The Effect of Digoxin on the Intermediary Metabolism of the Heart as Measured by Glucose-C$^{14}$ Utilization in the Intact Dog

By Gerald Alan Kien, M.S., and Theodore R. Sherrod, Ph.D., M.D.

Administration of a therapeutic dose level of digoxin in the intact dog resulted in an augmentation of the glucose utilization of the myocardium accompanied by a greatly increased rate of glycolytic and oxidative activity. The contribution of glucose to the total metabolism of the heart was increased, and the utilization of noncarbohydrate substrates was decreased. These metabolic changes occurred in the absence of changes in the dynamic functions of the heart, indicating that the metabolic alterations were due to a primary effect of the drug rather than an effect secondary to an altered state of cardiac activity.

Although great advances have been made in the basic biochemical and physiological mechanisms of cardiac contractility there still remains a general lack of agreement concerning the mechanism of action of the cardiac glycosides. A further knowledge of the mechanism of action of digitalis in returning the failing heart to a state of compensation would facilitate an understanding of the metabolism of the normal myocardium and the pathogenesis of cardiac failure.

Studies of the changes in the substrate utilization of the heart in congestive failure and after treatment with a cardiac glycoside have indicated a lack of significant alteration in the metabolic activity of the myocardium. There have been no direct studies of the intermediary metabolism of the heart to substantiate these findings. Based on the utilization of C$^{14}$ labeled substrates by dog ventricle strips, Wollenberger suggested an action of ouabain on metabolic processes.

The following experiments were specifically designed to correlate the cardiac hemodynamic action of digoxin with possible alterations in the dynamics of the intermediary metabolic pathways involved in the myocardial utilization of glucose-C$^{14}$ in the normal dog.

Methods

Cardiovascular Effects of Digoxin

The cardiovascular effects of 0.065 mg./Kg. digoxin, administered rapidly as a single intravenous dose, were determined in young male dogs weighing 10 to 16 Kg. The animals were anesthetized by the intravenous administration of pentobarbital sodium (25 to 35 mg./Kg.) to the point of loss of the lid reflex but presence of a corneal reflex. The trachea was cannulated and the animal was allowed to breathe room air which was supplemented with oxygen via a tracheal catheter in order to insure an arterial blood oxygen content of not less than 19 volumes per cent. The carotid artery was cannulated, and a polyethylene catheter was placed in the heart for measurement of blood pressure by means of a Statham transducer. Venous pressure was similarly measured by means of a catheter inserted via the jugular vein and positioned at the bifurcation of the inferior and superior vena cavae. Left ventricular pressure was obtained via a fine polyethylene catheter inserted into the left ventricular chamber by way of the carotid artery and nora. Cardiac output, coronary blood flow (in the circumflex branch of the left coronary artery), cardiac work, cardiac arterio-venous oxygen difference, and cardiac effi-

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The efficiency were measured according to methods previously described.5,6

The above measurements, in addition to the electrocardiogram, were recorded simultaneously by means of a multichannel recorder during the control period and at five minute intervals for 30 min. following the administration of the cardiac glycoside. In addition, the coronary venous, mixed venous, and arterial blood were analyzed for carbon dioxide7 and the total and cardiac respiratory quotients were calculated. The cardiac Tension-Time-Index (T.T.I.) was calculated according to the methods of Sarnoff et al.,8 and, in order to describe more fully ventricular function, the cardiac internal efficiency was calculated. All statistical analyses were made on the basis of a paired analysis of the difference between the control and experimental value for each animal.

Metabolic Effects of Digoxin

Young male dogs, between 1 and 2 years of age, were selected for the metabolic study. The dogs were maintained on a standard ration and observed periodically for a 1 month period prior to the experiment. The weights of the animals ranged between 7 and 12 Kg. The animals were anesthetized (in a postabsorptive state) as described above. Half of the animals received an intravenous dose of digoxin (0.065 mg./Kg.), administered via a jugular vein and a serial electrocardiogram was taken for one-half hour. The animals then received 20 /uC. of glucose-C14,UL in saline, injected intravenously. The glucose had a specific activity of 0,106 /uC./mg. (Nuclear Chicago). In the digoxin group, the isotope was injected one-half hour after the cardiac glycoside. At the time intervals of 1, 3, 5, 8 or 11 min. after injection of the isotope, in the respective experiments, the hearts of the animals were quickly removed. The chest wall was left intact until 5 seconds before removal of the heart. At a specified time interval after administration of the isotopic substrate, and anterolateral incision was completed in the fourth intercostal space and the heart rapidly excised and within 10 seconds submerged in liquid nitrogen.

