TABLE I.—g VALUES OF FOUR FREE RADICALS

	g Value
Prochlorperazine	2.0036
Chlorpromazine	2.0034
Triflupromazine	2.0034
Perphenazine	2.0029

determined by comparison with diphenylpicryl hydrazyl, whose resonant position in the magnetic field is indicated in each spectrum by an arrow.

It is seen that the nature of the substituent has almost no perceptible effect on the appearance of the spectrum and has only a minor effect on the *g* value. These spectra appear to consist of a single absorption line, different from that of phenothiazine and its ring-substituted derivatives, all of which give a spectrum of four well-resolved lines. The latter spectra have been interpreted in terms of the hyperfine splitting by the nitrogen nucleus together with the proton on this position in acid solution. In each of the present spectra, the slight inflection point suggests that the spectrum is the summation of three equally weighted broad component lines arising from splitting by the nitrogen nucleus. The breadth of the lines in this degree appears to be due to the hyperfine splitting by the protons in the side rings and in the complex chain attached to the nitrogen. Other contributory factors to the line breadth are the nature of the solvent system and the magnetic dipole-dipole interaction from neighboring molecules. However, these factors must not play an important role in the present case, as the conditions were comparable with those under which four well-resolved lines were obtained with phenothiazine and other compounds.

Further discussion of these effects will appear in a subsequent publication in which a series of N-alkyl-substituted radicals are studied (2).

Further work is planned to ascertain the concentration of free radical under various conditions of solvent and acidity, and therefrom the semiquinone formation constants (dismutation constants) will be computed.

REFERENCES

(1) Tuck, L. D., and Schieser, D. W., *J. Phys. Chem.*, in press.
(2) Tuck, L. D., and Tozer, T. N., to be published.

Simplified Method for the Measurement of Actomyosin Contraction

By DUANE G. WENZEL and HARBANS LAL

ACTOMYOSIN, the principal contractile protein in muscle, exists in the form of contractile filaments. It is possible to extract these filaments, fragmented transversely but otherwise undamaged, from muscle fiber. The microscopic filaments may then be realigned into a three-dimensional network capable of sustaining tension. It was demonstrated some years ago that threads formed from this actomyosin responded to the addition of adenosine triphosphate (ATP) with spectacular shortening (1). These first threads, however, were composed of poorly oriented actomyosin filaments and did not develop any appreciable tension (2). Close packing of the filaments by treatment with glycerol, drying, and stretching produced threads capable of developing tension (3). It was later demonstrated that relatively strong fibers could be formed by lateral compression of surface-spread layers of actomyosin (4).

The actomyosin model is similar, in many respects, to its intact muscle counterpart. Histologically, of course, the original muscle fiber also possesses a sheath or sarcolemma. The response of the model to ATP is, for all practical purposes, the same as that of the muscle fiber. Because of the relative simplicity of this system and because a specific muscle component may be studied independently, the actomyosin threads or fibers have become popular models for the study of muscle pharmacology.

Received August 17, 1961, from the University of Kansas, School of Pharmacy, Lawrence.
Accepted for publication September 7, 1961.

A number of methods have been employed to measure the contractile response of actomyosin. A simple procedure introduced by Hayashi (5) and later modified by Robb, *et al.* (6), employs a vertical fiber supporting a glass weight. This fiber was prepared in a Langmuir trough and then transferred to a beaker containing the bath solution. The response of the system was determined by measuring the movement of the weight with a microscope having a calibrated eyepiece. This method has several disadvantages. First, the actomyosin fiber must be transferred from the trough to the contraction chamber and must further be extensively manipulated in order to be loaded. Second, the vertical position of the fiber can affect the response to ATP through variable buoyancy and the weight of the thread itself.

In an attempt to eliminate the above disadvantages, a new method for the preparation of the actomyosin fiber and a new measuring apparatus were introduced (7). In this method the surface film of actomyosin is not completely compressed to a thick fiber, but instead is formed into a wider band of a constant width and minimal thickness. This assures a uniform diffusion of ATP into the center of threads or fibers which are still thick enough to support a weight. It has been suggested that during the isotonic contraction of actomyosin, the "unplasticized" core of the fiber not reached by ATP may interfere with the shortening (8). This method utilizing the wide band of actomyosin also permits horizontal attachment of the band between a fixed support extending from the bottom of the

trough and an elongated arm of a 500-mg. capacity Roller-Smith torsion balance. Comparison of the results obtained using vertical fibers to those using horizontal bands indicates a definite reduction in the scatter of observation with the latter method (7). Loading of the band is accomplished by moving the balance arm to the desired number of milligrams. Contraction of the actomyosin band then initiates an electromechanical feed-back mechanism causing the trough to move in a direction equal to and opposite to that of the contraction. Movements of the trough are recorded with an oscillograph after suitable amplification.

The purpose of the present study was to determine the feasibility of measuring the contraction of horizontal actomyosin bands with a microscope having a calibrated eyepiece.

