A BIOSYNTHETIC ORNITHINE DECARBOXYLASE IN *ESCHERICHIA COLI*[1]

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PUTRESCINE (1,4-DIAMINOBUTANE) IS FOUND IN *E. COLI*, UNDER A VARIETY OF GROWTH CONDITIONS, AT A LEVEL OF APPROXIMATELY 15 MICROMOLES PER GRAM OF WET WEIGHT. IN SPITE OF THIS RELATIVELY HIGH INTRACELLULAR CONCENTRATION, VERY LITTLE IS KNOWN ABOUT ITS BIOSYNTHESIS. TABOR, ROSENTHAL AND TABOR (1958) DEMONSTRATED THE INCORPORATION OF ^{14}C-ORNITHINE INTO PUTRESCINE IN INTACT *E. COLI*, INDICATING THAT ONE OF THE LATE INTERMEDIATES OF THE PATHWAY OF ARGININE BIOSYNTHESIS IS A PRECURSOR OF PUTRESCINE. GALE (1940) DEMONSTRATED AN INDUCED ORNITHINE DECARBOXYLASE IN *E. COLI* WHICH CATALYZED THE FORMATION OF PUTRESCINE. HOWEVER, SINCE HIGHLY SPECIALIZED CULTURE CONDITIONS ARE REQUIRED FOR THE FORMATION OF THIS ENZYME, IT CANNOT BE RESPONSIBLE FOR THE LARGE AMOUNTS OF PUTRESCINE WHICH ARE SYNTHESIZED DURING GROWTH IN MINIMAL MEDIUM. THIS INDUCED ORNITHINE DECARBOXYLASE PROBABLY SERVES A CATABOLIC FUNCTION.

IN THIS PAPER WE REPORT THE PRESENCE OF AN ORNITHINE DECARBOXYLASE IN *E. COLI* WHICH APPEARS TO FUNCTION IN A BIOSYNTHETIC RATHER THAN A CATABOLIC CAPACITY. THIS BIOSYNTHETIC ENZYME IS DISTINCT FROM THE INDUCED DECARBOXYLASE DESCRIBED BY GALE, DIFFERING IN ITS pH OPTIMUM, HEAT STABILITY AND CONDITIONS FOR FORMATION.

METHODS AND MATERIALS

MOST OF THE STUDIES DESCRIBED HERE WERE CARRIED OUT WITH STRAIN 3050-ARG 4 OF *E. COLI* K-12 (OBTAINED FROM DR. W. MAAS). THIS IS AN HFR STRAIN WHICH REQUIRES ARGININE DUE TO A BLOCK BETWEEN N-ACETYLGLUTAMIC ACID AND N-ACETYLGLUTAMIC SEMIALDEHYDE. THIS ORGANISM WAS ROUTINELY GROWN ON MEDIUM E, DESCRIBED BY VOGEL AND BONNER (1956). THIS MEDIUM WAS SUPPLEMENTED WITH TRACE ELEMENTS (AMES, GARRY AND HERZENBERG, 1960), THIAMINE (0.5 μGRAMS PER ML.) AND ARGININE (50 μGRAMS PER ML.). FOR INDUCTION OF

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THE GALE DECARBOXYLASE, THE CELLS WERE GROWN AS DESCRIBED BY SHER AND MALLETTE (1954) WITHOUT THE ADDITION OF LYSINE.

THE BACTERIA WERE DISRUPTED BY GRINDING IN A MORTAR WITH ALUMINA (APPROXIMATELY 2.5 TIMES THE BACTERIAL WEIGHT). THE MIXTURE WAS THEN SUSPENDED IN 0.05 M POTASSIUM PHOSPHATE, pH 6.7, CONTAINING 10^{-3} M EDTA AND 10^{-3} M CYSTEINE (ABOUT 10 TIMES THE VOLUME OF THE PACKED CELLS). THE SUPERNATANT OBTAINED AFTER TWO CENTRIFUGATIONS FOR 15 MINUTES EACH AT 18,000 X G WAS USED AS A SOURCE OF ENZYME. IN THE EXPERIMENTS DESIGNED TO DEMONSTRATE AN ABSOLUTE PYRIDOXAL PHOSPHATE REQUIREMENT, DIALYSIS WAS CARRIED OUT AT 4° C. AGAINST THE ABOVE BUFFER. THE DIALYSIS TUBING (VISKING DIVISION, UNION CARBIDE CORPORATION) WAS PREPARED BY HEATING TO 60° C. FOR THREE HOURS IN 10^{-3} M EDTA AND 1% SODIUM BICARBONATE AND WASHING TWICE FOR THREE HOURS AT 60° C. WITH DEIONIZED WATER. ESTIMATES OF PROTEIN CONCENTRATION WERE MADE BY THE METHOD OF LOWRY, ET AL. (1951).

