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.

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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).