GLUCOSE CATABOLISM IN FETAL AND ADULT MAMMALIAN HEART

by

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INTRODUCTION

The cells of growing and developing organisms are generally characterized by a higher rate of protein synthesis than is found in the cells of mature organisms, and it has been repeatedly shown (20) that increases in rate of protein synthesis are associated with increasing RNA content, though the exact nature of the relationship is still unclear (33). Brachet (20, p. 242), in his review of the subject, states that "--- all the organs which synthesize large amounts of proteins, whether for growth or multiplication, are always rich in RNA, which is localized in the nucleolus and cytoplasm; all other cells and tissues have a much lower content of RNA and much less conspicuous nucleoli." The high rate of cellular RNA synthesis in rapidly proliferating or actively secreting tissues will require a proportionately high rate of ribose synthesis (39) (57). One could expect then, that enzymes necessary for ribose synthesis would be in greater supply in such tissues than in non-secreting tissues or those with a low rate of proliferation. Thus metabolic pathways leading toward ribose should be much more in evidence in glandular organs, synthesizing large amounts of protein, such as

liver, salivary gland, pancreas, gastric mucosa, mammary gland, adrenal gland, and such rapidly proliferating tissues as embryonic organs and neoplasms, than in other tissues such as kidney, brain, lung, cardiac and skeletal muscle.

Recent work (41, 60, 67, 79) has shown that, in the tissues tested, two pathways of ribose synthesis are utilized; both pathways being portions of the pentose cycle* (Fig. 2) (42, 77). In one, ribose is derived from glucose via the direct oxidative route (Fig. 3), in the other, ribose is obtained by a reversal of the pentose cycle (Fig. 4). The results obtained by a number of workers indicate that these pathways are quantitatively more significant in proliferating and secretory tissues than in others. Glock and McLean (34) surveyed various tissues for the activity of pentose cycle enzymes. They found skeletal and cardiac muscle to be low in activity, while adrenal or mammary glands, liver, and embryos were high. Kelly (56) found all the pentose cycle enzymes to be highly active in rat adrenal cortex, an active secretory gland. At the same time tissues such as cardiac muscle were found to have virtually no activity.

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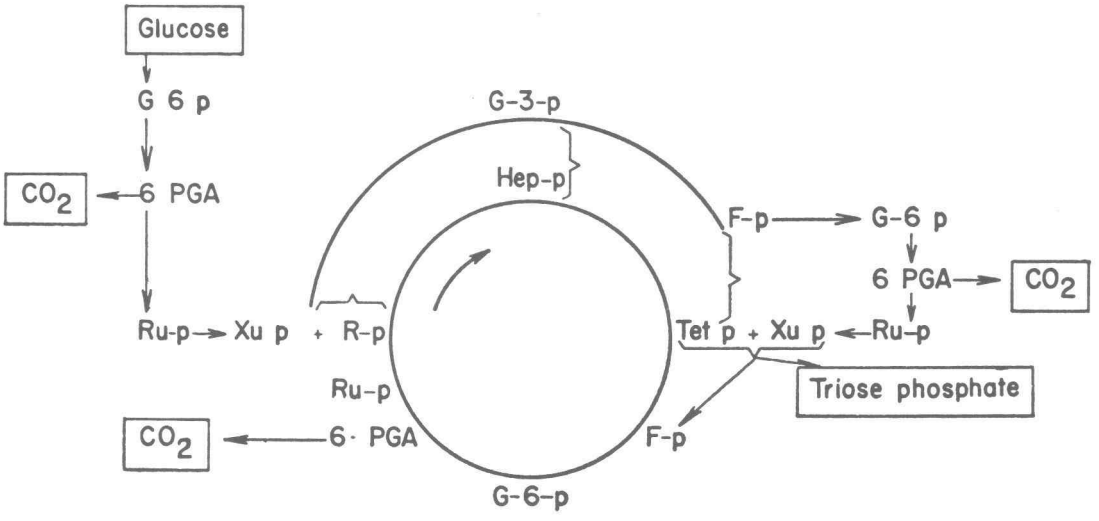
* Variously referred to also as the Direct Oxidative Pathway, Phosphogluconate oxidation pathway, pentose phosphate pathway, Hexose monophosphate oxidative pathway, and Hexose monophosphate shunt.

FIGURE 1



Recycle: 2 triose-p → FDP → F-p → 3 CO_2 + triose-p

G-3-p	= Glyceraldehyde phosphate	F-p	= Fructose-6-phosphate
Tet-p	= Tetrose-4-phosphate	G-6-p	= Glucose-6-phosphate
R-p	= Ribose-5-phosphate	6 PGA	= 6 Phosphogluconate
Ru-p	= Ribulose-5-phosphate	FDP	= Fructose diphosphate
Xu-p	= Xylulose-5-phosphate	Hep-p	= Sedoheptulose-7-phosphate

Glucose → $6CO_2$

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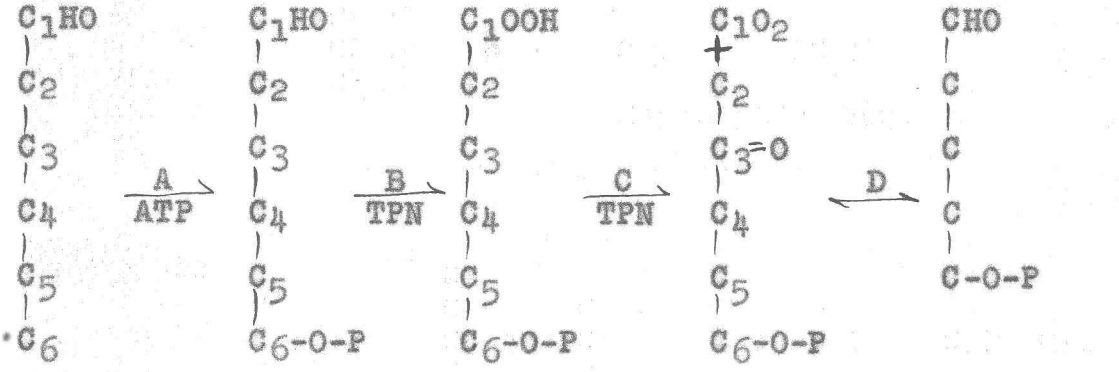
FIGURE 2

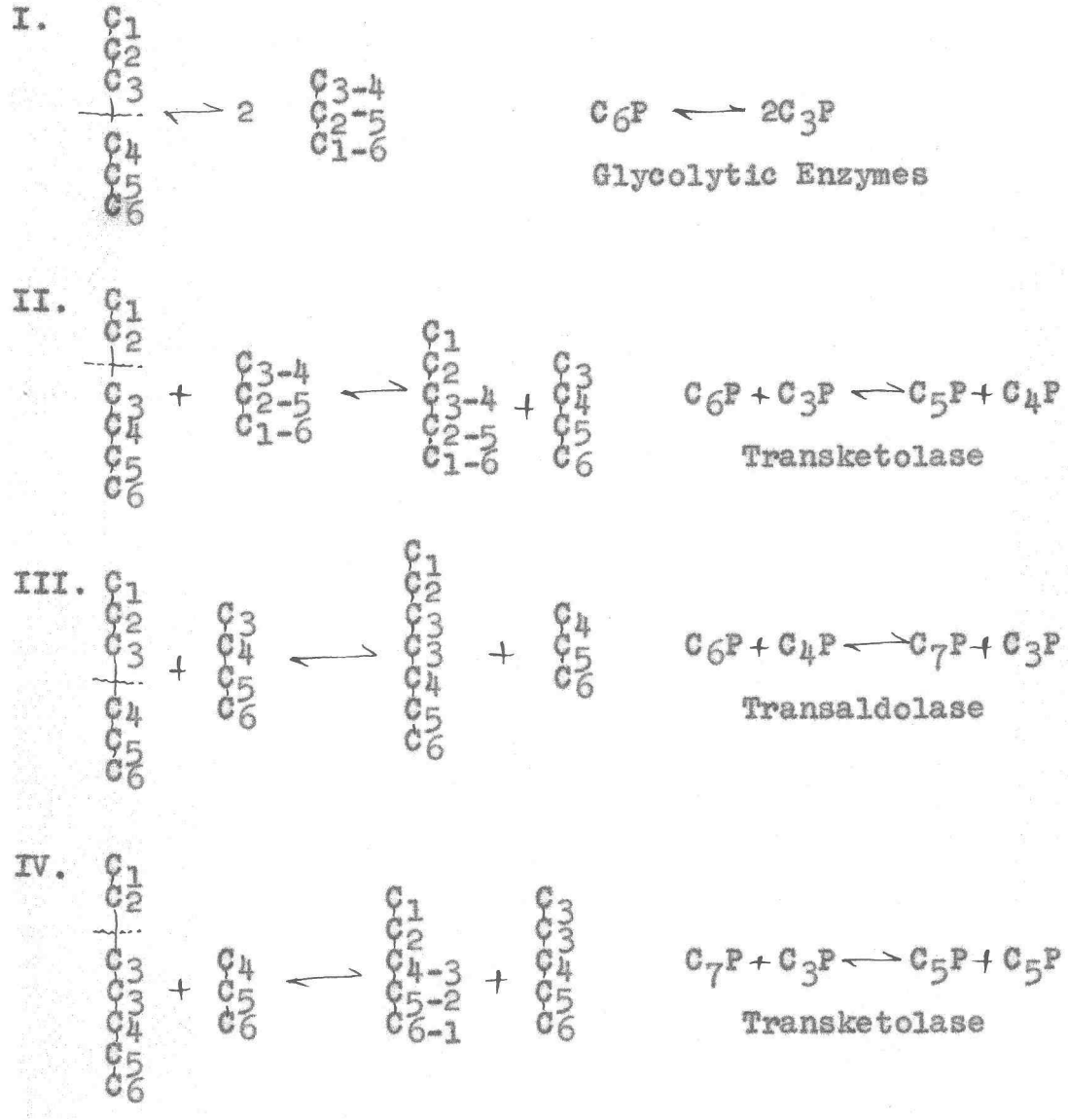
Formation of Pentoses from Hexoses by Way of Hexose Monophosphate Oxidation



A, Hexokinase; B, Glucose-6-Phosphate Dehydrogenase; C, 6-Phosphogluconate Dehydrogenase; D, Pentose Phosphate Isomerase

FIGURE 3

Formation of Pentoses from Hexoses by Reversal of the Pentose Cycle



In other work Glock and McLean (37) concluded that, in rat mammary slices, a considerable amount of glucose follows the phosphogluconate oxidation pathway especially during lactation. This agrees with similar findings of other workers (1), who reported that as much as 60% of glucose oxidation by mammary tissues proceeds by way of phosphogluconate oxidation.

In most of the work dealing with the relative importance of pathways of glucose metabolism, use is made of one or both of two approaches. One, generally used with *in vitro* studies, is to determine the relative rates of recovery of $C^{14}O_2$ from the individually-labeled carbon atoms of glucose (or other carbohydrate molecules). Another approach, used with *in vivo* studies, is to determine to what extent label from injected or ingested substrates is incorporated into liver or muscle glycogen of the rat. In studies of oxidative pathways, use is commonly made of the first-named approach. In its simplest form, this approach involves use of glucose labeled in carbon 1 or carbon 6. If glucose is catabolized by way of the Embden-Meyerhof pathway, symmetrical cleavage to trioses occurs, followed by Krebs cycle oxidation, so that carbons 1 and 6 will be evolved in exactly equal amounts.

If the hexose monophosphate oxidative pathway is followed, carbon 1 will be rapidly evolved as CO_2

carbon 6 only very slowly. When both pathways are utilized, there will be an excess of C-1 evolved. If one desires to know the fraction of glucose catabolized via each pathway, additional information is necessary, such as data on $C^{14}O_2$ production from labeled lactate and incorporation of glucose carbon atoms into fatty acid molecules.

Bloom and Stetten in 1953 (13, 14) reported no evidence of non-glycolytic catabolism of glucose in the intact rat (respiratory CO_2 studies) or in rat diaphragm; they found a small amount of an alternate pathway in kidney and reported that, in liver, the alternate route (direct oxidative path) predominated. Their conclusions, though not their data, were challenged by Katz and Chaikoff (53) who calculated that not more than 20% of the glucose catabolized by rat liver slices followed an alternate pathway. Their own work indicated that 83-98% of the CO_2 is formed glycolytically and 61-94% of the glucose is catabolized glycolytically (54). Another group (3) obtained results generally supporting those of Bloom, finding that rat kidney and diaphragm displayed essentially only the Embden-Meyerhof (glycolytic) pathway, while liver slices possessed a very active direct-oxidative route. It should be noted, however, that these workers found fetal and regenerating liver to have a somewhat less active oxidative path than

normal liver. One might rationalize this result, in the light of the earlier hypothesis, by the assumption that the secretory activity (thus protein synthesis, RNA synthesis, and ribose requirement) of normal adult liver far exceeds that of fetal or regenerating liver. In 1955 Bloom and Stetten (15, 16), admitting the correctness of the position taken by Katz and Chaikoff (53), derived new expressions utilizing data on fatty acid labeling as well as $C^{14}O_2$ production. They reported that, in rat liver slices, about 50% of glucose catabolism was by way of the direct oxidative route; in fasted and diabetic rat livers (17) this value dropped to about 35%. On the other hand, Bernstein et al.(11) relying on glycogen degradation data, attributed but a minor role to the alternate pathway in the overall glucose utilization by intact rat liver. Wenner and Weinhouse (88) also concluded that, in rat liver slices, most of the added glucose is metabolized via the Embden-Meyerhof process. Ashmore et al (5) also minimized the phosphogluconate oxidation path in rat liver slices, finding that only 3-11% of the total utilized glucose follows this path. They emphasized that over 50% of glucose utilization involves conversion to glycogen and that oxidation is not the primary fate of glucose taken up by the liver; others (66) concluded similarly. Muntz and Murphy (70), on

the basis of liver glycogen degradation data, report that, in the intact rat liver, 29-38% of the glucose is metabolized via the phosphogluconate oxidation pathway. In perfused rat livers these same authors (71) found that the direct oxidative route accounts for 56% of the glucose oxidized.

In 1956 Bloom and Stetten (18), in a revision of their earlier conclusion, reported, on the basis of glycogen degradation studies, the active utilization of a non-glycolytic pathway in the liver of the intact rat. They reasoned that, in earlier respiratory CO_2 studies with intact rat, the non-glycolytic catabolism of glucose had been masked by the much larger Embden-Meyerhof mechanism predominant in skeletal muscle. Black et al.(12) found that, in the lactating dairy cow liver or mammary gland, the pentose cycle route accounts for 60% of the glucose oxidation.

