THE EFFECT OF RIBONUCLEIC ACID AND ITS HYDROLYZATES ON THE UPTAKE OF GLYCINE-1-C^{14} BY PROTEINS OF NORMAL AND TUMOR TISSUES

I. B. Zbarskii and N. P. Karuzina

From the Biochemical Laboratory (Head — Prof. I. B. Zbarskii) of the P. A. Gertsen State Oncological Institute (Director — Prof. A. N. Novikov), Moscow

(Received February 6, 1959. Presented by Active Member AMN SSSR A. E. Braunshtein)

It is nowadays accepted that ribonucleic acid (RNA) plays a direct part in protein synthesis [6]. The stimulating effect of RNA or its hydrolyzates on the inclusion of radioactive amino acids in proteins has, however, been demonstrated in only a few investigations; for example, in damaged staphylococcal cells [7], in plants [10], and in sections of normal organs of the rat [3]. In the last of these investigations it was shown that the stimulation is inconstant in the case of cells of Ehrlich's ascitic cancer and practically absent in sections of rat sarcoma.

In the present research the effect of RNA on the incorporation of labeled glycine-1-C^{14} in protein was investigated mainly in minced normal organs and tumors of rats and also in sections of these tissues, and the relationship between this effect and the degree of hydrolysis of the RNA, the ages of the animals, and the time elapsing after transplantation of the tumor was also studied.

In connection with A. A. Khadzhiolov's findings that RNA hydrolyzates stimulate the respiration of yeasts [5], in some experiments the oxygen absorption was simultaneously estimated.

EXPERIMENTAL METHOD

The investigation was carried out on the liver, kidneys, spleen, brain, pancreas, and thymus, and also on transplanted tumors (cholangioma and M-1 sarcoma) of rats. The tissue was minced finely with scissors in the cold for $1\frac{1}{2}$-2 minutes.

RNA was used both in the form of neutralized hydrolyzates obtained by hydrolysis with 2.5N KOH at 37° for various periods of time, and also in the form of an unhydrolyzed preparation of sodium ribonucleate (from yeast). The preparation did not give the biuret reaction for protein and did not contain inorganic phosphorus. When dried over P_2O_5 in a vacuum exsiccator, the preparation contained 13.8% nitrogen and 7.2% phosphorus (ratio N/P = 1.97).

Experimental samples (usual volume 2 ml) consisted of 1.6 ml of Robinson's medium [9] of 0.1% glucose; 0.3 ml of RNA solution or hydrolyzate (final concentration 0.05%); an average of 0.1 ml of a solution of glycine-1-C^{14} (100 000 impulses per minute) and 150-250 mg of sections or mince in the case of normal tissues or 300-350 mg (in view of the higher water content) in the case of tumor tissue.

Incubation was carried out in Warburg flasks for 50 minutes in an atmosphere of O_2 at 38°.

Controls were set up without RNA and containing RNA or its hydrolyzates but not incubated. After incubation, trichloroacetic acid was added to give a final concentration of 5 %. Trichloroacetic acid was added to the unincubated control samples immediately after the sections or mince had been placed in the medium containing

TABLE 2

Duration of hydrolysis (in hours)	Number of experiments	Stimulation (in %)
0	4	+36.3
3	2	+12.5
6	6	+70.6
9	1	+34.0
12	5	+30.7
18	1	+77.0
24	1	+66.0
48	2	+29.4
Average	22	+44.6±8.5

Stimulation of Uptake of Radioactivity of Glycine-1-C^{14} into the Proteins of Minced Rats' Pancreas from Animals of Different Ages, Depending on the Duration of Hydrolysis of RNA

TABLE 1

The Influence of RNA Hydrolyzates on the Uptake of Glycine-1-C^{14} into the Proteins of Sections of Normal Organs of Rats $\left(\frac{\text{imp/min/g protein}}{\text{imp/min/ml medium}}\right)$

Organ	Number of experiments	With RNA	Without RNA	Change (in %)
Liver	10	0.63	0.59	+8.8±6.2
Kidney	10	1.43	1.28	+13.0±15.0
Spleen	7	1.66	1.90	−8.2±7.0

all the components of the test sample. The residue was ground and washed with 5% trichloroacetic acid, the nucleic acids were extracted with 5% trichloroacetic acid at 90° for 20 minutes, and the lipids with a mixture of alcohol and ether; the usual method was used to determine the radioactivity of 10 mg of protein on aluminum disks [2] by means of an end-type counter on a B apparatus.

The radioactivity was expressed in relative values: $\frac{\text{impulses per minute per g protein}}{\text{impulses per minute per ml medium}}$. The numbers of impulses in the control samples (not incubated) was close to the background level. As an example, in the experiment with minced brain, the experimental sample gave 584 impulses per minute, the control 27 and the background level 25; in the experiment with minced pancreas the corresponding values were 2500 impulses per minute with 32 for the control and 23 for the background.

