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An Experimental Study of Myocardial Protection with Special Reference to Cold Blood Potassium Cardioplegia: II. The Mechanism of Ischemic Damage

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Introduction

Several means have been employed to estimate the ischemic myocardium and to evaluate the effectiveness of myocardial preservation techniques. These include the measuring of amounts of intermetabolites during anaerobic glycolytic pathway such as lactate, pyruvate, glucose 6-phosphate, etc., and the assay of high energy phosphate compounds such as creatine phosphate (CP) and adenosine triphosphate (ATP) from the excised myocardium. An electron microscopic study is also performed as usual. In the previous biochemical and electron microscopical studies[6,37], related to the present one, superiority of asanguineous cardioplegia (cold blood potassium cardioplegia (CBKC)) to asanguineous cardioplegia (glucose-insulin-potassium cardioplegia (GIKC)) for myocardial protection during ischemic arrest was confirmed. Unfortunately, however, even with myocardial protection afforded by CBKC, it was impossible to prevent completely a reduction in high-energy phosphates or destruction of cellular structure[38] of the ischemic myocardial cells when periods of ischemia extended. Furthermore, the previous study,[6] using polarography to measure the level of oxygen tension within the myocardium, confirmed that, during intermittent CBKC, the level of oxygen tension in the myocardium, which at first fell off dramatically, began to climb again when the period of ischemia reached 150 minutes. At the same time, when oxidation-reduction reaction $NAD^+ \rightleftarrows NADH$ was measured by means of microfluorometry, it was possible to ascertain that transformations in the direction of either oxidation (NAD^+) or reduction (NADH) had ceased and remained suspended even with further infusion of the cardioplegic solution. It seems reasonable to interpret this phenomenon as a condition in which, as the result of the near cessation of oxidation-reduction reaction in the mitochondria, oxygen is no longer being reduced: the process of electron transport has been disrupted. What precisely, then, is the area of the electron trnasport chain where this disruption takes place?

Coenzyme Q_{10} (CoQ_{10}) is a component of the process of electron transport; it is held to be the only component which can move freely in the inner membrane of mitochondria. The research

Key word: Active oxygen, Lipid peroxide, Free radical scavenger, Coenzyme Q_{10}, Vitamin E.
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described in this paper has taken this characteristic of CoQ as a point of departure; it examines what variation occurs in the level of myocardial CoQ_{10} with prolonged ischemia, and considers what the significance of these variation may be. In addition, this study investigates whether or not lipid peroxide is in fact produced during ischemia, and also what variation occurs in the amount of vitamin E (VE), which is held to be an antioxidant, present in the myocardium.

Meterials and Methods

In this series of experiments six mongrel dogs weighing 12–17 kilograms were used. They were anesthesized by intravenous injection of 25 mg/kg of pentobarbital sodium. In each case, a dog was placed under intermittent positive pressure breathing by a Harvard respirator. The thoracic cavity was opened by a bilateral incision in the fourth intercostal space. After an intravenous injection of 3 mg/kg heparin, cannulation for the transmission of blood into the body and for removing blood from the body was performed into the right common carotid artery and into the right atrium respectively, and extracorporeal circulation was begun with non-blood prime at a final hematocrit of 25–30%. Bubble oxygenators, such as Temptrol Q_{130} manufactured by Bentley Laboratories or those made by Japan Medical Supply Co., Ltd. were used in these experiments. Blood was sent back to the body at a rate of 80 ml/kg/min by means of a roller pump made by Sarns Inc.. After core cooling to the point where the esophageal temperature reached 32°C. the ascending aorta was cross-clamped and 20–30 ml of diluted potassium chloride solution (150–200 mEq/l), cooled to 4°C, was immediately injected manually into the aortic root so as to arrest the heart in diastole. Topical cardiac cooling was employed simultaneously. Care was taken to ensure that the myocardial temperature, as measured at the apex of the heart by a thermistor probe, did not subsequently exceed 15°C. At this point, extracorporeal circulation was stopped and the blood was drawn off into the oxygenator so that it could be used in the CBK solution. Immediately after achieving diastolic cardiac arrest, the first specimen of the myocardium was removed from the apex of the left ventricle. Each tissue

Table 1. Composition of cardioplegic solutions.

	Solution I	Solution II
Substrate	5% Glucose 500 m*l*	Oxygenated heparinized blood 500 m*l*
Regular insulin	10 units	10 units
Potassium chloride	10 m*l*	10 m*l*
7% sodium bicarbonate	10 m*l*	10 m*l*
Na (mEq/*l*)	18	125±8
K (mEq/*l*)	18	24±2
Ca (mEq/*l*)	0	3.7±0.6
Mg (mEq/*l*)	0	1.4±0.2
pH	7.9	7.6±0.1
PO_2 (mmHg)	195	316±98
PCO_2 (mmHg)	15	32±7
Osmorality (mOsm/*l*)	362	347±14

