METABOLISM OF GLUCOSE-U-C^{14} BY BONE

A Thesis

Presented to

the Faculty of Graduate Studies

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Helen Katherine Wedel

Department of Oral Biology

May 1964

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Abstract. The metabolism of glucose-U-C^{14} was studied in vitro by incubating calvarial preparations from one- to two-day old Long Evans rats with varying levels of this substrate. Incubations, generally lasting for two hours, were carried out at 37°C. in air with calcium-free phosphate- or THAM-buffered medium (pH 7.4). Carbon dioxide, either metabolic alone, or total after addition of acid to the tissue, was collected, chemically determined by manometric procedures, and assayed for radioactivity. Glucose and lactate were obtained from the medium after incubation by elution with a linear HCl gradient from Dowex-1-Cl anion exchange resin columns. Glucose was determined by the glucose oxidase-3,3'-dimethoxybenzidine (o-dianisidine) procedure, and lactate by the DPN-lactic dehydrogenase procedure. Like carbon dioxide, these compounds were assayed for radioactivity: all activity determinations in these experiments were carried out either with a thin window gas flow tube or by liquid scintillation. In some of the experiments, after incubation, the skulls were extracted with various solvents so as to determine the distribution of activity in different components of the tissue. Paper chromatography and radioautography were used to identify and estimate the quantity of radioactivity in these components of the tissue, and in metabolic intermediates found in the medium after incubation.

Two preliminary experiments, in which calvaria were incubated with glucose-U-C^{14} in phosphate-buffered medium (pH 7.4) for two hours, were performed. From the oxygen uptake of 14.34 μmoles/gram wet weight/hour,

and the carbon dioxide production of 16.73 μmoles/gram wet weight/2 hours, an R.Q. of 0.58 was determined. The inverse relation between the specific activity and production of lactic acid indicated varying degrees of endogenous dilution. On the basis of previous work by Dowse et al, 1963, it was likely that glycogen was the source of this dilution. The specific activities of carbon dioxide and lactate were considerably lower than that of glucose, but the specific activity of the CO_2 was much like that of the lactate. Therefore, much more dilution was occurring in the glycolytic cycle than between pyruvate and the decarboxylation steps of the TCA cycle. In one instance the specific activity of the CO_2 actually exceeded that of the lactic acid: this would be possible if the hexose monophosphate shunt were more active in this case.

In experiment 2 calvaria were incubated as in the preliminary experiments. The quantity of glucose utilized and its utilization rate were determined. Glucose uptake was constant, as was the fraction of utilized activity in lactate and CO_2. The specific activity and production of lactate were not individually constant, but were inversely related to each other. From the above results it could be seen that, at a given phosphate level, the conversion of glucose-C^{14} to lactate-C^{14} was unaffected by the varying degrees of endogenous dilution which were occurring.

In experiments 7, 8, and 10, calvaria were incubated with varying concentrations of glucose-U-C^{14} for two hours in air. Phosphate and THAM buffers (pH 7.4) were used in experiments 7 and 8, and 10 respectively. The specific activity of the glucose in the medium after incubation was lower than that of the original specific activity, and the effect was

more marked in phosphate than in THAM buffer. Within a given experiment, in which the phosphate level was constant, the specific activity of the lactate rose with increasing levels of glucose in the medium. Hence, at a given phosphate concentration, the production of lactate-C^{14} from glucose-C^{14} was directly dependent on the glucose concentration. With similar initial glucose, and therefore similar glucose-1-phosphate concentrations, on the basis of specific activities, more dilution of the lactate occurred in phosphate than in THAM buffer. The above results supported the hypothesis already developed, that endogenous dilution was occurring, and that glycogen was likely responsible for this dilution. The hydrolysis of glycogen is directly dependent on the ratio of $\frac{\text{inorganic phosphate}}{\text{glucose-1-phosphate}}$.

