lected during this time which assayed 220 γ/ml, thus accounting for about 30% of the polymyxin administered. Details of these and other studies will be presented at a later time.

Summary. A filter paper disc method for the determination of polymyxin in blood and urine is described. Using ¼-inch diameter discs, 0.25 μg of polymyxin per ml of blood may be determined in triplicate with samples of blood as small as 0.05 ml.

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Effect of Digitoxin on Contractility of Ventricular Muscle Strips.

A. M. WEDD AND H. A. BLAIR.

From the Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

In a summary of his extensive studies on digitalis action, Gold[1] has concluded from the reactions of the papillary muscle of the cat heart that increased systolic force caused by direct action on heart muscle may be taken as the primary mechanism by which digitalis relieves heart failure. Variability of digitalis effect on muscle strips is indicated in numerous reports. Cattell and Gold[2] in carrying out earlier experiments selected as a "therapeutic" dose concentrations which produced augmentation of beat in approximately 50% of trials. Salter and White[3] who used the same procedure found that fresh muscles failed to respond to relatively high concentrations of ouabain, and in a later paper[4] they described alteration of the Ringer solution in addition to prolonged stimulation, so that the muscle might be more readily "fatigued." In their study of digoxin on strips of turtle ventricle, Wedd, Blair, and Dwyer[5] rarely observed pronounced or sustained increase in amplitude of contraction and argued that the constant shortening of systole, electrical and mechanical, might be a factor in reducing contraction height. It is apparent from such studies that augmentation of contractility by digitalis is not due to primary stimulation of that function, such as occurs with epinephrine or veratrine. Further observations indicate that to be enhanced contractility must have first been impaired and experiments to be cited suggest that such effect will occur only after some alteration of muscle metabolism. The present report deals with the effect of digitoxin on strips of turtle ventricle when contractility has been diminished by various physical and chemical means.

Method. Details of the method used in this laboratory to study drug effects on heart muscle have been described.[5] Ventricular strips suspended in a bath are rhythmically stimulated by condenser discharges and contraction recorded on a drum. The Ringer solution was phosphate buffered and had a pH of 7.2; no carbonate or sugar was present. The usual driving rate was 13.5 per minute. Tension was adjusted to record maximum contraction; it was altered if necessary during the control period, but not after the drug was added. It made no difference whether digitoxin was added to the original bath or a fresh one containing the drug was substituted. A concentration of 1:2 million was found generally satisfactory and was used in all experiments.* For each group in which con-

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* The digitoxin used in these experiments was Digitalline Nativelle, generously supplied by the Varick Pharmacal Co.

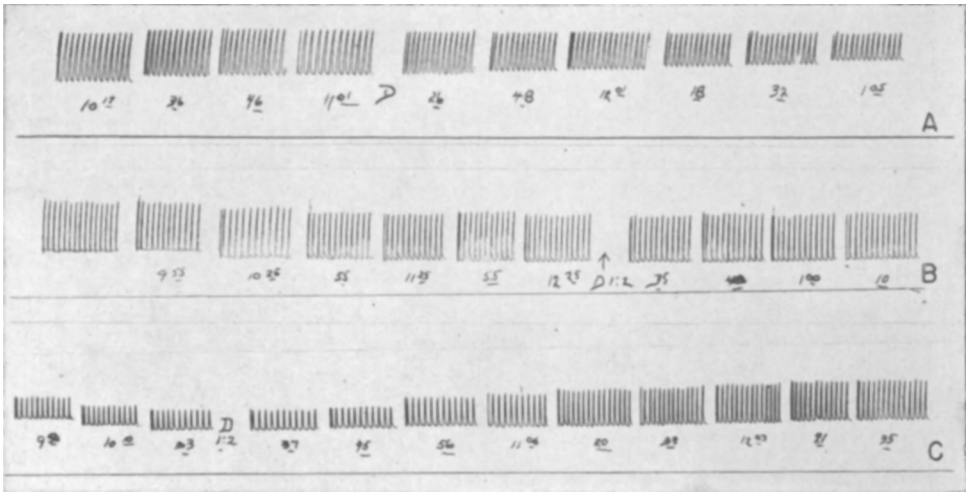

FIG. 1.
Illustrating the types of response of untreated muscle strips to digitoxin; concentration 1:2 mil in each instance. Lower figures indicate time.

tractility was artificially depressed at least 4 experiments were performed. When a positive inotropic response was obtained increase in contraction height ranged from 15 to 50%. The maximal effect restored beat size to its original level but never raised it beyond that. Relationship of electrical to mechanical systole which was also studied in these experiments will not be considered in detail, but it may be stated here that most of the agents used to depress contractility simultaneously shortened electrical systole, measured by the Q-T interval of the electrogram.

