Formation of Pentose Phosphate from 6-Phosphogluconate[1]

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Early studies on the oxidation and degradation of 6-phosphogluconate appeared to establish the formation of pentose phosphate by yeast enzymes. Although it was cautiously suggested by Dickens (*3*) that ribose-5-phosphate was produced, the identities of the pentose and of the intermediates were not established. In view of the importance of the problem of the origin of ribose in nucleic acids and coenzymes, and of the nature of the shunted metabolism in virus-infected bacteria (*1*), we have reinvestigated this system.

TABLE 1

RF VALUES FOR SUGAR PHOSPHATES

Compound	Solvents: 80% ethanol containing 0.8% acetate at pH 3.5		Solvents: 80% ethanol containing 0.64% boric acid
Glucose-6-phosphate	0.35		0
Fructose-6-phosphate	0.38		0
Glucose-1-phosphate	...		0
Glucose-4-phosphate	0.45		0
Ribose-5-phosphate	0.50		0
D-Arabinose-5-phosphate ..	0.54		0.25
D-Xylose-5-phosphate	0.55		0, 0.25
Ribose-3-phosphate	0.50		0, 0.19
Xylose-3-phosphate	0.53		0, 0.23
Glyceraldehyde-3-phosphate	0.73		0.92
	0.83	fluorescence	0.87
6-Phosphogluconate	0.89		.....

The action of yeast enzymes, prepared according to the method of Dickens and McIlwain (*4*), on 6-phosphogluconate was studied in the presence of TPN (triphosphopyridine nucleotide), phenazine as a hydrogen carrier, a 0.01 M phosphate buffer at pH 7.0, and 0.0067 M NaCN which promotes the oxidation. At the end of the reaction slightly more than 0.5 mole of O_2 was consumed per mole of substrate, and 0.5 mole of CO_2 was produced. It was found that 0.25–0.40 mole of new pentose accumulated in this system.

The end products and intermediates were fractionated as follows: Protein was removed with 5% trichloroacetic acid and the phosphate esters were isolated as Ba salts by precipitation in 80% ethanol. The esters were analyzed by paper chromatography in 80% ethanol containing 0.8% acetic acid at pH 3.5 or 0.64% boric acid. In Table 1, it may be seen that characteristic R_f values were obtained for a large number of sugar phosphates. Boric acid has, in addition, provided a tool for distinguishing *cis*-hydroxyls, inhibiting the movement of ribose-5-phosphate but permitting the migration of D-arabinose-5-phosphate. In this medium, it was shown (Fig. 1) that 50% of the Bial-reactive phosphate formed does not have characteristics of either ribose- or arabinose-5-phosphate. A substance having R_f values of ribose-5-phosphate was also observed. The formation of glyceraldehyde-3-phosphate and the disappearance of 6-phosphogluconate was also demonstrated as the reaction progressed.

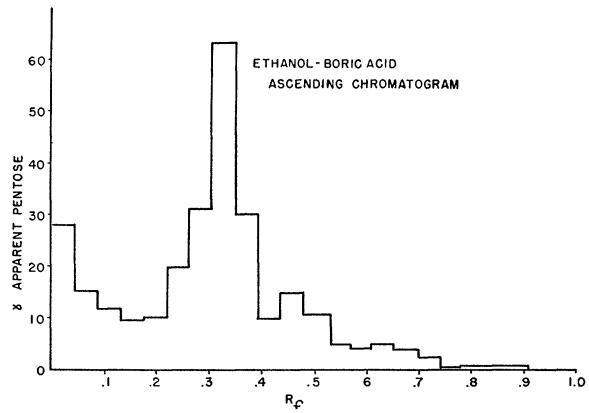

FIG. 1. Distribution of phosphorylated Bial-reactive carbohydrate in eluates of 1-cm strips of an ethanol-boric acid ascending paper chromatogram. The chromatogram was obtained on the isolated Ba salts after partial enzymatic degradation of 6-phosphogluconate. In this experiment the reaction was stopped after 40% of the theoretical O_2 has been consumed.

The pooled phosphate esters were hydrolyzed with alkaline phosphatase. The acids were adsorbed at pH 7 on the anion exchange resin, Amberlite IRA-400. Elution with 0.1 *N* HCl permitted the separation of gluconate and 2-ketogluconate. A 2-ketonic acid was observed in the eluates. Small amounts of a substance with the R_f values and chemical reactivities of 2-ketogluconic acid were observed in paper chromatograms, after hydrolysis of the esters isolated at intermediate stages of the reaction.

The neutral sugars in the resin filtrate were found to contain substances with the R_f values of ribose and possibly D-arabinose as determined on chromatograms with various solvents. R_f values were established by color reactions or estimations of pentose in eluates of cuts of the chromatograms. Direct fermentative analysis in Warburg manometers of this filtrate by means of *E. coli* strains specifically adapted to ribose or D-arabinose (*2*) revealed ribose amounting to 25% of the pentose. By this method ribose was also found in eluates of the appropriate cuts of the chromatograms.

