Role of Calcium and Lanatoside C in Increasing Oxygen Consumption in Human Myocardial Tissue Slices

By Lamar Crevasse, M.D., and Myron W. Wheat, Jr., M.D.

With the technical assistance of Dianne Cuppy

A large number of studies to determine whether cardiac glycosides have a demonstrable effect on myocardial metabolism have produced conflicting results. In vitro studies with homogenates of myocardium from various species have consistently shown no increase in oxygen consumption. Recently, Wollenberger and Finkelstein have clearly demonstrated an increase in oxygen consumption in dog and cat myocardial tissue slices incubated with 1 \times 10^{-6} M glycosides, ouabain, digitoxin, and scilliroside, the intact cell being essential for this acceleration. Furthermore, Finkelstein demonstrated a necessity of the calcium ion for this increase in oxygen consumption in cat heart tissue slices. Burdette, utilizing biopsy of the atrial appendage at thoracotomy in five cases, was able to show a two- to threefold increase in oxygen consumption in tissue slices incubated with 0.5 mg. per cent Lanatoside C (5 \times 10^{-6} M) utilizing glucose and pyruvate as substrates. There is, however, a paucity of information about the direct mode of action of the glycosides on human myocardial metabolism.

With the advent of open-heart surgery, a source of fresh myocardial tissue has been provided by means of atrial and ventricular biopsy. The principal purpose of this investigation was to re-explore the effects of the cardiac glycoside, Lanatoside C, on oxygen consumption in human myocardium, and to establish the relationship of Lanatoside C to the calcium ion which has been shown to be intimately related to the glycosides in the areas of contractility. Kohn, utilizing the isolated perfused rabbit heart, observed a cessation of contraction in a calcium-free medium. Only when calcium was available as bound ethylenediaminetetra-acetate could contractility be restored by digitalis.

Methods

Atrial strips (0.5 cm. \times 3 cm.) and the atrial appendage were removed from the auricle at the onset of bypass during open-heart surgery in 25 patients. Ventricular biopsies were obtained from ventriculotomy sites. The results with each tissue were essentially the same. Only the results with atrial strips are reported here. The muscle was placed in precooled oxygenated Krebs Ringer phosphate for transport and then promptly sliced with a Stadie-Riggs slicer into 0.3-mm. thick slices weighing approximately 60 mg.

Oxygen consumption was determined by the direct method of Warburg with oxygen as the gas phase at 37 C. Carbon dioxide was absorbed in the center well with 0.2 cc. of 20 per cent KOH. Krebs Ringer phosphate, with and without calcium, was the buffer medium. The composition of the medium was as follows: 0.093 M NaCl, 0.0048 M KCl, 0.00125 M CaCl$_2$, 0.0012 M MgSO$_4$, 0.033 M sodium-phosphate buffer of pH 7.4. Each Warburg flask contained 2.7 or 3 cc. of media. The manometers and flasks were gassed for five minutes with 100 per cent oxygen and stabilized for 10 minutes prior to reading. All substrates were of 10 mM concentration and were contained in the phosphate buffer. After stabilization, oxygen uptake was recorded at 10-minute intervals for 90 minutes. At this point, crystalline Lanatoside C was added in buffer media (0.3 cc.) was added from the side arm with a final concentration of 1 \times 10^{-6} M. Oxygen uptake was then recorded for an additional 90 minutes. In this manner, each tissue slice provided its own control. Oxygen consumption, as a function of time in figure 1 and figure 6 (single experiment), is expressed as microliters of...
Figure 1

Oxygen uptake is recorded at 10-minute intervals, and after 90 minutes of incubation, Lanatoside C is added from the side arm of the vessel to both the calcium and noncalcium-containing media. No Lanatoside is added to the control. Note the striking increase in oxygen consumption reaching a maximum in 40 minutes. There is no difference in the control and calcium-deficient Lanatoside-containing media.

Results

In figure 1, the temporal relations of oxygen consumption to the addition of Lanatoside C from the side arm of the Warburg vessel, are illustrated after 90 minutes of incubation. The glycoside was added to each of two vessels, with and without calcium, containing 10 mM glucose as substrate in Krebs Ringer phosphate buffer. The remaining vessel acted as the overall control. In the vessel containing calcium, there was a threefold acceleration of oxygen consumption as contrasted with the calcium-deficient Lanatoside C-containing vessel and the control. The acceleration of oxygen consumption began in the first 10 minutes, was maximal in 40 minutes, and maintained a stable two- to threefold increase throughout the remaining 50 minutes of the experiment.

In the following charts are the average of eight determinations and a single standard error of the mean. The light right-hand bars indicate the flask containing 1 × 10⁻⁶ M Lanatoside C, with and without calcium, as contrasted with the solid black controls.

