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Reversibility of Digitalis Action.*

GEORGE H. PAFF AND BEN B. JOHNSON. (Introduced by S. R. M. Reynolds.)

From the Department of Anatomy, Long Island College of Medicine, Brooklyn, N. Y.

In Cushny's monograph on digitalis one reads the following regarding drugs of the digitalis group, "The action of these drugs is therefore reversible only in the earlier stages, and that slowly and with difficulty; later the process becomes irreversible, and the poison can be displaced neither during life nor by the use of chemical solvents after death."¹ Again, Straub,² speaking of the excised adult heart treated with digitalis, states "the final effect, after it has once set in, cannot be removed by any amount of washing. The reaction of the digitalis glucoside with the heart muscle is not reversible, and the heart is killed." This opinion is widespread.

In contrast to the idea of irreversibility of digitalis action, Kingisepp³ demonstrated the ability of electrically driven isolated frog ventricles to respond to digitalis wash-out. Arrested ventricles were made to return to their original amplitude of response as measured "by the isochoric response." In the present work further evidence as obtained from the study of the embryonic heart is given. After complete stoppage it has been possible to revive the hearts and return them to entire normality. Before presenting our data we wish to express a warm sense of appreciation for Doctor Torald Sollmann's kindly aid.

Method. Three drugs were used: ouabain in 1:100,000 dilution; tincture of digitalis in 1:100 dilution made from Reference Digitalis Powder according to U.S.P. XI; and digitoxin, as much as would dissolve in Tyrode solution in an hour's time. In each case the diluent was glucose-free Tyrode solution at pH 7.4.

The ventricle and conus of chick embryos (hereafter called ventricle), incubated for 48 hours, were excised and placed in a drop of Tyrode solution in a well slide. A coverslip was set over the well

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¹ Cushny, A. R., *The Action and Uses in Medicine of Digitalis and Its Allies*, p. 80, Longmans, Green and Co., New York, 1925.

² Straub, W., *Stanford University Publications, Medical Sciences*, 1929, **3**, 45.

³ Kingisepp, G., *J. Pharm. and Exp. Therap.*, 1935, **55**, 377.

to prevent excessive evaporation and the preparation was placed in a warm chamber at 38°C. After the ventricle had reached incubator temperature the rate of beat was taken. Next, Tyrode solution around the ventricle was pipetted off and replaced by a drop of the desired glucoside diluted with Tyrode solution. After being completely stopped by the glucoside, the ventricle was removed with a platinum loop to a petri dish of Tyrode solution; then to a second dish; and finally removed to a well slide containing Tyrode solution, covered, and returned to the warm chamber. This constituted what we shall call a "wash". In some cases this procedure was repeated (digitalis, wash, digitalis, wash, etc.). Rates of beat, time required for stoppage in the glucoside, time of appearance of first beats after washing was started, time required for the return of normal beating, and other pertinent data were recorded.

Observations. For purposes of comparison 10 experiments with ouabain in 1:100,000 dilution in Tyrode solution were conducted. Straub⁴ demonstrated the reversibility of this glucoside in adult frog hearts. Paff and Johnson⁵ noted its reversibility in the embryonic heart. Table I summarizes the data. In the strength used the drug stops the ventricles completely in about 3.5 min (the median). The mean is really 4.6 min for the 10 experiments but discrepancies in preparations 8 and 10 raise the average stop time considerably. Here, as in all later experiments, this is partly due to the fact that the first rate of beat was taken with the ventricle in Tyrode solution and this was replaced by the glucoside. It was not

TABLE I.
Ventricles in 1:100,000 Ouabain.

No.	Rate per min	Min in drug	First beat after wash	Recovery	
				Time	Rate/min
1	103	2 + 3*	9 min	12 min	138
2	141	2 + 3	1	53	155
3	111	4 + 1	5	17	157
4	160	1 + 4	13	19	149
5	133	3 + 2	10	18	137
6	61	4 + 1	8	12	88 (irregular)
7	72	4 + 1	7	15	119
8	126	9 + 3	4	14	152
9	116	3 + 2	6	8	145
10	92	14 + 1	7	33	92
Mean:	112/min	4.6 + 2.1 min	7 min	20 min	133/min

*First number is time of complete stop. Second number is time left in drug after stop.

⁴ Straub, W., *Biochem. Z.*, 1910, **28**, 392.

⁵ Paff, G. H., and Johnson, J. R., *Am. J. Physiol.*, 1938, **122**, 753.

desirable to remove all the Tyrode solution for fear that the ventricle would be damaged. Even small amounts can produce sufficient variation in concentration to alter appreciably time of appearance of stoppage. The ventricles were left in the ouabain 2.1 min (mean) after complete stoppage. The time when first beats occurred after washing was started averaged 7 min. These first beats were not always regular in rate. In the table the time after washing when the beats did become regular and deep is indicated under the heading "Recovery". The rate of 6 and 7 after recovery are qualified by "(irregular)". Both of these showed periods of slight acceleration followed by periods of slower rate of beat. They are not significant since controls sometimes show the same thing.

The next group of experiments involved the use of tincture of digitalis in 1:100 dilution. Tincture was made from Reference Digitalis Powder (1 cc contained 1 U.S.P. XI unit). In Table II results are summarized. The average time of ventricular stoppage was 3.4 min. After stop the preparation was left in the diluted tincture 2.7 min on the average. The first beats after wash was started appeared in 15 min (average). A difference between tincture and ouabain was noticed here however. The first ouabain beats were usually of a nature involving the entire ventricle. In the tincture the first beats were usually slight "jiggles" near the end of the ventricle from which the atrium had been cut. These gradually increased in depth and involved more and more of the ventricle until the entire preparation was beating. It became obvious that the tincture was more difficult to remove than ouabain since irregularities usually persisted so long as to make a second wash advisable. The mean recovery time was 46 min and the average rate of beat was 134 per min. Ventricles 4 and 5 showed

TABLE II.
Ventricles in 1:100 Tincture Digitalis (U.S.P. XI).

