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

Incorporation of Isotopic Formate into the Nucleotides of Ribo- and Desoxyribonucleic Acids[1]

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Experiments utilizing isotopic formate were carried out for the purpose of gaining some insight into the apparent relationship of folic acid function and nucleic acid metabolism. The *in vivo* incorporation of formate into mononucleotides isolated from purified visceral ribo- and desoxyribonucleic acids of the rat and chick on complete diets, and the folic acid deficient chick, was investigated. The results indicate that formate is assimilated directly into the methyl group of thymidylic acid, as well as into the purines of both ribo- and desoxyribonucleic acids. No evidence was found for an inability of the folic acid deficient chick to utilize formate for nucleic acid synthesis, lending support to the hypothesis that folic acid may be more directly concerned with the formation of formate from glycine and serine. Differences in activity among the nucleotides of ribo- and desoxyribonucleic acids from the control and deficient chick, as well as those from the rat and chick, are discussed.

It is well known, from investigations with pigeons,[3,4] that carbons 2 and 8 of uric acid may be derived from formate or from biological precursors of "formate" such as serine,[5] glycine[6] or the methyl group of choline.[7] There is, as yet, but little available information on the distribution of isotopic formate in the nucleotides of intact nucleic acid. Recently Elwyn and Sprinson[8] found that the β carbon of serine and the methylene carbon of glycine were extensively incorporated into the purines of both ribonucleic acid (RNA) and desoxyribonucleic acid (DNA). Of the pyrimidines, thymine alone was found to contain isotopic carbon, the activity residing in the methyl group. There is much evidence that folic acid is concerned with one-carbon metabolic intermediate(s) related to formic acid, glycine and serine.[9,10] It is possible that the vitamin functions either to promote utilization of formate by precursors of the purines and thymine or to promote the formation of formate which may be utilized independently of the action of the vitamin. The present investigation was undertaken in an attempt to clarify some of the relationships among formate metabolism, the nucleic acids, and the metabolic function of folic acid. The incorporation of isotopic formate into RNA and DNA nucleotides was studied *in vivo* in the rat and chick on complete diets as well as in the folic acid deficient chick.

The data summarized in Table I show the activities recorded for the nucleotides isolated from the pooled viscera (exclusive of the intestinal tract) of the experimental animals and birds after injection of isotopic formate. It may be seen that the chick viscera nucleotides were found to contain activities quantitatively similar to those of the rat. If a correction for the relative quantities of radioactivity injected be made, the chick ribocytidylic, -adenylic, and -guanylic, and the desoxycytidylic acid activities are somewhat higher than those from the rat. On the other hand, the chick desoxyadenylic and -guanylic acids gave fewer counts than those from the rat. The thymidylic acids had about the same activities. The activities of the uridylic acids were too low to give reliable values.

Attention should be called to the ratios of activities of riboadenylic and riboguanylic acids. If, as seems probable from other work,[3,4] adenylic and guanylic acids be equally labeled in the 2- and 8-positions the specific activities of the two give some indication of "turnover" rate. In the rat, the adenylic acids had significantly higher specific activities than the guanylic acids. The ratio between the activities is very similar to the ratios for the rat found by Volkin and Carter[11] in similar experiments using radioactive phosphate. In these experiments, chick riboadenylic and riboguanylic acids had the same activities, within the limits of error of the methods used. This result is in accord with the phosphate labeling experiments with rabbits.[11]

The ratios between the specific activities of the ribo- and the desoxyribonucleotides are of interest. The rather high ratio found for the adult rats used in these experiments is in accord with the results of Elwyn and Sprinson[7] and differ from those of Furst, *et al.*[12] Such results do not support the hypothesis of a comparatively limited turnover rate for desoxyribonucleic acid in adult animals.

Hydrolyses of the nucleotides from rat ribonucleic acids have been carried out and determinations made of the specific activities of the purines and pyrimidines isolated from the hydrolysates. It was found that a negligible proportion of the activity of the adenylic acids could be assigned to the carbohydrate portion of the molecule, while in the pyrimidine ribonucleotides, which have about one-tenth the activity of the purine nucleotides, approximately 25% of the total activity resided in the ribose moiety.

These results indicate that formate is directly assimilated into the purines while the activities of cytosine, uracil, and pentose and desoxypentose may be derived from formate by a more circuitous route, probably through carbon dioxide.

Thymine, which contained all the activity of rat thymidylic acid, was isolated and degraded to urea, acetol and carbon dioxide. The acetol portion, which contains the methyl group of thymine, accounted for over 70% of the initial activity.

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No activity was found in the urea or carbon dioxide.

The results obtained with folic acid deficient chicks are somewhat surprising. As may be seen from Table I, no evidence was found for a gross inability of the deficient chicks to utilize formate for nucleic-acid synthesis. When a correction for the differences in the amounts of radioactive formate injected is made (μc./kg.) the relative molar activities of ribocytidylic, desoxyadenylic and desoxyguanylic acids, and the thymidylic acid are only slightly low in the deficient chicks as compared with the controls. The desoxyribocytidylic acid activity appears to be markedly low while the riboguanylic acid is significantly elevated. In general, the ratio of desoxyribonucleotide to ribonucleotide activity was lower in the deficient chicks than in the controls. The chicks on the folic acid deficient diet were losing weight, however, and a lowered DNA/RNA ratio might be expected under such circumstances. The data do not necessarily provide evidence that the lack of growth was a consequence of an inability to produce DNA because of failure to utilize formate.

