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Estimation of the Pentose Cycle Contribution to Glucose Metabolism in Tissue *in Vivo**

Karl Y. Hostetler† and Bernard R. Landau‡

ABSTRACT: A method is illustrated for estimating the contribution of the pentose cycle to glucose metabolism in individual tissues in an intact animal. The method depends upon the presentation to the tissues of glucose-2-^{14}C and the measurement of the randomization of the ^{14}C in its conversion to glucose-6-P derivatives in the tissues. The assumptions required in making the estimations are detailed. Glucose-2-^{14}C is infused into fasted, anesthetized rats. From the randomization of ^{14}C in glucose from glycogen of diaphragm, skeletal muscle, heart, thyroid, brain, liver, and kidney, and in the glycerol from triglycerides of adipose tissue, the contribution in each of these tissues is estimated. In muscle, *in vivo*, the pentose cycle contributes no more than a few per cent to over-all metabolism; in thyroid, brain, liver, and kidney, 3–5%; and in adipose tissue, in a single determination, 34%. These estimates are generally in good agreement with estimates made *in vitro*.

Various approaches have been employed to estimate quantitatively the contribution of the pentose cycle to glucose metabolism by tissues, *in vitro*. Most of these approaches depend on the unique metabolism of carbons 1 and 6 of glucose in the cycle, as compared to other pathways, during their conversion to CO_2, lactate, and other triose-P derivatives (Katz, 1961; Wood *et al.*, 1963). The estimates require many assumptions. Moreover, these approaches cannot be used for estimations *in vivo* unless the yield of the CO_2, lactate, or other product formed in the intact animal can be assigned to an individual tissue. However, the yields of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C in expired air may be used to estimate the over-all contribution of the pentose cycle to metabolism, *in vivo* (Segal *et al.*, 1961).

Thus far, no method for determining the contribution of the pentose cycle in individual tissues, *in vivo*, has been described. The unique randomization of carbon 2 of glucose during its metabolism by the pentose cycle has been used to estimate the contribution of the cycle *in vitro* (Wood *et al.*, 1963). This method is applicable to estimations for tissues in the intact animal. This is so because the product examined, a glucose-6-P derivative, usually glycogen, is retained within and can be isolated from the individual tissues. This report describes the results of this application.

Methods

Three male albino rats of the Wistar strain, each weighing between 160 and 205 g, were fasted for 14–16 hr. They were anesthetized with pentobarbital (5 mg/100 g body weight) by intraperitoneal injection; additional pentobarbital was injected as required to maintain anesthesia during the course of the experiment. Glucose dissolved in glass-distilled water (0.28–0.33 M) was infused by means of a constant infusion pump at the rate of 1 ml/hr into a femoral vein of each rat. During the first 20 min of infusion, the glucose was unlabeled and during the remainder of the infusion it was labeled with glucose-2-^{14}C (40 μc/ml) (purchased from New England Nuclear Corp., Boston, Mass.).

* From the Departments of Medicine and Biochemistry, Western Reserve University, School of Medicine, Cleveland, Ohio 44106. *Received July 5, 1967.* This work was supported by Grant AM-09495 from the National Institutes of Health.

† U. S. Public Health Research Postdoctoral Trainee in Medicine (Grant AM-1005)

‡ Present address, to which inquiries should be sent: Merck Institute for Therapeutic Research, Rahway, N. J. 07065.

The labeled glucose was infused into two of the rats (designated rats 1 and 2) for 1 hr and in the third rat (rat 3) for 2 hr. With the completion of the infusions, the abdomens of the rats were opened. The rats were then exsanguinated by withdrawing blood from their vena cavas into syringes containing heparin.

