Influence of Digitoxin on Labile and Inorganic Phosphates, Lactate, Glycogen, Potassium and Sodium in Dog Ventricle

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This communication deals with the effects produced by digitoxin on the metabolism of the dog's ventricles as indicated by changes in labile and inorganic phosphates, lactate, glycogen, potassium and sodium content of the myocardium immediately frozen after excision. The results suggest increased turnover of high energy phosphate and glycogen by nontoxic doses.

The metabolic action of cardiac glycosides on the intact mammalian heart has not been clearly demonstrated. For example, Wollenberger in his review cites several instances where increased, decreased or unchanged glycogen levels have been noted following glycoside administration. In hearts given sufficient amounts of glycoside to cause arrhythmias, Wollenberger was unable to detect any change in high energy phosphate content which could be attributed to a direct metabolic effect of the drug. Little change in potassium content follows digitalis administration according to Calhoun and Harrison, although a significant increase has been reported by Boyer and Poindexter.

In the present investigation, the concentrations of several chemical constituents were determined in dog hearts digitalized for 2 and 4 days.

Methods

Thirty-five adult mongrel dogs that survived digitoxin treatment may be divided into two groups, depending on whether they were given nontoxic or toxic doses. In each group, single or daily injections were given. And, with each dosage plan, animals were sacrificed 2 and 4 days after the initial injection. Dosage regimens were generally varied in successive experiments and, at suitable intervals, hearts from 13 normal, nondigitalized animals were examined.

The amount of digitoxin administered was based on the finding that 80 per cent of the minimal lethal dose (MLD, 0.575 mg./Kg.) decreased coronary flow over a period of several days whereas 30 per cent of the MLD had no apparent influence. For our single nontoxic injection, 20 per cent of the MLD was chosen and for the daily nontoxic dose, 12.5 per cent of the MLD was administered per day. Eighty per cent of the MLD was selected for the single toxic dose and 50 per cent of the MLD was given per day for the daily toxic injection.

Digitoxin powder* was dissolved in 95 per cent alcohol and diluted with water. The injections were given very slowly to the unanesthetized animal by way of the saphenous vein. At the appropriate time, the dogs were anesthetized with sodium pentobarbital (Nembutal) given intraperitoneally. Artificial respiration was applied, the chest opened quickly, the heart was excised rapidly and dropped into a beaker containing dry ice-ether freezing mixture.

The analytic procedures were identical to those used previously. Inorganic phosphate (IP) was precipitated with calcium from a neutralized trichloroacetic acid extract, phosphocreatine (PC) was hydrolyzed with molybdic acid at room temperature for 30 min., and the labile phosphate of adenosine polyphosphate (APP) was hydrolyzed by heating at 100 C. in 1 N HCl for 8 min. Inorganic phosphate in each instance was determined by the method of Fiske and SubbaRow and the concentrations of PC and APP were determined by difference. Lactic acid was determined on aliquots of the unneutralized trichloroacetic acid extract by a slight modification of the method of Barker and Summerson. Glycogen was separated from an alkaline digest, purified, and the acid-hydrolyzed glucose was estimated according to the method of Nelson. Potassium and sodium were analyzed in solutions diluted from a nitric acid digest with a Perkin Elmer model 52 flame photometer, using lithium as an internal standard.

Results

No animals were lost as a result of the nontoxic injections. In the toxic group, 9 out of 19 animals died within 4 days after a single injec-

* Generously supplied by Abbott Laboratories, North Chicago, Ill.
tion of 80 per cent of the MLD. A similar mortality, 4 out of 9 animals, was seen following 2 daily toxic injections by which time 100 per cent of the MLD had been given. In the group given daily toxic injections for 4 days, 12 out of 13 animals died. As a result, no data are available from this group. Dogs that survived the toxic injections were extremely nauseated although 4 days after a single toxic injection, the condition of the animals was much improved over that of the 2 day group. In contrast, animals given nontoxic doses were not visibly different from the nondigitalized animals.

The data have been arranged primarily in the order of increasing cumulative doses in figures 1–3.

