

Glucose Utilization by Chick Embryo Heart Homogenates

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ABSTRACT Homogenates of early chick embryos and homogenates of early chick embryonic hearts utilized the phosphogluconate pathway of glucose catabolism to a greater extent, relative to the glycolytic-Krebs cycle pathway, than did homogenates of hearts from older chick embryos or adult chicks. An abrupt drop in the relative participation by the phosphogluconate pathway in embryo heart homogenates occurs at about 5 to 7 days of incubation. Heart homogenates from adult chicks catabolize glucose almost entirely by the glycolytic-Krebs cycle pathway, with negligible participation by the phosphogluconate pathway.

INTRODUCTION

The relative contributions of the pentose phosphate pathway and the Embden-Meyerhof (glycolytic) pathway to the utilization of glucose have been investigated in a wide variety of organisms and tissues. When interpreting the relative contributions of the two pathways, it is important to consider the needs of the organism or tissue for metabolic products other than energy, CO_2 , and H_2O . In actively secreting and rapidly proliferating tissues, characterized by a high rate of protein synthesis, the content of ribonucleic acid and the activity of pentose cycle enzymes are high (1). It has been reasoned that such systems, requiring rapid formation of ribose and deoxyribose for nucleic acid synthesis, might utilize the more direct phosphogluconate oxidative route to pentose phosphate to a greater extent than the somewhat less direct, non-oxidative reactions of glycolysis and the pentose cycle (2).

The early chick embryo is a rapidly growing system in which the increase in protein and nucleic acid is exponential relative to time (3-5). Solomon (6) found that the RNA:DNA ratio was much higher in the chick embryo blastoderm and in the 10-somite stage than in most other tissues of the chick embryo at later stages. He concluded that the rapid rate of cell division and nucleic acid synthesis during embryogenesis during the first 3 or 4 days of incubation precluded a proportionately rapid increase in protein and wet weight of the embryo. In the chick embryo heart, which begins beating at

about the 36th hour of incubation, and which reaches its adult morphological form by the 8th day, the protein and DNA contents become constant at about the 8th day of incubation. However, Baker and Newburgh (7) found that the RNA:DNA ratio decreases continually from the 4th day of incubation to hatching. Consequently, one might expect that the relative importance of the phosphogluconate pathway to the utilization of glucose would be greater in the early embryo than in the older embryo or the adult chick. Such a trend might be particularly noticeable in heart tissue.

By comparing the yields of $C^{14}O_2$ resulting from the metabolic oxidation of glucose-1- C^{14} (G-1- C^{14}) and glucose-6- C^{14} (G-6- C^{14}), Krahl (8) concluded that the phosphogluconate pathway accounts for much more glucose oxidation than does the glycolytic-Krebs cycle pathway in early sea urchin embryos. Furthermore, the relative contribution of the phosphogluconate pathway decreases rapidly as the sea urchin embryo develops. Jolley *et al.* (2) obtained qualitatively similar results in the case of fetal pig heart, using similar methods. Needham and Lehmann (9) demonstrated that carbohydrate is the principal energy source for early chick embryonic development. Klein (10) confirmed this finding by assessing the $C^{14}O_2$ produced from embryos in intact eggs following replacement of the yolk or albumin by media containing C^{14} -labeled glucose, amino acids, fatty acids, or proteins.

The present work involved attempts to demonstrate a change in the relative contributions of the two pathways of glucose catabolism during the development of the chick embryo heart similar to the change noted by Krahl (8) in the sea urchin embryo and by Jolley *et al.* (2) in the mammalian fetal heart. The work entailed collection and measurement of radioactive CO_2 arising from C^{14} -labeled glucose added to homogenates of heart tissue of embryonic and adult chicks.

MATERIALS AND METHODS

Embryos were obtained from White Leghorn eggs, purchased from Hanson Leghorn Farm, Corvallis, Oregon. The eggs were incubated under conditions of controlled temperature and humidity in a commercial incubator. Adult chicken hearts were removed from Leghorn hens, directly after slaughtering at Mutual Produce Company, Corvallis, Oregon, or from 1- to 2-day-old chicks.

D-Glucose-1- C^{14} , D-glucose-6- C^{14} , and D-glucose-U- C^{14} (G-U- C^{14}) were purchased from New England Nuclear Corporation. D-Glucose-2- C^{14} (G-2- C^{14}) and Δ -gluconolactone-1- C^{14} were purchased from Volk Radiochemical Company. Adenosine triphosphate, diphosphopyridine nucleotide, and triphosphopyridine nucleotide were purchased from Sigma Chemical Corporation.

