CCXI. OXIDATION OF PHOSPHOHEXONATE AND PENTOSE PHOSPHORIC ACIDS BY YEAST ENZYMES

I. OXIDATION OF PHOSPHOHEXONATE

II. OXIDATION OF PENTOSE PHOSPHORIC ACIDS

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IN a preliminary note [Dickens, 1936] a scheme of oxidation of carbohydrate was outlined in which the first stages suggested were the esterification of the hexose to hexosemonophosphate, oxidation of this first to the phosphohexonate, then to the 2-ketophosphohexonate, decarboxylation to a pentose phosphoric acid and by a continuation of the process finally pyruvic acid, which would be completely combusted.

At that time the evidence in favour of this scheme was slight. No proof of the formation of pentose phosphoric acids by oxidation of hexosemonophosphate or of phosphohexonate existed. The theory of the formation of 2-ketophosphohexonic acid was first advanced by Lipmann [1936] on the basis of O_2 uptake experiments only. Lipmann found that phosphohexonic acid was oxidized by yeast macerate, and that for each mol. phosphohexonate $\frac{1}{2}O_2$ was consumed and 1-1.5 CO_2 appeared, the CO_2 being only about 1 mol. in presence of bromoacetate. On the basis of this evidence, Lipmann advanced the theory that 2-ketogluconic acid was first formed ($\frac{1}{2}O_2$ consumed), this was then decarboxylated ($1CO_2$ liberated) and the further process consisted in fermentation of the theoretically expected *d*-arabinosemonophosphate, this stage being the one inhibited by bromoacetate. Some evidence tending to show keto-acid formation was given by experiments [Dickens, 1936] in which HCN appeared to act as ketone fixative, and the addition of a preparation of carboxylase to a carboxylase-free enzyme preparation caused a further oxidation and CO_2 evolution with phosphohexonate.

None of these results, however, was sufficiently clear to provide a basis for this theory. Warburg & Christian [1936, 1] showed, by using a more active enzyme preparation, that the "end-point" in Lipmann's experiments was only apparent and was in reality due to a gradual destruction of the activity of the enzyme. The experiments with HCN and carboxylase [Dickens, 1936] were too indirect. The critical points which had to be shown were:

1. Pentose phosphoric acids must be shown to arise from the oxidation and decarboxylation of hexose phosphoric acids.
2. It must be shown that pentose phosphoric acids are readily oxidized and fermented by yeast extracts.

Both these points are established in the work to be described. In the first part of this paper the oxidation of phosphohexonic acid is described, and confirmation of the occurrence of 5-C phosphoric esters giving the pentose reaction [Warburg & Christian, 1937] is obtained. Further oxidation of the phosphohexonic acid to a

4-C phosphoric ester is shown to occur. In the second part, it is shown that pentose phosphoric acid can be readily oxidized by yeast enzyme in the presence of coenzyme II.

In the subsequent paper [Dickens, 1938] it will be shown that pentose phosphoric acid is readily fermentable by yeast enzymes in the presence of coenzyme I and inorganic phosphate.

During this work, the important point arose that the pentose phosphoric acid most readily attacked both oxidatively and by fermentation, is not the *d*-arabinose-5-phosphoric acid which would be expected to arise from simple 2-oxidation and decarboxylation of *d*-glucose, but is the stereoisomeride *d*-ribose-5-phosphoric acid. Since *d*-ribose is the naturally occurring pentose of animal and yeast cells, this fact is of great physiological interest, and may perhaps give a key to the metabolism of pentoses in living cells. *d*-Ribose is not itself fermentable, either by yeast cells or yeast extract, nor is it oxidized by brain slices or by yeast extract. Apparently direct phosphorylation of ribose presents difficulty in these materials, and the theory is advanced that *d*-ribose-5-phosphoric acid may originate from hexoses by a process of phosphorylation, oxidation and decarboxylation. Indirect evidence is presented in these papers which makes this assumption probable, and direct evidence will be sought in future experiments.

I. OXIDATION OF PHOSPHOHEXONATE

Enzyme. Prep. A. The enzyme preparation used in most experiments was made from Lebedew yeast maceration fluid by the method of acetate precipitation already described [Dickens & McIlwain, 1938] and the general methods for experiment were the same as detailed there, except that phosphohexonate was used as substrate. During the course of this work we have used three different yeasts: (1) Newcastle Brewery Co. top fermentation yeast. (2) Messrs Tennent, Glasgow, bottom yeast. (3) Löwenbräu, Munich, bottom lager beer yeast. Although there was little difference in the activities of the macerates from these, successful precipitation of enzyme without excessive dilution of the Lebedew juice was obtained only with the German yeast, presumably owing to its lower content of protective hop resins, which held up the precipitate with the British yeasts.

Prep. B. In earlier experiments a preparation was made from Tennent's yeast Lebedew juice by diluting 50 times with distilled water, passing CO₂, centrifuging from the inactive precipitate after 10 min., passing CO₂ to saturation and keeping in a stoppered bottle in the refrigerator overnight [cf. Warburg & Christian, 1933]. The centrifuged precipitate, dried *in vacuo* over H₂SO₄ and KOH was used: yield c. 250 mg. per 100 g. dried yeast; 5–20 mg./vessel were needed. This preparation attacked hexosemonophosphate more rapidly than phosphohexonate; otherwise there was little difference between A and B, but both differ from "Proteins I and II" of Warburg & Christian [1937] in that the precipitated enzyme is washed with acid in our two preparations, and this may account for some differences between the results of Warburg & Christian and those to be described.

The activities of the original Lebedew juice and of Preps. A and B are shown in Table I. It is evident that much activity is lost in the precipitation, and this passes into the acid supernatant liquid, from which Warburg & Christian [1937] prepare their enzyme. This loss is compensated for to some extent by the simplicity of the method and by the greater stability of the acid-precipitated enzyme.

Table I. *Activities of original Lebedew juice and Preps. A and B*All experiments at 38°, O₂, 0.2 ml. KOH in inner cup.*Original Lebedew juice.* Diluted to 2.2 ml. with *M*/100 phosphate containing 0.1 mg. coenz. II, 0.3 ml. *M*/10 substrate, and 0.2 ml. *M*/100 phenazine methochloride.

