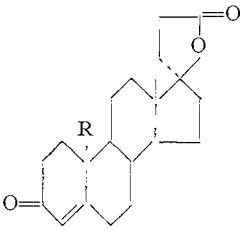
with metallic lithium[4] thereby causing reduction of both the triple bond and the aromatic A ring. Hydrolysis with mineral acid followed by chromatography yielded 3-(3-oxo-17β-hydroxy-19-nor-4-androsten-17α-yl)propionic acid γ-lactone (V), m.p. 126.5–127° (137–138°), $[\alpha]_D$ +22.7° ($CHCl_3$); *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.49; H, 8.34.



III R = CH_3
V R = H

Bioassay[5]—reversal of the effect of desoxycorticosterone (DOC) on the urinary Na–K ratio of adrenalectomized rats was used as a measure of potency. If progesterone, recently described as capable of reversing the effect of DOC,[6] is assigned an activity of 1, then III and V were found to have an activity of approximately 8 and 27, respectively. In similar tests III and V also reversed the electrolyte effects of aldosterone.

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CYCLIZATION IN GAMMA RAY IRRADIATED HIGH DENSITY POLYETHYLENE

Sir:

Material balance in the irradiation of polyethylene has never been convincingly attained, especially at liquid nitrogen temperatures where $G(H_2)$ and G(—CH=CH—) are essentially equal to their values at room temperature while cross link formation is sharply reduced.[1]

As adapted to the high density polyethylene, Marlex-50, and considering no chain scission, the material balance equation can be written

$$[H_2] + [Vi^0 - Vi] = {}^1/_2[R] + [Vl] + [C.L.] + [E.L.] + [S.L.] + [R.L.] \quad (1)$$

where brackets signify moles per gram of substances produced during the irradiation except for $Vi^0 - Vi$ which represents moles/g. of vinyl groups destroyed. R·, Vl, C.L., E.L., S.L. and R.L. signify free radicals, vinylene groups, cross, end,[2] square and ring links, respectively.

By "square" links we mean double cross links formed by a reaction such as

$$2\text{—CH=CH—} \longrightarrow \begin{matrix}\text{—CH—CH—}\\ \quad|\quad\ \ |\\ \text{—CH—CH—}\end{matrix} \quad (2)$$

Such a square link is assumed in Eq. (1) to constitute one cross link as well as one square link. By "ring" links we mean intramolecular bonds formed by reactions such as

$$\epsilon + \text{—}CH_2CH_2CH_2CH_2\overset{+}{C}H_2\text{—} \longrightarrow \text{—}\underbrace{CHCH_2CH_2CH_2CH}\text{—} + H_2 \quad (3)$$

(ϵ represents the electron). Equation (3) is similar to a reaction proposed earlier.[3] Other ring forming reactions could be proposed.

Cross links and vinylene groups formed by irradiation have been recognized since 1950,[3] but ring or square links have never before been observed in the irradiation of any substance as far as we know. It is the purpose of this note to present evidence for cyclization during irradiation.

1,2-Disubstituted cyclopentane and cyclohexane derivatives absorb[4] in the infrared approximately at wave lengths of 10.2 μ with extinction coefficients varying between 6 and 28 1. cm^{-1}. $mole^{-1}$. In Marlex-50 the initial absorption at 10.0 and 10.98 μ due to the vinyl group rapidly falls with irradiation at room temperature and *in vacuo* (initial G(-vinyl) equal to 7.7), revealing the growth of another absorption at 10.1 μ which is approximately the wave length at which *trans* 1,2-disubstituted cyclo pentanes and hexanes absorb. We have attributed the growth of this peak to the formation of ring links of this size (other ring or square links which might absorb at this wave length are not excluded). A similar growth in the peak at 10.1 μ was found also in the case of a Ziegler-type polyethylene.

An order of magnitude estimate of G (*trans* ring links) is 1.0 which is of sufficient magnitude to show the necessity of considering cyclization in attempting to achieve a material balance.

In the case of a low density polyethylene, the vinylene peak at 10.3 μ is broader than in the case of Marlex-50 and possibly obscures the peak due to the ring systems. The 10.3 μ peak is unsymmetrical, however, which suggests that ring links are produced in low as well as high density polyethylenes.

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(4) "Catalog of Infrared Spectral Data" of the American Petroleum Institute Research Project, Carnegie Institute of Technology, Pittsburgh, Pennsylvania.
(5) Fulbright Fellow, 1956.
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ENZYMATIC OXYGEN FIXATION INTO ACETATE CONCOMITANT WITH THE ENZYMATIC DECARBOXYLATION OF L-LACTATE

Sir:

The enzymatic incorporation of molecular oxygen into various organic substrates recently has been

reported from several laboratories.[1] These "oxygenases"[2] are generally concerned with the transformation (hydroxylation, ring cleavage, cyclization) of various ring compounds such as aromatic amino acids, phenols and steroids. In this communication we wish to report a novel type of decarboxylation reaction in which atmospheric oxygen is incorporated into a simple aliphatic compound.

