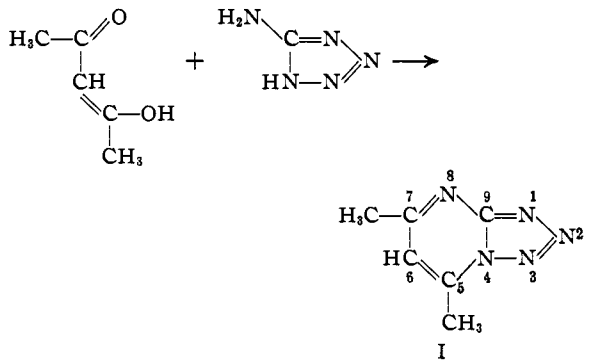
ignated as above according to the system employed in the "Ring Index."[2] It has not been studied further since its preparation from 5-aminotetrazole and acetylacetone in the presence of piperidine, the method which was also employed in this work.[3]



I

From an examination of the spectra of 5,7-dimethyltetrazolo[a]pyrimidine shown in Fig. 1,[4] a tremendous batho- and hyperchromic shift is to be noted in comparing the spectrum at *p*H 13 with that in neutral and acidic solutions. As the

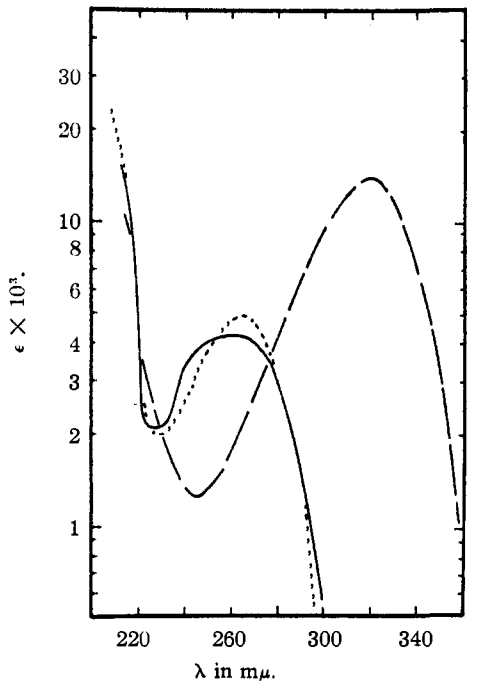
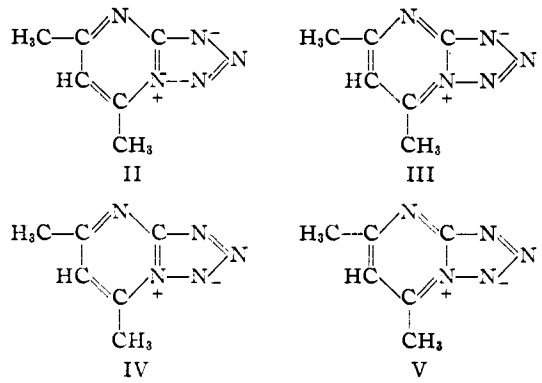


Fig. 1.—Spectra of 5,7-dimethyltetrazolo[a]pyrimidine in — ethanol (95%), ---- 0.01 *N* HCl, — — 0.01 *N* NaOH.

nitrogen atoms do not bear any hydrogen [atoms], a classical picture on the basis of tautomeric shifts cannot be developed. However, if one admits ionic structures,[5] this behavior can apparently be explained satisfactorily. The dipolar ions ("Zwitterions") shown in (II) to (V) contribute to the arrangement of the compound in acid, and, to a somewhat lesser extent, in neutral solution. These considerations would lead one to expect that the resulting spectrum, with respect to the position of the maximum, should be similar to that of a benzenoid ring with a conjugated double bond. Indeed, a measure of similarity to the spectra of styrene,[6] indole,[7] benzotriazole[8] and even benzoxazole[9] is observable. In alkaline medium, however, this polarization tendency, due to the addition of protons in position 1 or 3, disappears and the structure (I) is the only one remaining. Such a spectrum should more closely resemble that of a polyene and shift the absorption maximum toward the visible range. This is borne out by the similarity of the spectrum of 5,7-dimethyltetrazolo[a]pyrimidine in alkali with that of 1,3,5,7-octatetraene which was studied by Kovner[10] and Hausser.[11] Both of the compounds have four conjugated double bonds and a "maximum extinction coefficient" beyond 300 mμ.