Source	Per vessel ml.	μ l. O ₂ consumed per hr. with substrate		
			Hexosemono- phosphate	Phospho- hexonate
Newcastle Brewery Co.	0.20	34	226	287
	0.05	4	56	63
Löwenbräu, Munich	0.20	—	225	311
	0.05	—	63.5	141

Acetate precipitates (A). Dissolved in *M*/50 phosphate of pH 7.4, pH c. 6.5. 0.1 mg. coenz. II; 0.3 ml. *M*/10 substrate; 0.2 ml. *M*/100 phenazine methochloride.

Source	Mg.	Corrected for blank (c. 10 μ l./hr.)	
Löwenbräu, Munich	5	55	50
	10	90	90
	20	121	141
	50	155	180

CO₂ precipitate (B):

Tennent, Glasgow	5	207	42
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Numerous attempts to prepare stable extracts from the acid supernatant liquid proved unsuccessful: Warburg & Christian [1937] have since overcome this difficulty by warming the $\frac{1}{2}$ -sat. ammonium sulphate solution of the enzyme to 50°, thus "stabilizing" it, but the work described in this section was done before the publication of this method. Presumably destructive enzymes are removed by both methods.

Phosphohexonate. In a few earlier experiments, but not in any of the isolation experiments, the phosphohexonate was made from the prep. of muscle hexosemonophosphate described in the preceding paper [Dickens & McIlwain, 1938] by the method of Robison & King [1931]. Later Prof. Robison kindly gave a prep. of the neutral Ba salt of 6-phosphogluconate containing some 6-phosphomannonate with <1.5% of reducing hexosephosphate. Analysis (Prof. Robison): H₂O, 7.0%. Found (dry subs.): total P, 6.34%; inorg. P, 0.06%; H. and J. titn. with added NaOH, 0.5; $[\alpha]_{5491} - 1.2^\circ$. Calc. for (C₆H₁₀O₁₀P)₂Ba: P, 6.42%.

The solution of Na salt was prepared by grinding the Ba salt (288 mg.) with a slight excess of Na₂SO₄, 10H₂O (295 mg.) and centrifuging from Ba₂SO₄ after making up to 6 ml. with water and addition of 0.055 ml. *N* HCl. The concentration of ester P was estimated on this solution (c. 0.09 *M*).

Coenzyme. Coenzyme II of purity 1 was given by Prof. Warburg. Coenzyme II of purity 0.2 was prepared from horse blood corpuscles [Warburg & Christian, 1936, 2] and its activity checked by comparison with the Warburg preparation: yield 80 mg. from 2.2 l. washed corpuscles. This preparation contains also coenzyme I and an adenine nucleotide [Warburg & Christian, 1936, 2]. Usually 0.02 mg. of purity 1, or 0.1 mg. of purity 0.2 was used per vessel. Unlike the hexosemonophosphate oxidation, that of phosphohexonate is not always maximal with these amounts, and in some "end-point" experiments they were increased fourfold. Possible reasons for this are indicated later.

Carriers. Flavin enzyme and phenazine methochloride were used as previously [Dickens & McIlwain, 1938]. The O₂ uptake with phenazine methochloride was corrected for blank without substrate (autoxidation of phenazine methochloride + blank oxidation). The blank was small with Preps. A and B except in

a few "end-point" experiments where high concentrations of phenazine methochloride were used to ensure a rapid reaction. With flavin enzyme the blank was usually negligible.

Measurement of O_2 uptake. Warburg manometers filled with O_2 and containing KOH in the inner cup were used at 37.5° . Where HCN was present it was added as freshly prepared neutral solution, and instead of KOH, KCN-KOH mixture [Krebs, 1935] was used to avoid absorption of HCN. CO_2 evolved was measured (in absence of HCN) by a second vessel without KOH, which was acidified at the end of the exp. to drive out bound CO_2 , with a similar pair of vessels without substrate as controls.

pH-activity curve. The titration curve of the enzyme (Prep. A, 20 mg./ml. in $M/100$ phosphate buffer) was first determined and the contents of each vessel after cooling in ice were adjusted to the required pH by addition of the required vol. ice cold $N/100$ NaOH or, for the vessel at pH 4-7, $N/50$ acetic acid. Stronger phosphate buffer cannot be used since it inhibits the reaction (see below). The enzyme system with phenazine methochloride as carrier showed a broad pH optimum at pH 6.3-7.5 (Fig. 1).

Fig. 1. pH optimum of phosphohexonic system.

Fig. 2. Phosphate inhibition of phosphohexonic system.

Phosphate inhibition. Conditions were as in the preceding exp. except that pH was kept constant and varying concentrations of phosphate buffer were used. During the first 30 min. of the exp. the inhibitions with 0.05, 0.1 and 0.25 M phosphate were respectively 16, 32 and 57%. Fig. 2 shows that, as with the hexosemonophosphate system [Theorell, 1935], the velocity of oxidation varies inversely as the phosphate concentration. Comparison with the data of Theorell shows less inhibition for a given phosphate concentration with the phosphohexonic system than with the hexosemonophosphate one. This also is shown in Fig. 1 of the preceding paper [Dickens & McIlwain, 1938].

Attempted separation of the phosphohexonic and hexosemonophosphate systems. The above behaviour towards phosphate inhibition is typical of the slight differences between these two systems, which makes their separation difficult. As yet attempts to obtain an enzyme which attacks only phosphohexonate have been uniformly unsuccessful, whether by dialysis, ammonium sulphate precipitation, acetone precipitation or partial inactivation by incubation with alkali. On the other hand, the "Zwischenferment" of Negelein & Gerischer [1936] is free from the phosphohexonic enzyme [Dickens, 1936; Warburg & Christian, 1937]. It is probable that our enzymes, like those of Warburg & Christian [1937], are mixtures of several components, as is shown by the different substrates attacked and the multiple products formed.

Table II shows that the acid-precipitated enzymes require coenzyme II for their activity, purified cozymase being without effect. Gluconic acid is not oxidized unless phosphorylated, and the preparations contain no active carboxylase. An experiment with Warburg "Zwischenferment" is included to show its relative actions on hexosemonophosphate and phosphohexonate. In this exp. (Table II) the less pure sample of phosphohexonate was used, and the small O_2 uptake is probably due to traces of reducing hexosephosphate in the substrate.

Table II. *Action on various substrates: coenzyme required*

Enzyme in $M/100$ phosphate of pH 7.4; 0.04 mg. coenzyme II of purity 1; or 0.6 mg. purified cozymase; 0.5 ml. 0.8 N/HCN . Side bulb, 0.2 ml. 10% flavin enzyme. Inner cup, KCN-KOH. 0.4 ml. $M/10$ substrates. O_2 . 37.5°.

