case since another TPNH-generating system, D-isocitrate and TPN, is also capable of producing a marked stimulation of fatty acid and cholesterol synthesis (Table 1).

That the well-established defect in fatty acid synthesis seen in the diabetic state is likewise due to a lack of glucose oxidation via the hexosemonophosphate shunt is demonstrated in Table 1. The lesion observed in the intact animal and in the liver slice (*1*) is also demonstrable in liver homogenates (see also *10*, *11*). By stimulation of the hexosemonophosphate shunt, however, fatty acid synthesis in the diabetic can be restored approximately to the same level as it is in many normal livers. This increase represents a stimulation of diabetic lipogenesis of at least 100- to 700-fold. As in the normal liver, further enhancement of fatty acid synthesis is seen when both pathways are stimulated. For reasons which are not yet clear, cholesterol synthesis in the diabetic, while stimulated by hexosemonophosphate oxidation, was not relatively depressed by the addition of Embden-Meyerhof glycolysis.

That the defect in lipogenesis found in the diabetic liver is likewise primarily due to a deficiency of TPNH is supported by the fact that the alternate TPNH-generating system, D-isocitrate and TPN, will largely correct this lesion (Table 1). It would follow, therefore, that the primary diabetic block in fatty acid synthesis is at the site of action of TPNH—namely, at the reduction of crotonyl-CoA to butyryl-CoA (*9*). The location of this diabetic block at some point prior to the involvement of butyryl-CoA was previously indicated by the finding of Shaw, Dituri, and Gurin that butyryl-CoA can stimulate fatty acid synthesis in diabetic liver (*11*).

Finally, evidence that the conclusions drawn from these *in vitro* studies are applicable to the intact animal is the observation that diabetic acidosis is characterized by an accumulation of the ketone bodies, beta-hydroxybutyric and acetoacetic acids, the CoA derivatives of which are two of the fatty acid precursors preceding the blocked TPNH-requiring step.

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2 August 1957

Significance of the Malate Synthetase Reaction in Bacteria

An alternate pathway of malic acid synthesis has been discovered in our laboratories. An enzyme, named "malate synthetase," has been obtained from *Escherichia coli,* strain E26, that is grown on acetate. This enzyme converts equimolar concentrations of acetate and glyoxylate to malic acid (*1*).

Purification of malate synthetase by several ammonium sulfate precipitations and treatment with calcium phosphate gel and protamine sulfate resulted in a 50-fold purification with an approximate 30 percent yield. The final product was free of fumarase, Ochoa's condensing enzyme, isocitritase, and glyoxylate reductase. Experiments conducted with such preparations revealed that for each mole of acetyl phosphate and glyoxylate that disappears, 1 mole of malate is formed. With acetyl phosphate as substrate, coenzyme A (CoA) (*2*) and phosphotransacetylase are required. These can be replaced with acetyl-CoA. The formation of malic acid can thus be formulated as follows:

$$\text{Acetate} + \text{ATP} \rightarrow \text{acetyl phosphate} + \text{ADP} \quad (1)$$

$$\text{Acetyl phosphate} + \text{CoA} \xrightarrow{\text{transacetylase}} \text{acetyl-CoA} + \text{PO}_4 \quad (2)$$

$$\text{Acetyl-CoA} + \text{glyoxylate} \xrightarrow[\text{synthetase}]{\text{malate}} \text{malic acid} + \text{CoA} \quad (3)$$

To demonstrate the initial reactants in reaction 3, acetyl-CoA was synthesized chemically from thiolacetate and CoA and was incubated in the presence of glyoxylate and malate synthetase. Analysis of the reaction mixture, after the incubation period, revealed that malate was quantitatively formed. Magnesium ions were routinely added, but it has not yet been definitely ascertained that they are required.

The equilibrium of the over-all reaction is overwhelmingly in favor of malate synthesis. Attempts to demonstrate the reversibility of this reaction have failed thus far.

Malate synthetase appears to be an adaptive enzyme, for it was found only in cells that have been grown on acetate as the major carbon source. It has thus far been found in acetate-grown *E. coli, Aerobacter aerogenes, Corynebacterium creatinovorans,* and *Pseudomonas fluorescens.* When any of these organisms were grown on substrates other than acetic acid, malate synthetase could not be detected.