Untreated strips. Three types of response to digitoxin have been observed for untreated ventricular strips tested at varying times up to 96 hours after removal from the animal. A concentration of 1:3 million usually produced no effect on the beat. The standard test concentration, 1:2 million, in some strips caused a decrease in beat and considerable diastolic shortening, illustrated in Fig. 1, A. While there is not necessarily any relation between muscle contraction and recovery, it is possible that under certain conditions shortening the duration of systole may itself lead to a decrease in beat size and then such beat decrease is not an expression of toxicity. Shortening of diastolic length is perhaps more often seen in the turtle than in mammalian strips; the presence of varying amounts of smooth muscle in the turtle ventricle may be a factor. For other strips the standard concentration was without appreciable effect, Fig. 1, B. Certain turtles presented hearts whose muscle appeared to be in poor condition and the maximal beat was feeble for the size of the strip. It was in such strips or in muscles that showed progressive decline during the control period that contraction was significantly augmented by digitoxin, Fig. 1, C. There were also instances in which a poor beat was unchanged or continued to decline after the drug. Transient improvement of beat followed by decline and shortening such as Gold described and related to toxic dosage was not seen with the concentration used in these experiments.

Fatigue. It was found difficult to produce a decline in beat in fresh strips by stimulation alone. However, this was accomplished in a few by driving at double the usual rate. Digitoxin then caused further decline and some shortening or there was no appreciable change, results similar to those already described for strips driven at the slow rate. But in older strips a slight beat increase or at least arrest of decline followed digitoxin. In one experiment the strip which had been out of the animal for 72 hours showed a decrease of only 1.5 mm after driving for 3 hours; the following day, after 96 hours, there

