The original non-methylated DNA, in contrast, when treated identically was not appreciably hydrolyzed; 0.23 equivalents of secondary phosphoric acid groups were liberated, and 90% of the hydrolytic products were found to be undialyzable. The amount of dialyzable material, 10%, was again generally in accord with the calculated mononucleotide content of the hydrolytic products.

It is evident, therefore, from these results, that when methylated as described DNA becomes much more sensitive to alkaline hydrolysis than is normally the case. The data imply that methylation and hydrolysis are essentially quantitative but confirmation of the suggested mechanism or elucidation of the exact course of these transformations along other lines will have to await chemical identification of the hydrolytic products, on which work is currently in progress.

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Incorporation of radioactive uridine-5'-monophosphate into ribonucleic acid by soluble mammalian enzymes

Recent work from this laboratory has indicated the requirements for the degradation of uracil by enzymes of rat liver[1]. In addition, the existence in this tissue of enzyme systems which can convert uracil to uridine, uridine-5'-mono-, di-, and tri-phosphates (UMP, UDP and UTP respectively)[2] has been demonstrated. Uracil, uridine and UMP have been shown, under certain conditions, to be incorporated into the ribonucleic acid (RNA) of rat liver slices at rates comparable to those obtained with orotic acid[3]. A logical extrapolation of this work was to attempt to obtain a cell-free, and if possible, a soluble system from rat liver capable of incorporating UMP into RNA. Recently this has been achieved. The object of this communication is to describe the experimental conditions and the requirements of the system.

Dialyzed acetone powder extracts of the particle-free cytoplasmic fraction of rat liver[1] have been used throughout these experiments. The UMP-4-^{14}C was prepared from orotic acid-4-^{14}C (obtained from Tracerlab Inc.) by the method already described for the preparation of UMP-2-^{14}C[3]. At the end of the incubation period, perchloric acid was added to the incubation mixture; the precipitate obtained was washed[4] and incubated overnight in 0.2 *N* KOH at 38°; the resultant solution was acidified to 0.1 *N* with respect to perchloric acid. After centrifugation, the supernatant fraction was heated at 100° for one hour to hydrolyze the purine nucleotides, and the uridylic acid was isolated by paper chromatography[5]. Because of the low amounts of RNA present in the acetone powder extract, carrier RNA was added to the acid-denatured incubation mixture, when the extract was incubated in the absence of microsomes. This was done in order to facilitate the subsequent isolation of the uridylic acid from RNA. It was found most convenient to add the carrier RNA in the form of a known volume of rat liver microsomes to the already denatured incubation mixture. The uridylic acid was then isolated as previously described. A correction for the added carrier RNA was applied to the calculation of the specific activity of the uridylic acid isolated from RNA.

As can be seen from Table I, the microsomes alone showed no incorporation of UMP. Some activity was obtained when the microsomes were incubated in the presence of the acetone powder extract, while the latter alone showed maximal activity. Pre-incubation of microsomes alone for 10 minutes, followed by a further 10 minute incubation with the extract showed no incorporation of UMP into RNA; this value should be compared to that obtained when the extract was incubated with microsomes and ATP. This suggests that a factor is released from the microsomes which inhibits the incorporation of UMP into RNA. High-energy phosphate is essential; although ATP showed maximal activity in this system, ADP was also active.

A time curve of UMP-incorporation (not presented) into RNA in the presence and absence of microsomes shows that in the former system the specific activity attained a maximum at 10 minutes, which was then followed by a sharp decline. In contrast, in the latter system, the specific activity attained a maximum at 20 minutes; this was followed by a slower decline. It may be suggested that the inhibition exerted by the microsomes, and the decrease in the specific

TABLE I

INCORPORATION OF URIDYLIC ACID-4-^{14}C INTO RNA UNDER VARIOUS CONDITIONS

All samples contained 0.2 μmole UMP-4-^{14}C with 195,000 counts per minute, 10 μmoles Mg^{++}, 0.5 ml dialyzed acetone powder extract[1], 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.4, in a volume of 1.0 ml and were incubated for 10 minutes.

Additions	*Specific activity of uridylic acid isolated from RNA c.p.m. per μmole*
Extract	11.3
Extract + 3.2 μmoles AMP	11.6
Extract + 3.2 μmoles ADP	1760
Extract + 3.2 μmoles ATP	2250
Extract + microsomes + 3.2 μmoles ATP	150*
Microsomes + 3.2 μmoles ATP	12.5*
Microsomes + 3.2 μmoles ATP incubated for 10 minutes, then extract added and incubated for 10 minutes more	11.3*

* The specific activity of the uridylic acid isolated from RNA has not been corrected for the RNA content of the microsomes.

activity of the uridylic acid in the RNA are attributable to the action of ribonuclease present in these fractions[6]. A similar explanation may be offered for the rapid inactivation of the microsomes in the amino acid incorporation experiments of ZAMECHNIK AND KELLER[7].

The following evidence indicates that the UMP-4-^{14}C is incorporated in nucleotide linkage in a high molecular weight polymer. The washed precipitate[4] was extracted with 10% NaCl and the RNA precipitated with alcohol. This precipitate was then dissolved in 3% NaCl and reprecipitated with alcohol. The final precipitate was then dissolved in 3% NaCl and dialyzed against three 4-liter changes of 3% NaCl for 24 hours. One portion of this dialyzed solution upon treatment with ribonuclease yielded radioactive uridylic acid. The other portion after alkali hydrolysis yielded either uridylic acid 2'- or 3'-phosphate, or both, as identified by the chromatographic procedure of COHN[8]. The fact that the radioactivity was isolated in the form of uridylic acid after digestion with ribonuclease and that it was further shown to be uridylic acid 2- and/or 3-phosphate after alkali treatment provides evidence for its attachment in nucleotide linkage. Since this bound radioactive uridylic acid is acid and alcohol insoluble, is extractable with hot NaCl and is non-dialyzable, it may be concluded that it constitutes a portion of a high molecular weight polymer which has the properties of RNA. Further details on fractionation, effect of other di- and tri-phosphates, etc., will be published at a later date.

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