Enzyme and coenzyme	Enzyme wt. per vessel mg.	Time min.	O ₂ uptake (μl.) with substrate		
			Hexosemono-phosph.	Phospho-hexonate	Gluconic acid
Enz. B.:					
Coenz. I	8	30	—	— 2	0
		60	—	— 5	0
		90	—	— 6	—
Coenz. II	8	30	— 73.5	— 49.5	— 0.5
		60	— 125	— 78	— 1
		90	— 155	— 101	—
Warburg "Zwischenferment":					
Coenz. II	0.1	30	— 246	— 13.5	—
		60	— 313	— 17.5	—

Enzyme B in $M/100$ KH_2PO_4 , 0.2 ml. $M/5$ Na pyruvate, CO_2 evoln. (90 min.) + 2.5 μ l.

Action of HCN. In the earlier experiments which were made with acid-precipitated enzyme and coenzyme II of purity 1, HCN (0.12 M) was necessary for the reaction to continue [Dickens, 1936]. This result (Fig. 3) was obtained many times when the pure coenzyme was used with small quantities of the enzymes A or B. By increasing the amount of enzyme, however, it was no longer apparent, the reaction continuing even in the absence of HCN. In the later work more care was taken to prevent destruction of enzyme during its precipitation and washing, by using ice cooling and quick manipulations, and it is possible that this may account for the difference between earlier and later preparations in their need for HCN. We are unable to account for the results otherwise.

End-point with pure coenzyme II. Table III shows experiments with and without HCN for both hexosemonophosphate and phosphohexonate. In all these, coenzyme II of purity 1 was used; different end-points obtained with cruder coenzyme are described later.

The results of Table III show that under the conditions used, i.e. with pure coenzyme and vigorous enzyme preparations, the reaction proceeds either in presence or absence of HCN to an end-point which with phosphohexonate is

Fig. 3. Effect of HCN on O_2 uptake with phosphohexonate (earlier prep. of enzyme).

Table III. *End-point with pure coenzyme and acid-precipitated enzyme*

Phosphohexonate or hexosemono- phosphate			Enzyme prep. type	$\mu\text{l. O}_2$ uptake after hr.							End-point. % of 1 mol. O_2 per mol. substrate
No.	ml. 0.09 <i>M</i>	$\mu\text{l.}^*$		$\frac{1}{2}$	1	2	3	4	5	6	
With HCN (0.12 <i>M</i>): Phosphohexonate											
1	0.4	810	A	130	222	329	360	380	—	390	48
2	0.2	402	B	56.5	122	181.5	—	—	—	—	45
3	0.1	202	B	56	77	85	87.5	—	—	—	43
4	0.1	202	B	33	64	99.5	104	—	104	—	51
5	0.05	101	A	35	54	57.5	56	—	—	—	51
Hexosemonophosphate											
6	0.1	202	B	51	89	154	166	172	—	—	85
7	0.05	101	A	33	62	87.5	92	—	—	—	90
Without HCN: Phosphohexonate											
8	3.0	6050	A	—	—	—	—	—	—	3115	51.4
9	0.2	401	B	40.5	81.5	136.5	—	157	—	167	42
10	0.1	202	B	37	61	78	98	107	—	—	53
11	0.05	101	B	31	43	53.5	64	66	—	—	65
Hexosemonophosphate											
12	0.1	202	B	51	89	166	172	—	—	—	85
13	0.05	101	B	—	87.5	101	117	—	—	—	115

* 1 *mM.* = 22400 $\mu\text{l.}$

close to that required for $\frac{1}{2}\text{O}_2$ per mol., with hexosemonophosphate 1O_2 per mol. substrate. Although some very slow residual oxidation may remain, the figures of Table III, and still better the curves of these experiments, only one of which (Fig. 4) is reproduced, show the definite change in reaction rate at these points.

Fig. 4. End-point of phosphohexonic oxidation with coenzyme II of purity 1.

It seems most unlikely that this could be due in every case to destruction of enzyme coinciding with the different reaction times. Exp. No. 2, Table III, in which the enzyme with substrate and carrier, but without coenzyme, was incubated in the vessel for $1\frac{1}{2}$ hr. at 37.5° before the coenzyme was tipped in, still gave a similar end-point corresponding to 90% of the O_2 uptake required for $\frac{1}{2}\text{O}_2$ per mol. phosphohexonate.

The end-point observed corresponds fairly closely with the oxidation of both substrates to a stage corresponding with the formation of ketophosphohexonate.

H₂O₂ formation. H₂O₂ results from the autoxidation of the flavin enzyme when hexosemonophosphate is oxidized by the purified enzyme [Warburg & Christian, 1933]. H₂O₂ did not accumulate in the present experiments since (1) the acetate-precipitated enzyme contained very active catalase (25 mg. enzyme, 0.2 ml. 0.3 % H₂O₂, 114 μ l. O₂/5 min.) (2) HCN did not affect the end-value although it would have inhibited the catalase. Accumulation of H₂O₂ would have changed the interpretation of the end-point, but the above experiments show that it did not occur.

Other ketone fixatives. The action of HCN which was seen with the earlier enzyme preparations, might have been due to its action as ketone-fixative, as in the experiments of Green & Brosteaux [1936] with lactic dehydrogenase. As these authors found for the lactic system, semicarbazide (*M*/20) and hydrazine hydrate (*M*/8) were without effect in the phosphohexonic system. Hydroxylamine and phenylhydrazine (*M*/8) caused irregular pressures; with sulphite and bisulphite no combination with keto-substance could be demonstrated by I₂ titration. Increases of O₂ uptake and CO₂ output equal to the O₂ consumed were regularly observed [Dickens, 1936] on the addition of crude carboxylase [Axmacher & Bergstermann, 1934], but this may be attributable perhaps to other enzymes or coenzymes contained in the carboxylase and should be repeated now that purified enzyme and cocarboxylase [Lohmann & Schuster, 1937] are available. Unphosphorylated 2-keto-*D*-gluconic acid is attacked only very slowly by active carboxylase (Table IV), but the phosphorylated compound may of course behave differently.

Table IV. *Action of yeast carboxylase*

50 mg. carboxylase prep., in 2 ml. *M*/50 phosphate buffer. Side bulb 0.1 ml. 2.5 *N* HCl. Air. 37.5°.

