acetyl·X serving as hydrogen acceptor. Molecular hydrogen is subsequently formed and acetyl·X regenerated as in scheme A. It is suggested that acceptors other than hydrogenase may act as oxidants for the acetaldehyde derivative in which case molecular hydrogen would not be produced. The rate of CO_2 evolution in the presence of such oxidants could be construed as a measure of the "formic dehydrogenase" activity which is always observed in active hydrogenlyase preparations. Both sequences are represented in terms of formate decomposition; for purposes of clarity, the reverse reactions, which could account for the synthesis of formate from CO_2 and H_2 are not indicated.

Reaction (3) is common to both schemes which emphasizes the suggested role of C_2 derivatives as intermediate hydrogen carriers. In this connection, it is of interest that the inference may be drawn from a recent report by PEEL AND BARKER[4] that the reduction of vinylacetate by H_2, catalyzed by dried cell preparations of *Cl. kluyveri*, involves acyl derivatives in a similar role.

Acceptance of Scheme A implies that formation of H_2 and CO_2 from pyruvate by *E. coli* and *Cl. butylicum* occurs through a common pathway. Thus both organisms would activate pyruvate to $[C_3]$ and thence through reactions (2) and (3) to acetate, H_2 and CO_2. The inability of *Cl. butylicum* to convert formate to H_2 and CO_2 could be explained as due to absence of reaction (1). Reversal of reaction (1) would be visualized as one of the initial stages in the classical phosphoroclastic split of pyruvate (to acetate and formate), which can be readily demonstrated in *E. coli* when the organism is grown so as not to produce H_2 and CO_2 from formate or pyruvate.

The nature of the C_2 derivatives in the above schemes is not specified. Conceivably, any of the currently postulated C_2-coenzyme compounds (C_2 lipoic, C_2 lipothiamide, or C_2 cocarboxylase)[5,6,7], may be involved. At the present time there appears to be no definitive evidence which excludes the possibility that these C_2 derivatives can be generated without intermediation of acetyl coenzyme A[8].

The work described here can account for the observation that traces of fermentable carbohydrates stimulate the hydrogenlyase activity of intact cell preparations of *E. coli*[9]. Our results suggest that these activations may have been mediated through formation of a C_2 derivative.

The authors are indebted to Professor L. O. KRAMPITZ for his continued active interest in this investigation which was supported by a grant (Contract No. AT(30-1)-1050) from the Atomic Energy Commission.

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Received April 29th, 1953

ON THE DISSIMILATION OF DL-ALANINE-1-^{14}C BY RAT BRAIN HOMOGENATES

by

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In a recent article, we reported that brain tissue dissimilates DL-alanine-1-^{14}C at a rate about one third of that exhibited by the kidney[1]. This interpretation was based on measurements of $^{14}CO_2$ produced from this amino acid by homogenates. It was of interest to learn whether there is a preferential utilization of one optical isomer since KREBS[2] has shown that members of the D-series are

* Aided by a grant from the National Foundation for Infantile Paralysis.

more rapidly deaminated by kidney slices than are the corresponding members of the L-series. When an inert isomer is added to the radioactive racemic mixture prior to incubation with the tissue the isomer should decrease by isotope dilution the amount of radioactive CO_2 produced if the isomer is identical with that member of the racemic mixture which the homogenate selects for dissimilation. The results given in the table would suggest that *the kidney utilizes both the* L *and* D *forms of alanine while the brain utilizes mainly the* L*-form**. KREBS[2] showed that deamination of L-amino acids but not that of D-amino acids is inhibited by octyl alcohol. One would expect, therefore, that this alcohol inhibits the activity of kidney homogenate partially and that of brain homogenate totally. We observed, however, no inhibition in either case. From the table it is also evident that the dissimilation of DL-alanine is accelerated by addition of members of the polycarboxylic acid cycle and inhibited in the absence of oxygen.

TABLE I

INFLUENCE OF VARIOUS SUBSTANCES ON $^{14}CO_2$ PRODUCTION FROM DL-ALANINE-1-^{14}C BY KIDNEY AND BRAIN HOMOGENATES

Substance added	*Relative radioactivity of CO_2 produced*	
	Kidney	*Brain*
Control, buffer only	100†	100†
10 mg inert L-alanine	16	39
10 mg inert L-alanine	16	16
10 mg inert L-alanine	18	17
10 mg inert D-alanine	23	123
10 mg inert D-alanine	13	98
10 mg inert D-alanine	18	93
0.03 ml *n*-octyl alcohol	121	79
0.03 ml *n*-octyl alcohol	109	113
Nitrogen atmosphere to replace oxygen atmosphere	12	19
1 mg α-ketoglutarate	143	264
3 mg α-ketoglutarate	177	308
1 mg sodium succinate	138	230
3 mg sodium succinate	154	211

† This activity averaged 2.0% of administered counts eliminated as $^{14}CO_2$ for kidney homogenates and 0.6% of administered counts eliminated as $^{14}CO_2$ for brain homogenates.

Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 phosphate buffer (pH 7.4) and 0.1 mg (1.2×10^{-3} mc) of DL-alanine. The flasks were incubated at 37° C for 45 minutes in an oxygen atmosphere.

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Received April 9th, 1953

* In order to check this conclusion by a different procedure, L-alanine-1-^{14}C was prepared from DL-alanine-1-^{14}C by exposure to hog D-amino acid oxidase[3] and subsequent isolation of this L-isomer by two-dimensional paper chromatography using phenol-water and butanol-propionic acid-water as solvents. When the DL-form is dissimilated by kidney and brain homogenates, kidney is three times more active than brain, but when the L-form is used there is little difference between the rates of dissimilation by these two tissues.