STUDIES ON THE METABOLISM OF HUMAN CARDIAC MUSCLE OBTAINED BY AURICULAR APPENDECTOMY

PRELIMINARY REPORT*

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In a previous report[4] on extirpation of the auricular appendage it was suggested that the cardiac muscle removed at such an operation could profitably be used for *in vitro* metabolic studies. Since that time cardiac muscle removed at the time of auricular appendectomy in two patients has been used for this purpose, and results of these studies are reported here.**

METHODS†

The auricular appendage was removed from the right side in both patients. Other than arteriosclerosis in both cases and left axis deviation in the second case no abnormality of the cardiovascular system was noted before operation. Both patients had carcinoma of the lung, and an exploratory thoracotomy was done in the hope that a resectable lesion would be found. In each case the lesion was too extensive to permit resection, the pericardium was opened, and in a few minutes the appendage was removed before the thoracotomy wound was closed. An old pericarditis was encountered in the first case. In neither case was there any untoward effect of the operation. The first patient died from extension of the neoplasm one month later after receiving nitrogen mustard therapy. The second patient is now receiving palliative treatment after recovering satisfactorily from the operation.

Immediately after the muscle was removed it was placed in a petri dish resting in ice in order to prevent depression of respiration from anoxia.[3] Slices of the tissue 0.5 mm. in thickness were quickly made, and oxygen consumption was measured by the direct method of Warburg, using 100% oxygen as the gas phase. Readings were taken at intervals of 15 minutes. In addition to Krebs-Henseleit physiological saline buffered with phosphate[10] containing half the original concentration of calcium, substrates were added at zero time after the vessels had been equilibrated for 10 minutes to make the following final concentrations: 0.02 M sodium pyruvate, 1.0 mM glucose, 2.0 mM sodium iodoacetate, and 0.01 mg. % lanatoside C.‡ For one of the samples the concentration of potassium was 0.4 mM rather than the usual 5.0 mM potassium concentration of Krebs-Henseleit physiological saline. The respiratory quotient of the tissue was determined in the second case by the second method of Dickens and Šimer,[7]

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** In the course of these studies the auricular appendage has now been removed from five patients without deleterious effect. The operation is technically not difficult, but it seems indicated as a biopsy only when a thoracotomy is done for some other reason. The muscle has been obtained either when it was removed in the surgical treatment of cardiac disease or when a malignant lesion was found which was not resectable. Whether sufficient information may be obtained from the excised muscle to justify operation for that purpose alone will rest on further observations.

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using the Dixon-Keilin type of vessels. The results are given in Tables 1 and 2 where oxygen consumption is expressed as cu.mm. of oxygen consumed per mg. dry weight of the tissue per hour.

Results

In the second case the Q_{O_2} values varied from 6.39 to 2.28 during the first three hours when Krebs-Henseleit buffer was present alone; in the first case, however, the corresponding values were much lower, ranging

Table 1

Respiration of Human Cardiac Muscle

(Case 1)

	No. of determinations	Q_{O_2}					
		30′	*60′*	*90′*	*120′*	*150′*	*180′*
Saline	1	0.49	0.98	1.23	0.74	0.98	1.23
Pyruvate	1	5.57	4.51	4.51	3.98	3.98	3.18
Iodoacetate	1	2.42	0.66	1.32	0.44	0.44	0.44
Saline (0.4mM K)	1	4.30	2.15	2.80	1.72	1.72	1.08

Table 2

Respiration of Human Cardiac Muscle

(Case 2)

	No. of determinations	Q_{O_2}					
		30′	*60′*	*90′*	*120′*	*150′*	*180′*
Saline	2	6.39	3.21	2.28	2.94	3.22	3.07
Pyruvate	1	10.83	5.99	3.42	5.13	3.99	5.42
Glucose	1	9.30	4.80	2.10	3.60	2.60	3.40
Iodoacetate	1	3.08	1.63	0.97	.00	.00	.00
Lanatoside C	1	9.18	12.49	13.26	9.44	9.44	5.35

from 1.23 to 0.49. When 0.4 mM potassium was present in the saline in the first case, the initial level was higher, but it declined from 4.30 to 1.08 in three hours. The average dry weight was found to be 15.2% of the weight of the wet samples.