The frozen organ was broken into small pieces within a plastic bag, thus permitting gross separation of the fat and pericardium from the myocardial tissue. Cold (5 C.) 1 N perchloric acid (2 nil. /Gm. heart tissue) was added to the heart tissue in a Waring blender. The tissue was allowed to remain in the sealed blender jar for one-half hour during which time the myocardium was homogenized intermittently for a total duration of 3 min.

The collection of carbon dioxide was accomplished by continuously passing a stream of carbon dioxide-free air through a side arm in the blender jar over the myocardial tissue during the homogenization and trapping the collected carbon dioxide in a series of 4 gas washing towers, each filled with 50 ml. of N sodium hydroxide. The sodium carbonate thus formed was precipitated as insoluble barium carbonate.9 The precipitate was placed in the shallow well of a stainless steel planchette10 and its radioactivity measured in a windowless gas-flow Tracorlab Autoscaler at infinite thickness.

The acid supernate was neutralized with potassium hydroxide and the precipitated potassium perchlorate removed by centrifugation at 5 C. The supernatent solution containing the metabolic intermediates was brought to pH 1.5 with hydrochloric acid and washed on a Dowex-50 (hydrogen form), 4 per cent cross linked, 200 to 400 mesh anion exchange resin column (1 X 20 cm.).11 The amino acids were washed from this column as a single peak with N ammonium hydroxide. For the separation of amino acids this fraction was adjusted to pH 11.0 and placed on a Dowex-1 (acetate form), 8 per cent cross linked, 200 to 400 mesh anion exchange resin column (1 X 20 cm.). The amino acids were eluted from this column by means of gradient elution with 4 N acetic acid. Fifty fractions of 5 ml. each were collected at the rate of 1 ml./min. by an automatic fraction collector. An aliquot from each tube was removed and assayed for amino acid content12 and radioactivity. Alanine emerged from the column as a distinct peak in tubes 16 to 20. Glutamic acid appeared in tubes 25 to 35 and aspartic acid in tubes 35 to 45. The respective fractions in each peak were pooled and chromatographed on paper using a butanol-formic acid-water13 and a phenol-water14 solvent system. The chromatograms thus obtained were assayed for radioactivity with a Nuclear Chicago Chromatogram Scanner and sprayed with ninhydrin.
Table 1

<table>
<thead>
<tr>
<th>Function measured</th>
<th>Control ±S.E.</th>
<th>Digoxin ±S.E. (30 min. after injection)</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure (Mean) mm.Hg.</td>
<td>111.3±28.5</td>
<td>112.5±30.2</td>
<td>12</td>
</tr>
<tr>
<td>Heart Rate beats/min.</td>
<td>138±30.4</td>
<td>137±39.1</td>
<td>13</td>
</tr>
<tr>
<td>Venous Pressure (peak) cm. H2O</td>
<td>5.1±1.7</td>
<td>5.1±1.7</td>
<td>8</td>
</tr>
<tr>
<td>Cardiac Output ml./min.</td>
<td>1584±147</td>
<td>1584±147</td>
<td>7</td>
</tr>
<tr>
<td>Cardiac A-V Difference vol./100 ml.</td>
<td>14±1.9</td>
<td>14±1.9</td>
<td>6</td>
</tr>
<tr>
<td>Ventricular Pressure (peak) mm. Hg.</td>
<td>126±23.4</td>
<td>126±23.4</td>
<td>6</td>
</tr>
<tr>
<td>Coronary Blood Flow ml./min.</td>
<td>34.6±10.3</td>
<td>36.5±10.3</td>
<td>6</td>
</tr>
<tr>
<td>Respiratory Quotient</td>
<td>.76±.02</td>
<td>.76±.03</td>
<td>13</td>
</tr>
<tr>
<td>Cardiac Respiratory Quotient</td>
<td>.723±.017</td>
<td>.734±.040</td>
<td>13</td>
</tr>
<tr>
<td>Duration of Systole (mean) seconds</td>
<td>1.01±.034</td>
<td>1.00±.034</td>
<td>13</td>
</tr>
<tr>
<td>Cardiac Oxygen Utilization cc./min.</td>
<td>5.4±0.9</td>
<td>5.4±0.9</td>
<td>6</td>
</tr>
<tr>
<td>Caloric equivalent of Oxygen Used</td>
<td>10.3±.31</td>
<td>10.3±.33</td>
<td>6</td>
</tr>
<tr>
<td>Tension-Time-Index mm. Hg.</td>
<td>2.5±.07</td>
<td>2.5±.07</td>
<td>12</td>
</tr>
<tr>
<td>Cardiac Work Kg.M./min.</td>
<td>2.5±.22</td>
<td>2.5±.26</td>
<td>7</td>
</tr>
<tr>
<td>External Efficiency</td>
<td>24%</td>
<td>24%</td>
<td>6</td>
</tr>
<tr>
<td>Internal Efficiency</td>
<td>24%</td>
<td>24%</td>
<td>6</td>
</tr>
</tbody>
</table>