The average percentage of utilized activity in the lactate formed in phosphate buffer (experiment 8) was 29% as compared to 40% for that formed in THAM buffer (experiment 10). In both experiments, 2-4% of the utilized activity was recovered in the CO_2. Up to 58% of the utilized activity was calculated to be in compounds other than glucose and lactate in the medium after incubation in experiment 8, and up to 34% in experiment 10. 14-38% of the total counts utilized were calculated to be in the tissue of both experiments.

Paper chromatography and radioautography were used to identify and estimate the amount of activity in various components of calvaria. The ethanol extract of tissue incubated with glucose-U-C^{14} in phosphate buffer (pH 7.4) for 2 hours in experiment 3 contained labeled phosphorylated glycolytic intermediates, amino acids, and organic acids. Glutamic

and lactic acids were more heavily labeled than any of the other metabolic intermediates. In experiment 5 radioautograms were prepared from ethanol extracts of skulls incubated with glucose-U-C^{14} in phosphate buffer (pH 7.4) for 15 minutes, 30 minutes, 1 hour, 2 hours, and 3 hours respectively. After 15 minutes there was more activity in the phosphorylated glycolytic intermediates than in the amino acids, but after 3 hours, there was a much greater quantity of activity in amino acids such as glutamate than in the phosphorylated intermediates. This showed that, with time, much of the activity from the glucose had moved on through the glycolytic and tricarboxylic acid cycles to form labeled glutamic acid, which is used in protein synthesis.

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INTRODUCTION

Little comprehensive work has been done on carbohydrate metabolism in calvaria. Investigators in this field have generally preferred to use metaphyseal bone preparations (Borle, Nichols, and Nichols, 1960a, 1960b; Lekan, Laskin, and Engel, 1960; Cohn and Forscher, 1961, 1962a, 1962b; Vaes and Nichols, 1961, 1962a, 1962b). However, a biochemical study of calvaria from newborn rats was carried out by Dowse et al, 1963. The metabolic behaviour of calvaria was found to be qualitatively similar to that of metaphyseal preparations of bone. In the present report an attempt was made to elucidate further the metabolic behaviour of calvaria by incubating the tissue with glucose-U-C^{14} in vitro and tracing the products of metabolism.

I. PURPOSE OF THE STUDY

The metabolism of glucose-U-C^{14} by calvaria in different buffers at 37°C. in air was investigated. The object of the study was to (1) determine, by the use of paper chromatography, the distribution of radioactivity in the components of the medium after incubation and in various extracts of the skulls after incubation of the latter with glucose-U-C^{14}; (2) determine the utilization of oxygen, and the production and specific activity of metabolic CO_2 during the incubation of calvarial preparations; (3) determine the utilization of glucose by

calvaria and to assess the specific activity of the glucose in the medium before and after incubation; (4) assess the production and specific activity of lactic acid formed during the incubation of calvarial preparations; (5) determine the fate of glucose-U-C^{14} when calvarial preparations were incubated in media containing different levels of substrate and different buffers.

From the information obtained on the above parameters in normal calvaria, it should be possible to study more intelligently the effect of parathyroid extract on the metabolism of glucose-U-C^{14} by calvaria.

II. RELATION OF THE STUDY TO CARBOHYDRATE METABOLISM IN BONE

Although much work has been done on the effects of parathyroid hormone on carbohydrate metabolism in metaphyseal bone preparations, the results have not always been too meaningful, due to an inadequate understanding of carbohydrate metabolism in normal metaphyseal bone preparations. Cohn and Forscher (1962b) incubated epiphyseal-metaphyseal slices from the femurs of control and parathyroid extract-treated rabbits with glucose-U-C^{14}, glucose-1-C^{14}, and glucose-6-C^{14}. They found an increase in the total activity incorporated into CO_2, but did not know whether this increase was due to net changes of carbon dioxide or simply to increases in specific activity. In the present investigation information was to be obtained on the specific activity of metabolic CO_2 produced by calvaria from normal rats.

Experiments were to be performed to determine the percentage of utilized activity which would be present in various intermediates