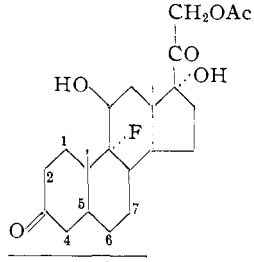
with one mole of bromine and the resulting bromoketone was dehydrohalogenated *via* semicarbazone formation. Reversal[5] of the semicarbazone with pyruvic acid in aqueous acetic acid gave in addition to (I) and (II) the ketone (III) [m.p.[6] *ca.* 237°; $[\alpha]_D$ +34.9° (acetone) λ_{max}^{MeOH} 222 mμ (log *E* 4.03); λ_{max}^{nujol} 2.77, 2.98 μ (OH), 5.73, 5.78 μ (acetylated side chain), 6.05 μ (unsaturated ketone); Found: C, 65.66; H, 7.05]. (III) possessed about 60% of the activity[4] of hydrocortisone acetate by the one-day oral mouse liver glycogen assay.

Bromination of (II) with two moles of bromine[7] followed by dehydrohalogenation with collidine afforded the dienone (IV) [m.p. *ca.* 237°; $[\alpha]_D$ +100.9° (acetone); $\lambda_{max}^{CH_3OH}$ 239 mμ (log *E* 4.19); $\lambda_{max}^{H_2SO_4}$ (2 hr.) 310 mμ (4.06), 262.5 mμ (4.18); λ_{max}^{nujol} 2.92, 3.02 μ (OH), 5.74, 5.82 μ (acetylated side chain), 6.0 μ (unsaturated ketone), 6.12, 6.21 μ (diene system); Found: C, 65.66; H, 6.74] and the isomeric dienone (V) [m.p. *ca.* 208°[6]; $[\alpha]_D$ +106° (acetone), $\lambda_{max}^{CH_3OH}$ 281 mμ (log *E* 4.40), λ_{max}^{nujol} 2.85, 3.06 μ (OH), 5.70, 5.81 μ (acetylated side chain), 6.12, 6.18 μ (conjugated dienone system); Found: C, 65.86; H, 6.99]. 1-Dehydro-9α-fluorohydrocortisone acetate (IV) possessed about 25 times the activity of hydrocortisone acetate in the mouse liver glycogen assay and in the rat systemic granuloma inhibition test.[4] It is, therefore, the most potent glucocorticoid known. It is of interest to note that enhanced glucocorticoid activity was reported recently[8] for 1-dehydrocortisone and 1-dehydrohydrocortisone acetate, which possess the same chromophoric system as (IV).



I, Double bond between C_4–C_5.
II, H at C_5 formulated as "α".
III, Double bond between C_1–C_2; H at C_5 formulated as "α".
IV, Double bonds between C_1–C_2 and C_4–C_5.
V, Double bonds between C_4–C_5 and C_6–C_7.

(5) W. F. McGuckin and E. C. Kendall, THIS JOURNAL, **74**, 5811 (1952).

(6) Taken on a micro hot-stage m.p. apparatus.

(7) See for instance C. Djerassi and C. R. Scholz, THIS JOURNAL, **69**, 2404 (1947).

(8) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Pearlman and M. M. Pechet, *Science*, **121**, 176 (1955).

R. F. HIRSCHMANN
R. MILLER
R. E. BEYLER
L. H. SARETT
M. TISHLER
MERCK & CO., INC.
CHEMICAL DIVISION
RAHWAY, N. J.

RECEIVED MAY 7, 1955

EFFECT OF IONIZING RADIATION ON AQUEOUS ETHYLENE OXYGEN SOLUTIONS

Sir:

In the course of our studies on the effects of ionizing radiations on hydrocarbons in aqueous solution we have noted oxidation of ethylene along with hydrolysis to ethanol. This oxidation leads to a production of acetaldehyde with *G* values as high as 60 when solutions equilibrated with 1–1 ethylene–oxygen mixtures under a pressure of 120 p.s.i. are irradiated with γ-rays from cobalt-60 at a dose rate of 2×10^5 r./hr.[1] Ethanol and acetic acid are also produced later in the reaction with much smaller G values (Fig. 1).