The direct oxidative mechanism was found by Emmelot (28) to account for up to 70% of glucose oxidation in mouse tumors. Another group (2) reported that little or no glucose was metabolized via direct oxidation in mouse livers but that a considerable fraction is so metabolized in mouse hepatomas. Wenner and Weinhouse (89) also noted that, in normal tissues, little glucose follows the alternate pathway and even in neoplastic tissues, where the alternate pathway is

active, 77-94% of the glucose is converted to lactic acid via glycolysis. Kit (59) reported the existence of a direct oxidative pathway in lymphatic tumors, while Villavicencio and Barron (83) also found an active direct oxidative pathway (estimated 25% of catabolized glucose) in lymphosarcoma cells, but not in normal lymphatic cells. In erythrocytes, which contain no mitochondria, Huennekens (47) showed that all oxidation of glucose proceeds by way of the direct oxidative pathway. The direct oxidative route is also reported to be characteristic of the mammalian cornea (58).

Among non-mammalian tissues, sea urchin eggs have been shown (62) to utilize a direct oxidative pathway, which, in fact, predominates over the glycolytic route during the first stages following fertilization. The pentose cycle has also been found in the house fly (24), egg and larval stage of the blowfly (69), the pea aphid (72) and the intestinal parasite ascaris (29). In the last named organism, however, no evidence was found for the presence of a transaldolase, an essential enzyme of the complete pentose cycle.

Cardiac muscle has thus been consistently found to utilize almost exclusively the Embden-Meyerhof mechanism in catabolism of glucose. In the light of the earlier hypothesis this is reasonable, since cardiac muscle is

known to be extremely low in RNA (thus ribose) content (23, 31, 50, 65). Agranoff and Brady (4) noted no disappearance of ribose when ribose was incubated with rat heart slices or homogenates, while the disappearance with other tissue, especially liver, was very high. They concluded that heart contained little if any ribokinase. If one considers the pentose cycle, as operative in mammalian cells, not as a mechanism for energy production but rather as a combination of interlocking schemes for ribose synthesis, then the complete predominance of the Embden-Meyerhof process in cardiac muscle is quite reasonable.

In fetal cardiac tissue, however, with a higher rate of protein synthesis than found in adult cardiac tissue, one could expect a higher RNA content than in the adult. To quote Leslie (65, p. 44) "A higher proportion of RNA to protein than is present in adult or resting cell populations is found in embryonic, regenerating, and neoplastic tissues." Borghese (19, p.334) in his review of histochemical evidence, reached essentially the same conclusion, "Cytoplasmic RNA is much more frequently detected during embryonic development than in adult organs, where it is limited to well-defined types of cells, such as the nervous or secretory ones." This, as suggested by Glock and McLean (36) and O'Connor (73), would also imply an equally high concentration of

systems for ribose synthesis, though it seems one should not overlook the possibility that fetal heart might utilize ribose obtained from the circulation after synthesis elsewhere in the organism.

In studies with chick embryo heart, Leslie and Davidson (64) found that the RNA content of the organ increased with the increasing heart size during incubation, but that the RNA per cell was at a maximum value during the phases of rapid multiplication and protein synthesis. Thereafter, the RNA content per cell falls gradually. If an analogous situation can be supposed to exist in mammalian heart development, then the RNA per cell should be at a maximum during the fetal period and then fall toward the lower adult values. Pentose cycle activity in the heart, then, can also be expected to reach a peak during the fetal period and then to decline as the animal approaches maturity.

The work described in this thesis was undertaken in an effort to determine whether such a change in enzymatic activity actually occurs. The results indicate that pentose cycle activity is, indeed, much greater in fetal mammalian heart than in adult heart. They also indicate that, in the adult heart, glucose catabolism proceeds almost, if not entirely, by way of glycolysis, while, in fetal heart tissue, some glucose follows the hexose monophosphate oxidative pathway.

METHODS AND MATERIALS

(1) Procurement of heart tissue.

Pig heart tissue was obtained through the courtesy of the D. E. Nebergall Meat Co., of Albany, Oregon. Adult heart tissue was taken from the apical portion of the ventricle. Fetal heart tissue for homogenate studies included the entire ventricular portion of the heart. For perfusion studies the whole heart and adjacent portion of the aorta were taken. At the time of removal of fetal hearts the crown-rump lengths of the fetuses were measured. This was later correlated with age and per cent gestation time by reference to the findings of Warwick (86). His work shows that the average length of the pig fetus at 30 days gestation time was one inch and that at parturition (114 days) was 10.3 inches. Between these two extremes, length and age were found to be roughly proportional.

(2) Preparation of homogenates.

Heart tissue, obtained immediately after slaughtering of the animals was immersed in ice cold physiological saline until used in the laboratory. A portion of ventricular tissue, freed as much as possible from fat and connective tissue, was weighed, finely minced with a razor, and homogenized with buffer in a pre-cooled,

hand-operated Dounce homogenizer (27). In order to obtain the least possible destruction of cells and cell components, only three or four strokes of the loose-fitting pestle were made in preparation of homogenates. To eliminate connective tissue the homogenate was strained through a stainless steel sieve, a spatula being used to force as much tissue through as possible. The pH of the homogenate was then adjusted with 10% KOH to 6.8 - 7.0. The initial pH of the entire reaction mixture generally fell in the range 6.9 - 7.1. Five-tenths ml. of the homogenate was pipetted into a weighed planchet, dried under an infra-red light, and weighed to determine solids content.

A drop of the homogenate was smeared on a glass slide, dried, stained with Wright stain, and examined under high power. The percentage of cell rupture in the adult preparations was rather low, since the bulk of the tissue appeared to have been broken down to fragments averaging 0.5 to 1.0 mm. in diameter. The destruction of the much more delicate fetal heart tissue, especially that of the younger fetuses, was far more complete. Even with only two strokes of the loose pestle, there were few fragments of tissue left, and it appeared that a considerable percentage of the cells were ruptured. Nuclei, however, appeared to be undamaged, and it seems unlikely that the fetal mitochondria suffered any damage. Dounce

(27) expresses the opinion that liver mitochondria are undamaged even by the tight-fitting pestle. Comparisons of the relative effects of gentle and vigorous homogenization were made with both fetal and adult heart homogenates. With adult heart the degree of homogenization appeared to have little effect on glucose oxidation. In an experiment with fetal heart, however, vigorous homogenization was found to result in a 20% decrease in the rate of oxidation of carbon-6 of glucose, but little, if any, effect on the C-1/C-6 ratio. Vigorous homogenization caused a slight increase in gluconolactone oxidation in both fetal and adult homogenates.

The medium for homogenization was that of Wenner and Weinhouse (87) plus 0.001 M versene. Versene was thought desirable in view of Slater's findings (80) that its presence helped to maintain a high level of oxidation and phosphorylation by heart sarcosomes. The phosphate buffer medium contained KH_2PO_4, 0.0124M; KCl, 0.99%; versene, 0.001M; adjusted to pH 7.4. The Tris buffer medium contained Tris (hydroxymethyl) aminomethane, 0.02M; KCl, 0.99%; versene, 0.001M; adjusted to pH 7.4. All solutions were made up in glass-distilled water.

(3) Preparation of soluble enzyme fractions from heart.

For soluble preparations, homogenization was continued

vigorously with 20 or 30 strokes of the loose-fitting pestle followed by an equal number of strokes with the tight-fitting pestle. This resulted in virtually complete disintegration of cells in the adult as well as fetal heart homogenates. The pH was then adjusted to 6.8 - 7.0. A preliminary centrifugation in the Servall centrifuge was followed by centrifugation in the Model L Spinco ultracentrifuge, in most cases for one hour at 100,000x g, this being sufficient to remove all particulate matter.

An estimate of protein concentration was made by turbidimetric assay. A 0.20 ml. aliquot of enzyme solution was diluted to 2.0 ml., 3 ml. of 5% trichloroacetic acid added, and, after 40 seconds, optical density was read at 540 mμ. This was referred to a standard curve obtained with known weights of crystalline egg albumin.

The reducing sugar concentration of soluble preparations was estimated by the method of Folin and Malmros (32). Twenty fetal "solubles" gave an average figure of 13.6 ug. of reducing sugar/mg. of protein; ten adult "solubles" gave an average of 15. μg./mg. protein. There will thus be little difference between fetal and adult preparations in the degree of dilution of labeled compounds by endogenous sugars.

(4) Reaction mixture components.

Certain components of the reaction mixtures were present in all manometric and radiochemical experiments with both homogenates and "solubles". These were present in approximately the same concentrations found to be optimal by Wenner and Weinhouse (87). Thus $MgCl_2$, 3×10^{-3}M; KCl, 0.14 M; phosphate buffer pH 7.4, 6×10^{-3} M; cytochrome c, 3×10^{-5} M. In addition, nicotinamide, not used by the above-named authors, was present at approximately 0.06 M. This component has been found to stabilize the oxidative capacity of homogenates by inhibition of pyridine nucleotidases (75, 90). A few experiments utilizing a DPNase assay (90) served to establish that a DPNase (equally active against TPN) was present at approximately the same levels in fetal and adult beef heart. Nicotinamide had the expected highly inhibitory action. In other experiments with beef heart homogenates, it was found (Table I) that added nicotinamide succeeded in preventing the levels of oxygen consumption and CO_2 evolution from declining due to the destruction of pyridine nucleotides. Isonicotinic acid hydrazide (91), at the same concentration, had a similar but less marked effect.

(5) Radiochemical experiments (homogenates and soluble preparations).

TABLE I

Effect of Nicotinamde (NA) and Isonicotinic Hydrazide (INH) on Oxygen Consumption (Endogenous) and $C^{14}O_2$ Production From C^{14}-labeled Glucose by Adult Beef Heart Homogenate

	O_2 Consumption at 1.5 hours (µl)	O_2 Consumption at 4 hours (µl)	$C^{14}O_2$ Recovery from Glucose-1-C^{14} (cpm)	$C^{14}O_2$ Recovery from Glucose-6-C^{14} (cpm)
No Additions	84	137	64	36
100 um NA	96	273	1336	1230
100 um INH	82	190	536	560

Flask Components:

5	µmoles $MgCl_2$
0.07	µmoles cytochrome c
10	µmoles phosphate buffer, pH 7.4
230	µmoles KCl
0.36	µc labeled glucose
0.5	ml homogenate in phosphate buffer

NA or INH as noted; Final volume 1.80 ml.
Incubation temperature, 37°C

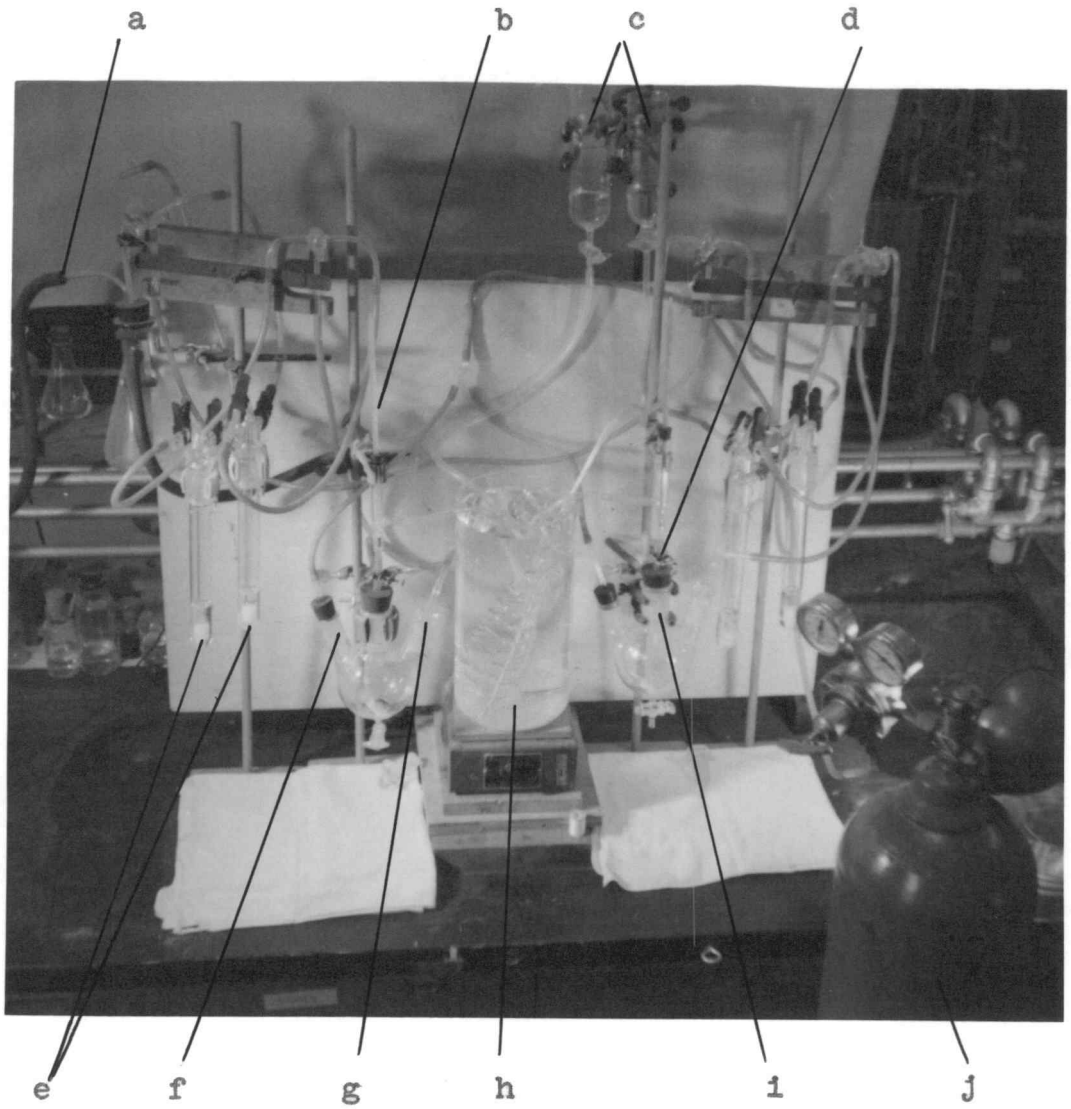
In these experiments an apparatus known as a radiorespirometer was used. This apparatus, first described by Wang et al. (84), and subsequently modified (85), allows evolved $C^{14}O_2$, swept continuously from a reaction flask to a trap containing NaOH solution, to be collected over any desired intervals throughout the course of the experiment. This permits one to follow the rate of $C^{14}O_2$ production from labeled substrates by determining the interval recovery of $C^{14}O_2$. As many as ten flasks can easily be run simultaneously on the apparatus, which utilizes a Warburg bath and shaking mechanism. Use of a calibrated gas flow meter permits exact regulation of the flow rate through each individual flask.