METHODS

Embryonic chick hearts were dissected and placed immediately in an ice cold homogenizing medium consisting of 0.25 M sucrose or 0.14 M KCl, 0.001 M disodium ver-

sene, and 0.006 M potassium phosphate, adjusted to pH 7.4 with KOH. Adult chicken hearts were kept in ice until minced with a razor blade at 0 to 5°C., then placed in the above homogenizing medium. Fat and connective tissue were discarded. Homogenization was performed with the Dounce glass homogenizer. Following homogenization, 1 ml of homogenate was added to 2.0 ml of a reaction medium which contained the following components: KCl, 0.14 M; MgCl₂, 0.003 M; disodium versenate, 0.001 M; cytochrome *c*, 3×10^{-5} M; ATP, 0.004 M; nicotinamide, 0.06 M; and potassium

TABLE I
EFFECTS OF ATP, DPN, AND
TPN ON GLUCOSE OXIDATION IN ADULT
HEART TISSUE HOMOGENATES

Substrate	Cofactor addition	O ₂ consumed	C ¹⁴ recovered as C ¹⁴ O ₂	G ₁ :G ₂
		<i>μliters</i>	<i>per cent</i>	
G-1-C ¹⁴	None	333±86*	1.5	1.30±0.38*
G-6-C ¹⁴	None		1.1	
G-1-C ¹⁴	DPN, 3 μ moles	1259±26	27.9	1.13±0.02
G-6-C ¹⁴	DPN, 3 μ moles		24.7	
G-6-C ¹⁴	TPN, 3 μ moles	1231±10	35.2	1.54±0.01
G-6-C ¹⁴	TPN, 3 μ moles		22.8	
G-1-C ¹⁴	ATP, 20 μ moles	1398±17	29.6	1.13±0.01
G-6-C ¹⁴	ATP, 20 μ moles		26.2	
G-1-C ¹⁴	DPN, 1.5 μ moles; ATP, 10 μ moles	1282±10	31.9	0.97±0.02
G-6-C ¹⁴	DPN, 1.5 μ moles; ATP, 10 μ moles		32.8	
G-1-C ¹⁴	DPN, 1.5 μ moles; TPN, 1.5 μ moles	1243±3	55.1	2.40±0.05
G-6-C ¹⁴	DPN, 1.5 μ moles; TPN, 1.5 μ moles		22.9	

The contents of each flask were the same as those described in Methods, with the exception of the cofactors, which are indicated.

Phosphate buffer, 30°C, 3 hours.

* Maximum range of values in different experiments.

phosphate or tris buffer, 0.02 M, pH 7.4. Two μ moles of C¹⁴-labeled glucose were added to each flask to initiate the reaction.

The radiorespirometric method described by Wang *et al.* (11) was used for collection of C¹⁴O₂ at half hourly or hourly intervals. In early experiments, C¹⁴O₂ was collected in 10 ml of 2 N CO₂-free NaOH, and counted as BaC¹⁴O₃ on aluminum planchets in a thin mica window Geiger-Müller counter. In later experiments, C¹⁴O₂ was collected in 15 ml. of 0.125 N ethanolic hyamine hydroxide. A 5 ml aliquot of each C¹⁴O₂ sample was added to 10 ml of a solution containing 6 gm PPO and 150 mg

POPOP per liter of toluene. Samples were counted in a Tracerlab liquid scintillation counter and corrected for minor quenching effects due to the ethanol and the hyamine.

RESULTS

A study of the effects of adding ATP, DPN, and TPN to the reaction mixture was performed in the hope of improving the oxidative capacity of the chick heart homogenates without altering the ratio of radiochemical yields of CO_2 from G-1- C^{14} and G-6- C^{14} . Data in Table I indicate that the addition of ATP, DPN, or TPN greatly increases the radiochemical yields of C^{14}O_2 and the