Tissues were rapidly removed in the order: liver, kidneys, diaphragm, skeletal muscle (from the legs), and heart. The thyroid was also removed from rat 1. These tissues were plunged into hot 30% KOH. Glycogens were isolated from the digests after addition of carrier and purified; glucoses from the glycogens were isolated and degraded using *Leuconostoc mesenteroides* (Merlevede *et al.*, 1963). In some cases the CO_2 obtained on combustion of the glucoses had more radioactivity than glucosazones prepared from the glucose, indicating the presence of a ^{14}C contaminant in the glucose. These samples were purified further by preparative chromatography on Whatman 3MM paper using as solvent 1-butanol–acetic acid–water (4:1:5, v/v) (Partridge, 1948) before degradation.

After exsanguination of rat 3, and before removing the tissues just listed, the head of the rat was guillotined into isopentane cooled to $-140°$ in liquid nitrogen. While frozen in liquid nitrogen the brain was chisled free and powdered. The powdered brain was treated with hot 30% KOH and after the addition of carrier glycogen, the glycogen was isolated and purified by the method of Kerr (1938). The glycogen was hydrolyzed and the glucose formed was purified on paper as just described and then degraded. The epididymal fat tissue of rat 3 was also removed and glycerol from triglycerides in the tissue was isolated, purified, and degraded (Landau *et al.*, 1966). The carbons of the glucoses from glycogens and the glycerol, isolated as CO_2, were assayed for ^{14}C activity to yield the relative specific activities in each carbon. Incorporation of ^{14}C into glycogens from epididymal fat tissue, adrenal, and testis was found to be too low, under the conditions of the experiment, to permit adequate assay of the distribution of ^{14}C in the glucoses from the glycogens.

Blood was collected from the tails of the rats midway in the glucose-2-^{14}C infusions. The concentrations of glucose in these bloods and the bloods withdrawn at exsanguination were determined using the method of Somogyi (1952). The concentrations midway and at exsanguination in the bloods of rat 1 were 176 and 112 mg/100 ml, respectively, in those of rat 2 they were 97 and 94 mg/100 ml, and in those of rat 3 they were 69 and 98 mg/100 ml. The corresponding specific activities of the blood glucoses, determined by assay of glucosazones prepared from aliquots of the Somogyi filtrates, were 1.3 and 1.7 $\times$ 10^5 cpm/mg of glucose for rat 2 and 1.1 and 1.4 $\times$ 10^5 cpm/mg of glucose for rat 3. The specific activities of the glucose in the bloods from rat 1 were not determined. Aliquots of the Somogyi filtrates of the bloods obtained at exsanguination and of the blood obtained after 30 min of infusion of glucose-2-^{14}C into rat 1 were deionized by passage through a column of anion- and cation-exchange resins (Duolite A-4, Diamond Alkali Co., Western Division, Redwood City, Calif., and IR-120, Rohm and Haas, Philadelphia, Pa.). The effluents were concentrated, applied to, and chromatographed on paper as previously described; the glucoses were eluted from the paper and degraded.

The fraction of glucose metabolized *via* the pentose cycle (PC) has been calculated from the formula C-1/C-2 = 2PC/(1 + 2PC), where C-1 and C-2 are the specific activities of carbons 1 and 2 of glucose-6-P or a derivative reflecting these activities (Wood *et al.*, 1963).

Results

The data, with the estimates of PC, are recorded in Table I. Glucose in the bloods of each of the three rats at the completion of the infusions and in the blood of the first rat, 30 min after beginning the infusion, was essentially labeled only in carbon 2. Thus, less than two-hundredths of the activity in carbon 2 was in any other single carbon. Degradation of glucose by *L. mesenteroides* is not completely specific and as much as $^1/_{100}$ of the activity in glucose-2-^{14}C, obtained commercially, can be recovered in carbons other than carbon 2 (Merlevede *et al.*, 1963; Bernstein *et al.*, 1955). Part of the small quantity of ^{14}C in carbons other than carbon 2 of the blood glucoses may also be due to the release of labeled glucose into the circulation from glucose-forming tissues, primarily liver, following randomization in these tissues of the carbons of the glucose entering from the circulation.