The significance of the occurrence of malate synthetase in nature (*3*) stems from the fact that it fills in a significant gap in our knowledge of carbohydrate metabolism in that it explains the long baffling problem concerning the mechanism by which bacteria can grow on two-carbon compounds such as acetic acid. Assume that cells, when first exposed to acetate as the sole carbon source, contain, as they do, minimal catalytic amounts of oxalacetate. This being the case, the first event to occur would be a combination of acetate and oxalacetate to form citrate. The latter could then be cleaved by way of isocitrate to succinate and glyoxylate. The glyoxylate formed would in turn condense with another molecule of acetate via the malate synthesis reaction, forming a *new* C_4 unit. The net result during this process, assuming no drainage to supply carbon skeletons for amino acid synthesis, would be a gain of one C_4 unit, as is shown in Fig. 1. Thus, by assuming the presence of even *one* molecule of oxalacetate, all acetate carbon could be converted to C_4 units as follows:

$$\text{Isocitrate} + \text{acetyl-CoA} \rightarrow \text{malate} + \text{succinate}$$

Further, since growth occurs on acetate as the sole source of carbon, intermediates are constantly being drained from the tricarboxylic acid cycle and utilized for amino acid synthesis. Under these conditions, rapid net synthesis of C_4 dicarboxylic acids is therefore required to provide the acceptor for the C_2 units entering the cycle. The malate synthetase reaction meets that need completely. This reaction, *in vitro,* at least, is rapid and proceeds almost exclusively in the direction of malate formation. In addition, as was pointed out earlier, the system is adaptive—that is to say, malate synthetase forms only when cells are grown on acetate as the sole carbon source. This suggests the enzyme is important primarily when C_2 intermediates are involved. Energy for synthesis during growth is, as illustrated in Fig. 1, un-

doubtedly provided by the conventional oxidation of isocitrate via the tricarboxylic acid cycle reactions. The predominant flow of any one of these pathways would of course be determined by the total economy of the cell at any moment during growth.

Now, suppose that the hypothetical situation exists in which no endogenous oxalacetate is available to the cells. Under these conditions, growth on acetate could be explained if an enzyme were present in bacteria which would bring about the direct conversion of acetate to glyoxylate via glycolate, not involving a C_6 intermediate, such as isocitrate. If this were the case, cells exposed to acetate would initially convert enough of this C_2 unit to glyoxylate in order to provide the appropriate C_2 unit for the formation of malic acid via the malate synthetase reaction. Once this process has been initiated, it could continue until all the acetate is utilized for both synthesis and energy. However, here again, when bacteria are grown on acetate, intermediates are being drained from the tricarboxylic acid cycle for synthetic reactions. Rapid synthesis of C_4 acids is therefore again required to provide acceptors for the C_2 units entering the cycle.

It is known that the condensation of CO_2 with pyruvate is one of the intermediate reactions in the synthesis of C_4 compounds. However, the quantitative significance of this reaction during growth of bacteria on C_2 units is not altogether apparent. Neither is the formation of the C_3 unit via a two-carbon compound and CO_2 apparent. Therefore, the direct formation of glyoxylate from acetate and the subsequent condensation of the latter acid to malate would indeed provide a source of readily available C_4 units.

A portion of this problem, the formation of malic acid via malate synthetase, has been solved. However, the conversion of acetate to glyoxylate not involving a C_6 intermediate has not yet been adequately demonstrated. That this reaction occurs in bacteria and yeast has been claimed by Bolcato and coworkers (*4*). However, their data do not completely exclude the possibility that glyoxylate arises by presently known mechanisms involving C_6 intermediates. Thus, with resting cells, which these investigators have used, acetate could readily combine with endogenous oxalacetate to form citrate. The latter acid, via isocitrate, could be cleaved to glyoxylate and succinate. Thus, we have the formation of glyoxylate from acetate. The data of Bolcato do not exclude this possibility.