Min.	μ l. CO ₂ evolved at			
	<i>pH</i> 7.4		<i>pH</i> 6	
	Addition: 0.2 ml. <i>M</i> /10 Na salt			
	Pyruvate	2-Ketogluconate	Pyruvate	2-Ketogluconate
5	+ 30	0	+ 228	0
30	+ 152	0	+ 363	+ 5
60	+ 173	0	+ 391	+ 7.5
120	—	—	—	+ 9

Product from the oxidation of phosphohexonate with $\frac{1}{2}$ O₂. A larger scale exp. was made to determine the nature of the product from the oxidation of phosphohexonate in presence of pure coenzyme II.

195 mg. enzyme A, well washed with acetate buffer [Dickens & McIlwain, 1938], were dissolved in 2.2 ml. water and the *pH* adjusted to 7.3 with ice cold *N*/100 NaOH (7.3 ml.). 0.3 mg. coenzyme II of purity 1 and 3 ml. 0.09 *M* Na phosphohexonate were added, the mixture was placed in two large Warburg manometer vessels containing 1.5 mg. phenazine methochloride and 0.5 ml. KOH in the side bulb and inner well of each vessel. O₂, 37.5°.

After 5 hr. the reaction became very slow and 3680 μ l. O₂, corr. for autoxidation of the carrier, had been consumed. The oxidation thus went a little beyond the consumption (calc. 3050 μ l.) of $\frac{1}{2}$ mol. O₂ per mol. phosphohexonate, perhaps because of traces of coenzyme contained in the large amount of enzyme used. The contents were precipitated with trichloroacetic acid and the supernatant liquid was neutralized with Ba(OH)₂, the resulting precipitate, together with that from addition to the mother liquor of 2 vol. abs. alc. was dried *in vacuo*; wt. 149 mg. This was placed in 5 ml. water, with *N*/10 HCl to make weakly acid. After centrifuging from the undissolved residue, the clear solution was neutralized with Ba(OH)₂ and was poured into 2 vol.

abs. alc. The precipitate ("Ba salt I") was well washed with 75% and abs. alc., then with ether and was dried *in vacuo* over P_2O_5 ; wt. 91 mg. = 60% of the amount of Ba phosphohexonate taken. Analysis (Weiler): Dried at 60° *in vacuo*, loss of wt. 7.26%. Found, for dry subs.: C, 15.50; H, 2.23; P (as phosphomolybdate) 7.04; Ba, 37.1%. Calc. for $C_6H_9O_9PBa$: C, 15.75; H, 2.36; P, 8.13; Ba, 36.1%. Calc. for $C_6H_{10}O_{11}PBa_{1.25}$: C, 15.62; H, 2.18; P, 6.73; Ba, 37.20%. The second formula is that of an acid Ba salt of 2-ketophosphohexonic acid; the first is that of the carboxylic acid derived from 2-ketophosphohexonic acid by oxidative decarboxylation, i.e. a phosphopentonic acid.

It seemed very probable that Ba salt I might be a mixture of these two compounds. Unfortunately their separation could not be achieved on the specimen available, and the repetition of the exp. has been left for a future occasion. An attempted purification by dissolving in HCl and making more alkaline than previously with $Ba(OH)_2$, resulted in a Ba salt containing much less P and Ba, these elements now being in the ratio 0.96:1. Found (Weiler): loss of wt. at 60° *in vacuo*, 5.02%. Dry subs. P, 5.23%; Ba, 24.4%. Some decomposition appeared to have occurred and the mixture could not be separated. Solution of a sample of the original Ba phosphohexonate in HCl and its reprecipitation by $Ba(OH)_2$ at similar alkalinity caused no such decomposition.

Oxidation of phosphohexonate in presence of coenzyme of purity 0.2: end-point and CO_2 formation. In the presence of the less pure coenzyme the oxidation with enzyme preps. A and B proceeds further than in the preceding experiments. Provided that sufficiently active enzyme is used in the presence of plenty of coenzyme, the O_2 uptake slightly exceeds 1 mol. O_2 per mol. phosphohexonate

Fig. 5. End-point of phosphohexonic oxidation with coenzyme II of purity 0.2.

(Fig. 5) and the CO_2 formed is almost exactly equivalent to the O_2 uptake when the reaction has gone to completion. If however the reaction is stopped by acidification before the end-point is reached, the CO_2 formed then exceeds the O_2 consumed (Table V).

These results seem to indicate the occurrence during the earlier stages of the oxidation of an excess of decarboxylation over O_2 uptake. This could occur in two different ways: (1) initial stage; $\frac{1}{2}O_2$ consumed, keto-acid formed, keto-acid decarboxylated forming pentose phosphoric acid with evolution of 1 mol. CO_2 ; later slower oxidation by a similar type of mechanism of the pentose phosphoric acid formed. (2) Pentose phosphoric acid formed as in (1), but then undergoing cleavage with oxidation of the cleavage products formed.

Table V

Enzyme: 20 mg. per vessel Prep. A dissolved in 1.5 ml. *M*/50 phosphate buffer at *pH* 7.4, initial *pH* of mixture 6.22; 0.2 ml. = 0.1 mg. coenzyme of purity 0.2; 0.2 ml. *M*/100 phenazine methochloride in main part of vessel. Temp. 37.5°. O₂. Amount of substrate (from P estimation) = 404 μl./vessel.

Vessel no.	...	1	2	3	4
Enzyme, coenz., carrier, ml.		1.9	1.9	1.9	1.9
Bulbs:					
I. <i>M</i> /10 phosphohex., ml.		0.2	0.2	No substrate	
II. 2.5 <i>N</i> HCl, ml.		—	0.1	—	0.1
Inner cup, ml.		0.2 KOH	—	0.2 KOH	—
μl. O ₂ or (free) CO ₂ after min.		O ₂	CO ₂	O ₂	CO ₂
20		- 67.5	+ 60.5	- 0.5	+ 0.5
40		- 101	+ 93	- 0.5	+ 0.5
60		- 160	+ 147.5	- 1.5	+ 0.5
120		- 237	+ 206	- 4.5	+ 2
Total CO ₂ on acidification		—	+ 329 uncorr. + 310.5 corr. for No. 4	—	+ 18.5

Hence when 0.58 mol. O₂ per mol. phosphohexonate has been consumed, the ratio CO₂/O₂ = 1.31.

At present it is not possible to decide between these alternatives, but it will be shown that both oxidation and fermentation of pentosemonophosphate can occur, and that a 4-C monocarboxylic-monophosphoric acid is formed oxidatively under conditions similar to those in the above exp.