II III IV V

(2) Patterson and Capell, "The Ring Index," Reinhold Publishing Corp., New York, N. Y., 1940.

(3) The authors wish to express their appreciation to Miss R. Pauline Brundage for her preparation of the compound.

(4) All spectral determinations were made with a Beckman quartz spectrophotometer, Model DU, Serial No. D-377, as in earlier work [*cf.* Ewing and Steck, THIS JOURNAL, **68**, 2181 (1946)].

(5) The authors are grateful to Dr. Elmer J. Lawson for his helpful discussion on the formation of dipolar ions.

(6) Elliott and Cook, *Ind. Eng. Chem., Anal. Ed.*, **16**, 20 (1944); Rodebush and Feldman, THIS JOURNAL, **68**, 896 (1946).

(7) Johnson, Bruce and Dutcher, *ibid.*, **65**, 2005 (1943).

(8) Specker and Gawrosch, *Ber.*, **75B**, 1338 (1942).

(9) Ramart-Lucas and Vantu, *Bull. soc. chim.*, [5] **146**, 1165 (1936).

(10) Kovner, *Acta Physicochim. (U.R.S.S.)*, **19**, 385 (1944). *Cf.* also Ferguson and Branch, THIS JOURNAL, **66**, 1467 (1944).

(11) Hausser, *Z. techn. Physik*, **15**, 10 (1934).

THE STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, N. Y. RECEIVED APRIL 20, 1948

Remarks on the Physico-Chemical Mechanism of Muscular Contraction and Relaxation

BY JACOB RISEMAN AND JOHN G. KIRKWOOD

The physico-chemical processes underlying the contraction and relaxation of muscle have been the subject of much speculation. Recently significant analogies between the elastic behavior of muscle and that of rubber and synthetic elastomers have

been investigated by Bull[1] and others. That the structure of striated muscle, and possibly smooth muscle, is much more ordered than those of elastomeric cross-linked high polymers is clearly demonstrated by the studies of Schmitt and his collaborators.[2] Nevertheless, important aspects of structural similarity exist. The basic structural unit of the muscle fibril is considered to be the linear polypeptide chain of the myosin or actomyosin molecule. Like the segments of a polymer network, the long polypeptide chain possesses many internal rotational degrees of freedom which allow it to gain configurational entropy on contraction. Therefore it is probable that the elastic modulus of a structure composed of such elements is in part determined by the dependence of their configurational entropy upon elongation.

The contraction or relaxation of a muscle segment under constant stress is the consequence of a change in the elastic modulus arising from alteration of its structural units. Following certain ideas suggested by the work of K. H. Meyer,[3] we wish to examine the hypothesis that the essential alteration of the structural unit, leading to a change in modulus, is a change in its net electric charge. A structural unit consisting of a long polypeptide chain can gain or lose electric charge in several ways in response to changes in its physico-chemical environment. If the absolute magnitude of the charge, whatever the sign, is increased, electrostatic repulsion between the elementary charges comprising the increment will decrease the elastic modulus by destroying the balance between the external stress and the contractile force arising from configurational entropy. Conversely, if the magnitude of the charge is decreased, the modulus will increase. According to this view, the relaxed state of muscle is an electrically charged state and the contracted state one in which the polypeptide chains are in an uncharged or "isoelectric" condition. We shall presently make some rough estimates of the change in elastic modulus produced by electrostatic repulsion between charges distributed at intervals along a polypeptide chain, after discussing possible mechanisms by which it might gain or lose charge.

Since a polypeptide chain is an ampholyte, it can gain or lose charge in response to a change in the *p*H of its environment. This mechanism was proposed by K. H. Meyer[3] as a basis for the analysis of the energetics of muscular relaxation and contraction. Meyer's proposal was rejected by Weber[4] on grounds which still must be regarded as inconclusive. Adsorption of cations, for example potassium, by actomyosin segments provides a second method of charging, which the work of St. Gyorgi suggests may play a role.[5] Nevertheless, both of these charging mechanisms leave obscure the manner in which the chain of carbohydrate oxidation reactions supplies free energy for the muscular processes.

