Equilibrium cannot be assumed to be attained in either normal or pathologic states unless consecutive blood samples show little or no variation either in volumes of distribution or concentration of D_2O. It is the normal variation, rather than edema formation following burns, which necessitates a longer time interval for equilibration.

Summary. 1. The average time to equilibrium following intravenous injection of D_2O in 16 normal adult mongrel dogs was 129.4 minutes. Equilibrium was established in thermally injured dog in 148.1 minutes. There were no statistically significant differences between animals before and after thermal injury. 2. Equilibrium should not be assumed to be complete at 2 hours in either normal or pathologic states. Consecutive blood samples should be taken over a longer period of time, preferably up to 5 hours. A point of equilibrium should be selected when variation is within 5% of final volume of distribution at 5 hours. 3. Because of wide variation in time to equilibrium in both normal and pathologic states, any conclusions regarding changes in "efficiency of total circulation" should be studied carefully and interpreted cautiously.

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Effects of Rhythm and Rate Changes on Inotropic Action of Ouabain.* (22844)

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These studies were performed to determine the relationship of changes in rhythm and rate to the inotropic action of digitalis glycosides. In the experiments on rhythm, the relationship of the inotropic action of ouabain to postextrasystolic potentiation was studied. This latter phenomenon was first described by Woodworth(1) in the dog heart. He found that when an extra contraction was interpolated in a series of regular contractions, the regular contraction immediately following the extra contraction was augmented. Cattell and Gold(2,3) subsequently redirected attention to this phenomenon and clarified many of its aspects. Recently several other studies of this response have been reported(4-8) in both atrial and ventricular musculature of different species. The term postextrasystolic potentiation was first used by Hoffman *et al.*(8). The occurrence of postextrasystolic potentiation indicates that there are sources of muscular power which can be demonstrated by alterations in the pattern of stimulation, and it was of interest to determine whether the inotropic action of a glycoside involved these sources of energy. In the second part of the experiment, the re-

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lationship of the glycoside action to the rate of stimulation at regular frequencies was investigated. There is a significant latent period after administration of a glycoside before an increase in force is seen, and a longer period before maximum effect is produced, although there are differences between individual glycosides. The duration of the latent period may be related, either to the number of contractions which take place before the glycoside effect is seen, or to the amount of time which elapses.

Method. The cat papillary muscle technic of Cattell and Gold(9) was used, with a phosphate-buffered Ringer's solution containing glucose, 1.8 g/L and, bubbled continuously with oxygen. In the first part of the study, the 14 preparations were stimulated at a base rate of 30 per minute, and an extra beat interpolated at intervals of 250, 300, 350, 400, 450 and 500 milliseconds, using the technics described earlier(5,7). The effects of rest periods of 10 seconds, with and without the interpolated beat were recorded and measured. These measurements were repeated after failure of the contractile force, and upon recovery after ouabain, 1 to 10 million. In analysing the data, all changes were compared to the initial control values to make possible a ready differentiation between changes due to a modification of the postextrasystolic potentiation and changes produced by the failure or recovery alone. In the second part of the experiment, 2 muscles were removed from each heart, and set up in separate chambers. One muscle of a pair was stimulated at a rate of 12 per minute and the other at a rate of 60 per minute. After contractile force had diminished to about half of the initial value, ouabain was added to each preparation to produce a concentration of 1:5 million. Measurements of contractile force were made at 5 minute intervals thereafter. In these experiments, 28 muscles were used.

Results. In the studies on postextrasystolic potentiation, the degree of potentiation fell gradually during the period of failure, and continued to fall despite the recovery of the muscle under the influence of ouabain

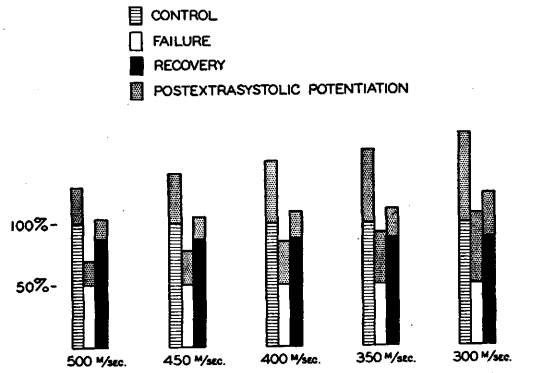

FIG. 1. Relationship of postextrasystolic potentiation to failure, and ouabain-induced recovery.