TABLE I

SPECIFIC ACTIVITIES OF RNA AND DNA NUCLEOTIDES AND DERIVATIVES SIXTEEN HOURS AFTER INJECTION OF C^{14}-LABELED FORMATE INTO RATS AND CHICKS

Nucleotide	Rat[a] DNA c./min./μ mol.	Rat[a] RNA c./min./μ mol.	Control chick[b] DNA c./min./μ mol.	Control chick[b] RNA c./min./μ mol.	Folic acid def. chick[c] DNA c./min./μ mol.	Folic acid def. chick[c] RNA c./min./μ mol.
Cytidylic acid	18[d]	81	22[d]	80	13[d]	145
Adenylic acid	622	1285	167	797	315	1985
Uridylic acid	...	93	...	...	...	..
Guanylic acid	543	767	214	807	476	3292
Thymidylic acid	263	..	121	...	221	..
Adenine	597	1350	...	...	...	..
Uracil	...	70	...	...	...	..
Acetol	138	..	...	...	...	..
CO_2 From thymine	0					
	0					

[a] Received 70 μc. per 100 g. of animal. [b] Received 34 μc. per 100 g. of bird. [c] Received 82 μc. per 100 g. of bird. [d] The activity of the desoxycytidylic acids may reside exclusively in the small amount (1–4% of the total desoxycytidylic acid) of 5-methyldesoxycytidylic acid present in these fractions,[13,14] through methyl labeling in this latter compound.

Recently, Plaut and co-workers have found that rats fed succinylsulfathiazole are unable to incorporate formate into the serine of visceral protein except when treated with folic acid.[15] The period of time permitted for the incorporation was only three hours as compared with the sixteen of the present experiments. It is possible that the longer period of time might obscure rate relationships in our experiments. The action of folic acid may be more directly concerned with the breakdown of glycine[16] and serine[10] to formate. Direct evidence is available that both these reactions are impaired in folic acid deficient animals.

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Experimental

Each of three young adult rats weighing about 150 g. was injected with 100 μc. of radioctive sodium formate (1.76 mg.). After 16 hours, the animals were sacrificed and the liver, lungs, spleen and kidneys removed. The pooled viscera were then homogenized in 4 volumes of 0.02 *M* *p*H 6.8 phosphate buffer, 0.15 molar with respect to sodium chloride. RNA was isolated from the homogenate by the procedure described by Volkin and Carter.[11] DNA was obtained by the method of Mirsky and Pollister.[17]

The isolated RNA was hydrolyzed to mononucleotides by treatment with 0.5 *N* sodium hydroxide for 17 hours at 37°. DNA was converted to mononucleotides in about 65% yield by the action of desoxyribonuclease followed by alkaline phosphatase in the presence of arsenate as described by Volkin, Khym and Cohn.[18]

For the chick experiments four folic acid deficient birds weighing a total of 490 g. and three control chicks weighing a total of 876 g. each received 100 μc. of the sodium formate. After 16 hours the isolation and degradation of the nucleic acids were carried out exactly as described for the rat experiments.

The folic acid deficient chicks were reared for four weeks on a purified diet with a composition similar to that used by Keith, *et al.*[9] The control birds received the same diet with 2 mg./kg. of added folic acid.

The mononucleotides were isolated by the anion-exchange chromatography method of Cohn.[19] Aliquots of the nucleotide solutions were evaporated on aluminum plates and counted with a proportional counter. The values obtained are reported in Table I as counts per minute per micromole of nucleotide. Less than 1 mg. of solid per plate (7 sq. cm.) was necessary to obtain an adequate counting rate and no correction for weight has been made.

Ribo- and desoxyriboadenylic acids were hydrolyzed by treatment with 1 *N* hydrochloric acid for two hours on the steam-bath. Pyrimidine nucleotides were hydrolyzed with 70% perchloric acid for 40 minutes. After neutralization the products were separated by anion-exchange chromatography.[19]

Additional carrier was added to the thymine which was then degraded by conversion to bromohydroxyhydrothymine. After hydrolysis of the latter with sodium bicarbonate,[20] the mixture was treated with a solution of 2,4-dinitrophenylhydrazine in hydrochloric acid and warmed for two hours on the steam-bath. The acetol dinitrophenylosazone which separated in 70% yield was filtered and washed. The recrystallized derivative was burned to carbon dioxide and the activity determined with a vibrating reed electrometer.

A separate aliquot of the thymine was similarly treated except that to the hydrolyzed material was added xanthydrol in acetic acid. The dixanthydryl urea was filtered, washed and dried. No activity was recovered in this derivative.

A similar experiment in which hydrolysis was carried out with barium hydroxide permitted the recovery of carbon dioxide which also proved to be inactive.

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