specimen was divided into its outer and inner layers and then used for measurements of levels of VE and CoQ_{10} in the myocardium. Subsequently, 10 ml/kg of either GIK or CBK solution, the composition of which is shown in Table 1, was infused every 30 minutes by gravity. During this procedure, specimens of myocardial tissue were taken every 60 minutes until the period of ischemia reached 180 minutes. In parallel experiments, when the method of cardiac protection described above were employed on other mongrel dogs, about ten gram of tissue each was excised from the apex of the left ventricle, and the mitochondria was removed from each of these specimens according to TYLER[47] method. Measurements were taken of the amounts of lipid peroxide (in this paper tribarbituric acid reactive substance (TBARS) was measured as the representative of lipid peroxide) and CoQ_{10} in the mitochondria, the method followed being that of PACKER[34] for the former measurements and that of ABE and associates[1] for the latter. Values for TBARS and CoQ_{10} were expressed in terms of the levels of proteins in the mitochondria as measured by the procedure of LOWRY and associates[23]. The amounts of CoQ_{10} in the myocardium were measured by means of high performance liquid chromatography (HPLC) under conditions for analysis proposed by ABE and associates[1]. Measurements of the amounts of myocardial VE were also carried out with the aid of HPLC under analytical conditions proposposed by KATO and associates;[18] the specimens of myocardial tissue were prepared before measurement according to the method developed by ABE and associates[2].

Values were expressed as a mean$\pm$standard error (M$\pm$SE). Statistical analysis was performed by means of Student t test, with $P<0.05$ considered significant.

Results

1. Variations in the level of TBARS present in the mitochondria (Fig. 1)

TBARS in the mitochondria was found to be 0.27 n moles/mg prot. (for the following figures the units of measurement will be omitted) just after the aorta had been clamped off. As the period of ischemia continued, this value increased: after 60, 120, and 180 minutes, the values obtained were 0.39 ± 0.06, 0.63 ± 0.18, and 0.67 ± 0.15, respectively, for the GIK group and 0.49 ± 0.04, 0.66 ± 0.06, and 0.65 ± 0.12, respectively, for the CBK group. In the case of the GIK group, the deviation was so great that no significant change could be confirmed, but in the CBK group the values obtained after ischemia had lasted 180 minutes were high enough to be considered significant when compared with the values measured immediately after the aorta had been clamped off. Unfortunately, a significant difference between the two groups could not be recognized.

2. Variations in the level of CoQ_{10} in the mitochondria (Fig. 2)

Immediately after the aorta was clamped shut, CoQ_{10} was measured in the mitochondria at a level of 13.8 ± 0.07 μg/mg prot. (for the following figures the units of measurement will be omitted). As the period of ischemia progressed, there was a tendency for this value to decline: after 60, 120, and 180 minutes, the values obtained were 12.8 ± 1.0, 11.9 ± 0.5, and 7.5 ± 0.5, respectively, for the GIK group. As these results show, the value obtained at 180 minutes

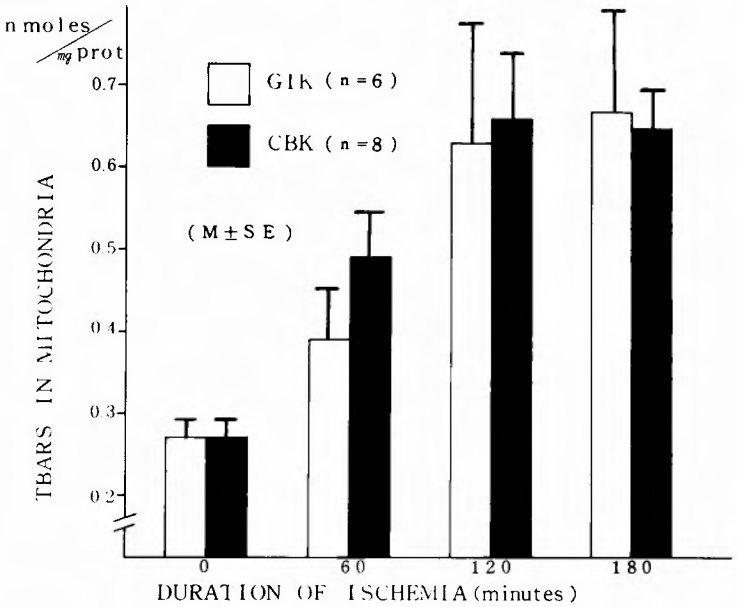


Fig. 1. Changes in TBARS in mitochondria are shown. The TBARS values are significantly increased beyond 60 minutes after aortic clamping, but there are no significant difference between both groups.

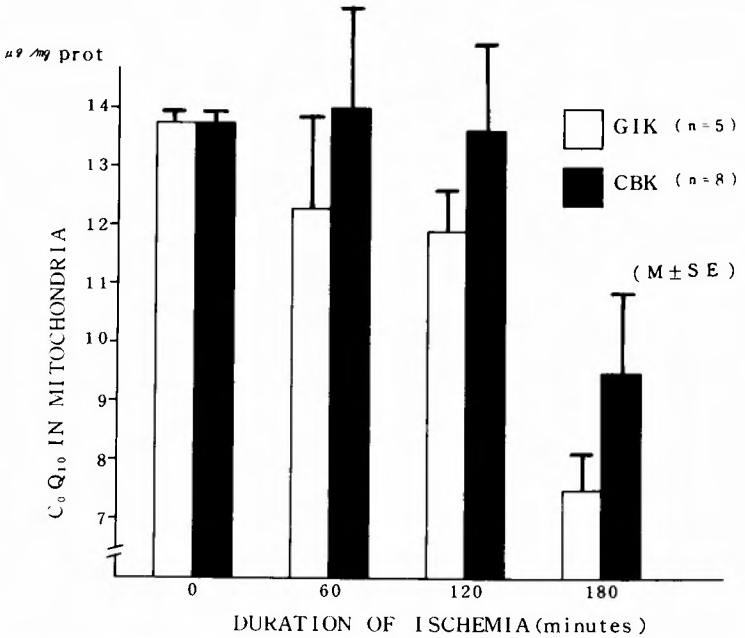


Fig. 2. Changes in mitochondrial CoQ_{10} values are shown. They are significantly decreased at 180 minutes after onset of ischemia in both groups. The CoQ_{10} values of CBK group always slightly higher than those of GIK group.

