

smaller amounts, yielded mass spectra in which only the molecular ion and an M-15 peak (if a methyl substituent was present) were evident. On the basis of this evidence and results from the coinjection of standards, these compounds are tentatively assigned the structures 2,6-dimethylnaphthalene, 1,3-dimethylnaphthalene, diphenylmethane, 1,4-dimethylnaphthalene or 2,3-dimethylnaphthalene, or both, fluorene, and 1-methylphenanthrene.

Molecular ions were obtained for nine other compounds, but standards were not available to enable us to establish their identities. In a few cases, tentative structural assignments were made on the basis of molecular weight and the presence or absence of an M-15 peak. It was also possible to rule out several possible compounds as responsible for these unknown peaks by the coinjection of standards. In this manner, it was established that peak 5' is not *trans*-stilbene, peak 5 is not acenaphthylene or biphenylene, and peak 13 is not a methylbiphenyl.

Gas chromatography of the eluates from the thin-layer bands showed that all of the compounds appearing in the chromatograms in Fig. 1 were contained in the first three thin-layer bands. The eluate from band 1 contained compounds from naphthalene up to phenanthrene, the eluate from band 2 contained phenanthrene and its methyl homologs, and the eluate from band 3 contained fluoranthene and pyrene. Ultraviolet spectra of the eluates from bands 2 and 3 helped to confirm the presence of phenanthrene, fluoranthene, and pyrene (Fig. 2, A and B).

Direct probe analysis of the eluate from band 4 produced a mass spectrum with a major molecular ion at a mass-to-charge ratio m/e of 228 and minor molecular ions at m/e 242, 244, 254, 256, and 258 whose total intensity was less than half that of m/e 228. These mass spectral data suggest that this band may be primarily chrysene or one of its isomers, and the R_f value of band 4 is identical with that of a chrysene standard. However, the ultraviolet spectrum of the eluate from band 4 does not resemble that of any of the chrysene isomers (Fig. 2C). Furthermore, gas chromatography of this eluate on the DC-430 column showed that a group of at least 12 peaks of almost equal intensity emerge in the region between the triphenylene and chrysene peaks. There was insufficient material to enable us to obtain mass spectra of these peaks, whose total in-

tensity amounted to less than 1 percent of that of phenanthrene (Table 1).

The distributions of aromatic compounds in the two Murchison samples are different. For instance, there are more compounds of higher molecular weight relative to those of lower molecular weight in sample I than in sample II; the unidentified peak 5' does not appear in sample II; and peak 5 does not appear in sample I. The degree of inhomogeneity in the distribution of organic matter within the whole meteorite is not known.

The composition of both aromatic fractions is relatively simple if we consider the number of aromatic and alkyl aromatic compounds possible. In both samples there is a noticeable lack of long- or even short-chain alkyl-substituted aromatic compounds. By far, most of the sample is composed of unbranched, polynuclear compounds containing an even number of carbon atoms. In these respects the Murchison aromatic compounds are similar to those produced by the pyrolysis of methane (4). This similarity suggests that the aromatic compounds in this meteorite may be the product of an essentially thermal, high-temperature synthesis.

The simple composition of the aromatic

fractions contrasts with the apparent complex composition of the aliphatic hydrocarbon fractions reported earlier (7), but the significance of this difference in complexity is not clear. Future work should include an exploration of the various synthetic conditions which might duplicate those that led to the formation of the Murchison hydrocarbons.

KATHERINE L. PERING

CYRIL PONNAMPERUMA

National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California 94035

References and Notes

1. E. Gelpi, J. Han, D. W. Nooner, J. Oro, *Geochim. Cosmochim. Acta* **34**, 965 (1970); C. Ponnampuruma, *N.Y. State J. Med.* **70** (No. 10), 1169 (1970).
2. P. Swings and L. Haser, *Atlas of Representative Cometary Spectra* (University of Liège Astrophysical Institute, Louvain, 1956); L. E. Snyder, D. Buhl, B. Lukerman, P. Palmer, *Phys. Rev. Lett.* **22**, 679 (1969).
3. J. M. Hayes, *Geochim. Cosmochim. Acta* **31**, 1395 (1967).
4. J. Oro and J. Han, *Science* **153**, 1393 (1966).
5. M. H. Studier, R. Hayatsu, E. Anders, *Geochim. Cosmochim. Acta* **32**, 151 (1968).
6. F. M. Johnson, *NASA SP 140* (1965), p. 229; B. Donn, *Astrophys. J.* **152**, L129 (1968).
7. K. Kvenvolden, J. Lawless, K. Pering, E. Peterson, J. Flores, C. Ponnampuruma, I. R. Kaplan, C. Moore, *Nature* **228**, 5273 (1970).
8. We thank Dr. J. Lawless for the low-resolution mass spectra, Dr. C. Folsome for performing the gas chromatographic analysis on the DC-430 column, and G. Rogers for recording the ultraviolet spectra.