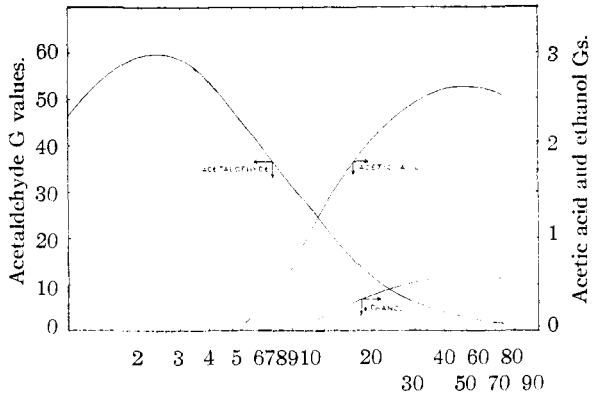


Fig. 1.—*G* values for ethanol, acetic acid and acetaldehyde production as a function of radiation dose.

Hydrogen peroxide is formed in water equilibrated with ethylene–oxygen mixtures under atmospheric pressure at an initial rate corresponding to a *G* of 20 (*Energy dissipitation in the solution was calculated from the rate of oxidation of ferrous ion in the Fricke dosimeter using a *G* value of 15.5). These *G* values for hydrogen peroxide production increase with increasing gas pressure, rising to an order of magnitude comparable to the aldehyde values at 120 p.s.i.

These *G* values contrast with those obtained with most organic materials in aqueous solution, which are an order of magnitude lower (Weiss[2]). Detailed studies to elucidate the nature of the processes leading to this chain utilization of oxygen are in progress.

(1) E. J. Henley, *Nucleonics*, **11**, no. 10, 41–43 (1953).

(2) J. Weiss, *Chem. & Ind.*, **13**, 358–9 (1955).

DEPARTMENT OF CHEMICAL ENGINEERING
COLUMBIA UNIVERSITY
NEW YORK 27, N. Y.
ERNEST J. HENLEY
JANE P. SCHWARTZ

RECEIVED APRIL 27, 1955

A NEW PTERIDINE IN URINE REQUIRED FOR THE GROWTH OF THE PROTOZOON *CRITHIDIA FASCICULATA*

Sir:

Work carried out jointly in these Laboratories and the Haskins Laboratories[1] established that the Trypanosomid flagellate *Crithidia fasciculata* (*Herpetomonas culicidarum*) could be grown in a chemically-defined medium in the presence of a "high" amount of pteroylglutamic acid (PGA), 1 γ/assay tube. It was then found that the amount of PGA required for growth of *C. fasciculata* was markedly spared by a variety of natural materials including certain liver fractions and human urine (adult males), or by certain 2-amino-4-hydroxy-6-substitued pteridines. These relationships are illustrated in Table I.

By procedures of adsorption and solvent distribu-

(1) J. Cowperthwaite, M. M. Weber, L. Packer and S. H. Hutner, *Ann. N. Y. Acad. Sci.*, **56**, 972 (1953).

tion about 20 mg. of a solid was isolated from 4000 liters of urine that supported half-maximum growth of *C. fasciculata* at a concentration of 0.05 millimicrograms per ml. The name biopterin is suggested for this substance. The material precipitated as pale yellow spheres from hot water and decomposed without melting at 250–280°. It was optically active, $[\alpha]^{25}D$ −50 (0.1 *N* HCl; *C*, 0.4). It was insoluble in the common organic solvents, slightly soluble in water, soluble in dilute acid and alkali. Analysis calculated for $C_9H_{11}N_5O_3$: C, 45.6; H, 4.64; N, 29.5; mol. wt., 237. Found on two separate samples, C, 42.8, 43.6; H, 5.00, 4.84; N, 29.3; S, negative.

The ultraviolet absorption spectrum of biopterin was typical of 2-amino-4-hydroxy-alkylpteridines with maxima at 254 mμ and 363 mμ in 0.1 *N* NaOH. Assuming biopterin has the same molecular extinction as 2-amino-4-hydroxy-alkylpteridines, a molecular weight of 282 was calculated from the ultraviolet absorption. The infrared spectrum measured on a KBr pellet showed strong bands at 3650–3450, 3310, 2975–2930, 1695–1680, 1538, 1490, 1418, 1370, 1295, 1245, 1173, 1130, 1063 and 823 cm.$^{-1}$.

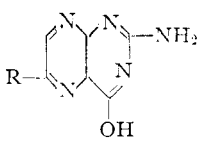
Biopterin titrated with periodate consumed 1.7 and 4.5 oxidation equivalents per molecular weight of 237 at *p*H 2 and 8.5, respectively. Formaldehyde, formic acid and ammonia were not detected in the oxidation mixtures. Upon adjusting the oxidation mixtures to *p*H 5, yellow precipitates came down, and the ultraviolet absorption spectra of these indicated that the material from the *p*H 2 oxidation was 2-amino-4-hydroxy-6-formylpteridine and the material from the *p*H 8.5 oxidation was 2-amino-4-hydroxy-6-carboxypteridine. Thus biopterin appears to consume about two more periodate oxidation equivalents in alkali by the pteridine aldehyde being oxidized to the corresponding acid. From these data and studies on models it is concluded the structure of biopterin is 2-amino-4-hydroxy-6-(1,2-dihydroxypropyl)-pteridine.

