IMP. 2′- and 3′-IMP have been identified as products of hydrolysis of the IMP polymer by alkali and 5′-IMP by snake venom phosphodiesterase preparations.[7] This identification is based on (a) paper chromatography with the Krebs and Hems[5] and C80A[8] solvent systems, (b) liberation of P_i on hydrolysis for 20 minutes at 100° with 1.0 HCl,[9] and (c) behavior toward 5′- and 3′-specific nucleotidases.[7] These results suggest that 5′-mononucleotide units are linked to one another either through 2′- or 3′-phosphoribose ester bonds, or both, as in nucleic acid. Similar polymers have been obtained with the other nucleoside diphosphates so far tried (ADP, UDP).

TABLE I

STOICHIOMETRY OF REACTION WITH IDP OR IMP-POLYNUCLEOTIDE

In experiment 1, 1.29 mg. of purified *Azotobacter* enzyme (SA, 10) as incubated with 24.8 μM. IDP, in the presence of 12 μM. $MgCl_2$ and 90 μM. Tris buffer, *p*H 8.1; final volume, 2.5 ml. Reaction was stopped by heating 1 minute at 100°. The IDP remaining in an aliquot of the supernatant was removed by hydrolysis to IMP and P_i with an excess (0.08 mg.) of purified ox liver IDPase[10] for 40 minutes and the enzyme destroyed by heating 1 minute at 100°. In experiment 2, an aliquot of the IDPase supernatant was incubated with 0.65 mg. of the *Azotobacter* enzyme (at *p*H 7.4). In experiment 3, 10.4 μM. (as mononucleotide) of a dialyzed solution of the IMP polynucleotide (isolated by TCA precipitation after incubation of IDP with *Azotobacter* enzyme as in experiment 1) was incubated with 0.65 mg. of enzyme (SA, 9) in the presence of 7 μM. $MgCl_2$ and 80 μM. Tris buffer, *p*H 8.1; final volume, 1.4 ml.; temp., 30° throughout. Values are expressed in μM. per ml. of reaction mixture. IDP was determined as the P_i liberated by IDPase; P_i was determined by the method of Lohmann and Jendrassik[11]; the polynucleotide was precipitated with TCA, dissolved in buffer, and determined from the light absorption at wave length 247 mμ at *p*H 7.0. This was corrected for the absorption ratio mononucleotide/polynucleotide (factor, 1.2) and expressed as mononucleotide. ε 247 for IMP was taken to be 13.2 at acid *p*H.[12]

Experiment	Incubation, min.	IDP	Pi	Polynucleotide
1	0	9.76	1.06	
	90	4.30	7.10	4.96[a]
	Δ	−5.46	+6.04	+4.96
2	0	0	14.2[b]	3.19
	60	2.3	12.0	1.20
	Δ	+2.3	−2.2	−1.99
3	0	0.69	8.10	7.50
	90	1.96	6.74	6.21
	Δ	+1.27	−1.36	−1.29

[a] Corrected for losses. [b] Some P_i contributed by *Azotobacter* enzyme solution.

(7) We are indebted to Dr. C. E. Carter and Dr. L. A. Heppel for generous gifts of snake venom preparations containing phosphodiesterase and 5′-nucleotidase (J. M. Gulland and E. M. Jackson, *Biochem. J.*, **32**, 590, 597 (1938); R. O. Hurst, and G. C. Butler, *J. Biol. Chem.*, **193**, 91 (1951)), and of 3′-nucleotidase (L. Schuster and N. O. Kaplan, *J. Biol. Chem.*, **201**, 535 (1953)). The latter enzyme was a gift of Dr. Kaplan to Dr. Heppel.

(8) L. A. Heppel, personal communication. This solvent consists of 800 ml. saturated ammonium sulfate, 180 ml. sodium acetate, and 20 ml. isopropanol.

(9) C. E. Carter, ref. 6.

(10) G. W. E. Plaut, *Federation Proc.*, **14**, 263 (1955). We are indebted to Dr. G. W. E. Plaut for a generous gift of this enzyme. It catalyzes the hydrolysis of IDP, GDP, and UDP but is inactive on ADP and CDP.

(11) K. Lohmann and L. Jendrassik, *Biochem. Z.*, **178**, 419 (1926).

