groups[20,21] and on other nutritional factors not well known as yet.[22-26]

Summary. Two dogs with occlusion of all pancreatic ducts showed extensive fatty changes of the liver about 12 months after operation. Two dogs with occlusion of the main pancreatic duct in which only a very small fraction of the pancreas had remained functioning showed no fatty changes of the liver after 7½ and 8½ months respectively. The development of fatty liver is evidently not due to a pancreatic hormone but due to the lack of the external pancreatic secretion and a very small fraction of the normal pancreas and its secretion seems to be sufficient to prevent it.

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Bacterial Assimilation of Acetic and Succinic Acids Containing Heavy Carbon by *Aerobacter indologenes.**

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Slade *et al.*[1] have shown the fixation of $C^{13}O_2$ in the carboxyl group of acetic acid by *Aerobacter indologenes* and *Clostridium welchii*. The present report provides evidence for the occurrence of the reaction by a cleavage of a C_4-dicarboxylic acid. Participation of C_2-compounds in the synthesis of 2,3-butylene glycol by *Aerobacter indologenes* is also shown to occur.

Two types of acetic acid were synthesized, (1) $CH_3 \cdot C^{13}OOH$ and (2) $C^{13}H_3 \cdot C^{13}OOH$[2]. Succinic acid ($C^{13}OOH \cdot CH_2 \cdot CH_2 \cdot COOH$) was recovered from various bacterial fermentation. Salts of the acids were added to cell suspensions of *A. indologenes* in the presence of glucose under an atmosphere of nitrogen. The concentration of C^{13} was determined by mass spectrometer analysis. The normal complement of C^{13} is 1.09%.

The distribution of C^{13} on the addition of the acids is shown in Table I. Succinic acid formed in the presence of $CH_3 \cdot C^{13}OOH$ contains C^{13} exclusively in the carboxyl carbons (Table II). Succinate was not formed by a synthesis of pyruvate by C_1 and C_2 addition, followed by C_3 and C_1 addition to oxalacetate, for the succinate formed would have contained C^{13} in the methylene carbons. Thus, succinic acid is formed by means of a carbon to carbon linkage involving the carbon† atom originally present in the methyl group of acetic acid. The general reaction is:

$$\begin{matrix} CH_3 \cdot COOH \\ + \\ CH_3 \cdot COOH \end{matrix} \rightleftharpoons \begin{matrix} CH_2 \cdot COOH \\ \cdot \\ CH_2 \cdot COOH \end{matrix} + 2H$$

The reaction is presented with the reservation that acetic acid may or may not be the actual C_2 compound involved in the condensation reaction.

Succinate formed in the presence of $C^{13}H_3 \cdot C^{13}OOH$ contains C^{13} equally distributed between the methyl and carboxyl carbons (Table II). This is in agreement with the above reaction and proves that the acetate was not oxidized to $C^{13}O_2$ and the succinate formed by the Wood and Werkman reaction; otherwise the C^{13} would have been found exclusively in the carboxyl carbons.

The formation of C^{13}-acetate from succinate demonstrates that the condensation reaction is reversible. There is no mechanism known for the oxidation of succinate by which single carbon atoms may be split off leaving a C_2-residue containing a carboxyl carbon of the original succinate.

* Journal Paper No. J. 1026 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 451.

1 Slade, H. D., Wood, H. G., Nier, A. O., Hemingway, Allan, and Werkman, C. H., *J. Biol. Chem.*, 1942, **143**, 133.

† Thanks are expressed to Dr. H. G. Wood for assistance in the preparation of this acid.

TABLE I.
Fermentation of Glucose Plus Organic Acids by *A. aerogenes.*

	Acid added	CO_2	Acetic acid	Succinic acid	Ethyl alcohol	2,3-Butylene glycol
$CH_3 \cdot C^{13}OOH$	2.39	1.08	2.01	1.21	1.64	1.21
$C^{13}H_3 \cdot C^{13}OOH$	4.51	1.12	3.27	1.33	2.19	1.24
$CH_2 \cdot C^{13}OOH$ · $CH_2 \cdot COOH$	1.57	1.09	1.22	1.37	1.18	1.15

Values expressed as per cent C^{13}.

TABLE II.
Location of Heavy Carbon of Assimilated Acetic Acid in Compounds Synthesized by *A. indologenes.*

	$CH_3 \cdot C^{13}OOH$ added	$C^{13}H_3 \cdot C^{13}OOH$ added
Succinic acid	1.21	1.33
methylene carbon	1.10	1.32
carboxyl carbon	1.25	1.29
2,3-Butylene glycol	1.21	1.24
methyl carbon	1.10	1.23
hydroxyl carbon	1.27	1.22
Ethyl alcohol	1.64	2.19
methyl carbon		2.16
hydroxyl carbon		2.17

The 2,3-butylene glycol formed in the presence of $CH_3 \cdot C^{13}OOH$ contains C^{13} exclusively in the hydroxyl carbons (Table II). Thus, a carbon to carbon linkage is created in the synthesis of the glycol which involves the carbon atom originally present in the carboxyl group of acetic acid. The glycol formed in the presence of $C^{13}H_3 \cdot C^{13}OOH$ contains C^{13} equally distributed between the methyl and hydroxyl carbons. Also the addition of C^{13} succinate results in the formation of glycol containing C^{13}. This result is comparable to those in which acetate was added because of the cleavage of succinate to acetate.

Hence, direct proof has been obtained for the participation of a C_2-compound, probably acetaldehyde, in the synthesis of 2, 3-butylene glycol by the intact cell of *Aerobacter.* It is possible that either (1) two molecules of acetaldehyde or (2) a molecule of acetaldehyde and a molecule of pyruvic acid are involved. The results of Green *et al.*[2] support the latter possibility.

Ethyl alcohol containing C^{13} is formed in the presence of acetic and succinic acids (Table I). On the addition of $C^{13}H_3 \cdot C^{13}OOH$, the alcohol formed contained C^{13} equally distributed between the methyl and carboxyl carbons, consequently a reduction of acetic acid to ethyl alcohol has occurred.

Approximate calculations indicate that the condensation of acetate to succinate and the reverse reaction, the condensation of acetate to 2, 3-butylene glycol, and the reduction of acetate to ethyl alcohol are reactions which possess quantitative significance under the experimental conditions.

[2] Green, D. E., Westerfield, W. W., Vennesland, B., and Knox, W. E., personal communication.