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	Atom % excess O^{18} Cell material—CO_2	Atom % excess O^{18} 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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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.

(1) A. F. Coburn, C. E. Graham and J. Haninger, *J. Exp. Med.*, **100**, 425 (1954).

(2) D. A. Long and A. J. P. Martin, *Lancet*, 464 (1956).

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

(4) A. F. Coburn and L. V. Moore, *Am. J. Dis. Child.*, **65**, 744 (1943).

(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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(9) W. W. Umbreit, "Metabolic Maps," Burgess Publishing Co., Minneapolis 15, Minnesota, 1952, p. 263.

We wish to thank Dr. D. A. Long for sending us a sample of his material to aid in the establishment of the biological assay. We are indebted to Mr. R. N. Boos and his associates for the microanalyses and to Mr. R. W. Walker for the determination and interpretation of the infrared absorption spectra.

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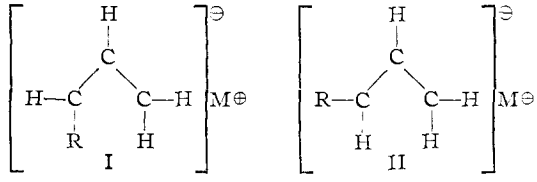
STRUCTURE OF ALLYLIC ANIONS

Sir:

The vibrational spectra of a large number of allylic organoalkali compounds are consistent with the proposition that these ions have a planar structure (C_{2v} symmetry) with angles of approximately 120° about the carbon atoms of the allylic system. That is, the ions have sp^2 hybridization as might be expected from a simple molecular orbital treatment if the double bond is considered to be completely delocalized.[1] On the basis of the observed spectra, the allylic ions of potassium, sodium and lithium have the same structure which may be represented approximately by I and II. Considerable evidence has been presented indicating Grignard reagents to be essentially covalent[2,3] (dicyclopentadienylmagnesium however, is reported to be ionic[4]) but the spectra of allylic magnesium compounds differ little from those of allylic organoalkali compounds.



I II

Alkenylsodium and potassium compounds were prepared by metalation of olefins with amylsodium and amylpotassium.[5] Alkenyllithiums were prepared by interchange of sodium compounds with lithium chloride and by addition of butyllithium to butadiene. Magnesium compounds were prepared from reaction of allylic halides with magnesium in ether and by interchange with sodium compounds and subsequent extraction. Grignard reagents prepared in ether were heated at reduced pressure to free them of ether.[6] Solvent-free Nujol mulls were prepared under dry-box conditions and spectra were determined on a Perkin–Elmer infrared spectrophotometer. Except for the regions obscured by Nujol, excellent spectra were obtained with most of the allylic compounds studied.

Allylic ions should have a considerable energy barrier to rotation about the allylic carbon–carbon bonds, hence the existence of two distinct isomers, I and II, is possible. None of the compounds examined had bands in the normal double bond stretching region, but all the compounds investigated did show very strong bands in the 1500–1560 cm.$^{-1}$ range. Those ions where R = H should have only one isomer but where R is some alkyl substituent configurations I and II are possible. Symmetrical compounds such as allyl-, isobutenyl- or cyclohexenylsodium show only one very strong band at 1535, 1520 and 1522 cm.$^{-1}$, respectively. Compounds such as pentenylsodium have two very strong bands, one about 1525 cm.$^{-1}$ and the other about 1560 cm.$^{-1}$. No evidence has been found for structures of the type ($R—CH{=}CH{=}CHCH_3)^{\ominus}M^{\oplus}$ (III). Carbonation of allylic organoalkali compounds yields acids derivable from structures I and II.[5] Indeed, attempts to prepare a system like III by metalation of heptene-3 gave a spectrum identical with that from heptene-2. Although heptene-1 was not available, the spectra of heptenylsodium prepared from heptene-2 and -3 showed only minor differences when compared with compounds prepared from pentene-1 and -2, hexene-1 and -2 or octene-1 and -2. However, since olefins are easily isomerized by organoalkali compounds it is rather difficult to determine which isomeric olefin is actually metalated.[7]

From a fairly complete analysis of the vibrational spectra of allyl- and perdeuteroallylsodium, the band at 1535 cm.$^{-1}$ in allylsodium has been assigned to the carbon–carbon unsymmetrical stretching frequency.[8] Analogy with substituted olefins suggests that the band around 1525 cm.$^{-1}$ in the substituted allylic ions belongs to structure I and the band around 1560 cm.$^{-1}$ belongs to structure II.[9] This assignment is supported by the position of the band in cyclohexenylsodium at 1522 cm.$^{-1}$ since this ion can exist only in a form geometrically equivalent to structure I.

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TRANSFER OF MOLECULAR OXYGEN BY PEROXIDASE

Sir:

The metabolic function of peroxidase is unknown. We wish to report observations, based upon the use of O^{18} as a tracer, which support the view that ferroperoxidase activates molecular oxygen.

The system consisting of horseradish peroxidase, dihydroxyfumarate and oxygen, catalyzes non-