ribonucleoprotein did not influence the purine/pyrimidine nucleotide ratio.

Analyses for phosphorus and nitrogen in the ribonucleic acids prepared by the guanidine salt method yielded N/P ratios which compared favorably with the values of 1.74 to 1.77 calculated from mononucleotide analyses of ribonucleic acids (other than thymus and pancreas, the latter two having a ratio of 1.86). The postulate of a statistical tetranucleotide does not coincide with the results of mononucleotide analysis of mammalian ribonucleic acids by ion-exchange chromatography, in agreement with previously reported analyses.[17] The pig liver ribonucleic acid analyzed by a chromatographic method[17] had molar proportions of cytidylic, adenylic, uridylic and guanylic acids of 2.1, 1.4, 1 and 2.4, respectively, values in fair agreement with those reported here for liver ribonucleic acids from other species. The sedimentation constants shown in the table reveal no striking differences among those preparations analyzed. It should be noted that the sedimentation constant of rabbit liver ribonucleic acid almost doubles as the *p*H is lowered from 6.8 to 4.8. This probably indicates an increased state of aggregation in more acid solution. Data concerning the physical-chemical homogeneity of mammalian ribonucleic acids prepared by other techniques[7,8,9,10] is available only for the non-dialyzable residue of ribonuclease-treated pancreas ribonucleic acid.[18]

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[CONTRIBUTION FROM THE BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY]

The Incorporation of Isotopic Phosphate in the Mononucleotides of Liver Nucleic Acids[1]

BY E. VOLKIN AND C. E. CARTER

Investigations have been carried out on the *in vivo* incorporation of isotopic phosphate into the component mononucleotides of liver ribonucleic acid (RNA) and desoxyribonucleic acid (DNA). Mononucleotides were isolated by anion-exchange chromatography from hydrolysates of highly purified nucleic acids. This work reveals that a considerable variation in distribution of the isotope occurs among the nucleotides of DNA, thymidylic acid containing the highest activity. The distribution among the nucleotides in rabbit liver ribonucleic acid is essentially homogeneous, while rat liver and mouse liver ribonucleic acids exhibit a heterogeneous distribution of activity among the mononucleotides. These results, in conjunction with those of other workers, suggest that the evaluation of studies on nucleic acid turnover rates may be considered with regard to the metabolic turnover of the substituent components of nucleic acids, as well as the nature of the isotopically labeled precursor.

Introduction

The metabolic turnover of nucleic acid phosphorus is generally studied on nucleic acid fractions of tissues with the assumption of homogeneous distribution of isotopic phosphorus among the substituent nucleotides. A study of the specific activity of the mononucleotides derived from RNA and DNA isolated from mammalian liver following *in vivo* assimilation of inorganic isotopic phosphate is reported in this paper.

The results of this investigation which are summarized in the table show an essentially homogeneous distribution of isotopic phosphate among the nucleotides of rabbit liver RNA isolated from an animal sacrificed 180 minutes following injection of the isotope. In order to obtain high nucleic-acid activities in shorter incubation periods smaller animals, rats and mice, were employed. Under the conditions described in the table (20-min. incubation period) isolated ribonucleotides are unequally labeled with isotopic phosphorus. In all cases each pair of the isomeric purine nucleotides is found to be equally active[2a,b]; adenylic acid exhibits the highest activity and, in the case of mouse liver, guanylic acid has a significantly lower specific activity than other mononucleotides. A striking variation of distribution of isotopic phosphorus is found among the desoxyribonucleotides derived from mouse and rat liver desoxyribonucleic acids. In both species thymidylic acid exhibits the highest specific activity. In the mouse, desoxyadenylic acid closely approximates the activity of thymidylic acid while in the rat it is about half the thymidylic acid value in both the normal and regenerating livers. A further species difference is found in the distribution of activity in the purine desoxyribonucleotides; in the rat, essentially equal activities are found, whereas in the mouse the activity of desoxyadenylic acid is about twice that of desoxyguanylic acid. In experiments employing isotopic formate, species differences in the distribution of activity among the nucleotides of chick and rat nucleic acids have also been found.[3]

The experiments with normal and regenerating rat liver were not performed under comparable conditions so that the absolute specific activities are not applicable. However, it may be seen that the relative values show no significant differences in the nucleotide distribution of phosphate in the two groups.

A satisfactory degradation of mouse hepatoma DNA to mononucleotides was not achieved, consequently, a comparison of this category with normal liver is not available. The ribonucleotides from the two tissues exhibited no significant differences of activity under the conditions employed.

The foregoing data do not impair the value of experiments which utilize the incorporation of isotopic phosphate into nucleic acids to assess the metabolic activities of these compounds under various conditions. However, there is considerable evi-

(1) Work performed under Contract #W-7405-eng-26 for the Atomic Energy Commission.

(2) (a) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950); (b) W. E. Cohn, *ibid.*, **72**, 1471 (1950).