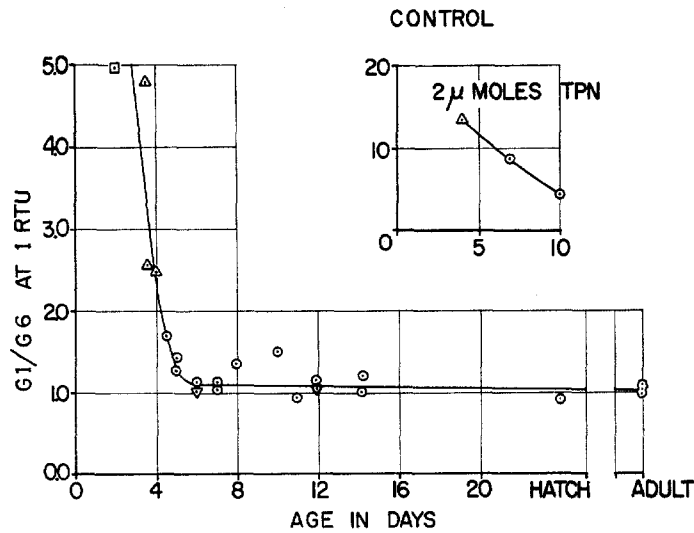


FIGURE 1. Ratio of C^{14}O_2 collected from glucose-1- ^{14}C and glucose-6- ^{14}C . Insert shows the effect of added TPN on this ratio. \circ = homogenate of heart, \square = homogenate of whole embryo, ∇ = intact heart, \triangle = homogenate of heart plus small amount of surrounding tissue.

oxidative capacity of the homogenate. The addition of TPN tended to increase the C^{14}O_2 yield from G-1- C^{14} more than that from G-6- C^{14} . This effect was even more marked in the presence of both TPN and DPN. Wenner and Weinhouse (12) estimated that 0.001 M TPN shifted the per cent participation of the pentose cycle in glucose catabolism from 47 to 65 per cent in rat liver preparations. Thus, TPN would be an undesirable stimulant of oxidation. DPN might be equally undesirable, in view of its selective requirement for glycolytic reactions and most of the Krebs cycle reactions, as opposed to its non-participation in the pentose cycle reactions. Thus, the choice of ATP, alone, to enhance the oxidation of glucose was made because it should have had little direct effect on the ratio of C^{14}O_2 evolved from G-1- C^{14} and G-6- C^{14} .

The yields of C^{14}O_2 arising from G-1- C^{14} and G-6- C^{14} metabolized by

embryonic and adult chick homogenates, whole embryo homogenates, and intact embryonic hearts are expressed as ratios in Fig. 1. The terms G_1 and G_6 indicate the radiochemical yields of $C^{14}O_2$ arising from G-1- C^{14} and G-6-

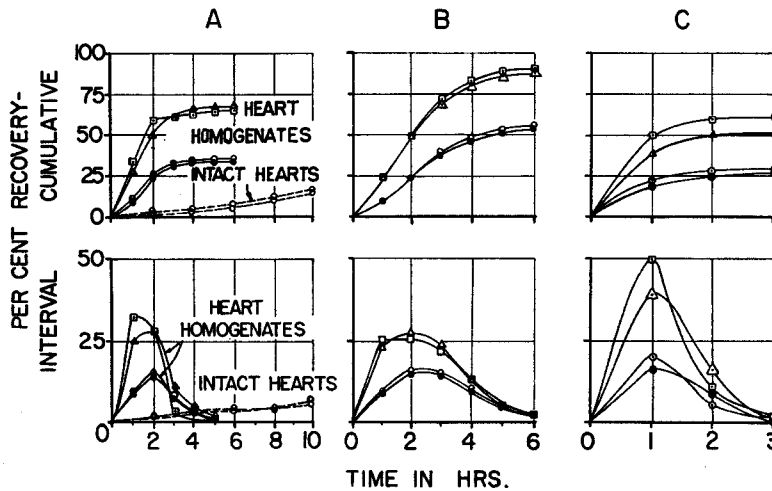


FIGURE 2. Per cent recovery in $C^{14}O_2$ from C^{14} -labeled glucose. A, 12 day embryo heart; B, 14 day embryo heart; C, adult heart. \circ = glucose-1- C^{14} , Δ = glucose-2- C^{14} , \bullet = glucose-6- C^{14} , \square = glucose-U- C^{14} .

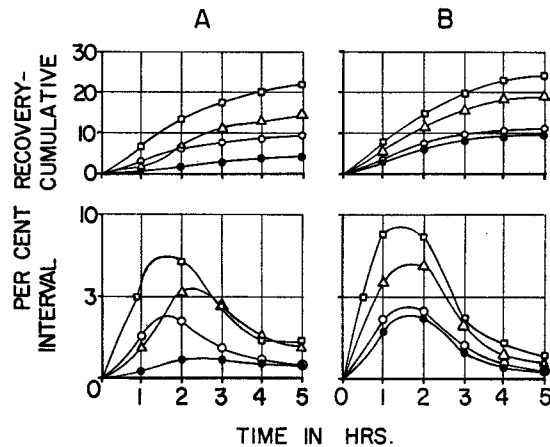


FIGURE 3. Per cent recovery in $C^{14}O_2$ from C^{14} -labeled glucose. A, 4 day whole embryo; B, 7 day embryo heart. Symbols same as in Fig. 2.