12; lactic acid in tubes 15 to 25 and malic acid, in tubes 45 to 50. Glucose was found in the neutral effluent of the column. An aliquot from each tube was removed for quantitative analysis and for radioactivity determination. Glucosamine was determined according to the method of Elston and Morgan,23 lactic acid according to the method of Barker and Summerson,10 glucose according to the Glucostat method,22 and the malic acid was titrated. A sample from each peak was chromatographed using the solvent systems previously mentioned. The chromatogram was assayed for radioactivity with a chromatogram scanner and sprayed for glucosamine with ninhydrin18 for lactic and malic acid with 2,4-dichlorophenylindophenol,21 and for glucose with periodate-permanganate.23 The individual pooled peaks were characterized in a manner similar to that for the amino acids.

Each point on the charts represents the mean of the isotope concentrations obtained in five experiments at each time interval. Statistical analysis of each time interval revealed that the respective standard deviation of the mean (S.E.) of each value was less than 10 per cent, and in most cases less than 5 per cent of the mean value.

Results

Cardiovascular Effects of Digoxin

The intravenous administration of digoxin to the normal dog in a dose of 0.065 mg./Kg. had no effect on the cardiovascular dynamics or the oxygen utilization of the heart. Table 1 lists the various cardiovascular functions measured, their values and standard errors, and the number of experiments in which each parameter was measured. No significant cardiovascular changes occurred at any time during the 30 min. following digoxin administration. No changes were observed in the calculated values of cardiac work, time tension index or oxygen metabolism, and hence, there was no significant change in the calculated external or the internal efficiency of the heart. This dose of digoxin when administered to animals in heart failure was effective in improving cardiac function.23

Metabolic Effects of Digoxin

The half-time of absorption of glucose-C14 from the blood was less than 15 sec. for both the control and the digoxin treated animals. No significant differences were observed between glucose-C14 disappearance from the blood of the control and the digoxin treated groups. Within 30 sec. after administration of the labeled glucose, only 1/20 (850 cpm/ml. whole blood) of the total radioactivity as glucose-C14 was present in the blood (assuming a blood volume of 10 per cent body weight). The circulation time from the left external jugular vein to the coronary ostia is approxi-
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Thus, 10 sec. after injection, the glucose-C\textsuperscript{14} reached the coronary arteries in maximal concentration. The isotope which did not enter the coronary arteries subsequently entered the general circulation. The circulation time from the aorta to the vena cava is approximately 15 sec. and again, from the vena cava to the area of the coronary ostia, another 10 sec. Thus, the time delay for the reperfusion of the coronary arteries with isotopic glucose is about 25 sec. The total time for reperfusion of the coronary arteries with glucose-C\textsuperscript{14} was approximately 35 sec. after the initial injection. After this time, however, the isotope concentration of the arterial blood was less than 1/25 of its initial concentration.

On this basis, one can assume that 70 to 80 per cent of the isotope entered the myocardium within 10 sec. after the initial injection of the glucose-C\textsuperscript{14}, and the remainder of the isotope entered within the next several reperfusions.