Fig. 1. Concentrations of phosphate compounds following digitoxin administration. Each bar, mean of 5 digitalized hearts and mean of 13 normal hearts. Open and hatched bars, left and right ventricles, respectively. Standard errors of the mean, at the top of each bar. At bottom, S and D refer to single and daily injections; 2 and 4, indicate days after the initial injection. Total dose expressed as the fraction of M.L.D. Statistically significant differences indicated by dots above bars. In the left ventricle, the p value is less than 0.05 for IP in the toxic, D-2 group; all other dots represent p values of 0.01 or less. In the right ventricle, the p value is less than 0.01 for lactate in the toxic, S-4 group; all other circles represent p values of 0.05 or less.

Fig. 2. Concentrations of lactate and glycogen following digitoxin administration. Symbols as in figure 1. In left ventricle, p value is less than 0.05 for lactate in nontoxic, D-4 group; all other dots represent p values of 0.01 or less. In the right ventricle, the p value is less than 0.01 for glycogen in the toxic, S-4 group; all other circles represent p values of 0.05 or less.

Fig. 3. Concentrations of potassium and sodium following digitoxin administration. Symbols, same as in figure 1. Numbers immediately below the bar graphs, sums of potassium and sodium. The p value is less than 0.01 for left ventricular K of the nontoxic, S-4 group and for right ventricular Na in the toxic, S-2 group; all other dots represent p values of 0.05 or less.
Phosphate Compounds. The most prominent change following digitoxin administration was the decrease in PC concentration which was observed in all cases (fig. 1). Low levels were found in left ventricles 4 days following single nontoxic injections, demonstrating that the drug action is of considerable duration. In several animals given injections of alcohol solution without digitoxin, significant change from the normal was not observed in PC, APP and IP concentrations. Toxic doses did not appear to promote greater change than that seen with nontoxic doses; 4 days after single injections and with 2 daily injections, the PC level was significantly higher ($p < 0.05$) than in the corresponding groups given nontoxic doses where comparable schedules were followed. This suggests that some other change may have taken place at higher drug levels. The values in the right ventricle appear to be higher in many cases ($p < 0.01$ for the differences in the means of nontoxic groups, S-4 and D-4) indicating that the right ventricle may be less sensitive to digitoxin.

Wollenberger has observed a small but significant increase in PC content of dog ventricles when digoxin or ouabain was administered continuously over a period of a few hours until arrhythmias appeared. The change in PC was attributed to the associated decrease in heart rate since experimentally induced bradycardias in the heart-lung preparation resulted in similar findings. His results may represent an early effect of toxic doses as defined in the present study since the nontoxic doses we have employed do not seriously affect heart rate. In one series of 5 animals in the present study, hearts were examined 1 hour after single nontoxic doses of digitoxin were administered and no change in PC, APP or in IP was noted. The reason that no change in PC content was observed in rat hearts by Kimura following 3 days of digitalization is not clear to us.

In the present experiments, there was no significant change in APP content noted except in a few instances when single or large injections of the drug increased the APP level. This indicates that the rate of formation of high energy phosphate did not fall far behind its utilization rate in all of these hearts and that higher than normal APP levels may occur under conditions of strong activation. As might be expected under conditions where high energy phosphate content decreases, IP tended to increase in most groups although only a few values attained statistical significance. Some of the changes may have been obscured by the decrease in the sum of the three measured phosphate fractions with time, the animals in the 4 day groups all showing a lower total than the 2 day groups by approximately 5 mg. per cent phosphorus.

Lactate. Associated with the phosphate changes were increases in muscle lactate following the administration of nontoxic doses (fig. 2). The highest lactate accumulation occurred in left ventricles examined 4 days after single nontoxic injections. In contrast, 4 days after a single toxic injection or with daily toxic doses for 2 days, muscle lactate values were not significantly different from the normal. These observations generally compare with the PC results as does the finding that the right ventricle in most cases is affected to a smaller extent than the left.

