# BIOSYNTHESIS OF RADIOACTIVE RNA AND DNA PYRIMIDINES FROM THYMIDINE-2-C-$^{14}$

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NEUROSPORA CRASSA APPEARS TO LACK THE THYMIDINE PHOSPHORYLATING MECHANISM WHICH ENABLES MANY ORGANISMS TO UTILIZE EXOGENOUS THYMIDINE FOR DNA SYNTHESIS (CHAKRABORTY AND LORING, 1960). ACCORDINGLY, IN WORKING WITH THE PYRIMIDINE-LESS NEUROSPORA MUTANT 36601 (KINDLY PROVIDED BY M. B. MITCHELL), IT SEEMED OF INTEREST TO FIND THAT THYMIDINE COULD STIMULATE GROWTH SIGNIFICANTLY IN THE PRESENCE OF SUBOPTIMAL LEVELS OF URIDINE (TABLE I).

TABLE I

GROWTH OF NEUROSPORA MUTANT 36601 FROM CONIDIAL SUSPENSIONS INCUBATED IN 125 ML ERLENMEYER FLASKS AT 25° FOR 5 DAYS WITH 20 ML OF MINIMAL LIQUID MEDIA (HOROWITZ AND BEADLE, 1943) SUPPLEMENTED AS NOTED

| SUPPLEMENTS (μMOLES/20 ML) | | DRY WT. OF MYCELIA (MG) |
|---|---|---|
| URIDINE | THYMIDINE | (TRIPLICATE FLASKS) |
| 0 | 1 | 0, 0, 0 |
| 1 | 0 | 25, 23, 26 |
| 1 | 1 | 34, 32, 36 |
| 2 | 0 | 54, 56, 56 |

AS AN INITIAL STEP IN A METABOLIC STUDY OF THIS STIMULATORY EFFECT, THE NON-RADIOACTIVE THYMIDINE EMPLOYED IN THE GROWTH EXPERIMENTS WAS REPLACED WITH THYMIDINE-2-C$^{14}$ (NEW ENGLAND NUCLEAR CORP., BOSTON, MASS.). IN THE RADIOCARBON EXPERIMENTS THE MYCELIAL PADS WERE PRESSED BRIEFLY BETWEEN LAYERS OF DRY FILTER PAPER AND IMMEDIATELY UTILIZED FOR PREPARATION OF A CRUDE NUCLEIC ACID FRACTION BY FOLLOWING EARLY STEPS IN THE PROCEDURE OF KIRBY AS MODIFIED BY KIT (1960).

THIS INVOLVED HOMOGENIZATION WITH P-AMINOSALICYLATE, STIRRING WITH PHENOL, ADDITION OF COLD ETHYL CELLOSOLVE TO THE AQUEOUS LAYER, AND WASHING THE PRECIPITATE WITH COLD ETHANOLIC SALINE. THE NUCLEIC ACID PRECIPITATE WAS TAKEN UP IN WATER (FINAL VOLUME 350 μL), ADJUSTED TO PH 6-7, AND INCUBATED UNDER TOLUENE FOR 12 HOURS AT 28° WITH 3.6 UMOLES OF $MgSO_4$ AND 0.3 MG OF DEOXYRIBONUCLEASE (WORTHINGTON BIOCHEMICAL CORP., FREEHOLD, N. J.). TWO MG OF LYOPHILIZED *CROTALUS ADAMANTEUS* VENOM (ROSS ALLEN REPTILE INSTITUTE, SILVER SPRINGS, FLA.) WERE THEN ADDED, FOLLOWED BY AN ADDITIONAL INCUBATION PERIOD OF 8 HOURS AT PH 8-9 AND 37°. THE ENZYMES WERE PRECIPITATED BY TREATING IN THE COLD WITH 3 VOLUMES OF ETHANOL, AND THE ALCOHOLIC SOLUTION OF NUCLEOSIDES WAS SUBJECTED TO TWO-DIMENSIONAL PAPER CHROMATOGRAPHY, EMPLOYING AS SOLVENT 1: T-BUTYL ALCOHOL, METHYL ETHYL KETONE, WATER AND AMMONIUM HYDROXIDE (40:30:20:10) AND AS SOLVENT 2: THE UPPER LAYER FROM T-BUTYL ALCOHOL, S-BUTYL ALCOHOL AND WATER (1:5:5.6).