The $C^{14}O_2$ was precipitated as $BaC^{14}O_3$ and counted with a thin window counter. In this counter 0.3 μcurie of $BaC^{14}O_3$ was found to give a rate of 43,600 counts per minute. The amount of radioactivity added per flask was 0.3 μc. in all experiments with the radiorespirometer; consequently, all values for per cent recovery of radioactivity are based on this figure.

(6) Heart perfusion experiments.

These experiments were performed with the duplicate heart perfusion apparatus shown in Fig. 1. The oxygen was drawn from the cylinder by a vacuum, resulting in

FIGURE 4



a. Vacuum line; b. Thermometer; c. Reservoir for medium

d. Entrance of medium into cannula; e. CO_2 traps;

f. Oxygen flow entrance; g. Oxygen flow exit;

h. Warm water bath; i. Position of cannula;

j. Oxygen cylinder

slightly lower than atmospheric pressure in the flasks, thus preventing leaks to the outside. Two CO_2 traps were associated with each perfusion flask in order to facilitate interval recovery without losses or extended interruption of the oxygen flow. The perfusion medium, at a hydrostatic head of 50 to 60 cm., enters the cannulae at 36-38°C and drips from the cannulated heart into very dilute phosphoric acid solution in the bottom of the flask. The oxygen passes through a sparger immersed in the solution. At the end of each interval this solution is drained from the flask and fresh solution added. The rate of flow of medium through the heart ranged from 80 to 120 ml. per hour. The length of the interval was arbitrarily chosen, and varied from 30 to 60 minutes.

Rat hearts were removed after nembutal anesthesia; fetal pig hearts were removed at the slaughterhouse, flushed with cold physiological saline and immersed in cold saline until cannulation.

The medium for perfusion was made up in glass-distilled water. That used in the earlier experiments contained Ca^{++} ions in addition to K^{+}, Na^{+}, Mg^{++}, and phosphate ions. This allowed rat hearts (but not fetal pig hearts) to continue beating during the experiment. However, it was thought that differences in beating rate

and vigor might accentuate differences in rate of glucose metabolism; consequently, the Ca^{++} ions were omitted in later experiments.

The results are presented as an average of the specific activities obtained for four or five intervals during an approximately three hour run. The $C^{14}O_2$ was precipitated without addition of carrier sodium carbonate. It was reasoned that if the rates of utilization of glucose were different in the two hearts (due to possible differences in effectiveness of the cannulation as well as to inherent differences in the hearts) then this would be compensated by reporting specific activities of the evolved $C^{14}O_2$. This is probably valid with the fetal hearts which were very nearly identical. However, with the rat hearts, differences in levels of endogenous glucose, for example, would not be compensated in terms of specific activities.

An attempt was made to test the duplicate perfusion apparatus to determine to what extent actual duplication was obtained when rat hearts were used. The results are summarized in Table II. The values given are the ratios of specific activities (with the higher specific activity arbitrarily set as the numerator) of pairs of rat hearts perfused with the same labeled glucose, generally glucose-6-C^{14}. In theory, the ratio for each perfusion would be 1.00, but in practice, the variation was considerable.

TABLE II

Control Experiments to Test the Duplicate Heart Perfusion System with Adult Rat Heart Pairs Perfused with Glucose Labeled in the Same Position

Expt. No.	Spec. act. Ratio* (Theoretical value 1.00)
P25[1]	1.10
P27	1.17
P31[2]	1.01
P32	1.05
P33	1.60
P34	1.36
P35	1.21
P36	1.26
P37[3]	1.26
P38	1.30
P39	1.41
P40	1.18
Average	1.24

* Specific activity of $C^{14}O_2$ from labeled glucose perfusing one heart / Specific activity of $C^{14}O_2$ from labeled glucose perfusing other heart

(Higher specific activity always placed in numerator of ratio)

1) Components in P25, 27 as in TableXVI . No pyridine nucleotide.

2) Components in P31-36 as in Table XVI , except: no pyridine nucleotides; $MgSO_4 \cdot 7H_2O$ 0.34 gm/l; 240 μmoles glucose/l.

3) Components in P37-40 as in Table XVII . No pyridine nucleotide.

This is presumably due in large part to individual differences between the two hearts of each pair. The rat hearts were not necessarily from littermates but were merely from rats of the same sex and approximately the same weight. Other factors, such as small differences in the temperature of the medium entering the hearts, and faulty cannulation, could also contribute to the variation.

It is difficult to assess the per cent recovery of radioactivity in the perfusion experiments. On the basis of the total amount of radioactivity passing through a heart during a perfusion run, the per cent recovery as $C^{14}O_2$ varied from less than 1% to around 5%, depending on the type of heart, beating action, if any, and the amount of carrier glucose. However, it is evident that the use of such a basis is not really justified, since the heart enzymes are not exposed to all the labeled glucose during the entire experiment. At the rates of perfusion used, most of the glucose passes through the heart without penetrating the cells. If one were to take the volume of the heart as the basis, assuming the heart to be exposed only to its own volume of medium throughout the experiment, one could be equally in error, since there is actually no depletion, but rather a constantly-renewed supply of substrate and during the course of the experiment the heart utilizes far more substrate than

would be present in it at any one time.

(7) Reagents.

ATP (adenosine-5-triphosphate, K salt), ribose-5-phosphate (Ba salt), fructose-6-phosphate (Ba salt), glucose-6-phosphate (Ba salt), 6-phosphogluconate (Ba salt), DPN (diphosphopyridine nucleotide), TPN (triphosphopyridine nucleotide), and cytochrome c were all obtained from Sigma Chemical Co. L-Thyroxine was obtained from Nutritional Biochemicals Corp.

Glucose-2-C^{14} (2.20 μc./mg.), Glucose-U-C^{14} (uniformly labeled glucose) (1.9 μc./mg.), and glucose-6-C^{14} (2.32 μc./mg.) were obtained from the National Bureau of Standards. Glucose-1-C^{14} (3.96 mc./mM), D-glucono-Δ-lactone-1-C^{14} (1.78 mc./mM), sodium acetate-1-C^{14} (5.18 mc./mM), and D-ribose-1-C^{14} (2 mc./mM) were obtained from Nuclear Instruments, Inc. Sodium pyruvate-2-C^{14} (3.17 mc./mM) was obtained from Volk Radiochemical Co.

(8) Spectrophotometric Studies.

The rate of reduction of TPN was followed on the Beckman Model B spectrophotometer. The rate was reported as units. A unit was defined as a change of optical density (at 340 mμ) of .001/minute/mg. protein.

RESULTS

A. Studies with Soluble Enzyme Preparations

(1) Spectrophotometric studies.

It is clear from Table III that the two dehydrogenases of the direct oxidative pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase, as well as hexose phosphate isomerase, are present in both fetal and adult soluble systems, but that the fetal "solubles" average 3 to 4 times as active as the adult.

An estimate of the entire pentose cycle system of the enzymes was made by observing the rate of reduction of TPN with ribose-5-phosphate as substrate. This is possible, since in order to effect reduction of TPN the substrate must be converted to hexose monophosphate via the pentose cycle (40,43,56,68), and thus must be acted upon, in turn, by:

(1) pentose phosphate isomerase (7,25,26)
(2) phosphoketopentoepimerase (6,25,49)
(3) transketolase (46)
(4) transaldolase (44)
(5) hexose phosphate isomerase
(6) glucose-6-phosphate dehydrogenase
(7) 6-phosphogluconic dehydrogenase

As shown in Table III the average rate of TPN reduction with ribose-5-phosphate as substrate - thus the overall activity of the pentose cycle enzymes - was over 10 times as great in the fetal heart preparations as in

TABLE III

Rate of Reduction of TPN by Various Substrates with Pig Heart Soluble Preparations

		Rate of Reduction of TPN (units)			
Enzyme	Size	Ribose-5-phosphate	Fructose-6-phosphate	Glucose-6-phosphate	6-phosphogluconate
2/13	Adult	0.00	6.00	6.90	10.87
2/14	"	0.42	5.28	7.20	8.10
77	"	0.38		9.00	8.21
79	"	0.25			
9/17	"	0.29			7.01
91A	"	0.09	6.47		6.48
83					5.16
Average (Adult)		0.24	5.94	7.68	7.61
2/17	1"	11.40		63.8	53.5
11/14	1"	3.50			
2/17	2"	4.65	30.2	39.4	31.0
78	3"	2.78			
2/17	4"	2.28		35.2	26.2
92	4"	3.80			35.6
P48	4-½"	1.15		17.0	15.6
P50	4-½"	1.90		18.5	15.5
74	5"	3.33			
1/31	5"	2.25		14.4	18.0
82	8"	4.76		27.6	24.0
81	8"	3.56	21.4		
91F	8"	2.49	21.7		28.8
P49	4"			21.8	
Average (Fetal)		3.50	24.4	28.5	26.6

Components of system: 2 μmoles substrate (except 4 μmoles Ribose-5-phosphate) 2 μmoles TPN: 2 μmoles Versene (EDTA); 264 μmoles KCl; 40 μmoles Tris buffer, pH 7.4; 0.30 ml. enzyme; Total volume 2.70 ml. 5 umoles $MgCl_2$ added when Ribose-5-phosphate was substrate Incubation temp: Room temp. (approx. 20°C), except 37°C when Ribose-5-phosphate was substrate

the adult heart preparations. Within the fetal group the data are too limited to indicate a definite correlation between size (i.e., age) of the fetus and pentose cycle activity.

It will be noted that the rate of TPN reduction with G-6-P, 6-PG, or F-6-P as substrate is many times greater than the rate with R-5-P. Thus the hexose phosphate isomerization and dehydrogenation steps are not rate-limiting in either the fetal or adult systems. The "bottleneck" must lie in one of the first four steps. That this "bottleneck" may lie at the isomerization and epimerization steps is suggested by Kelly et al. (56). The results obtained by Dickens and Williamson (25) also support this idea although they - apparently inadvertently - stated the opposite conclusion. Other work described in this thesis (Section B (5)) makes it seem likely that the transketolase and transaldolase are not the rate-limiting enzymes.

(2) Radiochemical Studies

Soluble preparations from adult and fetal pig heart together with added ATP and TPN have been found to produce $C^{14}O_2$ from various labeled substrates: Δ-gluconolactone-1-C^{14}; glucose-1-C^{14}; glucose-2-C^{14}; glucose-6-C^{14}; ribose-1-C^{14}.

TABLE IV

Cofactor Requirement for Gluconate Oxidation by Adult Pig Heart Soluble System (Dialyzed)

$C^{14}O_2$ Recovery (cpm) from Gluconolactone-1-C^{14}				
no added cofactors	TPN + DPN	ATP	ATP + TPN	ATP + TPN + DPN
181	769	1177	5429	6301

Components of system:
- 460 µmoles KCl
- 20 µmoles Phosphate Buffer pH 7.4
- 200 µmoles Nicotinamide
- 10 µmoles $MgCl_2$
- 0.14 µmole cytochrome c
- 0.30 µc. Gluconolactone-1-C^{14}
- 2.0 ml. dialyzed enzyme

Cofactors where indicated:
- 20 µmoles ATP
- 3 µmoles DPN
- 3 µmoles TPN

Final Total volume: 4.20 ml.

Temperature 37°C.

Production of $C^{14}O_2$ from Δ-gluconolactone-1-C^{14} was found to require both ATP and TPN (Table IV) and is simply evidence for the presence of a gluconokinase and 6-phosphogluconic dehydrogenase. Considerable purification of a specific hog kidney gluconokinase has recently been reported (63). Partial separation of the hexokinase and gluconokinase activities of pig heart was obtained. When saturated ammonium sulfate solution at pH 7.6 was used as precipitant, the bulk of the hexokinase activity was found at 0-50% saturation, while most of the gluconokinase activity lay in the 50-75% fraction. When solid ammonium sulfate was used, hexokinase came out in the 0-30% fraction, gluconokinase in the 30-50% fraction. Further purification of the pig heart gluconokinase was not attempted, but it is evident that the gluconokinase is not identical with hexokinase.

Production of labeled CO_2 from glucose-1-C^{14} bespeaks the presence of hexokinase and both dehydrogenases of the direct oxidative pathway.

In order to account for the recovery of label from ribose-1-C^{14} and glucose-2-C^{14}, it is necessary to postulate the presence of the entire pentose cycle schemes of enzymes. Only the kinases would be different for the two substrates. The labeled carbon atoms of the two substrates are homologous. Thus, the glucose-

TABLE V

Pyridine Nucleotide Requirement for $C^{14}O_2$ Production from Labeled Substrates by Fetal Pig Heart Soluble Preparations

Expt.	Length of Fetus	Total $C^{14}O_2$ Recovery (cpm)							
		Glucose-1-C^{14}		Glucose-2-C^{14}		Glucose-6-C^{14}		Ribose-1-C^{14}	
		-TPN	+TPN	-TPN	+TPN	-TPN	+TPN	-TPN	+TPN
24*	4"	264	8760	33	278	72	145		
25	3"			72	1797	77	142	87	1710
27	1-3/4"			107	2524	52	160	270	1368
29	5"			15	15500	14	32	420	5721
				-DPN	+DPN	-DPN	+DPN		
29	5"			15	17	14	27		

* 10 μmoles carrier glucose added.

Flask components: 460 μmoles KCl; 20 μmoles phosphate buffer, pH 7.4; 200 μmoles nicotinamide; 10 μmoles $MgCl_2$; 0.14 μmoles cytochrome c; 20 μmoles ATP; carrier as noted; 3 μmoles pyridine nucleotide as indicated; 0.30 μc. labeled substrate; 1 ml. enzyme (in phosphate buffer); Final volume: 3.0 ml; Incubation temperature: 37°C.

2-C^{14}, after an initial decarboxylation at C-1, becomes a C-1-labeled pentose which can enter the pentose cycle, leading back to C-1-labeled glucose and subsequent decarboxylation.

For glucose-6-C^{14} the situation appears to be more complex. One pathway would involve the dehydrogenases and transketolase, yielding a C-3-labeled triose phosphate. Then by way of triose phosphate isomerase, aldolase, and hexose diphosphatase, a hexose phosphate would be obtained with some labeling in C-1. The C-1 label would then be removed via the direct oxidative mechanism. It also seems possible that glycolytic breakdown to triose units and resynthesis of hexose phosphate by reversal of that route would give sufficient randomization to account for the recovery of label from carbon 6, which, in any case, was extremely low. The first alternative, i.e., via transketolase, may be the more likely route, however, since the presence of excess TPN would make rapid decarboxylation, rather than glycolysis, the probable fate of a newly-phosphorylated glucose molecule.