L-Lactate was incubated with a crystalline lactic oxidative decarboxylase prepared from *Mycobacterium phlei*[3] in an atmosphere of O_2^{18} and with H_2O^{16} as a medium. Potassium acetate isolated from the incubation mixture was found to have incorporated approximately one atom of atmospheric oxygen (Table I). The O^{18} enrichment of the incorporated oxygen atom corresponded to approximately 82% of that of the atmospheric oxygen used in this experiment, assuming only one atom was inserted into the product. On the other hand, when the reaction was carried out in the medium of H_2O^{18} and with O_2^{16} as a gas phase, potassium acetate isolated did not contain appreciable O^{18}. Carbon dioxide did not contain O^{18} in the first case, but was highly enriched in the second experiment. This incorporation of O^{18} from H_2O^{18} into carbon dioxide probably was caused by a non-enzymatic exchange reaction as reported by Cohn and Urey[4] and more recently by Rothberg and Steinberg in their studies of various microbial decarboxylases.[5]

TABLE I

ENZYMATIC INCORPORATION OF O^{18} INTO ACETATE

Experiment I: Crystalline lactic oxidative decarboxylase[3] (3,350 units), 2 millimoles of DL-lithium lactate and 2 millimoles of potassium phosphate buffer (*p*H 6.0) were incubated in a total volume of 50 ml. of water in a special flask designed for this type of experiment.[a] An O_2^{18}-helium gas mixture (2:3) was used as a gas phase.[b] Experiment II: The same reaction components as in Experiment I were employed except that H_2O^{18} was used as a solvent and O_2^{16}-helium gas mixture (2:3) was used as a gas phase. The incubation was carried out at 37° for 30 minutes with vigorous shaking. After the oxygen and carbon dioxide were removed for mass spectrometric analyses, the incubation mixture was immediately chilled at 4° and *p*H was adjusted to 3.5 with several drops of 2*N* H_2SO_4. The solution was then frozen and water and acetic acid were distilled from the frozen state. The distillate was neutralized with 1*N* KOH to *p*H 6.5 and water was removed by lyophilization. The residue dried over P_2O_5 was pyrolyzed[c] and the O^{18} content determined with a mass spectrometer.[d]

Expt.	Type	Atom % excess	Cpd. analyzed	Atom % excess found
I	O_2^{18}	6.31868	KOAc	2.59578
			CO_2	0.00452
II	H_2O^{18}	1.3380	KOAc	0.01772
			CO_2	1.32422

[a] Details will be published elsewhere. See also 1b. [b] 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. We are indebted to Dr. Y. Saito and Mr. L. Wartofsky for the preparation of O_2^{18} gas. [c] The mass spectrometric analyses were carried out in collaboration with Mr. W. E. Comstock of this Institute. [d] Pyrolysis was carried out at 400° for 1 hour with $HgCl_2$ as a catalyst according to D. Rittenberg and L. Ponticorvo (*Intern. J. Appl. Radiation and Isotopes*, **1**, 208 (1956)).

The enzyme which catalyzes "oxidative" decarboxylation of L-lactate has been described from various microörganisms.[6] The over-all reaction involves oxidation and decarboxylation of L-lactate to form stoichiometric quantities of acetate and carbon dioxide, but the mechanism of this unique enzymatic reaction has not yet been completely understood.

The available evidence suggests that at least one atom of atmospheric oxygen was incorporated into acetate when the C_1–C_2 bond of lactate was cleaved and that the other atom of the oxygen molecule probably was reduced to water

$$CH_3—CHOH—COOH + O_2^{18} \longrightarrow CH_3COO^{18}H + CO_2 + H_2O^{18}$$

The mechanism of this reaction may be analogous to that of the so-called "mixed function oxygenase"[1e,f] except that instead of reduced pyridine nucleotides, the substrate itself is providing electrons to reduce one atom of oxygen.

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POLYNUCLEOTIDE PHOSPHORYLASE IN LIVER NUCLEI

Sir:

Polynucleotide phosphorylase has been described in bacteria.[1] It catalyzes the reversible synthesis of polynucleotides from nucleoside diphosphates as well as the phosphorolysis of isolated ribonucleic acids. Thus, adenylic polynucleotide (Poly A) reacts with orthophosphate (P_i) to form adenosine diphosphate (ADP)

$$\text{Poly A} + n\text{P}_i \rightleftharpoons n\text{ADP}$$

An enzyme has now been fractionated from guinea pig liver nuclei which converted up to 30% of the adenylic acid units of Poly A to ADP (Table I). Part of the ADP then formed ATP and AMP due to the presence of adenylate kinase. The rest of the polymer was utilized by a competing hydrolytic enzyme, shown to occur in liver nuclei.[2] ADP was identified by its R_f in four solvent systems, electro-

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