Figures 1 through 8 represent the time course of the changes in the intermediary metabolism of the myocardium following the injection of 0.065 mg./Kg. digoxin. One minute after the injection of glucose-C\textsuperscript{14}, the myocardial isotope concentration of both the control and the digoxin-treated animals was at its maximum measured value (fig. 1). In the control animals the isotope concentration averaged 4750 (S.E. = ± 350) cpm/Gm. heart tissue and in the digoxin treated hearts the isotope concentration was 4890 (S.E. = ± 290) cpm/Gm. heart tissue. There was no significant difference between the 2 groups.

Analysis of the myocardium at later time periods indicated a progressive fall in the isotope concentrations of the heart tissue as a consequence of metabolism of the labeled substrate with the subsequent formation and release via the coronary venous blood, of carbon-C\textsuperscript{14} dioxide and labeled intermediary compounds. Integration of the curves (by determination of the area under each curve) representing isotope loss from both the normal and the digoxin-treated heart indicated that the extent to which the isotope was lost from digitalized heart was 290 per cent greater than the control group. Furthermore, after 11 min., 73 per cent of the total original isotope (3460 cpm/Gm. heart tissue as compared to the peak value of 4720) still remained in the myocardium of the normal dog while only 27 per cent of the total original isotope (1240 cpm/Gm. heart tissue as compared to the peak value of 4810) remained in the myocardium of the digoxin treated group.

The extent to which the isotope loss from the myocardium can be attributed to carbon-C\textsuperscript{14} dioxide production is represented in figure 2, in which the rate of production of
The change of specific activity of carbon dioxide in the myocardium as a function of time after the administration of glucose-C\textsuperscript{14}.

The rate of glucose-C\textsuperscript{14} utilization by the heart tissue as a function of time after its administration. Each point represents the total counts of radioactivity per gram of heart tissue (as glucose-C\textsuperscript{14}) utilized in the respective time periods.

carbon-C\textsuperscript{14} dioxide in cpm/Gm. of heart tissue is plotted against the time after injection of the labeled substrate. The slopes of the carbon-C\textsuperscript{14} dioxide production curves both became asymptotic after 3 min. at which time the formation of carbon-C\textsuperscript{14} dioxide by the myocardium appeared to have equilibrated with its rate of loss from the tissue to the coronary venous blood. Prior to the three minute period, however, the slopes of the curves were proportional to the rate of formation of carbon-C\textsuperscript{14} dioxide by the myocardium. Since the coronary blood flow remained constant after treatment with the cardiac glycoside, the rate of carbon-C\textsuperscript{14} dioxide accumulation in the myocardium becomes a function of its rate of production and its rate of diffusion from the tissue. At the greater rate of production, however, the rate of removal from the tissue is expected to be greater and therefore, the difference in the slopes of the curves would be somewhat less than the true change. The difference in the areas represented by the curves thus represents the minimum value for the increased rate of carbon-C\textsuperscript{14} dioxide formation subsequent to digoxin administration. Integration of these curves indicates an increase in the rate of carbon-C\textsuperscript{14} dioxide formation to the extent of 250 per cent in the digoxin treated animals. On this basis, the increased rate of carbon-C\textsuperscript{14} dioxide production after digoxin accounts for at least 90 per cent of the difference in total isotope lost from the myocardia of treated animals (fig. 1).

In view of this increase in the rate of carbon-C\textsuperscript{14} dioxide production it is of interest to note that the total content of carbon dioxide in the myocardia of the control and the treated animals was essentially the same. In the control group the carbon dioxide content was 26.3 (S.E. = ± 0.7) as compared to 27.5 (S.E. = ± 0.6) µM/Gm. of heart tissue for the digoxin treated group. Figure 3 is an illustration of the change in the specific activity of carbon dioxide as a function of time for both the control and the digoxin treated group. Calculation of the comparative rate of change of specific activity of carbon dioxide during the first 3 min. after substrate administration revealed a 250 per cent increase in specific activity following digoxin administration.

