tion of the σ bonds, four orbitals have the required symmetry (A_1) to participate in the bonding of the hydrogen atom. Besides the 1s orbital of hydrogen (ϕ_H) and the $3d_{z^2}$ orbital of the cobalt (ϕ_{Co}), one has a molecular orbital formed from a properly oriented p orbital of each of the three symmetrically equivalent carbon atoms plus a similar orbital involving the oxygen atoms. In the LCAO approximation these latter are:

$$\phi_C = \frac{1}{\sqrt{3}}\{p_C(1) + p_C(2) + p_C(3)\}$$

$$\phi_O = \frac{1}{\sqrt{3}}\{p_O(1) + p_O(2) + p_O(3)\}$$

If we place one electron in each of these four orbitals, there are just enough electrons left over to fill the remaining bonding and atomic orbitals. The problem then is how the orbitals ϕ_H, ϕ_{Co}, ϕ_C and ϕ_O combine to form bonds. The overlap integrals suggest that the cobalt–hydrogen distance is just under 2A, and m.o. calculations of the approximate energies and the approximate form of the bonding and antibonding orbitals were made for this location. The resultant orbitals are classified on the basis of relative energies as two which are bonding and two which are antibonding—leading nicely to the formation of a diamagnetic molecule.

The bonding orbitals, in the order of decreasing stability, are

$$\Psi_I = 0.612\phi_H + 0.416\phi_C + 0.396\phi_O + 0.005\phi_{Co}$$

$$\Psi_{II} = 0.625\phi_H + 0.005\phi_C - 1.000\phi_O - 0.001\phi_{Co}$$

The electron pair in Ψ_I is shared almost exclusively between the hydrogen, carbon and oxygen atoms while the pair in Ψ_{II} is concentrated on the hydrogen and the oxygen atoms. The charge on the hydrogen atom due to both Ψ_I and Ψ_{II} amounts to 1.6 electrons. Thus, the hydrogen is immersed in a sheath of negative charge in excellent agreement with the findings from the proton magnetic resonance spectrum (H. S. Gutowsky, private communication). The reason ϕ_{Co} participates to only a small extent in the bonding orbitals is its unfavorable energy position above ϕ_C, ϕ_O and ϕ_H. These results indicate that the bridge structure is plausible but do not prove its existence.

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RECEIVED OCTOBER 3, 1955

THE ROLE OF A POLYNUCLEOTIDE IN OXIDATIVE PHOSPHORYLATION[1]

Sir:

As previously shown, the enzyme system that catalyses phosphorylation linked to the oxidation of DPNH in extracts of *Alcaligenes faecalis* can be separated into three components.[2] These are a particulate DPNH oxidase, a soluble heat labile component, and a soluble heat stable factor. The tentative characterization of the latter as a polynucleotide is the subject of this communication.

The activity of the heat stable factor was assayed in a phosphorylating system containing the other two components isolated as previously described. The energy rich phosphate formed during DPNH oxidation was trapped as glucose-6-phosphate, which was determined enzymatically.[3] Heat stable factor was prepared by centrifuging out the DPNH oxidase from crude extracts at 105,000 × *g*. The precipitate was washed by centrifugation, taken up in water and heated to 100° for 6 minutes. The supernatant solution following centrifugation was used as a source of the factor. Boiled extracts so prepared were active in phosphorylation. They had a nucleotide absorption spectrum with a single peak at 260 mμ, and a minimum at 230. The 280/260 ratio was 0.50. Active heat stable factor could be precipitated with 4% trichloroacetic acid, and redissolved in water at slightly alkaline *p*H. The activity could also be recovered by precipitation with ammonium sulfate or with two volumes of alcohol in the presence of 0.1 *M* $MgCl_2$ at a *p*H of 5.5. Activity was not lost on prolonged dialysis against water, but it was completely destroyed by incubation with 1 *N* HCl at 100° for 20 minutes or with 0.3 *N* KOH at 37° for 15 hours.

Incubation of the heat stable factor with an excess of crystalline RNAase and DNAase (Worthington), in the presence 0.01 *M* $MgCl_2$, in phosphate buffer at *p*H 7.4 for 2.5 hours did not decrease its activity. Following enzymatic treatment the factor was dialyzed, precipitated with alcohol and again dialyzed. The effect of such partially purified factor on oxidative phosphorylation is shown in Table I.

TABLE I

OXIDATIVE PHOSPHORYLATION DEPENDENT ON THE ADDITION OF PARTIALLY PURIFIED HEAT STABLE FACTOR

The reactions were carried out in a Warburg apparatus under general conditions outlined in reference 1. DPNH was formed in the experimental vessels by tipping 0.8 μM. of DPN and 100 μM. of alcohol into the main space which already contained excess crystalline alcohol dehydrogenase as well as the phosphorylating system. The DPN control vessels lacked alcohol and alcohol dehydrogenase. 0.4 μM. of ATP was present in each vessel. The amount of heat stable factor used was determined spectrophotometrically and expressed as μM. of ADP giving the same optical density at 260 mμ. Phosphorylation due to the factor is the increment of glucose-6-phosphate found in the DPNH vessel over the DPN control in the presence of added factor minus this increment in the absence of added factor.

Heat stable factor added	μM Glucose-6-phosphate formed DPN	DPNH	μM. phosphorylation due to factor
None	0.7	0.8	...
Crude equivalent to 3.15 μM. ADP	0.6	1.2	0.5
Partially purified equivalent to 1.14 μM. ADP	0.5	1.2	0.6
Partially purified equivalent to 0.25 μM. ADP	0.7	1.1	0.3

A sample of similarly prepared factor was chromatographed by the ascending paper technique with isoamyl alcohol layered over 2% disodium phosphate.[4] A single ultraviolet light absorbing spot

(1) This work was supported by a Grant from the National Science Foundation, and by an Equipment Loan Contract with the Office of Naval Research. The able assistance of Miss A. Gunilla Adner to this project is gratefully acknowledged.

(2) G. B. Pinchot, *J. Biol. Chem.*, **205**, 65 (1953).

(3) E. Racker, *ibid.*, **167**, 843 (1947).

(4) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

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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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).