● No glycolaldehyde added.
□ ----- □ At A, 3 micromoles of glycolaldehyde were added.

The lower portion of the Figure represents an experiment in which 0.20 micromoles of TPN were present. At R 0.80 micromoles of R-5-P were added; at G 6 micromoles of glycolaldehyde were added.

The protein-free filtrate was analyzed enzymatically, and contained 0.185 micromoles of triose phosphate, 0.092 micromoles of fructose diphosphate and 1.59 micromoles of hexose-6-phosphate. Since each mole of hexose is derived from two moles of pentose, the total recovery of the 3-carbon portion of ribose is 4.1 micromoles or 74% of the amount phosphorylated.

In another experiment, four mixtures were prepared and, after incubation with the protein fraction and deproteinization, the barium salts insoluble in 80% ethanol were isolated. Any unphosphorylated sugar would remain in the supernatant fluid, while phosphorylated compounds were included in the precipitated salts. The first mixture was the usual one used in the ribokinase test; in the second, ATP was present and ribose omitted; in the third, ribose was present and ATP omitted and in the fourth, both ATP and ribose were omitted and authentic R-5-P added. The salts were suspended in water, barium removed with H_2SO_4 and the solutions neutralized and subjected to paper chro-

* The R-5-P and other phosphorylated substances are precipitated with this reagent. The filtrates contain only free ribose.

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matography. Reducing sugar was found to be present only in the products obtained from the first and fourth incubation mixtures. This excluded the possibility that the extracts had formed R-5-P from ATP.

The existence in muscle extract of a phosphoribomutase[1] catalyzing the reaction, ribose-1-P $\rightleftharpoons$ ribose-5-P, and the fact that yeast galactokinase[33] produces galactose-1-phosphate suggested the possibility that the primary product of the ribokinase reaction might be R-1-P. If that were the case the protein fraction would have to contain phosphoribomutase in order to explain the results obtained. However, the phosphoribomutase activity of the fraction is negligible under the conditions of the ribokinase test. The protein fraction also contains the enzyme[34] which catalyzes the reduction of TPN by R-5-P (*cf.* Fig. 3), but addition of R-1-P did not lead to any reduction of TPN. These findings as a whole suggest that R-5-P is the product of the ribokinase reaction. Ribokinase of ribose-adapted *E. coli*[35] also produces R-5-P. This fact is of significance in the chemistry of ribose, since it indicates that the sugar reacts either in the aldehyde or furanose form, presumably the latter. This form is probably present in aqueous solutions in more than trace amounts and would be in mutarotation equilibrium with the pyranose form[36]. Hydrolytic nucleosidases present in bakers' yeast[37] and brewers' yeast*, as well as phosphatases, probably produce significant amounts of ribose which may be the substrate for the enzyme in the living cell.

Pentose degradation in rabbit brain. Whole brain was homogenized with 10 volumes of water in a Waring blendor and stored at −15°. Before use 5 ml of the homogenate were dialyzed for 2 hours against 8 liters of water at 2°. A series of tubes were prepared containing aliquots of a solution of R-5-P, 0.90 ml dialyzed homogenate, 0.02 *M* tris (hydroxymethyl) aminomethane pH 8.0 0.1 *M* NaF, final volume 2.5 ml. The mixtures were incubated at 30°, and fixed by addition of 0.5 ml of a solution 2.5% $HgCl_2$–0.5 *N* HCl. Mercury was removed with H_2S, and the filtrates aerated and neutralized with solid $NaHCO_3$. These solutions were used for pentose determinations and for a terminal analysis of inorganic phosphate (Fig. 4). Approximately 60% of the pentose had disappeared before a stationary phase was reached.

The filtrates were also analyzed enzymatically. Hexose mono- and diphosphates were present in about the same proportions as in the filtrates obtained from degradation of R-5-P by yeast. In the final sample (201 minutes' incubation) the amount of these two esters corresponded to 1.13 micromoles of R-5-P, or 47.5% of the pentose which disappeared.

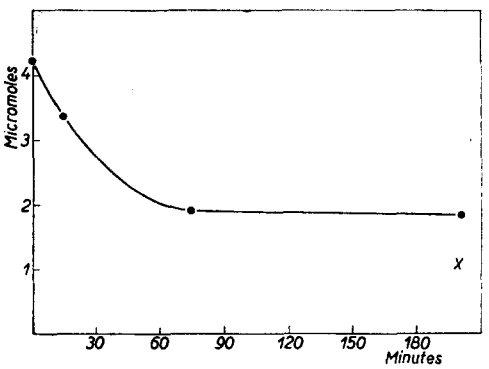

Fig. 4. Anaerobic degradation of R-5-P by a water extract of rabbit brain. Experimental details in text. The single point (x) at the level of 1 micromole represents the increase in inorganic phosphate in 201 minutes' incubation.

