Action of Sugars on Methionine Transfer in Ehrlich Ascites Carcinoma Cells

The entrance of nutrients into the cell should be considered the initial step of their further transformation. In some conditions, transport process may be the rate-limiting factor of metabolic reactions, thus controlling and regulating them. The availability of energy can limit carrier-mediated active transport[1], and the metabolic effect of several hormones consists as well in nutrient-transfer regulation through membranes[2]. These considerations led us to investigate the regulating effect of some sugars on amino acid transport.

Various sugars may act differently on amino acid transfer in divers tissue preparations[3–5]; moreover, there are many contradictory findings in this field[1,3]. Therefore, we undertook our experiments on a more simple model, viz. Ehrlich ascites carcinoma cells, using methionine, which, according to OXENDER and CHRISTENSEN[6], shares several carrier systems with other amino acids in its transport. We attempted to study many aspects of a simple system, reporting in the present paper our first results.

The method used was essentially as described by JOHNSTONE[7]. Ehrlich ascites carcinoma cells were incubated in Krebs-Ringer phosphate-buffer (pH $\doteq$ 7.4), with L-^{75}Se-selenomethionine-labelled L-methionine. The experimental data are given in the Figures 1 and 2. We followed the dynamics of transfer into the cells during the first 7 min of incubation, opposite to similar experiments which showed the effect of sugars in steady state, after 30–60 min incubation. The distribution ratio was calculated, the intercellular space being $20 \pm 2\%$. The results shown are the averages of triplicate determinations.

Sugars, when intracellular, stimulated the active transport of methionine by about 50%, as shown in Figure 1. Contrarily, the same sugars, if present in the medium (in 30 m*M* concentration), cause only a very slight inhibition on methionine transfer (in average $12 \pm 4\%$).

The exchange diffusion of L-^{75}Se-selenomethionine at 15 °C – the cells being preloaded with 10 m*M* unlabelled L-methionine – is not influenced as well by the presence in the medium (in 30 m*M* concentration), neither of glucose, nor of galactose ($5 \pm 2\%$ inhibition in average).

There was no significant difference ($\pm 4\%$), between the total water content – measured by drying to constant weight at 110 °C – of cells preloaded or not with sugars.

D-galactose and D-xylose, if present in the medium, did not alter the transfer of methionine at 60 min time intervals, opposite to D-glucose and D-mannose, which showed an increasing effect (Figure 2).

The mechanism of the stimulating effect on amino acid transfer of sugars, if present in the cell, remains to be determined.

The ineffectiveness of sugars, if present in the medium, on methionine influx and exchange diffusion, is consistent with the evidence[8], that active transport of amino acids and sugars is mediated by separate carrier mechanisms, thus excluding a carrier-mediated exchange diffusion, between amino acids and sugars.

MUNCK[9] emphasized in intestinal preparations the influence of water shift on amino acid transfer, caused by sugars, especially glucose. In our experiments, sugars did not increase the water content of cells, in agreement with the findings of SAUNDERS et al.[3]. In this way, the stimulating effect on methionine influx of sugars inside the cell, cannot be explained by the increase in water space.

Hexoses may increase amino acid transfer by energy derived from their metabolism. Thus, the increasing effect of glucose and mannose, if present extracellulary, on methionine transfer at 60 min time intervals (Figure 2), may be due to their metabolic action. In similar conditions, non-metabolized sugars such as xylose and galactose show no effect. Therefore, the stimulating effect of xylose and galactose, if present intracellularly, cannot be explained by their metabolic effect.

In a speculative manner, an allosteric effect of sugars on the amino acid carrier may be supposed, causing pos-

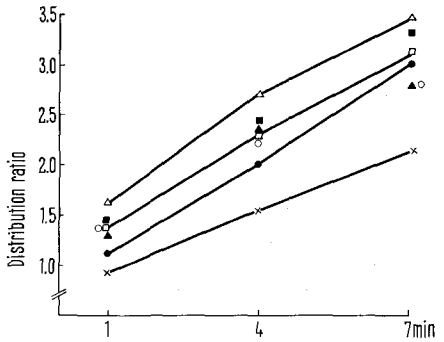

Fig. 1. Effect of sugars, intracellulary, on methionine influx into Ehrlich ascites carcinoma cells. Preloading with sugar in 30 m*M* concentration at 37 °C, 60 min. L-methionine concentration, 2 m*M*. L-^{75}Se-selenomethionine activity, 2 μc. 37 °C. × no sugar, □ D-glucose, ■ D-fructose, ▲ D-mannose, ● D-galactose, ○ D-xylose, △ D-glucosamine·HCl.

