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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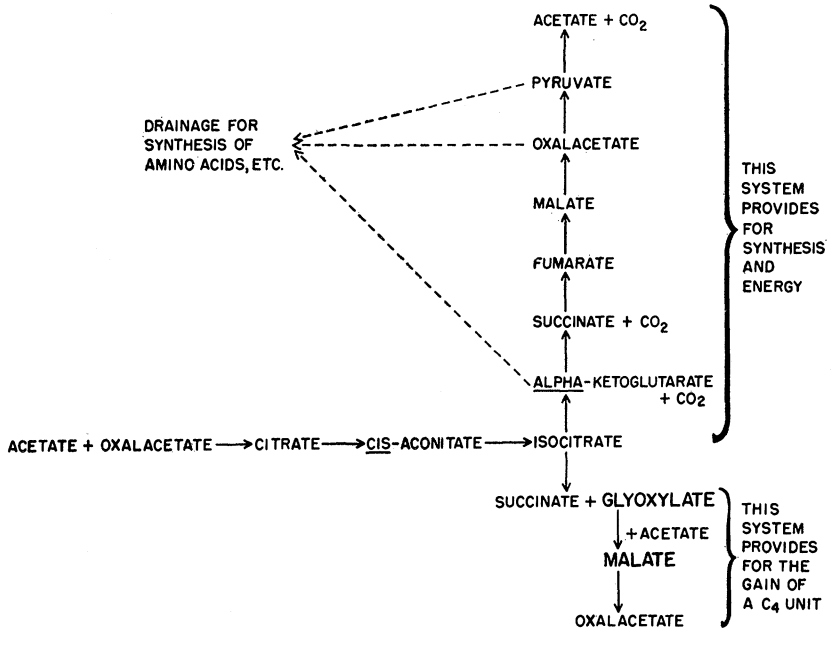

Fig. 1. Growth of bacteria on acetate.

References and Notes

1. D. T. O. Wong and S. J. Ajl, *J. Am. Chem. Soc.* 78, 3230 (1956).
2. The following abbreviations are used in this report: ATP, adenosine triphosphate; ADP, adenosine diphosphate; and CoA, coenzyme A.
3. While this manuscript was in preparation, a review paper by Kornberg and Krebs entitled "Synthesis of cell constituents from C_2 units by a modified tricarboxylic acid cycle" appeared in *Nature* [179, 988 (1957)] which includes several observations and interpretations reported here. We are deeply indebted to both Kornberg and Krebs for the opportunity they have extended to us in the reading of several of their manuscripts on this subject prior to publication.
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5 July 1957

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.

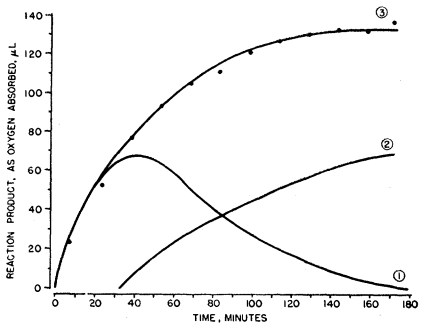

Fig. 1. Oxidation of serotonin in the presence of ceruloplasmin. Reaction rates were derived from spectrophotometric and oxygen-consumption data. Curve 1, product of the first oxidation (p_1); curve 2, product of the second oxidation (p_2); curve 3, total oxygen consumption ($p_1 + 2p_2$); the dots show observed oxygen consumption.

Ceruloplasmin concentrates were obtained by further alcohol and salt fractionation of Cohn's fraction IV-1, method 6 (*4*), from pooled human plasma. Preparation 49, which was used in the experiments described here, contained 0.23 g of copper and 1.23 g of protein per 100 ml. Thus, the enzyme had a purity above 50 percent (*2*). However, its catalytic properties were identical with those of more highly purified (90 percent) concentrates.

Three-tenths of a milliliter of enzyme solution and 2.2 ml of 0.1*M* acetate buffer, *p*H 6.00 ± 0.01, were placed in Warburg flasks and equilibrated at 37°C for 15 minutes before 0.5 ml of serotonin creatinine sulfate (6 μmole) was tipped in. The reaction was allowed to proceed in air for various intervals and was then stopped by the addition of 1 ml of 10 percent trichloroacetic acid. Spectra of suitably diluted filtrates were determined on the Beckman DU spectrophotometer.

