5. Oudin, J., *Methods in Med. Research,* 1952, v5, 335.
6. Halbert, S. P., Swick, L., Sonn, C., *J. Exp. Med.,* 1955, v101, 539.
7. Gordon, J. E., Janny, J. H., *J. Pediatrics,* 1941, v18, 587.
8. Lancefield, R. C., *J. Exp. Med.,* 1928, v49, 91.
9. Rantz, L. A., Randall, E., *Stanford Med. Bull,* 1955, v13, 290.
10. Fuller, A. T., *Brit. J. Exp. Path.,* 1938, v19, 130.

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Influence of Resting Tension on Contractility and Morphology of the Papillary Muscle.* (25240)

LESLIE P. MCCARTY AND FREDERICK E. SHIDEMAN
Dept. of Pharmacology and Toxicology and Cardiopulmonary Research Labs., University of Wisconsin, Madison

The papillary muscle preparation has become a popular tool in the field of cardiac research. A wide variety of factors, *e.g.*, resting tension, manner of recording, composition of bathing media, have been studied with respect to their effects on contractility or morphology. A review of the literature discloses only 4 publications(1-4) in which resting tension was stated as an absolute value. Bennett(5) made a detailed study of the effect of tension on physiological response but did not examine the muscles for any associated morphologic changes. Tanz *et al.*(6,7) reported alterations in morphology of papillary muscles exposed to drugs, but failed to indicate the resting tension to which their preparations were subjected. In a preliminary report(8) experiments were described in which a study was made of the relationships between initial resting tension, stability of initial contractile amplitude or tension, and morphology of working papillary muscles. The present report includes studies on nonworking as well as working muscles.

Methods. Cats were anesthetized with ether, the heart removed, and placed in warm, oxygenated, modified Tyrode's solution of the following composition: (meq/l) Na, 145.00; K, 5.34; Ca, 4.75; Mg, 1.20; Cl, 129.55; PO_4, 1.79; SO_4, 1.20; HCO_3, 25.00; glucose, 5.0. A thin papillary muscle, 1.0 mm or less in diameter, was selected from the right ventricle and ligatures were placed around it at its junction with the ventricular wall and the chorda tendineae. The muscle was then removed from the ventricle and placed in a muscle holder, care being taken not to apply tension to the muscle. The holder was immersed in a bath containing the previously described Tyrode's solution maintained at a temperature of 37.5°C and aerated by a rapid stream of 95% oxygen and 5% carbon dioxide entering through a sintered glass disk in the bottom of the bath. A supramaximal stimulus of 1 to 2 millisec in duration was provided as a square wave from an American Electronic Laboratories, Inc., Model 751, stimulator at the rate of 1/sec. In isotonic preparations, the upper ligature was attached to a light heart lever adjusted to give the desired resting tension and record on a smoked drum. For isometric studies, the ligature was attached to the pin of a RCA 5734 transducer tube in a bridge circuit similar to that described by Ranney(9). This arrangement allowed the transducer to be calibrated with weights and the resting tension to be adjusted to that desired. A total of 30 muscles were studied, the tension ranging from 1.6 to 4.0 g. Some muscles were merely subjected to static tension and not stimulated. The muscles were removed from the bath after 6½ hours and fixed in 10% formalin. After fixation the muscles were dehydrated and embedded in paraffin. Sections approximately 5 micra in thickness were cut and stained with hematoxylin and eosin.

Results. Muscles subjected to tensions of

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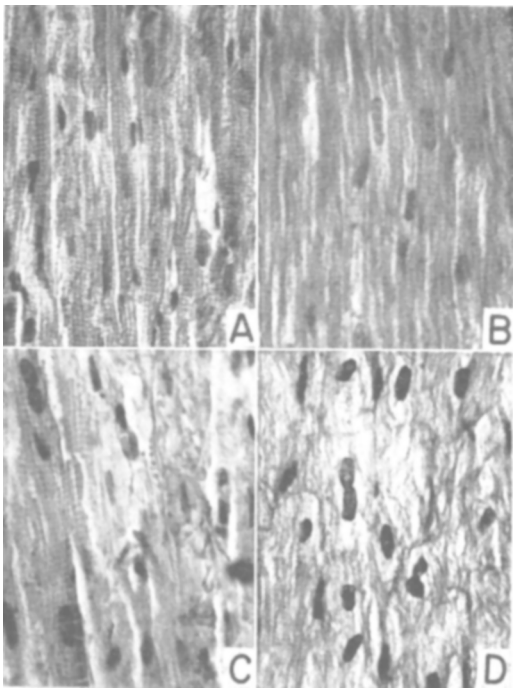

FIG. 1. Effect of loading on histologic appearance of papillary muscle of the cat. A. Fresh tissue, B. 1.6 g load, C. 3 g load, D. 4 g load. Loaded muscles were fixed following 6½ hr of isotonic contraction.

less than 3.0 g. (Fig. 1B) were morphologically similar to those (Fig. 1A) fixed immediately after removal from the animal. After being subjected to a tension of 3.0 g, muscles exhibited areas of early degeneration interspersed with areas of normal morphologic appearance (Fig. 1C). After application of 4.0 g. of tension, severe degeneration was evident (Fig. 1D). Warped and pycnotic nuclei and foamy cytoplasm were present and striations were absent. Work did not appear to be a significant factor in determining degree of morphologic change since muscles which were not stimulated to contract showed essentially the same morphologic alterations as working muscles subjected to an equal amount of tension. However, the degenerative changes noted in isotonic preparations appeared to be more pronounced than those observed in isometric preparations under the same conditions of tension and activity.