represents only 55% of the value measured just after the aorta was cross-clamped. In the case of the CBK group, the corresponding values were 14.0±1.5, 13.6±1.3, and 9.5±1.3--here, too, after 180 minutes there has been a decrease, in this case to 69% of the value seen just after a clamp was placed on the aorta. Values for the CBK group remained constantly higher than those of the GIK group, but not to a degree which could definitely be considered significant.

3. Variations in the level of myocardial CoQ_{10} (Fig. 3, Table 2 Table 3)

Fig. 3 presents the chromatograms obtained as CoQ was measured. CoQ_9 as well as CoQ_{10} is indicated in this case, but the retention time of CoQ_{10} is short—only 11 minutes. Table 2 gives a chronological list of changes in the amount of myocardial CoQ_{10} recorded when the myocardium was protected by GIKC Values of CoQ_{10} obtained for the outer layer of the myocardium at the onset of ischemia, and then as ischemia reached the 60-, 120-, and 180-minute marks, were 440±60 μg/gww (units of measurement omitted in the following figures), 439±61, 367±49, and 330±48, respectively; corresponding values for the inner layer of the myocardium equaled 465±66, 379±46, 325±42, and 262±36 at the same points in time. Both the inner and outer layers of the myocardium showed a decrease in CoQ_{10} values with the passage of time, but the rate of decrease in the inner myocardial layer was striking: after 60 minutes of ischemia, a statistically significant decrease had already occurred, the figure having dropped to 83% of the value recorded just after the aorta was cross-clamped. This decline continued subsequently, and the reading obtained at the 180-minute mark amounted to only 58% of the

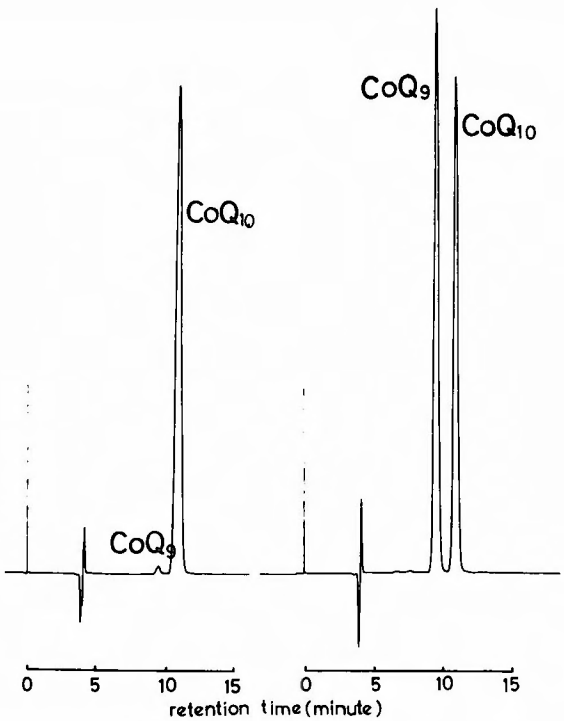


Fig. 3. Chromatograms of CoQ_{10} in the myocardium obtained from two dogs. CoQ_9 as well as CoQ_{10} was indicated in the second case.

Table 2. CoQ_{10} values in the myocardium protected by glucose-insulim-potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	240	200	137	140	238	190	128	112
2	688	568	430	425	706	536	411	314
3	447	492	371	293	406	365	317	239
4	427	526	485	475	536	446	435	401
5	299	266	309	233	310	290	274	241
6	537	580	471	416	595	445	382	267
M±SE	440±60	439±61	367±49	330±48*	465±66	379±46*	325±42*	262±36*

* $P < 0.05$

Per cent change in CoQ_{10} value of the ischemic myocardium protected by glucose-insulin-potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	100	83	57	58	100	80	54	47
2	100	83	63	62	100	76	58	44
3	100	110	83	66	100	90	78	59
4	100	123	114	111	100	83	81	75
5	100	89	103	78	100	94	88	77
6	100	108	88	77	100	75	64	45
M±SE	100	99±6	85±8	75±7	100	83±3	71±5	58±6

Significant decrease is found in the subepicardium at 180 minutes of the ischemic time and in the subendocardium after 60 up to 180 minutes of aortic clamping.
There is no significant difference between both layers.

first value. In the case of the outer layer of the myocardium, on the other hand, the decline in CoQ_{10} values remained relatively small: at the point the duration of ischemia had reached 180 minutes, the value of CoQ_{10} had only declined to 75% of the original value. Table 3 indicates variations in the amount of CoQ_{10} in the myocardium during myocardial protection by means of CBKC. With this method, a decrease over time is again evident for both the inner and outer layers of the myocardium, but it is slight in comparison with the figures for GIKC. When the period of ischemia had reached 180 minutes, the value of CoQ_{10} for the outer layer of the myocardium was 89% of the first value recorded, and 74% in the case of the inner layer—in other words, the decrease was 15% less than that observed with GIKC.