The characteristic ultraviolet absorption of serotonin rapidly diminished, becoming eventually about half as intense as it was initially. In the visible region, a broad absorption band centered at about 530 mμ developed, but this was later obscured by more rapidly increasing absorption at shorter wave lengths. In this, as in other experiments with varying concentrations of enzyme and substrate, two atoms of oxygen per molecule of substrate were consumed. The shape of oxygen consumption and time-absorbance curves suggested that two consecutive oxidation steps might be occurring and that the second step gained no appreciable velocity until the first was essentially complete. Therefore, for mathematical analysis of the data, it was assumed that, during the earlier part of the oxidation, the only species present which absorbed visible light was the first oxidation product, P_1. Relating oxygen consumption to absorbance yielded extinction coefficients at two wavelengths for P_1. Here,

$$A_{400} = \varepsilon_{1\text{-}400}\, p_1$$

and

$$A_{530} = \varepsilon_{1\text{-}530}\, p_1$$

where A is absorbance, $\varepsilon_{1\text{-}400}$ and $\varepsilon_{1\text{-}530}$ are extinction coefficients at $\lambda = 400$ mμ and 530 mμ, respectively, and p_1 is observed oxygen consumption in microliters.

At completion, when only the second product P_2 was presumably present, oxygen consumption was related to absorbance to obtain extinction coefficients for P_2. In this case

$$A_{400} = \varepsilon_{2\text{-}400}\, p_2'$$

and

$$A_{530} = \varepsilon_{2\text{-}530}\, p_2'$$

where p_2' is one-half of the observed oxygen consumption at completion of the reaction and is equal to one atom of oxygen per molecule of serotonin originally present.

At any time during the oxidation

$$A_{400} = \varepsilon_{1\text{-}400}\, p_1 + \varepsilon_{2\text{-}400}\, p_2$$

and

$$A_{530} = \varepsilon_{1\text{-}530}\, p_1 + \varepsilon_{2\text{-}530}\, p_2$$

Simultaneous solution of these equations gave values for p_1 and p_2 in terms of microliters of oxygen absorbed; these values are plotted against time in Fig. 1. Total oxygen consumed up to any time would equal the amount of P_1 present (p_1) plus twice the amount of P_2 present ($2p_2$) at that time. This sum yielded the calculated oxygen consumption curve in Fig. 1, which fits the observed values well.

By varying the ratio of enzyme to substrate, it was possible to estimate the velocity constant and Michaelis' constant for the first reaction. From the rate of appearance of P_2 shown in Fig. 1, these constants for the second reaction were calculated. In this way it was found that the first reaction was about twice as rapid as the second and that the affinity of the enzyme for serotonin was some 3.6 times as great as it was for the first oxidation product. Since Michaelis' constant is not identical with the association constant, this latter figure is at best a rough estimate of the true ratio of affinities.

Ceruloplasmin has no monoamine oxidase activity. Neither does it catalyze the oxidation of monoamine oxidase substrates such as tyramine and phenethylamine, nor does it liberate ammonia in the course of the catalyzed oxidation of serotonin.

Like serotonin, 2-methylserotonin absorbed 2 atoms of oxygen per molecule, which indicates that the 2-position is probably not a point of oxidative attack. Polymerization of oxidized serotonin to melaninlike pigments is not likely in view of the report (*5*) that only benzene-oxygenated indoles which are unsubstituted in position 3 react in this way.

The oxidation of serotonin exhibits certain similarities to the tyrosinase-catalyzed oxidation of catechol (*6*), where oxidation to *o*-quinone is followed by hydroxylation. The two reactions differ in several respects, one being that in the tyrosinase-catechol reaction, more than two oxygen atoms can be consumed, depending upon the ratio of enzyme to substrate (*7*).

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26 June 1957

Action of New Steroids in Blocking Effects of Aldosterone and Deoxycorticosterone on Salt

Aldosterone may be of etiological significance in salt and water retention of congestive heart failure, nephrosis, liver cirrhosis, and toxemia of pregnancy (*1*). We wish to report aldosterone-blocking activity of 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone (SC-5233) and its 19-nor analog (SC-8109 (*2*). Such compounds may serve as useful agents in elucidating the role of aldosterone in edema and may be of value in the treatment of edema. Structures of the steroids are as follows: