Modification of Mitosis by Chemicals[1]

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The mitotic chromosomes of guayule (*Parthenium argentatum*) are difficult to study because they are small, numerous, and tangled. Treatment with colchicine, found advantageous with *Phlox* (*1*), failed to facilitate the study of guayule chromosomes. It was found however, that *p*-dichlorobenzene could be used in a technic which permits precise chromosome studies not only in guayule but also in other plants (*2*).

Preliminary work showed that 8 chemicals reported to induce polyploidy could shorten and straighten the chromosomes of *Crepis capillaris* (2n = 6). The most effective concentration of each chemical was determined, and the relative percentages of diploid (2X) metaphases, tetraploid (4X) metaphases, and anaphases were found after each chemical treatment (Table 1).

TABLE 1

PER CENT OF METAPHASES AND ANAPHASES IN ROOT TIPS OF *Crepis capillaris* (2n = 6) AFTER 2-HR CHEMICAL TREATMENTS

Chemical	Concentration of chemical*	Mitotic divisions†		
		Metaphases		Anaphases
		2x	4x	
Acenaphthene	1.00	83	4	13
Chloral hydrate	0.10%	76	13	11
Chloroform-water	0.10	70	4	26
Colchicine	0.20%	98	2	0
Mercuric chloride	0.00005	80	1.5	18.5
p-Dichlorobenzene	1.00	89	6	5
Sanguinarine nitrate	0.005	78	4	18
Sulfanilamide	1.00	96	4	0

* Proportion of a saturated solution in distilled water at room temperature, except concentrations given in %.

† Forty mitotic divisions in each of 10 root tips, or 400 mitotic divisions, classified for each chemical treatment.

The chloral hydrate treatment produced far more tetraploid metaphases than did the other chemical treatments; apparently this drug allowed sister chromatids to separate from each other in spite of the absence of a normal, anaphase-producing spindle. The chloral hydrate and chloroform-water treatments sometimes caused the sister chromatids of some, but not all, of the chromosomes to separate from each other. This gave chromosome numbers intermediate between the diploid and tetraploid numbers; these divisions were classed as tetraploid.

No anaphases were found after the colchicine and sulfanilamide treatments. Perhaps sulfanilamide could be used to induce polyploidy during the current shortage of colchicine. The chloroform-water treatment gave a high percentage of anaphases. There are no comparable data on the percentage of anaphases without treatment.

Some late prophase chromosomes showed distinct minor coils after the treatments with chloroform-water, colchicine, *p*-dichlorobenzene, and sulfanilamide. Sister telophase nuclei were sometimes connected by chromatin bridges after the chloral hydrate, colchicine, and mercuric chloride treatments. Some of these bridges were formed by lagging chromosomes, while others may have been due to chromosome breakage and recombination.

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[1] Studies leading to this paper were made while the writer was employed by the Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, USDA.

On the Nature of the Interaction Between Actomyosin and ATP

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There is at present considerable interest in the nature of the interaction between adenosine triphosphate (ATP) and actomyosin (Weber's myosin). This interest originates in experiments which Engelhardt, *et al.* (*5*) performed on actomyosin threads manufactured by squirting a thin stream of concentrated protein solution into water. These investigators found that ATP induces an increase in tensile strength of actomyosin threads made in this manner. Subsequently, Szent-Györgyi and his collaborators (*3*) have reported that threads of actomyosin which are floating freely in water "contract" upon treatment with ATP. Furthermore, Buchthal (*2*) has emphasized the possible importance of the ATP-actomyosin interaction in the living muscle cell. He maintains that ATP can produce a contraction in a single muscle fiber. This contraction is associated with an action potential and is accompanied by a reversible decrease in birefringence of the fiber. Indeed, Buchthal (*1*) has stated that the "breakdown of ATP is the reaction nearest in time to the physical process of contraction."

Unfortunately, agreement is not general on the nature of the effect of ATP on the actomyosin molecule. Thus, in a study of actomyosin dispersed in KCl solution, rather than in the form of threads, Needham and co-workers (*3*) observed that both ATP and high concentrations of KCl bring about a drop in viscosity and flow birefringence of the protein solution. They concluded that these changes are the result of a coiling up of the actomyosin molecules. Contrariwise, Szent-Györgyi considered quite similar data to indicate that both ATP and high KCl concentrations

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effect a dissociation of the dispersed actomyosin molecules into actin and myosin.