11 February 1971

Glycerinated Muscle Fibers: Relation between Isometric Tension and Adenosine Triphosphate Hydrolysis

Abstract. *The isometric tension of glycerinated muscle fibers and the adenosine triphosphatase activity of homogenates were determined as a function of the concentration of adenosine triphosphate without the addition of divalent cations. These two phenomena are not parallel; large tensions can be developed with negligible hydrolysis of adenosine triphosphate. It is concluded that the large negative free energy change of the hydrolysis is not required for shortening or development of tension.*

In "living" muscle and in the model glycerinated muscle fiber system adenosine triphosphate (ATP) is hydrolyzed during some stage of the contractile process (1). The necessary catalysis is provided by the fibrous protein myosin acting as an enzyme. The specific role and function of ATP in this process has not been established definitely (2), and two widely divergent views have evolved (3). One argument is that the large negative free energy change which accompanies the ATP hydrolysis is the direct cause of shortening or tension development. Hence, it is claimed that the two processes

are directly coupled with one another (4). Alternatively, it has been proposed that the intact ATP induces contractility, with hydrolysis taking place subsequently (5).

When the contractile process is mediated by Mg^{2+} or Ca^{2+} , there is a striking lack of parallelism between shortening and enzymatic activity (6). Bowen (6) has reported that although Mg^{2+} accelerates the ATP-induced shortening of myosin threads and glycerinated muscle fibers the dephosphorylation is retarded. The introduction of Ca^{2+} results in the opposite effect; shortening is retarded, but

the hydrolysis is accelerated (6). The expected complementary results are obtained when the isometric tension is studied in these media (7). Recognizing that the results obtained with the divalent cations might reflect specific chemical effects rather than the contractile process (3), we have measured the isometric tension and adenosine triphosphatase activity as a function of ATP concentration in the absence of Mg^{2+} and Ca^{2+} . We used conventional experimental techniques (7, 8) to measure isometric tension in glycerinated rabbit psoas fibers and the enzymatic activity of their homogenates.

The experimental results are summarized in Fig. 1. The isometric tension becomes perceptible when the concentration of ATP is about 10^{-4} mole/liter. With a further increase in the concentration of ATP a very rapid increase in the tension is observed, and a value of about 1 kg/cm^2 is attained at $10^{-2}M$ ATP. A comparison with previous experiments on shortening (9, 10), under conditions of zero force and a similar supernatant composition, indicates the required parallelism between these two types of experiments.

On the other hand, the rate of ATP hydrolysis follows a quite different pattern. The rate of splitting and the isometric tension are parallel at ATP concentrations up to 10^{-3} mole/liter, where the adenosine triphosphatase activity attains its optimum value. As the ATP concentration is further increased, however, the enzymatic activity precipitously drops to very low levels. Concomitantly, over this same concentration range, the tension increases to very large values. These two observations are thus distinctly divergent. Thus, it is demonstrated that large tensions can be induced by ATP in the muscle fiber system without the attendant large decrease in free energy which accompanies the hydrolysis.

It may be that there is a unique kind of coupling between hydrolysis and force generations which does not manifest itself in an enhanced rate of splitting at high concentrations of ATP. However, the results obtained with the system of muscle fiber and ATP finds an almost exact analogy in the behavior of the more simply constituted fibrous proteins (11, 12). For example, when the fibrous protein collagen is immersed in an aqueous medium large tensions can be developed, and controlled, by the addition