*Nature of the products formed from the oxidation of phosphohexonate
in presence of crude coenzyme*

Warburg & Christian [1937] have investigated the products formed when phosphohexonate is oxidized by flavin enzyme, pure coenzyme II and a yeast enzyme (prepared without washing with acid: "Protein of protein fraction I, 4th step") in the presence of methyl alcohol. This preparation differs from ours in its action. With our enzyme, and with phenazine methochloride as carrier and coenzyme of purity 0.2, the oxidation of phosphohexonate becomes slow when 1 mol. O₂ is absorbed and 1 mol. CO₂ has been evolved. With the Warburg & Christian system, the reaction stops when 0.6 mol. O₂ has been consumed and 0.7–0.8 mol. CO₂ has been liberated.

In view of these differences, the products formed under our conditions were investigated. The fractionation of the products followed the description given by Warburg & Christian [1937, p. 293] exactly, and details of fractionation are therefore omitted.

1.86 g. enzyme prep. A was dissolved in 130 ml. water + 28 ml. ice cold *M*/50 NaOH. 120 mg. phenazine methochloride and 9.5 mg. coenzyme II (purity 0.2) were added, and the mixture was shaken at 37.5° in O₂ with 10.0 ml. 0.09 *M* phosphohexonate; *pH* of mixture 6.4. An aliquot part of the mixture was used to measure O₂ consumption, with a control using water instead of substrate, this exp. running concurrently.

Manometric readings: 1.5 ml. enzyme solution (containing the coenzyme and phenazine methochloride) in main part of vessels. 0.1 ml. 0.09 *M* phosphohexonate (or water) in side bulb. 0.2 ml. KOH in inner cup. O₂. 37.5°.

		μl. O ₂ consumed after min.					
Vessel no.	Add at <i>t</i> ₀	30	60	120	240	1100	1200
1	Phosphohex.	64	100	144	195	266.5	267.5
2	Water	6	9	11	16	25.5	26.5
Difference μl. O ₂ : (1–2)		58	91	133	179	241	241

Calc. for 0.1 ml. (*M*/10) × 0.91 phosphohexonate (P analysis): 204 μl. O₂ if 1 mol. phosphohexonate consumes 1 mol. O₂.

The trichloroacetic acid filtrate was worked up by Warburg & Christian's method. The filtrate contained 28.7 mg. combined P; amount of ester P added as phosphohexonate = $0.91 \times 31.0 = 28.2$ mg. Evidently little breakdown of organic P occurred. As in Warburg & Christian's exp., neutral and basic lead acetate yielded distinct precipitates of Pb salts; neutral (after removal of inorg. P): wt. 315 mg. Fractionated by Hg salt; Hg precipitable converted into Pb salt; wt. 85 mg. "A". Hg supernatant fluid reprecipitated with neutral Pb acetate; wt. 76 mg. "B". Basic Pb acetate precipitate from supernatant liquid of neutral Pb acetate precipitation, converted into Ba salt.; wt. 60 mg. "C". Salts "A" and "B" were redissolved and reprecipitated for analysis.

Analyses. (Weiler, except colorimetric P estimations on Pb but not on Ba salts. All figures are expressed on subs. dried at 40° *in vacuo* unless otherwise stated.)

Subs. "A". Found: Pb, 63.40; P, 6.26%. Warburg & Christian's Pb salt 1, prepared similarly from the Hg precipitate, had Pb, 61.5; P, 6.2%. The two were not identical however since Pb salt "A" gave the orcin test and contained more C (ratio C:P:Pb = 4.7:1.01:1.53). Consistent analyses were not obtained and the fraction was probably a mixture.

Subs. "B". Found: C, 8.92; H, 1.43; P, 5.60; Pb, 57.0%. Calc. for $C_4H_6O_8PPb_{1.5} + H_2O$: C, 8.86; H, 1.47; P, 5.71; Pb, 57.3%. Subs. "B" dried *in vacuo* at 100° . Found: C, 9.47; H, 1.56%. Calc. for $C_4H_6O_8PPb_{1.5}$: C, 9.16; H, 1.15%.

Subs. "B" thus corresponds closely with the monohydrated neutral Pb salt of a monophospho-erythronic or -threonic acid: $Pb_2OOC.(CH.OH).(CH.OH).CH_2O.PO_3Pb + H_2O$. In the corresponding fraction from their oxidation, Warburg & Christian [1937] obtained a Pb salt, $C_5H_6O_9PPb_{1.5}$ containing (CH_2O) in excess of our Pb salt. Taken in conjunction with the results described here, this can be taken as strong presumptive evidence that this salt of Warburg & Christian was the next higher member, namely the Pb salt of the phosphopentonic acid. These facts, and the isolation of the pentose-containing fraction "C" described below, together provide strong experimental support for the existence of a system of progressive oxidative breakdowns of the sugar-monophosphoric acids, such as was previously tentatively suggested [Dickens, 1936].

It is particularly interesting that in our series, where the oxidation proceeded further, the O_2 uptake being 1 mol. per mol. substrate, instead of 0.5 mol. as in Warburg & Christian's exp., the degradation of the C-chain has proceeded one stage further. As a variety of stages of oxidation are simultaneously present, it is not possible to correlate the O_2 uptake more than roughly with the extent of breakdown. But it is noteworthy that the formation of fraction "B" in our exp. required the consumption of only half the amount of O_2 (2 mol.) calc. from the following equations:

Similarly for the formation of Warburg & Christian's Pb salt 1, the consumption of 1 mol. O_2 would be required, whereas only half this amount was observed as the total O_2 uptake in their exp. (The CO_2 outputs in the two series of exp. were also about half those calc. from the above equations.) Since the calc. O_2 uptakes (and CO_2 outputs) were in excess of the observed amounts, it is evident that the excess oxidation of this stage must be accompanied by the formation of less highly oxidized products. That this was in fact the case in our exp. is shown by the analysis of Ba salt "C".

Subs. "C". Found: C, 23.72; H, 3.02; P, 5.21; Ba, 24.80. Calc. for $C_{11}H_{21}O_{15}P\text{Ba}$: C, 23.50; H, 3.74; P, 5.52; Ba, 24.50 %. This formula corresponds empirically with the Ba salt of a compound containing 1 mol. hexose, 1 mol. pentose and 1 mol. H_3PO_4 , with the addition of 1 atom O and the loss of 1 mol. H_2O . Since it gives an intense pentose reaction (see below) it is apparently a combination of 1 mol. pentose with 1 mol. phosphohexonic acid, or alternatively of 1 mol. pentose phosphoric acid with 1 mol. hexonic acid. The fate of the lost phosphoric acid mol. is unknown: since the inorganic P did not appreciably increase during the exp., either H_3PO_4 was lost during the isolation, or else was used during the incubation to phosphorylate another molecule.

