(molecules of HD/100 e.v.) was observed to be equal to $G(-D_2)$ in samples irradiated to 10 per cent. exchange, at least. G(HD) values for such samples averaged 11 ± 1 molecules per 100 e.v. and were independent of deuterium pressure and virtually independent of temperature in the ranges employed.

G(HD) corresponds to that for dissociation of one water molecule according to the stoichiometry implicit in $D_2 + H_2O = HD + DOH$. A reasonable mechanism can be written as

$$H_2O \longrightarrow H + OH \quad (1)$$

$$H + D_2 \longrightarrow HD + D \quad (2)$$

$$OH + D_2 \longrightarrow DOH + D \quad (3)$$

$$D + D \xrightarrow{M} D_2 \quad (4)$$

Thus, $-d(D_2)/dt = d(HD)/dt = k_1(H_2O) = k_0$. G(HD) is directly proportional to k_0, since the energy absorbed by the water is proportional to time. At vapor densities employed (0.78 g./cc.) over 99% of the tritium β-particles are absorbed by the water.[2] G(HD)'s were calculated using 12.46 years for the tritium half-life and 5.69 kev. for the average β-particle energy. Virtual absence of a temperature coefficient is good evidence against the possible chain-propagating step, $D + H_2O \rightarrow H + DOH$. Absence of dependence of G(HD) on deuterium pressure indicates that deuterium at mole fractions of 10^{-3} to 10^{-2} is a completely efficient scavenger for the reactive species involved.

Comparison of G(HD) in this work with that obtained by Gordon and Hart[3] in Co^{60}-irradiated liquid water saturated with deuterium shows that approximately four times as many hydrogen atoms are available for reaction with deuterium when water vapor is irradiated. Hart's measurement of the water decomposition yield in liquid tritium–water using formic acid and oxygen as free-radical scavengers provided $G(-H_2O) = 3.8$.[4] Assuming a similar mechanism for formation of reactive species in water vapor, as above, an average value of 9 e.v. per radical pair is obtained in the present work. If roughly 30 e.v. are required per ion pair formed in water vapor, a total of three measurable radical pairs appear to be formed per ionization. It is suggested that the two additional pairs measurable in the vapor recombine in liquid water via $H + OH \xrightarrow{M} H_2O$ in track regions inaccessible to solute molecules in times necessary for recombination.

The author is indebted to E. J. Hart and S. Gordon for helpful discussion and for preparation of the tritium–water and to L. Pobo and H. Rest for the mass spectrometer analyses.

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HETEROGENEITY IN THE DINITROPHENOL UNCOUPLING OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION[1]

Sir:

Previous experiments by Ochoa,[2] Lehninger,[3] Copenhaver and Lardy,[4] and others, established that phosphorylations are coupled with the transport of electrons by the respiratory enzyme chain localized in mitochondria. Concurrently, Loomis and Lipmann,[5] Hunter and Hixon,[6] and others demonstrated that dinitrophenol uncouples these phosphorylations. We have now observed that the sensitivity of the phosphorylation mechanism to a given dinitrophenol concentration is not uniform.

The mitochondria for the present experiments were obtained from flight muscle of house flies, *Musca domestica*, by a modification[7] of techniques previously described.[8] The isolation medium was 0.25 *M* sucrose–0.005 *M* EDTA, *p*H 7.2. Table I shows the effect of two concentrations of dinitrophenol on oxidative phosphorylation with four respiratory substrates.

TABLE I
HETEROGENEITY OF DNP ACTION DEMONSTRATED BY THE OXIDATION OF DIFFERENT SUBSTRATES

Substrate	DNP added, *M*	ΔO μ atoms	ΔP μ moles	*P/O*
α-Ketoglutarate	.....	5.5	11.6	2.1
	5×10^{-5}	5.4	6.5	1.2
	1×10^{-4}	5.0	1.2	0.2
α-Glycerophosphate	.....	6.7	9.6	1.4
	5×10^{-5}	6.3	7.7	1.2
	1×10^{-4}	6.8	5.7	0.8
Succinate	.....	5.7	8.5	1.5
	5×10^{-5}	5.5	6.7	1.2
	1×10^{-4}	5.1	2.5	0.5
Glutamate	.....	4.9	11.0	2.2
	5×10^{-5}	4.9	10.1	2.1
	1×10^{-4}	5.2	9.0	1.7

Measurements for oxygen consumption and inorganic phosphate uptake were made by the methods reported in an earlier paper.[8] The reaction mixtures contained 30 μM. K phosphate buffer, *p*H 7.4; 20 μM. $MgCl_2$; 10 μM. ADP; 50 μM. glucose; 2% bovine serum albumin; 150 K.M. units hexokinase; and 0.5 ml. of mitochondrial suspension. The quantities of substrate used in their respective experiments were as follows: α-ketoglutarate, 30 μM.; glutamate, 30 μM.; succinate, 90 μM.; α-glycerophosphate, 100 μM.; 0.15 ml. of 5 *N* KOH placed in center well. Total fluid volume of Warburg flask made to 2.5 ml. with 0.25 *M* sucrose; time 30 minutes, temperature 25°. Each set of comparative *P/O* values for the various substrates was obtained with the same mitochondrial suspension. The mito-

(1) The following abbreviations have been used: DNP for 2,4-dinitrophenol, ADP for adenosine diphosphate, EDTA for ethylenediaminetetraacetate, P/O for the ratio of the micromoles of inorganic phosphate esterified to the microatoms of oxygen utilized.
(2) S. Ochoa, *J. Biol. Chem.*, **151**, 493 (1943).
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(4) J. Copenhaver and H. Lardy, *ibid.*, **195**, 225 (1952).
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chondria were used immediately after isolation. All data are based on from two to five independent determinations.