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TABLE I

SPECIFIC ACTIVITIES OF THE MONONUCLEOTIDES OF LIVER RIBO- AND DESOXYRIBONUCLEIC ACIDS

Source	Injection dose, mc. per animal	Time, minutes	Specific activity; counts/minute/μg. phosphorus RNA[a] Cyt.	Ade.	Ury.	Gua.	DNA[a] Cyt.	Ade.	Thy.	Gua.
Rabbit liver	1.0	180	10.2	10.3	10.3	10.0				
Rat liver	1.0	20	4.3	6.4	4.4	4.1				
Rat liver	1.3	60					...	8.1	15.9	5.5
Rat, regenerating liver	1.0	20	12.1	19.1	13.8	13.7	5.0	4.7	8.5	4.3
Mouse liver	1.0	20	36.1	39.1	33.6	28.2	4.0	7.1	7.5	2.8
Mouse hepatoma	1.0	20	36.0	38.6	33.6	33.4				

[a] The mononucleotide analytical composition of the ribonucleic acids may be found in Volkin and Carter.[9] Small but significant differences in composition exist among the various ribonucleic acids, the purine/pyrimidine ratios occurring between 1.03 and 1.22. The DNA mononucleotide yields (60 to 70%) were not sufficiently high to justify calculation of composition.

dence which suggests refinement of the concept of nucleic acid turnover in favor of more detailed studies of the metabolic construction of this class of compounds. It is evident that relative rates of turnover of RNA and DNA are dependent not only upon growth and cell division but also on the nature of the isotopically labeled precursor,[3,4,5,6] and that these rates represent an average of heterogeneously distributed activity among the substituents of these compounds.

Experimental

The experimental animals included the rabbit (normal liver), mouse (normal liver and transplantable hepatoma[7]) and rat (normal and regenerating livers). To obtain sufficient quantities of purified nucleic acids, it was necessary to pool the livers of three rats and those of five mice for each experiment. The regenerating rat liver tissue was obtained from animals which 48 hours previously had been subjected to subtotal hepatectomy. Young adult animals were employed in all cases. Inorganic orthophosphate containing ^{32}P was injected intrapleurally under the conditions stated in the table.

After sacrificing the animals, the tissues were immediately removed and homogenized in a Waring blendor with four volumes of cold 0.15 *M* sodium chloride 0.02 *M* phosphate, *p*H 6.8. DNA was purified from the insoluble material collected by centrifugation at 3000 g. for 30 minutes, according to the procedure of Mirsky and Pollister.[8] RNA was prepared from the supernatant solution by precipitating the crude ribonucleoprotein with 2 *M* guanidine hydrochloride and further purifying as described by Volkin and Carter.[9] All nucleic-acid preparations were purified to constant specific activity and were free of protein and cross contamination with each other. Forty- to sixty-milligram quantities were employed for degradation to mononucleotides.

RNA was hydrolyzed by incubating in 0.5 *N* sodium hydroxide at 37° for 17 hours, using 10 mg. of RNA per ml. of alkali. Under these conditions approximately 95% of the nucleic acid was recovered as mononucleotide.[9] The hydrolysis of DNA was carried out with a combination of the enzymes, desoxyribonuclease and alkaline phosphatase, the details of the procedure being reported elsewhere.[10] The presence of sodium arsenate in the digestion with phosphatase inhibits the hydrolysis of mononucleotides by this enzyme and allows a mononucleotide yield of about 60 to 70%.

Separation of mononucleotides was carried out on an ion-exchange column as described by Cohn.[1] The resin column (Dowex-1, bed size 8 cm. × 0.72 sq. cm.) was previously converted to the chloride form with 100 cc. of 2 *N* HCl and excess hydrochloric acid removed by washing with water. The alkaline digest of RNA was first diluted with water to a concentration of 0.02 *N* sodium hydroxide, then run through the column. The DNA digest was made 1 molar with respect to ammonium hydroxide before analysis. After allowing the digest to pass through the column, the resin was washed with enough water to remove excess hydroxyl ion, then 0.01 *M* ammonium chloride introduced until the effluent reached *p*H 6, a procedure which removes bases and nucleosides. The elution of mononucleotides from the anion-exchange column was effected by increasing concentrations of hydrochloric acid in the wash.[2] Quantitative determination of the mononucleotides in the effluent fractions by ultraviolet spectrophotometry were based on the following molar extinction coefficients of the nucleotides at 260 mμ in 0.01 *N* hydrochloric acid: adenylic acid, 13,900; guanylic acid, 11,800; cytidylic acid, 12,750; uridylic acid, 9930; and thymidylic acid, 8400.

Specific activity measurements were based on spectrophotometric determinations of the nucleotides which were then converted to equivalent phosphorus contents. These values were occasionally checked with direct determinations of organic phosphorus and were found in agreement. The ^{32}P content was measured by counting aliquots of solutions slowly dried on porcelain capsules. For specific activity determinations, the fractions used corresponded to the peak fractions of each nucleotide curve. The specific activities of the RNA and DNA component mononucleotides given in the table are the average of four to six determinations and do not vary from the corresponding highest and lowest observed values by more than 6%.

Although the nucleic acid preparations were purified to constant specific activity the possibility remains that they were contaminated to some extent with inorganic isotopic phosphate. In the case of DNA, contaminating inorganic phosphate would necessarily be present since the enzymatic hydrolysis degrades a portion of the mononucleotides to nucleosides and phosphate.[10] In order to determine at which point inorganic phosphate is eluted from the ion-exchange column, a control run consisting of 10,000 counts of inorganic isotopic phosphate in a solution of RNA was hydrolyzed and treated on the ion-exchange column in the usual manner. Though all the inorganic phosphate remained on the resin when the alkaline digest was passed through, 8500 counts were removed by the 0.01 *M* ammonium chloride wash, and essentially all the remainder was removed in the initial fractions of 0.001 *N* hydrochloric acid before the elution of mononucleotides had begun. As a further check on the presence of contaminating inorganic phosphate, fractions from different points on the mononucleotide elution curves were counted and the specific activities found to agree with those from the peaks of the corresponding curves.

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