C^{14} , respectively. One relative time unit (1 RTU) has been defined by Chel-
delin, Wang, and King (13) as "the time required to complete the initial
catabolic reaction with respect to the administered substrate." The end of
the time unit is usually indicated by the rapid decrease in interval recovery of
 $C^{14}O_2$ from C_3 , C_4 , or C_2 of glucose (11). The value for 1 RTU in the ex-

periments reported in Fig. 1 varied from 2 to 5 hours. At the end of this time the $G_1:G_6$ ratio was much higher in the preparations from two- to four-day embryos than in older embryos or adults. The average $G_1:G_6$ ratio for adult hen heart homogenates was 1.03, while the ratio for embryonic hearts was significantly greater than unity in most cases. Ratios similar to those of the adult are seen in homogenates from embryos as early as 5 to 7 days, however, and suggest a changing pattern of glucose metabolism at about this time. The addition of TPN caused a marked increase in the $G_1:G_6$ ratio. This increase was less in the older embryo heart homogenates than in the younger ones. This increase was mainly due to a pronounced increase in the $C^{14}O_2$

TABLE II
 $C^{14}O_2$ YIELDS FROM Δ -GLUCONOLACTONE-1- C^{14} ,
 G-1- C^{14} , AND G-U- C^{14} METABOLIZED BY CHICK
 EMBRYO HEART HOMOGENATES

Age	Yield of $C^{14}O_2$ at 1 RTU as per cent of administered C^{14}			Ratios of yields	
	Δ -Gluconolactone-1- C^{14}	G-1- C^{14}	G-U- C^{14}	Δ -gluconolactone 1- C^{14} : G-1- C^{14}	Δ -gluconolactone 1- C^{14} : G-U- C^{14}
<i>days</i>					
4	95.2	8.2	19.4	11.3	4.9
7	83.4	9.7	20.9	8.6	4.0
8	48.9	16.3	20.6	3.0	2.4
14	81.2	48.1	81.4	1.7	1.00

Radiorespirometric experiments. All flask components were the same as those described in Methods, with the exception of Δ -gluconolactone-1- C^{14} , which was added to 2 μ moles per flask where indicated.

Tris buffer, 30°C.

yields from G-1- C^{14} , as the yields from G-6- C^{14} were not affected or only slightly depressed from the control yields.

In the two experiments with intact chick embryonic hearts (6 + 12 days), the $G_1:G_6$ ratios are similar to those for the heart homogenates of embryos of corresponding age, suggesting that the pathways of carbohydrate metabolism have not been altered by homogenization.

The kinetics of glucose- C^{14} decarboxylation by preparations from representative ages of chick embryo and adult are presented graphically in Figs. 2 and 3. These figures resemble those for microorganisms which utilize the phosphogluconate and the glycolytic-Krebs cycle pathways for the utilization of glucose (13). It is apparent that the initial rate of $C^{14}O_2$ arising from G-1- C^{14} is greater than from G-6- C^{14} , and that differences in total yields from these two substrates are greatest in the 4-day embryo homogenate. Intact hearts from 12-day embryos metabolized G-1- C^{14} and G-6- C^{14} to $C^{14}O_2$ at almost equal rates, as did the homogenates of 12-day hearts, but the extent

of oxidation by the intact hearts was much less than that by the homogenates during the time period used.

As seen in Table II, the ratio of $C^{14}O_2$ yields from gluconolactone-1- C^{14} :G-1- C^{14} and from gluconolactone-1- C^{14} :G-U- C^{14} is highest in homogenate preparations from early embryos. The ratios decrease as the age of the embryo increases.

DISCUSSION

The ratios of $C^{14}O_2$ yields from G-1- C^{14} and G-6- C^{14} provide strong evidence that the phosphogluconate pathway contributes more to the utilization of glucose in early chick embryo homogenates and early chick embryo heart homogenates than in heart homogenates from older embryos, and that this pathway is minor or insignificant in adult chick heart homogenates. The similarity between intact hearts and homogenized hearts in the decarboxylation of G-1- C^{14} suggests that the homogenates provide a true picture of the relative importance of the two pathways.

