METABOLIC CONVERSION OF THYMINE-2-C^{14} AND ITS INCORPORATION INTO NUCLEAR RNA OF ENDOSPERM NUCLEI OF *COCOS NUCIFERA*, LINN.*

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Received October 21, 1963

While studying the effect of the plant growth hormone indole acetic acid on deoxyribonucleic acid metabolism of endosperm nuclei of the coconut (*Cocos nucifera* Linn.) with thymine-2-C^{14}, it was observed that nuclear RNA had incorporated considerable C^{14}. In these experiments free endosperm nuclei from very young green fruits were isolated by centrifugation of 2-3 litres of coconut milk at 3000xg. The nuclei were then resuspended in a small volume of the supernatant at pH 6.0 containing 10 µmole/ml sucrose, 5 µmole/ml $MgCl_2$ and 10 µmole/ml thymine-2-C^{14}(sp. activity 0.1 µcurie/ml) with or without 10^{-5}M (0.01 µmole/ml) IAA at 25°C for 12 hrs. The nuclei were macerated with glass powder in a mortar and RNA and DNA extracted by Kirby's phenol method[1]. RNA and DNA were estimated after colour development with orcinol and diphenylamine at 660mµ and 600mµ respectively, using a Hilger spectrophotometer. Radioactivity meas-

* The following abbreviations have been used : IAA, indole-3 acid; DNA, deoxyribonucleic acid; DNase, deoxyribonuclease. RNA, ribonucleic acid; UMP, uridylic acid.

urements were performed with a Tracerlab windowless gas-flow counter. It is apparent from Table I that thymine-2-C^{14} had found its way not only into nuclear DNA but also into RNA. This is not due to any contamination of thymine -2-C^{14} with uracil -C^{14} since the preparation of thymine -2-C^{14} used was radiochemically pure as revealed by chromatographic analysis in which entire activity was found to be concentrated in a single spot which co-chromatographed with authentic thymine.

Table-1

Treatment	cpm/mg RNA	cpm/mg DNA
Control	7,800	19,800
IAA, 10^{-5} M	12,000	24,500

It is however possible that some C^{14} -labelled DNA was extracted along with the RNA. To eliminate this possibility a part of the isolated RNA was reprecipitated, redissolved in buffer and incubated with 20 µg/ml DNase at pH 7.4 containing 3 µmole/ml $MgCl_2$ at 28° C for 24 hrs. The contents were centrifuged and the supernatant precipitated with 4 vol. of alcohol. The precipitate was washed several times with 80% alcohol, dissolved, plated and counted. It is clear from table II that the specific activity of the RNA remained practically unaffected.

Table-II

Treatment.	C^{14} counts cpm/mg RNA (DNase treated)
Control	7,160
IAA, 10^{-5} M	11,670

To provide additional evidence that the C^{14} activity resided exclusively in uracil, the RNA was hydrolysed in 0.5N KOH at 30°C for 24 hrs. The sample was acidified with $HClO_4$ and centrifuged. The acid soluble fraction was, neutralised with KOH and the $KClO_4$ was removed by centrifugation in cold. The hydrolysate was then chromatographed in isopropanol : HCl: H_2O (78:20:22). A single radioactive spot was obtained. The spot was eluted with 0.01N HCl, dried *in vacuo*, dissolved in water and the ratio of optical densities at 280 mμ and 260 mμ was calculated. It was found to be close to 0.30 which is characteristic of UMP.

Table-III

Treatment	O.D. at 280 mμ / O.D. at 260 mμ	C^{14}-activity cpm/mg UMP.
Control	0.33	11,500
IAA, 10^{-5} M	0.34	19,200
UMP	0.30	-

It thus appears that thymine-2-C^{14} has been demethylated to uracil-2-C^{14} and incorporated into UMP of RNA in the endosperm nuclei of coconut (*Cocos nucifera* Linn.) Sells[2] had observed that the presence of thymidine considerably decreased the incorporation of uracil into the nucleic acids by intact cells of *Bacillus cereus*; this was interpreted to imply conversion of thymidine to some uracil containing material. The investigations of Friedkin *et al*[3] on chick embryos and Reichard[4] on liver tissue on the other hand indicate that thymidine apparently is not metabolised

to compounds which may contribute towards the composition of RNA. Similar observations were recorded in Escherichia coli and Salmonella typhimurium by Schaechter et al[5] and in E. coli and Enterococcus stei by Wacker[6] although conversion in the reverse direction i.e., deoxyuridine[7] or Uracil[8,9] to thymidine has been reported by several investigators.

The investigations described above clearly indicate a stimulation of the synthesis of nuclear DNA and RNA by IAA. This is in full agreement with the earlier observations of Biswas and Sen[10] and Roychoudhuri and Sen[11] concerning P^{32} incorporation in the nucleic acids of oat and rice coleoptiles as also pea internodes. Incorporation of uracil-2-C^{14} into the RNA of coconut milk nuclei has also been found[11] to be enhanced by IAA and the stimulation is over 70%. The promotion in the utilisation of thymine-2-C^{14} for RNA synthesis in presence of IAA as reported here is more than 50%. Whether IAA favours the demethylation of thymine to uracil, or the incorporation of uracil in RNA, or both, are questions which enzymological studies in progress at present are expected to answer. IAA favours not only the synthesis but also the release of nuclear RNA[11] and the regulation of cell metabolism[12,13,14] by IAA through its effects on nucleic acid and protein synthesis is a possibility which certainly merits serious consideration.

We are thankful to Dr. D.M.Bose, Director, and Dr. P.K. Bose, Head of the Dept. of Chemistry for laboratory facilities and to Dr. B.B.Biswas for his interest and encouragement.

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