endogenous release of glucose seen following intubation with a 1.2% sucrose solution.

Incidentally, preliminary experiments had demonstrated the necessity of administering a relatively dilute solution of sucrose to prevent masking this effect by providing a surplus of sugar in the intestines. The crucial period for sampling was during the first 30 min post-dose; at later time periods blood glucose levels were directly attributable to the sugar ingested.

Conclusions. It appears that this study is a direct link between the works of SAMOLS et al.[1] and MCINTYRE et al.[2]. As a result of the 3 combined studies we can postulate the apparent sequence of events occurring immediately following the ingestion of sucrose or glucose. First, by a mechanism yet unknown there is a secretion of glucagon which in turn stimulates a release of endogenous glucose. Then assuming that the release of glucose was in a phosphorylated form from glycogen stores, the increase in phosphorylated glucose in the blood would stimulate the secretion of insulin by the pancreas.

Résumé. On a examiné l'élevation de la glycémie chez les rats des 2 sexes après administration orale de sucrose. Une sécrétion de glucose semble prendre place en même temps que l'absorption de cette substance. On suggère un mécanisme basé sur l'interaction du glucagon, du glucose-6-phosphate et de l'insuline suivant l'administration de sucre. Les expériences témoins semblent éliminer la possibilité que ces résultats soient dus au stress.

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Polyribosome Patterns in EMC-Virus Infected Krebs-2 Cells and Cell Extracts

The rate of cell protein synthesis in picornavirus-infected cells is sharply decreased[1,2]. This decrease is accompanied by disintegration of cellular polyribosomes[3]. Yet the rate of incorporation of amino acids into protein in cell extracts is not so deeply inhibited as the rate of protein synthesis in intact cells[4,5]. This phenomenon still has no satisfactory explanation.

We have studied the distribution of polyribosomes in sucrose density gradient after a short pulse label of EMC-virus-infected Krebs-2 cells and after a similar labelling of cell extracts in vitro.

Krebs-2 mouse ascites carcinoma cells were obtained through courtesy of Dr. BRADA (Institute of Oncology, Brno, CSSR). EMC virus was obtained from Pasteur Institute, Paris. The virus was propagated as described by MARTIN et al.[6] with slight modifications. Cells were infected at a multiplicity of 10–15 IU/cell, washed, suspended in a maintenance medium at a concentration of 4×10^6 cells/ml and incubated at 37 °C with constant stirring. Viral hemagglutinin reached the maximal level in the cells 7 h after infection. The rate of protein synthesis at 4.5 h after infection was 3–4 times lower than in control (uninfected) cells (Figure 1).

The distribution of label and of optical density at 260 nm in sucrose density gradient after 4 min pulse label of Krebs-2 cells 4.5 h after infection and of normal cells is shown in Figure 2. In the infected cells the optical density is decreased in polyribosome area and C^{14} amino acids incorporation is correspondingly reduced. Specific activity of polyribosomes (CPM/OD_{260}) is not changed. The relation of optical density of polyribosomes to total optical density of ribosomes (P) is diminished in the infected cells. This result indicates that in EMC-virus infected Krebs-2 cells a partial degradation of cellular polyribosomes occurs. The unbroken polyribosomes are functioning at a normal rate. Similar results were reported for another picornavirus system[3,7].

In spite of the destruction of polyribosomes in the infected cells, the incorporation of C^{14} amino acids into protein in cell extracts is only slightly inhibited (Table). The inhibition is stronger if the time of incubation with the label is short. With longer periods of incubation, the incorporation in the extracts of infected cells is almost equal to the incorporation in the extracts of normal cells. In the intact cells at this stage of infection, the rate of protein synthesis is deeply inhibited (Figure 1).

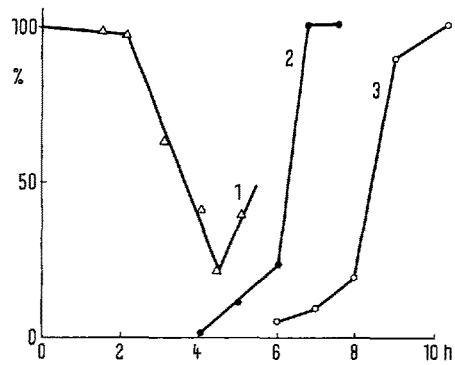

Fig. 1. Rate of protein synthesis in EMC-infected Krebs-2 cells at different time intervals after infection. (1) C^{14} amino acids incorporation into protein (the incorporation in normal cell is taken for 100%). (2) Accumulation of viral hemagglutinin in the cells (% of maximal level). (3) Cell death (% of dead cells as determined with trypane blue staining).

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In the next series of experiments we used sucrose density gradient centrifugation after a short pulse label of cell extracts (in vitro). The extracts were labelled for 2.5 min and centrifuged in 5–20% sucrose density gradient. The distribution of optical density and of radioactivity (Figure 3) was close to the pattern obtained with in vivo labelling.

