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.

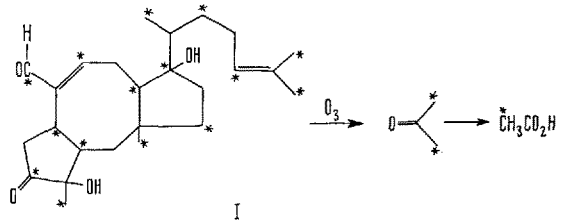
ВЫВОДЫ. РНК, эстрагированная из вирионов болезни Ньюкасла (ВБН), образует вирус-специфические полисомы в инкубационной смеси, содержащей рибосомы куриных эмбрионов, фракцию S100 и остальные компоненты бесклеточной белок-синтезирующей системы. В процессе трансляции происходит деградация вирусной РНК и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

V. Zhdanov jr. and V. M. Zhdanov

D. I. Ivanovsky Institute of Virology,
USSR Academy of Medical Sciences,
Gamaleya St. 16, Moskwa D-98 (USSR), 7 May 1971.

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

[1] a) *Studies on Biosynthesis.* Part V. for Part IV, see M. Anchel, A. K. Bose, K. S. Khanchandani and P. T. Funke, Phytochem. *9*, 2135 (1970). b) Presented in part at the 3rd Natural Products Symposium, University of West Indies, Jamaica, January 1970.

[2] D. Groger, W. Maier and P. Simchen, Experientia *26*, 820 (1970).

[3] J. P. Kutney, J. F. Beck, V. R. Nelson, K. L. Stuart and A. K. Bose, J. Am. chem. Soc. *92*, 2174 (1970).

[4] J. R. Gear and A. K. Garg, Tetrahedron Lett. (*1968*), 141.

[5] A. K. Garg and J. R. Gear, Tetrahedron Lett. (*1969*), 4377.

[6] A. K. Garg and J. R. Gear, Chem. Commun. (*1969*), 1447.

[7] A. K. Bose, K. G. Das, P. T. Funke, I. Kugajevsky, O. P. Shukla, K. S. Khanchandani and R. J. Suhadolnik, J. Am. chem. Soc. *90*, 1038 (1969).

[8] A. K. Bose, K. S. Khanchandani, R. Tavares and P. T. Funke, J. Am. chem. Soc. *90*, 3593 (1968).

[9] A. K. Bose, K. S. Khanchandani, P. T. Funke and M. Anchel, Chem. Commun. (*1969*), 1347.

[10] L. Canonica, A. Fiecchi, M. Galli Kienie and A. Scala, Tetrahedron Lett. (*1966*), 1211.

[11] S. Nozoe, M. Morisaki, K. Tsuda, Y. Litaka, N. Takahashi, S. Tamura, K. Ishibashi and M. Shirasaka, J. Am. chem. Soc. *87*, 4968 (1965).

[12] L. Canonica, A. Fiecchi, M. Galli Kienie, S. M. Ranzi and A. Scala, Tetrahedron Lett. (*1966*), 3035. – S. Nozoe, M. Morisaki, K. Tsuda and S. Okuda, Tetrahedron Lett. (*1967*), 3365. – L. Canonica, A. Fiecchi, M. Galli Kienie, B. M. Ranzi and A. Scala, Tetrahedron Lett. (*1967*) 3371. – L. Canonica, A. Fiecchi, M. Galli Kienie, B. M. Ranzi and A. Scala, Tetrahedron Lett. (*1967*), 4657.

[13] Two days after incubation as a shake culture, the mycelia were centrifuged, washed and resuspended in 2% sucrose solution with added labeled substrate at PH 4.5; ophiobolin B was harvested after 2 days[12].

[14] T. A. Geissman and D. H. G. Crout, *Organic Chemistry of Secondary Plant Metabolites* (Freeman, Cooper and Co., San Francisco 1969).

Table II. Isotope incorporation from labeled compounds

Substrate	Amount μc	Added mg	Spec. activity of substrate (dpm/mM: 1s)	Spec. activity of metabolite (dpm/mM: 1 m)	Overall isotope dilution[15]: 1s/1m
Metabolite: Ophiobolin B [a]					
[2-^{14}C] Acetate	10	2.8	667×10^6	110×10^5	61
[1-^{14}C] Acetate	10	2.6	700×10^6	259×10^4	250
[2-^{14}C] Glycine	10	2.4	690×10^6	165×10^5	42
	5	1.1	757×10^6	102×10^5	73
	10	2.2	755×10^6	97×10^5	78
[1-^{14}C] Glycine	10	2.3	724×10^6	462×10^3	1,580
	5	1.25	666×10^6	418×10^3	1,600
	5	1,25	666×10^6	306×10^3	2,180
[1-^{14}C] Glycine	5		166×10^6	592×10^2	286
[2-^{13}C] Glycine	^{13}C = 50%	5		^{13}C = 3%	17
DL-[3-^{14}C]	10	2.3	101×10^7	153×10^5	66
DL-[1-^{14}C] Serine	10	2.3	101×10^7	293×10^3	3,300
[^{14}C Formate]	10	2.1	719×10^6	205×10^4	340

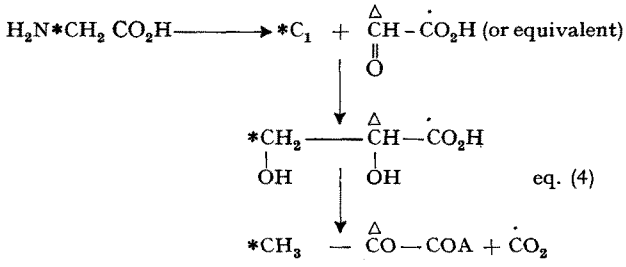
[a] The procedure described in ref.[13] was used for the incorporation experiments.