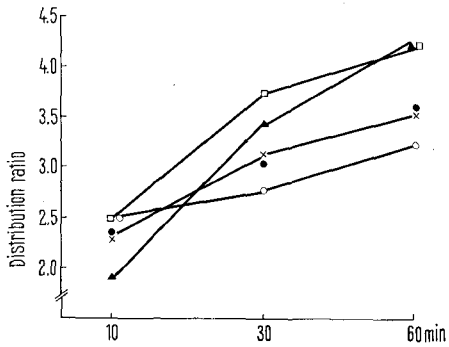

Fig. 2. Longer time intervals effect of sugars present in the medium on methionine influx into Ehrlich ascites carcinoma cells. Sugar concentration, 30 m*M*. L-methionine concentration, 2 m*M*. L-^{75}Se-selenomethionine activity, 2 μc. 37 °C. × no sugar, □ D-glucose, ▲ D-mannose, ● D-galactose, ○ D-xylose.

[1] H. NEWEY and D. H. SMYTH, Nature *202*, 400 (1964).
[2] S. GOLDSTEIN and W. J. REDDY, Biochim. biophys. Acta *150*, 733 (1968).
[3] S. J. SAUNDERS and K. J. ISSELBACHER, Nature *205*, 700 (1965).
[4] J. K. BINGHAM, H. NEWEY and D. H. SMYTH, Biochim. biophys. Acta *120*, 314 (1966).
[5] S. THIER, M. FOX, L. ROSENBERG and S. SEGAL, Biochim. biophys. Acta *93*, *106* (1964).
[6] D. J. OXENDER and H. N. CHRISTENSEN, J. biol. Chem. *238*, 3686 (1963).
[7] R. M. JOHNSTONE and P. G. SCHOLEFIELD, Biochim. biophys. Acta *94*, 130 (1965).
[8] S. G. SCHULTZ and R. ZALUSKY, Nature *205*, 292 (1965).
[9] B. G. MUNCK, Biochim. biophys. Acta *150*, 82 (1968).

sibly its dissociation from methionine, at the inner surface of membrane, in agreement with ALVARADO's hypothesis[10]. It can also be assumed that sugars act on the coupling between energy supplying mechanisms and transport, probably regulating it. The approach of this aspect of the problem, concerning hormonal action upon this process, is object to further investigation.

Zusammenfassung. Bei mit Zucker aufgeladenen Ehrlich-Ascites-Tumorzellen steigert sich das Eindringen des Methionins in die Zelle. Wenn sich dieselben Zucker im extrazellulären Raum befinden, bleibt dieser Effekt aus. Die möglichen Ursachen dieses Effektes werden besprochen.

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[10] F. ALVARADO, Science *151*, 1010 (1966).

1-^{14}C-Acetyl-Coenzyme A as Steroid Precursor in the Monkey Adrenal in vitro

^{14}C-Activity from sodium 1-^{14}C-acetate has been shown to be incorporated into steroids by various endocrine tissues[1]. The present paper reports the isolation of ^{3}H/^{14}C double labelled pregnenolone (3β-hydroxy-pregn-5-ene-20-one), DHEA (dehydro-epiandrosterone = 3β-hydroxy-androst-5-ene-17-one) and progesterone (pregn-4-ene-3,20-dione) after in vitro incubation of monkey adrenal slices with 1-^{14}C-acetyl-Coenzyme A and ^{3}H-pregnenolone. Evidence is presented of different pathways for the biosynthesis of pregnenolone and DHEA.