The oxygen absorption was estimated by Warburg's standard method [4] and expressed as μ liters O_2 per 100 mg raw tissue during the time of the experiment.

EXPERIMENTAL RESULTS

In the majority of experiments with sections of liver, kidneys, and spleen from rats (weighing from 40 to 170 g) in the presence of RNA hydrolyzates (duration of hydrolysis 19, 24 and 48 hours) the incorporation of the radioactivity of the glycine into the proteins was slightly higher than that in the absence of RNA, although the difference was not statistically significant (Table 1).

The experiments on the pancreas, as an organ characterized by a high intensity of protein synthesis, were carried on further. Since we were unable to obtain satisfactory sections of rats' pancreas, these and the subsequent experiments, including those on other organs, were carried out on minced preparations.

In the experiments with minced rats' pancreas, both RNA and its hydrolyzates definitely stimulated the uptake of the radioactivity of the glycine into the protein. This effect, however, did not depend on the duration of hydrolysis of the RNA, as in the experiments on sections of liver, kidney, and spleen described above (Table 2).

For the experiments on minced preparations, the pancreas and brain were taken from rats of different ages. Stimulation was observed in rats of various weights, but the younger the animal, the more marked the stimulation (Table 3). Similar results were obtained in minced brain.

In the experiments on minced preparations of other organs of rats, RNA and its hydrolyzates also had a stimulating action on the uptake of the radioactivity of glycine-1-C^{14} into the proteins. Table 4 shows the results of these experiments treated statistically.

In the experiments on tumors we used M-1 sarcoma and a cholangioma — an alveolar carcinoma of the liver, originally induced by 2-acetylaminofluorene, a slowly growing tumor with a small number of necrotic areas that appeared only in the later stages of its growth.

TABLE 3

The effect of RNA Hydrolyzate (Hydrolysis for 6 Hours) on the Uptake of Radioactivity of Glycine-1-C^{14} into the Proteins of Minced Pancreas of Rats of Different Ages $\frac{\text{(imp/min/g protein)}}{\text{(imp/min/ml medium)}}$

No. of rats	Weight of rat (in g)	With RNA	Without RNA	Change (in %)
5	16—18	5.71	2.92	+95.0
3	17—25	7.29	3.34	+118.0
3	26—31	6.94	4.02	+72.7
9	28—34	4.32	3.54	+22.0
6	49—67	5.99	5.65	+6.0

TABLE 4

The effect of RNA and its Hydrolyzates on the Uptake of Glycine-1-C^{14} into the Proteins of Minced Normal Organs of the Rat $\frac{\text{(imp/min/g protein)}}{\text{(imp/min/ml medium}}$

Organ	Number of experiments	With RNA	Without RNA	Change (in %)
Pancreas	22	6.07	4.44	+44.2±8.5
Liver	4	1.60	1.09	+45.1±8.5
Kidney	4	2.00	1.50	+40.1±16.9
Spleen	4	1.55	1.08	+61.3±30.1
Brain	20	0.76	0.48	+85.0±18.2
Thymus	5	2.45	2.17	+13.2±11.0

In the case of minced tumor preparations, the effect of RNA and its hydrolyzates on the uptake of the radioactivity of the glycine into the proteins was slight and occasionally even amounted to depression of the uptake. For instance, in 6 experiments on minced sarcoma M-1, the uptake in samples without RNA averaged 0.65, and with the addition of RNA the uptake was 0.56. The average change was 7.1 ± 17.7%. The duration of hydrolysis of RNA, and also the length of time after transplantation of the tumor did not infulence the observed effect.

The results of the next experiments, in which the oxygen demand was determined in addition to the uptake of radioactivity in the same minced preparations, are shown in Table 5 for sarcoma M-1 and in Table 6 for cholangioma. In the case of the sarcoma M-1, as in the previous experiments, RNA produced no statistically significant changes either in respiration or in the uptake of radioactivity. The time elapsing after the transplantation of the tumor was also without effect. In the case of the cholangioma, in early stages of growth (15-22 days after transplantation) some degree of stimulation of incorporation of the amino acid took place, but this was shown less clearly than in the normal organs. At later stages of growth (30th-48th day after transplantation) the uptake of radioactivity in the experimental and control (no RNA added) samples was practically identical. RNA behaved in the same way towards the respiration of the minced cholangioma. The effect on respiration was shown very feebly, but comparison of the respiration and the uptake of radioactivity in the same experiments indicates a slight positive correlation between those two metabolic indices.