4. Variations of VE in the myocardium (Fig. 4, Table 4, Table 5)

Fig. 4 depicts the conditions for the analysis of VE and gives the chromatogram. Each homologue of VE is clearly separated and indicated; the retention time of these homologues is short—only 9 minutes. Changes over time in the values of myocardial α-tocopherol (α-tocopherol was chosen to represent VE becasue it is the most active biochemically of the

Table 3. CoQ_{10} values in the myocardium protected by cold blood potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	746	878	689	641	656	665	481	393
2	332	269	304	387	325	309	256	340
3	635	505	712	549	790	658	674	528
4	308	268	234	224	298	261	214	212
5	363	357	337	323	378	345	324	292
6	429	391	375	347	453	406	313	288
M±SE	469±67	445±86	442±77	412±58	483±74	441±66	377±64**	342±41*

* $P < 0.05$ ** $P < 0.005$

Per cent change in CoQ_{10} value of the ischemic myocardium protected by cold blood potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	100	118	92	86	100	101	73	60
2	100	81	92	117	100	95	79	105
3	100	94	112	86	100	83	85	67
4	100	87	76	73	100	88	72	71
5	100	98	93	89	100	91	86	77
6	100	91	87	81	100	90	69	64
M±SE	100	95±5	92±4	89±6	100	91±2	77±3	74±6

It is noted that CoQ_{10} values in the subendocardium are significantly decreased at 120 and 180 minutes after aortic clamping, but there is no significant difference between the subepicardium and subendocardium at each ischemic time.

homologues) are shown during protection of the myocardium by GIK or CBK cardioplegia in Tables 4 and 5, respectively. In the case of GIKC, as ischemia continued for 60, 120, and 180 minutes, the values at these points in time for the outer layer of the myocardium were 16.5 μg/gww (units of measurement omitted in the following figures), 15.2±2.2, and 15.1±2.2, while the corresponding values for the inner layer of the myocardium equaled 15.2±2.0, 14.0±2.0, and 13.6±2.2—in either case, hardly any change took place. Results with CBKC, at the points in time mentioned above, were 17.0±4.3, 17.3±4.5, and 16.3±3.6 for the outer layer of the myocardium, and 16.0±3.9, 16.9±4.7, and 16.8±4.7 for the inner layer. Again, there was practically no change discernible.

Discussion

Normally, the mammalian myocardium metabolizes through aerobic pathways, utilizing all substances as fuels. These include free fatty acids, glucose, ketones, lactate, pyruvate, etc. These substances ultimately transferred to the mitochondria, where the electron energy is released

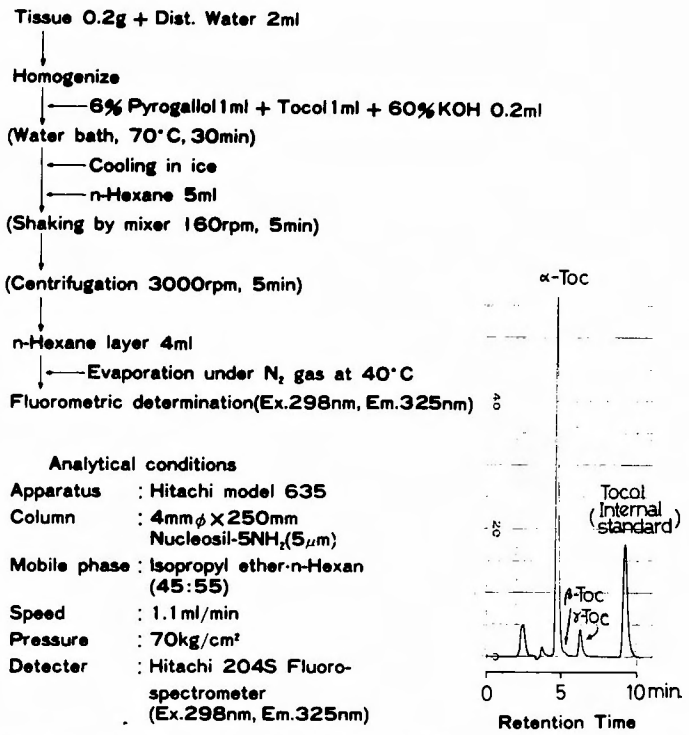


Fig. 4. HPLC analysis of tocopherols in the myocardium. Each homologue of vitamin E is well separated and indicated in the chromatogram.

through the TCA cycle and the respiratory enzyme chain. Such energy is trapped in orderly fashon and ultimately incorporated into ATP. The mitochondria comprise almost 40 to 50% of the average mammalian myocardium. The electron transport system is very active in the heart mitochondria. It is suspected that the mitochondria produce active oxygen species such as superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxy radical ($HO\cdot$) during oxidative metabolism[4]. These oxygen species are very reactive and moreover cytotoxic. Living organism are kept from the attack from these active oxygen radicals by metabolizing them to non-toxic chemical substances such as water and molecular oxygen.

By the way, it is generally recognized that, with the onset of ischemia, the level of CP and ATP in the myocardium falls off rapidly[5,16,17]. It is also well known that, if ishcemia is prolonged, the cellular structure disintegrates[10,11,40]. Exactly, what processes take place in the interior of the cell to produce such an outcome?

