Glucose Metabolism By Rat Embryos *in Vitro*[1] (34985)

T. TANIMURA AND T. H. SHEPARD
with the technical assistance of Glenda Bass
(Introduced by J. K. Lauber)

Central Laboratory for Human Embryology, Department of Pediatrics, School of Medicine, University of Washington, Seattle, 98105

Embryonic nutrition has held the interest of biologists since the start of the scientific age. With the advent of isotope-labeling techniques and introduction of a superior method for *in vitro* culture of mammalian embryos (New, 1), quantification of embryonic metabolism of glucose has become possible. Previous measurements of glucose metabolism in the embryonic chick (2) and rat (3) followed disruption of normal physiologic states. The present work describes glucose utilization in early somite rat embryos during their maintenance in a continuously growing state and reaches the conclusion that the very high rate of glucose utilization during somite formation drops with aging, a decline associated with reduced lactate production.

Materials and Methods. The embryos were obtained after cervical dislocation of Sprague–Dawley rats on days 10 through 12. The details of animal care and breeding have been given previously [Fink *et al.*, (4)].

The technique and apparatus for culturing embryos were practically identical with those used by New (1). Three embryos (two in the case of day 12) with yolk sacs intact were anchored on rayon fabric rafts and placed in the glass circulator. Human serum as a medium proved in preliminary experiments to be the same as rat serum for support of growth of rats in 22-hr periods.

To the medium was added ^{14}C-glucose, and the volume of the medium was adjusted to 10 ml. Uniformly labeled ^{14}C-glucose, 1-^{14}C-glucose, or 6-^{14}C-glucose with specific activities of 40–260 μCi/mmole was used to give a final concentration of 0.25–1.25 μCi/ml of medium.

The gas phase used was 95% air and 5% CO_2 for day-10 embryos, and 95% O_2 and 5% CO_2 for day-11 and day-12 embryos. The higher concentration of O_2 is detrimental to day-10 embryos for 22 hr of culture while day-11 and day-12 embryos require 95% O_2 for survival as previously reported (5). The gas flow was controlled at a rate of approximately 18–20 ml per minute, and the temperature was 38° ± 0.3°.

The culture period was limited to 3 hr. During this short time yolk sac circulation, heart beat, and normal increase in somite number were observed.

At the close of the culture period, 3 hr after the addition of isotope, the conceptuses were washed in ice cold Hanks' balanced salt solution and their somite number, length, and shape of extremity were recorded. Growth during the culture period was assessed by comparing these parameters to those of control littermates examined at the start. The cultured embryos were then digested in *p*-(diisobutyl-cresoxyethoxyethyl) dimethylbenzylammonium hydroxide (hydroxide of hyamine) overnight. During the entire period of culture, CO_2 in the outflow gas was trapped in hydroxide of hyamine. By the method of Cuppy and Crevasse (6), $^{14}CO_2$ was liberated from the medium, and lactate in the serum was separated by the method of Barker and Summerson (7). Glucose level in the serum was determined by the glucose oxidase method (8).

Radioactivity was measured by standard liquid-scintillation methods and quenching effect was calculated by the addition of the internal standard.

Protein content of embryos was used as a chemical denominator for comparison of

[1] Supported by U.S. Public Health Service Grants HD 02392, HD 00180, and HD 00836.

TABLE I. Age, Somite Number, and Protein Content of Rat Embryos.

Age (day)	No. of embryos	Somite	Protein content (μg) Embryo	Extra-embryonic membranes
10	33	11.3 ± 0.3[a]	45 ± 2[a]	133 ± 6[a]
11	48	25.3 ± 0.2	236 ± 5	546 ± 8
12	16	35.3 ± 0.5	730 ±43	1322 ±91

[a] Mean ± standard error.

stages of development. Protein content of the cultured embryos was measured (9) or calculated by comparison with measurements of uncultured embryos of corresponding somite number (Table I). Protein and somite increment *in vitro* was the same as *in vivo* during the 3-hr culture period. Histologic examination of cultured embryos revealed no cell necrosis.

For calculations of glucose utilization the sum of the ^{14}C counts from the tissue, CO_2, and lactate was expressed as "utilized." The amount of radioactivity added to the serum at the start represented 100%. On the assumption that cold glucose is utilized in the same manner as labeled glucose, the glucose utilization (μmoles glucose per gram protein per hour, Gu) was calculated by the following formula: $Gu = U \times g \times 55{,}600/P$ where U is percentage of ^{14}C utilized; g is mg of glucose in the medium; 55,600 is a constant; and P is total protein content of embryos and membranes in μg.

