solvated aluminum hydride as follows: A solution of aluminum hydride in diethyl ether is prepared in the usual fashion[3] and filtered promptly (before polymerization can occur) through sintered glass under nitrogen into an inert liquid, which is not a solvent. Pentane and ligroin have been found to be suitable. It is essential that the hydride solution be rapidly mixed with a relatively large volume of this inert liquid (at least 100 ml. for each gram of aluminum hydride); a satisfactory method is to run the solution in a thin film down a wire while the precipitant is vigorously stirred by a magnetic stirrer. Precipitation is instantaneous and a very fluffy product results.

The bulk of the ether–precipitant mixture is removed by vacuum, and the apparently dry residue is subjected to high vacuum at room temperature. At least twelve hours are ordinarily required to remove all volatile material from the product. During evacuation, it is beneficial to grind and stir the product by means of the magnetic stirrer.

Aluminum hydride samples prepared in the above manner were analyzed for aluminum by precipitation as the 8-hydroxyquinolinate and for hydrogen by measuring the gas evolution produced by a water-dioxane mixture.

Anal. Calcd. for AlH_3: Al, 89.93; H, 10.07. Found, for a sample precipitated by ligroin: Al, 89.28; H, 9.96.

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ENZYMATIC SYNTHESIS AND BREAKDOWN OF POLYNUCLEOTIDES; POLYNUCLEOTIDE PHOSPHORYLASE[1]

Sir:

In the course of experiments on biological phosphorylation mechanisms[2] it was found that extracts of *Azotobacter vinelandii* catalyze a rapid exchange of P^{32}-labelled orthophosphate with the terminal phosphate of ADP,[3] IDP, UDP, CDP and (less rapidly) GDP. There is no reaction with the corresponding nucleoside triphosphates or monophosphates (tried ATP, ITP, AMP, IMP). The exchange is accompanied by the liberation of P_i and requires Mg^{++}. Employing the rate of the ADP-P_i exchange as an assay, the enzyme activity has been purified about 40-fold through ammonium sulfate fractionation and $Ca_3(PO_4)_2$ adsorption steps. The ratio of the rates of ADP-P_i exchange to P_i liberation remained constant.

On incubation of the purified enzyme with IDP, in the presence of Mg^{++}, 50–60% of the nucleoside diphosphate disappears with liberation of a stoichiometric amount of P_i. The missing nucleotide is accounted for by a water-soluble, non-dialyzable product which is precipitated by TCA or alcohol. Its solutions are rather viscous and exhibits a typical nucleotide ultraviolet absorption spectrum. Judging from its chromatographic behavior on Dowex anion exchange columns[4] the material is strongly acidic. It yields IMP (Fig. 1) on mild alkaline hydrolysis[6] and thus appears to be an

