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The pathophysiological effects of brain death on potential donor organs, with particular reference to the heart

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Summary

Major electrocardiographic, haemodynamic, and histopathological changes take place during the development of brain death; myocardial and pulmonary injury may result. Significant depletion of certain circulating hormones occurs, resulting in an inhibition of mitochondrial function, leading to reduced aerobic metabolic oxidative processes, affecting the body as a whole. Major organ energy stores are therefore diminished, leading to deterioration of function. Replacement of the depleted hormones, in particular triiodothyronine (T_3), cortisol, and insulin, leads to rapid replacement of organ energy stores, associated with a return to normal function. T_3 alone leads to reactivation of the mitochondria, stimulating aerobic metabolism. Hormonal therapy to brain-dead potential organ donors has been shown to lead to metabolic and haemodynamic stability, resulting in no wastage of organs, and in improved function after transplantation.

Introduction

While developing a system of hypothermic perfusion storage of the heart *(1,2)*, we made an interesting observation which strongly suggested that brain death was deleterious to cardiac function. Using continuous hypothermic perfusion, storage of hearts (taken from healthy anaesthetised baboons) for periods of up to 48 h was followed by immediate good function and the long-term survival of recipient baboons into whom these hearts were orthotopically transplanted. When hearts taken from human brain-dead donors were stored by the same system, however, following heterotopic transplantation a delay of several hours in the return of good myocardial function was observed. As the storage system utilised in these patients was identical to that used in our animal studies, it appeared that this delayed function must presumably result from a loss of myocardial energy stores and/or other reversible damage sustained during and following the onset of brain death, this being the only obvious difference between the two groups.

These observations led us to undertake a series of experiments in the baboon and pig to elucidate the effects on myocardial function of brain death and the subsequent management of the brain-dead donor.

Methods of induction of brain death in the experimental animal

Two different methods of inducing brain death were employed. Both were followed by almost identical haemodynamic changes, and therefore the differences in technique of induction of brain death do not appear important. In the majority of our studies in the baboon, brain death was induced by a sudden increase in intracranial pressure brought about by inflation of the balloon of a Foley catheter placed within the skull. In the majority of our studies in the pig, and also in a small group of baboons, brain death was induced by clamping or ligation of the two major arteries which arise from the arch of the aorta and supply the upper part of the body; as both the carotid and vertebral arterial supplies were interrupted, this brought about sudden ischaemia of the brain.

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Pathophysiological changes occurring during the development of brain death

Two major effects of brain death have been observed in these studies *(3)*. The first is a series of major electrocardiographic, haemodynamic, and histopathological changes which take place during and immediately following the agonal period, and which result from the 'autonomic storm' (ie intense autonomic nervous system activity) that occurs during the development of brain death. The second consists of significant changes in circulating levels of certain hormones, which in turn result in major changes in body metabolism; major organ energy stores are diminished, leading to deterioration of function.

'AUTONOMIC STORM'

The sudden increase in intracranial pressure brought about by inflation of an indwelling Foley balloon, or the sudden onset of ischaemia brought about by ligation of the major vessels to the brain, led to a series of major pathophysiological changes which may be collectively referred to as the 'autonomic storm'. Though there is a brief initial period of excessive parasympathetic activity, evidenced by a marked bradycardia, most of the effects of this autonomic stimulation are brought about by the sympathetic nervous system *(4)*; the terms 'sympathetic' or 'catecholamine' storm have also been used to describe these events.

There was a large increase in circulating catecholamines in the few minutes after induction of brain death, which was associated with an increase in myocardial activity, together with the appearance on the electrocardiogram (ECG) of ventricular arrhythmias *(3)*. Circulating catecholamines increased significantly 5 min after inflation of the Foley catheter balloon. Adrenaline concentration rose elevenfold over baseline levels ($P<0.001$), noradrenaline threefold ($P<0.01$), and dopamine twofold ($P<0.05$). These levels returned to control values 10 min later. By the 3rd hour, catecholamine levels had decreased further, below baseline values; only the reduction in noradrenaline, however, was significantly below control value ($P<0.05$).

