UTILIZATION OF [U-^{14}C]GLUCOSE BY THE HUMAN UMBILICAL CORD *IN VITRO*. EFFECTS OF INSULIN

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1. Introduction

At the end of pregnancy, the umbilical cord comprises two thick-walled arteries and one vein, deeply embedded in Wharton's jelly. The latter contains large amounts of glycosaminoglycans (GAG) and collagen; it probably represents the fused adventitial layers of the three large vessels [1].

Thus, this organ may be considered as a vascular tissue, but also presents some foetal characteristics. These two latter types of tissues have been the object of relatively few metabolic studies in the human species, owing to obvious ethical reasons. Therefore, investigations on the umbilical cord, which is at the same time vascular and foetal, might provide more insights into the physiology and biochemistry of such structures, and furthermore it is easily available.

We have followed the fate of [U-^{14}C]glucose into umbilical cord slices, recovering radioactivity from the GAG, glycogen, proteins, lipds and CO_2.

Significant amounts of ^{14}C could be recovered from these various fractions, thereby indicating a rather large diversification of glucose metabolism in this tissue.

Furthermore, the effect of insulin at a supramaximal concentration was also tested. It could be shown that the effects of this hormone are most prominent when glucose concentrations in the incubation medium are in a physiological range, and less so at higher glucose levels.

2. Material and methods

The umbilical cords were freed of contaminating blood and sliced as previously described [2]. Batches of 8 slices (ca. 1 g) were incubated in 10 ml Krebs–Ringer phosphate buffer [3] containing 5 or 20 mM glucose (or none in the controls). A similar series of flasks contained also 1000 μU/ml of crystalline insulin/ml.

Lipids were extracted from all the slices according to Folch et al. [4].

Glycogen was extracted from 2 defatted slices by the technique of Le Barron [5], and reprecipitated thrice.

For GAG, four slices and 1/2 of the incubation medium were digested overnight at pH 1.5 with Pepsin (Merck, Darmstadt, W. Germany), followed by a 2 hr step with trypsin at pH 8.5 (same dealer). They were precipitated with cetylpyrridinium chloride (CPC) according to Scott [6].

Both glycogen and GAG were dissolved in a few drops of 5 M HCl at 90° until the solution was clear. It was then brought to 2 ml with distilled water and to 15 ml with Insta-Gel (Packard, Benelux).

Proteins were precipitated from 2 slices and 1/4th of the medium with trichloroacetic acid (TCA), and further washed several times with the same solution. The "classical" hydrolytic step (15 min at 90°) was avoided, as it solubilized a large amount of collagen,

Table 1
Recovery of radioactivity from [U-^{14}C]glucose into various fractions of the human umbilical cord (in nmoles glucose/g wet weight/3 hr).

	Glucose 5 mM			
	Insulin: 0 μU/ml	Insulin: 1000 μU/ml	Difference	*P*
CO_2	14.10 ± 0.90	15.30 ± 0.92	+1.20	N.S.
Glycogen	28.9 ± 3.3	37.5 ± 2.0	+8.6	$P < 0.05$
Glycosaminoglycans	69.0 ± 8.1	118.8 ± 12.8	+49.8	$P < 0.01$
Lipids	7.5 ± 0.4	6.9 ± 0.9	−0.6	N.S.
Proteins	48.8 ± 4.6	62.2 ± 4.8	+13.4	$P < 0.05$
	Glucose 20 mM			
CO_2	45.6 ± 1.21	46.2 ± 1.40	+0.6	N.S.
Glycogen	88.5 ± 5.5	99.9 ± 5.0	+11.4	N.S.
Glycosaminoglycans	199.4 ± 29.1	305.5 ± 30.5	+106.1	$P < 0.01$
Lipids	20.1 ± 1.2	21.7 ± 1.4	+1.6	N.S.
Proteins	150.6 ± 19.5	162.2 ± 20.0	+11.6	N.S.

14 cords were incubated in bicarbonate buffer with 0.1 μCi of labelled glucose/ml, with or without insulin. Values are means ± SEM. N.S. : non significant.

even in this short lapse of time. The precipitate was dissolved in Soluene-100 (Packard, Benelux), and counted in Insta-Gel (total volume: 15 ml).

Labelled CO_2 was determined separately in a standard Warburg apparatus. The contents of the central well (including the filter paper wick) were transferred quantitatively in scintillation counting vials with 15 ml Insta-Gel.

All counting procedures were performed in a Nuclear Mark I scintillation counter, internal standards were used for quenching corrections.

3. Results and conclusions

The results are presented in table 1, from which it is evident that the pathways studied are mainly controlled by the levels of glucose in the incubating medium.

The CO_2 output is low, and is not influenced by insulin; but these experiments were conducted in phosphate buffer, which is well known to mask some insulinic effect [7].

The more active of the metabolic routes tested is the incorporation of radioactivity from glucose into the GAG fraction. This was not surprising, since the GAG represent nearly 10% of the dry weight [8] of the cord tissue; but surprisingly enough, the biosynthesis of these compounds had never been studied heretofore in the umbilical cord, although the latter is the major source of commercial hyaluronic acid. Such a high rate of synthesis, if sustained during the whole pregnancy, would lead to the accumulation of about twice the amounts of GAG present in the tissue at term [8]. This finding suggests that the turnover rate of these polymers must at best be very slow.

The recovery of radioactivity in glycogen could also be expected, since electron microscopy reveals numerous glycogen clusters in the cells of Wharton's jelly [9]. However, its mere presence does not imply an active synthesis: it has been shown that the placenta contains large amounts of glycogen, but has lost at term the glycogenic potentialities it had earlier in pregnancy [10].

Lipids are also labelled from [U-^{14}C]glucose. This fact is also consistent with the presence of sparce lipid droplets in the cells of the jelly [9]. Although the rate of incorporation is low, it surely warrants further fractionation and dynamic studies: indeed the easily available umbilical cord might then prove a useful tool in the study of atherosclerosis and other angiopathies.

All of the metabolic pathways studied are enhanced by insulin, excepted CO_2 (already discussed) and lipids, when the surrounding glucose concentrations are in the "normal" range. By contrast, when exogenous glucose levels are higher, insulin loses its significant

effects excepted for the biosynthesis of GAG.

It is well known that the umbilical cords of diabetic pregnancies are usually grossly oedematous, and contain an excess of hexosamines [11], which reflects an overproduction of GAG with their high water-binding capacity. The maternal hyperglycaemia, partially transmitted to the foetal blood, might account for this fact. However, this hyperglycaemia can induce a foetal insulinic response, which might potentiate its effects on GAG oversynthesis. Although it is doubful that foetal insulin ever reaches levels sufficient to exert such an action, we cannot definitely rule out this hypothesis at present.

One pitfall in the present study is that the cord tissue evidently lacks an adult homologue as a control. Even aorta cannot be a safe reference tissue, since both the site of sampling [12], as well as ageing [13] modify its metabolic profile. Furthermore, the experimental conditions vary according with the authors, who do not express their data in similar units. Thus any comparison with those results can only lead to very rough estimates.

Nonetheless, the glucose metabolism by the umbilical cord tissue seems much more active and diversified than in aortic tissue. Such a finding, together with a low citric acid cycle activity [14], and a very active aerobic glycolysis [2], suggest that the cord tissue at term has retained at least a large part of its foetal metabolic potentialities.

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