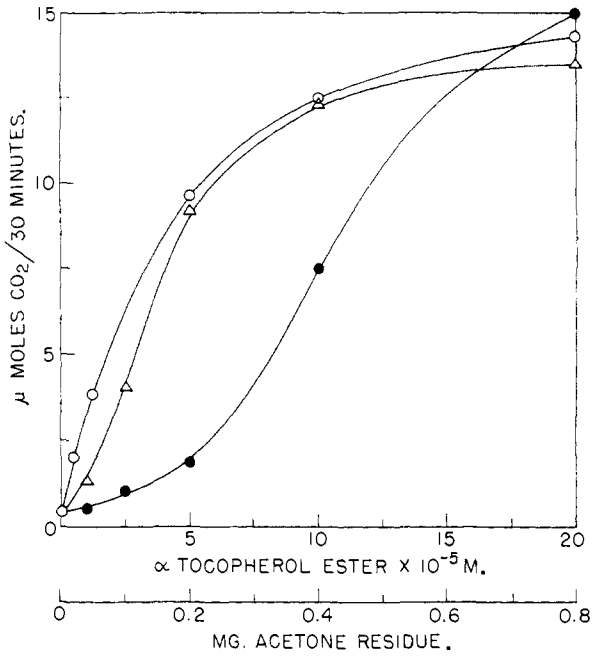

Fig. 1.—The rate of pyruvate oxidation as a function of the concentration of the α-tocopherol ester or acetone residue. The complete system contained the same additions listed in Table I plus the indicated amount of α-tocopherol phosphate (△—△—△), α-tocopherol succinate (●—●—●) or acetone residue(O—O—O).

α-Tocopherol and α-tocopherol acetate when added to the enzyme assay either from a concentrated alcoholic solution or "suspended" in bovine serum albumin[4] were not active. Other compounds which were tested and found to be without activity were vitamin K_1, menadione, vitamin A, carotene, vitamin C, vitamin D, lipoic acid, ethylenediamine tetraacetate, phenylphosphate, di- and triphosphopyridine nucleotides, flavin adenine dinucleotide, riboflavin, riboflavin monophosphate and Tween 80. Bovine serum albumin and *n*-butyl stearate, both of which activate the diphosphopyridine nucleotide–cytochrome C reductase system in the absence of α-tocopherol,[7,8] do not activate this pyruvate oxidation system.

TABLE I

REQUIREMENTS FOR FERRICYANIDE LINKED PYRUVATE OXIDATION

The complete system contained 100 μmoles of potassium phosphate buffer *p*H 6.0, 10 μmoles of $MgCl_2$, 0.1 μmole of thiamin pyrophosphate, 50 μmoles of potassium pyruvate, 25 μmoles of potassium ferricyanide, 0.7 mg. of the soluble enzyme and 0.75 mg. of an acetone extract of the particulate fraction in a total volume of 1 ml. Incubation was carried out at 30° under nitrogen in standard Warburg vessels for 20 minutes.

Additions	Products: CO_2, μmoles	Products: Ferricyanide,[a] μmoles/2	Products: Acetate[b] μmoles
1. Complete system	9.5	10.1	8.3
2. 1-Thiamin pyrophosphate	0.3	0.5	0.5
3. 1-Pyruvate	0.2	0.9	0.9
4. 1-Ferricyanide	0.1	0.0	0.0
5. 1-Enzyme	0.2	0.6	0.7
6. 1-Acetone extract of the particulate fraction	0.5	1.2	0.7
7. 6 + 0.2 μmoles of α-tocopherol phosphate	7.9	8.5	7.8
8. 6 + 0.2 μmoles of α-tocopherol succinate	8.2	9.0	7.8

[a] Determined colorimetrically by a modification of the Park and Johnson assay for reducing sugars.[5] [b] Determined enzymatically using the acetokinase assay[6] after steam distillation.

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Acknowledgments.—This work was supported by grants from the National Science Foundation and the United States Public Health Service. The author gratefully acknowledges the technical assistance of Mr. William Morris and Mrs. Caroline Fenn.

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RECEIVED AUGUST 2, 1957

INCORPORATION OF ATMOSPHERIC OXYGEN INTO THE CELL CONSTITUENTS OF A GROWING CULTURE OF *Pseudomonas*

Sir:

Studies on various oxygenases with O_2^{18} and H_2O^{18} have recently shown that a number of enzymes from animal, plant and microbial sources catalyze incorporation of atmospheric oxygen into organic substrates.[1,2] However, the significance of these oxygenases in the metabolism of intact cells has not yet been explored. When *Pseudomonas sp.*, a strictly aerobic microörganism, was grown with benzoic acid, phenylalanine or tryptophan as the carbon source, from 4 to 9% of the oxygen of cell constituents was found to be derived from atmospheric oxygen (Table I). CO_2 as well as water, which was in complete isotopic equilibrium with CO_2,[3,4] contained much less O^{18}, indicating that most of the incorporation of O^{18} into the cell material did not involve conversion of the atmospheric oxygen to either CO_2 or water. On the other hand when a more oxygenated substrate such as glucose was used as a carbon source, O^{18}-enrichment was about 0.4% of that of the atmospheric oxygen used in the experiment under similar growth conditions. These observations are consistent with the previous findings that these aromatic compounds are degraded by enzymatic reac-

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TABLE I

INCORPORATION OF O_2^{18} INTO THE CELL CONSTITUENTS[a] OF *Pseudomonas* AND *E. coli*