(Fig. 1). Similar results were noted at all the interpolation intervals, although, as reported earlier(5,7) the shorter the interval, the greater the potentiation. As an example, the results obtained at an interpolation interval of 350 m/sec may be considered. During the control period, postextrasystolic potentiation was 58% (S.E. 10.3, $N = 8$) of control force. After the failure of the regular contractions to 50% of the control (S.E. 3.7, $N = 8$) the postextrasystolic potentiation was 42% (S.E. 7.7, $N = 8$). After ouabain, there was an average recovery of regular contractile force to 87% of the control (S.E. 8.8, $N = 8$). At the peak of this recovery, however, the postextrasystolic potentiation had fallen to 24% (S.E. 6.4, $N = 8$). At any individual interpolation interval, the difference between postextrasystolic potentiation during the control interval and at the time of failure, and the difference between the potentiation at failure and after recovery are not statistically significant. However, since similar results were seen at all 5 interpolation intervals (Fig. 1), it is likely that these changes are meaningful. The major findings, which are apparent from Fig. 1 are that the rate of failure of the regular contractions differs from the rate of failure of the postextrasystolic potentiation, and that ouabain does not improve postextrasystolic potentiation.

In the experiments on differing rates, the speed of failure at a stimulation rate of 60 per minute was found to be only slightly greater than at 12 per minute. At the slower

stimulation rate, it took an average of 215 minutes for the muscles to fail to 39.5% of maximum force (S.E. 4.0, N = 14). At the more rapid stimulation rate, it took an average of 172 minutes to fail to 39.5% of maximum force (S.E. 2.5, N = 14). The muscles were observed continually, and it was clear that the time course of failure was similar in the two series. No significant differences were seen. After the addition of ouabain, the contractile force improved at a slightly faster rate in the muscles stimulated at 12 times per minute. At 60 minutes after ouabain they had improved 23.1% (S.E. 4.4, N = 13) while the more rapidly stimulated muscles improved 20% (S.E. 3.5, N = 14). At 90 minutes, the improvement was 33.7% (S.E. 6.2, N = 12) for the slowly stimulated preparations and 27.0% (S.E. 6.0, N = 13) for the rapidly stimulated preparations. At 120 minutes, the improvement was 44.5% (S.E. 7.7, N = 13) for the slowly stimulated preparations, and 32.2% (S.E. 7.0, N = 13) for the rapidly stimulated preparations. The average maximum improvement attained at the stimulation rate of 12 per minute was 56.5% (S.E. 5.7, N = 14) in an average of 141 minutes. At the faster rate, the maximum improvement was 42.9% (S.E. 6.1, N = 14), reached in an average of 138 minutes.

Discussion. Since ouabain produced significant improvement in the force of the regular contractions after failure, but did not produce any improvement in postextrasystolic potentiation, it may be that in the latter phenomenon, additional contractile mechanisms are involved.

Rosin and Farah(4) and Katzung and Farah(6) have postulated the existence of a "potentiating substance" to account for postextrasystolic potentiation. Our experiments neither confirm nor contradict their hypothesis, but do suggest that if such a substance is present, its production gradually falls off in the isolated tissue, and is unaffected by ouabain.

