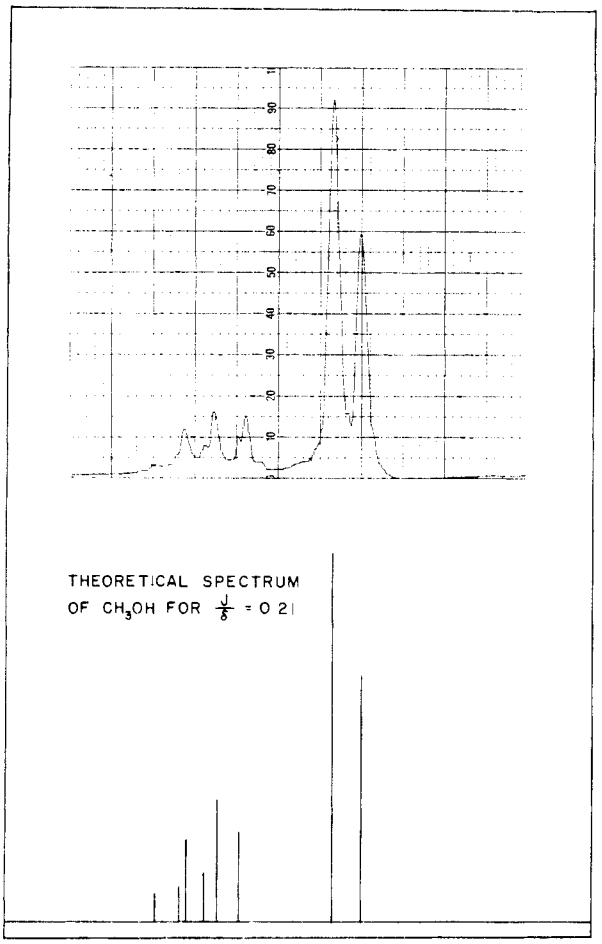

Fig. 1. - Proton magnetic resonance spectrum of methyl alcohol at 40×10^6 sec^{-1}.

with the theoretical spectra; (b) in some instances, molecules which are free to exchange and molecules which are complexed to acetone can be observed simultaneously and intensity measurements provide a measure of the number of molecules in each state; (c) the effect of acetone concentration on exchange rate can be studied.

A full report on these experiments is being prepared and will be published in the near future.

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RECEIVED APRIL 26, 1958

EVIDENCE FOR AN INTERMEDIATE IN THE HYDROLYSIS OF ATP BY MUSCLE PROTEINS

Sir:

Muscular contraction has been shown to involve the interaction of an energy source, ATP, with the muscle proteins actin and myosin,[1] but the nature of the interaction has remained obscure. Isotopic studies reported here give evidence on the existence and properties of an intermediate formed during superprecipitation of actomyosin, the gel analogy of muscular contraction.

(1) (a) A. Szent-Gyorgyi, "Chemistry of Muscular Contraction," New York, 2nd edition, 1951; (b) S. V. Perry, *Symposia Soc. Experimental Biol.*, **9**, 203 (1955); (c) J. Hanson and H. E. Huxley, *ibid.*, **9**, 228 (1955); (d) H. H. Weber, *ibid.*, **9**, 271 (1955).

In Table I the results of experiments in which ATP was hydrolyzed by muscle proteins in H_2O^{18} are presented. Superprecipitation was observed during reaction with the actomyosins. It is seen that the phosphate produced in the reactions with intact lobster muscle, purified actomyosin, synthesized actomyosin and myosin had an O^{18}-content greatly in excess of the value (0.25) to be expected for simple cleavage of the terminal bond. That the exchange occurred at a stage intermediate

TABLE I

O^{18}-CONTENT OF H_3PO_4 PRODUCED IN THE HYDROLYSIS OF ATP (ADENOSINE TRIPHOSPHATE) BY MUSCLE PROTEINS

Conditions: 0.05*M* tris, 0.01*M* $MgCl_2$, 0.005*M* ATP, 0.1*M* KCl, *p*H 7.3, atom % excess of medium H_2O, *ca.* 1.0