In rats 1 and 2, the small randomization of ^{14}C in glucoses from glycogens of diaphragm, skeletal muscle, and heart cannot be attributed to metabolism *via* the pentose cycle in these muscles, since similar randomization existed in the blood glucoses of these animals. Even if the randomization were attributed to the cycle, the contribution of the cycle to total glucose metabolism by the muscles would be less than 1%. For rat 3, while randomization was more extensive, the contribution would still be less than 2%.

Randomizations of ^{14}C in glucoses from glycogens of thyroid, brain, liver, and kidney were more than in muscle and correspond to a pentose cycle contribution of 3–5%. Again, in these estimates no correction has been made for the degree of randomization found in the blood glucoses. Randomization of ^{14}C was extensive into glycerol from triglycerides of epididymal fat tissue and corresponds to a pentose cycle contribution of 34%.

Discussion

Carbon 2 of glucose is randomized into carbons 1 and 3 of glucose-6-P and its derivatives *via* the pentose cycle. The randomization of ^{14}C of glucose-2-^{14}C into carbon 1 relative to carbon 2 can be used to estimate the contribution of the cycle to over-all glucose metabolism (Wood *et al.*, 1963). The estimates of PC from the specific activities of carbons 1 and 2 are probably maximal values since ^{14}C, introduced by

TABLE I: Distribution of ^{14}C in Blood Glucose, Glucose from Glycogen in Various Tissues, and Glycerol from Adipose Tissue and the Pentose Cycle Contributions.

Tissue	Blood				Diaphragm			Skeletal Muscle		
Rat	1[a]	1	2	3	1	2	3	1	2	3
C-1	0.6	1.0	1.8	1.1	1.0	1.4	3.3	0.7	1.5	2.5
C-2	100	100	100	100	100	100	100	100	100	100
C-3	1.0	0.9	1.0	1.2	0.9	0.7	7.5	1.2	0.9	5.7
C-4	0.3	0.2	0.4	0.2	0.0	0.2	2.3	0.3	0.2	2.1
C-5	0.4	0.9	1.8	1.3	0.9	1.0	2.7	1.1	1.7	9.1
C-6	0.5	0.6	1.3	0.8	0.6	0.6	1.4	0.3	2.1	1.2
% Recovery[c]	90	91	108	92	93	120	103		125	94
PC					0.005	0.007	0.017	0.004	0.008	0.013

	Heart		Thyroid	Brain	Liver			Kidney		Adipose
	2	3	1	3	1	2	3	2	3	3[b]
C-1	0.5	3.6	6.1	5.4	5.5	4.8	5.4	5.5	9.0	17.6
C-2	100	100	100	100	100	100	100	100	100	100
C-3	1.4	4.3	12.4	5.5	2.4	3.1	4.0	6.5	9.5	40.5
C-4	0.6	0.5	0.7	1.3	0.7	0.6	0.9	1.0	0.5	
C-5	2.8	2.9	4.0	5.1	3.8	5.9	4.5	11.9	7.4	
C-6	0.3	0.5	2.4	1.9	2.9	3.7	1.9	5.4	0.2	
% Recovery[c]	87	75	91	86	90	121	99	133	96	84
PC	0.003	0.018	0.032	0.029	0.029	0.025	0.029	0.029	0.049	0.34

[a] Glucose from blood 30 min after beginning infusion of glucose-2-^{14}C. Remaining degradations are of glucoses in bloods at the completion of the infusions. [b] C-1 and C-3 of glycerol are derived, respectively, from C-3 and C-1 of glucose-6-P. [c] Per cent recovery is the per cent of the ^{14}C activity in the entire glucose molecule, determined by combustion, which was recovered, as determined by summing the activities found in each of the carbon atoms of the glucose (Merlevede *et al.*, 1963).