Since the supply both of oxygen and of energy substrates ceases when the myocardium experiences ischemia, the oxygen density in the vicinity of the mitochondria, which naturally has a low oxygen tension to begin with, falls even lower. Would any kinds of radicals be generated also in ischemia?

Demopoulos[8] has proposed the following hypothesis: since during ischemia there is a shortage of oxygen to serve as an acceptor of electrons, a surplus of electrons builds up in the

Table 4. α-tocopherol value of the ischemic myocardium protected by glucose-insulin-potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	22.6	21.5	18.4	15.2	22.5	16.4	14.0	12.7
2	13.4	12.9	11.0	14.2	13.0	12.3	11.2	11.7
3	12.0	13.1	12.4	12.9	11.8	13.0	12.4	11.8
4	28.5	25.1	25.8	26.6	29.4	25.8	24.5	25.4
5	12.7	13.3	12.5	11.9	12.4	12.6	11.7	10.6
6	11.3	13.0	10.8	9.7	11.8	11.2	10.4	9.1
M±SE	16.8±2.6	16.5±2.0	15.2±2.2	15.1±2.2	16.8±2.8	15.2±2.0	14.0±2.0	13.6±2.2

Per cent change in α-tocopherol value of the ischemic myocardium protected by glucose-insulin-potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	100	95	81	67	100	73	62	56
2	100	96	82	106	100	95	86	90
3	100	109	103	108	100	110	105	100
4	100	88	91	93	100	88	83	86
5	100	105	98	94	100	102	94	85
6	100	115	96	86	100	95	88	77
M±SE	100	101±4	92±3	92±6	100	94±5	86±5	82±6

There is no significant difference.

electron transport system and radicals of CoQ_{10} and flavin adenine dinucleotide (FAD) are formed; the polyunsaturated fatty acids of the cell lipids become acceptors of the CoQ_{10} and FAD radicals; a hydrogen atom is then abstracted from the vicinity of the double bond and alkyl radicals are formed. Since the unsaturated fatty acid radicals, which are unstable and highly reactive, react easily with the small quantity of ground state oxygen which exists in the mitochondria, a peroxidation chain reaction is initiated inside the membrane, with the result that such lipid peroxides as hyroperoxide and endoperoxide are produced and the chain reaction comes to an end, but not before the structure and functions of the membrane suffer damage. The results of the experiments described in the present paper lend support to DEMOPOULOS' hypothesis, for they indicate that TBARS is produced in the ischemic mitochondria. KOGURE[19,20], however, holds that lipid peroxides are not formed during ischemia, but rather when blood and oxygen are again supplied to the cells which have experienced ischemia, i.e. lipid peroxides are formed, in KOGURE's view, at the time of reperfusion (post-ischemic peroxidation).

According to KOGURE and associates[21], the pathological series of biochemical changes covered by brain ischemia can be classified into three phases. In the first phase, glycolysis in the cytoplasm is restricted, but there is as yet no disruption of the electron transport system.

Table 5. α-tocopherol value of the ischemic myocardium protected by cold potassium blood cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	9.7	8.5	11.2	11.1	10.5	9.9	10.8	10.5
2	37.7	38.9	41.1	35.8	39.0	36.2	41.7	41.7
3	7.4	8.3	7.2	8.9	7.9	7.5	7.5	7.4
4	16.3	18.8	16.6	16.8	17.0	16.5	16.9	17.4
5	13.3	13.1	13.5	12.7	11.5	11.2	10.9	10.1
6	15.4	14.3	14.0	14.2	15.8	14.6	13.8	13.5
M±SE	16.6±4.0	17.0±4.3	17.3±4.5	16.6±3.6	17.0±4.2	16.0±3.9	16.9±4.7	16.8±4.7

Per cent change in α-tocopherol of the ischemic myocardium protected by cold blood potassium cardioplegia.

	Subepicardium				Subendocardium			
	Time after aortic clamping (minutes)							
No.	0	60	120	180	0	60	120	180
1	100	88	115	114	100	94	103	100
2	100	103	109	95	100	93	107	107
3	100	112	97	120	100	95	95	94
4	100	115	102	103	100	97	99	102
5	100	98	102	94	100	97	95	88
6	100	93	91	92	100	92	87	85
M±SE	100	102±4	103±3	103±4	100	95±1	98±3	96±3

No significant difference is found.

Within this period, if blood flow is restored, NADH within the cytoplasm would be oxidized by means of the shuttle system and the electron transport system in the mitochondria and become NAD^+. The oxidation of substrates would recommence smoothly and, since electrons could then be transferred to the oxygen molecules in sufficient quantitites through the electron transport system, it can be supposed that practically no active oxygen would be generated. In the second phase, glycolysis in the cytoplasm comes almost to a standstill, but the electron transport system still remains intact. Within this period, even if blood flow is restored, it would not be possible to oxidize NADH in the cytoplasm, since the shuttle system would not function; accordingly, with no electrons flowing in the electron transport system of the mitochondria, oxygen within the mitochondria could only be partially reduced, and such types of active oxygen as $\cdot O_2^-$, H_2O_2, and $HO\cdot$ would be generated. In the third phase, the electron transport system in the mitochondria is damaged so that even if blood flow is restored and oxygen is again provided, no reduction of oxygen takes place because electrons are not being transported. KOGURE states, moreover, that if one can assume that peroxidation of lipids takes place owing to radicals lacking one electron which have come loose from the electron transport system in accordance with the mechanism postulated by DEMOPOULOS, then this peroxidation is part of the third phase of the

pathological series of biochemical changes subsumed by the term ischemia.