Expt. no.	Protein	$\frac{O^{18}\text{ atom \% excess } H_3PO_4}{O^{18}\text{ atom \% excess } H_2O}$
1	Actin, rabbit muscle	0.004[a]
2	Myosin, rabbit muscle	0.77
3	Actomyosin, synthesized from actin and myosin preparations	0.52
4	Actomyosin, isolated from rabbit muscle	0.52
5	Lobster muscle strips[5]	0.71

[a] O^{18}-content of phosphate in unhydrolyzed ATP.

between the starting material and final product was shown by the (a) absence of exchange (less than 0.01 atom % excess) with inorganic phosphate added alone or during ATP hydrolysis and (b) absence of O^{18} in unhydrolyzed ATP (less than 0.02%) isolated after stopping the reaction at *ca.* 50% hydrolysis. Thus, the intermediate cannot be in rapid mobile equilibrium with either the starting material or the product. Furthermore, the high exchange in these experiments with Mg^{++} and the lack of exchange in previously reported[2] experiments with Ca^{++} show that a striking change in the properties of the intermediate is caused by the activating metal ion.

Despite the extensive purification of the individual proteins, the possibility of a contaminant required special attention in view of the known scavenging properties of myosin[3] and the O^{18}-exchanges in other systems.[4] The formation of actomyosin from myosin increased the rate of hydrolysis tenfold. If an impurity unrelated to actin were causing the O^{18}-exchange in myosin the amount of exchange in actomyosin prepared from it should be decreased by approximately this factor. Accordingly, actomyosin was synthesized[1a] from the purified actin and myosin preparations and the decrease in exchange was found to be too small to be accounted for by such an impurity (*cf.* experiments 1, 2 and 3, Table I). Further support for this conclusion was obtained by the agreement in exchange rates with intact lobster muscle,[5] isolated actomyosin and synthesized actomyosin.

(2) D. E. Koshland, Jr., Z. Budenstein and A. Kowalsky, *J. Biol. Chem.*, **211**, 279 (1954).

(3) H. M. Kalckar, *ibid.*, **153**, 358 (1944).

(4) M. Cohn and G. R. Drysdale, *ibid.*, **216**, 831 (1955); P. D. Boyer, A. B. Falcone and W. H. Harrison, *Nature*, **174**, 401 (1954); M. Cohn, *J. Biol. Chem.*, **230**, 369 (1958).

(5) D. E. Koshland, Jr., and E. Clarke, *ibid.*, **205**, 917 (1953).

The simplest explanation for these facts is that a phosphorylated intermediate, capable of exchanging oxygen with water, is formed in the myosin portion of the actomyosin. The actin can then attack this intermediate with formation of an actin–myosin bond which holds the protein in contracted form.

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RECEIVED MARCH 28, 1958

FORMATION OF VOLATILE COMPOUNDS BY Pb^{212} RECOILING FROM ALPHA DECAY[1]

Sir:

The discovery that tritium and halogen atoms, recoiling from nuclear processes, undergo substitution reactions in the gas phase in high yield has prompted us to investigate the possibility of gas-phase reactions for metallic atoms undergoing nuclear recoil.[2–4] Our experiments demonstrate the formation of volatile organo-lead compounds by Pb^{212} atoms from the alpha decay of Po^{216} in a methane atmosphere.

The thoron (Em^{220}) daughter activity in equilibrium with Th^{232} was removed from thorium nitrate solution by sweeping with carrier gas. The carrier gas flowed through a cold trap, a 200-ml. storage bulb, and then was vented. After the system reached equilibrium, the bulb was shut off and bypassed, and became a vessel for reaction of thoron decay products with sweep gas. The steady-state concentration of Em^{220} in the bulb was determined from a gas aliquot taken immediately after isolation from the flow system.