We are inclined to the view that phosphorylation of the hydroxy amino acid residues of the myosin or actomyosin molecule by adenosine triphosphate provides a charging mechanism in best accord with known facts. From its amino acid analysis, myosin is known to contain a large proportion of hydroxy amino acid residues; serine, 3.9%; and threonine, 4.95%.[6] Myosin is also considered to be one of the enzymes involved in the dephosphorylation of ATP to ADP and inorganic phosphate. Assuming that the first step in the dephosphorylation of ATP consists in the phosphorylation of the $-OH$ groups of the hydroxy amino acid residues of the myosin molecule, we conclude that at a *p*H of 7, the approximate *p*H of the sarcoplasm, each $-H_2PO_4$ group will be approximately singly ionized to $-HPO_4^-$. Thus the phosphorylation process would impart to the neutral sites originally occupied by $-OH$, approximately one unit of negative charge. The extension of the myosin chain and that of the structure of which it is the unit, resulting from electrostatic repulsion between the negatively charged $-HPO_4^-$ groups, stores up the free energy, released in the degradation of the high energy phosphate bond of ATP, in the form of negative configurational entropy of extension. Subsequent dephosphorylation of the myosin molecule with release of inorganic phosphate ion to the sarcoplasm would remove negative charge from the molecule and release the stored free energy as mechanical work in contraction of the structure. The coupling between the carbohydrate oxidation process and the mechanical processes of muscle activity is thus clarified by the proposed mechanism. ATP, regenerated in the chain of oxidation reactions, serves as a carrier of free energy released in these reactions to the myosin units of the muscle structure.

In order to examine the quantitative implications of our hypothesis, we will make some rough estimates of the change in elastic modulus E of a flexible linear molecule produced by attaching electric charges of equal magnitude at equal intervals along its length. If the terminal groups of the molecule are separated by a distance L and n charges of the same sign and magnitude e are attached to the chain at points separated by equal numbers of bonds, the random coil model of a flexible linear molecule leads to the following approximate estimate of the increment in elastic modulus ΔE, produced by the charge increment ne

(1) H. B. Bull, THIS JOURNAL, **67**, 2047 (1945).

(2) F. O. Schmitt, M. A. Jakus and C. E. Hall, *Biol. Bull.*, **90**, 32 (1946); M. A. Jakus and C. E. Hall, *J. Biol. Chem.*, **167**, 705 (1947).

(3) K. H. Meyer, *Biochem. Z.*, **214**, 1 (1929).

(4) H. H. Weber, *ibid.*, **217**, 430 (1930).

(5) A. St. Gyorgi, "Chemistry of Muscular Contraction," Academic Press, Inc., N. Y., 1947, p. 30.

(6) M. L. Anson and J. T. Edsall, "Advances in Protein Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1944, p. 310.

$$\Delta E = -\frac{8}{3}\frac{N\rho}{M}\frac{n^2e^2}{D_eL}$$

where D_e is an effective dielectric constant, N is Avogadro's number, M the molecular weight of the linear molecular unit of the structure and ρ is the density of the structure, regarded as a three-dimensional network with L the average distance between net points. Since electrostatic interactions between the charges of neighboring chain segments are neglected and other gross approximations have been employed in its derivation, Eq. (1) is intended to provide no more than an estimate of the order of magnitude of ΔE.

The elastic modulus of muscle[1] is of the order of magnitude 10^5 dynes/sq. cm. In order to produce a change of this order of magnitude by charging the structural units, we estimate from Eq. (1) a magnitude of n of the order of 100, with the rough assignments of value $\rho = 1$, $M = 10^6$, $L = 10^4$A, $D_e = 100$ to the other parameters. The values of L and M are those determined for the myosin or actomyosin molecule in solution, and D_e is assumed to be of the order of magnitude of the dielectric constant of water. The estimated number of charges would require phosphorylation sites situated at intervals of 100 Å. along the myosin or actomyosin molecule. This value is not inconsistent with the hydroxy amino acid content of myosin.