Pentose degradation in skeletal muscle. It seemed desirable to study the fate of R-5-P in muscle as a preliminary to further study of the phosphoribomutase system. A rabbit was killed by intravenous injection of nembutal, the muscle rapidly excised, ground in a chilled meat grinder and then extracted twice with cold water,

* A. J. GUNRINO, AIDA T. DE CORI, AND H. Z. SABLE: Unpublished experiments 1951.

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1 ml water being used per gram of muscle for each extraction. The extract was pressed through folded gauze and then filtered. The resulting turbid solution was stored at —15°.

Experiments to study the degradation of R-5-P were carried out as outlined in the section on brain, except that deproteinization was accomplished with perchloric acid. A typical result is shown in Fig. 5. Between 20 and 25% of the pentose disappeared in various experiments, and on prolonged incubation no further disappearance of pentose was observed*.

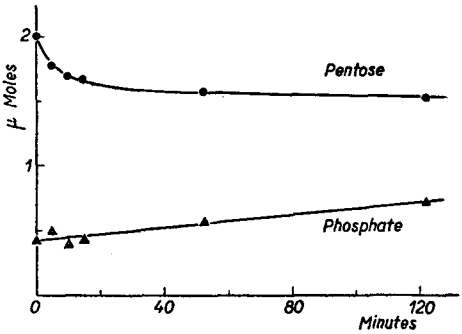

Fig. 5. Degradation of R-5-P by a water extract of rabbit skeletal muscle. Experimental details in text.

Disappearance of R-5-P in muscle extract was not accompanied by an accumulation of substances giving the fructose colour reaction. When the muscle extract was incubated in a spectrophotometer cuvette with DPNH, the addition of fructose diphosphate caused rapid reoxidation of the reduced coenzyme, indicating the presence of aldolase and glycerophosphate dehydrogenase[38]. Addition of glyceraldehyde phosphate also led to the oxidation of DPNH, indicating the presence of triose phosphate isomerase. Thus if either triose phosphate were formed, DPNH should be oxidized. However, when R-5-P was incubated with muscle extract, there was no formation of substances which reoxidized DPNH. In another experiment when TPN, zwischenferment, muscle extract and R-5-P were incubated together, no TPNH was formed, indicating that hexose-6-phosphate was not produced. This was verified in another experiment when R-5-P was incubated with muscle extract, and the reaction stopped with $HgCl_2$–HCl. The mercury-free filtrates were analyzed enzymatically, and again there was no formation of hexose phosphates.

While pentose disappeared, the reducing power of the reaction mixtures did not change. However, concurrent with the decrease in pentose content there was an accumulation of material which reduced the alkaline copper solution at 30° (Table III). This is a property of sugars with a free carbonyl group[39] such as dihydroxyacetone. This finding suggested the formation of a 2-ketopentose-5-phosphate, which could not form a monomeric 5- or 6- membered lactol ring, and would thus have an essentially free carbonyl function. The production of ketose would occur by a mechanism analogous to the isomerization of triose-3-phosphates and hexose-6-phosphates. R-5-P was not affected by partially purified glucose-6-phosphate isomerase of muscle** or of yeast, by phosphomannose isomerase of muscle[40], nor by crystalline aldolase.

Support for the hypothesis that a ketopentose phosphate is formed was obtained by periodate oxidation of the product of the reaction (Table IV). EULER *et al.*[41] found that the oxidation of R-5-P by periodate was complete in 1½ hours. In the present work an oxidation period of 3 hours was chosen. Control experiments showed that there was no over-oxidation of the reaction products by excess periodate under the conditions

* In similar experiments carried out with A-5-P and X-5-P there seemed to be a very slow disappearance of pentose over a period of 3 hours, the rate being about 1/50 that observed with R-5-P. The maximum disappearance in the case of the two former compounds was 5–6%. This is so close to the limit of experimental error that it is not entirely certain that these compounds are affected by the muscle extract.

** This enzyme was purified according to an unpublished procedure of Dr ARDA A. GREEN.

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TABLE III

RIBOSE-5-PHOSPHATE DEGRADATION IN MUSCLE EXTRACT*

Time Minutes	*Pentose*** *micromoles*	*Reducing sugar**** *micromoles*	*"Cold reduction" Colorimeter reading§*
0	3.11	3.42	8
5	3.01	3.18	21
20	2.77	3.19	28
60	2.74	3.21	40
120	2.60	3.24	31

* Experimental details in text.
** By orcinol-HCl reaction.
*** By Nelson's method, authentic ribose as a standard, heating 20 minutes at 100°.
§ With Nelson's Reagent, 2 hours at 30°. When the test is carried out at 100° with these aliquots a colorimeter reading of 40 corresponds to 0.37 micromoles of ribose. The colorimeter reading is reported because there is no absolute standard of reference.