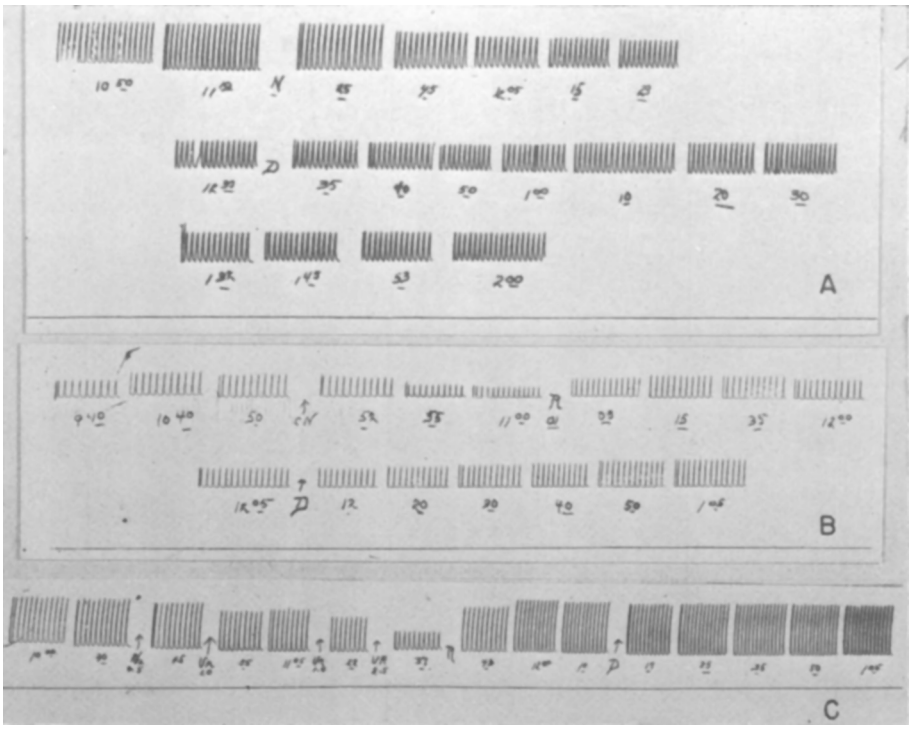

FIG. 2.
A: Depression of beat by nitrogen and improvement following digitoxin added at D. B: Depression by sodium cyanide; recovery in fresh Ringer solution, R; further increase after digitoxin, D. C: Depression by urethane; complete recovery in fresh Ringer solution, R; no further change after digitoxin.

was a considerable decrease in beat, followed by a slight increase after digitoxin. In another strip tested after 72 hours the beat decreased 40% in 45 minutes; there was a gradual increase after digitoxin so that at the end of 2 hours the beat size was up 25%. These experiments suggest that decline in beat due to rapid stimulation is not necessarily related to metabolic changes which will be favorably influenced by digitoxin, and that for a positive inotropic response "fatigue" alone is insufficient.

Cold. Cooling the bath containing the muscle from room temperature, 21-22°C, to 9° reduced the size of the beat by half and digitoxin caused no increase. Further lowering of temperature resulted in alternation and then alternate response.

Increased Acidity. By addition of HCl the pH of the bath was decreased to 6.5 and to 6.0. Such changes caused a decrease in contraction height which digitoxin could in part offset. The drug prevented further decline or increased the size of the beat, though it never restored it to the original height.

Other Ionic Changes. Ringer solution which contained half the normal amount of calcium caused little or no change in beat. In a calcium free solution the beat decreased greatly and that effect was not counteracted by digitoxin. The addition of a very small amount of calcium promptly restored the beat. In a bath of isotonic sodium chloride, alone or with phosphate buffer, the beat was greatly reduced and not improved by digitoxin. Excess potassium caused the beat to decline, and again digitoxin did not increase it. Increasing potassium to 7 times the normal also caused alternation and alternate response. In one experiment the bath was first changed to a chloride-phosphate solution and the beat fell to half; it further declined after digitoxin and then still further on adding the normal quantity of potassium; with the addition of the

normal amount of calcium the beat promptly increased, almost to its original level.

Nitrogen. The bubbling of pure nitrogen through the bath instead of air caused a prompt and progressive decline in beat. In one instance only was digitoxin able to offset the depressant effect while that gas was still present, Fig. 2, A. If after an hour in nitrogen the bath was again aerated recovery of beat occurred. Often recovery was incomplete, and then digitoxin regularly caused further increase in beat size.

Chemical Depressants. The action of sodium cyanide, sodium azide, hydroxylamine and urethane was similar. Each in suitable concentration depressed contractility. It was necessary to permit recovery in fresh Ringer solution; otherwise the addition of digitoxin caused further decline and shortening. When recovery was partial or not sustained digitoxin increased the beat; the response to digitoxin after recovery from depression by sodium cyanide is illustrated in Fig. 2, B. Urethane was a potent depressant and following its removal recovery was often complete; then digitoxin caused no further increase in the beat, Fig. 2, C. Concentrations of iodoacetate and of sodium fluoride sufficient to cause an appreciable decrease in beat likewise tended to produce much diastolic shortening. There was little tendency to recover from the shortening and the change to fresh Ringer solution was often followed by additional shortening; that reaction was further increased by the addition of digitoxin. None of the foregoing substances, which were tried for their property of interfering with enzyme systems, gave reactions which were sufficiently specific to indicate which phase of metabolic activity might have been disturbed. Even cyanide is thought to have an effect on contractility independent of its interference with oxygen utilization. The results of the group in general were similar to those obtained with nitrogen gas. The effect of quinidine was to depress contraction in strips driven at the rapid rate; digitoxin did not improve the beat in the presence of the former drug; in fresh Ringer solution recovery was incomplete and then digitoxin increased the beat.