The haemodynamic changes observed in these animals reflect the body's attempts to compensate for the intracranial changes taking place during 'coning' (Cushing's reflex) *(5)*. Significant and often massive increases in systemic vascular resistance (SVR) and mean arterial pressure (MAP) occurred (Fig. 1), and were almost certainly the direct result of a great increase in sympathetic nervous activity, which produced an extreme degree of peripheral vasoconstriction *(6)*. Blood was therefore redistributed into the capacitance vessels, leading to a rapid accumulation within the great veins and right atrium. Due to a combination of low pulmonary vascular resistance (PVR), high pulmonary vessel compliance, and pulmonary capillary reserve recruitment, associated with a higher degree of right ventricular compliance compared to the left ventricle, the right ventricle was able to adjust to this increased venous return, and increase its output, demonstrated by a statistically significant increase ($P<0.01$) in pulmonary artery (PA) flow compared with aortic flow at this time.

In the majority of animals studied, the left atrial pressure (LAP) actually exceeded the PA pressure for a matter of seconds during the period of peak peripheral

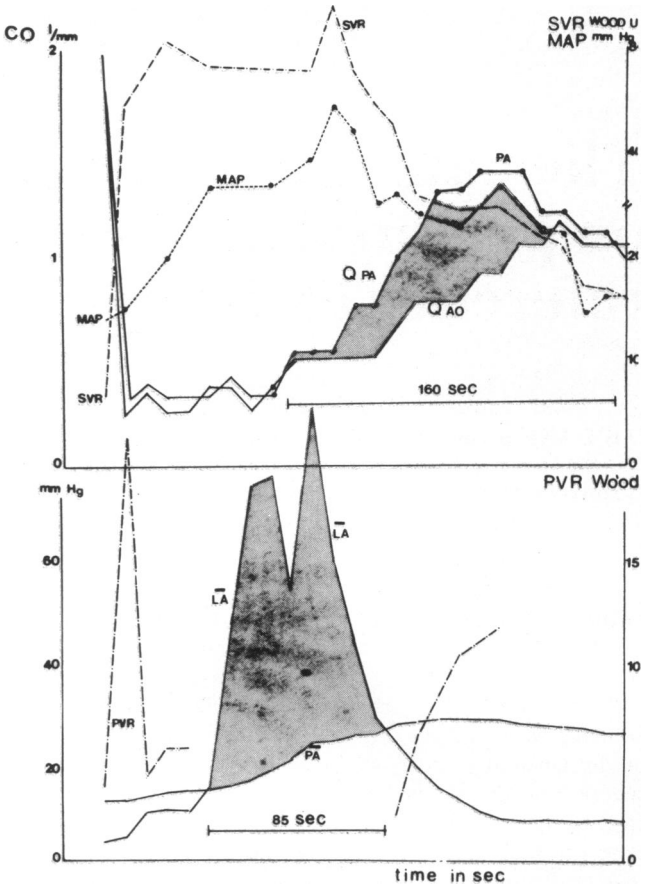

FIG. 1 Systemic and pulmonary haemodynamic data during induction of brain death in the Chacma baboon.

The upper graph shows changes in systemic vascular resistance (SVR) (Wood units), mean atrial pressure (MAP) (mmHg), pulmonary artery blood flow (PA) (l/min) and aortic blood flow (AO) (l/min). The discrepancy between pulmonary artery and aortic blood flows (shaded area) represents the period and extent of blood pooling within the lungs; in this case, blood pooling extended for a period of 160 s.

In the lower graph, changes in mean left atrial pressure (LA) (mmHg), mean pulmonary artery pressure (PA) (mmHg) and pulmonary vascular resistance (PVR) (Wood units) are shown. The shaded area represents the period of 85 s during which the left atrial pressure exceeded the pulmonary artery pressure.

vasconstriction (Fig. 1). This remarkable and surprising observation implies that the pulmonary capillary blood flow temporarily ceased entirely. It would seem likely that it is during this period that disruption of the normal pulmonary capillary anatomy could occur. As the peak LAP far exceeded the normal hydrostatic pulmonary capillary pressure, capillary integrity within the lungs would be disrupted, resulting in pulmonary oedema with high protein content and interstitial haemorrhage; this, in fact, occurred in 36% of cases *(7)*.

During the agonal phase, major structural damage also occurred to the myocardium (Fig. 2) *(3, 6)*. Evidence would suggest that this tissue damage was related to the release of endogenous catecholamines from sympathetic nerve endings in the myocardial cells, possibly leading to increased calcium uptake within the sarcoplasmic reticulum. In addition, there was almost certainly a temporary decrease in coronary blood flow during the period of tachycardia, associated with ECG features of ischaemia.