The TPN requirement for $C^{14}O_2$ production from these labeled substrates is evident from Table V. The total recovery of radioactivity, as $C^{14}O_2$, from ribose-1-C^{14} and glucose-2-C^{14} averaged 12% and 7%, respectively, for adult preparations; 28% and 26% for

FIGURE 5

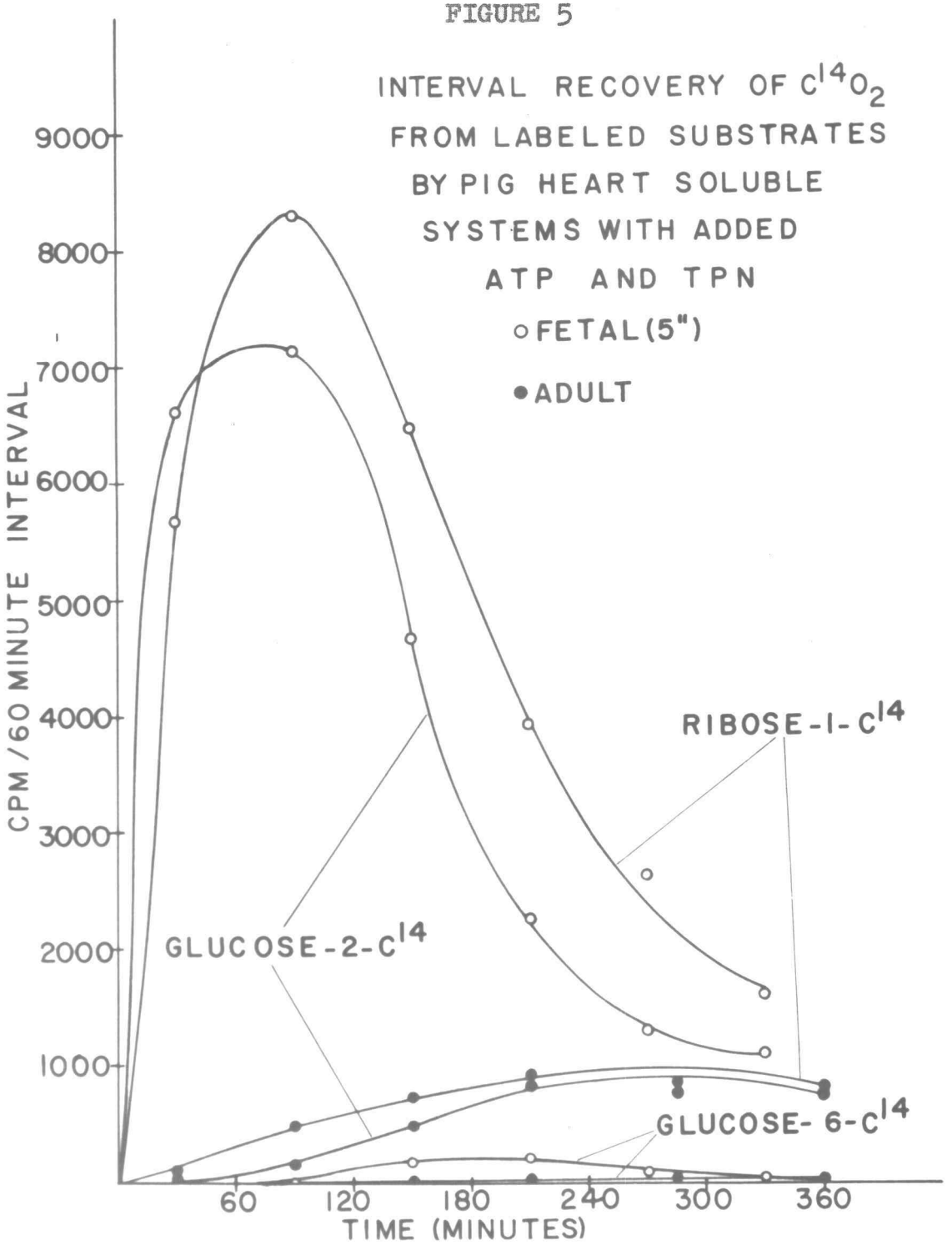

INTERVAL RECOVERY OF $C^{14}O_2$
FROM LABELED SUBSTRATES
BY PIG HEART SOLUBLE
SYSTEMS WITH ADDED
ATP AND TPN
○ FETAL (5")
● ADULT
CPM / 60 MINUTE INTERVAL
9000
8000
7000
6000
5000
4000
3000
2000
1000
RIBOSE-1-C^{14}
GLUCOSE-2-C^{14}
GLUCOSE-6-C^{14}
60
120
180
240
300
360
TIME (MINUTES)

TABLE VI

Recovery of $C^{14}O_2$ from Labeled Substrates by Soluble Pig Heart Preparations with Added ATP and TPN

Enzyme	Size	Carrier Glucose (G) or Ribose(R)	$C^{14}O_2$ Recovery from Indicated Substrate in cpm/mg. Protein: Ribose-1-C^{14}	Glucose-1-C^{14}	Glucose-2-C^{14}	Glucose-6-C^{14}
		µmoles				
24[1,2]	4"	10 G		8760	278	145
25[1,2]	3"	1 R; 1 G	450		473	37.4
27[1,2]	1-3/4"	0.5 G; 0.5 R	976		1800	114.0
28[1,2]	1"	0.4 R; 0.4 R	250		815	35.7
29[1,2]	5"	0.5 R; 0.5 G	1100		2980	6.2
34[1,2]	1-1/8"	0.5 G; 0.5 R	461		2320	45.0
58	3"	0	1170			
53	3-1/4"	0			271	
	1"	0			6310	
76	5"	0	2510		2030	50.5
89	4"	0	980		855	
	5"	0	915		796	
93F	4"	0			2660	
77	Adult	0	312		231	8.9
80	Adult	0	248		195	13.1
93A	Adult	0			73	

1) 20 µmoles ATP; 2) 3 µmoles TPN

Components of the system: 460 µmoles KCl; 20 µmoles phosphate buffer, pH 7.4; 200 µmoles nicotinamide; 10 µmoles $MgCl_2$; 0.14 µmoles cytochrome c; 30 µmoles ATP (except where noted); 4 µmoles TPN (except where noted); carrier as noted; 1-2 ml. enzyme; 0.30 µc. labeled substrate; Final volume, 3-4 ml; incubation temperature, 37°C.

fetal. Recovery from glucose-6-C^{14}, however, was quite low for all soluble preparations, averaging around 0.4%.

Figure 5 and Table VI show that $C^{14}O_2$ production from soluble systems is several times as great as that from the adult systems; the ratio of fetal to adult activities (in cpm/mg. protein) averaging about 5 for ribose-1-C^{14}, 10 for glucose-2-C^{14}, and 5 for glucose-6-C^{14}. These results generally corroborate those obtained in the spectrophotometric studies and it is clear that the fetal solubles possess at least 5 times as much pentose cycle activity as do the adult.

B. Studies with Pig Heart Homogenates

(1) Catabolism of glucose by adult heart homogenates.

In contrast to the relatively low level of pentose cycle enzymes in the adult soluble preparations, the level of overall oxidative ability in adult homogenates is very high. Manometric studies of the endogenous respiration in homogenates show (Fig. 6: Table VII), that the endogenous rate of oxygen consumption by adult pig heart homogenates is around 3 times that for fetal heart tissue when calculated as µl/min/mg. dry wt. Addition of exogenous glucose was found to have, if anything, a slight depressant effect on the oxygen consumption rate in homogenates, possibly by inhibition of phosphorylase. Only after a considerable time of

FIGURE 6

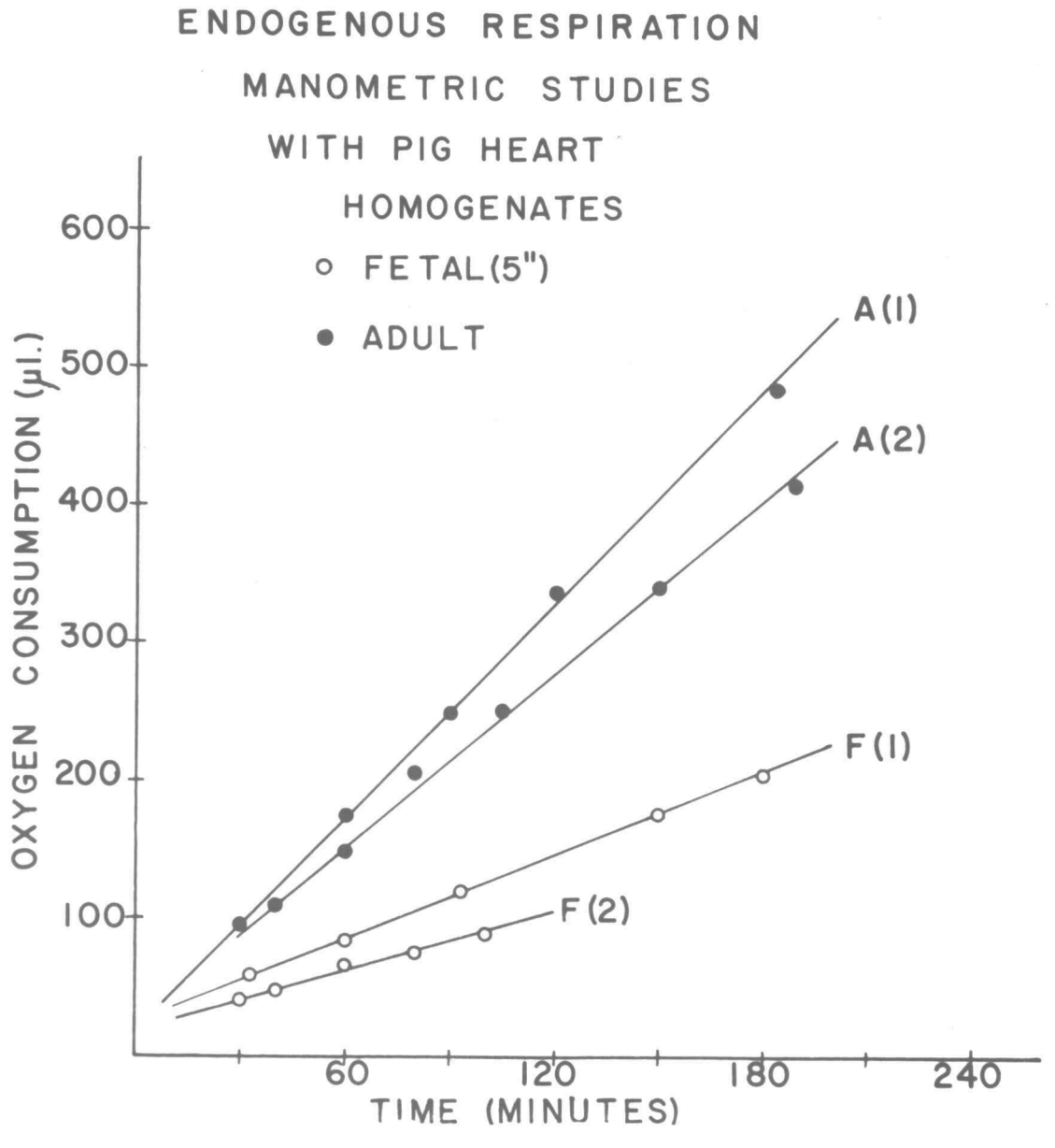

ENDOGENOUS RESPIRATION
MANOMETRIC STUDIES
WITH PIG HEART
HOMOGENATES
FETAL(5")
ADULT
A(1)
A(2)
F(1)
F(2)
OXYGEN CONSUMPTION (µl.)
600
500
400
300
200
100
60
120
180
240
TIME (MINUTES)

TABLE VII

Endogenous Respiration in Pig Heart Homogenates

Expt.	Size	O_2 Rate of Consumption µl/min.	Specific Rate of O_2 Consumption, µl/min/mg. dry wt.
A(1) (W35)	Adult	2.60	0.15
A(2) (W36)	Adult	2.12	0.13
F(1) (W25)	5" Fetus	1.02	0.049
F(2) (W27)	5" Fetus	0.73	0.035

Flask Components:
- 5 µmoles $MgCl_2$
- 230 µmoles KCl
- 10 µmoles phosphate buffer, pH 7.4
- 100 µmoles nicotinamide
- 0.07 µmoles cytochrome c
- 10 µmoles ATP
- 3.3 µmoles AMP (fetal Expts. only)
- 0.5 ml. Homogenate
- 0.2 ml. 20% KOH in center well

Final volume: 2.0 ml.
Incubation temp. 37°C.

incubation, when the endogenous substrate has been largely consumed, does exogenous glucose tend to maintain a high level of oxygen consumption.

Similarly, the rate of $C^{14}O_2$ production by adult heart homogenates from uniformly-labeled glucose is over three times that from fetal heart homogenates (Tables VIII and IX).

As can be seen from these tables and from Fig. 7, adult/fetal ratio of oxidation rates ($C^{14}O_2$ recovery) is around 2.5/1 for carbons 3 and 4, 3.5/1 for carbons 2 and 5, and over 5/1 for carbons 1 and 6. CO_2 from C-3,4 is derived from oxidative decarboxylation of pyruvate, while C-2 (and C-5), corresponding to the carboxyl of acetate, are evolved in a single turn of the TCA cycle, C-1 and C-6, corresponding to the methyl carbon of acetate, are evolved at a slower rate.

The peaks in the recovery curves for adult heart homogenate (Fig. 7) mark the onset of disappearance of substrate from the medium, followed by a rapid drop in recovery rates. The average total recovery of radioactivity, as $C^{14}O_2$, from labeled glucose (Fig. 8), acetate, and pyruvate was rather high (60-95%) for adult heart homogenates, and roughly one-third as high for fetal homogenates, ranging, in these, from 16% recovery from glucose-6-C^{14} to 76% recovery from glucose-U-C^{14}.

TABLE VIII

Rate of Production of $C^{14}O_2$ from Various Labeled Substrates by Adult Pig Heart Homogenate

Expt.	µmoles carrier or inhibitor	Rate of Recovery of $C^{14}O_2$ (cpm/hr/mg.dry wt.) Glucose-U-C^{14}	Glucose-3,4-C^{14} (calc.)	Glucose-2-C^{14}	Glucose-6-C^{14}	Acetate-1-C^{14}	Pyruvate-2-C^{14}
4[1]	50 µm glucose			38.3	25.1		
5[1]	20 µm glucose	62.5	109	67.8	43.3		
69	0	261					
69	10 µm glucose	154					
69	20 µm glucose	100					
91A	10 µm glucose	143	148	140	110		
67[2]	0			146			113.5
67[2]	40 µm Fluoroacetate			33.6			46.3
68[2]	0					580	85.5
68[2]	80 µm Fluoroacetate					108.8	47.2
70[2]	20 µm acetate 0					107.4	
70[2]	20 µm acetate 200 µm malonate					2.2	

1) 20 µmoles ATP added; 2) 40 µmoles ATP added
Flask components: same as in Table IX. Homogenates for Experiments 4 and 5 in phosphate buffer; all others in Tris buffer.