Figure 4 represents the rate of utilization of glucose-C\textsuperscript{14} by the myocardium (expressed as cpm/Gm. heart tissue). Integration of the respective curves indicates a 55 per cent increase in the rate of glucose metabolism by the myocardium following digoxin treatment. Analysis of the glucose content of the heart tissue revealed that the total pool size of glucose did not change as a result of digoxin treatment. Glucose was found in the myocardium of the control and digoxin treated group in a concentration of 3.83 (S.E. = ± 0.9) and 3.46 (S.E. = ± 1.0) µM./Gm. heart tissue, respectively.
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The rate of change of specific activity of glucose in the myocardium is illustrated in figure 5. The utilization of glucose-C\textsuperscript{14} by a tissue does not decrease the specific activity of glucose in that tissue per se. The decrease in specific activity of glucose in the myocardium represents only the dilution of the non-metabolized isotopic substrate by the entry of non-radioactive glucose from the coronary arterial blood. The greater rate of glucose metabolism by the myocardium of the digoxin treated animal could increase the myocardial extraction ratio for glucose and hence give rise to the greater rate of decrease of specific activity (since total glucose remained constant). It is conceivable, however, that the cardiac glycoside could have increased the permeability of this tissue for glucose and, in this manner, increased its rate of metabolism. Calculation of the quantity of non-radioactive glucose necessary to dilute the specific activity of the total glucose pool of the myocardium (as represented in fig. 5) indicates that the myocardial glucose utilization has been increased from 3.8 to 6.2 mg./100 Gm./min. (60 per cent) as a consequence of digoxin administration. This calculated value represents a minimum value, however, since the glucose pool of the heart was diluted by blood containing some glucose-C\textsuperscript{14}.

Figure 6 represents the rate of change of isotope concentration of the total isolated intermediary metabolites of glucose. These curves represent the sum of the isotope concentration in glucosamine, lactic acid, malic acid, aspartic acid, glutamic acid, and alanine. Quantitative determination of these substances at each time interval revealed that the total amounts of each intermediary compound was the same before and after treatment with digoxin. Analysis of the difference in the rate of change of slope of the curves to the point of greatest isotope activity (the rate of synthesis of the intermediary compounds) revealed an 86 per cent increase in the rate of isotope incorporation following digoxin treatment. The slopes of isotope activity curves became negative after 3 min. in the digoxin group and after 5 min. for the control group. Analysis of the rates of decrease of isotope activity after these time intervals indicate a 400 per cent increase in the rate of isotope transformation after digoxin treatment. Thus, combining the rates of change of isotope activity in the total intermediary metabolic compounds represented by these curves, an apparent 5-fold increase in the turnover rates of the isolated compounds is noted.

Figures 7 and 8 represent the specific activity curves of the isolated intermediary compounds in the control and digoxin treated
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TIME MINUTES

Figure 7
The change of specific activity of the individually isolated intermediary compounds as a function of time after administration of glucose-C\(^{14}\) in the control heart. Gl. = glucosamine; L. = lactic acid; G. = glutamic acid; M. = malic acid; Al. = alanine; As. = aspartic acid.

TIME MINUTES

Figure 8
The change of the specific activity of the individually isolated intermediary compounds as a function of time after the administration of glucose-C\(^{14}\) in the digoxin treated heart. Abbreviations see figure 7.

hearts. Comparison of the curves for glucosamine, an alternative metabolic pathway of glucose utilization, indicates that the total isotope incorporation into this compound was decreased by a factor of about one-half. The rate of change of slope of the curves before and after peak isotopic activity was the same in both the control and the digoxin group.

Thus, although the total activity of this fraction was greatly reduced, the rate of change of radioactivity in the compound was similar before and after digoxin. After 11 min., 200 cpm/\(\mu\)M./Gm. heart tissue remained (as glucosamine) in the control hearts (fig. 7) while only 25 remained in the digoxin treated hearts (fig. 8). These data indicate a marked reduction in the synthesis of glucosamine from glucose.

A comparison of the curves representing lactic acid in the control and digoxin groups indicates that there was no significant difference in the maximum amount of isotope incorporated in this fraction. The turnover rates in the 2 groups are greatly different, however. Since the maximum isotope concentration was attained before the one min. time period in the digoxin treated group, the slope of the curve representing lactic acid synthesis was at least 5.0 compared with 0.5 for the control group. This represents a 10-fold increase in the rate of isotope incorporation into lactic acid. The slope of the curve representing lactic acid in the digoxin treated group became negative after the one minute time interval. Its average value, during the duration of the experiment, was \(-0.22\) compared with \(-0.08\) for the control group. Thus, digoxin caused a marked increase in the turnover rate of lactic acid, and the flux of isotope activity "through" lactic acid was increased approximately 13 times its control value.