Figure 2 represents the changes in qO₂ with 10 mM glucose as the substrate in the presence and absence of calcium in Krebs Ringer phosphate. In the calcium-deficient media, the qO₂ declined from 2.8 to 2.0 after the addition of Lanatoside C from the side arm. In the presence of calcium, the qO₂ sharply increased from 2.5 to 7.5, a threefold increase.

In figure 3, the substrate was 10 mM pyruvate, and the qO₂ declined from 2.7 to 2.2 after the addition of the glycoside in the calcium-deficient media, in contrast to an increase of qO₂ from 3 to 5.2 in the calcium-containing media.

In figure 4, no exogenous substrate was present, and the tissue was respiring in Krebs Ringer phosphate with and without calcium. In the absence of calcium, the qO₂ declined from 3.3 to 2.3. In the presence of calcium, the qO₂ rose sharply from 2.4 to 6.4 with no exogenous substrate available.

In figure 5, 10 mM succinate was the exogenous substrate. The qO₂ without calcium, and on the addition of Lanatoside C, remained stable, 8.2 and 8.1. In the presence of calcium, the qO₂ increased slightly from 9.7 to 11.0. This increase in qO₂ is not statistically significant.

To explore the high qO₂ with succinate in tissue slices and the inability of Lanatoside C to accelerate oxygen consumption in the presence of succinate, the succinoxidase reaction in the human myocardium was studied. By means of homogenization, it is possible to disperse cellular components so that endogenous respiration ceases as a result of dilution effects of respiratory enzymes and their cofactors. The restoration of a particular reaction can be accomplished by the addition of appropriate substrates and soluble cofactors.

The technique of Schneider and Potter was utilized to study the isolated succinoxidase system in three biopsies of human myocardial tissue. In this manner, succinate qO₂ of the isolated system can be compared with the qO₂ of tissue slices. Ten per cent homogenates were prepared in buffer, and 0.2 ml added to 0.1
MPO₄ pH 7.4, 0.5 M Na-succinate pH 7.4, 1 × 10⁻⁴ M cytochrome C, 4 × 10⁻⁴ M CaCl₂, and 4 × 10⁻³ M AlCl₃ to final volume of 2.7 cc. Oxygen consumption was measured for 90 minutes; at this point, a final concentration of 1 × 10⁻⁶ M Lanatoside C was added from the side arm, and oxygen consumption measured for an additional 90 minutes. The qO₂, prior to addition of Lanatoside C, averaged 18.8 and during the period after the addition of the glycoside 14.5. Since this reaction measures succinate +½ O₂—fumarate and H₂O, the high qO₂ in tissue slices respiring in succinate can be accounted for by the succinioxidase reaction. Lanatoside C has been demonstrated not to influence this reaction per se.

In figure 6 are the metabolic interrelations of calcium and Lanatoside C on tissue slices respiring in 10 mM glucose over a prolonged time interval (6½ hours).

This experiment was designed (1) to evaluate the duration and stability of acceleration of oxygen consumption; (2) to determine the responsiveness of the control (containing calcium) to the addition of Lanatoside C after a five-hour period of respiration; and (3) to establish if the restoration of calcium to the calcium-free Lanatoside C-containing control could now accelerate oxygen consumption to the sustained level induced by Lanatoside C in the calcium-containing media. After stabilization at 90 minutes, Lanatoside C increased oxygen consumption as previously demonstrated in the calcium-containing media. The vessel containing Lanatoside C, but no calcium, remained unchanged as did the control containing calcium but no addition of Lanatoside C. At 300 minutes, the consumption of oxygen in the calcium-Lanatoside C medium was twofold greater than in the calcium-free medium and the control. At this point, a final concentration of 2.5 mEq./L of calcium chloride was restored to the Lanatoside C-calcium-free vessel, and oxygen consumption rose rapidly to the existing elevated level of the Lanatoside C-calcium-containing vessel. Lanatoside C, added at this point to the control containing calcium but no previous Lanato-

**FIGURE 2**

The changes in qO₂ in tissue slices respiring in 10 mM glucose, with and without calcium, are illustrated. The light right-hand bars indicate the flask containing 1 × 10⁻⁶ M Lanatoside C, with and without calcium, as contrasted with the (dark) controls. Note the threefold increase in oxygen uptake induced by the glycoside in the presence of calcium. There was no alteration in qO₂ by the glycoside in the calcium-deficient flasks. \[ \text{S. E. M.} \]
FIGURE 3
The changes in $Q_{O_2}$ in tissue slices respiring in pyruvate as influenced by Lanatoside C (light bar), with and without calcium, in the media are shown above. Oxygen consumption is approximately doubled by Lanatoside C in the presence of calcium as contrasted to the controls.