The Entner-Douderoff pathway of heterolactic fermentation has never been observed in mammals or in higher plants (13), nor has it been reported in avian systems. Participation in glucose utilization by this pathway can reasonably be excluded from consideration in the chick embryo. In fact, this pathway, operating alone or in conjunction with the phosphogluconate pathway, would require C-1 of glucose to appear as $C^{14}O_2$ before C-2 of glucose, whereas the reverse was true in all the ages of chick embryos investigated. Madson was unable to demonstrate the glyoxylic acid cycle in the chick embryo (14).

Using the ratios of $C^{14}O_2$ yields as an indication of the relative importance of the phosphogluconate pathway to glucose utilization, it is apparent that the contribution of this pathway to glucose catabolism is greater the younger the embryo heart homogenate. It is also apparent that at early incubation times, the heart homogenate differs little from the whole embryo homogenate with respect to glucose catabolic pathways. A rather sharp drop in phosphogluconate pathway participation is indicated at incubation times of 5 to 6 days. This age corresponds roughly to that at which the morphological development of the heart has been nearly completed (3).

The supporting evidence for this contention lies with the data for the $C^{14}O_2$ yields from gluconolactone-1- C^{14} and from G-1- C^{14} plus TPN. It is assumed that the increase in the G_1 : G_6 ratio due to added TPN represents a proportional increase in the capacity of the phosphogluconate pathway. Similarly, it is assumed that differences in the ratio of $C^{14}O_2$ yields from gluconolactone-1- C^{14} :G-1- C^{14} represent proportional differences in the activities of the phosphogluconate pathway and the glycolytic-Krebs cycle pathway. When the yields for gluconolactone-1- C^{14} are compared with those for G-1- C^{14} or G-U- C^{14} , the participation of the direct oxidative route in the early embryo heart

homogenates is seen to be far more important than in the older heart homogenates. The abrupt change in participation by the two pathways of glucose metabolism is not evident from the gluconolactone-1-C¹⁴ data, but a change between incubation ages of 4 and 14 days is apparent. This conclusion is also obtained by the comparison of G₁:G₆ ratios in the presence of added TPN with the G₁:G₆ ratios.

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BIBLIOGRAPHY

1. BRACHET, J., *Biochemical Cytology*, New York, Academic Press, Inc., 1957, 266-295.
2. JOLLEY, R. L., JR., CHELDELIN, V. H., and NEWBURGH, R. W., Glucose catabolism in fetal and adult heart, *J. Biol. Chem.*, 1958, **233**, 1289.
3. LESLIE, I., and DAVIDSON, J. N., The chemical composition of the chick embryonic cell, *Biochim. et Biophysica Acta*, 1951, **7**, 413.
4. LEVY, M., Metabolic patterns in embryonic development, *Ann. New York Acad. Sc.*, 1952, **55**, 51.
5. SOLOMON, J. B., Increase of deoxyribonucleic acid and cell number during morphogenesis of the early chick embryo, *Biochim. et Biophysica Acta*, 1957, **23**, 24.
6. SOLOMON, J. B., Nucleic acid content of early chick embryos and the hen's egg, *Biochim. et Biophysica Acta*, 1957, **24**, 584.
7. BAKER, W. W., and NEWBURGH, R. W., Intracellular distribution of isocitrate-nicotinamide-adenine dinucleotide phosphate oxido-reductase activity during development of the chick embryo, *Biochem J.*, 1963, **89**, 17.
8. KRAHL, M. E., Oxidative pathways for glucose in eggs of the sea urchin, *Biochim. et Biophysica Acta*, 1956, **20**, 27.
9. NEEDHAM, J., and LEHMANN, W., Intermediary carbohydrate metabolism in embryonic life, *Biochem. J.*, 1937, **31**, 1210.
10. KLEIN, N. W., The metabolism of carbon-14-labeled nutrients by the chick embryo, Ph.D. dissertation, University of California, Davis, California, 1955.
11. WANG, C. H., STERN, I., GILMOUR, C. M., KLUNGSOYR, S., REED, D. J., BIALY, J. J., CHRISTENSEN, B. E., and CHELDELIN, V. H., Comparative study of glucose catabolism by the radiorespirometric method, *J. Bact.*, 1958, **76**, 207.
12. WENNER, C. E., and WEINHOUSE, S., An isotope tracer study of glucose catabolism pathways in liver, *J. Biol. Chem.*, 1956, **219**, 691.
13. CHELDELIN, V. H., WANG, C. H., and KING, T. E., in *Comparative Biochemistry*, (M. Florkin and H. L. Mason, editors), New York, Academic Press, Inc., 1962, **3**, 427.
14. MADSON, N. B., Test for isocitratase and malate synthetase in animal tissues, *Biochim. et Biophysica Acta*, 1958, **27**, 199.