EFFECTS OF SOME NUCLEIC ACID COMPONENTS AND OF THEIR PRECURSORS ON INCORPORATION OF [1-C^{14}] GLYCINE AND [1-C^{14}] TYROSINE INTO THE PROTEINS OF NORMAL AND TUMOR TISSUE

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The researches of the past few years have contributed valuable data to our knowledge of the role of nucleic acid in protein synthesis, and some of the mechanisms involved have been investigated. Although the first researches establishing a connection between the nucleic acid content of cells and the intensity of protein synthesis were begun less than 20 years ago, a number of postulates then advanced, on the basis of certain experimental findings, have since undergone revision. Thus, for example, the original view that deoxyribonucleic acid (DNA) is the directive agent in the synthesis of proteins has now been abandoned by the majority of workers, who consider that protein biosynthesis in the cytoplasm is connected directly with ribonucleic acid (RNA), and only indirectly with DNA.

Two recently published review articles [2,3] have been devoted to interrelations between protein synthesis and nucleic acids. Although many aspects of the role of nucleic acids in protein synthesis still await clarification, there can be no doubt that they are interrelated, and that the process proceeds at both the high-and the low-molecular level.

We were interested in investigating the part played by certain purine and pyrimidine bases of nucleic acids, and of their precursors, in the synthesis of protein. In the present research we examined the effects of aspartic acid, ureidosuccinic acid, and uracil (each of these substances being the precursor of the succeeding one) on incorporation of [1-C^{14}] glycine and [1-C^{14}] tyrosine into the proteins of slices of normal tissues, transplanted rat tumors, and ascitic tumor cells of rats. We also examined the effects of mixtures of pyrimidines (uracil, cytosine, thymine), and of each of these pyrimidines separately.*

METHOD

We examined the livers of normal rats and of rats with transplanted tumors, viz., rat sarcoma 45, and rat ascitic hepatoma (Sagedale strain). The experimental systems had a total volume of 5 ml, and contained 0.5 g of liver slices, 4 ml of Krebs-Ringer phosphate solution, 0.2 ml of various adjuvants, and 0.4 ml of [1-C^{14}] glycine or [1-C^{14}] tyrosine solutions, of an activity of 100 000-300 000 c.p.m. The systems were incubated in Warburg vessels for an hour at 38°. The slices were then washed with the same saline solution until the washings showed absence of radioactivity, when they were ground up with 2.5 ml of 0.2 N $HClO_4$. The control systems either did not contain additives, or these were introduced together with the $HClO_4$ before incubation. The homogenates were centrifuged, and the deposits were again treated with 0.2 N $HClO_4$. The supernatant solutions were made neutral with KOH, and the radioactivity of the acid-soluble extractives was measured. Nucleic acids were extracted from the sediments with 0.2 N $HClO_4$ at 90° for 30 min. Lipids were extracted by heating for 3 min with a 3:1 alcohol-ether mixture. The residues were washed with alcohol and ether, and dried.

Radioactivity was measured by means of a MST-17 end-window counter, in a Type B apparatus. The substances under examination were added to the systems at a concentration of $2 \cdot 10^{-3}$ M. Ureidosuccinic acid was synthesized according to Nyc and Mitchell [4], and its purity was checked by melting point, analytical, and chromatographic data [1].

* M.B. Sapozhnikova participated in the research.

RESULTS

The data of Table 1 illustrate the effects of ureidosuccinic acid on incorporation of [1-C^{14}] glycine into sarcoma 45 slices and into liver slices from tumor-bearing rats.

TABLE 1. Effect of Ureidosuccinic Acid on Incorporation of [1-C^{14}] Glycine into Proteins, Nucleic Acids, and Acid-Soluble Compounds of Slices of Liver and Rat Sarcoma 45 (counts per minute per 10 mg of protein)

Tissue	Protein		Nucleic acids		Acid-soluble compounds	
	Control	Experimental	Control	Experimental	Control	Experimental
Liver	130	52	37	32	384	291
	144	78	33	28	410	294
	249	181	55	47	549	405
	158	90	28	22	440	260
	215	167	66	64	866	671
Mean percentage inhibition	36.6 (60—22)		13.6 (21.4—3.2)		27.6 (41—22.6)	
Sarcoma 45	960	750	186	160	949	1 524
	505	369	99	90	990	1 084
	1 034	880	184	198	985	1 315
	988	630	159	160	1 015	1 621
	839	568	160	150	—	—
Mean percentage inhibition	26.4 (36.3—14.9)		5.8		+29	

Note: The figures in parentheses indicate the range of variation of percentage inhibition (the values for the control systems, not containing ureidosuccinic acid, are taken as 100).

The results show that ureidosuccinic acid inhibited incorporation of [1-C^{14}] glycine into the proteins of sarcoma 45 slices, and of the liver of tumor-bearing rats, but had practically no effect on incorporation of glycine into the nucleic acids of sarcoma 45 tissue, and only a slight effect on incorporation into liver nucleic acids. The opposite results were found for the acid-soluble fractions of the tissues under examination. Whereas incorporation of [1-C^{14}] glycine into this fraction of the liver of tumor-bearing rats was inhibited by 27.6%, it was activated to the same extent in sarcoma 45 tissue. This activation may possibly be ascribable to the relatively greater utilization of ureidosuccinic acid for the synthesis of acid-soluble nucleotides in tumor tissue than in liver.

In our further experiments we used [1-C^{14}] tyrosine, with the object of getting a clearer picture of the effects on protein metabolism; tyrosine differs from glycine in not being utilized for the synthesis of nucleic acid components.