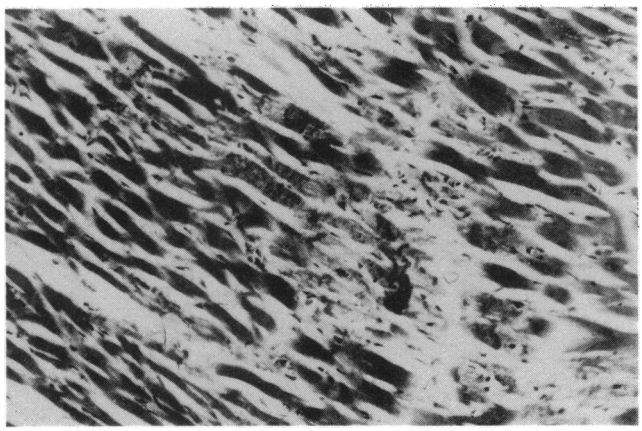

FIG. 2 Microscopic section of myocardium from brain-dead baboon showing widespread myocytolysis, smaller areas of coagulative necrosis and interstitial oedema, and occasional contraction bands. (Haematoxylin and eosin ×150.)

Injury was also observed in the conduction tissue of the heart *(7)*, and this may be responsible for the reported cases of donor heart failure from heart block or arrhythmia occurring soon after transplantation. Similar changes were also seen in the smooth muscle of the wall of the coronary arteries *(7)*, almost certainly reflecting severe vasospasm occurring during the catecholamine storm, and contributing both to the ischaemic changes seen on ECG and to histopathological damage of the myocardium; this may also be a factor in early failure of the donor heart after transplantation *(8)*.

ENDOCRINE CHANGES OCCURRING DURING BRAIN DEATH

In the baboon, the thyroid hormones plasma-free triiodothyronine (T_3) and thyroxine (T_4) fell sharply to 50% of control values ($P<0.05$) by the end of the 1st hour; no circulating T_3 or T_4 was detectable 16 h after the induction of brain death ($P<0.0001$) *(3)*. Thyroid stimulating hormone (TSH) showed no significant change from the baseline level.

Plasma cortisol level rose in all animals during the first 5 min, then declined progressively to 50% of baseline values at 1 h ($P<0.05$); by 16 h, a further decline had occurred ($P<0.0001$) *(3)*. Plasma insulin also fell in all baboons during the first 5 min; by 1 h the fall was significant ($P<0.05$), and by 13 h the level had reached 20% of the baseline level ($P<0.005$) *(3)*.

Antidiuretic hormone levels fell significantly in all animals, disappearing from the plasma within 6 h *(3)*. Unless fluid replacement was given actively, urine output ceased between the 2nd and 3rd hour of observation, by which time MAP had fallen to approximately 45 mmHg. When no fluid replacement was given, mean total urine output in baboons was 500 ml. If fluid replacement was given, the baboon continued to pass large quantities of urine throughout the experimental period, the mean total output reaching 8 litres in 24 h.

Blood levels of glucagon and ionised calcium showed no significant changes from baseline values *(3)*.

In the brain-dead pig we have also confirmed lower levels of plasma-free T_3, though cortisol and insulin appeared to be in the low normal range. Similar observations have been made in brain-dead potential organ donors, as will be discussed later.