TABLE IV

APPARENT FORMATION OF KETOPENTOSE PHOSPHATE FROM RIBOSE-5-PHOSPHATE

Two equal aliquots of a ribose-5-phosphate solution were incubated with the muscle extract at 30°, pH 8.0. For the "zero time" sample, perchloric acid was added to the reaction mixture before the extract was added. In the other sample, the reaction was stopped by addition of perchloric acid. The precipitated protein was removed and aliquots of the filtrate transferred to glass-stoppered flasks, adjusted to pH 6.5 with solid $NaHCO_3$, a solution of sodium periodate was added, and the mixture left at 30° for 3 hours.

Time Hours	*Pentose** *Micromoles*	*Periodate consumed Micromoles*	*Formaldehyde Micromoles*
0	33.2	101.9	4.5
2	26.3	102.4	13.3
Change:	— 6.9	+ 0.5	+ 8.8

* By orcinol-HCl

employed. It can be seen from the Table that while over 20% of the pentose disappears, the periodate consumption remains constant, indicating that there has been no cleavage of a carbon-carbon bond by the muscle enzyme. The periodate consumption at "zero time" also serves as a good check on the orcinol reaction, $^1/_3$ of periodate consumption being almost exactly equal to the initial amount of pentose. The fairly good agreement between decrease of pentose and increased production of formaldehyde by periodate oxidation means that a new hydroxymethyl group is generated. These data can be interpreted as follows:

$$\text{R-5-P} + 3\ IO^-_4 \rightarrow 3\ HCOOH + 3\ IO^-_3 + \text{glycolaldehyde-2-phosphate}$$

$$\text{2-ketopentose-5-phosphate} + 3\ IO^-_4 \rightarrow 2\ HCOOH + CH_2O + 3\ IO^-_3 + \text{glycolaldehyde-2-phosphate.}$$

The process is an isomerization, and the name *phosphoriboisomerase* is proposed for the enzyme which catalyzes this reaction. Recently HORECKER *et al.*[42,43,44] have described a phosphopentoisomerase in yeast, and have demonstrated the participation of ribulose-5-phosphate in carbohydrate metabolism in yeast and in mammalian tissues. It is

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probable that ribulose-5-phosphate is produced in all tissues which degrade R-5-P, and that the enzymes which catalyze subsequent steps are present in very small amounts, if at all, in muscle.

Pentose degradation in heart muscle. Rabbit heart muscle was freed of fat and connective tissue with scissors, and an extract prepared by pounding 4 grams of muscle in a cold mortar, with gradual addition of 16 ml of 0.05 *M* phosphate buffer, pH 7.5. Finally the mixture was stirred for 5 minutes and then centrifuged for 5 minutes at 7000 r.p.m. on a Sorvall supercentrifuge. The clear red supernatant fluid was dialyzed for 2 hours against 8 liters of distilled water at 2°. In an experiment arranged similarly to those with brain, the rate and extent of R-5-P degradation and the production of inorganic phosphate resembled the experiment with skeletal muscle shown in Fig. 5. Neither triose phosphates nor hexose mono- or diphosphates were demonstrable in the filtrates.

Phosphoribomutase. Some of the properties of this enzyme have been described. It was first demonstrated that R-1-P which is very labile to acid was converted to an acid stable ester*, and that R-5-P was converted to R-1-P[1]. It remained only to prove that the acid-stable ester formed from R-1-P was R-5-P. This was done in the following way: 8 micromole aliquots of R-1-P were incubated with skeletal muscle extract in the presence of 0.007 *M* $MgCl_2$ and 0.001 *M* glutathione buffered with 0.04 *M* tris (hydroxymethyl)aminomethane at pH 7.5. The samples were fixed with $HgCl_2$–HCl at 0, 45 and 180 minutes, and the neutralized mercury-free filtrates were analyzed. DICKENS[45] described an enzyme in brewers' yeast which catalyzes a reaction between R-5-P and TPN. Some purification of this enzyme has been achieved[34] but purification could not be carried to the point where the enzyme might serve as a quantitative analytical tool. It has been found possible, however, by choice of the proper conditions, to use the enzyme in qualitative and semiquantitative determination of R-5-P. In this way it was found that at 45 minutes 10 to 15% of the R-1-P had been converted to R-5-P, and in 180 minutes about 30% had been converted.

DISCUSSION

The anaerobic degradation of R-5-P in yeast and brain extracts occurs in a manner similar to that seen in other mammalian tissues and micro-organisms. In the fermentation of labelled xylose by *L. pentosus*[46] carbon atoms 1 and 2 are recovered as acetic acid, indicating that here too there is a cleavage of pentose into 2- and 3-carbon moieties. This anaerobic reaction is an alternate mechanism for the production of triose phosphate from hexose phosphate, since glucose-6-phosphate can form R-5-P as a result of oxidative processes[42,45,47]. In spite of the widespread distribution of enzymes which can form R-5-P from hexose, recent work[48] indicates that ribose in the animal organism may also be formed by a reversal of the 3–2 cleavage.