In the experiments on an earlier precursor of nucleic acid components we took aspartic acid, and, for an assessment of the specificity of its action, we compared the effects with those given by equimolar concentrations of glutamic, malic, and succinic acids (Table 2).

The data of Table 2 show that aspartic acid caused enhanced incorporation of [1-C^{14}] tyrosine into the proteins of sarcoma 45 tissue, in all the experiments, whereas (except for one experiment) the remaining acids gave practically no activation of tyrosine uptake, or even depressed it.

In our next experiments we examined the effect on incorporation of [1-C^{14}] tyrosine into protein of addition of nucleic acid pyrimidine bases, separately or together; for this purpose we used uracil, thymine, and cytosine, and their mixtures in equimolar proportions (Table 3).

The data of Table 3 show that of the pyrimidine bases examined, only uracil had any activating effect on incorporation of [1-C^{14}] tyrosine. The mixture of pyrimidine bases did not show any additive action.

TABLE 2. Effects of Various Acids on Incorporation of [1-C^{14}] Tyrosine into the Proteins of Slices of Rat Sarcoma 45 Tissue (c.p.m. per 10 mg protein)

Control (without additives)	Acids			
	aspartic	glutamic	malic	succinic
780	1 229	1 106	944	1 125
865	1 144	723	683	587
250	328	228	232	200
135	228	150	163	164
70	105	79	79	77

TABLE 3. Effects of Various Nitrogenous Bases of Nucleic Acids, and of TheirMixtures, on Incorporation of [1-C^{14}] Tyrosine into Proteins of Rat Sarcoma 45 and Rat Ascitic Hepatoma (c.p.m. per 10 mg protein)

Number of experiment	Control (without additives)	Uracil	Thymine	Cytosine	Mixture
1	67	98	60	70	77
2	84	102	70	90	72
3*	573	680	583	577	680

* Rat ascitic hepatoma cells were used in this experiment.

TABLE 4. Effects of Aspartic Acid, Ureidosuccinic Acid, and Uracil on Incorporation of [1-C^{14}] Tyrosine into the Proteins of Sarcoma 45 Slices (c.p.m. per 10 mg protein)

Number of experiment	Control (without additives)	Acids		
		Aspartic	Ureidosuccinic	Uracil
1	105	224	72	205
2	80	170	101	246
3	85	200	74	232
4	70	90	48	213

TABLE 5. Effect of Uracil on Incorporation of [1-C^{14}] Tyrosine into the Proteins of Liver Slices from Normal Rats (c.p.m. per 10 mg protein)

No. of experiment	Control	Experiment
1	110	93
2	61	78
3	81	90
4	81	87

In our next series of experiments we compared the effects of adding two pyrimidine precursors (aspartic and ureidosuccinic acids), and of adding uracil, on incorporation of [1-C^{14}] tyrosine into the proteins of sarcoma 45 slices (Table 4).

The data of Table 4 indicate that aspartic acid activated incorporation of tyrosine into proteins to a greater extent than in the experiments of Table 2; the activating effect of uracil was even greater, incorporation being as much as three times that of the control systems, in some of the experiments. The effect of ureidosuccinic acid on incorporation of [1-C^{14}] tyrosine into proteins was much the same as for [1-C^{14}] glycine (see Table 1), viz., incorporation of tyrosine was considerably inhibited.

We examined the effect of uracil on incorporation of [1-C^{14}] tyrosine into the proteins of liver slices from control rats (Table 5), for purposes of comparison with its effect on tumor tissue.

The data of Table 5 show that uracil has practically no effect on the incorporation of [1-C^{14}] tyrosine into the proteins of normal liver.

Ogata et al. [5] found that some of the pyrimidine bases of nucleic acids promote incorporation of [1-C^{14}] glycine into ribo- and deoxyribonucleoproteins of regenerating rabbit liver; this effect was much less pronounced in normal liver. Considerable stimulation of incorporation was given by orotic acid, which is a pyrimidine precursor. These authors attributed the effect to utilization of pyrimidines by proliferating tissue as precursors of nucleic acids.

We also found that in transplanted rat tumors uracil activates protein biosynthesis, as shown by incorporation of [1-C^{14}] tyrosine, whereas this activating effect was not found with normal rat liver. These findings are in agreement with published reports of the slight utilization of pyrimidines by normal tissues, as contrasted with their utilization by tumor tissue. Rutman et al. [6] found that active incorporation of labeled uracil took place in tumor tissues, but not in normal tissues. Of the compounds examined by us, uracil was the most active in stimulating protein synthesis. The effect of aspartic acid closely resembled that of uracil, both qualitatively and quantitatively; aspartic acid is one of the direct precursors of the pyrimidine bases of nucleic acids. Activation of protein biosynthesis in tumor tissue by some of the precursors of nucleic acids should, perhaps, be regarded as a consequence of stimulation of

nucleic acid production in such tissues, this, in turn, stimulating protein synthesis, rather than as a direct result of the action of the precursors.

An unexpected result was the absence of any stimulating effect of ureidosuccinic acid (a precursor of orotic acid) on incorporation of [1-C^{14}] glycine into proteins of tumor tissue; this finding may be due partly to utilization of glycine for the synthesis of nucleic acids.

SUMMARY

The effect on incorporation of [1-C^{14}]-glycine and -tyrosine into rat liver and rat sarcoma 45 proteins of nucleic acid pyrimidine bases, and of their precursors, aspartic and ureidosuccinic acids, has been investigated. Of the substances examined, only aspartic acid and uracil were found to activate incorporation of labeled amino acids into proteins.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