CBK cardioplegia is a method of myocardial protection which intermittently resupplies oxygen to the myocardial cells undergoing ischemia. Results of the experiments presented in this paper make clear that, with this method, TBARS does increase as the period of ischemia is prolonged. On the basis of these experiments, however, it cannot be asserted that with this method production of TBARS definitely occurs to a greater extent than in the case of GIK cardioplegia, which povides almost no supply of oxygen. In sum, results which would lend positive support to KOGURE's views were not forthcoming in the experiments presented here. This outcome may possibly be due to suppression of TBARS production resulting from the profound low myocardial temperature incidental to CBKC and GIKC, or it may be due to the difficulty of properly comparing the results here with KOGURE's since the experimental models were completely different. At any rate, there in no question as to the fact that lipid peroxides are generated inside cells once they have fallen into a state of ischemia. Lipid peroxides are known to cause cleavage of the polymerized polypeptides and also chemical changes in amino acids;[35,36] the importance of preventing the formation of lipid peroxides is readily understandable in view of the damage they cause to subcellular organelles[26,45].

Coenzyme Q (CoQ) was discovered by MOORE and colleagues[31] in 1940; in 1957 CRANE and associates[7] isolated it from lipids in beef heart mitochondria. It is now known to exist widely in almost all living organisms. Its basic molecular structure is 2,3-dimethoxy-5-methyl-1,4-benzoquinone with an isoprenoid side chain at C-6 (Fig. 5). CoQ_9 occurs in mammals together with CoQ_{10}, but in man this coenzyme is present only in the form CoQ_{10}[22]. CoQ, which in the electron transport system intermediates between the flavoprotein and cytochrome chains, is believed to function as a coenzyme promoting the production of ATP.

According to MICHELL, protons inside the inner membrane of the mitochondria are shifted to a position just inside its outer wall, where they increase the proton density; when the protons along the outer wall, with the help of ATPase, move to the inner wall of the inner membrane, ATPase is activated and the synthesis of ATP results. MICHELL takes the view that, as part of this mechanism, CoQ moves about freely in the inner mitochondrial membrane, transporting protons to both sides of the membrane (proton motive Q cycle)[29,30].

Given this interpretation of the function of CoQ, it is logical to suppose that, if CoQ were to be adversely affected in any way, then at that point production of ATP would cease. In the

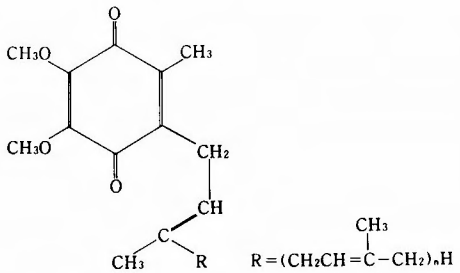


Fig. 5. Molecular structure of CoQ

course of the research described here, measurements of the amounts of CoQ_{10} in the myocardial cells made during myocardial protection by either GIKC or CBKC demonstrated, first, that with either method the levels of CoQ_{10} decreased with time; second, that this decrease was smaller in the case of CBKC than with GIKC; and thirdly, that the percentage of decrease was greater in the inner myocardial layer than in the outer. Here it may be noted that both YAMASAWA[49] and YAMAGAMI[48], with their respective colleagues, have confirmed, as a result of inducing cardiac infarction experimentally, that the level of CoQ_{10} in the affected areas of the myocardium was quite low compared with the unaffected areas; this observation agrees with the results obtained from the experimental model described here, which involved global ischemia.

How, then, is this decrease in CoQ_{10} to be interpreted? CoQ_{10} is not a vitamin, though it functions like one[39]. Unlike a vitamin, it is synthesized within the living organism from such materials as tyrosine, phenylalanine, and various vitamins and minerals. In ischemic myocardial cells, it may be assumed that, since substrates for the synthesis of CoQ_{10} are not being supplied, there is no production of CoQ_{10} going on. This being the case, the fact that CoQ_{10} decreases suggests that CoQ_{10} is being consumed. Quite possibly cleavage of the isoprenoid side chain takes place, so that a transformation results either into some types of CoQ other than CoQ_9 and CoQ_{10}, or into a different chemical substance. While the reason why it was possible to maintain a higher level of CoQ_{10} with CBKC than with GIKC is not clear, it may be that the constituents of the blood include substances from which CoQ_{10} is synthesized. Furthermore, the somewhat larger decrease in the amounts of CoQ_{10} observed in the inner myocardial layer in comparison with the outer layer suggests that there is a difference in the metabolism of CoQ_{10} between these two muscle layers.