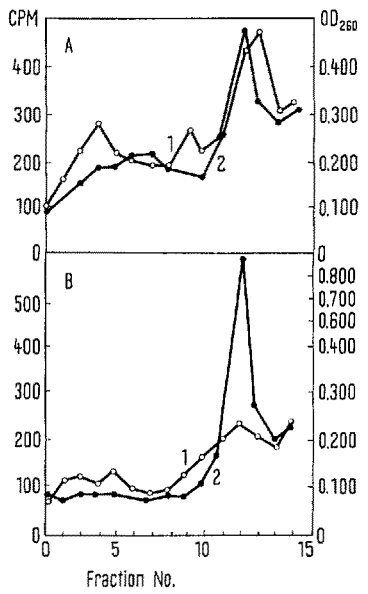

Fig. 2. Fractionation of ribosomes in sucrose density gradient after a short pulse label of intact Krebs-2 cells. (A) Uninfected cells. CPM/OD_{260} 1249; *P*, 0.60. (B) Cells 4.5 h after EMC-virus infection. CPM/OD_{260} 1333; *P*, 0.37. (1) radioactivity; (2) optical density. Cells were labelled for 4 min with a mixture of C^{14} amino acids and destroyed in a glass-tephlone homogenizer in 0.01 *M* $MgCl_2$. *Tris*-buffer, sucrose and KCl were added to the homogenate (final concentrations: 0.15 *M* sucrose, 0.025 *M* KCl, 0.05 *M* *Tris*-buffer pH 7.6). Nuclei and mitochondria were deposited and sodium deoxycholate added (final concentration 0.5%). The extract was layered on top of 5–20% sucrose density gradient and centrifuged in Spinco L SW-25 rotor for 90 min.

C^{14} amino acids incorporation into protein in cell extracts of EMC-infected and uninfected Krebs II cells

No.	Time of incorporation	C^{14} amino acids incorporation (CPM) %		
		Extract of uninfected cells	Extract of infected cells	
1	4	3232	1820	57
	40	9135	8281	90
2	4	1080	765	69
	25	1675	1420	85
3	50	2370	2230	94

Reaction mixture (total volume 1.1–1.3 ml) contained 3 μM of ATP, 0.05 μM of GTP, 6.25 μM of *Tris*-buffer pH 7.6, 25 μM of KCl, 6.25 μM of NaCl, 5 μM of $MgCl_2$, 75 μM of sucrose, 80 μM of NH_4Cl, 10 μM of phosphoenolpiruvate, 50 μg of piruvate kinase, a mixture of C^{14} amino acids and an amount of postmitochondrial cell extract containing 1.5 mg of protein. After incubation for different time intervals at 37 °C, the samples were treated with equal volume of 10% trichloroacetic acid, washed and counted in liquid scintillation counter.

It was suggested[8] that in picornavirus-infected cells ribosomes fail to get attached to cellular mRNA, because initial codons are blocked by a component of viral origin. One may assume that in an in vitro system this component dissociated from mRNA molecules, or that in the conditions we used translation in vitro may be started from codons other than initial. In any of these cases the translation in the extracts of infected cells should be approximately as effective as in the extracts of normal cells. A difference in the rate of protein synthesis will be registered only at the beginning of incubation, when the attachment of ribosomes to mRNA is not completed. Our results are in good agreement with this assumption.

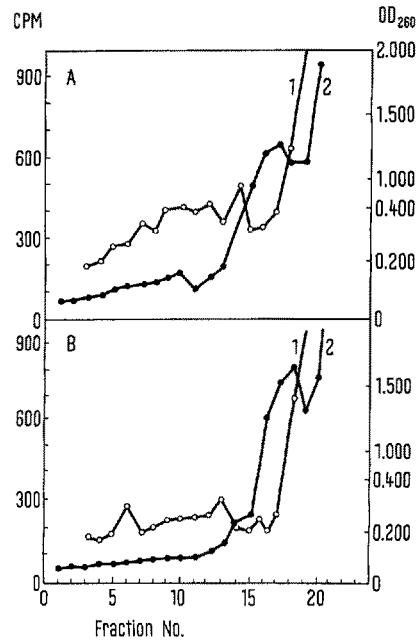

Fig. 3. Fractionation of cytoplasmic extracts of Krebs II cells in sucrose density gradient after in vitro incorporation of C^{14} amino acids. (A) Extract of uninfected cells. *P*, 0.30. (B) Extract of infected cells. *P*, 0.17. (1) Radioactivity; (2) optical density.

Выводы. В клетках Кребс-2, зараженных вирусом энцефаломиокардита, наблюдается распад клеточных полирибосом. При включении C^{14}-аминокислот в белок in vitro в экстрактах зараженных и незараженных клеток разница в величине включения (а также в величине оптической плотности в районе полириосом) при короткой импульсной метке оказывается близкой к картине, наблюдаемой при синтезе белка in vivo, в интактных клетках. При длительной инкубации с меткой различие в величине включения аминокислот в белок в экстрактах зараженных и незараженных клеток почти исчезает.

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Ivanovsky Institute of Virology and Institute of Molecular Biology, Moskwa (USSR), 16 May 1967.

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