The data imply that this system contains enzymes for at least two reaction steps. At least one oxidation involving the disappearance of 0.5 mole of 6-phosphogluconate did not result in pentose formation. However, oxidation corresponding to the remaining 0.5 mole of substrate was followed by decarboxylation and pentose phosphate for-

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mation. The finding of ribose among these products indicates an inversion at some level in this series of reactions. The nature of the unknown Bial-reactive phosphate is of interest in this connection. Whether the triose phosphate arose from the pentose phosphate (*5*) or some other product is being investigated.

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Transplantation of Rabbit Blastocysts at Late Stage: Probability of Normal Development and Viability at Low Temperature[1]

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Following previous investigations on transplantation of rabbit ova at different stages (*1*, *2*), the following experiment was performed. Rabbit blastocysts at late stage, 6 days after mating, were recovered from superovulated does (*7*) by flushing the excised uterus with fresh rabbit serum diluted with an equal volume of 0.9% NaCl. The percentage of recovery was very high, 75%–100%, as checked by counting the number of corpora lutea. The highest number of blastocysts recovered from one doe was 62. Most of the blastocysts measured 3 mm in diam, but a few of them measured 1 mm, probably due to the large number of ova produced by superovalation. Most of them were round in shape, and the germ disk appeared in the large ones. After recovery they were kept at 30° C in a watch glass placed inside a Petri dish no more than 45 min before transplantation.

Transplantation was performed by making a 5-mm longitudinal incision on the exposed uterus and by placing the blastocysts into the uterus with a pipette 4 mm in diam. The incision was closed with catgut sutures. Fifty blastocysts were transferred to seven recipients at the sixth day of pseudopregnancy, and six of the recipients gave birth to 21 normal young genetically resembling the donors. The gestation period was 26–27 days, and the percentage of blastocysts which developed into young in the pregnant recipients was 47.7.

The blastocysts, placed in a small tube containing serum diluted with 0.9% NaCl, were stored for 1 or 2 days at 10° C (at room temperature for 30 min before storage) or at 0° C (with acclimatization at 10° C for 2 hr). They were then cultured at 38° C in a Carrel flask containing undiluted serum. The results are presented in Table 1. After storage for 1 day at 10° C about one-third of them were shrunk, their round shape lost, but practically none shrank at 0° C for 1 day. After storage for 2 days, either at 10° or at 0°, most of them were collapsed, with separation of trophoblast and albumin coat, and had sunk to the bottom of the tube. In culture, it took about 12 hr for the shrunken blastocysts, or about 24 hr for the collapsed blastocysts, to recover their round shapes. Their growth was observed by enlargement in size and appearance of the primitive streak (or neural groove in two cases) after 3 days' culture.

TABLE 1

VIABILITY OF RABBIT BLASTOCYSTS AT LOW TEMPERATURE

Storage temperature in °C	Storage time in days	No. stored	% Shrunk or collapsed	No. cultured	No. recovered	No. grown
10	1	36	36	15	11	8
	2	39	87	25	19	6
0	1	37	3	9*	5	3
	2	11	91	6	0	0

* Eight of nine shrank in culture on the first day, five of eight recovered their round shape on the second day.

It is interesting to note that no blastocysts recovered in culture after storage at 0° C for 2 days, and that some intact blastocysts after storage at 0° C for 1 day shrank in culture on the first day and recovered on the second day. Very few of them resumed their growth. A temperature of 10° C is therefore better for the storage of blastocysts at the late stage, just as in the case of ova at an early stage (*1*).

Following these observations, 18 blastocysts after storage at 0° C for 1 day (all intact) were transferred to three recipients at the fifth and sixth days of pseudopregnancy. None was diagnosed as pregnant by palpation. At laparotomy, one small swelling on the uterus (indicating maternal placental formation) of one recipient was observed. Twenty-three blastocysts after storage at 10° C for 2 days (18 shrunk, five intact ones) were transferred to three recipients. Three swellings of different sizes but without normal embryos (indicating degeneration of embryos at different stages after placental formation) were observed in two of the recipients. Twenty-eight blastocysts after storage at 10° C for 1 day were transferred to five recipients. The first, which received four blastocysts, did not become pregnant; the second, which received four intact ones, had two large swellings as well as two small swellings at laparotomy and gave birth to two young at term; the third, which received three intact and four shrunken ones, had six normal embryos, 7–9 mm, when examined 6 days later. The last two animals each gave birth to two normal young 28 days after transfer. The percentage of development of blastocysts in the pregnant does was therefore 50.

The viability of blastocysts at different stages *in vitro* may not be the same. Most of the blastocysts which

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