I. Ozonization was employed to obtain acetone which was oxidized to acetic acid to determine this pattern.

A number of pathways from glycine to acetyl CoA can be conceived[16,17]. Our findings reported here exclude the possibility that glycine is deaminated to acetic acid or some other two-carbon acid which is converted directly to acetyl CoA.

The labeling pattern observed by us is inconsistent with the formation of 1 mole of serine from 2 moles of labeled glycine[16]. The observed labeling pattern, however, is in accord with eq. (4).

This pathway is consistent with the observation by GOODWIN et al.[18] that [2-^{14}C] glyoxylic acid is an efficient precursor for introducing radioactivity in β-carotene in chloroplasts but [1-^{14}C] gloxylic acid is not. Incorporation



eq. (4)

of radioactivity into I from sarcosine, formate and methionine which are known precursors of one carbon units, is also in agreement with eq. (4).

Since it is believed that amino acids are among the first organic compounds to be formed in prebiotic times, the easy incorporation of carbon from glycine and serine into a terpenoid is not surprising.

Studies are in progress using mixtures of ^{14}C- and ^{13}C-labeled substrates to obtain a more detailed understanding of the biosynthesis of isoprenoids from amino acids.

Zusammenfassung. Mit doppelt markiertem Glycin konnte gezeigt werden, dass das Kohlenstoffatom der Methylgruppe viel besser als das Kohlenstoffatom der Karboxylgruppe in die Isoprengruppe des Ophiobolins B aus *Cochiobolus miyabeanus* eingebaut wird. Die Radioaktivität wird in Ophiobolin B eingebaut, wenn die folgenden Verbindungen verwendet werden: [3-^{14}C]-Serin, [^{14}C-Methyl]-Sarcosin, [^{14}C-Methyl]-Methionin, und [^{14}C] Formate.

A. K. BOSE, K. S. KHANCHANDANI and B. L. HUNGUND[19]

Department of Chemistry and Chemical Engineering, Stevens Institute of Technology Hoboken (New Jersey 07030, USA), 14 June 1971.

Table I. Incorporation of radioactivity in Ophiobolin B from labeled compounds in presence of glycine

^{14}C-labeled compound [a]	Incorporation (%)
[2-^{14}C] Acetate	2.7
[2-^{14}C] Glycine	1.7
[3-^{14}C] Serine	4.2
[^{14}C-Methyl] Methionine	1.5
[^{14}C-Methyl] Sarcosine	2.9
[2-^{14}C] Pyruvate	4.1
[3-^{14}C] Pyruvate	6.8
[2-^{14}C] Acetate [b]	1.1
[3-^{14}C] Serine [c]	3.3

[a] Each flask contained 5–25 μc of ^{14}C-labeled compound and 5–10 mg of [2-^{13}C] glycine. [b] Glycine was omitted but 5 mg of [2-^{13}C] acetate was added to each flask. [c] Glycine was omitted but 5 mg of L-serine was added to each flask. The procedure described in ref.[13] was used for the incorporation experiments.

[15] The 'Overall isotope dilution' does not take into account the number (n) of sites in the metabolite that can bear the label; the true 'isotope dilution' will be n times larger.

[16] T. SATO, H. KOCHI, Y. MOTOKOWA, H. KAWASAKI and G. KIKUCHI, J. Biochem. *65*, 63 (1969). – S. P. J. SHAH and E. A. COSSINS, Phytochemistry *9*, 1545 (1970). – H. KOCHI and G. KIKUCHI, Archs Biochem. Biophys. *132*, 359 (1969). – E. A. COSSINS and S. K. SINHA, Biochem. J. *101*, 542 (1966).

[17] S. P. J. SHAH and L. J. ROGERS, Biochem. J. *114*, 395 (1969).

[18] S. P. SHAH, L. J. ROGERS and T. W. GOODWIN, Biochem, J. *103*, 52 P (1967).

[19] The support of this research by Stevens Institute of Technology is gratefully acknowledged. We wish to thank Dr. J. H. DAVIS and Prof. L. Z. POLLARA for their interest and encouragement and Drs. H. LEVY, P. T. FUNKE, M. S. MANHAS, P. K. BHATTACHARYYA and M. ANCHEL for valuable discussions and help with some of the experiments. We are particularly thankful to Dr. L. CANONICA for providing us with cultures of *Cochiobolus miyabeanus* that made our work on ophiobolin B possible.