EXPERIMENTAL

The apparatus employed consisted of three major components. A Langmuir trough of methyl methacrylate polymer,[1] similar to that of Bing and co-workers, was employed (7). The inner dimensions were 48 × 12.5 × 1.75 cm. A separate chamber in which the band contracts was formed by the addition of an inner wall about half the height of the trough. This chamber had the dimensions of 12.5 × 3.8 × 0.9 cm. A series of holes, 3 mm. in diameter and 0.5 cm. apart, were bored along the longitudinal axis of this chamber. These served to fix a small plastic[1] block, 6 mm. in height, which was fitted with a peg to be plugged into one of the holes. The trough was mounted on a platform fixed to a small scissors-type auto jack which was used to raise the chamber into position beneath the balance arm. A Roller-Smith torsion balance with a 500-mg. capacity was fastened on a rack over the trough, as shown in Fig. 1. The weighing pan of the balance was replaced with an aluminum spatula which extended vertically over the contraction chamber. The flat blade of the spatula was bent at a right angle to the shaft in order to provide support for the actomyosin band. Measurement of the movement of the band was accomplished with a stereoscopic microscope as described by Robb and Mallov (6). The system employed thus combines the simplicity of the visual method for microscopically measuring movement of the actomyosin fiber with the improved horizontal loaded actomyosin band.

Actomyosin was extracted from fresh dog heart in the usual manner and a band was formed in the trough as previously reported (7). This band was attached to the block fixed in the base of the bath and to the movable balance arm. A load of 10 mg. was placed on the band by movement of the weighing spring of the torsion balance. The zero point of the calibrated microscope eyepiece was then focused on the edge of the movable arm. Ten milliliters of the chamber solution were removed and replaced with a concentrated solution of the sodium salt of ATP (sigma) to make a final concentration of 0.005 *M*. Measurements were recorded at 2, 4, 8, 16, and 32 minutes following addition of the ATP. Five bands prepared from freshly extracted actomyosin were tested on the same day. The per cent shortening from the original length was determined for each band. Initial band lengths

[1]Tradename, Plexiglas.

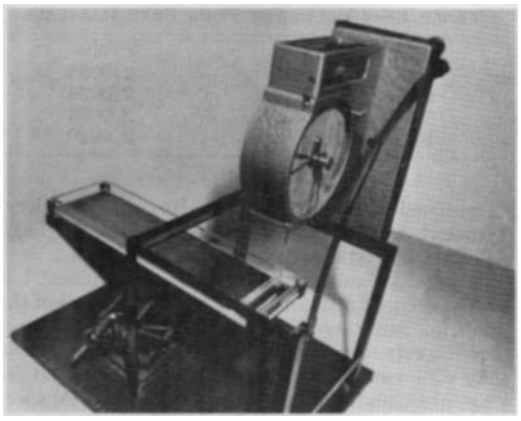

Fig. 1.—Apparatus for the measurement of actomyosin band contractions. Stereoscopic microscope used to perform actual measurements is not shown.

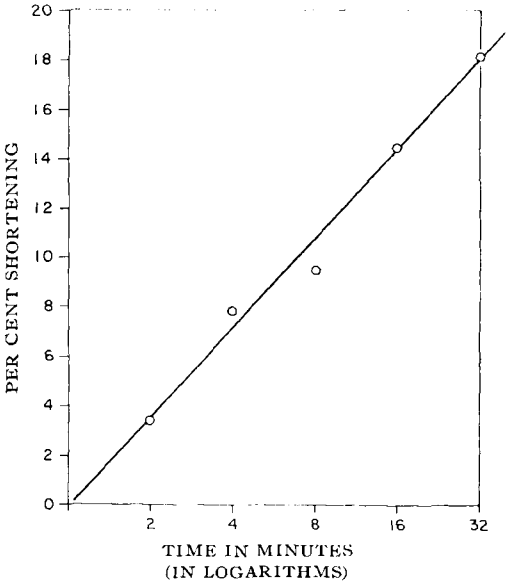

Fig. 2.—Mean per cent shortening of five actomyosin bands at several times.

for the five bands ranged between 20 and 25 mm. The mean per cent shortenings obtained with these five bands plotted against the logs of the times tested are shown in Fig. 2.

From the linearity of the response it is apparent that the method described can be employed to measure effectively the rate and extent of loaded, isotonic actomyosin contractions.

REFERENCES

(1) Szent-Gyorgi, A., *Studies Inst. Med. Chem. Univ. Szeged*, **1**, 17(1942).
(2) Buchthal, F., Deutch, A., Knaffeis, G. G., and Munch Peterson, A., *Acta Physiol. Scand.*, **13**, 167(1947).
(3) Portzehl, H., and Weber, H. H., *Z. Naturforsch.*, **5b**, 123(1950).
(4) Hayashi, T., *Federation Proc.*, **10**, 61(1951).
(5) Hayashi, T., *J. Gen. Physiol.*, **36**, 139(1952).
(6) Robb, J. S., Grubb, M., and Braunfelds, H., *Am. J. Physiol.*, **181**, 39(1955).
(7) Bing, R. J., and Dettli, L., *ibid.*, **187**, 145(1956).
(8) Weber, H. H., and Portzehl, H., *Ergeb. Physiol.*, **47**, 369(1952).