Effect of Urethan on the Glycogen and RNA Content of Liver Parenchymal Cells. A Cytophotometric Study

Urethan in a single dose decreases the liver RNA content of newborn and young adult mice[1,2] and also the liver glycogen content of rats[3]. However, no further information on the effect of chronic drug treatment on these compounds has been described.

Materials and methods. 30 male adult mice weighing about 20 g were divided into 2 groups. In the experimental group, urethan was administered i.p. (1.0 mg/g body weight) once daily. Control group was injected with redistilled water. Animals were killed by decapitation 24 h after the last injection on the 1st, 3rd, 5th, 7th and 9th day. Specimens of liver to be compared were embedded in paraffin wax at the same time. Sections of 7 µm thickness of pieces fixed in acetic-alcohol-formalin were mounted on the same slide and stained by periodic acid-Schiff (PAS) method[4]. Gallocianin-chrome alum technic[5] was used in sections of 3 µm thickness for RNA. Diastase[4] and ribonuclease[6] digestion was carried out in order to check the specificity of the histochemical reaction. The absorption values of individual cells were recorded using a Zeiss cytophotometer MPM under the following conditions: ×40 Apo. objective (N.A. 1.00); Z type condenser (N.A. 1.3); Köhler ilumination; light with wavelength of 540 nm for glycogen and 580 nm for RNA selected by a Zeiss graduated interference filter. For measurements an optical cross-section area of 3.2 µm in diameter was selected from the cytoplasm of each hepatocyte. At least 100 hepatic cells were measured on each section.

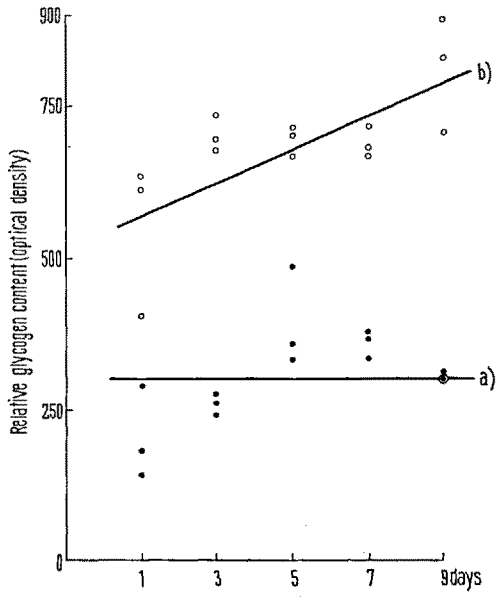

Fig. 2. Cytophotometric values of glycogen for control and urethan-treated group. a) Mean value for control group. b) Least squares linear regression for urethan-treated group. ●, Individual values for control group; ○, individual values for urethan-treated group.

Table I. Regression analysis for cytophotometric measurements of RNA in hepatocytes of urethan-treated mice

Source of variation	Sum of square	Degree of freedom	Mean square	F
Regression	0.0248	1	0.0248	49.075*
About regression	0.0041	3	0.0014	2.719
Within groups	0.0051	10	0.0005	
Total	0.0340	14		

* significant ($P < 0.01$)

Table II. Regression analysis for cytophotometric measurements of glycogen in hepatocytes of urethan-treated mice

Source of variation	Sum of square	Degree of freedom	Mean square	F
Regression	0.0757	1	0.0757	13.781*
About regression	0.0259	3	0.0086	1.574
Within groups	0.0549	10	0.0055	
Total	0.1566	14		

* significant ($P < 0.01$).

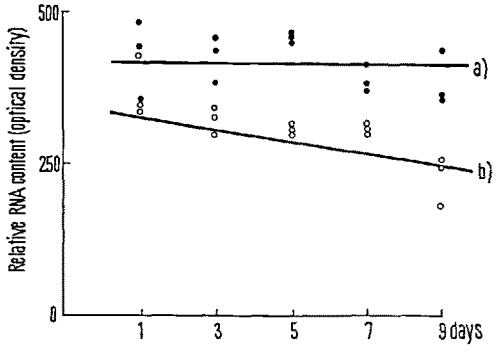

Fig. 1. Cytophotometric values of RNA for control and urethan-treated group. a) Mean value for control group. b) Least squares linear regression for urethane-treated group. ●, Individual values for control group; ○, individual values for urethan-treated group.

Results. The cytophotometric measurements of RNA and glycogen for control and urethan-treated mice were submitted to regression analysis. The basis for the variance analysis consisted of each time period. The individual values for RNA and glycogen are represented by diagrams (Figures 1 and 2). Tables I and II corresponding to the urethan-treated group give the values for linearity and regression tests for RNA and glycogen, respectively.

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Both regression tests were significant at a 1% level. The regression tests for the control group were not significant, so the respective lines for this group in diagrams represent only the average values.

