enzyme. The possibility should also be considered that changes can occur with other labile or reactive groups, such as free amino or hydroxy groups on the surface of enzyme proteins, in connection with other types of resistance, especially where the usual theories are not entirely applicable.

Results which are analogous to the postulated phenomenon of SH shift have been described. Ingbar and Kass(7) have shown that normal hemoglobin has two moles of SH per mole of hemoglobin while hemoglobin from sickle cell anemia has three moles of SH per mole. Hughes(8) has isolated two fractions of serum albumin, one fraction having a SH group and the other appearing to have no SH group. There are some instances where the same enzyme will function either in SH or in an apparent non-SH form depending on the tissue from which it is derived. The α-glycerophosphate dehydrogenase of rabbit thigh muscle is apparently non-SH and is not inhibited by furacin(4). The same enzyme from *E. coli* is inhibited by furacin(4). The presence or absence of a SH group in a given enzyme may not affect its ability to function on a given substrate. The presence of the SH group, however, may make the enzyme liable to inhibition by SH inhibitors.

Summary. In the presence of urea, *Micrococcus pyogenes var. aureus* produces a urease that is inhibited by furacin, p-chloromercuribenzoate and trivalent arsenicals. When the organisms are grown in the absence of urea or when furacin-resistant organisms are grown in media containing urea, ureases are obtained that are not affected by the above inhibitors. In explanation of these results as well as those previously reported involving the inhibition of dehydrogenases, it is suggested that some aspects of resistance to the nitrofurans can be explained by a shift of certain enzymes from the SH to a non-SH form.

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A Cofactor for the Formic Hydrogenlyase Enzyme System.* (19361)

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A survey of the literature concerning the effects of oleic acid on the growth of microorganisms reveals 3 major findings: 1) this fatty acid is essential for the optimal growth of some species of the genera *Lactobacillus, Corynebacterium,* and *Mycobacterium*(1-4); 2) oleic acid is required for certain strains of lactobacilli only in the absence of biotin which it apparently replaces(3,5); and 3) in sharp contrast to the growth stimulating effect, the toxic action of this fatty acid for various microorganisms has been recognized(2-4,6). These actions are not strictly limited to oleic acid, since some of these effects have been reported for other unsaturated fatty acids, chief-

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ly linoleic and linolenic. Recent studies designed to investigate the possible enzymatic functions of oleic acid, and the relationship of this fatty acid to biotin, have demonstrated that oleate is required for the optimal activity of the formic hydrogenlyase (FHL) and formic dehydrogenase enzyme systems(7).

The present paper reports further studies on the role of oleate in the FHL system and demonstrates the existence in certain natural materials of a substance more active than the fatty acid.

Materials and methods. The organisms employed were a biotinless mutant strain of *Escherichia coli* and a normal wild strain of *Aerobacter aerogenes* (D-1). The basal medium contained 0.2% each of KH_2PO_4, K_2HPO_4, NaCl, and $C_6H_5O_7Na_3 \cdot 2H_2O$; 0.4% $(NH_4)_2SO_4$; 0.5% acid-hydrolyzed vitamin-free casein; 0.1% each of $MgSO_4 \cdot 7H_2O$ and HCOOH; 0.01% $MnCl_2$; and 0.001% $Fe_2(SO_4)_3$. The medium was adjusted to pH 6.5 before autoclaving, inoculated with washed suspensions of the organism, and incubated at 30°C for 15-18 hours. The cells were harvested by centrifugation, washed once with H_2O, and resuspended in H_2O to give the desired cell concentration. *A. aerogenes* was grown in the basal medium, while the *E. coli* mutant was cultured in the basal medium containing 10^{-4} μg of biotin per ml. FHL was determined at 37°C in an atmosphere of N_2 by measuring H_2 production from formate manometrically. The Warburg flask contained KOH in the inner well to absorb the CO_2 produced; formate, phosphate buffer, water, and any other materials were placed in the main compartment; the cells were added to the side arm and were tipped into the main compartment after equilibration. When desired, formate disappearance was measured colorimetrically(8).