Pentose reaction. The orcin- $FeCl_3$ test was used [Tollens-Elsner, 1935, p. 111]. The phosphohexonate used gave a negative reaction, or at most the faintest trace of green which developed only very slowly (2-keto-*D*-gluconate behaved similarly). Pb salt "A" and Ba salt "C" gave intensely positive reactions (c. 1 mg. used for test); pigment sol. in amyl alcohol with absorption bands at 595–600 and 650–60 $m\mu$. As was to be expected from its analysis, Pb salt "B" gave no colour with the pentose test. Unfortunately the quantities were insufficient for quantitative pentose estimation; the colours in the micro-method of McCance [1926] were found to fade rapidly with pentose phosphoric acid (ribosephosphate) and to differ too much from one pentose to another.

II. OXIDATION OF PENTOSE PHOSPHORIC ACIDS

Since in the preceding part it has been shown that pentose phosphoric acids or compounds closely resembling these are formed in the oxidation of phosphohexonate by yeast enzymes, and that compounds representing still further oxidation stages are also present, particularly the substance analysing as a phosphoerythronic acid, the next step was evidently to study the oxidation of those pentose phosphoric acids that are available.

I am greatly indebted to Dr P. A. Levene, who has added so much to knowledge of pentose phosphoric acids, for generous gifts of synthetic esters, without which these experiments would have been very incomplete.

Pentose phosphoric acids

d-Ribose-5-phosphoric acid. (a) *Natural.* Ba inosinate (Laoköon, Lwów) was hydrolysed and the Ba salt isolated by the method of Levene & Jacobs [1911]. Yield 2.9 g. from 5 g. Ba salt. Recryst. from 10 ml. hot water (animal charcoal) with addition of 10 ml. alcohol to the mother liquor: yield 1.7 g. The white crystalline subs. was dried *in vacuo* over H_2SO_4 for 24 hr. Analysis (Weiler), dried at 100° *in vacuo* loss of wt. 8.64 %. Found (dry subs.): Ba, 37.7; P, 8.88 %. Calc. for $C_5H_9O_8P\text{Ba}$: Ba, 37.6; P, 8.49 %; $+2H_2O$ req. 8.97 %.

(b) *Synthetic*. Dr Levene's prep. of the synthetic Ba salt [Levene & Stiller, 1934].

d-Arabinose-5-phosphoric acid Ba salt (synthetic). Found (Dr Levene): Ba, 37.3; P, 8.77%. *Xylose-5-phosphoric acid (Ba salt)*. Synthetic, from Dr Levene [Levene & Raymond, 1934]. *2-Keto-d-gluconic acid (Ba salt)*. Prepared according to Ohle & Koller [1924].

Preparation of c. M/10 solutions of Na salts. These were prepared freshly, as on keeping in the ice box the solutions turned yellow. 77 mg. Ba salt ground with 65 mg. $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ were made up to 2 ml. with addition of 0.02 ml. *N HCl*. Found (ribose phosphoric acid): organic P, 0.756, 0.765 M/10.

The same enzyme preparations, carriers and coenzymes were used as in the preceding section, unless otherwise stated, and the experimental procedure was the same.

Oxidation experiments

1. *Oxidation by Lebedew extract*. For these experiments yeast macerate, either undiluted or with M/100 phosphate buffer, pH 7.4, pH of mixture c. 6.4, was placed in the main part of Warburg manometers, containing in the side bulb 0.3 M/10 substrate (Na salts of acids), and in the inner cup 0.2 ml. KOH. O_2 . 37.5°. In those exp. with extra carrier and coenzyme, 0.2 ml. M/100 phenazine methochloride and 0.1 mg. coenzyme II of purity 0.2 were added to the main contents.

Table VI. *Oxidation in Lebedew fluid*

Dilution with M/100 phosphate buffer of pH 7.4; pH of mixt. c. 6.5. 0.3 ml. M/10 substrates. KOH in inner cup. O_2 . 37.5°.

No carrier or coenzyme added:

Source	Vol. per vessel ml.	$\mu\text{l. O}_2$ uptake in 1st hr. with substrate					
		None (water)	Glucose	Gluconic acid	2-Keto-gluconic acid	Phospho-hexonic	Ribose-5-phosph. acid
Löwenbräu:							
(Fresh)	1	40	196	116	44	212	126
(Overnight at 0°)	1	40	137.5	—	—	—	163
„	0.2	11.5	68	—	—	—	51.5

With carrier and coenzyme added:

0.2 ml. Newcastle Brewery Lebedew fluid diluted to 2.2 ml. total vol. with M/100 phosphate; this vol. contains 0.2 ml. M/100 phenazine methochloride, 0.1 mg. coenz. II of purity 0.2; 0.3 ml. M/10 substrate. O_2 . 37.5°. KOH in inner cup.

$\mu\text{l. O}_2$ in 1st hr. with						
Water	Phospho-hexonic acid	Hexose mono-phosphoric acid	Xylose-5-phosphoric acid	d-Arabinose-5-phosphoric acid	d-Ribose-5-phosphoric acid	2-Keto-gluconic acid
42.5	186	—	—	46.5	146	—
29	—	205	27	34	108	32
					(synthetic)	
					92	
					(natural)	

Table VI shows that ribosephosphate is oxidized with both specimens of Lebedew fluid, whether diluted or undiluted, with or without the addition of the extra carrier and coenzyme. Lebedew fluid kept overnight in the ice box still oxidizes vigorously. Natural and synthetic ribosephosphates are both oxidized, but xylosephosphate and arabinosephosphate are little or not at all affected when phenazine methochloride is the main carrier present. The figures for keto-gluconic acid are included because of the suggestion that phosphoketogluconic

acid may be an intermediate in hexose oxidation. The unphosphorylated compound was not appreciably oxidized. Figures for phosphohexonate and hexosemonophosphate are included for comparison.

Fig. 6 shows the course of the reaction and the comparison between natural and synthetic ribose esters.

Fig. 6. Oxidation by Lebedew fluid (Newcastle Breweries). 0.3 ml. *M*/10 substrate + 0.2 ml. Lebedew fluid + phenazine methochloride + 0.1 mg. coenzyme to 2.2 ml. with *M*/100 phosphate. O_2 , 37.5°. *A*: hexosemonophosphate. *B*: ribosephosphate (synthetic). *C*: ribosephosphate (natural). *D*: 2-ketogluconic and *d*-arabinose phosphate. *E*: *d*-gluconic acid.

2. *Boiled enzyme.* Table VII shows that the activity of Lebedew fluid towards ribosemonophosphate is destroyed by boiling.

Table VII. *Boiled and unboiled Lebedew fluid*

0.3 ml. Löwenbräu Lebedew fluid diluted to 1.5 ml. with *M*/30 phosphate of *pH* 7.4; *pH* of mixt. c. 6.5. 1.5 ml. per vessel either untreated or placed in a boiling water bath for 10 min. 0.3 ml. *M*/10 ribose phosphate or 0.3 ml. water. O_2 , 37.5°.

μ l. O_2 uptake in 1st hr.				
Enzyme ...	Ribosephosphate		Water	
	Unboiled	Boiled	Unboiled	Boiled
	68.5	16	23	16.5

3. *Acetate precipitate from Lebedew fluid.* The enzyme used in the previous section (prep. A) is not suitable for the study of the oxidation of pentose phosphoric acids, since its activity is low and variable with this system at any rate when phenazine methochloride is used as carrier (Table VIII). That the preparation used was fully active towards hexosemonophosphate or phosphohexonate is shown by the control exp. with these substrates. Evidently different systems are concerned in the oxidation of pentose and hexose phosphoric acids. This explains the fact that the pentose esters were only in part further oxidized in our previous experiments with phosphohexonate.

Table VIII. *Acetate precipitate A*

20 mg. enzyme dissolved in 1 ml. *M*/30 phosphate buffer of pH 7.4; pH 6.4: 0.2 ml. *M*/100 phenazine methochloride; 0.1 mg. coenzyme I of purity 0.2. 0.2 or 0.4 ml. *M*/10 substrate. O₂. 37.5°. 0.2 KOH in inner cup.

	μ l. O ₂ uptake						
	Min.	Water	Ribose-5-phosph.		Arabinose-5-phosph. (synthetic)		Phospho-hexonate 0.2 ml.
			Synthetic 0.2 ml.	Natural 0.2 ml.	0.2 ml.	0.4 ml.	
2 months' old enzyme	60	4.5	7.5	13.5	—	—	147.5
Freshly prepared enzyme	60	0.5	—	36.5	5	4.5	135
	120	1.0	—	64	12.5	18	214
	180	1.5	—	77	16	23	256

4. *Dialysed Lebedew fluid*. Lebedew fluid was dialysed for various times in a "Visking" cellophane tube against running tap water in a shaking machine. The results are shown in Table IX.

Table IX. *Dialysed Lebedew fluid*

1 ml. dialysed Lebedew fluid; 0.1 mg. coenzyme II of purity 0.2, or 0.2 mg. purified cozymase; 0.3 *M*/10 ribose-5-phosphoric acid or 0.3 ml. water. O₂. 37.5°. 0.2 ml. KOH in inner cup.

Dialysis hr.	Exp. min.	μ l. O ₂ uptake						
		Water			Ribose phosphoric acid			
		Water	Coenz. I	Coenz. II	Water	Coenz. I	Coenz. II	Coenz. I + II
4	60	20.5	—	25	110	—	146	—
16	60	0.5	—	2	6	—	38	—
	100	1	—	2	10	—	62.5	—
16	60	—	0	—	1	4.5	53	50
	120	—	0	—	5	10	109	116

4 hr. dialysis do not sufficiently remove the coenzymes, although an increase is now detectable on addition of coenzyme II (purity 0.2 throughout this work). On the other hand after 16 hr. the oxidation of ribosephosphate is very small unless coenzyme II is added, when it is about the same as in the original Lebedew fluid (Table I) after subtraction of the corresponding blanks without substrate. The blank is now very small in the dialysed Lebedew fluid. Cozymase (tested for activity with the lactic dehydrogenase) caused only a trifling increase, which may well be due to a trace of coenzyme II still contained in the purified cozymase. Addition of cozymase and coenzyme II together did not give any appreciable increase over coenzyme II alone. (Note however that the coenzyme II employed in these experiments certainly contained some cozymase.)

5. *Dried enzyme from dialysed Lebedew fluid*. *Preparation*. 150 g. Löwenbräu, Munich, dried yeast was macerated with 450 ml. distilled water for 2½ hr. at 35°. The centrifuged Lebedew fluid (190 ml.) was dialysed as described above for 16 hr. against running tap water with shaking. After centrifuging from a considerable deposit, the supernatant, almost clear liquid was evaporated over H₂SO₄ in flat dishes in large vacuum desiccators evacuated with a Hyvac pump until the contents froze. The residue became almost dry in a few hr. The dry, light coloured, feathery powder weighed 6.2 g. For use, 80 mg. were taken in 1 ml. (equiv. 2 ml. original Lebedew fluid) distilled water per vessel. No carrier was added in these experiments, only substrate and 0.2 mg. coenzyme II being added. The solution

was centrifuged from a little denatured protein before use. The preparation retains its activity for long periods if kept cold and dry.

Activity of the dried preparation. Fig. 7 shows the activity with ribosephosphate, and that in the absence of coenzyme no significant O_2 uptake occurs. Since the O_2 consumed in Fig. 7 (0.1 ml. 0.076 M = 167 μ l. ribose) exceeds

Fig. 7. A: 0.3 ml. 0.076 M ribosephosphate. B: 0.1 ml. ribosephosphate. C: 0.3 ml. 0.076 M ribosephosphate, no coenzyme. D: no substrate, with coenzyme. E: no substrate, no coenzyme.

Fig. 8. Oxidation of various substrates (0.3 ml. $M/10$) by dried dialysed Lebedew fluid. A: phosphohexonic. B: hexosemonophosphate. C: ribosephosph. (Synthetic). D: *d*-arabinose phosph. E: xylosephosph. F: blank without substrate, with coenz. G: blank: no coenz., no substrate.

1 mol. O_2 per mol. ribosephosphate, it is evident that the oxidation of this compound proceeds beyond the phosphoribonic acid, and even beyond the ketoribonic acid, if this is indeed formed.

Fig. 8 shows the action on various substrates. With this preparation, unlike the system consisting of Lebedew fluid with phenazine methochloride as carrier (Table VI), xylosephosphate and arabinosephosphate are also oxidized, but at less than half the rate of the ribose ester, which in this experiment was also the synthetic compound. It is interesting to note that this is oxidized almost as rapidly as the two hexose phosphoric esters. The action on the arabinose and xylose compounds which did not occur with phenazine methochloride but occurs with the natural carrier may be compared with the different behaviours of Neuberg and Robison esters with phenazine methochloride and flavin enzyme already described [Dickens & McIlwain, 1938].