In conclusion, it can be said that the malate synthetase reaction provides a rational explanation for the mechanism by which bacteria such as *E. coli* grow on acetate as a sole source of carbon, provided that the assumption is made that the organisms have within them catalytic quantities of oxalacetate to initiate the following processes:

$$\text{Acetate} + \text{oxalacetate} \rightarrow \text{citrate} \rightarrow \text{isocitrate} \quad (4)$$

$$\text{Isocitrate} \rightarrow \text{succinate} + \text{glyoxylate} \quad (5)$$

$$\text{Glyoxylate} + \text{acetate} \rightarrow \text{malate} \quad (6)$$

Reactions 5 and 6 may be summarized as follows:

$$\text{Isocitrate} + \text{acetate} \rightarrow \text{succinate} + \text{malate}$$

On the other hand, assuming that catalytic amounts of oxalacetate are not available, a mechanism for C_4 acid-formation needs to be postulated. Known CO_2 fixation reactions alone do not explain the formation of a C_4 compound from a C_2 unit, although the participation of CO_2 in the formation of C_4 units from acetate has been recently suggested by the experiments of Kornberg (*5*).

The mechanism by which bacteria grow on C_2 carbon units other than acetate is now also being elucidated. Thus Krakow and Barkulis (*6*) discovered a reaction wherein two molecules of glyoxylate are involved in the formation of a C_3 unit, presumably hydroxypyruvate, and CO_2. Here the situation appears to be considerably less complex than in the case of acetate. Assuming that hydroxypyruvate can be readily converted to pyruvate via known CO_2 fixations, oxalacetate is formed, and a steady supply of C_4 acids is thus provided. Malate synthetase could play a role in the growth of bacteria on glyoxylate by providing an additional route of C_4 acid formation, but only after acetate has been produced from pyruvate.

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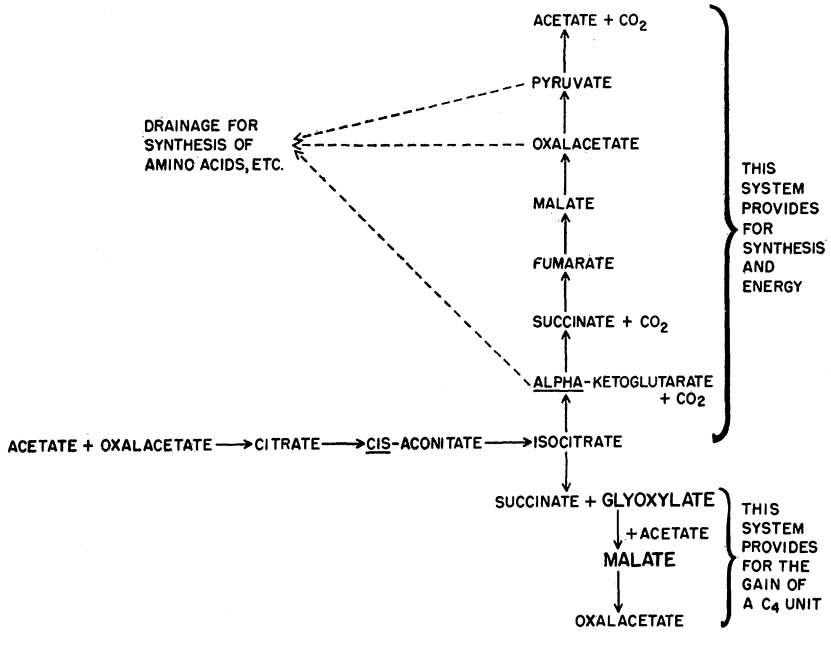

Fig. 1. Growth of bacteria on acetate.

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Oxidation of Serotonin in the Presence of Ceruloplasmin

The metabolism of serotonin leads to the formation of 5-hydroxyindoleacetic acid (*1*). However, there are other possible metabolic pathways which might bear a relationship to the physiological activity of serotonin. One of these is oxidation of the molecule to yield a *p*-quinone imine derivative, a reaction which should be catalyzed by the copper-protein enzyme, ceruloplasmin (*2*). Further oxidation or hydroxylation, enzymatic or "nonspecific" (*3*), of the *p*-quinone imine could result in compounds structurally related to adrenochrome.