Induction of coiling in actomyosin by ATP is consistent with the notion that the ATP-actomyosin interplay is of fundamental importance in muscular contraction. However, if ATP produces dissociation in actomyosin, it is not easy to understand the role that this interaction might play in contraction of the muscle cell.

To obtain information of possible value for a clearer understanding of the ATP and KCl effect, we have undertaken the light-scattering and electron microscope studies described here in a preliminary report.

The actomyosin used in our experiments has been prepared by a method slightly modified from that of Greenstein and Edsall (*6*). The preparation was purified by ultracentrifugation at 28,000 rpm.

The light-scattering measurements were made with a simple apparatus which employs the 546-mμ line of a mercury vapor lamp as light source and a photomultiplier tube for registering intensities of scattered light. We have determined the intensities of light scattered in the horizontal plane at angles of 45° and 135° with a beam of light transmitted through a 1% solution of actomyosin in 0.5 molar KCl and 0.09 molar KH_2PO_4–K_2HPO_4 buffer at pH 6.8. In 10 observations on three preparations of actomyosin, an average value of 3.4 was obtained for the ratio of the forward to backward scattering at the angles mentioned.

Debye (*4*) has pointed out that measurements of this ratio yield information concerning the size, shape, and behavior of macromolecules comparable in size with the wave length of light. Furthermore, Oster (*7*) has determined the limiting values of the ratio to be expected for macromolecules which are in the form of spheres, random coils, or stiff rods. In the light of Oster's calculations for the angles used here, the value of 3.4 which we have found for actomyosin indicates that this protein has a configuration intermediate to that of a stiff rod or random coil; that is, the actomyosin particles in 0.5 molar KCl at pH 6.8 are slightly coiled.

After addition of ATP in quantity sufficient to make its final concentration 1% in a solution of actomyosin in 0.5 molar KCl and buffer at pH 6.8, it was found that the ratio had increased to 4.7. This was observed in 5 experiments on two preparations of actomyosin. On the basis of the theory of light scattering (see 7) one should expect the ratio to decrease if the actomyosin were dissociated by ATP. However, the increase in ratio we have observed is entirely consistent with an increase in coiling of the actomyosin.

No change in pH of the actomyosin solution after addition of ATP was detectable as measured by glass electrode. Tenfold dilution of the ATP-actomyosin solution with 0.5 molar KCl did not alter the ratio of 4.7, nor was there a change in light transmission of the actomyosin solution after addition of ATP. The transmission was measured in a Klett-Summerson colorimeter using a blue filter. The latter finding may be interpreted as indicating that the change in ratio is not the result of aggregation or coagulation of the actomyosin.

Addition of KCl to actomyosin solution over a concentration range of 0.5 to 2 molar KCl failed to alter the ratio at the previously stated angles. This result, obtained in 5 experiments done on two preparations, constitutes good evidence that the actomyosin does not dissociate at higher concentrations of KCl. It is, rather, an indication that there is no change in the size or shape of the protein as a result of increasing the concentration of KCl. Thus, the drop in viscosity of actomyosin solution observed at high KCl concentrations demands an explanation different from those previously offered. It is conceivable that the decrease in viscosity is related to an electroviscous effect and is associated with the adsorption of potassium by actomyosin.

Electron micrographs demonstrate an effect of ATP on actomyosin which is consistent with the preceding interpretation based on light-scattering data.

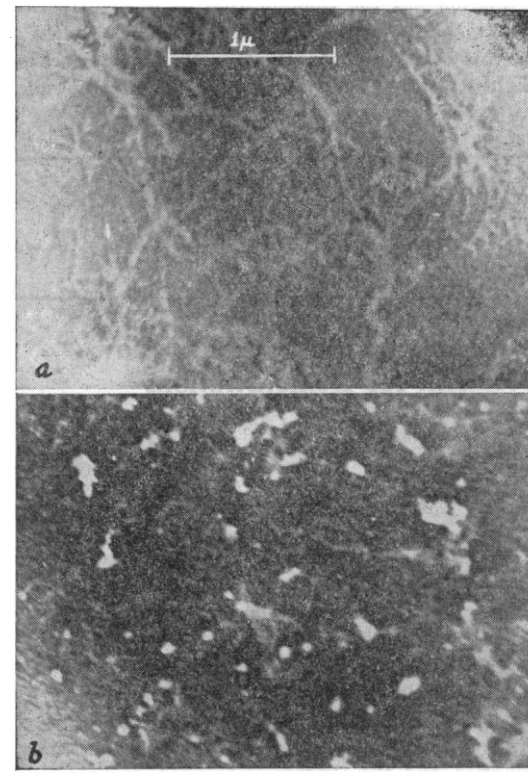

FIG. 1

Samples of untreated actomyosin were prepared by placing a drop of 0.01% actomyosin solution in 0.5 molar KCl on a collodion film supported by a copper screen. The sample was allowed to dry and was washed with distilled water to remove readily soluble salts. The specimen was then gold shadow-cast by the method of Williams and Wyckoff (*9*) to enhance photographic contrast. Fig. 1a is a representative picture of such a sample taken in an RCA console model electron microscope. The typical entangled, fibrillar structure of actomyosin is illustrated, along with the characteristically wide distribution of particle lengths.

Similar samples were then prepared from solutions of 0.01% actomyosin in 0.5 molar KCl to which ATP had been added in sufficient quantity to make its final concentration 0.01%. Fig. 1b is a picture of such an ATP-treated specimen. As can be seen in the figure, the bulk of the elongated fibrils of actomyosin have been changed into shortened, thickened irregularly shaped forms. They are of different sizes, as is to be expected from the wide distribution of particle size in the untreated specimen.

It was impossible to study the effect of varying KCl concentrations on actomyosin in the electron microscope. The changes in salt concentration which occur in the preparation of the sample make it impossible to know with any accuracy the effective KCl concentration.

Thus, we have obtained evidence from light-scattering observations which indicates that actomyosin dispersed in KCl solution has the configuration of a slightly coiled particle. Increasing the concentration of KCl does not affect this configuration. On the other hand, as shown by light-scattering data reinforced by electron micrographs, ATP increases the degree of coiling of actomyosin, an effect which is compatible with a fundamental role in muscular contraction for this nucleotide-protein interaction. Further studies on both the ATP and KCl effects are in progress.

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IN THE LABORATORY

Modification of the Kardos Shadow Experiment for Demonstrations of Color Mixing

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In an experiment first described by Kardos (*2*), a small disc is suspended in the center of a hole in a screen. The screen is illuminated from a strong source, but an object is interposed to cast a shadow on the disc, so that the edges of the shadow fall within the hole around the disc. With this arrangement, and using a light gray screen and disc, the latter appears to be black, or very dark gray, even though the observer can see the source of illumination and the shadow caster and, seated slightly to one side, can observe that the shape of the shadow falling on the wall to the rear of the screen is not of the same shape as the disc. The illusion is so compelling that even continued observations, alternately of the normal disc color without the shadow and of the disc in the shadow, do not serve in any way to decrease its strength. Descriptions of this and similar experiments may also be found in Brunswik (*1*) and Woodworth (*3*).

A different, but equally striking effect, can be produced by having the room in semidarkness and illuminating the disc alone with a brilliant source. In this case the disc appears to be a source of light itself, much as the moon seems to be. It was while investigating the effects of colored light on such a disc that the writer found the Kardos apparatus to be a most excellent device for demonstrating color mixing.

The screen used was of light gray cardboard, 35″ long and 23″ high. From the center a circular hole 8″ in diameter was cut. Within this hole a 4″ disc was suspended by means of thin black threads, crossed to suspend the disc steadily and fastened to the rear of disc and screen with cellulose tape. (In the Kardos experiment, the disc is of the same material as the screen, but for color mixing a white disc may be used if desired.) For illumination, two or more strong, narrow beams of light and suitable colored filters were used. With the screen about 4′ from the rear wall and the observers seated slightly to one side so that they can see where the edges of each light beam strike the wall, it is possible to examine the results of the mixture on the disc while being able to see an "analysis" of the colors used on the rear wall.

With this apparatus and good red and green filters, it is possible to produce a powerful, well-saturated yellow on the disc, quite unlike the dirty yellow usually achieved with a color wheel or the weak, streaky effect usually obtained with lights observed through milk glass. With blue and yellow filters or three sources—red, green, and blue, a bright white is easily achieved. Relative brightnesses can be regulated by polaroid filters, episcotisters, or distance of source, whichever may be convenient.

In addition to color mixing, a great variety of demonstrations of afterimages, simultaneous contrast, and induction of the complementary color in contrast shadows may be performed with this apparatus. For example,