Oxybiotin in the Bacterial Deamination of Aspartic Acid. (17982)

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Oxybiotin has been shown to have biotin-like activity for a variety of microbial and animal species(1-5). It is now accepted that, in the systems that have been studied, oxybiotin functions as such without conversion to biotin. The statement has been made that, "Since several widely different species can utilize oxybiotin as such, it seems reasonable to predict that oxybiotin can replace biotin in all biological forms"(6).

Among the functions in which biotin more recently has been implicated is that of stimulating the aspartic acid deaminase activity of bacterial cells that have been exposed to M phosphate buffer of pH 4(7-12). The effect of oxybiotin under these conditions has not been reported previously and this paper describes the ability of oxybiotin to replace biotin in the system.

Experimental. Procedures that have been employed in the aspartic acid deaminase experiments have been described in detail(12). Briefly an 18-hour culture of the organism in a yeast extract-tryptone-formate-phosphate medium was centrifuged, washed in water, recentrifuged, suspended in M phosphate buffer of pH 4 at 37°C for inactivation of the aspartic acid system, and neutralized. The effect of various adjuvants on the ability of the organism to deaminate added aspartic

TABLE I.
Protocol for Aspartic Acid Deamination.

	No. 1, ml	No. 2, ml	No. 3, ml	No. 4, ml
Bacterial suspension in pH 4 M phosphate buffer	.5	.5	.5	.5
Inactivated at 37°C for 30 min.				
Na_3PO_4 (saturated)*	.5	—	—	—
Aspartic acid† in Na_3PO_4 (saturated)*	—	.5	.5	.5
H_2O	1.0	1.0	—	—
Biotin‡	—	—	1.0	—
Oxybiotin§ or desthiobiotin ¦	—	—	—	1.0
Incubated at 37°C for 30 min.				
Trichloroacetic acid	.5	.5	.5	.5

Centrifuged and ammonia nitrogen determined by Nesslerization on an aliquot of each centrifugate.

* This addition brings the pH to 7.0.

† 266 mg DL-aspartic acid dissolved in 100 ml saturated Na_3PO_4. Calculated to give 0.005 M DL-aspartic acid in the final reaction mixture.

‡ 0.001 γ D-biotin/ml.

§ 0.001 γ or 1.0 γ D-oxybiotin/ml.

¦ 0.001 γ D-desthiobiotin/ml.

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TABLE II.
Summary of Aspartic Acid Deaminase Experiments.

Exp. No.	Ammonia nitrogen formed			
	No supplement, γ	Biotin, γ	Oxybiotin, γ	Desthiobiotin, γ
1	11.8	18.0 (.001 γ)	17.3 (.001 γ)	—
2	11.5	58.5 (.001 γ)	25.0 (.001 γ)	—
3	10.0	51.5 (.001 γ)	16.0 (.001 γ)	—
4	3.5	30.0 (.001 γ)	41.5 (1.0 γ)	—
5	13.0	57.5 (.001 γ)	—	15.0 (.001 γ)

Figures in parentheses indicate the quantity of supplement tested per 2 ml reaction mixture.

acid at pH 7 then was determined according to the protocol given in Table I. A strain of *Proteus vulgaris* was the organism employed. The oxybiotin* (O-heterobiotin)* and the desthiobiotin used were racemic mixtures. The quantities used in this paper are in terms of active D– component employed.

Results and discussion. The results that have been obtained are summarized in Table II. It is apparent from experiments 1-3, where biotin and oxybiotin were tested at equivalent levels, that oxybiotin is active in stimulating the aspartic acid deaminase system, although it is somewhat less active than is biotin. As demonstrated by experiment 4, when tested at a high level (1.0 γ/tube) oxybiotin can replace completely the functions of biotin in the bacterial deamination of aspartic acid. Desthiobiotin, as the results of experiment 5 indicate, is essentially inactive. Thus it appears that ring closure is essential for activity in the present system as it is in other systems involving biotin that have been studied previously.

Summary. Oxybiotin is less active than biotin in stimulating the aspartic acid deaminase system of bacterial cells that have been inactivated by exposure to M phosphate buffer of pH 4. In sufficient amount, however, oxybiotin duplicates completely the stimulation produced with biotin.

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Effect of Hepatectomy upon the Analgetic Action of 1 Methadone.* (17983)

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While it may be considered that the liver is probably the chief site of detoxification of 1 Methadone, it was of interest to test this hypothesis. It was proposed to determine this by examining the effect of partial hepatectomy upon the duration of analgesia following a standard dose of the drug administered to rats.

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Methods. Male albino rats (Red Bank) weighing 250 to 350 g were used. Analgesia was measured using a modification of the D'Amour Smith method(1,2) in which radiant heat is applied to the terminal portion of the rat's tail. One of the chief features

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