Discussion
It has been demonstrated that Lanatoside C in a concentration of $1 \times 10^{-6}$ M can increase oxygen consumption in human myocardial tissue slices respiring in glucose, pyruvate, or without exogenous substrate. No direct effect of this agent was demonstrated on the succinoxidase reaction. This acceleration of oxygen consumption by the cardiac glycoside Lanatoside C has been demonstrated to be a calcium-dependent phenomenon. Adequate data are not available from previous or current experiments to explain readily this

Circulation Research, Volume XI, October 1962
MYOCARDIAL OXYGEN CONSUMPTION

FIGURE 5
Lanatoside C does not significantly increase the qO2 in myocardial tissue slices respiring in 10 mM succinate with or without calcium present in the media.

acceleration of oxygen consumption in human myocardial tissue slices by the cardiac glycoside. In contrast to the previous animal experiments\(^1\)\(^-\)\(^5\) sited where only transient increases in qO2 were induced by the glycosides, consistent and sustained increases in qO2 have been found in human tissues. The observations of the greatest increase in qO2 occurring with glucose as the substrate is consistent with the previous animal experiments of Finkelstein and Bodansky.\(^2\) The high qO2 with succinate in myocardial tissue slices undoubtedly represents the succinoxidase reaction per se. Lanatoside C has been demonstrated not to influence this reaction. Of particular interest is the acceleration of oxygen consumption in the absence of added substrate. To account for this acceleration, endogenous substrates such as glycogen or fatty acids must be made available for oxidation.

Lee et al.\(^6\) have demonstrated, in isolated contracting cat papillary muscle, that the addition of 20 mM succinate produced an increase in oxygen consumption and yet had no effect on contractility undoubtedly related to the succinoxidase reaction per se, whereas the addition of 10 mM pyruvate increased oxygen consumption and contractility.

The dependency upon the calcium ion in accelerating oxygen consumption in relation to the cardiac glycosides may be related to the ability of the glycoside to produce an influx of calcium into the myofibrils. Holland and Sekul\(^7\) have demonstrated that ouabain increases calcium influx into the myocardium and that this effect of influx is enhanced by
an increase of calcium in the media. Kaye and Mombaerts\textsuperscript{8} have recently demonstrated that the membrane-control activation of glycolysis in resting muscle is contingent upon the presence of extracellular calcium. They postulate that, in the activation of glycolysis, the entry of the calcium ion is a determining factor. That this effect of the increase in oxygen consumption induced by the glycoside is independent of myocardial contractility and occurs in resting muscles has been previously demonstrated by Lee.\textsuperscript{9} The maximum increase in qO\textsubscript{2} occurring with glucose as the substrate suggests an augmentation of glycolysis under the influence of the glycoside. This concept has been suggested by the animal work of Kien and Sherrod.\textsuperscript{9} The mechanism of acceleration of oxygen consumption in human myocardial tissue slices and its relationship to contraction are not clear. The data demonstrate an in vitro effect of the cardiac glycoside on human myocardial metabolism and its dependency upon the calcium ion.

**Summary**

\[ 1 \times 10^{-6} \text{ M Lanatoside C has been demonstrated to produce a sustained increase in oxygen consumption in human myocardial tissue slices as previously demonstrated by Burdette. This acceleration of qO}_2 \text{ is independent of the substrates employed with the exception of succinate. This qO}_2 \text{ acceleration has been demonstrated to be calcium dependent in human heart tissue slices similar to the known interrelationship of calcium and digitalis on myocardial contractility. The observation that the greatest increase in qO}_2 \text{ occurred with glucose and without exogenous substrate suggests that the increase in qO}_2 \text{ induced by the glycoside is dependent upon a stimulatory effect on glycolysis.} \]

**References**

7. **HOLLAND, W. C., AND SEKHIL, A. A.:** Effect of ouabain on Ca\textsuperscript{45} and Cl\textsuperscript{36} exchange in isolated rabbit atria. Am. J. Physiol. 197: 757, 1959.

**Book Review**


The material in this book was presented at the Brown University Symposium, held on January 30 and 31, 1960. Nine papers by various contributors are included. The cutaneous vascular patterns, as well as the innervation of these vessels, are discussed, with support from major methods for studying blood vessels: perfusion with opaque substances, x-ray microscopy, and histochemical methods. The chapter on in vivo studies of the effect of heat on the undisturbed skin is an excellent one. A number of problems for future investigation are listed. The vasomotor innervation of the skin needs full description in terms of anatomical identification of the spinal outflows and of their regional distribution. The innervation of the sweat glands and the vascular smooth muscle provide conveniently accessible structures for the quantitative study of autonomic physiology.
Role of Calcium and Lanatoside C in Increasing Oxygen Consumption in Human Myocardial Tissue Slices
Lamar Crevasse and Myron W. Wheat, Jr.

*Circ Res.* 1962;11:721-726
doi: 10.1161/01.RES.11.4.721

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
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:
http://circres.ahajournals.org/content/11/4/721

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/