Results. The data plotted in Fig. 1 demonstrate two important points: 1) that cells grown in the presence of oleic acid have an active FHL enzyme system in contrast to cells grown without this fatty acid, and 2) that cells harvested from the medium containing biotin and exhibiting essentially no FHL activity were capable of being stimulated by

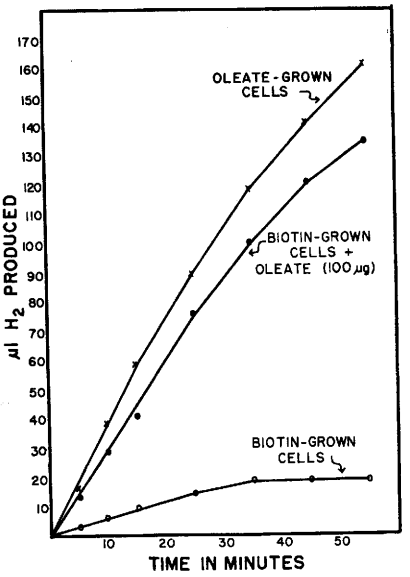

FIG. 1. Effect of oleic acid on the FHL activity of resting cell suspensions of *E. coli* (biotinless mutant). (Biotin-grown cells were harvested from the basal medium containing 10^{-4} μg biotin per ml; the oleate cells were grown in the basal medium plus 100 μg oleic acid per ml in place of biotin. Reaction run at 37°C in M/15 phosphate buffer pH 6. Cell concentration equivalent to .15 mg nitrogen. Formate added in M/10 conc. (.1 ml); final volume 3 ml).

oleate. These findings suggest that oleic acid, or some substance readily synthesized from it by the living cell, functions as a cofactor in this system.

The specificity of oleic acid was examined by studying the effects on the FHL system in resting cell suspensions of *E. coli* of each of 2 unsaturated fatty acids, linoleic and linolenic, and of several saturated fatty acids, stearic, palmitic, and pimelic. The experimental findings are presented in Table I. It may be seen that each of these substances was capable at times and at suitable concentrations of stimulating this system. The unsaturated fatty acids were more effective than the saturated members and oleic acid was the most active. On the other hand, acetate has been uniformly inactive. These data were interpreted to suggest that the living cell is capable, under certain conditions, of synthesizing a cofactor for the FHL enzyme from several fatty acids, and that of those tested oleic acid is the most suitable precursor.

TABLE I. Stimulation of FHL by Fatty Acids.*

Fatty acid	Concentration (μg) 100		10		1	
Oleic	13/15†	87‡	16/16	100	7/9	78
Linoleic	8/15	53	12/16	75	4/8	50
Linolenic	13/15	87	8/15	53	1/8	13
Stearic	7/14	50	2/15	13	1/7	14
Palmitic	9/15	60	4/16	25	1/6	17
Pimelic	5/15	33	2/15	13	1/8	13

* *E. coli* (biotinless mutant).
† Denominator = No. of experiments. Numerator = No. of positive experiments.
‡ % of experiments in which stimulation was recorded.

TABLE II. Comparative Activity of Oleate and Natural Materials.*

Additions	FHL activity†
0	27
Oleate, 10 μg	260
Yeast extract, 1 mg	960
0	0
Oleate, 100 μg	53
Yeast extract, 1 mg	940
0	550
Oleate, 50 μg	588
Yeast extract, 2 mg	1650
Liver " 2 mg	1120
0	150
Oleate, 100 μg	370
Yeast extract, 2 mg	610
Liver " 2 mg	550
0	350
Oleate, 50 μg	550
Yeast extract, 5 mg	1800
" " 1 mg	1400
" " .5 mg	950

* *E. coli* (biotinless mutant).

† Expressed as $Q_{H_2} = \frac{\mu l\ H_2 \text{ produced per hr}}{\text{mg cell N}}$.

Yeast and liver extracts are dried hot-water extracts.

Attempts were made to learn whether natural materials contain substances capable of stimulating this enzyme. That such substances exist in nature can be seen by examination of the data presented in Table II. Water extracts of yeast and liver were found to be more active than oleic acid. The organic nature of the stimulatory material was demonstrated in the following manner. The extracts were incinerated and the ash tested both by incorporating suitable amounts into the growth medium, and by the addition of the ash to the contents of the Warburg vessel. The results were uniformly negative.

Yeast extract was studied further in order to ascertain the stability of the stimulatory material. It was found that the activity of the yeast extract was increased greatly by hydrolysis at 121°C for 3 hours in 0.9 N H_2SO_4, and that hydrolysis at greater acid concentrations resulted in gradual destruction of this activity. Autoclaving without added acid resulted in a slight increase in activity. All samples were neutralized with $Ba(OH)_2$ before testing. The comparative activity of unhydrolyzed and hydrolyzed yeast extract may be seen by the titration plotted in Fig. 2.