TABLE IX

Rate of Production of $C^{14}O_2$ from Various Labeled Substrates by Fetal Pig Heart Homogenates

Expt.	Size	µmoles carrier	Rate of Recovery of $C^{14}O_2$ (cpm/hr/mg.dry wt.) Glucose-U-C^{14}	Glucose-3,4-C^{14} *	Glucose-2-C^{14}	Glucose-6-C^{14}	Acetate-1-C^{14}	Pyruvate 2-C^{14}
6[2]	3½"	10 µm glucose	38.7	85.0	28.6	7.6		
8	2"	5 µm glucose			37.4	8.1		
90[3]	6"	20 µm acetate			43.3		26.1	35.1
90[1,3]	6"	20 µm acetate					1.04	
91F	8"	10 µm glucose	40.3	62.0	39	20.4		

* calculated values; 1) 150 µmoles malonate; 2) 20 µmoles ATP; 3) 40 µmoles ATP

Flask components: 460 µmoles KCl; 20 µmoles phosphate buffer, pH 7.4; 200 µmoles nicotinamide; 10 µmoles $MgCl_2$; 0.14 µmoles cytochrome c; ATP (10 µmoles except as noted); carrier and inhibitors as noted; Homogenate, 1-2 ml; labeled substrate 0.3 µc. Final volume, 3-5 ml; Incubation temperature 37°C.

FIGURE 7

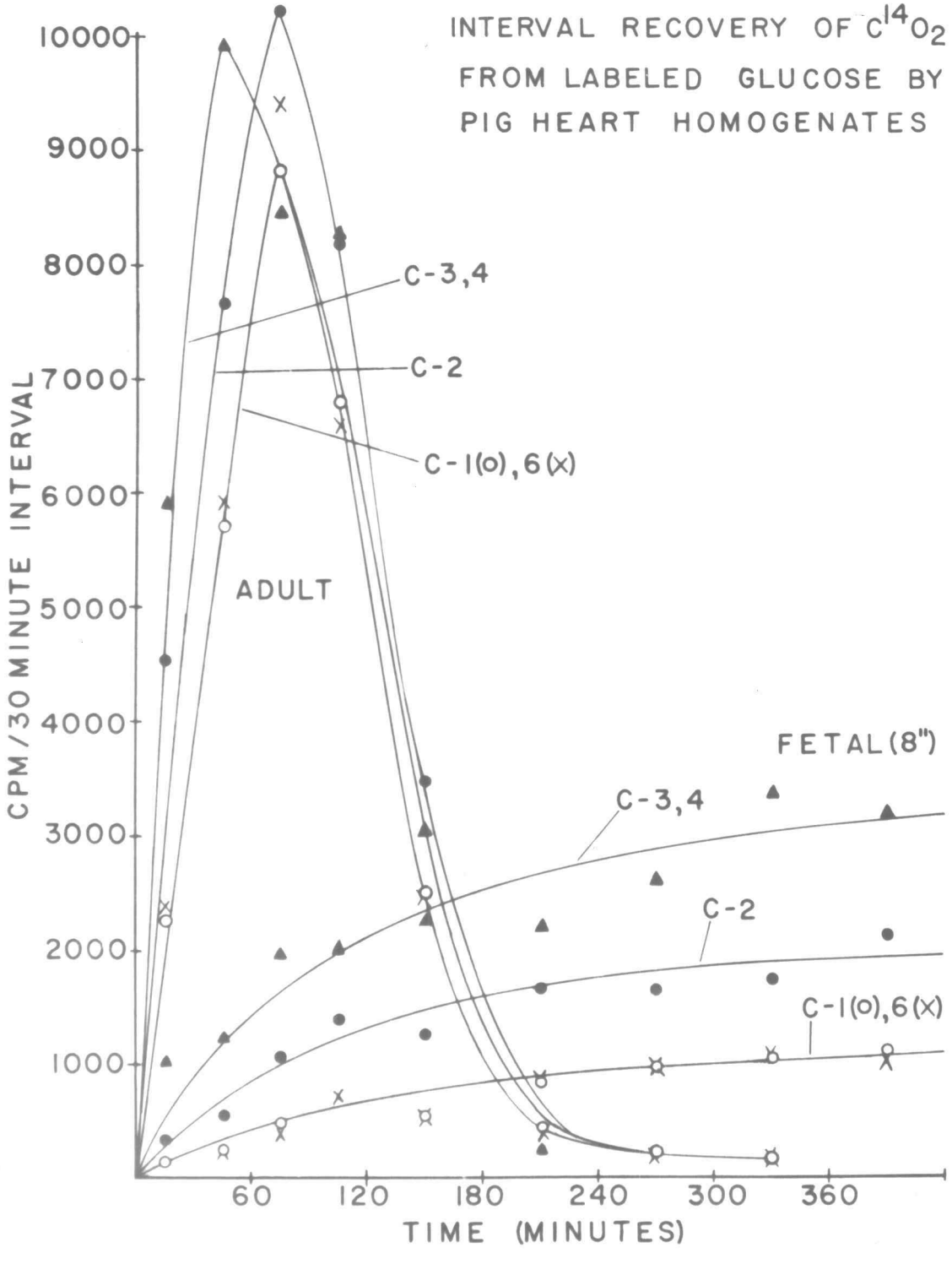
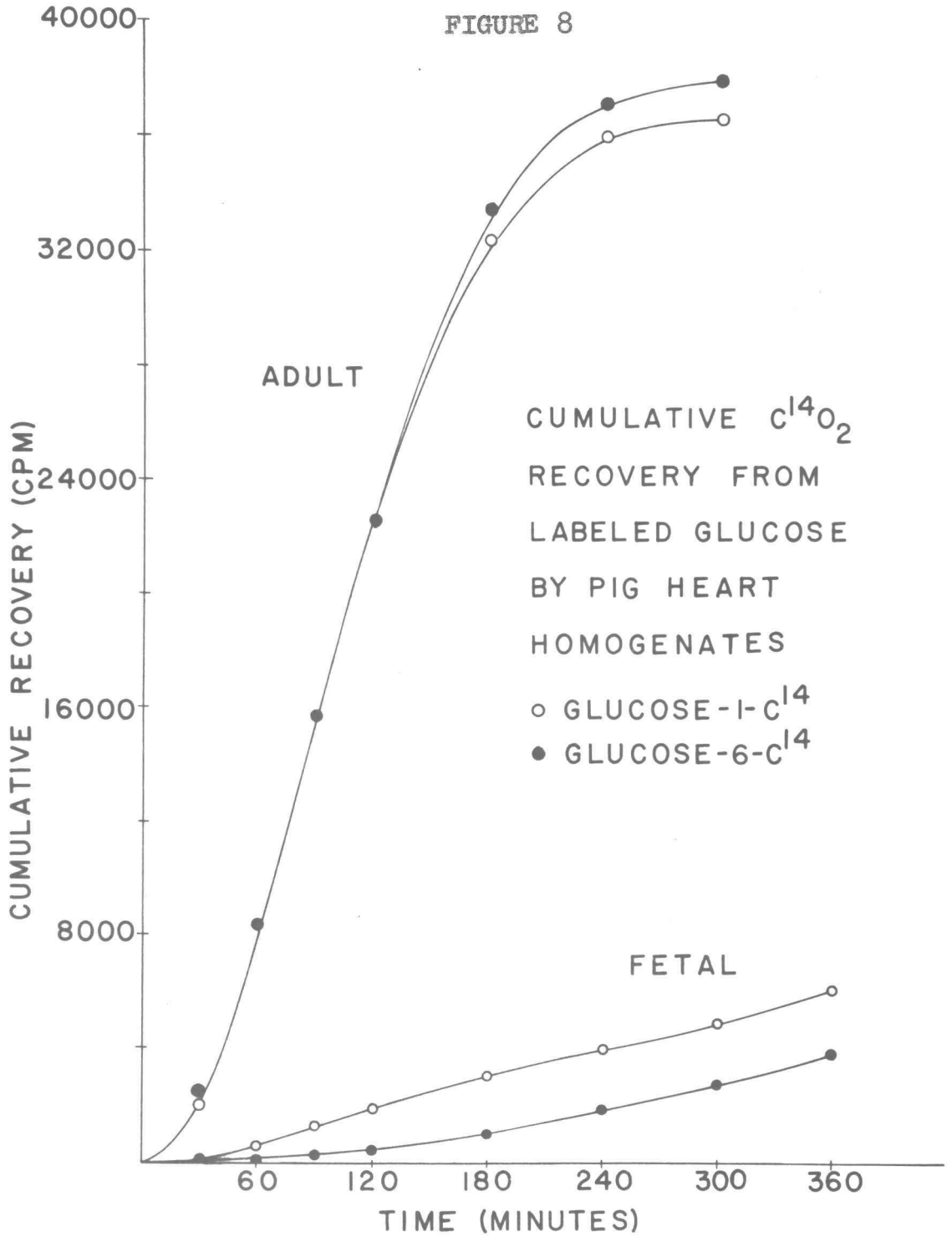

INTERVAL RECOVERY OF $C^{14}O_2$ FROM LABELED GLUCOSE BY PIG HEART HOMOGENATES
CPM/30 MINUTE INTERVAL
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
C-3,4
C-2
C-1(o),6(x)
ADULT
FETAL(8")
C-3,4
C-2
C-1(o),6(x)
60
120
180
240
300
360
TIME (MINUTES)

FIGURE 8


40000
32000
24000
16000
8000
CUMULATIVE RECOVERY (CPM)
ADULT
FETAL
CUMULATIVE $C^{14}O_2$ RECOVERY FROM LABELED GLUCOSE BY PIG HEART HOMOGENATES
○ GLUCOSE-1-C^{14}
● GLUCOSE-6-C^{14}
60
120
180
240
300
360
TIME (MINUTES)

Corroborative information is yielded by other experiments measuring rates of oxidation of acetate-1-C^{14}, pyruvate-2-C^{14} and glucose-2-C^{14}, in all three of which the labeled carbon atom is homologous as far as Krebs cycle oxidation is concerned. The rate of evolution of $C^{14}O_2$ from each of these three substrates (Tables VIII and IX) is roughly 3 to 4 times as great in the adult as in the fetal homogenate.

The limited oxidative ability of fetal homogenates thus can probably be attributed to lower mitochondrial activity, and, in particular, a lower Krebs cycle activity. It seems doubtful whether, with the gentle homogenization methods employed, there was any appreciable damaging of the fetal heart mitochondria. Nevertheless, the extent of cell rupture, with the resulting increased disorganization, was much higher in the fetal homogenates than in the adult homogenates, and the possibility of mitochondrial damage cannot be excluded.

Well-known inhibitors have the expected effect on Krebs cycle oxidation (Tables VIII and IX). Malonate at high concentrations abolishes acetate-1-C^{14} oxidation in both fetal and adult preparations. Fluoroacetate at 80 micromoles per flask cuts acetate-1-C^{14} oxidation to 1/5 but pyruvate-2-C^{14} oxidation only to 1/2 of control values. The effect on acetate oxidation is probably

more severe because of the direct competition of fluoroacetate with acetate for a site on the acetate-activating enzyme, though it has been suggested that fluoroacetate and acetate are not activated by the same enzyme, Peters (74).

Experiments were also carried out to determine the relative rates of $C^{14}O_2$ formation from carbons 1 and 6 of glucose (Table X) (Fig. 9). The average C-1/C-6 ratio for 15 experiments with adult homogenates in phosphate buffer was 1.04; that for 7 experiments with adult homogenates in Tris buffer was 1.00. This would indicate that practically all glucose catabolized by adult heart homogenates follows the Embden-Meyerhof and TCA cycle pathways, though a very small fraction does appear to follow an alternate pathway leading to decarboxylation at carbon-1, presumably the phosphogluconate oxidation pathway.

Studies with some common inhibitors showed that inhibition of glycolysis or the Krebs cycle, while greatly decreasing the absolute amounts of C-1 and C-6 oxidation, nevertheless increased the C-1/C-6 ratio (Table X). This effect was quite marked with iodoacetate (83), less so with fluoride and malonate. A possible explanation of this effect would be that these inhibitors cause an accumulation of substrate at the initial stages of glycolysis, resulting in a higher than

TABLE X

Rate of Production of $C^{14}O_2$ from Glucose-6-C^{14} by Adult Pig Heart Homogenates and Corresponding C-1/C-6 Ratios*