The microbiological response to biopterin is now obtained in the absence of added PGA.[3] The involvement of PGA in the metabolism of *C. fasciculata* was demonstrated indirectly, however, by adding an inhibitory amount of 2,4-diamino-5-*p*-chloro-phenoxy-6-ethylpyrimidine (I) to the culture medium. Under these conditions growth was obtained by adding sufficient PGA to counteract I only if biopterin was present (Table 2). The data of Tables I and II illustrate that *C. fasciculata* required both PGA and a pteridine for growth, implying that these structurally-related substances have independent metabolic functions for this organism.

We are indebted to Mrs. L. H. Smith for some of the microbiological assays and to Dr. S. H. Hutner, Haskins Laboratories, New York, N. Y. for his suggestions concerning the culturing of the test organism.[4]

TABLE I

THE EFFECT OF PGA ON THE GROWTH RESPONSE OF *Crithidia fasciculata* TO URINE OR TO CERTAIN 6-SUBSTITUTED PTERIDINES

Additions per tube (2.5 ml.) final strength medium[a]		PGA Additions to medium: None	10 mγ	100 mγ	1000 mγ
		Optical Density			
None		0.02	0.05	0.11	0.88
0.001 ml. urine		0.02	0.64		
0.01 ml. urine		0.05	0.98		
0.1 ml. urine		0.37	1.05		
					
R = CH_2OH[b]	10 mγ	0.02	0.25	0.65	0.87
	100 mγ	0.07	0.58	0.81	0.88

[a] Test conditions were those described by Nathan and Cowperthwaite[2] except that the adenosine was replaced by 100γ adenine, guanine and uracil per tube and the assay was carried out in slanted test tubes rather than flasks. [b] Microbiological findings with other pteridines tested with 10 mγ PGA in the medium were as follows: When R = H, OH or COOH, no activity at 10γ/tube, when R = CHO or CH_3, 0.3 to 0.1 as much activity as when R = CH_2OH (see table). 2-Amino-4-hydroxy-5-formyl-6-methyltetrahydropteridine, the methylpteridine moiety of leucovorin, was inactive at 10γ/tube.

TABLE II

TOXICITY OF 2,4-DIAMINO-5-*p*-CHLOROPHENOXY-6-ETHYLPYRIMIDINE FOR GROWTH OF *Crithidia fasciculata*: REVERSAL BY PGA[a]

Biopterin	PGA	2,4-Diamino-5-*p*-chlorophenoxy-6-ethylpyrimidine (I): 0 γ	1 γ	3 γ	10 γ
		Optical Density			
....	....	0.03	0.02	0.01	0.03
10 mγ	....	.43	.02	..	..
100 mγ	....	.46	.09	..	..
....	10 mγ	.03	..	..	..
....	100 mγ	.02	..	..	..
100 mγ	10 mγ	.49	.49	.04	..
100 mγ	100 mγ	.46	.45	.38	.03

[a] Test conditions as in footnote to Table 1; 2.5 ml. final volume.

(2) H. A. Nathan and J. Cowperthwaite, *Proc. Soc. Exptl. Biol. Med.*, **85**, 117 (1954).

(3) Within the past year the test organism apparently "lost" its nutritional requirement for preformed folic acid. Factors responsible for this change are unknown.

(4) ADDENDUM.—After this Communication was submitted for publication, the Editors informed us that Forrest and Mitchell have isolated and characterized what appears to be the same pteridine from wild type *Drosophila*.

NUTRITION AND PHYSIOLOGY SECTION — E. L. PATTERSON
AMERICAN CYANAMID COMPANY — H. P. BROQUIST
RESEARCH DIVISION — ALBERTA M. ALBRECHT
LEDERLE LABORATORIES — M. H. VON SALTZA
PEARL RIVER, NEW YORK — E. L. R. STOKSTAD

RECEIVED MARCH 24, 1955

BOND ORDER—LENGTH RELATIONSHIPS

Sir:

The importance of establishing reliable bond order-length relationships for as many atom-pairs as possible is generally recognized. There are several ways of defining bond order (w). Examples are the original method suggested by Pauling, Brockway and Beach, and the more recent method of molecular orbitals. Bond order length curves have been usually constructed by plotting