Endogenous Glucose Release Stimulated by Oral Sucrose Administration in Rats

Physiological chemistry and its alteration from the norm after various stimuli are the basis of the diagnosis of various disorders. An example of this method is the glucose tolerance in suspected diabetics. There, only the shape of the curve representing blood glucose levels has received much attention. A thorough search of the literature did not reveal any study concerning the source of glucose necessary for any rise in blood sugar levels. Apparently we have taken for granted that this rise is directly attributable to the ingested glucose and its absorption. However, the rapidity of this response appeared to us to raise some doubt as to this explanation.

Recent experiments by SAMOLS et al.[1] have shown an initial secretion of glucagon following a glucose challenge to fasted patients. This increase in plasma glucagon appeared to precede an elevation in plasma insulin and blood glucose. Another interesting report by MCINTYRE et al.[2] seems to be closely related to the work cited above (as well as to the work reported here). MCINTYRE and his coworkers[2] found a much higher plasma insulin level following an intrajejunal administration of glucose than following an i.v. administration of glucose. They concluded that there appears to be a mechanism in the intestinal wall which initiates a concomitant insulin release.

The following experiment was designed to test whether the rise in glycemia following oral ingestion of sugar is totally attributable to the absorbed sugars.

Materials and methods. Sprague–Dawley rats of both sexes weighing between 180 and 220 g were used throughout the experiments. They were fasted for 16 h.

All animals were dosed by oral intubation with 5.0 ml of a 1.2% uniformly labeled ^{14}C sucrose solution of known specific activity following the taking of 2, 20 λ blood samples to be used as time zero or control values. Additional samples were taken 10, 20 and 30 min after dosing. Again 2, 20 λ samples were taken at each time period, 1 sample for spectrophotometric assay for total blood sugar by the micro-micro-method reported by KNIGHTS et al.[3] and 1 for radiometric assay for the detection of that portion of the total blood sugar attributable to the ingested sucrose.

All blood samples were taken from the lateral tail vein of the rats, and where possible from a single incision to minimize the occurance of experimental stress.

Samples for radiometric assay were placed in 17 ml of XDC (Xylene, Dioxane and Cellosolve 1:3:3, containing 8% Naphthalene, 1% PPO and 0.05% POPOP) and counted in a Nuclear-Chicago liquid scintillation system following treatment with barium hydroxide and zinc sulfate to remove all extraneous substances. All samples were counted for at least 2, 20 min periods and the average cpm and channel ratios were used in calculating the increase in blood sugar attributable to the ingested meal as follows:

$$\frac{\text{cpm} - \text{Bkg}^{a}}{\text{Efficiency}^{b}} \times \frac{100\text{ ml}}{0.02\text{ ml}} \times \frac{1}{\text{Sp. Ac. (dpm/mg)}^{c}} = \text{mg/100 ml}.$$

[a] Background = cpm of time zero sample. [b] Efficiency was determined by channel ratio method using quenched standards. [c] Specific activity was determined for the dosing solutions as they were prepared.

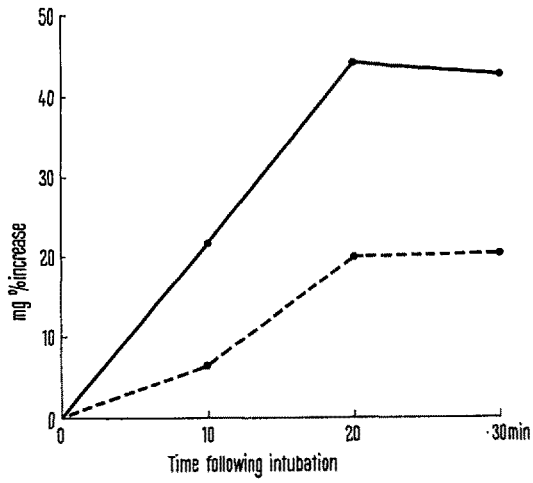
Results and discussion. The Table lists the results obtained in studies involving both the male and female rats. This comparison between the means for total increase and the increase due to absorbed glucose suggests that there is an initial release of endogenous glucose. This is confirmed by calculation of the p values obtained in Student's t determinations for paired data. The Figure graphically illustrates the results obtained with female rats. The results with male rats are similar.