The rate experiments suggest that the latent period in glycoside action on mammalian heart muscle is related to elapsed time, rather than to the number of contractions. The muscles stimulated at a rate of 12 per min. improved 23.1% in 60 minutes, or 720 beats, while the muscles stimulated at a rate of 60 per min. improved 20.0% in 60 minutes or 3,600 beats. If the latent period were related to number of contractions, the improvement after 3,600 would be significantly greater than after 720 beats. Since this is not the case, it is probable that the elapsed time is the important factor in the latency of the glycosides. Although there are differences between the two series they are slight, and not at all in the same proportion as the rate difference, which was 5 to 1. The small differences observed in these experiments probably result from slightly more rapid failure at rapid than at slow stimulation rates. Wilbrandt *et al.*(10) studying the frog heart arrived at different conclusions. They found that under the conditions of their experiments, the time required for glycoside effect depended on the number of contractions. In their studies, they used a calcium-poor solution. The difference between their findings and those reported here may result from the different solutions used, or from a difference in species. It has been pointed out several times(7,11,12) that there are marked differences between the responses to drugs of frog and mammalian hearts.

Summary. In failing cardiac muscle, postextrasystolic potentiation decreases at a slower rate than response in a series at a regular frequency. Postextrasystolic contraction is not improved by ouabain. The latent period of ouabain action is related to elapsed time, not the total number of contractions.

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Agglutination of Blood Cells in *Limulus polyphemus.** (22845)

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The horseshoe or king crab, *Limulus*, is as distinctive among the arthropods in its pattern of hemostasis as it is in other aspects(1). It appears to have neither fibrinogen nor the power of autotomy, relying on the toughness of its integument and a very large number of agglutinating cells to prevent blood loss. Not only does it possess many more of these cells than other arthropods(2), but the formation of the solid mass seems to differ qualitatively. Two cellular phases, agglutination and "gelation," have been distinguished(3), the latter of which is distinct from the process of gelation by polymerization of plasma fibrinogen which occurs in crustacea.

It has been difficult to study this phenomenon since procedures which stabilize vertebrate or even crustacean bloods—cooling, removal of calcium ions, addition of heparin, provision of hydrophobic surfaces, etc.—are largely ineffective in *Limulus*(4). By working quickly in the cold, both *Limulus* and crustacean cells can be separated before agglutination occurs but under these circumstances it is not possible to resuspend the centrifuged mass of cells. Agglutination is effectively prevented by formaldehyde fixation, a procedure which is used in counting blood cells or observing their structure(2). This drastic reagent would ordinarily be considered incompatible with any functional aspects but it was found to be effective at relatively low concentrations (0.05-0.10%). Higher concentrations (0.25-0.50%) resulted in some clumping and also precipitated some plasma protein. The cells separated from formaldehyde treated plasma could be readily resuspended in formaldehyde-free sea water or other salt solutions and maintained indefinitely without clumping.

However, these stable suspensions quickly agglutinated upon the addition of small quantities of *Limulus* plasma (0.01-0.05 volumes). In suspensions of 10-20 x 10^6 cells/cc this clumping began within 2 minutes and was complete in 10-15 minutes with large clumps of more than a hundred cells usually

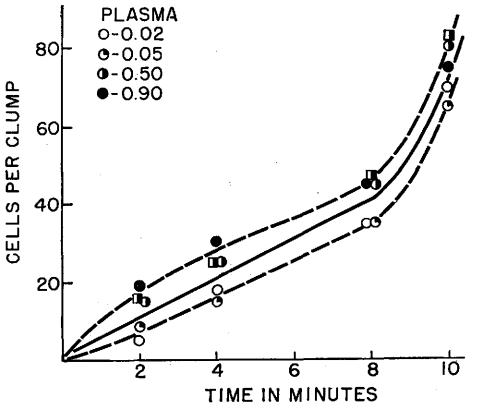

FIG. 1. The course of clumping of stabilized *Limulus* cells suspended in artificial sea water with various concentrations of added plasma; cell concentration, 14.8 × 10^6/ml; plasma concentrations as indicated (referred to whole plasma); temperature 22°; circles, fresh cells; squares, cells dried from the frozen state and resuspended. Ordinate represents avg No. of cells per clump.

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