It has been reported that CoQ_{10} has an inhibiting effect on the formation of lipid peroxides[27]. Accordingly, on the assumption that exogenous CoQ_{10} would be absorbed into the myocardial cells if administered, it is logical to suppose that it would inhibit the production of lipid peroxides which are thought to be generated during ischemia or reperfusion, and thereby make it possible to protect the functions and structure of the myocardial cells during ischemia or reperfusion. Fortunately it has been demonstrated that CoQ_{10} administered as a drug is incorporated into the inner membrane of the mitochondria;[32] furthermore, ABE and associates[3], MATSUNAGA and associates[25], and TAKEUCHI and associates[44] administered CoQ_{10} either into a vein or, along with the protective solution for the myocardium, into a coronary artery, and all reported success in alleviating a decrease in high-energy phosphate compounds. Furthermore, TANAKA and associates[43] confirm the effectiveness of CoQ_{10} for clinical use. Having administered CoQ_{10} orally to patients scheduled to undergo surgery for valvular disease, they found that, after valve replacement surgery, the group which had received CoQ_{10} showed a lower incidence of low cardiac output syndrome than the control group. Opinion remains divided, however, on the methods of administering CoQ_{10} and on the dosage, topics which should receive careful consideration in the future.

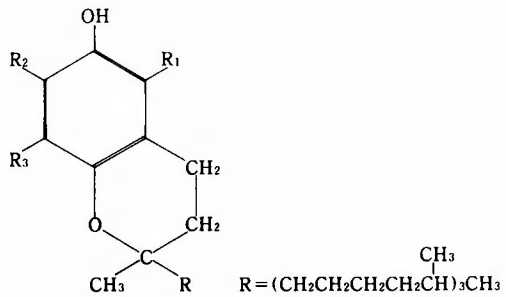
VE was first discovered as an anti-infertility vitamin by EVANS and BISHOP in 1922; since then, its homologues have been named α-, β-, γ-, and δ-tocopherol in the order of their effectiveness

in combating infertility. Their molecular structure are shown in the Figure 6. While the role of VE in metabolism is still not entirely understood, it is generally recognized to have an antioxidant effect. Among the more important observations upon which this conclusion is based are the following: 1) VE prevents the oxidation of such substances as unsaturated fatty acids and vitamin A; 2) certain other antioxidants are effective as substitutes for VE in some types of VE deficiency;[12] 3) the amount of lipid peroxides increases in animals suffering from VE deficiency;[42] 4) when VE is administered to animals, the amount of lipid peroxides in the liver cells decreases[24].

VE is one of the vitamins which are soluble in lipids. In the blood stream it is bonded to β-lipoprotein; in the cell membrane it is bonded to this lipoprotein and forms part of the cell membrane[33]. VE is thought to contribute to the stability of the cell membrane by eliminating active oxygen species and unsaturated fatty acid radicals and preventing thereby the formation of lipid peroxides[14,28,46] (Fig. 7).

As far as the results of the present experiments are concerned, it was not possible to confirm changes over time in the amount of α-tocopherol present in the myocardium during either CBKC or GIKC. In view of the fact the VE makes up part of the cell membrane, as was stated above, it was expected that a decrease in VE would take place about 150 minutes after the onset of ischemia, the time when the morphological disintegration of the cell membrane is observed, but such a decrease was not evident for reasons which are not clear.

VE and CoQ are similar in their chemical structures, but as part of the biochemical reactions which sustain life they perform completely different functions. VE is a chromanol; the quinone analogue of VE corresponding to CoQ is plastoquinone. This substance does not work like VE nutritionally, but is involved in photosynthesis. CoQ, on the other hand, is a quinone; chromanol of CoQ which corresponds to VE evinces no sort of biological activity. FOLKERS[13] has established that VE cannot take the place of CoQ in the function of coenzyme. He also states that: 1) in human beings, deficiency in VE causes widespread damage to lipids, including damage to



-Tocopherol	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3

Fig. 6. Molecular structure of vitamin E.

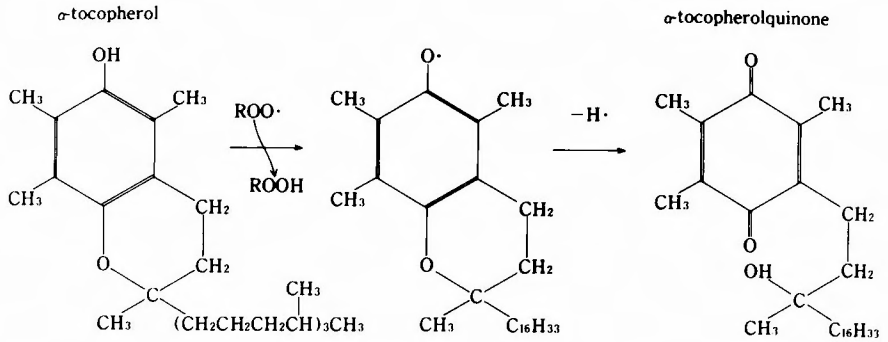


Fig. 7. Chemical reaction between α-tocopherol and a lipid peroxide free radical is shown. α-tocopherolquinone, a quinone form of α-tocopherol, is formed as a result of oxidation of α-tocopherol by the radical, which is reduced to a fatty acid.

CoQ and CoQ precursors, as a result of oxidation; 2) when VE deficiency is not accompanied by impairment of CoQ biosynthesis, the situation can be corrected by administering VE, which not only prevents the disintegration of CoQ and its precursors, but also eliminates damage stemming even from extensive lipid peroxidation; 3) if biosynthesis of CoQ is disrupted, a deficiency of VE intensifies the deficiency of CoQ and its precursors in VE deficient patients by administering VE in medicative dosage, CoQ is not fully restored in cases where biosynthesis of CoQ has been seriously impaired.