No.	Rate per min	Min in drug	First beat after wash	Recovery	
				Time	Rate/min
1	123	2 + 3	22 min	36 min	164
2	114	2 + 3	16	55	158
3	89	4 + 5	26	50	132
4	118	2 + 3	12	25	130 (irregular)
5	69	2 + 3	27	55	130
6	73	4 + 1	2	70	179
7	121	5 + 2	19	22	165
8	80	3 + 2	4	50	122
9	129	3 + 2	5	50	107
10	82	7 + 3	16	45	56
Mean:	100/min	3.4 + 2.7 min	15 min	46 min	134/min

irregularities, but here again they were of a nature comparable to those seen in the controls.

The results with a single stoppage by tincture encouraged the attempt at repeated stoppage and washing with the same ventricles. The mere mechanical manipulation alone involved in this process offers a real hazard to continued activity of the embryonic ventricle. Proof that it can be successfully carried out is seen, however, in the following experiment:

Ventricle, rate, 90/min. Stopped in tincture in 2 min 40 sec. Washed 17 min. Beat again 94/min (irregular). Stop in tincture again in 45 sec. After 48 min wash the rate was 110/min (irregular). Stopped the third time in tincture in 1 min 15 sec. Washed 20 min, and beat became regular at 160/min. Stopped a fourth time in tincture in 1 min 3 sec. After 50 min of washing beat returned and was irregular. No rate was taken at this time. Stopped a fifth time in tincture in 55 sec. Wash 25 min and irregular beat returned. Again no rate taken. A sixth stop occurred in 47 min, and after recovery the rate was irregular. Mean rate was 22/min. Four other ventricles gave comparable results.

In the third group of experiments digitoxin was used. The same technic applied successfully with tincture and ouabain failed in 4 out of 5 attempts with digitoxin even 150 min after the single wash had begun. It was necessary to change the technic. This consisted in repeated washes at intervals of 15 min until it was apparent that complete recovery would occur. Results are summarized in Table III.

Discussion and Further Observations. The tabulated results indicate that both digitoxin and tincture as well as ouabain can be washed out of the embryonic ventricles. Of the 3, digitoxin is the most difficult to remove. Tincture is next and, as was expected, ouabain was removed with ease. The short time in which the ventricles remained in the glucosides raises the question whether or not the drug had sufficient time to act. As proof that the time is ample it was noted that mechanical manipulation of the ventricles invariably

TABLE III.
Ventricles in Digitoxin.

No.	Rate per min	Min in drug	No. of washes (15-min intervals)	First beat after wash	Recovery	
					Time	Rate/min
1	104	5 + 2	8	64 min	142 min	111
2	99	9 + 2	6	80	85	133
3	90	13 + 2	7	82	92	115
4	116	2 + 3	6	56	119	90
Mean:	102/min	7.2 + 2.2 min	6.7	71	110	112/min

failed to elicit a response. More conclusive evidence is the fact that controls stopped by digitoxin, for example, remained absolutely quiescent to single make and break induction shocks. Furthermore the ventricles, when removed from the glucosides, continued to be affected as evidenced by the fact that they passed into extreme systole. This is especially true with tincture and digitoxin. The picture at this stage was that of a dead ventricle. Accompanying this reaction it was noted that the mesothelium at the surface of the heart seemed to become swollen beyond the amount explainable by the systole. The cells appeared rounded. We believe it also significant that the ventricles became sticky, so much so that they often adhered strongly to the glass or to the platinum loop used in transfer. When this last occurred, small portions of the heart were sometimes torn away, suggesting that the process seriously involved the muscle as well as the mesothelium.

These stages suggest marked degenerative changes. However, despite this suggestion, they are only apparent. As proof of this, ventricles stopped in tincture and in digitoxin were placed in chicken blood plasma in Carrell flasks along with controls. The results were conclusively in favor of removal. Not only did all the ventricles, both controls and glucoside-treated hearts (after recovery), beat for an observation period of 3 days but the beats, both as to rate and depth, were normal. Unless one knew which ventricles had been treated with the glucosides, it would be impossible to distinguish drugged hearts from controls. This is equally true of both physiological activity and structural integrity.

In the embryonic heart additional evidence is necessary before a reasonable hypothesis regarding the probable nature of digitalis action can be stated. The production of stickiness and rounded appearance of cells suggests an exchange of material between cell and environment accompanied by marked surface phenomena. It can be postulated, however, that no firm chemical union occurs between the digitalis and the embryonic heart muscle since the time necessary for recovery is so short.

Summary and Conclusions. 1. In embryonic ventricles stopped by ouabain, tincture of digitalis, and digitoxin the effects of the drugs can be removed by washing with Tyrode solution. 2. Of the 3 glucosides, the action of digitoxin is the most difficult of removal. Tincture, in turn, is more difficult to remove than ouabain. 3. With tincture, the process of stop, wash-out, stop, wash-out, etc., can be repeated at least 6 times in the same ventricle. 4. Digitoxin-stopped ventricles recover in blood plasma and are indistinguishable as to function and structure from controls in the same medium.