any mechanism into carbon 1 of glucose-6-P, will be ascribed to the pentose cycle. Several assumptions required in making these estimates in studies *in vitro* have been detailed (Wood *et al.*, 1963; Landau *et al.*, 1965) and additional ones are required in studies *in vivo*. Thus, the *in vivo* distribution of ^{14}C in carbons of the glucose-6-P, or its derivative glycogen, isolated from a tissue, must be attributable to metabolism in that tissue and not another tissue. To achieve this, either each tissue must be perfused with glucose-2-^{14}C or the extent of randomization of ^{14}C in the circulating glucose must be known and ideally must be negligible. By continuously infusing glucose-2-^{14}C, we have presented to the tissues glucose essentially labeled only in carbon 2, as evidenced by the degradations of glucose from the bloods. Thus, the carbons of the blood glucose were not significantly randomized by tissues, released into the blood, and then exposed to other tissues. A single injection of glucose-2-^{14}C would be expected to result in blood glucose extensively randomized within a few hours (Reichard *et al.*, 1963). While theoretically possible, correction of estimates of PC for the extent of this randomization would be difficult.

Incorporation into carbons 1 and 2 of the glucose from glycogen is assumed not to arise by formation of glucose from carbons of lactate first randomized in the Krebs cycle. Such incorporation would be expected to occur particularly in the gluconeogenic tissues, liver and kidney. Thus, lactate-2-^{14}C formed by the glycolysis of glucose-2-^{14}C by peripheral tissues should be converted in liver and kidney into glucose labeled primarily in carbons 1, 2, 5, and 6. To the extent that these reactions occur the contribution of the pentose cycle will be overestimated since incorporation into carbon 1 will be incorrectly attributed to the cycle.

Steady-state conditions are required for the estimations of PC. Thus, glucose concentration should be maintained constant, since the cycle's contribution can be a function of glucose concentration (Merlevede *et al.*, 1963; Hostetler *et al.*, 1966). In rat 1, the blood glucose concentrations differed markedly with time but were relatively constant in rats 2 and 3. Ideally the specific activity of blood glucoses should also have been kept constant. However, the gradual increase in specific activity is probably unimportant since the deposition of the glucose carbons in glycogen would be expected to have the same degree of randomization at any point in time, irrespective of the absolute specific activity of the carbons of the glucose-6-P.

In the calculation of PC, the pentose cycle is assumed to proceed only in the oxidative direction. Incorporation into carbon 3 of glucose-6-P should then be about one-half that of incorporation into carbon 1 (Wood *et al.*, 1963). This is the case for liver and adipose tissue (remembering that carbons 1 and 3 of glucose correspond to carbons 3 and 1 of glycerol), but not the remaining tissues. The additional incorporation into carbon 3 is probably attributable to reversal of the nonoxidative portions of the pentose cycle (Landau and Bartsch, 1966). It is not attributable in any significant degree to formation of glucose from lactate, as described above, since activity in carbon 4 compared to carbon 3 was small for all the degradations.

Glucose-6-P and fructose-6-P are assumed to be in complete isotopic equilibrium. This assumption is supported by the high activity of phosphohexose isomerase found relative to the activities of other enzymes of the pentose cycle and Embden–Meyerhof pathway in these tissues (Landau and Bartsch, 1966; Shonk and Boxer, 1964). In theory, the extent of equilibration can be estimated from the incorporation into carbons 1, 2, and 3 of the glucose unit of glycogen relative to that into carbons 6, 5, and 4 (Landau and Bartsch, 1966). However, in the present experiments this was not possible because of the low yields in carbons 4, 5, and 6, as well as in some cases in carbons 1 and 3, and the similar yields in the blood glucoses.

The contributions of the pentose cycle estimated in Table I are in general in good agreement with those estimated by incubation of the corresponding tissues *in vitro*, providing support for the adequacy of the method. Thus, using specific yields of CO_2 from glucose-1-^{14}C and glucose-6-^{14}C (Beatty *et al.*, 1966) and using the randomization of ^{14}C of glucose-2-^{14}C (Green and Landau, 1965) incubated with muscle preparations, the contribution of the cycle in muscle has been estimated to be no more than a few per cent. The distributions of ^{14}C in glucoses from glycogens of skeletal muscle are similar to those observed following the intraperitoneal injection of glucose-2-^{14}C into rats (Bloom *et al.*, 1956; Marks and Feigelson, 1957). Pentose cycle contributions of 2.5–4.0% have been estimated for thyroid, *in vitro* (Merlevede *et al.*, 1963), and 9–24% for adipose tissue (Landau and Bartsch, 1966), the higher values in the presence of insulin.

