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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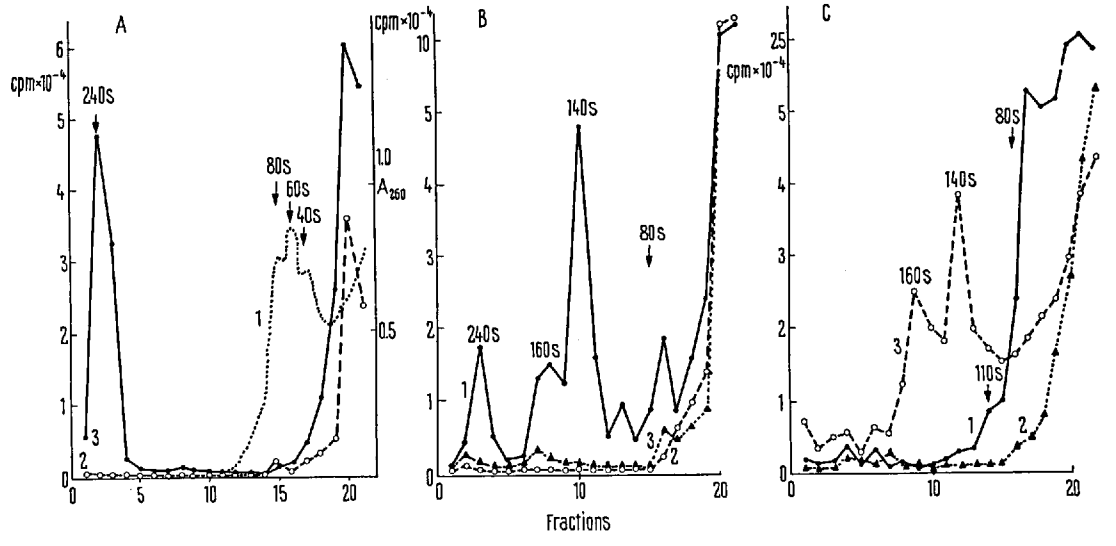

Fig. 1. Kinetics of formation and decay of virus-specific polysomes after incubation of NDV RNA with chick embryo ribosomes and C^{14}-amino acids in cell-free protein-synthesizing system at 32°C for 5 (A), 15 (B) and 45 min (C). Samples were centrifuged in linear 17–40% sucrose density gradients at 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).

precipitates were washed with 5% TCA on Millipore filters. Radioactivity was measured in a Packard-Tricarb liquid scintillation counter.

Figure 1 presents the results of this series of experiments indicating that preincubation of ribosomes is accompanied by disintegration of endogenous polysomes into monosomes and ribosomal subunits (Figure 1A–1) in which protein synthesis does not occur (Figure 1A–2). The addition of NDV RNA to the system is coincident with the formation of polysomes with a sedimentation coefficient of 240S (Figure 1A–3). 15 minutes later, a part of the heavy polysomes disappears and polysomes with sedimentation coefficents of 140 to 160S are prevalent (Figure 1B–1), which are destroyed in the presence of EDTA (Figure 1B–2) and ribonuclease (Figure 1B–3). Virus-specific polysomes break up in 45 min (Figure 1C–1); the addition of puromycin into the incubation mixture accelerates the process (Figure 1C–2) and the addition of cycloheximide slows it down (Figure 1C–3). Dissociation of polysomes is due to the synthesis of proteins, the amount of which increased in the upper gradient fractions.