Comparison of certain glycosides. The effect upon mechanical response and on electrical systole was studied for ouabain, digitoxin, digoxin and lanatoside C. While these substances produced practically equivalent shortening of the Q-T interval of the electrogram,[6] their influence on the mechanical response appeared unequal. Because of its relatively greater viability and perhaps variability of response the turtle ventricle is probably not so suitable for the study of comparative contractility effects as mammalian heart. However, certain results are in agreement with reported findings for these drugs. Of the 4 substances ouabain was most potent. A concentration of 1:10 mil usually produced no effect on beat size; in untreated strips, 1:7.5 mil and greater concentrations caused decline in beat and shortening. In strips that had been depressed by chemicals, 1:5 mil increased the beat without significant shortening; higher concentrations first increased and then depressed contraction. Digitoxin and digoxin in concentration of 1:2 mil appeared approximately equivalent to 1:5 mil ouabain, and the equivalent concentration of lanatoside C was 1:3 mil. While these experiments cannot be said to be conclusive the indicated concentration ratios are 1:1.65:2.5 for ouabain, lanatoside C, and digitoxin or digoxin. When compared on the basis of molar concentration the corresponding ratios are 1:1:1.9. Although ouabain appeared of considerably greater potency, in a series of trials on chemically depressed muscles effective concentrations did not act more promptly or cause greater increases in contraction height than digitoxin.

Summary. Study of the action of cardiac glycosides on strips of turtle ventricle indicates that the positive inotropic effect produced by those drugs does not result from primary stimulation of contractility but that it will occur only after there has been some suitable change in muscle metabolism. Beat depression caused by rapid stimulation, cold, calcium want, or excess potassium was not improved by digitoxin. Reduced contraction height due

[6] Wedd, A. M., and Blair, H. A., *J. Pharm. Exp. Therap.*, 1947, **90**, 211.

to increased acidity was partly offset by digitoxin. When contractility was depressed by various chemicals digitoxin usually increased the beat provided spontaneous recovery had been incomplete.

Ouabain produced effects comparable to digitoxin with approximately half the concentration. It did not act more promptly or cause relatively greater increases in beat.

16466 P

pH Stability, Response to Antibiotics and Factors Influencing Egg-Culture of Mumps Virus.*

M. L. WEIL, DOROTHY BEARD, AND J. W. BEARD.

From the Department of Surgery and the Department of Pediatrics, Duke University School of Medicine, Durham, N.C.

Like the agents of influenza the mumps virus grows in embryonated eggs,[1-4] reaching high concentrations in the chorio-allantoic fluid. Studies have been undertaken for the purification and examination of the agent by physical and chemical methods.

Preliminary to the development of purification procedures, studies have been made on (a) the conditions influencing the concentration of the virus in the chorio-allantoic fluid; (b) the effects of antibiotics on the virus and on (c) the pH stability of the virus. The results of this work are briefly given here, and the purification and characters of the virus are described in an accompanying report.[5]

Material and Methods. The virus was the Enders chick embryo adapted strain,[2] obtained through Dr. Lalla Iverson. The methods of inoculating the virus into the chorio-allantoic sac and harvesting the chorio-allantoic fluid were similar to those used in studies of influenza virus.[6] Virus infectivity was titrated in 7-day-old embryos. Hemagglutinative capacity[2,7] was measured by the method of Hirst[8] as used with the influenza virus.[9]

To avoid bacterial growth the use of antibiotics seemed desirable. The effects on the virus in chorio-allantoic fluid of penicillin (500 units per cc) and streptomycin (100 μg per cc) separately, and in combination in the same respective concentrations, were studied in *in vitro* experiments. The mixtures were stored at 4°C and titrated after 20 minutes, 7, 14 and 28 days.

The pH stability of virus infectivity was examined in a composite buffer[10] of borate, phosphate and phthalate (0.04 M total concentration) containing penicillin, 500 units per cc and streptomycin, 100 μg per cc. To 16 cc aliquots of the buffer adjusted to pH values at intervals of 1 pH unit over the range of 2.0 to 11.05, 4 cc of virus-containing chorio-allantoic fluid were added. The mixtures were

* This work was aided by a grant to Duke University from Lederle Laboratories, Inc., Pearl River, N.Y.

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