Malic acid reached maximal isotopic concentration before the one min. analysis interval in the digoxin treated group. Comparison of this slope with the malic acid curve in the control experiments indicates that there was a 13-fold increase in the flux of radioactivity into malic acid formation and a 65 per cent increase in the total isotope incorporation after digoxin treatment.

The total isotope incorporation into the glutamic acid fraction was the same in both groups of animals. The turnover rate of this compound was increased 10-fold, however, in the digoxin group. More marked changes were observed in the turnover rates of aspartic
acid. There was a 500 per cent increase in the total isotope incorporated into aspartic acid subsequent to digoxin treatment. In addition, the turnover rate was increased 10-fold. No significant difference was noted in the turnover rates or the total isotope incorporation into alanine.

Discussion

A definite pattern of changes in cardiac intermediary metabolism has been established subsequent to administration of digoxin: 1) the glucose utilization of the myocardium was increased, 2) the production of carbon-C14 dioxide by the myocardium was increased, 3) the total contribution of glucose to the carbon dioxide pool of the heart was increased, 4) the turnover rates of the intermediates of the glycolytic and tricarboxylic acid cycles, and of aspartic and glutamic acid were increased, and, 5) the total activity of an alternative metabolic pathway of glucose, glucosamine, was decreased. These changes in the intermediary metabolism of the myocardium occurred in the absence of significant changes in its dynamic function. Therefore, these metabolic alterations can be considered a primary effect of the drug rather than a secondary effect due to an altered state of cardiac function.

Since the myocardial pool size of carbon dioxide did not change after digoxin treatment, in spite of the greater rate of carbon-C14 dioxide formation, the increased radioactivity of the carbon dioxide pool indicates a greater contribution by glucose to that pool in the digoxin treated animal. Moreover, the contribution of carbon dioxide from the metabolism of substrates other than glucose (fats, amino acids, etc.) must have decreased. Calculations based on the rate of disappearance of isotope present in the heart as glucose and the rate of appearance of isotope in the heart tissue as carbon dioxide indicate that glucose contributed to the metabolism of the control hearts to the extent of 8 per cent while in the digoxin treated hearts, glucose contributed 23 per cent of the total myocardial metabolism.

A more precise explanation concerning the action of digoxin on the intermediary metabolism of the myocardium is possible. Clearly, the rate of glucose metabolism in the heart tissue has been increased by treatment with the cardiac glycoside. The increase in the rate of glucose utilization appears to have increased the turnover rates of the intermediates of the glycolytic and the tricarboxylic acid pathways. Since the total production of carbon dioxide did not change, the net alteration in the metabolism of the heart appeared to be only a shift in the substrate and its rate of metabolism by the cardiac tissue.

The mechanism by which the cardiac glycoside increased the carbohydrate metabolism of the heart is not clear. Whether it acts on the metabolism of glucose directly (e.g., hexokinase reaction or glucose permeability) or perhaps on a particular rate limiting step in the glycolytic chain has not been elucidated in these experiments. The studies of Wollenberger with dog ventricle strips indicate a greater rate of glucose metabolism to carbon dioxide subsequent to treatment with ouabain, i.e., an effect similar to that found in our studies with digoxin. The result of addition of C14-labeled substrates to his in vitro preparation indicated that the increased rate of carbohydrate metabolism was due to a greater activity of lactic dehydrogenase following addition of ouabain with a subsequent greater rate of oxidative decarboxylation of pyruvic acid. Segre has also presented evidence of a greater rate of pyruvic acid formation from lactic acid following treatment with strophanthin. The investigation of Rebar et al. suggests that the cardiac glycosides stimulate the hydrolysis of high energy phosphate with the subsequent increase in the intracellular levels of inorganic phosphate. Such an increase in the intracellular level of inorganic phosphate may be related to the increased metabolic rates found in our study.

The observations of many investigators indicating no significant changes in the arterio-venous differences of many substrates in cardiac failure or after treatment with the
cardiac glycosides can be explained on the basis of the very slight changes in total glucose metabolism observed in our experiments. However, these changes are sufficient to cause the very marked changes in the intermediary metabolism of the heart. Furthermore, a cardiac arterio-venous difference in a substrate concentration gives no indication of the turnover of that substrate within the myocardium, but merely indicates its net utilization and synthesis by the heart.