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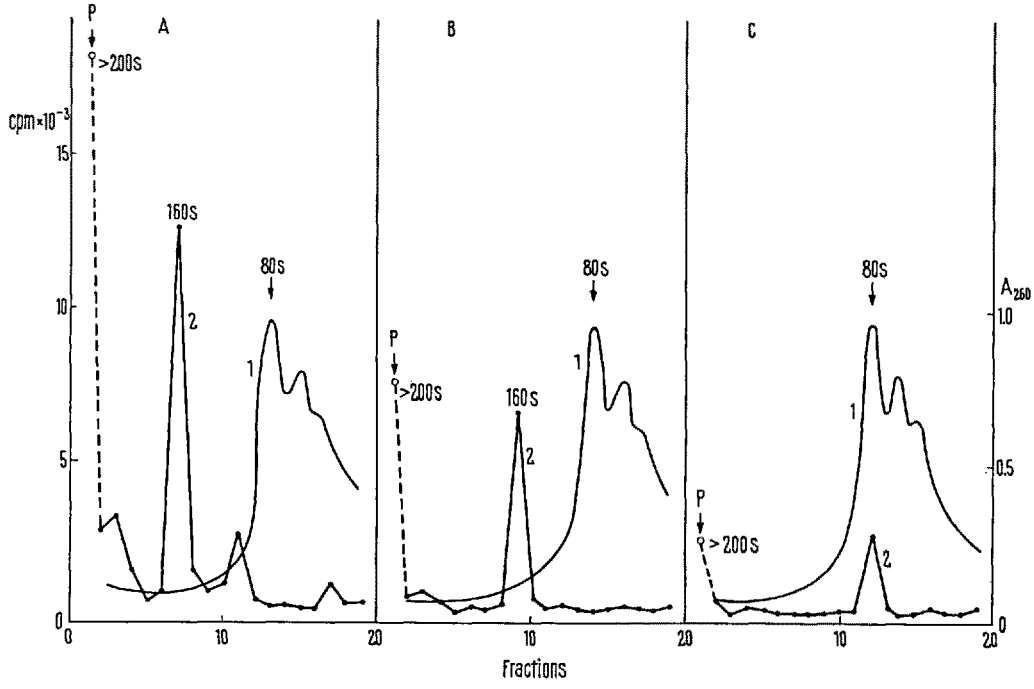

Fig. 2. Kinetics of formation and decay of virus-specific polysomes after incubation of NDV^{3}H-RNA with chick embryo ribosomes in protein-synthesizing system for 5 (A), 15 (B) and 45 min (C). Conditions of the experiment are the same as above. Designations: 1. Optical density at 260 nm; 2. Radioactivity of gradient fractions. The first gradient fractions are the pallets (shown by the arrows).

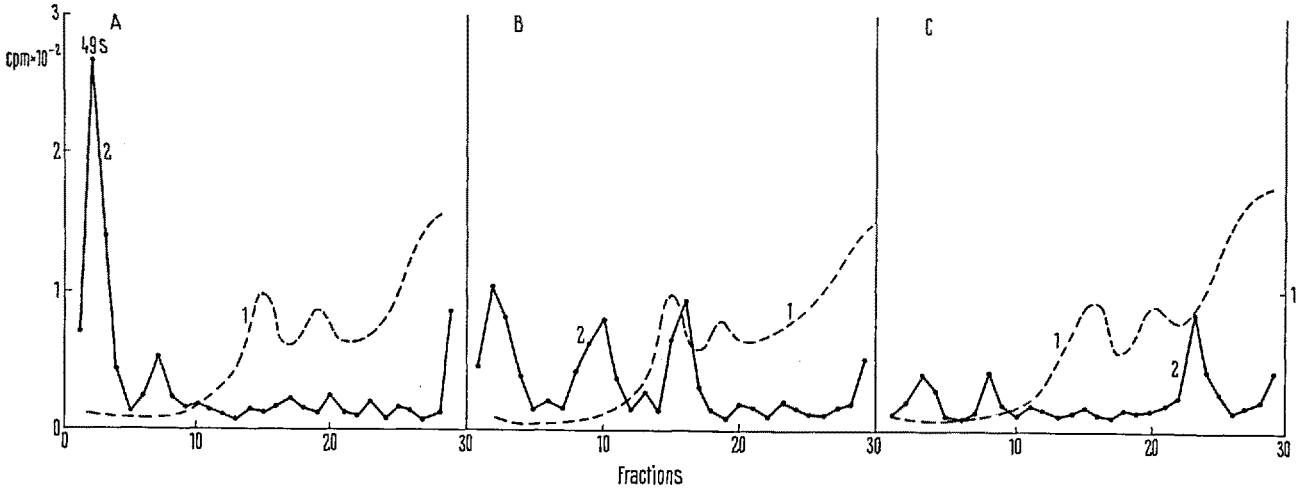

Fig. 3. Kinetics of degradation of NDV H^3-RNA inoculated with the cell-free protein-synthesizing system and extracted from it by phenol 1 (A), 15 (B) and 45 min (C) after incubation. Samples were centrifuged in linear sucrose density gradients at 18,000 g/min for 14 h. Designations: 1. Optical density at 260 nm. 2. Radioactivity of gradient fractions.