No quantitative estimate of the contribution of the pentose cycle has been reported in brain, although it has been considered to be small from the ratio of the yields of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C on incubation of brain slices in the absence of added triphosphopyridine nucleotide or other oxidation–reduction mediators. Moss (1964) prefused the heads of calves with glucose-1-^{14}C and glucose-6-^{14}C and from the yields of $^{14}CO_2$ concluded that the pentose cycle is of major importance in the metabolism of glucose by brain, but the contribution was not quantitated. Moss (1964) made no correction in the yields of $^{14}CO_2$ for the contribution of blood cells, other tissues in his system, and for $^{14}CO_2$ yielding impurities shown to be present in ^{14}C-labeled glucoses (Merlevede *et al.*, 1963). Our data from a single experiment indicate a small pentose cycle contribution. In kidney, based on the ratios of yields of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C, the contribution of the cycle has been considered to be negligible. Our data show a pentose cycle contribution of 3–5%. In liver, the contribution has been in considerable debate (Katz, 1961). From the randomization observed by others in glucose from glycogens of liver after intraperitoneal injection of glucose-2-^{14}C into fasted rats, Katz (1961) estimated contributions of 5% in good agreement with our estimates. The over-all estimate of a small contribution of the pentose cycle to glucose metabolism in fasting man (Segal *et al.*, 1961) would also seem in accord with our estimates for the individual tissues of the rat.

References

Beatty, C. H., Peterson, R. D., Basinger, G. M., and Bocek, R. M. (1966), *Am. J. Physiol. 210*, 404.

Bernstein, I. A., Lentz, K., Malm, M., Schambye, P., and Wood, H. G. (1955), *J. Biol. Chem. 215*, 137.

Bloom, B., Eisenberg, G., and Stetten, D., Jr. (1956), *J. Biol. Chem. 222*, 301.

Green, M. R., and Landau, B. R. (1965), *Arch. Biochem. Biophys. 111*, 569.

Hostetler, K., Cooperstein, S. J., Landau, B. R., and Lazarow, A. (1966), *Am. J. Physiol. 211*, 1057.

Katz, J. (1961), *in* Radioactive Isotopes in Physiology, Diagnostics and Therapy, Schwiegk, H., and Turba, F., Ed., Berlin, Springer-Verlag, p 705.

Kerr, S. E. (1938), *J. Biol. Chem. 123*, 433.

Landau, B. R., and Bartsch, G. E. (1966), *J. Biol. Chem. 241*, 741.

Landau, B. R., Bartsch, G. E., Williams, H. R. (1966), *J. Biol. Chem. 241*, 750.

Landau, B. R., Katz, J., Bartsch, G. E., White, L. W., and Williams, H. R. (1965), *Ann. N. Y. Acad. Sci. 131*, 43.

Marks, P. A., and Feigelson, P. (1957), *J. Clin. Invest. 36*, 1279.

Merlevede, W., Weaver, G., and Landau, B. R. (1963), *J. Clin. Invest. 42*, 1160.

Moss, G. (1964), *Diabetes 13*, 585.

Partridge, S. M. (1948), *Biochem. J. 42*, 238.

Reichard, G. A., Jr., Moury, N. F., Jr., Hochella, N. J., Patterson, A. L., and Weinhouse, S. (1963), *J. Biol. Chem. 238*, 495.

Segal, S., Berman, M., and Blair, A. (1961), *J. Clin. Invest. 40*, 1263.

Shonk, C. E., and Boxer, G. E. (1964), *Cancer Res. 24*, 709.

Somogyi, M. (1952), *J. Biol. Chem. 195*, 19.

Wood, H. G., Katz, J., and Landau, B. R. (1963), *Biochem. Z. 338*, 809.