Discussion. The longer exposure to urethan anaesthesia produced a statistically significant ($P < 0.01$) increase in the liver glycogen content of intact mice. Urethan in a single dose decreases the liver glycogen content of rats[3] being that effect interpreted as a consequence of the liberation of catechol amines. Urethan really stimulates the secretion of adrenaline from the adrenal medulla[7], and it is reasonable to suppose that under urethan intoxication there is a continuous release of catechol amines from the adrenal glands with a consequent reduction in the circulating catechol amines due to depletion of the gland. This fact may explain the results achieved for glycogen. Considering RNA, the decreased cytophotometric values observed are in accordance with the biochemical results obtained by other authors[1,2].

Résumé. Utilisant la méthode cytophotométrique on a observé l'accroissement du glycogène et la réduction de ARN dans le foie de souris soumises au traitement toxique par l'uréthane.

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Formation and Decay of Virus-Specific Polysomes in vitro

It has been shown earlier that RNA extracted from Newcastle disease virus (NDV) infected cells forms in vitro virus-specific polysomes after contact with chick embryo ribosomes[1]. However, a mixture of both viral and cellular RNAs was used in that study, which hampered interpretation of the results obtained. The present report records the study of the formation of polysomes of virion NDV RNA and their fate in the process of translation.

Experiments were performed with NDV RNA[2] extracted by the detergent phenol method[3] and precipitated by ethanol with 0.2% sodium acetate. The method for preparation of chick embryo ribosomes and protein-synthesizing system have been described elsewhere[1]. To destroy endogenous polysomes, ribosomes in all experiments were preincubated at 32°C for 40 min with amino acids, energy-regenerating system and cell fraction S100, containing transport RNA and other components of protein-synthesizing system.

In the first series of experiments, NDV RNA was added to the incubation medium for various intervals; the mixture was supplemented with C^{14}-algae hydrolysate and at the end of incubation period was rapidly chilled; polysome formation was analyzed by centrifugation in linear sucrose density gradients. Gradient fractions were sedimented with 10% trichloroacetic acid (TCA), the

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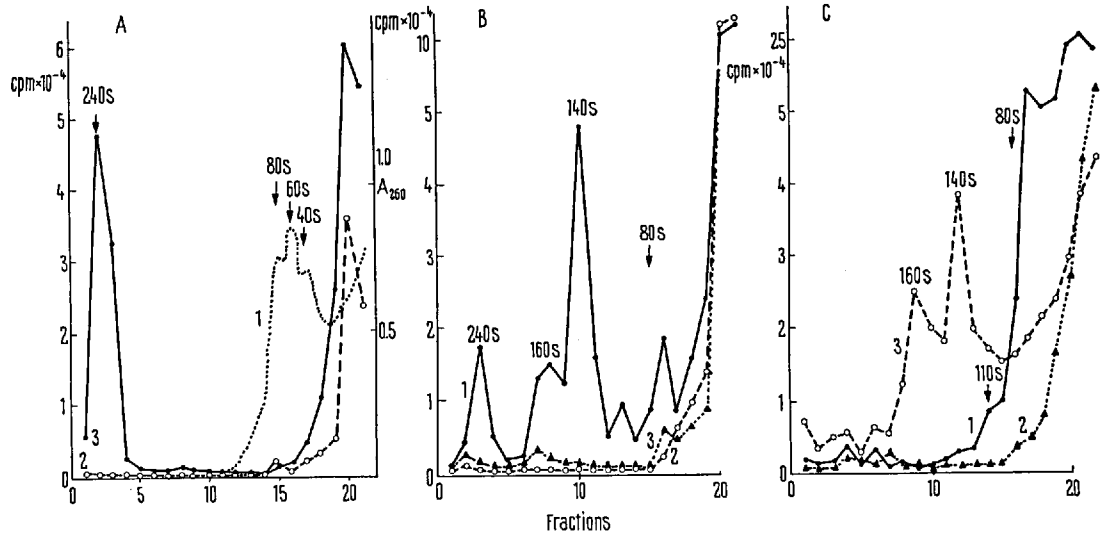

Fig. 1. Kinetics of formation and decay of virus-specific polysomes after incubation of NDV RNA with chick embryo ribosomes and C^{14}-amino acids in cell-free protein-synthesizing system at 32°C for 5 (A), 15 (B) and 45 min (C). Samples were centrifuged in linear 17–40% sucrose density gradients at 25,000 g/min for 1 h and 45 min. Designations: A) Optical density at 260 nm (I), radioactivity of fractions without (2) and with (3) the addition of NDV RNA into the system. B) Radioactivity of fractions with the addition of NDV RNA into the system (1) as well as 0.02 *M* EDTA (2) and 50 μg/ml of pancreatic ribonuclease (3). C) Radioactivity of fractions with the addition into the system of NDV RNA as well as 50 μg/ml of puromycin (2) and 100 μg/ml cycloheximide (3).