WHEN A CHROMATOGRAM FROM THE INITIAL EXPERIMENT WITH THYMIDINE-2-$C^{14}$ WAS SCANNED WITH A GEIGER COUNTER, IT WAS FIRST NOTED WITH SOME GRATIFICATION THAT UNDER THE CONDITIONS EMPLOYED THE NEUROSPORA MUTANT HAD APPARENTLY BEEN ABLE TO INCORPORATE A MODERATE AMOUNT OF THE ISOTOPE INTO DNA THYMIDINE, THUS PRESUMABLY ACCOUNTING FOR THE URIDINE-SPARING ACTION OF EXOGENOUS THYMIDINE. AS THE SCANNING PROCEEDED, HOWEVER, THE FEELING OF GRATIFICATION WAS CONVERTED TO SOMETHING APPROACHING MORTIFICATION, FOR THE PATTERN OF RADIOACTIVITY BEGAN TO SHOW A STRIKING SIMILARITY TO THAT FOUND IN URIDINE-2-$C^{14}$ EXPERIMENTS (TABLE II). RADIOTHYMIDINE HAS LONG BEEN UTILIZED AS A MEANS OF SPECIFICALLY LABELING DNA (FRIEDKIN, TILSON AND ROBERTS, 1956), THUS IT SEEMED ALMOST CERTAIN THAT THE RNA-LABELING IN THIS INSTANCE REPRESENTED SOME GROSS EXPERIMENTAL ERROR, E.G. A 50% CONTAMINATION OF THE COMMERCIAL RADIOTHYMIDINE WITH RADIOURIDINE.

A RIGOROUS CHROMATOGRAPHIC CHECK OF THE IDENTITY AND PURITY OF THE THYMIDINE-2-$C^{14}$ PREPARATION, HOWEVER, SHOWED IT TO CONTAIN NO MORE THAN TRACES OF OTHER RADIOACTIVE COMPOUNDS; REPETITION OF THE THYMIDINE-2-$C^{14}$

TABLE II

RADIOACTIVITY OF THE MAJOR PYRIMIDINE NUCLEOSIDES IN NUCLEIC ACIDS ISOLATED FROM *NEUROSPORA* MUTANT 36601 GROWN ON 0.7 μMOLE (1 μC) OF THYMIDINE-2-$C^{14}$ PLUS 1 μMOLE OF URIDINE (DUPLICATE EXPERIMENTS), OR ON 1 μMOLE (75 mμC) OF URIDINE-2-$C^{14}$

| SUPPLEMENTS | % RECOVERY OF ADMINISTERED $C^{14}$ IN: | | | |
|---|---|---|---|---|
| | URIDINE | CYTIDINE | DEOXYCYTIDINE | THYMIDINE |
| THYMIDINE-2-$C^{14}$ PLUS URIDINE | 8, 8 | 7, 8 | 1.1, 1.1 | 1.2, 1.1 |
| URIDINE-2-$C^{14}$ | 17 | 16 | 2.2 | 2.2 |

EXPERIMENTS WITH *NEUROSPORA* PROVED THAT THE RESULTS WERE REPRODUCIBLE; AND THE INTENSE LABELING OF RNA WAS FOUND NOT TO BE DEPENDENT UPON AUTOCLAVING OF THE SUPPLEMENTARY NUCLEOSIDES OR UPON THE ANALYTICAL PROCEDURE EMPLOYED. FOR EXAMPLE, FILTER STERILIZATION OF THE THYMIDINE-2-$C^{14}$ AND USE OF 1 M PIPERIDINE AT 55° (CRESTFIELD AND ALLEN, 1957) FOR DIRECT DIGESTION OF A PORTION OF THE RADIOACTIVE MYCELIA PROVIDED A HIGH YIELD OF $C^{14}$-LABELED URIDYLIC AND CYTIDYLIC ACIDS.

USE OF PERCHLORIC ACID (MARSHAK AND VOGEL, 1951) TO HYDROLYZE MYCELIA GROWN IN URIDINE PLUS THYMIDINE-2-$C^{14}$ YIELDED HIGHLY RADIOACTIVE PYRIMIDINE BASES, PROVIDING EVIDENCE THAT A MAJOR PROPORTION OF THE ISOTOPE WAS INCORPORATED INTO THE PYRIMIDINE RING OF THE NUCLEIC ACIDS RATHER THAN INTO THE CARBOHYDRATE MOIETIES. THE PYRIMIDINE LABELING SEEMED TOO EXTENSIVE TO REPRESENT A RE-UTILIZATION OF $C^{14}O_2$ FORMED BY CATABOLISM OF THE ADDED RADIOTHYMIDINE, AND THE ABSENCE OF DETECTABLE AMOUNTS OF RADIOACTIVITY IN THE NUCLEIC ACID PURINE NUCLEOSIDES ALSO SUGGESTED THAT THE LABELED CARBON OF THE ADDED THYMIDINE WAS UTILIZED RATHER SPECIFICALLY FOR THE FORMATION OF NUCLEIC ACID PYRIMIDINES. THE NATURE OF THE METABOLIC PATHWAY INVOLVED IN THIS PHENOMENON IS UNDER INVESTIGATION.

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