The possibility that stress causes the apparent endogenous release of glucose by the action of sympathetic stimulation was ruled out by additional control experiments in which the separate effects of serial sampling, restraint, mechanical intubation, stomach distention (intubated 5.0 ml distilled water) and a solution of 0.04% sodium cyclamate (approximate sweetness of a 1.2% sucrose solution) were measured. Although a slight rise in blood sugar was apparent in all of the experiments it was comparatively small in relationship to the proposed

Increase in blood sugar levels following intubation of 5.0 ml of a 1.2% sucrose solution to rats

No.	Time post-dose	Increase in blood sugar			
		Total	From ingested sucrose	Difference	P
15[a]	10 min	21.66	6.46	15.20	0.00587
15[a]	20 min	44.19	20.25	23.94	0.00000
16[a]	30 min	42.73	21.45	21.28	0.00003
15[b]	10 min	33.80	5.60	28.20	0.00000
15[b]	20 min	34.07	9.08	24.99	0.00007
15[b]	30 min	28.20	10.63	17.57	0.00336

[a] Female rats, [b] male rats.



Blood sugar levels in rats as affected by oral intubation of 5.0 ml of a 1.2% C-14 sucrose solution. —— total increase; - - - increase from ingested sucrose.

[1] E. SAMOLS, J. TYLER, G. MARRI and V. MARKS, Lancet *2*, 1257 (1965).

[2] N. MCINTYRE, C. D. HOLDSWORTH and D. S. TURNER, J. clin. Endocr. Metab. *25*, 1317 (1965).

[3] E. M. KNIGHTS JR., R. P. MACDONALD and J. PLOOMPUU, *Ultramicro Methods for Clinical Laboratories* (Grune and Stratton, New York 1957).

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).

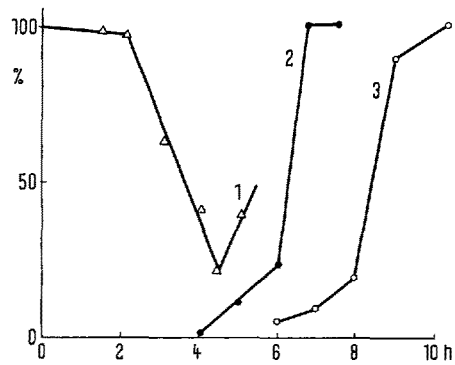

Fig. 1. Rate of protein synthesis in EMC-infected Krebs-2 cells at different time intervals after infection. (1) C^{14} amino acids incorporation into protein (the incorporation in normal cell is taken for 100%). (2) Accumulation of viral hemagglutinin in the cells (% of maximal level). (3) Cell death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

1 R. M. FRANCLIN and D. BALTIMORE, Cold Spring Harb. Symp. quant. Biol. *27*, 175 (1962).
2 E. F. ZIMMERMAN, M. HEETER and J. E. DARNELL, Virology *19*, 400 (1963).
3 S. PENMAN, K. SIERRER, Y. BECKER and J. E. DARNELL, Proc. natn. Acad. Sci. USA *49*, 654 (1963).
4 J. M. KERR, E. M. MARTIN, M. G. HAMILTON and T. S. WORK, Cold Spring Harb. Symp. quant. Biol. *27*, 259 (1962).
5 J. J. HOLLAND and J. A. PETERSON, J. molec. Biol. *8*, 556 (1964).
6 E. M. MARTIN, J. MALEC, S. SVED and T. S. WORK, Biochem. J. *80*, 585 (1961).
7 D. F. SUMMERS and J. V. MAIZEL, Information Exchange group 7, 678 (1966).