with an R_f of 0.77 was found, and a water eluate of this spot was active in the phosphorylation reaction. Another sample of factor treated with RNAase and DNAase and recovered by precipitation with alcohol was hydrolyzed with 1 N HCl for 20 minutes at 100°. Aliquots were chromatographed on paper using butanol NH_3 and 2-propanol HCl as solvents.[5] Roughly equal amounts of adenine and guanine, and smaller amounts of uridylic and cytidylic acids, were identified by their R_f values and spectroscopic characteristics.

The properties of the heat stable factor described above suggest that it is a polynucleotide, and that the activity is associated with a molecular size considerably smaller than that of nucleic acid. Polynucleotides other than those isolated from DPNH oxidase preparations of *Alcaligenes faecalis* were therefore assayed for phosphorylating activity. These were made by the enzyme polynucleotide phosphorylase from ADP.[6] One sample of such a polymer given us by Dr. Grunberg-Manago and made by the enzyme isolated from *Azotobacter vinlandii*, gave clear cut positive results, while other similar samples gave equivocal results. *Alcaligenes faecalis* extracts also contain polynucleotide phosphorylase.[7] Non-dialyzable material made by incubating ADP with these crude extracts also stimulated phosphorylation in some but not all cases. The reasons for this variability are not known, but other data suggest that molecular size of the polymer may be important. The homogeneity of the mononucleotides in the polymer may also play a role. In any case the positive results with enzymatically formed polynucleotides lend added weight to the hypothesis that polynucleotides play an important role in oxidative phosphorylation in *Alcaligenes faecalis* extracts.

(5) G. R. Wyatt, E. Chargaff and J. N. Davidson, "The Nucleic Acids," Academic Press, Inc., New York, **1**, 243 (1955).

(6) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955).

(7) M. Grunberg-Manago, personal communication.

DEPARTMENT OF MICROBIOLOGY
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THE ISOLATION OF A RAT PLASMA ALBUMIN WHICH IS ELECTROPHORETICALLY HOMOGENEOUS AT LOW *p*H VALUES[1]

Sir:

We recently reported the isolation of a rat plasma albumin[2] which exhibited a high degree of electrophoretic homogeneity at *p*H 8.5. Like other purified plasma albumins,[3] however, it was electrophoretically inhomogeneous at a *p*H close to or below the isoelectric point. We have since found that advantage may be taken of a differential precipitation with zinc to recover about two thirds of the total albumin in a form which is electrophoretically homogeneous (as judged by the appearance of a single peak) at low *p*H values as well as high.

(1) Supported by a grant (H-1620) from the National Institutes of Health.

(2) Alan Keltz and J. W. Mehl, THIS JOURNAL, **76**, 4004 (1954).

(3) See, for example, W. L. Hughes, pp. 678, in H. Neurath and K. Bailey, "The Proteins," vol. 2, Academic Press, New York, N. Y., 1954.

In the original method, Fraction V was refractionated by removing salts, including zinc and barium, with Dowex-50 and by dialysis. The salt-free, zinc-free Fraction V (500 mg.) was dissolved in 5 ml. of water, and 25 ml. of a cold solution containing 230 ml. of 95% EtOH per liter was added; 0.5 ml. of Solution 2 (containing 54.8 g./l. of zinc acetate) was then added, the solution being maintained at −5°. It was calculated that this contained about 17 moles of zinc per mole of albumin. When the amount of zinc was reduced to about $^1/_{20}$ of this, only about two-thirds of the albumin was precipitated, but this albumin was now homogeneous at low *p*H values.

The obvious inference may be drawn that the fraction of the albumin which is precipitated has a much higher affinity for the first mole of zinc than the remainder of the albumin, or that it forms a particularly insoluble salt with one mole of zinc. Equilibrium dialysis studies with the homogeneous albumin at *p*H 4.8 did indicate that the first mole of zinc bound per 64,000 g. of protein had a much higher apparent affinity constant (of the order of 4×10^6) than did subsequent zinc ions.

