Hexose monophosphate pathway in thyroid tissue

Many of the substrates of the tricarboxylic acid and EMBDEN-MEYERHOF cycles are oxidized by appropriately supplemented thyroid homogenates. However, lactate was the only one of these substrates which was oxidized by thyroid tissue slices[1]. Although the existence of glucose-6-phosphate dehydrogenase is suggested by some experiments[2], no report has yet appeared on the presence of an hexose monophosphate pathway in the thyroid. In this study, the consumption of glucose by thyroid slices is demonstrated, and evidence is presented for the existence in this tissue of an important oxidative pentose phosphate cycle.

[1-^{14}C]glucose and [6-^{14}C]glucose (Nuclear-Chicago Corporation) were diluted with non-labeled glucose. The radioactivities of washed recrystallized glucosazones and $BaCO_3$, plated on copper planchettes, were determined with a windowless automatic gas-flow counter (Tracerlab). Duplicate samples corrected for background were adjusted to a 5-mg standard[3]. Sheep muscles and thyroids were sliced with a Stadie-Riggs slicer, washed with Krebs–Ringer Bicarbonate, and distributed at random in duplicate Erlenmeyer flasks. One flask contained [1-^{14}C]glucose and another [6-^{14}C]glucose. The two experiments were directly comparable. After thorough flushing with O_2–CO_2 (95:5) for 7 min, the flasks were incubated in a shaker at 37.5° with this atmosphere for 30, 60, or 120 min. $^{14}CO_2$ was counted as $BaCO_3$ for 1,000 counts[4]. The mean of 4 different samples was used as the specific activity of CO in each flask. Three similar double experiments were made with sheep neck-muscle slices, and another control with two hemidiaphragms of a rat.

$^{14}CO_2$ is evolved when labeled glucose is incubated in the presence of thyroid slices (Table I). The ratio of $^{14}CO_2$ evolved from [1-^{14}C]glucose to that from [6-^{14}C]-glucose was 19.5 after 30-min incubation. When the incubation was prolonged, more CO_2 was evolved, but the ratio C_1/C_6 decreased markedly[5,6]. There is thus a preferential cleavage of the C_1 over the C_6 of glucose, indicative of an active hexose monophosphate pathway. If glucose were metabolized entirely via the glycolytic and citric acid pathway, a ratio of 1 would have been observed, as in muscle[6,7]. If one

TABLE I

Each flask contained 10 ml of Krebs–Ringer bicarbonate buffer (pH = 7.4), 124.6 μmoles glucose and approximately 1.40 g of tissue slices (wet wt.). Specific activities in counts/min/5 mg glucosazone were: [1-^{14}C]glucose, 28,000; [6-^{14}C]glucose, 107,940.

Tissue	*Incubation time (min)*	*Relative specific activity formed from**		*Ratio C_1/C_6***	*Number of experiments*
		[1-^{14}C]glucose	*[6-^{14}C]glucose*		
Thyroid	30	0.482 ± 0.040	0.024 ± 0.006	19.5 ± 5.2	5
Thyroid	60	0.657 ± 0.043	0.062 ± 0.010	10.7 ± 2.2	6
Thyroid	120	0.972 ± 0.145	0.265 ± 0.055	3.78 ± 1.0	5
Sheep muscle	60	0.147	0.162	0.91	1
Sheep muscle	60	0.165	0.130	1.27	1
Sheep muscle	60	0.172	0.112	1.55	1
Rat diaphragm	60	1.26	1.20	1.05	1

* Relative specific activity, expressed in per cent equals:
$$\frac{\text{Specific activity of } CO_2 \text{ in counts/min/}\mu\text{mole/g tissue}}{\text{Specific activity of glucose in counts/min/}\mu\text{mole glucose}}$$
** Ratio C_1/C_6: Means and standard errors of the ratios found for each pair of experiments.

makes the assumptions that there is no randomization of isotope from C_1 to C_4, C_5 or C_6, and that the oxidation of C_1 of glucose via the hexose monophosphate pathway predominates over that of carbon C_2 and C_3, we can calculate[8] that 75 % of the CO_2 produced from glucose would come via this pathway. However, these assumptions are doubtful[5,8] and this percentage is thus considered as semi-quantitative[9].

The existence of an active pentose pathway in the thyroid tissue implies a supply of reduced triphosphopyridine nucleotide, which is, in fact, required in the deiodination of iodotyrosines[2,3] by a microsomal enzyme and may be the most efficient coenzyme for iodide binding to protein in the thyroid homogenate[10].

Since the hexose monophosphate pathway seems to be an active metabolic route for glucose in the adrenal gland[7] and in testis[11], the possibility of this being a common property of endocrine glands should be considered.

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Department of Medicine, Harvard Medical School and the Thyroid Laboratory of the Medical Services of the Massachusetts General Hospital, Boston, Mass. (U.S.A.) J. E. DUMONT*

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* Present adress: Department of Experimental Medicine, University of Brussels (Belgium).

Intracellular ribonuclease from *Bacillus subtilis*

NISHIMURA AND NOMURA previously reported that *Bacillus subtilis* accumulated large amounts of ribonucleases in a culture medium; one of these enzymes was highly purified[1,2]. The specificity, molecular weight and immunological nature of these extracellular enzymes have also been described[3].

The present report deals with the presence of an intracellular RNase in this organism which has quite different properties from the extracellular RNases. A lysozyme lysate was prepared from bacteria which had been harvested from the culture medium (70 h) by centrifugation, and washed 3 times with 0.05 *M* phosphate buffer, pH 7.3. The lysate showed a small amount of RNase activity, measured by a modi-

Abbreviations: RNA, ribonucleic acid; RNase, ribonuclease; EDTA, ethylenediaminetetracetic acid.