THE DECARBOXYLATION OF ORNITHINE WAS MEASURED BY TRAPPING AND COUNTING THE $^{14}CO_2$ EVOLVED FROM U-^{14}C-ORNITHINE. THE STANDARD ASSAY MIXTURE FOR THE ESTIMATION OF THE BIOSYNTHETIC ORNITHINE DECARBOXYLASE CONTAINED 30 µMOLES OF TRIS-HCL BUFFER, pH 7.5, 2.22 µMOLES OF U-^{14}C-ORNITHINE (1.2×10^5 D.P.M./µMOLE), 0.012 µMOLES OF PYRIDOXAL PHOSPHATE AND AN APPROPRIATE AMOUNT OF ENZYME IN A TOTAL VOLUME OF 0.30 ML. THE REACTION WAS STARTED BY THE ADDITION OF ENZYME. THE INCUBATION WAS CARRIED OUT AT 37° C. IN A STOPPERED TUBE WHICH HAD INSERTED IN THE TOP A FLUTED PIECE OF FILTER PAPER, IMPREGNATED WITH 0.02 ML. OF HYDROXIDE OF HYAMINE (PACKARD INSTRUMENT CO.). AT THE END OF THE INCUBATION PERIOD, THE REACTION WAS STOPPED BY THE ADDITION OF 0.02 ML. OF 100% (W/V) TRICHLOROACETIC ACID. THE STOPPERED TUBE WAS THEN INCUBATED AT 37° C. FOR 20 MINUTES IN ORDER TO TRANSFER ALL OF THE $^{14}CO_2$ EVOLVED TO THE FILTER PAPER. THE LATTER WAS PLACED IN 15 ML. OF BRAY'S SCINTILLATION FLUID (1960) AND COUNTED IN A PACKARD SCINTILLATION COUNTER. UNDER THESE CONDITIONS, THE ENZYME WAS SATURATED WITH RESPECT TO BOTH ORNITHINE AND PYRIDOXAL PHOSPHATE AND THE FORMATION OF CO_2 PROCEEDED LINEARLY FOR AT LEAST 60 MINUTES.

U-^{14}C-ORNITHINE WAS SYNTHESIZED FROM U-^{14}C-ARGININE (VOLK RADIOCHEMICAL CO.) BY THE METHOD OF RIVAL AND CARTER (1955). THE SYNTHETIC MATERIAL CONTAINED LESS THAN 5% ARGININE AS JUDGED BY THIN LAYER CHROMATOGRAPHY.

RESULTS

THE LIBERATION OF CO_2 FROM ORNITHINE, CATALYZED BY A DIALYZED CRUDE

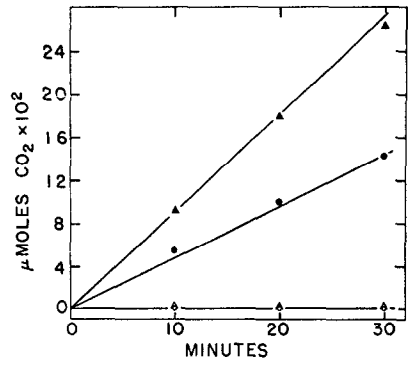

FIG. 1. ENZYMATIC DECARBOXYLATION OF ORNITHINE. THE DECARBOXYLATION OF ORNITHINE WAS MEASURED UNDER THE STANDARD ASSAY CONDITIONS DESCRIBED IN METHODS AND MATERIALS IN THE PRESENCE OF 0.10 MG. OF A CRUDE EXTRACT OF 30SO-ARG4 WHICH HAD BEEN DIALYZED OVERNIGHT (●—●). THE ACTIVITY WAS ALSO MEASURED IN THE ABSENCE OF PYRIDOXAL PHOSPHATE (△—△), IN THE ABSENCE OF ENZYME (○—○), AND IN THE PRESENCE OF 0.20 MG. OF THE DIALYZED CRUDE EXTRACT (▲—▲).

EXTRACT IS SEEN IN FIG. 1. THE RATE OF THE REACTION WAS PROPORTIONAL TO THE CONCENTRATION OF ENZYME, AND THE ENZYME HAD AN ABSOLUTE REQUIREMENT FOR PYRIDOXAL PHOSPHATE. THE OTHER PRODUCT OF THE REACTION WAS DEMONSTRATED TO BE PUTRESCINE BY THIN LAYER CHROMATOGRAPHY. THE AMOUNT OF PUTRESCINE FORMED WAS STOICHIOMETRIC WITH THE AMOUNT OF CO_2 EVOLVED (1.3 μMOLES OF PUTRESCINE PRODUCED, 1.5 μMOLES OF CO_2 EVOLVED, AND 1.5 μMOLES OF ORNITHINE LOST)[3].