(12) H. H. Kalckar, *J. Biol. Chem.*, **167**, 429 (1947).

The reaction catalyzed by the *Azotobacter* enzyme is readily reversible. In the presence of the enzyme and Mg^{++}, the IMP-polynucleotide undergoes phosphorolysis to IDP. Table I shows the stoichiometry of the reaction with IDP in both directions. Phosphorolysis by the purified enzyme of nucleic acid isolated from *Azotobacter* has been shown through the incorporation of P_i^{32} and chromatographic identification of radioactive GDP, UDP, CDP, and ADP. Further, the labelled GDP and UDP were specifically hydrolyzed by IDPase.[10] The above results indicate that the new enzyme (or enzymes) catalyzes the reaction.

$$n\text{X-R-P-P} \rightleftharpoons (\text{X-R-P})_n + nP_i$$

where R is ribose and X may be adenine, hypoxanthine, guanine, uracil or cytosine, and suggest that, in analogy with polysaccharides, reversible phosphorolysis may be a major mechanism in the biological breakdown and synthesis of polynucleotide chains. Studies of the reaction with mixtures of several nucleoside diphosphates, the distribution of the enzyme (already known to be present in other microörganisms), and further work on its behavior toward natural nucleic acids, are in progress.

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A NEW BIOLOGICALLY POTENT STEROID: 1-DEHYDRO-9α-FLUOROHYDROCORTISONE ACETATE

Sir:

In the course of a study concerned with the relationship between structure and physiological activity we have prepared four new 9α-fluorinated[1,2] steroids related to hydrocortisone acetate. The description of these compounds is the purpose of this communication.

9α-Fluorohydrocortisone acetate (I) was hydrogenated in methanol over 5% Pd–C. The reduction product, [m.p. 233–235.5°; $[\alpha]_D$ +65.4° ($CHCl_3$); end absorption only in the ultraviolet; λ_{max}^{nujol} 2.79, 2.99 μ (OH), 5.73, 5.79 μ (acetylated side chain), 5.95 μ (saturated 3-ketone); $\lambda_{max}^{CHCl_3}$ 2.82–2.96 μ (OH), shoulder at 5.75 μ, λ_{max} 5.79 μ (acetylated side chain), 5.87 μ (3-ketone); Found: C, 65.41; H, 7.67; F, 4.15] formulated as the *allo*dihydro-9α-fluorohydrocortisone acetate (II) by analogy with the reduction of hydrocortisone acetate,[3] did not show glucocorticoid activity upon systemic administration.[4] (II) was treated

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **76**, 1455 (1954).

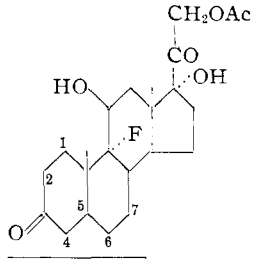
(2) J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer, and P. Numerof, *ibid.*, **77**, 1068 (1955).

(3) J. Pataki, G. Rosenkranz and C. Djerassi, *J. Biol. Chem.*, **195**, 751 (1952).

(4) The glucocorticoid activities were determined by Drs. C. A. Winter and C. C. Porter of the Merck Institute for Therapeutic Research to whom we are very much indebted. Details of these assays will be published elsewhere.

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).

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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).

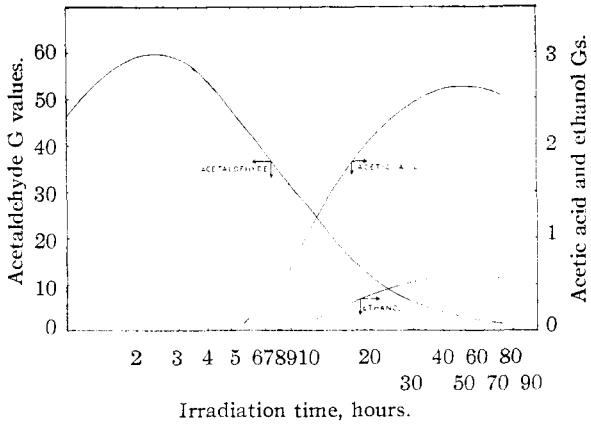

Fig. 1.—*G* values for ethanol, acetic acid and acetaldehyde production as a function of radiation dose.

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).

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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-substituted 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).