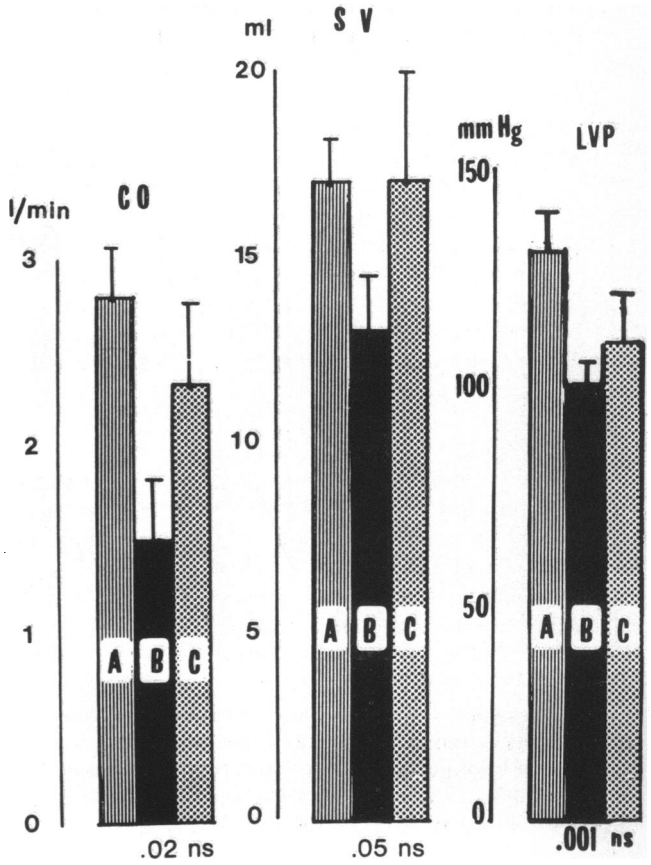

FIG. 3 Cardiac output (CO), stroke volume (SV), and left ventricular pressure (LVP) in (A) freshly excised hearts, (B) hearts taken from brain-dead pigs, and (C) hearts taken from brain-dead pigs which had received hormonal therapy. The statistical differences between the control group (A) and the two experimental groups (B) and (C) are shown.

Effects of brain death on myocardial function and metabolism

These effects were tested by excising pig hearts and functionally testing them on a system which has been described in detail previously *(1)*. The results obtained by this system of *ex vivo* functional testing of isolated hearts have been shown to reflect accurately the performance of the heart following orthotopic transplantation.

In selected hearts, the myocardium was biopsied immediately before undergoing functional testing, for the estimation of adenosine triphosphate (ATP), creatine phosphate (CP), lactate, and glycogen, using standard techniques *(9)*.

When the haemodynamic status of a pig was maintained for 4 h from the time of onset of brain death, significant deterioration of subsequent myocardial function occurred (Fig. 3) *(10)*. This was associated with a reduction in myocardial energy stores (CP and glycogen) (Fig. 4), though these appeared to be sustained in part by anaerobic metabolism, as evidenced by a rise in tissue lactate. It seems likely that ATP levels were sustained at the expense of CP.

It would appear, therefore, that brain death in pigs is followed by a consumption of myocardial energy stores which, despite anaerobic glycolysis, the brain-dead animal is unable to replenish. This is associated with reduced myocardial function. Studies in the baboon lend support to this conclusion *(3)*.

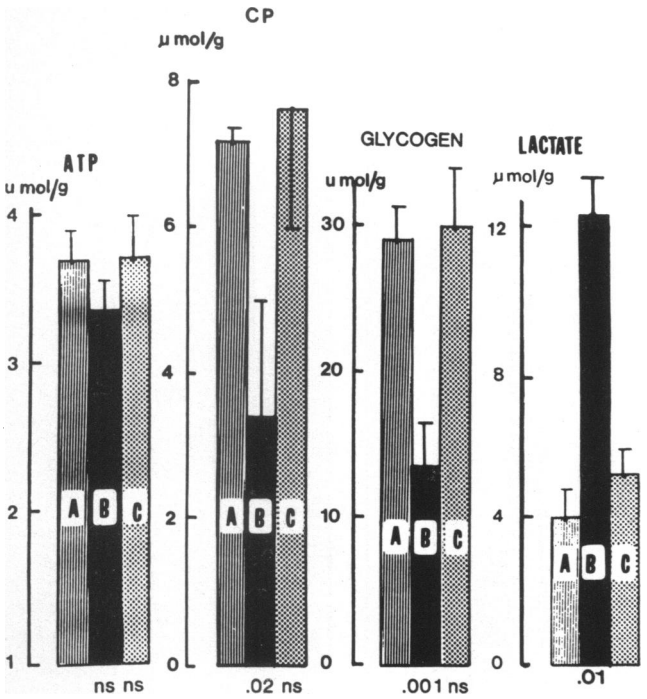

FIG. 4 Adenosine triphosphate (ATP), creatine phosphate (CP), glycogen, and lactate in (A) freshly excised hearts, (B) hearts taken from brain-dead pigs, and (C) hearts taken from brain-dead pigs which had received hormonal therapy. The statistical differences between the control group (A) and the two experimental groups (B) and (C) are shown.