Lactate content has been reported to be unchanged or decreased in dog hearts examined a few days after therapeutic doses of digitoxin. In rabbits, lactate was unchanged or increased within 2 hours following intravenous injection. It may be noted that the control lactate values in both of these studies were much higher than those in our experiments. Lactate has been reported to be unchanged after 14 daily injections.

Glycogen. Glycogen tends to decrease with lower doses of digitoxin as may be expected from the lactate findings (fig. 2). A curious result was the failure for significant change to be noted in hearts given daily nontoxic injections for 4 days whereas a marked lowering was seen after 2 days. In other words, the larger cumulative dose may have affected myocardial metabolism in some other manner. With toxic doses, the results were even more variable. In hearts examined 2 days following a single toxic dose, the glycogen content not only failed to show a decrease but was markedly increased. In animals given daily toxic doses for 2 days, glycogen content also failed to decline. Four
days after a single toxic dose, a decrease was observed which may have been due to partial recovery from the influence of large doses.

In connection with our present results, the studies of Bomskov and Kaulla appear to be particularly relevant. In rat hearts examined 36 hours after single doses of various glycosides, decreased glycogen levels were found. However, in a dose-response study, considerably less change was induced by large doses of Digilanid than by small doses. It has also been noted previously that digitoxin may increase glycogen levels under certain circumstances. Following an injection of 3.4 mg./Kg. of digitoxin, an increase to 140 per cent of the normal was observed in 12 hours in rat hearts. In dogs, Tscherkess observed an increase in left ventricular glycogen 2 days after a single dose of digitoxin (0.14 to 0.2 mg./Kg.) was given as well as under other conditions where even smaller dosages were administered. These results, in general, offer support to our observations that glycogen may vary in different directions depending on dosage and duration of drug exposure.

Potassium. Four days following a single nontoxic injection of digitoxin, K content in the left ventricle was significantly greater than the normal (fig. 3). This result is similar to that seen with phosphocreatine and lactate where the greatest change in all groups of animals studied occurred 4 days after a single nontoxic dose. On the other hand, daily injections for 4 days resulted in a significant decrease in K content. In the data of Calhoun and Harrison, K values may be seen to be either unchanged or increased after 2 and 4 daily doses of Digifoline whereas decrease in K was more generally observed in hearts digitalized over 30 days with comparatively small maintenance doses. Conn observed virtually no change in K after daily administration of digitoxin at a small dosage, 0.2 to 0.4 mg./dog/day for 10 to 14 days. It appears that with small daily doses of glycoside, the duration of the digitalization seems to be an important factor affecting K content. Our normal values are lower than those reported by others for which we have no satisfactory explanation. It may be pointed out, however, that both increase and decrease in K content also occurred from higher preinjection levels than ours in the experiments of Calhoun and Harrison.

With toxic doses, K content in the left ventricle was raised generally to about the same extent as with nontoxic injections following comparable dosage schedules. Boyer and Poindexter found increased K levels in cat ventricles 2 to 5 days after rather large doses of Digifoline (0.5 cat unit/3 Kg. cat/day) were administered. The decreased levels observed in dog ventricles given daily doses over a period of 1 to 3 weeks until toxic manifestations or death occurred may have been due to the longer exposure period. In contrast to left ventricle, the right ventricle in the present study appeared to respond differently to similar doses of digitoxin. Single doses appeared to be relatively ineffective while multiple injections caused significant increases.

Sodium. Significant change in tissue Na was not observed with nontoxic doses although the pattern of change in Na content in the left ventricle with the various dosage regimens appears to parallel the pattern seen with K content changes (fig. 3). With toxic doses, Na content was decreased except in the group examined 4 days following a single injection. A partial recovery from the influence of large doses may be responsible for this exceptional result. In cat hearts exposed to rather large doses of Digifoline, Boyer and Poindexter found no significant change in Na.

DISCUSSION

Digitoxin in nontoxic amounts significantly altered the metabolism of the normal dog myocardium. The nontoxic doses employed generally caused no nausea so that it is likely that heart rate and blood pressure were also not affected significantly. The present results may, therefore, be considered as metabolic effects of the cardiac glycoside uncomplicated by concomitant cardiovascular change.