Methods. Fresh adrenal slices (0.5 g; 0.5 mm thickness) from a Rhesus monkey were incubated[2] in 4 ml of Krebs-Ringer[3] phosphate buffer (pH 7.4) and 2 ml of heparinized blood plasma from the same monkey. The following substances were added: 50 μC of 1-^{14}C-acetyl-CoA (1.07 μM; 111×10^6 dpm; purchased from Tracerlab Inc., Waltham, Mass., USA); 1.5 μC of 7α-^{3}H-pregnenolone (0.0001 μM; 3.3×10^6 dpm; New England Nuclear, Boston, Mass.); 0.2 ml of propylene glycol; 8 mg glucose and 50,000 IU potassium penicillin G. After 6 h the tissue was homogenized in a virtis homogenizer. Free steroids were extracted with ether, free sterols with *n*-hexane. The isolation of free neutral steroids is described in detail elsewhere[2]. It included protein precipitation, purification by solvent partitions, fourfold thin layer or paper chromatography respectively and formation of acetates[4] and 2,4-dinitrophenylhydrazones[5]. To each of the fractions of pregnenolone, DHEA and progesterone 15 mg of standard steroids were added and twice crystallized from aqueous ethanol to constant specific activity (Table I).

Results and discussion. In the hexane extract containing most of the presterols, the free sterols and a few per cent of free steroids[6], 32,900 dpm ^{3}H and 4,350,000 dpm ^{14}C were found. The compounds of this fraction were not further characterized.

Table I contains the specific activities of pregnenolone, DHEA and progesterone after addition of 15 mg standards and twofold crystallization. Table II shows the distribution of total ^{3}H and ^{14}C activity in different fractions of the ether extract.

Acetyl-coenzyme A was isolated as an intermediate in the conversion of acetate to presterols and to cholesterol[1]. The present data show that the monkey adrenal in vitro can synthesize ^{14}C labelled pregnenolone, DHEA and progesterone from 1-^{14}C-acetyl-CoA also. Further results prove that acetyl-CoA is a preferred precursor over acetate under experimental conditions. This will be published later.

Table I. Specific activity (dpm/mg) of pregnenolone, DHEA and progesterone after chromatography in specified systems, addition of 15 mg standards and twofold crystallization from aqueous ethanol

Steroid	ML[a] I ^{3}H	^{14}C (R[b])	Cr[a] I ^{3}H	^{14}C (R)	ML II ^{3}H	^{14}C (R)	Cr II ^{3}H	^{14}C (R)
Pregnenolone	19,600	71 (277)	18,700	76 (254)	19,900	74 (268)	19,100	76 (250)
DHEA	3,680	61 (6.01)	3,200	58 (5.50)	3,400	67 (5.10)	3,100	62 (5.03)
Progesterone	7,300	182 (40.0)	6,700	95 (75.1)	6,550	75 (87.00)	6,600	71 (92.8)

[a] ML, mother liquor; Cr, crystals. [b] R = dpm ^{3}H/dpm ^{14}C.

Table II. Total ^{3}H and ^{14}C activity (dpm) in different fractions of free neutral steroids as calculated to 100% recovery

Fraction	^{3}H	^{14}C	^{3}H/^{14}C (dpm/dpm)
Pregnenolone	588,000	2,350	250.00
DHEA	111,000	22,000	5.03
Progesterone	212,000	2,280	92.80
X-P-1[a]	161,000	15,300	10.50
X-P-2[a]	358,000	133,000	2.69

[a] 2 fractions more polar than DHEA, which were not further characterized.

[1] R. I. DORFMAN and F. UNGAR, in *Metabolism of Steroid Hormones* (Academic Press, New York and London 1965).
[2] P. KNAPSTEIN, J. C. TOUCHSTONE and G. W. OERTEL, Steroids *12*, 191 (1968).
[3] H. A. KREBS, Hoppe-Seyler's Z. physiol. Chem. *217*, 191 (1933).
[4] I. E. BUSH, in *The Chromatography of Steroids* (Pergamon Press, New York 1961), p. 358.
[5] L. TREIBER and G. W. OERTEL, Z. klin. Chem. *5*, 83 (1967).
[6] J. KABARA and J. MACLAUGHLIN, J. Lipid Res. *2*, 283 (1961).