Preliminary sedimentation and diffusion data showed no difference between the two albumin preparations and yielded a molecular weight of 63,000. The value obtained from a few measurements of light scattering was 61,000. This material precipitating with one mole of zinc per mole was found, in one experiment, to bind 4 moles of *p*-chloromercuribenzoate per 64,000 g. It was not found possible to substitute mercury for zinc in the precipitation of the albumin from ethanol at *p*H 4.8.

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RECEIVED SEPTEMBER 26, 1955

THE MECHANISM OF ACTION OF MUSCLE ALDOLASE[1]

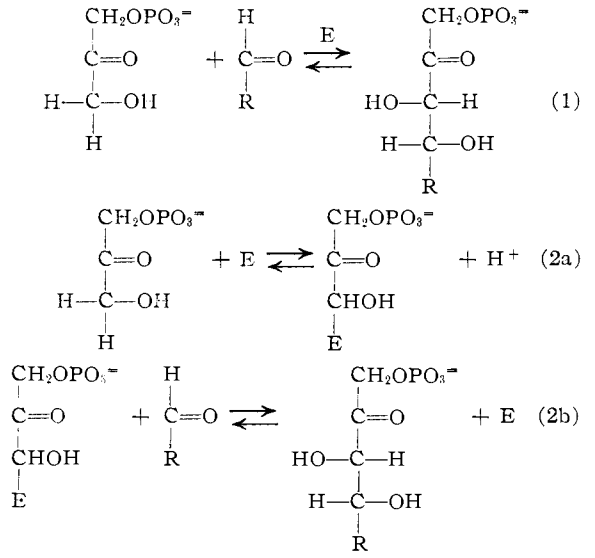
Sir:

In the reaction catalyzed by muscle aldolase, dihydroxyacetone phosphate (DHAP) is the specific substrate and condenses with glyceraldehyde-3-phosphate or with various non-phosphorylated aldehydes to give fructose-1,6-diphosphate (HDP) or a ketose phosphate.[2] This reaction leads to the formation of an asymmetric carbon in the condensation product by displacement of a hydrogen atom from the portion of the molecule derived from DHAP and may be expected to occur by (1) a displacement of hydrogen by the carbonyl carbon of the aldehyde, in which case the enzyme provides an active surface for the reaction, or (2) a two-step reaction in which the enzyme first displaces the hydrogen and then, in turn, is displaced by the aldehyde.

According to reaction 2a, the DHAP would exchange a hydrogen ion with the medium in the absence of acceptor aldehyde, whereas an aldehyde would be required for such an exchange if reaction 1 were operative. To establish the reaction mechanism, experiments were performed in which

(1) Aided by a grant from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

(2) O. Meyerhof, K. Lohmann and P. Schuster, *Biochem. Z.*, **286**, 301, 319 (1936).



DHAP, HDP, or a mixture of DHAP and HDP was incubated with 6 times recrystallized rabbit muscle aldolase[3] (free of triose-phosphate isomerase) in the presence of tritiated water and the reaction was stopped by the addition of Ag^+ ions. The DHAP and HDP were isolated by use of Dowex-1 (Cl^-) ion exchange resin and counted in a liquid scintillation counter. Experiment 1 of Table I shows that the DHAP became radioactive upon incubation with aldolase, but that no isotope incorporation

TABLE I

THE INCORPORATION OF TRITIUM INTO DHAP BY ALDOLASE

Each incubation mixture contained, in addition to the noted components, an amount of aldolase which, in experiment 1, would have split 75 μmoles of HDP, and in Experiment 2, 100 μmoles of HDP at 30° in the 30 minutes of the incubations. The total radioactivity of TOH was 6.53×10^6 c.p.m./ml. The final volume was 1.0 ml. and the *p*H was 7.4 (by adjustment). The reaction was stopped by adding $AgNO_3$ to a final concentration of 2×10^{-5} M and the DHAP and HDP were isolated by ion exchange on Dowex-1 (Cl^-). The samples were concentrated by lyophilization and counted (in a liquid scintillation counter made by Technical Measurement Corp., New Haven, Conn.) in a final mixture containing 0.5 ml. water, 5.0 ml. absolute ethanol, and 10 ml. of phosphor-toluene solution.[4]

Exp.	Additions (μmoles)	Sample counted (μmoles)	C.p.m. above background	Hydrogen exchanged (μmoles/μmole sample counted)
1	DHAP (14.5) with heated enzyme	DHAP (3.0)	0	0
	DHAP (14.5)	DHAP (4.0)	309	1.3
2	DHAP (10) + $AgNO_3$ (0.08)	DHAP (6.0)	0	0
	DHAP (10)	DHAP (4.6)	256	0.95
	HDP (8)	HDP (1.6)	7	0.07
	DHAP (10) + HDP (8)	DHAP (3.5) HDP (3.4)	173 2	0.84 0.01

occurred with heat-inactivated enzyme. This result supports reaction 2a. In experiment 2 of Table I it is shown that, under conditions in which the DHAP became labeled, the HDP was non-isotopic either in the presence or the absence of DHAP. It is also shown that Ag^+ ion, which completely inhibits the over-all reaction starting with HDP,[5] also inhibited the exchange of tritium with DHAP. It may be concluded from the non-labeling of the HDP that the enzyme is able to distinguish between the two hydrogens on the carbinol carbon of DHAP. That the labeling of DHAP is in excess of 1 μmole of exchangeable hydrogen may be attributed to isotope enrichment or experimental error.

The presence of small amounts of contaminating aldehyde in the incubation mixture of DHAP and enzyme could be responsible for the labeling of DHAP according to reaction 1. If this were true the addition of a condensation product to the incubation mixture containing DHAP and enzyme would stimulate the exchange reaction. On the other hand, according to reaction 2, the addition of a condensation product should not stimulate but could decrease the rate at which DHAP becomes labeled. The extent of this decrease would depend upon the relative "affinities" of the DHAP and condensation product for the enzyme. In a separate experiment the rate of labeling of DHAP was found to be much reduced by the addition of HDP to the incubation mixture. This finding further supports reaction 2 as the mechanism of action of aldolase.

These data do not permit one to distinguish between labilization of the hydrogen atom by the enzyme or actual displacement of it to form an enzyme-DHAP complex. However, in either case, the activation of the hydrogen atom is a feature common to the enzymatic and the base-catalyzed aldol condensations. Further work is in progress to elucidate the mechanism of the aldolase-catalyzed reaction.

The authors are grateful to Dr. Seymour Lipsky for the use of his scintillation counter, to Dr. E. Racker for a sample of α-glycerol phosphate dehydrogenase used in the assay of DHAP, to Dr. Melvin V. Simpson for a sample of triose phosphate dehydrogenase used in the assay of HDP, and to Mr. George Kalf for his help in the preparation of aldolase and DHAP.

(3) J. F. Taylor, A. A. Green and G. T. Cori, *J. Biol. Chem.*, **173**, 591 (1948).

(4) F. N. Hayes and R. G. Gould, *Science*, **117**, 480 (1953).

(5) D. Herbert, H. Gordon, V. Subramanya and D. E. Green, *Biochem. J.*, **34**, 1108 (1940).

DEPARTMENT OF BIOCHEMISTRY
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RECEIVED SEPTEMBER 19, 1955

THE ENZYMATIC SYNTHESIS OF β-ALANYL COENZYME A

Sir:

In the course of studies to determine whether acrylyl Co A[1] is an intermediate in propionyl Co A oxidation by extracts of *Clostridium propionicum*, it was observed that a reaction is catalyzed between acrylyl Co A and ammonia to form β-alanyl Co A.

$$CH_2{=}CHCOS\ CoA + NH_3 \longrightarrow CH_2NH_2CH_2COS\ CoA$$

The enzyme catalyzing this reaction has been purified about 10-fold by means of protamine and ammonium sulfate precipitations. Since the enzyme

(1) CoA and Pa are abbreviations used for Coenzyme A and Pantotheine, respectively.