Confirmation of change from aerobic to anaerobic metabolism in the brain-dead baboon

A series of experiments was performed to monitor aerobic and anaerobic metabolism in the baboon, both before and after brain death. Details of the experimental techniques have been described elsewhere *(11)* and will not be elaborated here, but the method was briefly as follows. The kinetics following single bolus injection of labelled ^{14}C–R glucose, pyruvate, and palmitate, and subsequent measurement of both plasma activity and of exhaled ^{14}C–CO_2 were used to study metabolite utilisation under conditions of (i) sedation and (ii) brain death in the same animal. Blood was also taken for serial measurements of serum lactate and plasma-free fatty acids.

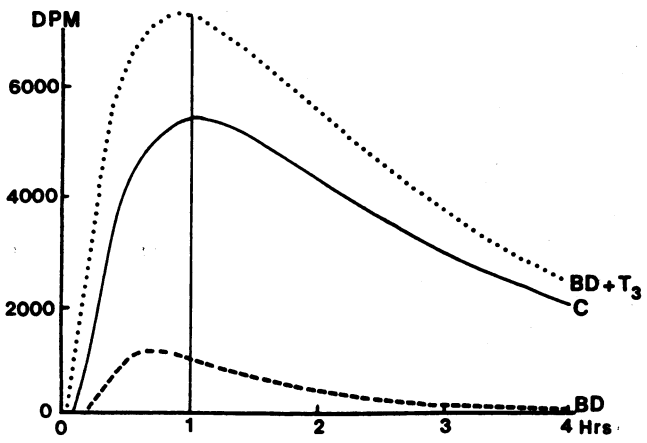

FIG. 5 Best fit data curves plotted for expired $^{14}CO_2$ following a single intravenous injection of ^{14}C-1-palmitate in the baboon. CO_2 counts were accumulated for 1 h. Carbon dioxide production was measured during (i) a period of sedation (C), (ii) 12 h after the induction of brain death (BD), and (iii) following the initiation of therapy with triiodothyronine 17 h after the induction of brain death (BD+T_3). (DPM=disintegration counts/min.)

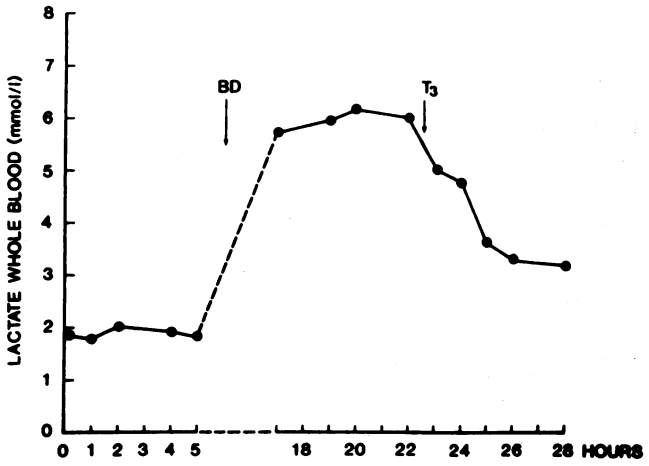

FIG. 6 Serum lactate levels in the baboon during (i) a period of sedation (0–5 h), (ii) 12 h after the induction of brain death (BD) (18–23 h), and (iii) following the initiation of triiodothyronine (T_3) therapy 17 h after the induction of brain death (23–28 h).

Our findings demonstrated that there was a major change in metabolic oxidative processes following brain death (Fig. 5) *(11)*. The rates of glucose, pyruvate, and palmitate utilisation were markedly reduced, and there was an accumulation of lactate (Fig. 6) and free fatty acids in the plasma. Pyruvate and palmitate can only be oxidised in the mitochondria; the reduction in their metabolism indicated mitochondrial inhibition.

These findings indicated an inhibition of aerobic metabolic rate, almost certainly from an inhibition of mitochondrial function, affecting the body as a whole, and correlated well with our previous findings related to metabolism in the heart alone. High-energy phosphates will be rapidly depleted (presumably from all major organs *(12)*) under this changed metabolic environment, almost certainly leading to deterioration in function of all organs.

Hormonal therapy in the brain-dead experimental animal

Noting the deterioration in cardiac function, depletion of myocardial energy stores, and lactic acidosis which occurred after brain death, consideration was given as to whether these effects resulted from the depletion in circulating hormones such as T_3, cortisol and insulin. A further group of pigs was studied in which these three hormones were administered after brain death *(10)*.