In isotonic preparations, contractile amplitude and degree of failure varied considerably, depending on tension applied. If the muscles were loaded with 1.6 g, contractile amplitude decreased slowly and in a fairly predictable manner. The results from several experiments are shown in Fig. 2. When tension was increased to 3.0 g, it was not possible to predict the course of the contractile amplitude. Some preparations with a resting load of 3.0 g behaved similarly to those loaded with 1.6 g while others failed rapidly, displaying a reduction of contractile amplitude of as much as 80% by the end of experiment. Lack of a uniform response was due, perhaps in part, to a variation in size of the individual muscles. Under a 4.0 g load, failure was rapid and complete within 2 hours.

Under isometric conditions papillary muscles displayed similar contractile characteristics with loads ranging from 1.6 to 4.0 g. Increasing the load from 1.6 to 4.0 g did not significantly affect rate or degree of failure. Fig. 2 depicts the average response for all muscles with the range of loads employed.

Discussion. The results of this investigation show that resting tension is important with respect to both contractile and morphologic characteristics of the papillary muscle preparation. Bennett(5) reported that muscles contracting isotonically with a load of 2.0 g exhibit a contractile response similar to that shown in Fig. 2 for muscles loaded with 1.6 g. Gold and Cattell(3) showed that after a short period, the contractile amplitude of the papillary muscle under 1.2 g of isometric tension stabilized and thereafter decreased very slowly

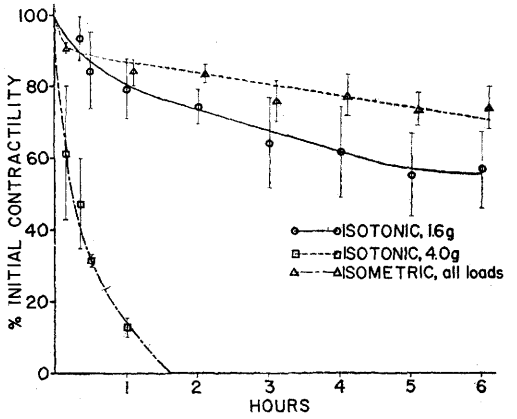

FIG. 2. Effect of loading on contractility of papillary muscle of the cat. Vertical lines represent stand. errors for each mean value. Each curve represents avg of results from 3 or more preparations.

over a period of 5 hours. Deterioration of contractility is much more rapid in a medium containing phosphate rather than bicarbonate as a buffer(4,10).

Our experiments indicate that isotonically contracting preparations of the papillary muscle are quite sensitive to the load applied and that the speed with which failure ensues is a function of this load. On the other hand, failure in isometric preparations does not appear to be correlated with tension. If one is interested in the effects of drugs, *e.g.*, the cardiac glycosides, on mechanically produced failure in the papillary muscle the type of preparation and degree of loading perhaps are not critical as long as these variables are maintained constant. However, if one wishes to examine the effects of drugs on a preparation which approximates the normal or non-failing heart, it appears that resting tension is an important factor and should be taken into consideration. These experiments also show that tension alone can result in morphologic changes, ranging from slight alterations to severe degeneration. The mechanism of this degeneration is not clear but such changes are not unique in as much as they are observed in tissue post mortem if fixation is not carried out rather promptly.

Summary. Papillary muscles of the cat subjected to various resting tensions exhibited morphologic changes, the severity of which could be correlated with degree of loading with loads of 3 g or greater. These changes were similar to those which occur post mortem in cardiac muscle and appeared whether the muscle was stimulated to contract or subjected to static tension. Morphologic alterations appeared in both isotonic and isometric preparations but appeared more severe in isotonic preparations. Rate and degree of failure of isotonically contracting muscles could be correlated with size of the resting load but such a correlation was not observed with muscles contracting under isometric conditions.

1. Bennett, D. R., Andersen, E. S., Andersen, M. V., Robertson, D. N., Chenoweth, M. B., *J. Pharmacol. Exp. Ther.*, 1958, v122, 489.

2. Hoffman, B. F., Bindler, E., Suckling, E. E., *Am. J. Physiol.*, 1957, v185, 95.

3. Gold, H., Cattell, M., *Arch intern. Med.*, 1940, v65, 263.

4. Faust, R. M., Saunder, P. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 351.

5. Bennett, D. R., *U. Mich., Dept. of Pharmacology Thesis*, 1958.

6. Tanz, R. D., *J. Pharmacol. & Exp. Therap.*, 1956, v116, 56.

7. Tanz, R. D., Whitehead, R. W., Weir, G. J., Jr. PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 258.

8. McCarty, L. P., Shideman, F. E., *J. Pharmacol. Exp. Ther.*, 1958, v122, 50A.

9. Ranney, R. E., *J. Appl. Physiol.*, 1954, v6, 513.

10. White, W. F., Salter, W. T., *J. Pharmacol. Exp. Ther.*, 1946, v88, 1.

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Thermal Sensitivity of Polioviruses Isolated during Oral Vaccine Field Trial: Comparison with Monkey Neurovirulence.* (25241)

ISAO YOSHIOKA,† JOHN T. RIORDAN, AND DOROTHY M. HORSTMANN
(Introduced by R. H. Green)
Section of Epidemiology and Preventive Medicine and Dept. of Pediatrics, Yale University School of Medicine, New Haven, Conn.

Recent observations by Dubes *et al.*(1,2), Lwoff and Lwoff(3), and Sabin and Lwoff(4), indicated that virulent strains of poliovirus grow readily at temperatures of 39° and 40°C, while multiplication of attenuated strains is greatly reduced at such relatively high temperatures. In the following experiments an effort was made to determine if this characteristic, here designated the thermal marker, T (for attenuated), I (for intermediate), and T^+ (for virulent), could be used for the fol-

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† Fellow of James Hudson Brown Memorial Fund, Yale Univ. School of Med.