The observation of Needham[7] that the flow birefringence of myosin solutions is diminished by the addition of ATP seems at first to be in contradiction with our hypothesis. However, it seems that the effect was observed under conditions leading to the dissociation of actomyosin into actin and myosin, according to St. Gyorgi.[5]

We have deliberately avoided placing undue emphasis on hypothetical structural details of muscle and on the detailed analogy between the elastic properties of muscle and elastomers. The essential qualitative aspects of our suggestions, (a) change in the elastic modulus of the structure due to alteration of the electric charge of the structural unit, considered to be a polypeptide chain rich in hydroxy amino acid residues; (b) charging of the structural unit through phosphorylation of the hydroxyl groups by ATP, are to a large extent independent of assumed structural details.

(7) J. Needham, Shih-Chang Shen, D. Needham and A. S. C. Lawrence, *Nature*, **147**, 766 (1941).

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CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

Purification of N-Hydroxymethylphthalimide through a Molecular Compound with Pyridine

BY EUCLID J. SAKELLARIOS

In connection with the synthesis of N-alkylated phthalimides, we have prepared N-hydroxymethylphthalimide. This compound was first prepared by Sachs[1] by the hydrolysis of N-bromomethylphthalimide. It was later prepared by Sachs[2] from formaldehyde and phthalimide in sealed tubes and by Buc[3] from the same reactants at atmospheric pressure. The melting points reported by these authors were 141–142°, 139–140° and 137–141°, respectively. Buc also reported that the melting point is not improved by crystallization from ethanol.

We attempted to eliminate the impurities by adding about 1 g. of fuller's earth per 100 ml. of solution in Buc's procedure. This yielded a product melting at 144–145° which was, however, still not pure.

A product of high purity melting at 149.5° was finally obtained through an unstable, previously unreported complex formed from N-hydroxymethylphthalimide and one mole of pyridine.

Chloro-, bromo- and iodomethylphthalimides were prepared from the pure compound and the appropriate halogen acid. In all cases the products had higher melting points than those previously reported.

Procedure.—A solution obtained by warming 17.7 g. of N-hydroxymethylphthalimide[3] in 30 ml. of pure pyridine was filtered, if necessary, and left to crystallize. If crystallization did not occur, seed crystals were obtained by placing a few drops of the solution in a desiccator over sulfuric acid. As soon as the first crystals appeared, they were added to the solution. The new compound crystallized in long, bright needles which after cooling in an ice-bath were filtered with suction.

To determine the pyridine the crystals were dried briefly on a porous plate over calcium chloride. A weighed sample was then dried in vacuum over sulfuric acid. The crystals gradually lost their brightness and came to constant weight after twenty-four hours.

Anal. Calcd. for $C_6H_7O_3N \cdot C_5H_5N$: pyridine, 30.58. Found: pyridine, 30.68.

The residue melts at 148.5–149°. One crystallization from acetone brings the m. p. to 149.5°.

Anal. Calcd. for $C_6H_7O_3N$: N, 7.91. Found: N, 7.88.

The halogenomethylphthalimides were prepared essentially according to Gabriel,[4] heating at 50° for one hour after the crystals separated. The crystals were filtered, washed with the appropriate acid and then dried over sulfuric acid and potassium hydroxide. The results obtained are summarized in the table.

TABLE I

N-HALOMETHYLPHTHALIMIDES

Compound	Yield crude, %	Crystn. solvent	M. p., °C. Obs.	M. p., °C. Prev.[a]
Chloro-	93	Ethyl acetate	136.5	132–133[4]
Bromo-	91.1	Ethyl acetate	151.5	149–150[5]
Iodo-	92.3	Benzene–ethyl acet.	155.5	153[5]

[a] Best previously reported.

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(1) Sachs, *Ber.*, **31**, 1231 (1898).
(2) Sachs, *ibid.*, **31**, 3230 (1898).
(3) Buc, THIS JOURNAL, **69**, 254 (1947).
(4) Gabriel, *Ber.*, **41**, 242 (1908).
(5) Gabriel, *Ber.*, **31**, 1229 (1898).