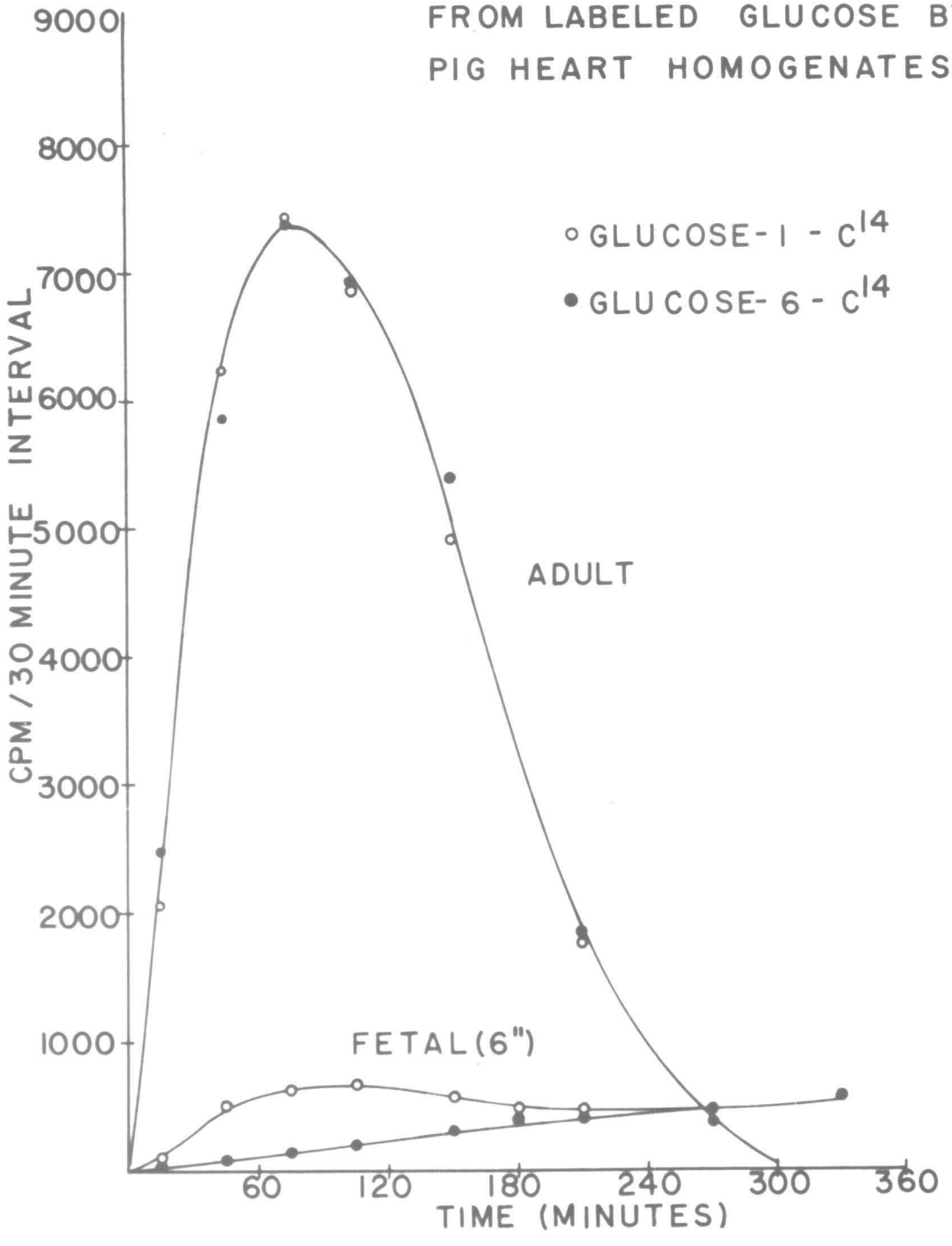
Expt. No.	Buffer	µmoles carrier glucose present	Rate of C-6 oxidation cpm/hr/mg dry wt.	C-1/C-6*
33	phosphate	0	104	1.09
36	"	0	85	1.01
39	"	0	80	1.04
7	"	10	127	1.08
10	"	10	50	1.09
12	"	10	88	1.02
20	"	10	47	1.04
61	"	10	128	0.91
62	"	10	56	1.00
64	"	10	18	1.15
73	"	10	112	0.91
5	"	20	43	1.03
2	"	50	15	1.15
4	"	50	25	0.99
49[1]	"	0	36	1.05
49[2]	"	0	37	1.03
103	"	10	2.5	2.56
124	"	10	46	1.08
615	"	10	3.6	0.59
626	"	10	48	0.88
72	Tris	10	60	1.05
73	"	10	118	1.02
75	"	10	136	1.05
79	"	10	109	0.94
83	"	10	109	1.00
79	"	50	30	1.01
727	"	10	6.1	1.05
758	"	10	3.5	1.10

* $\frac{C^{14}O_2 \text{ from glucose-1-}C^{14}}{C^{14}O_2 \text{ from glucose-6-}C^{14}}$

1) pH 6.4; 2) pH 7.1; 3) 10 µmoles Iodoacetate;
4) 20 µmoles Fructose-6-phosphate; 5) 40 µmoles Fluoride;
6) 60 µmoles Phosphate; 7) 40 µmoles Fluoride;
8) 200 µmoles Malonate
Components of the system: As in Table IX

FIGURE 9

INTERVAL RECOVERY OF $C^{14}O_2$ FROM LABELED GLUCOSE BY PIG HEART HOMOGENATES



normal concentration of glucose-6-phosphate. Thus the amount of glucose-6-phosphate diverted to the direct oxidative pathway would be increased. A simpler assumption is that the proportion going to the alternate pathway remains unchanged while that going via the EM-TCA cycle pathway is greatly reduced.

Addition of excess phosphate, raising the phosphate concentration from 0.01M to 0.025M, resulted in a decrease in C-1/C-6 from 1.00 to 0.88. This preferential oxidation of C-6 appears to be due to the phosphate-engendered inhibition of triose phosphate isomerase (10). Such an inhibition would prevent the dihydroxyacetone-phosphate moiety, containing the original carbon-1 of glucose, from being metabolized as rapidly as the glyceraldehyde-phosphate moiety.

(2) Catabolism of glucose by fetal pig heart homogenates.

The average value of C-1/C-6 for 12 experiments with fetal heart homogenates in phosphate buffer was 1.21. Eight experiments with fetal homogenates in Tris buffer gave an average of 1.25 (Table XI; Figs. 9 and 10). All the hearts in these two groups came from fetuses 6-1/2" or less in length (up to two thirds of gestation time). Hearts from large fetuses gave values more nearly resembling the adult. Thus, four 8" (approximately 90% of gestation time) fetus heart preparations gave

TABLE XI

Rate of Production of $C^{14}O_2$ from Glucose-6-C^{14} by Fetal Pig Heart Homogenates and Corresponding C-1/C-6 Ratios*

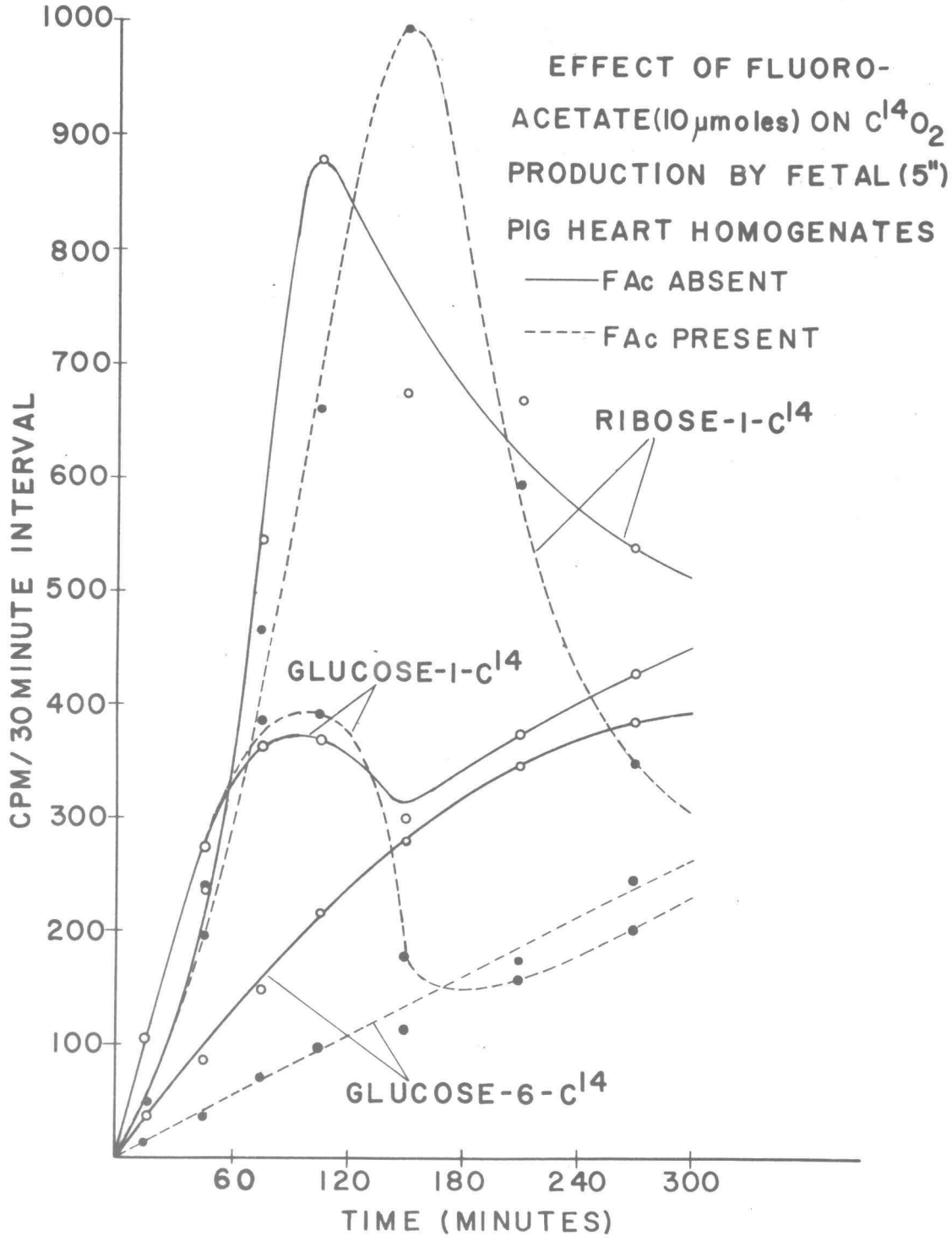
Expt. No.	Buffer	Length of Fetus	µmoles carrier glucose present	Rate of C-6 oxidation cpm/ hr/mg.dry wt.	C-1/C-6*
38	phosphate	6"	0	10.6	1.46
40	"	4-1/4"	0	19.5	0.79
48[1]	"	6-1/2"	0	10.2	1.01
48[2]	"	6-1/2"	0	6.7	1.26
30	"	3-1/2"	1	11.4	1.20
32	"	3-3/4"	1	7.4	1.11
8	"	2"	5	8.1	1.77
14	"	3-1/4"	5	20.8	1.05
16	"	5"	5	5.2	1.22
6	"	3-1/2"	10	7.6	1.46
60	"	3"	10	13.8	1.20
63	"	5"	10	12.0	1.07
65	"	6-1/2"	10	7.4	1.21
633	"	5"	10	1.1	1.95
65	Tris	6-1/2"	10	9.6	1.60
71	"	3-1/4"	10	12.2	1.17
74	"	5"	10	8.0	1.32
78	"	3"	10	18.1	1.14
84	"	1"	10	16.5	1.33
85	"	2"	10	11.1	1.11
86	"	5-1/2"	10	20.3	1.09
88	"	2-3/4"	10	15.0	1.27
744	"	5"	10	4.1	1.71
885	"	2-3/4"	10	16.1	1.40
81	"	8"	10	40.3	0.95
82	"	8"	10	20.2	0.99
87	"	8"	10	24.1	0.98
91F	"	8"	10	20.4	0.97
31	Excess phosphate	1"	1	-	0.61
35	"	1"	1	-	0.95
55	"	2"	0	-	0.98
57	"	1-3/4"	0	-	0.98
59	"	7"	0	37.2	0.93
60	"	1"	10	-	1.02

* $\frac{C^{14}O_2 \text{ from glucose-1-}C^{14}}{C^{14}O_2 \text{ from glucose-6-}C^{14}}$

1) pH 6.8; 2) pH 7.4; 3) 40 µmoles Fluoride; 4) 10 µmoles Fluoroacetate; 5) 50µg. L-Thyroxine

Components of the system as in Table IX.

FIGURE 10


EFFECT OF FLUORO-
ACETATE(10 μmoles) ON $C^{14}O_2$
PRODUCTION BY FETAL (5")
PIG HEART HOMOGENATES
FAc ABSENT
FAc PRESENT
RIBOSE-1-C^{14}
GLUCOSE-1-C^{14}
GLUCOSE-6-C^{14}
CPM/30 MINUTE INTERVAL
1000
900
800
700
600
500
400
300
200
100
60
120
180
240
300
TIME (MINUTES)

an average C-1/C-6 of 0.97.

The effect of high phosphate concentrations in favoring the oxidation of C-6 (by inhibition of triose phosphate isomerase (10) is shown by six preparations in which the ratio of phosphate concentration to enzyme concentration was great. Here the average C-1/C-6 ratio was 0.86. Another point for possible phosphate inhibition in these cases might be the glucose-6-phosphate dehydrogenation step. High phosphate concentration also inhibited ribose utilization, possibly by its effect on pentose phosphate isomerase (7).

Inhibitions of the EM -TCA pathway show a pronounced effect on the C-1/C-6 ratio (Table XI). Fluoride increases it from 1.07 to 1.95; fluoroacetate causes an increase from 1.32 to 1.71.

These results indicate that the direct oxidative pathway is definitely operative in the fetal heart homogenates, particularly in the younger fetuses, i.e., shorter than 8" (average length at parturition is approximately 10-1/2"). In any developing fetus there is presumably a continuous transition towards the glucose catabolism characteristic of the adult state, but individual differences from one litter to another will make this change clear only if a large number of litters are tested. A perusal of the limited data on fetal material (Table XI) shows that only at the

8" size level does the trend toward adult patterns become evident; below this size no correlation between fetal size and C-1/C-6 ratio is discernible, so all these data are averaged simply as "fetal". These observations with fetal heart are in line with those obtained by Glock and McLean (34) with whole rat fetuses. They estimated the pentose cycle activity of 10 day and 20 day fetuses, finding that the activity of the former was high, resembling that of adrenal tissue, while the older fetuses were less than half as active.

It is obvious that in fetal, as well as adult heart, the predominant pathway for glucose oxidation is the EM-TCA pathway. However, it does not seem that one would be justified in using these data from homogenates to calculate numerical values for the relative amounts of glucose following each pathway, or in assuming that such values would validly apply to intact heart. Fig. 10 clearly shows that we are not dealing with a steady state. Many of the experiments with fetal homogenates were characterized by an initial large excess of $C^{14}O_2$ from carbon 1, following which the C-1 rate dropped to the level of the C-6 rate and continued at or near that level for the remainder of the experiment. The initial peak of the C-1 rate was generally reached during the

second hour of incubation, and the C-1 rate had, in most cases, fallen to the C-6 rate by the end of three hours.

In the experiments with fetal homogenates, substrate was never limiting. One must then consider the enzymes, and specifically, the dehydrogenases of the direct oxidative pathway. It was found that incubation of an adult heart soluble preparation for three hours at 37°C. in a shaking Warburg flask caused a loss in activity of around 5%. If, then, it can be supposed that the loss of enzyme activity is not responsible for the drop in rate of C-1 evolution, the most likely assumption is that the drop is due to exhaustion of the coenzyme supply; in this case, the oxidized form of TPN. If there were no mechanism for reoxidation of the TPNH, the drop would be abrupt as in Fig. 10. If such a mechanism existed, there would be a constant renewal of the TPN supply, though perhaps not sufficient to replace the initial losses. This would cause a more gradual drop from the peak, or no drop at all. Such an effect was observed in many cases (Fig. 9) and in some of these cases, the C-1 rate remained somewhat higher than the C-6 rate throughout the experiment. Thus there may be some reoxidation of TPNH in these homogenates, although one might expect that mechanisms for such reoxidation would be rather sensitive to homogenization.