Although suitable hydrolysis of yeast extract resulted in a marked increase in its activity with respect to the FHL enzyme system, it is emphasized that the unhydrolyzed material exhibited considerable activity. This observation suggested either of two possibilities: 1) that 2 factors are present in yeast, one available before hydrolysis, and the other after hydrolysis, or 2) that there is one factor and it is available only after hydrolysis, but the living cell is capable of accomplishing enzymatic hydrolysis. These possibilities were tested experimentally by studying dried cell preparations of *A. aerogenes* to learn the lability to the drying process of the enzyme(s) essential for hydrolysis of the yeast extract. The cells were dried by 2 methods, 1) acetone and 2) *in vacuo* over Drierite in a desiccator. The acetone-dried cells were inactive. The vacuum-dried preparations were uniformly capable of carrying out the breakdown of formate to CO_2 and H_2, thus establishing that the FHL enzyme system is stable to vacuum drying. The results given in Table III, when compared to the data plotted in Fig. 2, demonstrate that unhydrolyzed yeast extract was considerably less active when tested in the

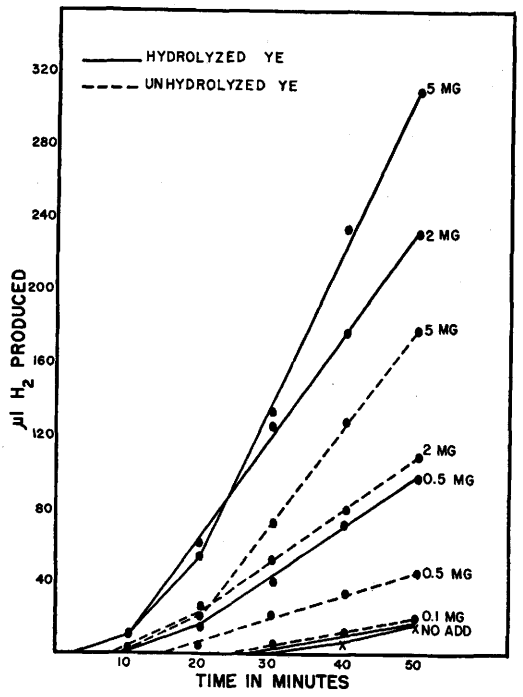

FIG. 2. Relative activity of hydrolyzed and unhydrolyzed yeast extract. (FHL activity in resting cell suspensions of *A. aerogenes*. Conditions of reaction as for Fig. 1 except .2 ml of M/10 formate added.)

dried preparation than in the living cell. In contrast to these findings, the acid-hydrolyzed extract maintained its ability to stimulate the FHL system. These results suggest that the cofactor present in yeast extract is released only after acid or enzymic hydrolysis, and that the degree of stimulation of the unhydrolyzed yeast extract is a reflection of the ability of the living cells to accomplish enzymic hydrolysis, an ability reduced greatly by vacuum drying of the cells. However, these results may also be interpreted to suggest that the dried preparations respond only to the hydrolyzed material because, in contrast to the unhydrolyzed yeast, it contains two factors. At present we are unable to distinguish between these two possibilities. It is suggested that the stimulatory material(s) present in natural substances are derived from a fatty acid precursor, possibly oleic acid.

Summary. 1. The formic hydrogenlyase enzyme system (FHL) is activated by oleic acid, thus representing an enzymatic function for a fatty acid. 2. Other fatty acids are capable of stimulating FHL activity in bacterial cell suspensions under certain conditions, but oleate is the most effective of those tested. 3. Yeast and liver contain a substance or substances more active than oleic acid. 4. The activity of natural materials is increased by acid hydrolysis. 5. Active dried cell preparations catalyzing the breakdown of formate to CO_2 and H_2 may be obtained by vacuum drying.

TABLE III. Stimulation of FHL Activity of Vacuum-Dried Preparations of *Aerobacter aerogenes*.

No additions	FHL activity* yeast extract (1-2 mg)	Hydrolyzed† yeast extract (1-2 mg)
170	340	840
1700	1900	2800
300	380	860
360	420	1000
1100	1300	2240
62	106	164
910	940	1400

* Expressed as $Q_{(H_2)}$; see Table II.

† 121°C, .9 N H_2SO_4, 3 hr.

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