Modified Method for Determining Myocardial Refractory Period. (30444)

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The isolated auricle technique of Dawes (1) is a useful method to study the effect of substances on the refractory period of cardiac muscle. Because of the uncertainty of the end point in Dawes' method, a modification has been developed which gives a more accurate end point utilizing the cat papillary muscle preparation. It is the purpose of this report to describe this modification and to show its advantage over the older method.

Methods. The use of the cat papillary muscle preparation in this laboratory has been previously described in detail (Tanz(2, 4); Tanz and Kerby(3)). Briefly, cats varying in weight from 2 to 3 kg were anesthetized with ether and sacrificed by cardiectomy. Two papillary muscles weighing between 2 to 6 mg were removed from the right ventricle. The muscles were individually dissected leaving the chorda tendinae and some valvular tissue attached to one end and a tab of myocardium at the other end. The papillary muscle was thus isolated without causing gross tissue injury.

The muscles were mounted on individual muscle holders and placed in 2 identical chambers containing a modified Krebs-Ringer's bicarbonate solution. A mixture of 95% oxygen and 5% CO_2 was bubbled through the solution by means of a sintered glass filter attached to the base of the muscle chamber. The perfusate was maintained at a temperature of 37.5°C. Stimulation frequency was 1/sec with suprathreshold voltages at a duration of 1 msec, using a Grass (model S-4) stimulator. Isometric contractile force recordings were obtained by employing Sanborn transducers (fta-30). The optimal tension for each muscle was individually determined, which, upon stimulation, yielded the maximum force of contraction. All drugs were prepared just prior to use, and whenever possible the perfusate was used as the solvent.

Only one muscle was used for any given drug. The second muscle remained unstimulated under slight tension in a separate bath for approximately 2½ to 3 hours. (There was no difference in the results obtained between the 2 muscles.) The tension is not critical in these experiments as it is with experiments designed to measure the direct action of a substance on contractile force.

The muscle was driven by repetitive stimuli of 1/sec for a 30-minute equilibration period prior to taking MFRP readings (MFRP refers to the minimal frequency refractory period). At the end of the 30-minute equilibration period the MFRP was determined by increasing the frequency of stimulation. This was accomplished by connecting the frequency control of the stimulator to a geared driving motor, which, when activated by a remote control switch, slowly and uniformly increased the stimulus frequency. As the frequency of electrical stimulation was gradually increased the muscle contracted to each stimulus up to a point at which it began to show pulsus alternans. With some muscles pulsus alternans was more evident than with others. A further minute increase in the frequency of stimulation results in the muscle adopting a 2:1 rhythm. The preparation at this point was no longer able to respond to every stimulus applied because the interval between stimuli was now less than the refractory period. The frequency at which the muscle adopted this 2:1 rhythm is referred to as the MFRP. Readings were taken at 5-minute intervals. Between readings the frequency was returned to 1/sec. Quinidine SO_4 was used as the standard for comparison.

Controls were established by taking 3 consecutive MFRP readings that were within a frequency range of 0.3 per second. This usually was attained within 3 to 6 readings (15-30 minutes). The control remained relatively stable over a long period of time provided nothing was added to the bath.

After establishing the control, the test sub-

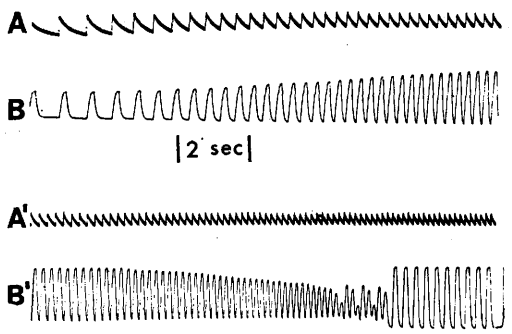

FIG. 1. Minimal frequency refractory period (MFRP) in cat papillary muscle preparation. A, A^1 = stimulation frequency; B, B^1 = contractile response.

stance was added to the bath at various concentrations and the test compound remained in the bath solution for a duration of at least 30 minutes. If a substance prolongs the refractory period (*e.g.*, quinidine), the MFRP readings decrease.

After measuring the change in MFRP produced by a substance, the muscle may be washed with fresh perfusate and a different concentration of the same drug may be tested after re-establishing controls. However, not more than two concentrations of the same drug were used on each muscle.

Results. Fig. 1 illustrates a recording of a typical papillary muscle response tracing as the frequency of stimulation was increased. The conversion from 1:1 to pulsus alternans to 2:1 rhythm is clear-cut and serves as an excellent biological end point. The mean of 30 individual papillary muscle MFRP controls was 8.50 (S.E. $\pm$ 0.21). The results obtained following administration of various agents in different concentrations (which increase myocardial refractory period), are plotted in Fig. 2.