6. CO_2 formation. Fig. 9 shows that CO_2 formation and O_2 consumption are nearly equimolecular.

Fig. 9. CO_2 formation and O_2 uptake with dried dialysed Lebedew fluid + 0.2 mg. coenzyme. A: 0.3 M/10 ribosephosph. B: 0.1 M/10 ribosephosph. C: no added substrate.

For this exp. R.Q. vessels [Dickens & Šimer, 1930] were used with enzyme, coenzyme and substrate in the inner part, $\text{Ba}(\text{OH})_2$ as CO_2 absorbent, and HCN in the side bulb. The readings were extrapolated for the 1st 10 min. of temperature equalization. During this exp. inorganic P increased by about 0.4–0.5 of the equivalent of the O_2 uptake.

7. *Oxidation by laked blood cells.* Each vessel contained the cells from 2 ml. horse blood, washed three times with 0.9% NaCl, and diluted to 2 ml. with water. 0.3 ml. M/10 substrate was added to each, and 0.1 mg. methylene blue dissolved in 0.2 ml. water was tipped in from the side bulb when equilibrium was reached. 0.2 ml. KOH in inner cup. Fig. 10 shows the O_2 uptakes corrected for the O_2 evolution in the blank [cf. Warburg & Christian, 1931]. Of the three pentose phosphoric acids, the ribose compound is the only one to be attacked. Curves for hexosemonophosphate and phosphohexonate are included for comparison.

8. *Oxidation in tissue slices.* In intact cells diffusion problems probably prevent the entry of phosphorylated products. On the other hand, pentoses are not apparently readily phosphorylated within the cell, though our evidence shows

that they may arise from phosphorylated hexoses which the cell is able easily to manufacture. Consequently, in intact cells oxidation of added pentoses is hardly to be expected, and in fact did not occur (Fig. 11).

Fig. 10.

Fig. 10. Oxidation by laked horse blood. A: hexosemonophosph. B: phosphohexonic. C: ribose-phosph. D: arabinosephosph. and xylosephosph.

Fig. 11.

Fig. 11. Oxidation in brain slices of rat. $M/90$ substrates in phosphate-Ringer solution. O_2 . 37.5° . A: glucose. B: *d*-ribose. C: *l*-arabinose. D: *d*-arabinose and without added substrate. E: *i*-erythritol.

Although not entirely relevant here, it may be mentioned that kidney slices can readily oxidize 2-keto-*d*-gluconic acid, as the following exp. shows.

Rat kidney slices. Phosphate-Ringer soln. O_2 . 37.5° . 0.2 ml. KOH in inner cup.

	Q_{O_2}		
	1st	2nd	3rd hr.
Control	-15.1	-10.9	-9.6
Na 2-ketogluconate $M/50$	-19.0	-16.7	-15.6

DISCUSSION

It is proposed to discuss these results in detail when a sufficient quantity of the oxidation products has been prepared for their identification. At present the results, particularly the fact that 5-C and 4-C compounds have been identified among the products, provide support for the scheme of progressive oxidation and decarboxylation previously advanced [Dickens, 1936].

The fact that *d*-ribose-5-phosphoric acid, and not the expected *d*-arabinose-5-phosphoric acid, should prove to be the physiologically oxidizable (and fermentable [Dickens, 1938]) substrate introduces a new factor into the problem. Evidently if *d*-ribose is to arise from *d*-glucose by removal of the terminal group, an epimerization must occur. It is recognized that inversion may occur in an asymmetrically disposed C atom with an attached H atom and an adjacent =CO group. A type of Walden inversion could theoretically occur either as a result of decarboxylation or by the introduction and subsequent removal of a second phosphoric acid group. These possibilities will be discussed when more direct evidence of this inversion has been sought.

SUMMARY

I. *Oxidation of phosphohexonate*

An enzyme has been prepared from Lebedew maceration fluid by precipitation at pH 4.6, washing with acid and drying. It has been used to study the oxidation of phosphohexonic acid. There is an optimum activity between pH 6.3–7.5. The action is inhibited by phosphate like the hexosemonophosphate system, but less powerfully. With pure coenzyme II oxidation of phosphohexonic acid proceeds until $\frac{1}{2}$ O₂ per mol. is consumed. At this stage the product, prepared as a Ba salt, appears to consist of a mixture of phosphoketohexonate and phosphopentonic acid, as judged by its analysis and mode of preparation.

When less pure coenzyme is used the reaction proceeds further, the end-products and end-point being altered. The O₂ uptake and CO₂ output are now each about 1 mol. per mol. substrate, an amount different from that seen in experiments of Warburg & Christian [1937] with a different preparation of enzyme. Among the end-products an analytically pure compound corresponding to a phosphorylated 4-C monocarboxylic dihydroxy-acid—possibly phosphoerythronic acid—was isolated. Two other fractions gave a definite reaction for pentose by Bial's test.

II. *Oxidation of pentose phosphoric acids*

Lebedew maceration fluid from dried brewers' yeast oxidizes *d*-ribose-5-phosphoric acid vigorously, and to a less extent *d*-arabinose- and xylose-5-phosphoric acids. When phenazine methochloride is the carrier the latter two substrates are not appreciably oxidized. The activity is destroyed by boiling. The acetate precipitate from Lebedew fluid is feeble towards pentosephosphates, at least with phenazine methochloride as carrier. An active dialysed preparation was made from Lebedew fluid; it can be kept as dry powder and is inactive without the addition of coenzyme II. With coenzyme II oxidation is vigorous with ribosephosphate, only about half as fast with the other pentosephosphates. CO₂ evolution and O₂ absorption are about equal to 1 mol. of each per mol. pentosephosphate. Laked horse blood oxidizes ribosephosphate slightly in presence of methylene blue; the other two pentose esters are not attacked.

Intact cells being impermeable to phosphoric sugar esters do not oxidize them, nor are free pentoses oxidized by brain slices. Kidney slices readily oxidize 2-keto-*d*-gluconic acid.

I wish to express my indebtedness to Dr P. A. Levene for the synthetic pentose phosphoric acids, to Prof. O. Warburg for a gift of pure coenzyme II, and to Prof. R. Robison for an analysed specimen of phosphohexonic acid prepared from Robison ester. Supplies of yeast were kindly given by Newcastle Breweries and Messrs Tennent.

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