THE DEPENDENCES ON PH OF THE RATES OF ORNITHINE DECARBOXYLATION BY EXTRACTS OF CELLS GROWN UNDER TWO DIFFERENT CONDITIONS WERE COMPARED (FIG. 2). THE EXTRACT OF CELLS GROWN ON MINIMAL MEDIUM SHOWED A SINGLE OPTIMUM AT PH 7.5. IN CONTRAST, WHEN E. COLI WAS GROWN UNDER CONDITIONS WHICH INDUCE THE GALE DECARBOXYLASES, TWO DISTINCT PH OPTIMA WERE OBSERVED. THE ACTIVITY OPTIMUM AT PH 7.5 CORRESPONDED TO THAT OF NON-INDUCED CELLS AND REPRESENTED THE BIOSYNTHETIC ORNITHINE DECARBOXYLASE ACTIVITY. THE OPTIMUM AT PH 5.3 WAS ONLY PRESENT IN INDUCED CELLS AND IS IDENTICAL TO THE PH OPTIMUM REPORTED BY GALE FOR THE ADAPTIVE ORNITHINE DECARBOXYLASE (1940).

THE STABILITIES OF THE BIOSYNTHETIC AND CATABOLIC ORNITHINE DECARBOXYLASES TO HEATING WERE DISTINCTLY DIFFERENT. IN THE EXPERIMENT

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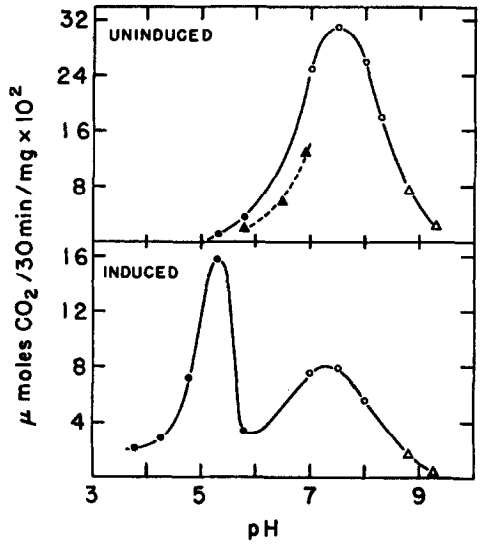

FIG. 2. DEPENDENCE OF BIOSYNTHETIC AND CATABOLIC ORNITHINE DECARBOXYLASE ACTIVITIES ON pH. EXTRACTS WERE PREPARED OF 3080-ARG 4 WHICH HAD BEEN GROWN ON MEDIUM E (UNINDUCED CELLS) AND ON THE SHER AND MALLETTE MEDIUM (INDUCED CELLS). THESE EXTRACTS WERE ASSAYED IN THE STANDARD REACTION MIXTURE BUFFERED BY 0.1 M SODIUM ACETATE (●—●), POTASSIUM PHOSPHATE (▲—▲), TRIS-HCL (○—○), OR AMMEDIOL-HCL (△—△) AT THE INDICATED pH VALUES. THE SOLID LINE BETWEEN pH 6-7 IN THE UPPER CURVE (UNINDUCED CELLS) WAS OBTAINED BY CORRECTING FOR THE 50% INHIBITION BY PHOSPHATE (▲—▲).

SHOWN IN FIG. 3, AN EXTRACT OF AN INDUCED CULTURE OF E. COLI WAS SUBJECTED TO 55° C. AND ASSAYED AT pH 5.3 AND pH 7.5 AFTER VARIOUS TIMES OF EXPOSURE. THE ACTIVITY AT pH 7.5, WHICH WAS DUE TO THE ACTIVITY OF THE BIOSYNTHETIC ENZYME, WAS STABLE OVER A 10 MINUTE PERIOD. ON THE OTHER HAND, THERE WAS A MARKED DROP IN THE ACTIVITY OF THE ADAPTIVE ENZYME, AS MEASURED AT pH 5.3. THE STABILITIES OF THE BIOSYNTHETIC ENZYMES IN BOTH UNINDUCED AND INDUCED CELLS WERE IDENTICAL.