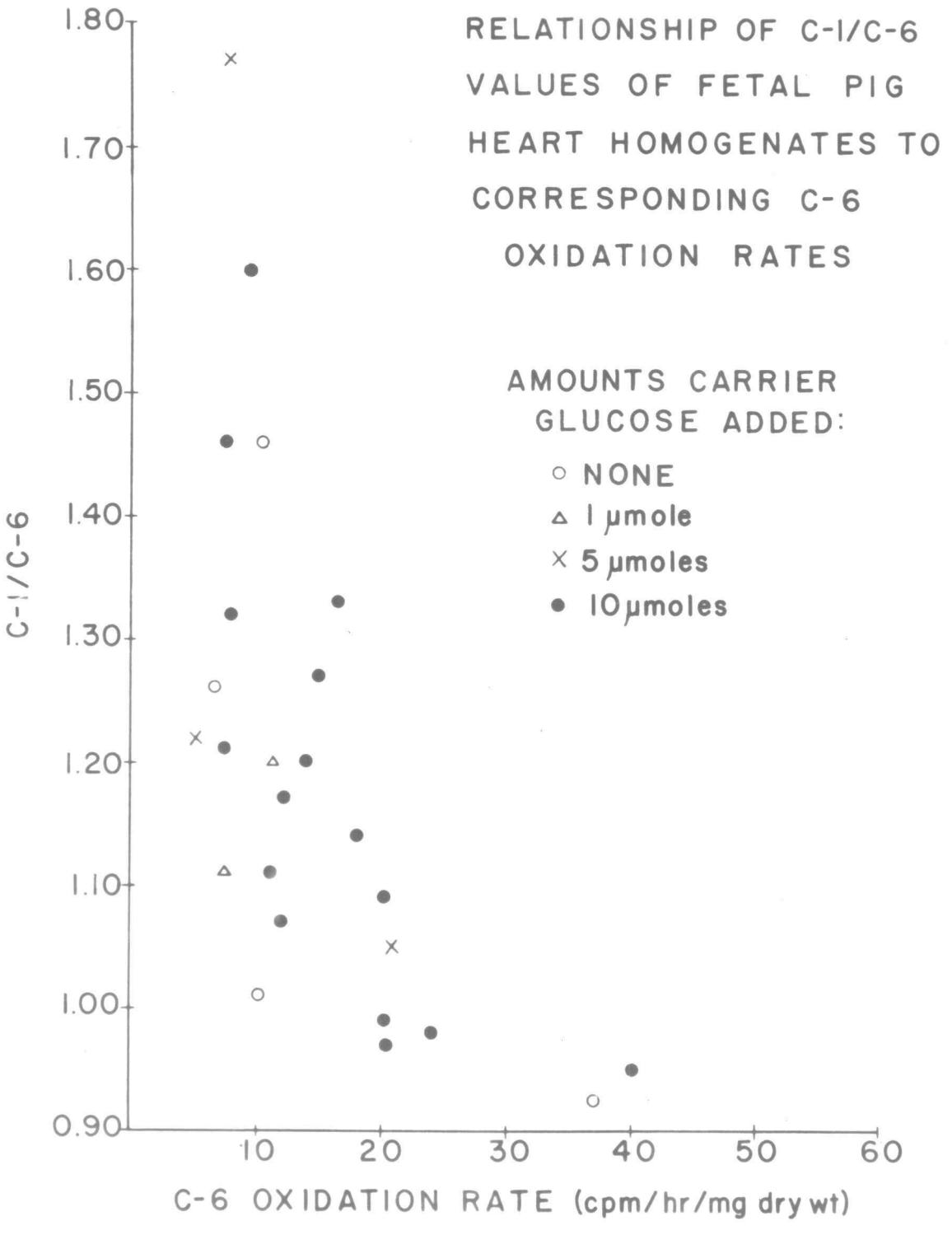
The pH used in both fetal and adult homogenate

studies was pH 7.0±0.1. This appeared to be an optimum pH for mitochondrial activity, thus giving the maximum recovery of $C^{14}O_2$. On the other hand, the pH optimum of the pentose cycle enzymes are such that the overall optimum for that system lies between 7.4 and 7.6. The use of pH 7 thus will not allow the direct oxidative mechanism to operate at maximum activity. Since, however, this limitation applies to all the homogenate studies, there remains a basis for comparison of fetal and adult tissue, if not for calculation of numerical values for relative contributions of the two pathways.

The capacity of fetal homogenates to oxidize carbon 6 of glucose can be used as a measure of glucose-oxidizing capacity. These values, expressed as cpm/hour/mg. dry wt., range from about 7 to 40 with an overall mean at 13 for homogenates in phosphate buffer (final phosphate concentration, approx. 0.01M), and 18 for homogenates in Tris buffer (approx. 0.005M phosphate) (Table XI). The respective averages for adult homogenates are 78 and 107, thus six times as great as the values for fetal homogenates (Table X).

There appears to be some correlation (negative) between the carbon 6 oxidizing capacity of fetal heart homogenates and the corresponding C-1/C-6 ratios. A plot of these values for the various fetal heart

FIGURE 11


RELATIONSHIP OF C-1/C-6 VALUES OF FETAL PIG HEART HOMOGENATES TO CORRESPONDING C-6 OXIDATION RATES
AMOUNTS CARRIER GLUCOSE ADDED:
○ NONE
△ 1 µmole
× 5 µmoles
● 10 µmoles
C-1/C-6
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
10
20
30
40
50
60
C-6 OXIDATION RATE (cpm/hr/mg dry wt)

preparation (Fig. 11) would lead one to suspect that the higher C-1/C-6 ratios are associated with lower overall oxidative capacities, and that as the oxidative capacity (via the EM -TCA pathway) rises toward the adult level, the C-1/C-6 ratio drops toward the characteristic adult level.

(3) Pyridine nucleotide effects on glucose catabolism.

Wenner and Weinhouse (88) have described the effect of added TPN in shifting the pattern of glucose oxidation in liver homogenates in favor of the direct oxidative pathway. Similarly, in heart, experiments have shown that the C-1/C-6 ratios can be changed by addition of pyridine nucleotides, especially TPN (Table XII). In an adult homogenate the addition of DPN caused an increase from 1.00 to 2.29. In a fetal homogenate, DPN increased the ratio from 1.09 to 1.47, while TPN increased it from 1.09 to 14.24. The effect is thus much more marked in the fetal heart. The DPN effect in the fetal preparation is probably due to the presence of a transhydrogenase which quickly reoxidizes reduced TPN at the expense of the added DPN. The presence of a transhydrogenase in adult pig heart was demonstrated, and has been previously reported by other workers (48, 52).

It is likely that the TPN effect represents chiefly an activation of the two TPN-requiring dehydrogenases

TABLE XII

Effect of Pyridine Nucleotides on the C-1/C-6 Ratio* in Pig Heart Homogenates

		C-1/C-6		
Expt.	Size	No Pyridine Nucleotides	DPN	TPN
CW 83	Adult	1.00	1.02	2.29
CW 86	Fetal (5½")	1.09	1.47	14.24

* C-1/C-6 $= \frac{\text{Rate of Production of } C^{14}O_2 \text{ from glucose-1-}C^{14}}{\text{Rate of Production of } C^{14}O_2 \text{ from glucose-6-}C^{14}}$

Flask Components:
- 460 μmoles KCl
- 20 μmoles phosphate buffer, pH 7.4
- 200 μmoles nicotinamide
- 10 μmoles $MgCl_2$
- 0.14 μmoles cytochrome c
- 10 μmoles ATP (K salt)
- 10 μmoles glucose
- 3 μmoles pyridine nucleotide
- Homogenate (Tris), 2 ml.
- Labeled substrate, 0.30 μc.
- Final volume 4.15 ml.
- Incubation temp. 37°C.

of the direct oxidative pathway, these enzymes being 3 to 4 times as active in the fetal heart as in the adult. It must be recalled, however, that in the preparations of the homogenates, the extent of cell rupture in the adult preparations was small relative to that in the fetal homogenates, thus cell permeability to TPN may have been a factor in the great difference of response to TPN.

Brin and Yonemoto (21) recently reported the stimulating effect of Methylene Blue on the direct oxidative pathway in erythrocytes. They accounted for this in terms of the electron-accepting properties of the dye, which presumably acts by reoxidizing reduced TPN. Wenner and Weinhouse (88) suggested that coenzyme concentrations might have a decisive influence on patterns of glucose oxidation. Cahill et al. (22) similarly concluded that the rate of oxidation of TPNH limits and controls the direct oxidative pathway in liver. Other than direct mitochondrial reoxidation of TPNH (51), oxidation by way of TPNH-cytochrome c reductase, or mediation of TPNH oxidation through transhydrogenase, the latter being perhaps the most likely route in heart, certain cyclic mechanisms have been suggested whereby TPNH formed in direct hexose monophosphate oxidation can be utilized (and thereby reoxidized) for synthesis in other systems. Requirements

for TPNH in liver glutathione reductase (76), an enzymic deamination (8), and conversion of phenylalanine to tyrosine in liver and adrenal (55) have been established. In adrenal tissue, furthermore, which has a very active pentose cycle activity (56), it has been found (61) that TPNH is required in adrenal steroid synthesis. One could speculate that a similar cyclic mechanism for TPNH oxidation might also exist in mammary gland.

Glock and McLean (35) determined the pyridine nucleotide concentrations in a number of different rat tissues. In all organs the total DPN was at least three times the total TPN. Most of the DPN was in the oxidized form; most of the TPN was in the reduced form. Only in adrenal gland was there a considerable amount of oxidized TPN. Total TPN of heart was rather low - about 1/16 of the total DPN. The same workers (38) found the pyridine nucleotide levels in tumors to be lower than in normal tissues, TPN being especially low in tumors.

(4) Catabolism of Δ-gluconolactone-1-C^{14}

This substrate undergoes oxidation and decarboxylation in both adult and fetal heart tissue, but the fetal preparations are about 3-1/2 times as active as the adult preparations (Table XIII). Average total recovery of radioactivity was also much higher in the fetal preparations (ca. 100%) than in the adult (27%). As was

TABLE XIII

Rate of Production of $C^{14}O_2$ from Gluconolactone-1-C^{14} by Pig Heart Homogenates

Expt.	Size	Homogenization Buffer	µmoles carrier Glucose(G) or Gluconate (Gl)	Rate of oxidation of C-1 of Gluconate cpm/hr/mg. dry wt.
7[1]	Adult	phosphate	10 G	43.5
39	Adult	"	0.6 Gl	20.5
75[2]	Adult	Tris	0	109.0
			Average (Adult)	57.7
8	2" Fetus	phosphate	5 G	148.0
38	6"	"	0.6 Gl	183.0
40	4-1/4"	"	0.6 Gl	227.0
57	1-3/4"	"	0	234.0
74[2]	5"	Tris	0	183.0
			Average (Fetal)	195.0
14	3-1/4"	phosphate	5 Gl	49.3
14[5]	3-1/4"	"	5 Gl	52.8
16	5"	"	5 Gl	86.9
74[2,3]	5"	Tris	0	207.0
75[2,4]	Adult	"	0	34.4

1) 20 µmoles ATP added
2) 30 µmoles ATP added
3) 10 µmoles fluoroacetate added
4) 200 µmoles malonate added
5) vigorous homogenization

Components of system as in Table IX

noted earlier, 3-1/2 is also the ratio of activities of 6-PG dehydrogenase in fetal and adult soluble preparations. It seems likely that enzyme concentration rather than TPN concentration is the limiting factor here. If TPN is limiting, it is equally limiting in fetal and adult.

(5) Catabolism of Ribose-1-C^{14}

Ribose-1-C^{14} is actively metabolized by both adult and fetal homogenates (Tables XIV and XV). The rate of $C^{14}O_2$ evolution for adult heart averages about 50 cpm/hour/mg. dry wt. while the average rate for fetal heart homogenates is 19 cpm/hour/mg. dry wt.; the respective values for total recovery of radioactivity being 38% and 13%. Perhaps more significant is the ratio of the rate for ribose-1-C^{14} to that for glucose-6-C^{14}. The R1/G6 ratio is higher in the case of the fetal heart homogenates, where the ratio averages near 1.0, while it averages close to 0.5 for adult homogenates. In these homogenates the ribose probably follows a path (Fig. 12) involving phosphorylation and conversion to hexosemonophosphate via transketolase (with endogenous fructose-6-phosphate as donor substrate) and transaldolase (with endogenous triose phosphate as acceptor). From this point the bulk of the hexose monophosphate, labeled in position 3, follows the EM-TCA pathway which is particularly active in adult heart. In

TABLE XIV

Rate of Production of $C^{14}O_2$ from Ribose-1-C^{14} by Pig Heart Homogenates

Expt.	Homogenization Buffer	μmoles inhibitor or cofactor	Rate of oxidation of C-1 of Ribose, cpm/hr/mg.drywt.	Ratio of oxidation rates C-1 of Ribose / C-6 of Glucose
33	Phos.	0	34.0	0.33
46	"	0	25.1	
49	"	0	12.0	0.33
61	"	0	61.4	
64[2]	"	0	18.2	
73[1]	"	0	34.5	0.31
66[3]	"	0	38.7	
66[3]	Tris	0	51.3	
67[3]	"	0	41.4	
68[3]	"	0	33.1	
69	"	0	53.5	
70[3]	"	0	46.2	
72[1]	"	0	44.7	0.75
73[1]	"	0	47.1	0.40
75[2]	"	0	81.0	
94	"	0	81.8	
		Average (Adult)	44.0	0.42
46	Phos.	4 TPN	46.2	
94	Tris	4 TPN	96.4	
61	Phos.	40 Fluoride	4.4	
72[1]	Tris	40 Fluoride	5.9	
64[2]	Phos.	10 Fluoroacetate	16.7	
66[3]	"	40 Fluoroacetate	9.6	
66[3]	Tris	40 Fluoroacetate	12.7	
67[3]	"	40 Fluoroacetate	13.1	
68[3]	"	80 Fluoroacetate	6.2	
70[3]	"	200 Malonate	3.3	
75[2]	"	200 Malonate	7.6	

1) 10 μmoles carrier glucose; 2) 30 μmoles ATP;
3) 40 μmoles ATP

Components of system as in Table IX

TABLE XV

Rate of Production of $C^{14}O_2$ from Ribose-1-C^{14} by Fetal Pig Heart Homogenates