The data reveal that the uncoupling efficiency of a given concentration of DNP depends upon the substrate metabolized. At 5×10^{-5} *M*, DNP uncoupled approximately 50% of the phosphorylation associated with α-ketoglutarate oxidation. This same concentration of DNP had relatively little effect on the phosphorylation coupled with the oxidation of α-glycerophosphate, succinate and glutamate. At a higher level of DNP, 1×10^{-4} *M*, phosphorylation associated with α-ketoglutarate oxidation was almost completely eliminated. In contrast, phosphorylation coupled with glutamate oxidation was only slightly reduced. With the respiratory substrates, succinate and α-glycerophosphate, moderate decreases in the phosphate esterified were observed. Neither concentration of DNP caused marked alterations in the respiratory rate. Other experiments with a further five-fold increase in DNP, 5×10^{-4} *M*, completely uncoupled phosphorylation with all substrates.

These data indicate that phosphorylations coupled to electron transfer are not equally sensitive to DNP. This implies that during oxidative phosphorylation steady state concentrations of the components of the system are established and that this balance regulates the response to a given concentration of DNP. In our experiments the different substrates may have modified this equilibrium by influencing the steady state levels of respiratory enzymes[9] or by having different phosphorylation sites.[10] Other evidence demonstrates that the oxidation of these substrates in the fly is mediated by the same respiratory chain components as found in mammalian preparations.[11,12] In both organisms the initial electron acceptor for α-ketoglutarate and glutamate is pyridine nucleotide. This coenzyme is not a participant in the mitochondrial oxidation of α-glycerophosphate.[13] Thus it is unlikely that the apparent contrast in our results with some previously reported data with mammalian tissues[14] can be attributed to gross differences in the electron transport system. This comparison therefore suggests that the differing effects of a given concentration of inhibitor may be a reflection of differing equilibrium states.

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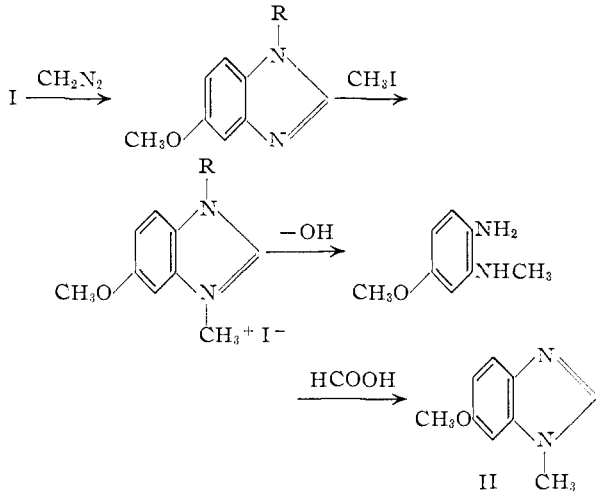
VITAMIN B_{12}. XXVII. STRUCTURE OF THE FACTOR III NUCLEOSIDE AND SYNTHESIS OF HYDROXY- AND METHOXYBENZIMIDAZOLE RIBOSIDES

Sir:

It has been reported previously that Factor III contains a 5- or 6-hydroxybenzimidazole moiety[1,2] and appears to differ from vitamin B_{12} only by having this moiety in place of 5,6-dimethylbenzimidazole.[3] The following evidence shows that the nucleoside of Factor III is a 5-hydroxybenzimidazole glycoside, probably an α-ribofuranoside.[4] Factor III was kindly supplied by Professor Dr. K. Bernhauer.

The nucleoside (I)[1] obtained by acid hydrolysis of Factor III was not isolated in crystalline form but was purified by paper chromatography using an *n*-butanol–acetic acid–water (4:1:5) system (R_f 0.46). The absorption spectrum in methanol showed λ_{max} at 249 and 296 mμ, and the $[\alpha]^{25}D$ was about −11°.

Since 5-hydroxybenzimidazole could result from degradation of either a 5- or 6-hydroxybenzimidazole glycoside the position of the hydroxyl group in I was shown as follows



The Factor III nucleoside I was methylated with diazomethane and the product was converted to the methiodide by refluxing in methanol with methyl iodide. The methiodide was cleaved to an *o*-phenylenediamine by treatment with hot methanolic sodium hydroxide, conditions known to cleave N,N′-dialkylbenzimidazolium salts to N,N′-dialkyl-*o*-phenylenediamines.[5] In the case of I, these conditions would also be expected to cause removal of the sugar moiety, giving a monomethyldiamine in which the unsubstituted amino group is derived from the original glycosidic nitrogen. For characterization, the diamine from I was converted into 1-methyl-6-methoxybenzimidazole (II), m.p. 67–68°, by reaction with formic acid. II was synthesized by first reacting 3-bromo-4-nitroanisole and methylamine to give N-methyl-6-nitro-*m*-anisidine. This was hydrogenated to the

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(2) W. Friedrich and K. Bernhauser, *Angew. Chem.*, **67**, 619 (1955).
(3) W. Friedrich and K. Bernhauser, *Z. Naturforschung*, **9b**, 686 (1954).
(4) By private communication, we have been informed by Professor Dr. K. Bernhauer that he and Dr. Friedrich have methylated Factor III directly, and then by degradation have obtained 6-hydroxy-1-methylbenzimidazole. Thus, they have established that the nucleoside of Factor III is a 5-hydroxybenzimidazole glycoside (W. Friedrich and K. Bernhauer, *Angew. Chem.*, in press).
(5) K. Hoffman, "Imidazole and Derivatives," Part I, Interscience Publishers, Inc., New York, N. Y., 1953, p. 280.