Exp.	Organism	Carbon source	Atom % excess O^{18} O_2	Cell material	CO_2
1	*Pseudomonas*	Benzoate	9.5148	0.8342	0.0542
2	*Pseudomonas*	Phenyl-alanine	7.5793	.5753	.0204
3	*Pseudomonas*	Tryptophan	9.3980	.3758	.0336
4	*Pseudomonas*	Glucose	9.6830	.0399	.0176
2	*E. coli*	Phenyl-alanine	8.0000	.0101	.0145
6	*E. coli*	Tryptophan	8.0580	.0089	.0113
7	*E. coli*	Glucose	9.3768	.0037	.0101

[a] Cells (*Pseudomonas* ATCC 11250, *E. coli* K12) were grown in a special flask designed for this type of experiment[6] at 25° for about 20 hours with vigorous mechanical shaking. Basal medium contained 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.1% Difco yeast extract. In addition, in exp. 1, 0.1% benzoic acid and 0.1% NH_4Cl; in exp. 2 and 5, 0.1% L-phenylalanine; in exp. 3 and 6, 0.1% L-tryptophan and in exp. 4 and 7, 0.1% glucose and 0.1% NH_4Cl were added as carbon and nitrogen sources. The gas phase was a mixture of nitrogen and oxygen in a ratio of 4:1. Highly enriched O_2^{18} gas was prepared by electrolysis of approximately 33% enriched H_2O^{18} purchased from The Weizmann Institute of Science, Israel. Cells were harvested by centrifugation, washed with 0.9% KCl and distilled water. Pyrolysis was carried out at 500° for 1 hour with $HgCl_2$ as a catalyst according to D. Rittenberg and L. Ponticorvo (*Internat. J. Appl. Radiation and Isotopes*, **1**, 208 (1956)). The mass spectrometric analyses were carried out in collaboration with Mr. W. E. Comstock of this Institute.

tions involving fixation of atmospheric oxygen.[5-7] When *E. coli*, a facultative aerobe, was grown under comparable conditions, O^{18}-enrichment in the cell material was approximately 0.1% or less of that of the atmospheric oxygen. Although the data indicate that *E. coli* also incorporates atmospheric oxygen, particularly when aromatic compounds are used as carbon sources, oxygenases seem to play a more important role in the metabolism of a strictly aerobic microörganism. Further studies are in progress in order to determine the distribution of oxygenases in various tissues and other microörganisms.

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RECEIVED AUGUST 26, 1957

THE IDENTIFICATION OF N-(2-HYDROXYETHYL)-PALMITAMIDE AS A NATURALLY OCCURRING ANTI-INFLAMMATORY AGENT

Sir:

Coburn, Graham and Haninger[1] recently reported that a phospholipid fraction prepared from egg yolk showed antiallergic activity in an assay in the guinea pig. The antiallergic factor of egg yolk was further purified by Long and Martin[2] to the extent of showing its marked biological and chemical similarity to a preparation obtained from arachis (peanut) oil,[3] and they also obtained what appeared to be a closely related substance from "vegetable lecithin." These reports have been of especial interest because of the earlier observation of Coburn and Moore[4] that feeding of dried egg yolk to underprivileged children prevented the recurrence of rheumatic fever in spite of repeated attacks of haemolytic streptococcal infection.

We have succeeded in isolating a crystalline anti-inflammatory factor from soybean lecithin[5] and identifying it as N-(2-hydroxyethyl)-palmitamide. The compound also was isolated from a phospholipid fraction[6] of egg yolk and from hexane-extracted peanut meal. The products obtained in the course of this work were tested by the use of a local passive joint anaphylaxis assay in the guinea pig.[7] The isolation procedure was adapted from that of Martin and Long[2] for the preparation of an active concentrate of the factor, and when applied to soybean lecithin yielded a partially purified fraction from which the homogeneous factor was obtained by crystallization from cyclohexane.

The crystalline material, m.p. 98–99°, was neutral, optically inactive, and possessed the formula $C_{18}H_{37}O_2N$. It showed no significant ultraviolet absorption spectrum, but bands in the infrared indicative of substituted amide (6.07 and 6.38 μ) and of OH or NH groups (3.05 and 3.25 μ) were observed. Hydrolysis of the factor to yield palmitic acid and ethanolamine permitted its identification as the known N-(2-hydroxyethyl)-palmitamide.[8] The compound readily was synthesized by refluxing ethanolamine with palmitic acid according to the literature procedure.

An investigation of the component parts of N-(2-hydroxyethyl)-palmitamide showed that the basic moiety is responsible for its anti-inflammatory activity. The nature of the acid group appears to be of no consequence, because in addition to ethanolamine itself, N-(2-hydroxyethyl)-lauramide, N-(2-hydroxyethyl)-salicylamide and N-(2-hydroxyethyl)-acetamide are all potent anti-inflammatory agents. O-Acetylethanolamine is also active. These pharmacological properties of ethanolamine appear to be quite specific, since the homologs D$_g$-1-amino-2-propanol and 1-amino-3-propanol did not show a response in the assay. A study of the members of the "choline cycle"[9] revealed that the anti-inflammatory properties of ethanolamine are shared by all other members of this cycle that are in the reduced form. Thus ethanolamine, choline, N-dimethylaminoethanol and N-methylaminoethanol all had the same order of activity. On the other hand the oxidized members of the choline cycle, glycine, serine, sarcosine and betaine, showed no response in the assay.

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(3) D. A. Long and A. A. Miles, *ibid.*, 492 (1950).

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(5) Alcolec-S, a product of the American Lecithin Co.

(6) We are indebted to Dr. A. F. Coburn, who kindly supplied us with this material.

(7) The biological assay was carried out by (O.H.G.), and was adapted from that described by Coburn, Graham and Haninger.[1] Details of the biological studies will be published elsewhere.

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