The formation of volatile Pb^{212} compounds was studied by isolating the reaction bulb until the decay of thoron to Pb^{212} was essentially complete ($\geq$ 15 min.). An aliquot of the gas then was examined for Pb^{212} activity. An appreciable amount of the Pb^{212} in methane sweep gas was transferred with the aliquot. The results of these experiments are summarized in Table I. In similar experiments

TABLE I

PER CENT. VOLATILE Pb^{212} FROM Po^{214} RECOIL IN GASEOUS ATMOSPHERE

Gas	Delay time min.	Volatile activity, %
Methane	15	38
	30	20
	45	14
	60	19
	330	5
Helium	15	0
	30	0

with helium carrier, no Pb^{212} activity entered the proportional counter. The range of Pb^{212} (128 k.e.v. recoil energy) is < 1 mm. in methane or helium at STP,[5] but long enough to ensure equilibrium in charge-exchange processes. Therefore, essentially no recoils will strike walls before thermalization, while all should be neutralized before chemical reaction.

The Em^{220} concentration was reproducible to $\pm$ 40%—each run was compared to a zero-delay run immediately preceding. The decay curve in each case showed the growth of Tl^{208} and Bi^{212} daughters of Pb^{212}, as well as Em^{222} from Ra^{226} in solution.

The nature of the organo-lead compound(s) has not been established—the lower volatile percentages with longer delay times probably are caused by further reactions of the original species, leading to less volatile compounds.

Volatile metallic products may prove useful for quick chemical separations of nuclear recoils from thin films. They may also help to explain low gaseous diffusion coefficients observed for Tl^{208},[6] and are important in measurements of bond-breaking accompanying β^- decay such as in $Pb^{210}(CH_3)_4$.[7] Other metallic recoil atoms are being studied.

(1) Research supported by A.E.C. contract No. AT-(11-1)-407.

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(4) J. Willard, *et al.*, *J. Chem. Phys.*, **20**, 1556 (1952); **25**, 904 (1956); THIS JOURNAL, **75**, 6160 (1953); **79**, 4609 (1957).

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(7) R. R. Edwards, J. M. Day and R. F. Overman, *J. Chem. Phys.*, **21**, 1555 (1953).

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RECEIVED APRIL 29, 1958

INTERCONVERSIONS OF POLYRIBONUCLEOTIDES AND NUCLEOSIDE TRIPHOSPHATES[1]

Sir:

Ribonucleoside diphosphates have been shown to be the precursors of polyribonucleotides in the polynucleotide phosphorylase reactions.[2] Enzymes catalyzing this reaction have since been demonstrated in extracts from a variety of microbial and plant sources,[3] and purified from several different bacteria.[3–5] An enzyme catalyzing the phosphorolysis of adenylic polynucleotide to ADP[6] has recently been isolated from nuclei of mammalian liver.[7] Some evidence has been accumulated, however, which suggests that the incorporation of AMP into polymeric material catalyzed by soluble extracts from mammalian sources may utilize ATP as the substrate.[8–11]

(1) Supported by grants-in-aid No. H-2177 from the National Heart Institute, USPHS, from the National Science Foundation, and the Eli Lilly Research Laboratories. Presented in part at the Meeting of the American Society of Biological Chemists, Philadelphia, April, 1958.

(2) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955); M. Grunberg-Manago, P. J. Ortiz and S. Ochoa *Science*, **122**, 907 (1955); *Biochim. et Biophys. Acta*, **20**, 269 (1956).

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(4) R. F. Beers, *Nature*, **177**, 790 (1956); *Biochem. J.*, **66**, 686 (1957).

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(6) Abbreviations used: tris,tris-(hydroxymethyl)-aminomethane, P_i, inorganic phosphate, PP_i, inorganic pyrophosphate, ATP, adenosine 5'-triphosphate, ADP, adenosine 5'-diphosphate, GTP, guanosine 5'-triphosphate, UTP, uridine 5'-triphosphate, CTP, cytidine 5'-triphosphate, RNA, ribonucleic acid or mixed polyribonucleotide, c.p.m., counts per minute above background, corrected for self-absorption.

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