Addition of sodium pyruvate resulted in an increased oxygen uptake in both cases, reaching levels as high as 5.57 in the first and 10.83 in the second. Only 7.2×10^{-5} M pyruvic acid was present at the conclusion of the experiment. When glucose was added the Q_{O_2} determination was 9.30 at the beginning and 3.40 at the end of three hours (Table 2). The presence of lanatoside C had a pronounced effect on oxygen uptake, the Q_{O_2} rising as high as 13.26 in the vessel containing it.

The Q_{O_2} levels were fairly well sustained after 90 minutes in all of the vessels except those containing sodium iodoacetate. This substrate caused rapid decrement in oxygen consumption reaching negligible proportions after 90 minutes. The respiratory quotient was found to be 0.96.

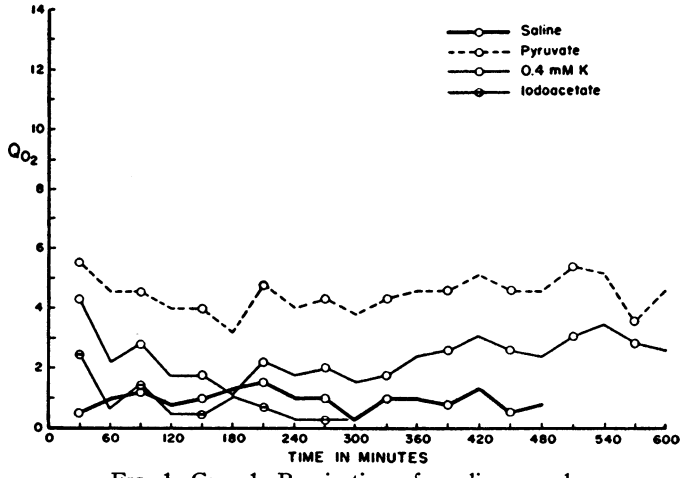

FIG. 1. Case 1. Respiration of cardiac muscle.

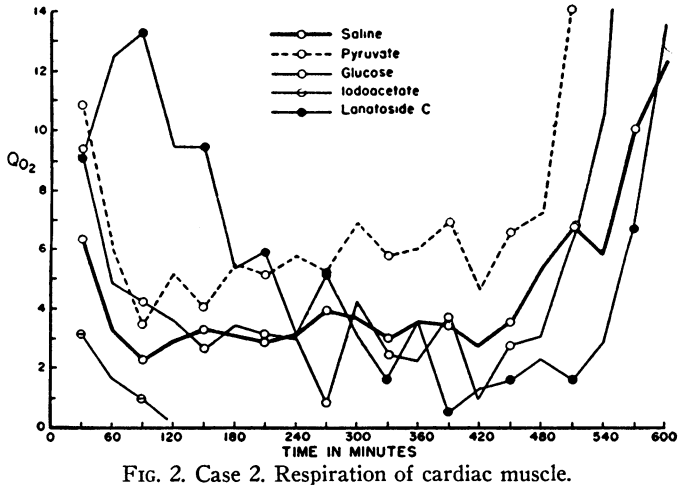

FIG. 2. Case 2. Respiration of cardiac muscle.

Curves for Q_{O_2} levels for Case 1 are presented in Figure 1, those for Case 2 in Figure 2. With two exceptions (saline in Case 1 and lanatoside C in Case 2) there was an initial decrement during the first hour. This decline continued when the muscle was poisoned with iodoacetate. In Case 2 the presence of lanatoside C was associated with an initial rise in Q_{O_2} followed by a fall after 90 minutes. This continued to a point below the level obtained with physiological saline and remained there after five hours.

The shape of the curves when pyruvate was added was very similar to that when saline was present alone except that the Q_{O_2} values were higher with pyruvate. The initial Q_{O_2} for the vessel containing glucose was at a higher level (than when no substrate was present) for only two hours.

Beginning about the seventh hour there was a marked rise in oxygen consumption in all vessels used for Case 2 (except the one containing sodium iodoacetate which was not observed after three hours). This increased respiration was observed until readings were discontinued at the end of twelve hours. Cultures taken at that time were positive for *Alcaligenes faecalis* and *Staphylococcus albus*. Subsequent inoculation of Warburg vessels with strains of these organisms under the same conditions, except for the addition of cardiac muscle, resulted in similar changes in manometer readings, although of lesser magnitude. This occurred after six hours, and it is assumed that the late changes in manometer readings described are attributable to the presence of these organisms.