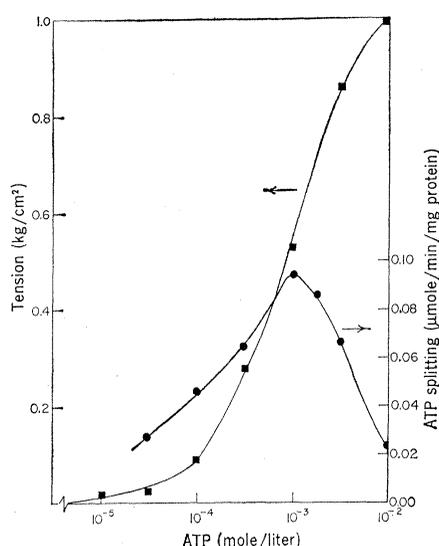


Fig. 1. Plot of isometric tension and hydrolysis of ATP as a function of ATP concentration. At $25^{\circ}C$ with $0.1M$ KCl added.

of certain species such as KSCN or LiBr (11, 12). It is well established that, for these situations, the tension is that required to maintain equilibrium between the ordered or crystalline state and the disordered one (11, 13). The shift in the equilibrium, with the added species, results from a greater preferential interaction or "binding" to the constituent macromolecules in the disordered state. Hence, from both a theoretical and experimental point of view there is no a priori necessity to involve chemical reactions in the development of tension of fibrous macromolecular systems. The results obtained here, for muscle fiber and ATP, appear to fall into the same general category.

For the system of muscle fiber and ATP we therefore conclude that the preferential interaction of the nucleotide with fibrous myosin in the disordered state is the basis for the isometric tension that is developed or for shortening. Since this event must involve a change in the native structure of myosin, we postulate that it is the new structure that is the enzyme. This would then account for the high level of adenosine triphosphatase activity that is observed approximately midway through the process, when it can be presumed that the optimum enzymatic structure exists. However, with a further increase in ATP concentration, the ordered structure will be completely destroyed and the myosin will develop a statistical or random conformation. Under these conditions,

although the particular local structure required for the enzymatic action would be lost, the tension will still increase. The mechanism outlined here is consistent with the data presented in Fig. 1, with the most general physicochemical considerations of the problem (12-14), and with the demonstration that the ordered structure, similar to that of α -keratin, characteristic of myosin in native muscle, disappears with large amounts of ATP-induced shortening (9).

In physiological situations, or in experiments which are limited to studying relatively small amounts of shortening, the enzymatic activity will be optimum. Upon hydrolysis the preferentially interacting species will be automatically removed from the system. Therefore the native myosin structure will be restored. Since shortening, hydrolysis, and the regaining of myosin structure will occur in rapid succession, the apparent anomaly that the usual morphological methods of analysis under these conditions do not indicate any gross structural changes in the myosin fibers or in thick filaments can be understood.

W. J. BOWEN*

L. MANDELKERN

National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland 20014, and Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee 32306

References and Notes

1. A. A. Infante and R. E. Davies, *J. Biol. Chem.* **240**, 3996 (1965).
2. P. Dreizen and L. C. Gershman, *Trans. N.Y. Acad. Sci.* **32**, 170 (1970).
3. M. Morales, in *Enzymes: Units of Biological Structure and Function*, O. H. Gaebler, Ed. (Academic Press, New York, 1956), p. 325.
4. H. H. Weber and H. Portzehl, *Adv. Protein Chem.* **7**, 161 (1952); *Prog. Biophys.* **4**, 60 (1954).
5. M. Morales and J. Botts, *Arch. Biochem. Biophys.* **37**, 283 (1952).
6. W. J. Bowen, *J. Cell. Comp. Physiol.* **49** suppl. 1, 267 (1957).
7. ——— and H. L. Martin, *Am. J. Physiol.* **195**, 311 (1958).
8. T. C. Evans, Jr., and W. J. Bowen, *Anal. Biochem.* **25**, 136 (1968).
9. L. Mandelkern, A. S. Posner, A. F. Diorio, K. Laki, *Proc. Natl. Acad. Sci. U.S.A.* **45**, 814 (1959).
10. W. J. Bowen and L. Mandelkern, *Physiol. Chem. Phys.* **2**, 227 (1970).
11. P. J. Flory and O. K. Spurr, Jr., *J. Am. Chem. Soc.* **83**, 1308 (1961).
12. M. M. Rubin, K. A. Piez, A. Katchalsky, *Biochemistry* **8**, 3628 (1969).
13. L. Mandelkern, *J. Gen. Physiol.* **50**, 29 (1967).
14. P. J. Flory, *Science* **124**, 53 (1956).
15. Supported in part by a contract with the Division of Biology and Medicine, Atomic Energy Commission.

* Deceased

4 March 1971