Decrease in phosphocreatine content along with an unchanging adenosine polyphosphate concentration indicates either a relatively greater utilization or a relative decrease in formation of high energy phosphate (HEP). Assuming that digitoxin does not uncouple ox-
dative phosphorylation, the associated increase in glycogenolysis suggests that formation of HEP, if anything, increases rather than decreases in rate. The finding that ouabain increases the rate of liberation of radioactive CO₂ from C¹⁴-labeled lactate in dog ventricle slices supports the view that the observed increase in lactate content is associated with increased energy production rather than merely representing accumulation of lactate. Thus, it would appear that nontoxic amounts of digitoxin cause an increase in both formation and utilization of HEP. A simple working hypothesis which may be proposed is that utilization of HEP is initially increased which, by increasing inorganic phosphate within the cell, stimulates glycogen breakdown and oxidative phosphorylation.

Potassium content in the left ventricle was increased by single nontoxic doses of digitoxin which is also indicative of a cellular effect of the drug. In contrast, 4 daily nontoxic doses caused a decrease in K content. A tentative explanation for these findings may be based on the observations that cardiac glycosides are said to inhibit K entry in many tissues. Moreover, in short term experiments on human hearts, the early effect of Cedilanid appears to be primarily on extracardiac tissues as judged by the increased serum K levels which occur when no significant alteration in myocardial K balance is noted. Thus, it seems possible that small doses of digitoxin may increase K content in the myocardium as an indirect consequence of K loss in extracardiac tissues. With continued daily injections, depression of K entry may occur in heart muscle as well. It is pertinent to note that in isolated hearts of the dog heart-lung preparation, an apparent decrease in K content was seen following therapeutic doses of digitalis.

In general, toxic doses appeared to produce smaller changes than nontoxic ones. Several factors might account for this: reduction in coronary flow, marked cardiac slowing, and changes in extracellular fluid.

**Summary**

Nontoxic doses of digitoxin lower phosphocreatine and raise lactate levels significantly in dog left ventricle. A tendency for glycogen to decrease was noted whereas adenosine polyphosphate and inorganic phosphate showed no marked change. These results suggest that the rate of turnover of high energy phosphate and of glycogen may be increased by nontoxic doses of cardiac glycoside.

Small doses of digitoxin increase whereas 4 daily nontoxic doses lower K content. These results may be due to a differential inhibition of K entry across cell membranes of extracardiac and cardiac tissues.

With toxic doses, nonspecific changes in cardiodynamic events or in fluid volume may be partly responsible for modifying the metabolic action of digitoxin.

**Acknowledgment**

Major credit for this contribution belongs to the late Dr. Arthur G. Mulder, who initiated the project and who unfortunately passed away before the investigation was completed. The authors are grateful to Dr. M. B. Visscher for reading the manuscript.

**Summario in Interlingua**

Doses nontoxic de digitoxina resulta in grados significative de reduction del nivello de phosphocreatina e de augmentation del nivello de lactato in le ventriculo sinistre del can. Esseva notate un tendentia decrecitori in le nivello de glycogeno, durante que nulle marcate alteracion occurreva in le nivello de polyphosphato de adenosina e de phosphato inorganic. Iste resultatos supporta le conclusion que le metabolismo de phosphato a alte energia e de glycogeno es possibilemente acce- lerate per doses nontoxic de glycosidos cardiac.

Parve doses de digitoxina augmenta le contento de kalium durante que 4 doses non- toxic per die reduce lo. Iste resultatos es possibilemente explicate per le presentia de un inhibition differential del passage de kalium a transverso le membranas cellular in tessutos extracardiac e cardiac.

Quando doses toxic es usate, alterationes nonspecific del cardiodynamica o del volumine de fluido es forsan partialmente responsabile pro le modification del efecto metabolic de digitoxina.

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Circ Res. 1957;5:504-509
doi: 10.1161/01.RES.5.5.504
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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