Results and Discussion. Utilization of uniformly labeled glucose by day 10–12 embryos is shown in Table II. It is decidedly high in the youngest embryos (day 10) and drops progressively as the embryo ages (days 11 and 12). The precipitous drop was due principally to a diminished ^{14}C-lactate output by the larger embryos. The $^{14}CO_2$ production did not differ significantly among the three age groups. Uptake and incorporation of ^{14}C by embryos and membranes, on the other hand, increased as the age advanced. An explanation for the high lactate production at the earlier stage may be the relative deficiency in day-10 embryos of lactate catabolism via the Krebs cycle. Such a theory is supported by the report of Aksu *et al.* (10) that these earlier embryos normally show low activities of the terminal electron-transport system that oxidizes the Krebs cycle intermediates. It is possible that glucose metabolism in these early somite embryos is comparable to the metabolic processes occurring in exercised skeletal muscle.

Factors which could influence the glucose uptake are: temperature (11), rate of perfusion (12), insulin content (13), and especially glucose level in the medium (13, 14, 15). The first two were controlled strictly. The third one, insulin content, was not measured

TABLE II. Glucose Utilization by Rat Embryos Cultured for 3 hr (experiments with U-^{14}C-glucose).

Day of gestation	No. of embryos	Glucose (μmoles/g protein/hr) mean ± SE[a] Total	Emb. + memb. (%)[b]	CO_2 (%)[b]	Lactate (%)[b]
10	15	731 ± 91	39 ± 4 (5.6)	33 ± 4 (4.6)	659 ± 88 (89.8)
		↓↓	↑↑	‖	↓↓
11	24	474 ± 32	54 ± 2 (11.6)	40 ± 2 (8.6)	381 ± 30 (79.8)
		↓	↑	‖	↓↓
12	8	312 ± 39	66 ± 5 (21.7)	34 ± 6 (10.7)	212 ± 30 (67.6)

[a] ‖ = $p > .05$; ↓ = $p < .05$; ↓↓ = $p < .01$.
[b] Percentage of total.

but serum from a single healthy donor was used throughout. Glucose uptake is known to be significantly increased by very high levels of glucose in the medium. However, no correlation between glucose utilization and glucose concentration in the medium was found in these experiments. The variation of glucose content was 69–108 mg/100 ml.

The extensive literature on the general subject of glucose metabolism was examined with special attention to studies of intact systems that gave the highest rate of glucose uptake, the object being to compare the glucose metabolism of living embryos with that of other tissues. To arrive at some common denominator to enable comparison, the data in these studies, originally given per unit wet weight, were converted to μmoles glucose per gram protein per hour based on the best estimate of protein content in the corresponding tissue (16). This conversion gave the following values in these tissues of the rat: perfused heart, 267 (11) and 515 (14); perfused liver, 118 (12); diaphragm, 66 (17); brain slice 107 (18); lens, 29 (19). A comparison of the glucose utilization of these tissues with those in Table II leads to the conclusion that rat embryos metabolize glucose at a very high rate.

A few studies, in addition to ours, are available on embryonic tissues. De Plaen (20) reported that isolated rat embryos used glucose at a rate of 302 μmoles/gram protein/hour when cultured in Warburg flasks for 3 hr. This value dropped to 23 for older embryos, both figures significantly lower than ours. Probably the disrupted state of his embryos as opposed to the growing state of ours, accounts for the decreased metabolism he found. Studies by Fridhandler *et al.* (21) showed that the glucose utilization by day-6 rabbit preimplantation blastocysts in 410 μmoles/gram protein/hour. Guidotti *et al.* (15) demonstrated that the hearts of day-5 chick embryos incubated at a glucose concentration of 4 μmoles/ml utilized 4.3 times more glucose than those of day 10. In summary, scattered studies using other vertebrate embryos are compatible with ours in the rat, namely, that glucose utilization is high in the young embryo and drops dramatically with age.

The ratio of CO_2 from C1- and C6-labeled glucose was 11.3 on day 10, 3.6 on day 11, and 4.6 on day 12 (Table III). Therefore, carbon 1 of glucose was more rapidly metabolized into $^{14}CO_2$ than carbon 6, suggesting a significant role of the pentose phosphate pathway especially at the earlier stage of rat embryo development. In most mature tissues the C1/C6 ratio is usually about one, indicating that almost all the glucose is metabolized via the Embden–Meyerhof pathway. Noted exceptions may be adipose tissue, mammary gland, and some endocrine glands (22, 23). In embryonic tissues it is generally assumed that the pentose phosphate pathway has an important role in glucose metabolism as compared with adult tissues. This pathway is considered to have direct connections with the synthesis of nucleic acids and the reduction of nicotinamide adenine dinucleotide

TABLE III. Comparison of 1-^{14}C-Glucose and 6-^{14}C-Glucose Utilization by Rat Embryos Cultured for 3 hr.