Nethalide is a beta-adrenergic blocking substance first described by Black and Stephenson(5). Imipramine is an anti-depressant which has been reported to possess direct antiarrhythmic activity by Fekete and Borsy(6). Tripelennamine, an antihistaminic, has also been reported to possess antiarrhythmic properties by McCawley *et al*(7) and others. The results illustrated in Fig. 2 show that on a molecular weight basis tripelennamine>imipramine>quinidine>nethalide insofar as prolonging the refractory period of cat papillary muscles.

Discussion. This report describes a simplified procedure for indirectly determining the refractory period of cat papillary muscles. The end point (conversion to 2:1 rhythm) can be exactly titrated. It is felt that this is a more reliable end point than the initial drop of a single auricular beat, as described by Dawes(1). Furthermore, the MFRP bares a linear relationship to the logarithm of the concentration of the substances thus far tested.

It is not the purpose of this report to discuss the mechanism of actions of individual substances which prolong the refractory period. Rather, the results obtained with the 4 substances known to possess antiarrhythmic properties, merely serve to illustrate the efficacy of the method.

Prolongation of the refractory period *per se,* is insufficient to justify classification of a substance as a useful antiarrhythmic agent. It is also necessary to consider its effects on conduction velocity, excitability and automaticity. Because several mechanisms may be involved in the initiation and maintenance of abnormal rhythms, a complete profile of effects upon all these properties of the heart should be evaluated.

Conclusions. A modification of Dawes' method is described for the bio-assay of potential antifibrillatory substances, based on

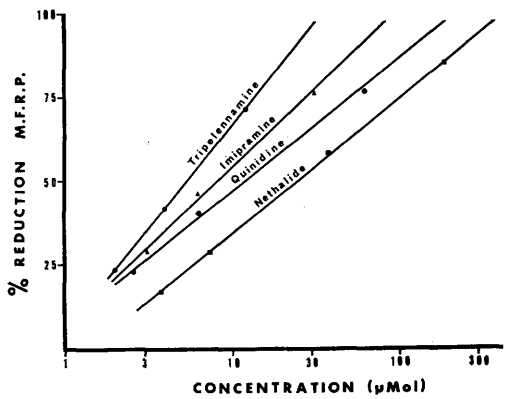

FIG. 2. Effect of various agents on minimal frequency refractory period at different concentrations. Number of individual experiments for each substance given in parentheses: Quinidine (18); Nethalide (20); Imipramine (15) and Tripelennamine (16).

their ability to prolong the refractory period of the isolated cat papillary muscle preparation.

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Comparison of Muscle Tissue from Normal and Dystrophic Chick at Different Stages of Development.* (30445)

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Explants of pectoralis muscle from hatched dystrophic chick show a greater deterioration of histological structure and a more pronounced spreading of cells than similar explants from normal chick muscle of the same age(1). These results, as well as biochemical analyses by Kaplan and associates(2), Cosmos(3) and Perkoff(4), indicate that in dystrophic chick muscle maturation is delayed. In the experiments reported here, it was intended to define further the beginning of this apparent developmental retardation in dystrophic muscle. Since the DNA level has been found to be higher in muscle of newly hatched dystrophic chick than in that of normal animals(5), determinations of the DNA content were carried out in the normal and dystrophic muscle at different stages of development. As a second index of the state of maturation, the rates of amino acid incorporation into muscle proteins were measured *in vitro* using muscle preparations immediately after dissection of the tissue or after explantation of muscle for several days.

Materials and methods. The origin of the normal and genetically dystrophic chick muscle tissue and the explantation technique were described previously(1). Only pectoralis muscle was used in the present tissue culture experiments. An explantation period of 5 days was chosen because only small changes occur in the measured incorporation on further *in vitro* maintenance up to 11 days. For the measurement of amino acid incorporation about 7 to 10 mg of a mince or of a single bundle of fibers (strap) were incubated in a Dubnoff shaker for 2 hours in 10 ml beakers with 1 ml of a tissue culture medium(6) from which glycine-C^{12} was omitted, adding 1 μC of glycine-1-C^{14} (Spec. Act. 5.49 mc/mM) to each sample. The freshly dissected material was suspended in the incubation medium. The tissue incubated with glycine-1-C^{14} after explantation remained attached to the coverslips on which it was explanted(1). The explants were washed before adding glycine-1-C^{14} by 2 ten-minute incubations in a Dubnoff shaker with 2 ml aliquots of the glycine-free medium in order to remove glycine present in serum. After incubation, soluble material was removed by 2 washings with cold 5% trichloracetic acid and one washing with hot alcohol ether (1:1).

The DNA was extracted with hot perchloric acid and its quantity determined according to the method of Burton(7). The residue was collected in 3 ml centrifuge tubes and 0.2 ml each of HCl and acetic acid were added. The centrifuge tubes were capped and kept at 95°. After 5 hours the acid mixture was driven off with jets of air while maintaining the tubes at about 110°C. The now easily soluble dry residue was taken up in water, plated on aluminum planchets, and

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