TABLE 5

Uptake of Glycine-1-C^{14} into Proteins $\left(\frac{\text{imp/min/g/protein}}{\text{imp/min/ml medium}}\right)$, and Respiration ($\mu$ liters O_2/100 mg fresh tissue during time of experiment) of Minced Transplanted Sarcoma M-1 of a Rat

No. of experiments	Respiration			Uptake		
	with RNA	without RNA	change (%)	with RNA	without RNA	change (%)
6	46.0	43.7	+9.6±5.5	0.86	0.81	+15.3±15.8

TABLE 6

Incorporation of Glycine-1-C^{14} into Proteins $\left(\frac{\text{imp/min/g/protein}}{\text{imp/min/ml medium}}\right)$ and Respiration (μ liters O_2 / 100 mg fresh tissue during time of experiment) of Minced Transplanted Cholangioma of a Rat at Different Stages of Growth of the Tumor

Time after transplantation	Respiration				Incorporation			
	Number of experiments	with RNA	without RNA	change (%)	Number of experiments	with RNA	without RNA	change (%)
From 15 to 22 days	8	18.7	16.6	+13.8±5.52	8	1.78	1.49	+14.8±3.84
From 30 to 48 days	4	11.4	13.6	−18.1±9.52	6	0.53	0.56	−1.3±11.7

The action of RNA hydrolyzates on the uptake of the radioactivity of the glycine into the proteins of the sections and minced preparations of normal organs and transplanted tumors did not depend on the duration of hydrolysis (from 0 to 48 hours). It was more marked in rats weighing less than 25-30 g than in older animals.

In the experiments on the cholangioma, the effect of RNA on the resoipration of the minced preparations was in general similar to its effect on the incorporation of glycine into the proteins. In individual experiments a correlation was observed between these two values.

The results obtained do not permit any conclusion to be drawn concerning the mechanism of stimulation of incorporation of glycine-1-C^{14} nor the active principle responsible for this effect. It is possible that this principle is not nucleotide in nature but is to some extent analogous to the factor described by Gale and Folkes [8]. There is also the possibility that the action of RNA on glycine uptake is due to the influence on respiration, with a corresponding increase in the energy metabolism [5]. Finally, it may be postulated that the observed effect depends on the stimulation of the synthesis of RNA itself [1, 3].

The absence of any effect of RNA on minced preparation of tumors indicates that their metabolism is of a different character. It is apparent that RNA has no effect on minced preparations of tumors in late stages, when partially necrotic, but it does affect recently transplanted, intensively growing tumors. In this respect a characteristic difference is seen between the early and late stages of cholangioma — a slowly growing tumor in which necrosis occurs only in late stages of growth — and the absence of effect on the various stages of growth of sarcoma M-1 — a tumor in which diffuse necrosis develops even in the early stages.

The fact that the effect is well marked in the minced preparation and is almost absent in the normal tissue slices evidently implies that the action of RNA is best shown when the cells are partially destroyed. The marked effect obtained in previous work on slices [3] probably depended on the fact that the slices were damaged more than in the present investigation, when they were prepared very carefully.

SUMMARY

Ribonucleic acid and its hydrolyzates exert a stimulating effect on the incorporation of glycine-1-C^{14} into the proteins of minced normal organs (pancreas, liver, kidneys, spleen, brain, and thymus) of rats and have almost no effect on the incorporation of glycine radioactivity into the proteins of minced sarcoma M-1 and transplanted cholangioma at late stages of its growth. At early stages of cholangioma growth some stimulation of radioactivity incorporation is noted. The effect of the stimulation is independent of the length of RNA hydrolysis.

LITERATURE CITED

[1] A. K. Belousov, Biokhimiya, 20, 4, 495 (1955).

[2] I. B. Zbarskii and K. A. Perevoshchilova, Doklady Akad. Nauk SSSR, 107, No. 2, 285 (1956).

[3] K. A. Perevoshchikova and I. B. Zbarskii, Doklady Akad. Nauk SSSR, 114, No. 1, 150 (1957).*

[4] V. V. Umbreit et al., Manometric Methods of Study of Tissue Metabolism. Moscow, 1951 [In Russian].

[5] A. A. Khadzhiolov, Izvest. Otdel. Biol. Med. Nauk Bolgarskoi AN, No. 1, 123 (1957).

[6] J. Brachet, Biochemical Cytology, New York, 1957.

[7] E. F. Gale and J. P. Folkes, Biochem. J., 1953, v. 55, N. 1, p. XI; N. 5, p. 721.

[8] E. F. Gale and J. P. Folkes, Nature, 1958, v. 182, p. 94-97; Biochem. J., 1958, v. 69, p. 611.

[9] I. R. Robinson, Biochem J., 1949, v. 45, p. 68-74.

[10] G. C. Webster and M. P. Johnson, J. Biol. Chem., 1955, v. 217, p. 641.

* Original Russian pagination. See C. B. Translation.