Probably R-5-P is not the compound which undergoes cleavage. It can be seen from Table II that although maximum disappearance of pentose-reactive material had occurred in 10 minutes, the recovery of the 3-carbon fragment derivatives was greater in a longer incubated material. This was a regular finding. The decrease noted in the final sample is probably due to the action of phosphatase. The delay in appearance of the 3-carbon fragment is good evidence for the interposition of one or more intermediate steps between R-5-P and triose phosphate.

* H. M. KALCKAR: Unpublished experiments 1950.

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SUMMARY

The anaerobic degradation of ribose-5-phosphate by extracts of yeast, brain, skeletal muscle and heart muscle has been investigated. In yeast and brain extracts cleavage occurs between carbon atoms 2 and 3 of the pentose, yielding triose phosphate and an unidentified 2-carbon fragment. With yeast extracts an apparent end-point is reached when 85% of the pentose reactive material has disappeared, and with brain the apparent end-point is reached when 60% has disappeared. In muscle extracts there appears to be no cleavage of a carbon-carbon bond, and the reaction is markedly slowed when 20–25% of the pentose-reactive material has disappeared. The product formed from ribose-5-phosphate by muscle extract is a 2-ketopentose-5-phosphate.

Skeletal muscle contains phosphoribomutase which catalyzes the reversible interconversion of ribose-1-phosphate and ribose-5-phosphate.

Evidence is presented which identifies the product of yeast ribokinase action (ribose + ATP) as ribose-5-phosphate. The yeast extracts used contain enzymes which catalyze the dismutation of glycolaldehyde. DPN and TPN appear equally effective in these reactions.

RÉSUMÉ

L'auteur a étudié la dégradation anaérobie du ribose-5-phosphate par des extraits de levure, de cerveau, de muscle strié et de muscle cardiaque. Dans des extraits de levure et de cerveau la rupture a lieu entre les atomes de carbone 2 et 3 du pentose; il en résulte du triose-phosphate et un fragment contenant deux atomes de carbone. Avec les extraits de levure un point final apparent est atteint lorsque le 85% de la matière à réaction de pentose a disparu, tandis que dans le cas de l'extrait de cerveau le point final apparent est atteint lorsque le 60% a disparu. Dans le cas des extraits de muscle il ne semble pas y avoir de rupture d'une liaison carbone-carbone et la réaction est considérablement ralentie lorsque le 20–25% de la matière à réaction de pentose a disparu. Le produit formé à partir du ribose-5-phosphate par l'extrait de muscle est le 2-cétopentose-5-phosphate.

Le muscle strié contient de la phosphoribomutase qui catalyse la transformation réversible du ribose-1-phosphate en ribose-5-phosphate.

Le produit de l'action de la ribokinase de levure (ribose + ATP) est identifié comme ribose-5-phosphate. Les extraits de levure employés contiennent des enzymes qui catalysent la dismutation du glycolaldéhyde. Le DPN et le TPN se montrent dans ces réactions aussi effectifs l'un que l'autre.

ZUSAMMENFASSUNG

Der anaerobe Abbau von Ribose-5-phosphat durch Extrakte von Hefe, Gehirn, Skelett- und Herzmuskel wurde untersucht. In Hefe- und Gehirnextrakten findet die Spaltung zwischen Kohlenstoffatom 2 und 3 der Pentose statt, wobei Triose-phosphat und ein nicht identifiziertes zwei Kohlenstoffatome enthältendes Spaltstück entstehen. Mit Hefeextrakten ist ein scheinbarer Endpunkt erreicht, wenn 85% des als Pentose reagierenden Materials verschwunden ist, mit Gehirnextrakten ist dies bei 60% der Fall. In Muskelextrakten scheint keine Spaltung einer Kohlenstoff-Kohlenstoff-Bindung stattzufinden und die Reaktion ist bedeutend verlangsamt, wenn 20–25% des als Pentose reagierenden Materials verschwunden ist. Das Produkt, welches aus Ribose-5-phosphat durch die Wirkung von Muskelextrakt entsteht ist 2-Ketopentose-5-phosphat.

Skelettmuskel enthält Phosphoribomutase, welche die reversibele Umwandlung von Ribose-1-phosphat in Ribose-5-phosphat katalysiert.

Das Produkt der Hefe-Ribokinase-Wirkung (Ribose + ATP) wurde als Ribose-5-phosphat identifiziert. Die verwendeten Hefeextrakte enthalten Enzyme, welche die Dismutation des Glycolaldehydes katalysieren. DPN und TPN zeigen sich in diesen Reaktionen gleich wirksam.

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