IN ADDITION TO THE RESULTS REPORTED HERE WITH A MUTANT STRAIN OF E. COLI K-12, WE HAVE ALSO OBSERVED APPROXIMATELY THE SAME LEVEL OF BIOSYNTHETIC ORNITHINE DECARBOXYLASE ACTIVITY IN WILD TYPE K-12 AND ALSO IN E. COLI B GROWN ON MINIMAL MEDIUM.

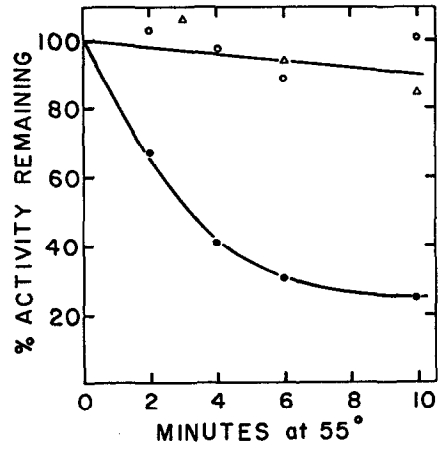

FIG. 3. HEAT STABILITIES OF THE BIOSYNTHETIC AND CATABOLIC ORNITHINE DECARBOXYLASES. AN EXTRACT OF 3080-ARG 4 GROWN UNDER THE CONDITIONS OF SHER AND MALLETTE (11 MG./ML., SPECIFIC ACTIVITIES 0.24 μMOLES/30 MINUTES/MG. PROTEIN AT PH 5.3, AND 0.13 AT PH 7.5) WAS HEATED AT 55° C. FOR THE INDICATED TIMES AND CENTRIFUGED AT 18,000 X G FOR 15 MINUTES. SAMPLES (0.05 ML.) WERE ASSAYED UNDER THE STANDARD CONDITIONS IN 0.1 M TRIS-HCL, PH 7.5 (O—O, BIOSYNTHETIC ACTIVITY) AND IN 0.1 M SODIUM ACETATE, PH 5.3 (●—●, CATABOLIC ACTIVITY) AND THE RESULTS PLOTTED AS PER CENT OF THE ORIGINAL ACTIVITY REMAINING. AN EXTRACT OF CELLS GROWN IN MEDIUM E (12 MG./ML., SPECIFIC ACTIVITY 0.29 AT PH 7.5) WAS HEATED UNDER THE SAME CONDITIONS. SAMPLES (0.01 ML.) WERE ASSAYED AT PH 7.5 (△—△) AND THE RESULTS PLOTTED AS ABOVE.

DISCUSSION

THE ABOVE RESULTS DEMONSTRATE THE PRESENCE OF TWO ORNITHINE DECARBOXYLASES IN *E. COLI*. ONE OF THESE ENZYMES APPEARS TO SERVE A BIOSYNTHETIC FUNCTION AND IS PRESENT IN CELLS GROWN UNDER A VARIETY OF CULTURE CONDITIONS. THE FORMATION OF THE OTHER ORNITHINE DECARBOXYLASE IN *E. COLI* REQUIRES SPECIAL CONDITIONS OF NUTRITION, PH AND AERATION (GALE, 1940). THIS ENZYME SEEMS TO HAVE A CATABOLIC ROLE. THESE TWO ENZYMES ALSO DIFFER IN THEIR PH OPTIMA AND HEAT STABILITIES. IN AN ANALOGOUS CASE, UMBARGER AND BROWN (1957) DEMONSTRATED THE PRESENCE OF A BIOSYNTHETIC THREONINE DEAMINASE IN *E. COLI* IN ADDITION TO THE CATABOLIC ENZYME DESCRIBED BY WOOD AND GUNSALUS (1949).

IF THE DECARBOXYLATION OF ORNITHINE IS THE SOLE PATH TO PUTRESCINE IN *E. COLI*, AN ANOMALY EXISTS. SINCE ARGINASE ACTIVITY HAS NEVER BEEN REPORTED IN *E. COLI*, MUTANTS WHICH CANNOT MAKE ORNITHINE SHOULD REQUIRE PUTRESCINE AS WELL AS ARGININE FOR GROWTH. THEREFORE, EITHER A HITHERTO

UNKNOWN MECHANISM IN E. COLI CONVERTS ARGININE TO ORNITHINE, OR A BIOSYNTHETIC PATHWAY FROM ARGININE TO PUTRESCINE EXISTS WHICH DOES NOT INVOLVE ORNITHINE AS AN INTERMEDIATE. EVIDENCE CONSISTENT WITH THE LATTER ALTERNATIVE WILL BE PRESENTED IN A SUBSEQUENT PUBLICATION[3].

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