EDWIN and associates[9] have reported that myocardial CoQ decreases in animals suffering VE deficiency, and similar results were obtained in research with VE deficient hamsters conducted by the present author (Table 6). Such observations support the view that there is a close relationship between VE and CoQ. SUGIYAMA and associates[41] state that in suppressing the formation of lipid peroxides CoQ_{10} has the greater effect in the myocardium, but in the liver VE is more

Table 6. Changes in the amounts of serum α-tocopherol, myocardial α-tocopherol, and myocardial CoQ_{10} of hamsters fed on either VE deficient or VE contained diets.

	α-tocopherol in serum (μg/ml)	α-tocopherol in myocardium (μg/g.w.w.)	coenzyme Q_{10} in myocardium (μg/g.w.w.)
Group I	0.40±0.08	ND	33.8±5.3
Group II	20.5±4.5	17.3±5.3	43.0±4.8
Group III	9.2±1.2	3.1±2.3	46.5±4.4

Each value is expressed as mean±SD.
Group I: fed on VE deficient diet (corn oil added) (n=6)
Group II: fed on VE added diet (corn oil added) (n=6)
Group III: fed on VE normal diet (corn oil added) (n=6)
ND: not detected

effective. Accordingly, the results of the experiments described in the present paper could be interpreted to mean that CoQ_{10}, in producing a greater suppressive effect on the formation of lipid peroxides in the myocardium, was consumed to a greater degree than VE.

In reporting on their work with rabbits, however, GUARNIERI and colleagues[15] state that, by administering VE, it proved possible: 1) to preserve mitochondrial function in hypoxic perfused rabbit heart muscle; 2) to limit the decrease in high-energy phosphate compounds; 3) to prevent reoxygenation injury at the time of reoxygenation. On the basis of these results, it may be suggested that, like CoQ_{10}, VE would be an effective drug for use in myocardial protection, provided that it can be incorporated into the myocardial cells when administered. (In research not yet published, the author of the present paper discovered that levels of α-tocopherol in the right atrial appendage approximately trebled as a result of the administration, orally, of α-tocopherol nicotinate.)

Summary

When ischemia of the myocardium was induced by occlusion of the aorta, and myocardial protection was carried out by means of either cold blood potassium cardioplegia or glucose-insulin-potassium cardioplegia, the following observations were made:

1. It was possible to confirm that an increase over time took place in lipid peroxides in the mitochondria of the myocardium. It could not, however, be definitely concluded that the formation of lipid peroxides was greater in the case of CBKC than in the case of GIKC.
2. A decrease over time was recognized in levels of CoQ_{10} in the myocardium and in the mitochondria. This decrease was especially marked in the case of GIKC, and was more conspicuous in the inner myocardial layer than in the outer.
3. The amount of VE in the myocardium showed almost no change.

From these results, it might be concluded that the formation of lipid peroxides and thereby the depletion of CoQ_{10} in the myocardium, especially in the inner myocardial layer, play an significant role to produce cellular damages caused by ischemia of the myocardium.

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和文抄録

心筋保護法に関する実験的研究について
特に Cold Blood Potassium Cardioplegia について
II 虚血による細胞障害機序

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好気性代謝を営む心筋細胞が虚血に陥った場合，クレアチン燐酸（CP）やアデノシン3燐酸（ATP）が急速に減少する．もし，虚血が長時間に及べば，細胞機能の低下や細胞構築の崩壊を余儀なくされる．

最近，このような虚血による細胞障害の発生機序の1つとして，フリーラジカルや過酸化脂質の関与が注目されている．

大動脈遮断下虚血心筋細胞の保護手段として数々の方法が試みられているが，今回，非血液性成分を用いた心筋保護法の1つとしてグルコース，インシュリン，カリウムから成る GIK cardioplegia（GIKC）を，また血液そのものを用い，cold blood potassium cardioplegia（CBKC）をおこない，虚血心筋細胞内の過酸化脂質，コエンザイム Q_{10}（CoQ_{10}），ビタミンE（VE）の含量を測定し，それらの経時的変化を検討したところ，以下の結果が得られた．

1) ミトコンドリア内の過酸化脂質は両法ともに経時的に増加し，虚血180分後には虚血直後値の2倍以上に達した．間歇的に虚血心筋細胞に酸素を与える CBKC が GIKC に比して過酸化脂質の生成が多いとは必ずしも言えず，低温によって過酸化脂質の生成が抑制される可能性があることを示唆していた．

2) ミトコンドリア及び心筋内 CoQ_{10} 含量は，両法とも経時的に減少するが，その減少率は GIKC の方が CBKC よりも約15%大きく，また，心内膜側心筋の減少率は心外膜側心筋のそれよりも著明だった．この結果は，CoQ_{10} の代謝に関して心筋内外両筋層間に差異があることを示唆するとともに，CoQ_{10} の維持という点では CBKC は GIKC よりも優れていることを示唆していた．

3) 両法とも，心筋内 VE 含量にはほとんど変化が認められなかった．この結果は，同じく遊離基消去物質でありながら CoQ_{10} と VE の消耗度は臓器によって異なり，心筋においては CoQ_{10} の方が消耗が大きく，より強い抗酸化作用を発揮していることを示唆していた．