Day of gestation	No. of embryos	^{14}C-Glucose	Glucose (μmoles/g protein/hr; mean ± SE)	Ratio (C1/C6)		
				Embryo and membranes	CO_2	Lactate
10	9	C1	871 ± 78	0.89	11.31	0.91
	9	C6	905 ± 13			
11	12	C1	498 ± 33	0.74	3.59	0.90
	12	C6	523 ± 37			
12	4	C1	418 ± 66	1.09	4.59	0.89
	4	C6	404 ± 32			

phosphate. Most data on mammals so far reported are based on experiments with fetuses in later gestation rather than with young embryos (22, 24, 25) and show a C1/C6 ratio much lower than found here. Coffey *et al.* (2) reported that the C1 to C6 ratio of chick embryo heart homogenate was very high (5 to 2) on days 2 to 4, falling sharply to almost one at a later stage. De Meyer and De Plaen (3) noted that the C1/C6 ratio in their disrupted rat embryos incubated for 30 min duration was 28 and stressed the relative importance to the embryo of the pentose phosphate pathway. The accumulated evidence then points to greater activity of the pentose phosphate pathway in embryos than in fetuses.

Studies on the C1/C6 ratio of $^{14}CO_2$ yield by preimplantation ova by Fridhandler (26) in rabbits, Brinster (27) in mice, and Brinster (28) in rabbits showed a relatively low ratio (1.1 to 1.4) at the blastocyst stage when incubated 1–4 hr. It may be assumed that the same case obtains in rat blastocysts although no data are available. Between the blastocyst and early somite stages there exists still a very great gap in knowledge of the pathways of glucose metabolism.

Summary. During the somite stages of rat embryogenesis glucose metabolism was high. Its sharp decline with advancing age was associated with reduced lactate production. By comparison of $^{14}CO_2$ evolution rates from 1-^{14}C and 6-^{14}C-glucose the pentose shunt pathway was shown to be active.

1. New, D. A. T., J. Embryol. Exp. Morphol. **17**, 513 (1967).

2. Coffey, R. G., Cheldelin, V. H., and Newburgh, R. W., J. Gen. Physiol. **48**, 105 (1964).

3. De Meyer, R., and De Plaen, J., Life Sci. **3**, 709 (1964).

4. Fink, B. R., Shepard T. H., and Blandau, R. J., Nature, (London) **214**, 146 (1967).

5. Shepard, T. H., Tanimura, T., and Robkin, M., Teratology **2**, 107 (1969).

6. Cuppy, D., and Crevasse, L., Anal. Biochem. **5**, 462 (1963).

7. Barker, S. B., and Summerson, W. H., J. Biol. Chem. **138**, 535 (1941).

8. Marks, V., Clin. Chem. Acta **4**, 395 (1959).

9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

10. Aksu, O., Mackler, B., Shepard, T. H., and Lemire, R. J., Teratology **1**, 93 (1968).

11. Shipp, J. C., Matos, O. E., and Crevasse, L., Metabolism **14**, 639 (1965).

12. Gordon, E. R., Can. J. Biochem. Physiol. **41**, 1611 (1963).

13. Crofford, O. B., and Renold, A. E., J. Biol. Chem. **240**, 14 (1965).

14. Vahouny, G. V., Katzen, R., and Entenman, C., Proc. Soc. Exp. Biol. Med. **121**, 923 (1966).

15. Guidotti, G. G., Loreti, L., Gaja, G., and Foá, P. P., Amer. J. Physiol. **211**, 981 (1966).

16. Long, C., "Biochemists' Handbook," Van Nostrand, Princeton, N.J. (1961).

17. Pocchiari, F., and Silano, V., Biochem. J. **107**, 305 (1968).

18. Smith, M. E., Nature, (London) **209**, 1031 (1966).

19. Farkas, T. G., and Roberson, S. L., Exp. Eye Res. **4**, 124 (1965).

20. De Plaen, J. L., "Aspects Dynamiques du Métabolisme Glucidique chez L'embryon de Rat. Action de Substances Hypoglycémiantes et Tératogènes," Édition Arscia S. A., Bruxelles (1969).

21. Fridhandler, L., Wastila, W. B., and Palmer, W. M., Fertil. Steril. **18**, 819 (1967).

22. Villee, C. A., and Loring, J. M., Biochem. J. **81**, 488 (1961).

23. Pon, N. G., *in* "Comparative Biochemistry" (M. Florkin and H. S. Mason, eds.), vol. 7, p. 1. Academic Press, New York (1964).

24. Jolley, R. L., Cheldelin, V. H., and Newburgh, R. W., J. Biol. Chem. **233**, 1289 (1958).

25. Beatty, C. H., Basinger, G. M., and Bocek, R. M., Arch. Biochem. Biophys **117**, 275 (1966).

26. Fridhandler, L., Exp. Cell Res. **22**, 303 (1961).

27. Brinster, R. L., Exp. Cell Res. **47**, 271 (1967).

28. Brinster, R., Exp. Cell Res. **51**, 330 (1968).

Received April 10, 1970. P.S.E.B.M., 1970, Vol. 135.