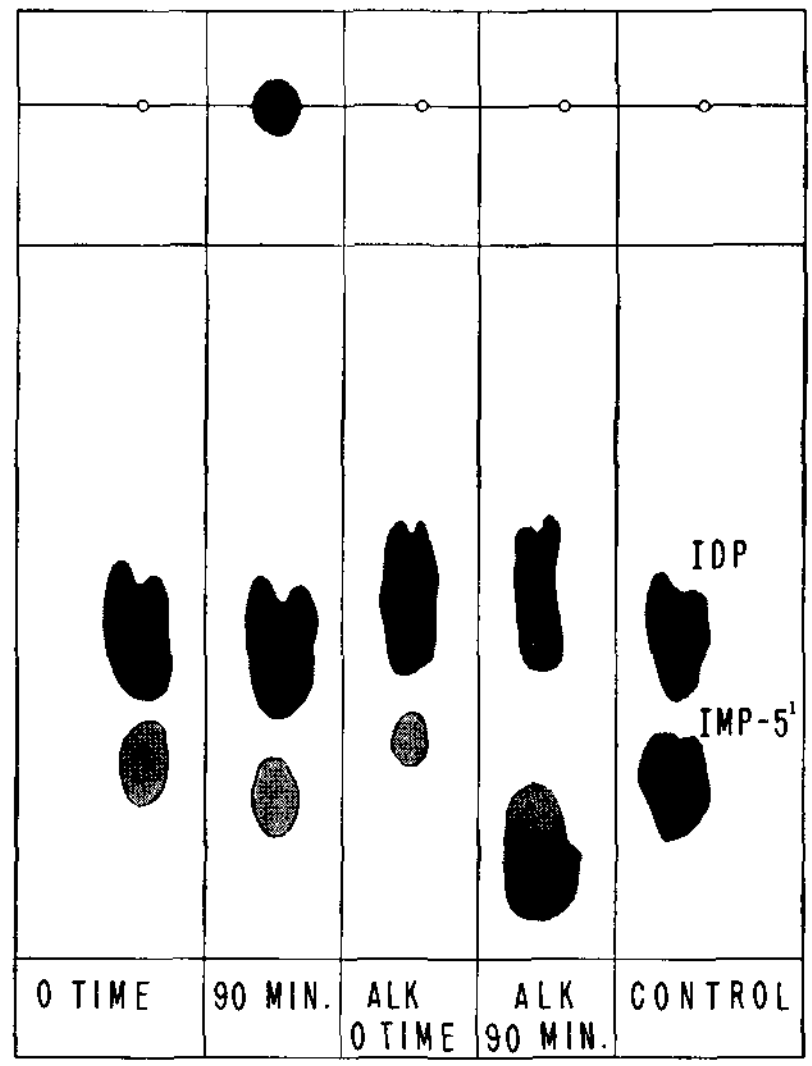

Fig. 1.—Identification of product of reaction of IDP with *Azotobacter* enzyme by paper chromatography. Solvent system of Krebs and Hems[5]; spots located by UV absorption. The three degrees of shading indicate strong, medium, and weak absorption respectively; 0.07 mg. of enzyme (SA, 12) incubated 90 minutes at 30° with 25 μM. IDP, in the presence of 12 μM. $MgCl_2$ and 90 μM. Tris buffer, *p*H 8.1; final volume, 2.5 ml. Mixture deproteinized by heating 1 minute at 100° and equal aliquots (without and with hydrolysis with 0.4 *N* KOH for 22 hours at 37°) used for chromatography. The IDP is contaminated with small amounts of 5'-IMP. Incubation results in decrease of IDP and appearance of an ultraviolet absorbing material which remains at the origin of the chromatogram. After alkaline hydrolysis, this material disappears and is replaced by a product migrating somewhat faster than 5'-IMP. This has been identified as a mixture of 2'- and 3'-IMP. The SA of the enzyme is defined as units/mg. protein. One enzyme unit catalyzes the exchange of 1.0 μM. of P_i^{32} in ADP in fifteen minutes at 30° under standard assay conditions. SA of initial enzyme extract was 0.3.

(1) Supported by grants from the U. S. Public Health Service, the American Cancer Society (recommended by the Committee on Growth, National Research Council), the Rockefeller Foundation, and by a contract (N6onr279, T.O. 6) between the Office of Naval Research and New York University College of Medicine. Presented at the April, 1955, meeting of the Federation of American Societies for Experimental Biology in San Francisco.

(2) M. Grunberg-Manago and S. Ochoa, *Fed. Proc.*, **14**, 221 (1955).

(3) Abbreviations: diphosphates of adenosine, inosine, guanosine, uridine, and cytidine, ADP, IDP, GDP, UDP, and CDP; orthophosphate, P_i; adenosine and inosine monophosphates, AMP, and IMP; inosine-2'- and 3'-monophosphates, 2'-IMP and 3'-IMP; inosine diphosphatase, IDPase; trichloroacetic acid, TCA; tris(hydroxymethyl)aminomethane, Tris; specific activity, SA; micromoles μM.

(4) Based on W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950).

(5) H. A. Krebs and R. Hems, *Biochem. et Biophys. Acta*, **12**, 172 (1953).

(6) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 715 (1948); C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

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.