A similar picture was observed when ^{3}H-uridine-labelled NDV RNA was added to the system. After 5-min incubation, virus-specific polysomes with sedimentation coefficents of 160S and 200S were distinctly manifested (Figure 2A); a considerable part of these degraded in 15 min (Figure 2B), and within 45 min debris of viral RNA was mainly found on monosomes (Figure 2C).

Figure 3 presents degradation of viral RNA in the process of translation. In a series of experiments NDV ^{3}H RNA was incubated in the cell-free protein-synthesizing system within the same time intervals, thereafter the reaction was stopped by rapid cooling. RNA was extracted from the incubation mixture by phenol and analyzed in sucrose density gradients. The data adduced indicate that RNA is homogeneous immediately after its inoculation into the incubation mixture and has a sedimentation constant of about 50S (Figure 3A). A considerable part of RNA breaks up into smaller fragments within 15 min (Figure 3B) and decays almost completely after 45 min incubation (Figure 3C).

Thus, NDV RNA forms polysomes after contact with ribosomes and their subunits, the decay of these polysomes in the process of translation is coincident with degradation of viral RNA.

ВЫВОДЫ. РНК, эстрагированная из вирионов болезни Ньюкасла (ВБН), образует вирус-специфические полисомы в инкубационной смеси, содержащей рибосомы куриных эмбрионов, фракцию S100 и остальные компоненты бесклеточной белок-синтезирующей системы. В процессе трансляции происходит деградация вирусной РНК и распад полисом.

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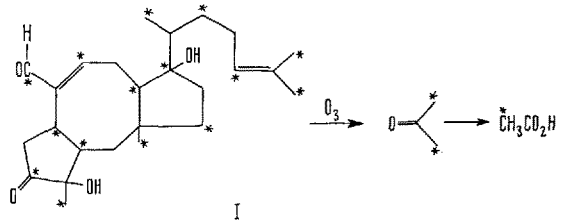
The Role of Glycine in the Biosynthesis of a Terpene[1]

Prompted by some recent publications[2-6] in this and other journals, we wish to record here our own findings in a related area. In the course of our studies on biosynthesis using stable isotopes and spectral methods[1,7-9] we became interested in testing whether under favorable circumstances glycine can have a significant role in the biosynthesis of terpenoids. For our investigation we selected ophiobolin B[10,11] (I), a sesterterpene with a regular isoprenoid skeleton devoid of *O*-Me groups or additional methyl or ethyl sidechains. That I can be derived from mevalonic acid has been conclusively demonstrated[12] by using ^{14}C, ^{3}H and ^{18}O labels.



I

We report in Table I our observations on the incorporation of some labeled substrates into I by the fungus *Cochiobolus miyabeanus* grown in a chemically defined medium[13]. Since acetic acid, serine, and pyruvic acid are known to be precursors of mevalonic acid via acetyl CoA[14], their ready incorporation into the mevalonoid terpene ophiobolin B is to be expected. It is noteworthy, however, that radioactivity is efficiently incorporated into I by [2–^{14}C] glycine, [^{14}C-methyl] methionine and [^{14}C-methyl] sarcosine.

The findings from a closer examination of the incorporation of a few substrates into I are listed in Table II. In seemingly identical experiments [2-^{14}C]-glycine appeared to be incorporated much better than [1-^{14}C] glycine. When a mixture of [2-^{13}C] glycine and [1-^{14}C] glycine was added to the substrates, the ^{13}C-incorporation[7] was one magnitude higher than the ^{14}C-incorporation.

Ozonization of ophiobolin B derived from [2-^{14}C] glycine gave acetone (spec. gct. 1.68×10^{6} dpm/m*M*) containing 14.4% (calcd. 13.6%) of the activity of I. Kuhn-Roth oxidation of this acetone produced acetic acid (sp. act. 0.81×10^{6} dpm/m*M*) with one half of the activity of the acetone. These observations indicating the alternate labeling pattern shown in I is further supported by the finding that Kuhn-Roth oxidation of a sample of I from [2-^{14}C] glycine produced 2.6 moles of labeled acetic acid (Canonica et al.[12]).

Radioactivity from [3-^{14}C] serine is incorporated efficiently and corresponds to the alternate pattern shown in

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