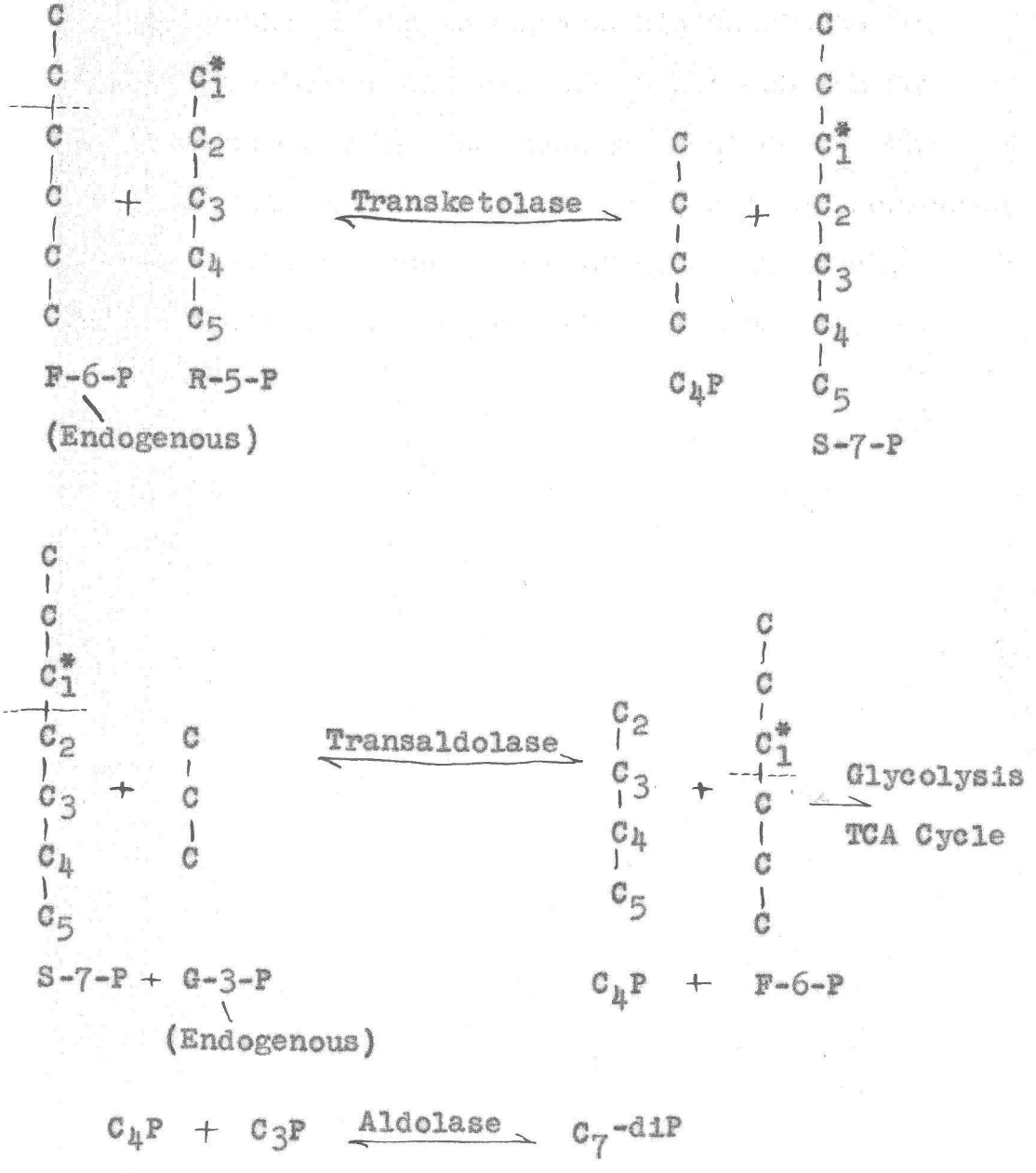
Expt.	Length of Fetus	Homogenization Buffer	µmoles inhibitor or cofactor	Rate of oxidation of C-1 of Ribose, cpm/hr/mg.dry wt.	Ratio of oxidation rates C-1 of Ribose / C-6 of Glucose
30	3-1/2"	Phosphate	0	7.8	0.68
38	6"	"	0	15.4	1.46
40	4-1/4"	"	0	12.7	0.65
63	5"	"	0	7.2	
65	6-1/2"	Tris	0	12.8	
74[1]	5"	"	0	13.5	
81	8"	"	0	17.5	
90[2]	6"	"	0	23.5	
92[2]	4"	"	0	27.7	
			Average (Fetal)	15.3	0.98
63	5"	Phosphate	40 µm. Fluoride	1.5	
74[1]	5"	Tris	10 µm. Fluoro-acetate	17.8	
90[2]	6"	Tris	150 µm. Malonate	12.7	
92[2]	4"	Tris	150 µm. Malonate	9.3	

1) 30 µmoles ATP; 2) 40 µmoles ATP

Components of the system as in Table IX

FIGURE 12

Suggested Pathway of Ribose-1-C^{14} Metabolism in Heart Homogenates



the experiments with soluble preparations, the added TPN held the fructose-6-phosphate at a low level (due to rapid decarboxylation at C-1) and thus prevented any extensive bypassing of these two steps. Hiatt (40) has suggested that, when a large amount of endogenous hexose is present (as in heart homogenates) fructose-6-phosphate of endogenous origin would be the expected 'active glycolaldehyde' donor in the transketolase reaction. He speculates that the tetrose-phosphate formed in these reactions might be further metabolized by condensation with dihydroxyacetone-phosphate to form sedoheptulose-1,7-diphosphate. This condensation, catalyzed by aldolase, has been reported (9, 45).

If ribose is metabolized by homogenates in the manner outlined in the foregoing paragraph, then the transketolase and transaldolase of adult as well as fetal heart must be quite active. This would make it unlikely that these two enzymes are rate-limiting in the pentose cycle activity of soluble heart preparations (Section A (1)).

The overwhelming importance of the EM-TCA pathway in ribose metabolism in the adult heart is emphasized by the severe effect of inhibitors of glycolysis and Krebs cycle on $C^{14}O_2$ evolution from ribose-1-C^{14} (Table XIV). Addition of TPN caused some increase in the rate of

$C^{14}O_2$ production. This result could be due to increased C-1 oxidation of 1,3-labeled glucose formed via the pentose cycle from the labeled pentose, or to an increase in the supply of xylulose-5-phosphate from endogenous hexose; the xylulose-5-phosphate acting as donor in formation of carbon 3-labeled hexose via transketolase and transaldolase.

The probable reason for the higher R-1/G-6 ratio in fetal homogenates is the more rapid conversion of labeled pentose to labeled hexose. Inhibitors such as malonate, fluoride, and fluoroacetate had markedly less effect on ribose oxidation in the fetal homogenates than in adult homogenates (Table XV). This is apparently due to the availability of the direct oxidative pathway which is not greatly affected by these inhibitors. It is difficult, however, to account for an increase in ribose oxidation in the presence of low fluoroacetate concentration, as shown in Fig. 7.

C. Heart Perfusion Studies

In studies with isolated perfused adult rat and fetal pig hearts, evidence has been gathered indicating a degree of support, at the level of the whole organ, for conclusions drawn from homogenate experiments.

(1) Pyridine nucleotide effects

The effect of pyridine nucleotides was examined with

adult rat hearts. When, with glucose-1-C^{14} as substrate, TPN was added to the perfusion medium of only one of a pair of hearts, the specific activity of the CO_2 collected from that heart was found to be about twice that from the heart without TPN (Table XVI).

To offset the effect of inherent differences in the hearts, three experiments were conducted in which TPN was added to one of the hearts midway during a 3-hour perfusion run (Table XVII). The ratio of specific activities of the two hearts was compared before and after TPN addition to the one. There was an average 75% increase in the ratio of specific activities in favor of the heart receiving TPN.

When glucose-6-C^{14} was the substrate, addition of TPN to the medium perfusing one of the hearts did not cause that heart to produce significantly more $C^{14}O_2$ than the other heart.

DPN appeared to have a small stimulating effect on $C^{14}O_2$ evolution from both Carbon 1 and Carbon 6 of glucose.

These effects of pyridine nucleotides seem to fall in line with results obtained with adult pig heart homogenates. They also imply that pyridine nucleotides, under the conditions of perfusion, are able to pass through the cell membrane, although other workers have

TABLE XVI

Effect of Pyridine Nucleotide on $C^{14}O_2$ Production from Labeled Glucose by Perfused Hearts

Expt. No.	Type of Heart	Labeled Substrate	Pyridine Nucleotide	Ratio*
P19	8" pig fetus	glucose-1-C^{14}	TPN	1.37
P20	Adult Rat	"	"	2.29
P26	Adult Rat	"	"	2.54
P21	Adult Rat	glucose-6-C^{14}	"	1.39
P22	Adult Rat	"	"	1.00
P23	Adult Rat	glucose-1-C^{14}	DPN	1.32
P24	Adult Rat	glucose-6-C^{14}	DPN	1.39

* Ratio $\frac{\text{Spec. Act. of } C^{14}O_2 \text{ from heart with pyridine nucleotide}}{\text{Spec. Act. of } C^{14}O_2 \text{ from heart without pyridine nucleotide}}$

Components per liter of medium: NaCl, 8.14 gm; KCl, 0.41 gm; $CaCl_2$, 0.33 gm; Nicotinamide, 9.7 mg; 0.1 M Phosphate Buffer, pH 7.2, 9.0 ml; 0.05 M Barbital Buffer, pH 7.2, 23 ml; no carrier glucose.
Concentration of pyridine nucleotide, 1.74×10^{-4} M.
Temperature, 37°C.

TABLE XVII

Effect of TPN on $C^{14}O_2$ Production from Glucose-1-C^{14} by Perfused Adult Rat Hearts

Expt. No.	a/b* Before (1)	a/b* After (2)	(2)/(1)
P 41	0.64	1.73	2.70
P 42	1.08	1.25	1.16
P 43	.77	1.13	1.47

* Ratio of specific activities of $C^{14}O_2$ from hearts a and b before (1) and after (2) TPN addition to Heart a.

Components per liter of medium: NaCl, 8.14 gm; KCl, 0.41 gm; Nicotinamide, 9.7 mg; phosphate buffer, pH 7.4, 9.0 ml; $MgSO_4 \cdot 7H_2O$, 0.34 gm; carrier glucose, 240 µmoles. Concentration of TPN, 1.74×10^{-4}M. Temperature, 37°C.

submitted evidence that this is not normally true for nucleotides (78).

(2) C-1/C-6 in perfused hearts

In experiments in which one of a pair of adult rat hearts was perfused with glucose-1-C^{14} and the other with glucose-6-C^{14}, equivocal results were obtained (Table XVIII). Two experiments indicated a preponderance of $C^{14}O_2$ from Carbon 1; two other experiments showed little if any excess of $C^{14}O_2$ from Carbon 1.

Perfusion of fetal pig hearts with labeled glucose yielded results of a more positive nature (Table XIX). Out of 8 pairs of fetal hearts perfused (each pair being litter mates), 7 pairs indicated a definite excess of $C^{14}O_2$ from Carbon 1 of glucose. Though statistically not conclusive*, these data lend strength to the idea of an operative hexose monophosphate oxidative pathway in intact fetal heart.

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* Determined at the 5% significance level.

TABLE XVIII

C_1/C_6 Ratios* from Perfused Adult Rat Hearts

Expt. No.	µmoles carrier glucose per ml. perfusion medium	C_1/C_6*
P16[1]	0	2.56
P17	0 L-thyroxine 3×10^{-5} M	3.35
P18	0	2.58
P44[2]	0.24	1.00
P45	0.24	1.20

* C-1/C-6 Ratio $= \frac{\text{Spec. Act. of } C^{14}O_2 \text{ from heart perfused with glucose-1-}C^{14}}{\text{Spec. Act. of } C^{14}O_2 \text{ from heart perfused with glucose-6-}C^{14}}$

1) Components of the system (P16,17,18): Same as Table XIV except 55 ml. 0.05 M Barbital Buffer, pH 7.2; no nicotinamide; no pyridine nucleotides.

2) Components of the system (P44,45): Same as Table XV except no pyridine nucleotides.

TABLE XIX

C_1/C_6 Ratios* from Perfused Fetal Pig Hearts

Ratios of specific activities of $C^{14}O_2$ from C_1 and C_6 of Glucose

Expt. No.	Size of Fetus	µmoles carrier glucose per ml. perfusion medium	C_1/C_6*
P13[1]	5"	0	3.95
P14	4"	0	1.79
P15	4"	0	4.76
		Average	3.50
P47[2]	4"	0.24	1.71
P48	4½"	0.24	0.81
P49	4"	0.24	1.39
P50	4½"	0.24	1.61
P51	4"	0.24	2.03
		Average	1.51

* C-1/C-6 Ratio $= \frac{\text{Spec. Act. of } C^{14}O_2 \text{ from heart perfused with glucose-1-}C^{14}}{\text{Spec. Act. of } C^{14}O_2 \text{ from heart perfused with glucose-6-}C^{14}}$

1) Components of the system (P13,14,15): Same as Table XIV except, 55 ml. 0.05 M Barbital Buffer, pH 7.2; no nicotinamide; no pyridine nucleotides.

2) Components of the system (P47-51): Same as Table XV except no pyridine nucleotides.

SUMMARY

(1) The rate of production of $C^{14}O_2$ from glucose-1, -2, -6, and -U-C^{14}, and pyruvate-2-C^{14} is roughly three to four times as high as that with adult pig heart homogenates than with fetal heart homogenates on a dry weight basis. Oxygen consumption follows a similar pattern.

(2) The rates of production of $C^{14}O_2$ from glucose-1-C^{14} and glucose-6-C^{14} are approximately equal in heart homogenates from adult pigs and late pig fetuses (i.e., in the last quarter of the gestation period), but with heart homogenates from earlier fetuses the rate of $C^{14}O_2$ production from glucose-1-C^{14} exceeds that from glucose-6-C^{14} by an average of 25%.

(3) Addition of TPN causes a 10-fold increase in the $C^{14}O_2$ production from glucose-1-C^{14} by fetal heart homogenates; a two-fold increase in that by adult heart homogenates.

(4) Both adult and fetal heart homogenates oxidize carbon-1 of ribose. The rate of oxidation was greater in adult homogenates, but the rate of oxidation of C-1 of ribose compared to that of C-6 of glucose was greater in fetal homogenates.

(5) The rate of $C^{14}O_2$ production from gluconolactone-1-C^{14} is three to four times as high in

fetal as in adult heart homogenates.

(6) Soluble enzyme fractions from heart can reduce TPN in the presence of glucose-6-phosphate, fructose-6-phosphate, 6-phosphogluconate, or ribose-5-phosphate. The rate of reduction by fetal preparations is over four times as great as that by adult preparations.

(7) Soluble enzyme fractions from heart are able, in the presence of ATP and TPN, to produce $C^{14}O_2$ from gluconolactone-1-C^{14}, glucose-1-C^{14}, ribose-1-C^{14}, and glucose-2-C^{14}. The activity of the fetal preparations is roughly four to five times as great as that of the adult preparations.

(8) Perfused fetal pig hearts have been found to produce $C^{14}O_2$ at a higher rate from glucose-1-C^{14} than from glucose-6-C^{14}.

(9) With perfused adult rat hearts, addition of TPN to the perfusion medium has been found to cause an increase in the oxidation of carbon-1 of glucose.

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