Discussion*

Probably more reliance may be placed in the results reported for the second case than those of the first case where pericardial adhesions were present, fewer determinations were done, and where the Q_{O_2} with 0.4 mM potassium was higher than with 5.0 mM potassium. The Q_{O_2} values for human muscle are somewhat lower than similar values in experimental animals from this and other laboratories,[1, 16, 18] especially the low Q_{O_2} determinations in Case 1 when physiological saline was present alone.

The presence of sodium pyruvate was clearly associated with a pronounced and sustained elevation of oxygen consumption. This was not a non-specific effect, since pyruvate actually disappeared from the medium. There is general agreement that pyruvate initiates an increase in oxygen consumption in experimental animals,[1, 2, 5, 14, 15, 17, 18] and Goodale, *et al.*[8] have reported lower concentrations of pyruvate in human coronary venous blood than in arterial blood.

The proximity of the respiratory quotient (0.96) to unity, an indication of the metabolism of carbohydrate, is additional evidence for the importance of carbohydrate utilization by the tissue which was studied. However, this R.Q. is considerably higher than the value found by Powers and Bing[8] who made determinations on coronary blood.

The findings with glucose should be interpreted cautiously,[11] particularly in view of previous experience[5] and that of others[1, 2] in failing to find an increase in Q_{O_2} with glucose substrate in the rat. Although initially the determinations were elevated, the remainder of the readings showed little change from the control. It should be noted, however, that numerous authors have shown that glucose can be used for the energy of contraction

* Suggestions of Dr. A. E. Wilhelmi during preparation of the manuscript were very helpful.

under certain conditions.[8, 13, 17] The effect of lowering the concentration of potassium may likewise be considered equivocal (Table 1).

Sodium iodoacetate, which inhibits oxidation and reduction, effectively blocked the respiration of the slices. The importance of phosphocreatine in transferring chemical energy to muscular work has been inferred by several workers;[6, 9, 12] the results obtained with sodium iodoacetate may therefore mean that no energy is available for the resynthesis of phosphocreatine due to inability to utilize sugar because of inhibition of the oxidative phosphorylation of phosphoglyceraldehyde to phospho-glyceryl-phosphate. However, this is not conclusive evidence that the phosphocreatine cycle is operating, since iodoacetate inhibits a number of enzyme systems.

The increase in Q_{O_2} following contact with lanatoside C is impressive although it was not prolonged much beyond the fourth hour. The effect of the glycoside on respiration of the slices, of course, is not necessarily related to inotropic effects of the drug.[13] It should also be noted that the effect described was obtained without addition of substrate, which suggests that this drug acts in some manner other than by facilitating entrance of exogenous substrate into the cell.[18] The danger of drawing hasty conclusions from a single observation deters further speculation.

The data presented are necessarily scanty because of the limited source of the tissue, and, even if the number of samples were of desirable magnitude, the chemical events during contraction could scarcely be analyzed accurately from the information. The extent to which results using the auricle may apply to ventricular muscle also awaits further study. Of more importance than the data presented is the continued use of cardiac biopsy for study of the metabolism of human cardiac muscle. Through the use of this method it should be possible to learn about the normal metabolic processes involved in contraction and their alteration in disease. The relation of phosphate bonds to energy transfers is of particular interest. Also the combination of the method described here with the use of labelled compounds and with catheterization of the coronary sinus appears especially promising. There are certain problems which ultimately can be solved accurately only in human tissue, and confirmatory evidence in man of the findings in experimental animals is always desirable.

The data presented are compatible with the idea that the muscle tested was respiring aerobically, utilizing carbohydrate with adequate metabolic reserve to respond to additional pyruvate and to the presence of lanatoside C, respectively, with increased Q_{O_2}, and that the tricarboxylic and phosphocreatine cycles are operative in the intermediary metabolism of the tissue. Further studies are desirable in order to verify and extend these observations.

Summary

1. Slices of human cardiac muscle which were obtained by excising the auricular appendage in two patients were studied in the Warburg apparatus.

2. The presence of sodium pyruvate resulted in an increased Q_{O_2}.

3. Lanatoside C in the absence of exogenous substrate caused the greatest increase in oxygen consumption of the tissue slices.

4. Sodium iodoacetate blocked the respiration of the muscle.

5. The respiratory quotient was found to be 0.96.

6. The use of cardiac biopsy for obtaining and studying fresh cardiac muscle from the living patient should be a valuable adjunct to the study of human cardiac physiology in health and disease.

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