It is fully realized that any hypothesis concerning the therapeutic actions of the cardiac glycosides based on the present experiments would be an extrapolation of the actions of this drug in the normal animal. If cardiac failure is characterized by a "lesion" in the metabolic pathways of the myocardium, particularly the phase of energy production dealing with carbohydrate metabolism, an increase in the glucose utilization of the heart should reverse the cardiac failure. Gremels has pointed out that failure in the dog heart-lung preparation is preceded and accompanied by a decrease in the cardiac glucose utilization. The experiments by Bayliss and Visselier have shown that the addition of insulin or glucose to the failing heart-lung preparation was able to restore normal cardiac function. Also, Lorber has reported an increase in the cardiac respiratory quotient subsequent to administration of Lanatoside-C in the failing heart-lung preparation. The addition of glucose augments the contractile response of the isolated heart muscle strip to ouabain.

It has been shown that treatment of the "failing heart" with Lanatoside-C results in an increase in the per cent extraction of lactic and pyruvic acid and a decrease in the cardiac utilization of free amino acids. This was explained on the basis of a "protein sparing" effect of the increased carbohydrate utilization. If cardiac failure is associated with a deranged carbohydrate metabolism one might suggest that the changes in the myocardial contractile protein, actomyosin, which are evident in cardiac failure, are secondary to a defect in protein metabolism resulting in an increase in the utilization of free amino acids to maintain the oxidative pathways of the heart. Thus, one would expect to find no direct action of the cardiac glycosides on the actomyosin extracted from the failing heart as has been found by Benson and Stutz.

Our investigations indicate that the cardiac glycosides exert their effect on the myocardium by augmenting the metabolism of glucose, thus supplying the glycolytic and oxidative pathways with an increased level of intermediary substrates which in turn results in a markedly elevated rate of intermediary metabolism. Augmentation of a depressed metabolic rate of the heart appears to be involved in the mechanism of the therapeutic action of the cardiac glycosides.

**Summary**

The effect of a therapeutic dose level of a cardiac glycoside, digoxin, on the intermediary metabolic pathways involved in the myocardial utilization of glucose-C has been studied in the normal dog.

Digoxin increased 1) the rate of glucose utilization by the myocardium (60 per cent), 2) the contribution of glucose to the total metabolism of the myocardium (from 5 to 23 per cent), 3) the rate of carbon dioxide production from glucose (250 per cent), and 4) the turnover rates of the intermediary metabolites of the glycolytic and respiratory pathways. These changes occur in the absence of any significant changes in the dynamic functions of the myocardium.

The results of this investigation suggest that the cardiac glycosides exert their effect on myocardial metabolic processes involved in energy production.

The therapeutic role of the cardiac glycosides in congestive cardiac failure is discussed.

**Summario in Interlingua**

Le effecto de un nivello terapeutico de dosage de un glycosido cardiac, i.e. digoxina, super le viss metabolico intermediari interesse in le utilisation myocardial de glucosa a C14 esseva studiate in canes normal.

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DIGOXIN ON CARDIAC METABOLISM

Digoxina aumentava (1) la intensità del utilizzazione di glucosa per il myocardio (50 pro cento), (2) la contribuzione di glucosa al metabolismo total del myocardio (ab 8 a 23 pro cento), (3) la intensità del production de bioxido de carbon a C\textsuperscript{14} (250 pro cento), e (4) la intensità del transition del metabolites internocellulai in le vins glycolytic e respiratori. Iste alterationes ocurrova in le absentia, do alternation de glucosa per myocardio (50 pro cento), (2) de congestive discompansation cardiac es discutite. solites interinodari in le vins glycolytic e respiratori. del production de bioxido de carbon a C\textsuperscript{14} (250 pro cento), (4) de congestive discompansation cardiac es discutite. Le results del presente investigation suggere que le glycosidos cardiac exerce lor effecto super le processos metabolic in le myocardio le quales participa in le production de energin. Le rolo therapeutice del glycosidos cardiac in casos de congestive discompansation cardiac es discutite.

References

7. VAN SIYVKE, D.


The Effect of Digoxin on the Intermediary Metabolism of the Heart as Measured by Glucose-C\textsuperscript{14} Utilization in the Intact Dog

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