The administration of the hormonal mixture of T_3, insulin and cortisol led to improvement in myocardial function (Fig. 3) *(10)*. It is particularly notable that cardiac output returned to normal.

Creatine phosphate and glycogen also returned to normal levels, and lactate levels were correspondingly reduced (Fig. 4) *(10)*; hormonal therapy therefore stimulated the rate of aerobic metabolism in the myocardium, which is reduced in brain-dead pigs. By stimulating aerobic metabolism, myocardial high-energy stores were replaced and maintained.

Effects of T_3 therapy on aerobic metabolism in the brain-dead baboon

Using the same technique of single bolus injection of labelled glucose (^{14}C–R) (as described earlier) *(11)*, the effects of T_3 therapy in the brain-dead baboon were studied. Only T_3 was administered, as it seemed likely that it was this hormone that had the major effects on cellular metabolism.

The administration of 2 micrograms of T_3 at hourly intervals to the brain-dead baboon resulted in a dramatic reversal in the rate of metabolite utilisation (Fig. 5), and in reductions in plasma lactate (Fig. 6) and free fatty acids *(11)*.

These changes indicated stimulation of aerobic metabolism, resulting in a reversal from anaerobic to aerobic metabolism in the brain-dead animal. This observation correlated well with the earlier studies which showed replacement of myocardial energy stores and improvement in myocardial function following hormonal therapy.

Results of hormonal therapy in human brain-dead potential organ donors

Following these experimental observations, all brain-dead potential donors referred for heart transplantation at Groote Schuur Hospital, Cape Town (and subsequently at Baptist Medical Center, Oklahoma City), have been treated with hormonal therapy. An initial series of haemodynamic and metabolic observations was made in 21 brain-dead potential donors who received hormonal therapy (Group B) and compared with those in 26 who did not (Group A) *(13)*. Donors in both groups were treated by standard therapy, though those in Group B received additional intraveneous T_3 (2 micrograms), cortisol (100 mg), and insulin (10–30 IU) when first seen. This therapy was repeated at hourly intervals, depending on the condition of the donor and his response to the therapy, until the heart was excised.

Table I shows the significant changes that were noted during the course of management of the donors. Group B donors showed significant improvements in cardiac function compared with those in Group A *(13)*.

These observations provided further evidence of the impairment of aerobic metabolism which occurs in brain-dead donors. The increasing anaerobic metabolism (as evidenced by a low pH, large base deficit, rise in serum pyruvate and lactate, and repeated and increasing need for bicarbonate administration) was associated with diminished myocardial function (as evidenced by low cardiac output, low MAP, high central venous pressure, appearance of abnormalities on ECG, and need for inotropic support).

Hormonal therapy for even as short a period as 3 h was followed by evidence of increasing aerobic metabolism and improving myocardial function. The optimum dosage of T_3 is still uncertain, but recent experience would suggest that it may be more than the 2 micrograms/hr given in this series. Dosage should be judged by the haemodynamic and metabolic response obtained. The great improvement in acid–base balance and associated improvement in the haemodynamic status of the potential donor following hormonal therapy have enabled us to salvage some donor hearts which would previously have been unusable, and also to transplant all hearts in as good a condition as possible.

TABLE I *Initial and final haemodynamic and metabolic observations in brain-dead donors at Groote Schuur Hospital*

Group	*n*	*Timing of Observations*	*Temperature (°C)*	*MAP (mmHg)*	*CVP (cmH_2O)*	*Heart rate (beats/min)*	*Dopanine requirement (μg/kg/min)*	*Base deficit (mmol/l)*	*Bicarbonate requirement (ml (8.4%))*	*Serum lactate (mmol/l)*	*Serum pyruvate (mmol/l)*
A (Received no hormonal therapy)	26	Initial	33.7 (0.64)	79.9 (6.81)	9.1 (2.70)	72.5 (6.30)	14.4 (3.71)	−12.0 (2.03)	113 (31)	—	—
		Final	33.3 (0.70)	82.4 (3.26)	11.8 (2.24)	83.6 (7.40)	19.3 (2.90)	−7.4 (1.40)	225 (33)	—	—
		P	NS	NS	NS	NS	<0.05	<0.005	<0.0003	—	—
B (Received hormonal therapy	21	Initial	32.5 (0.71)	56.1 (14.4)	11.3 (6.36)	67.4 (10.61)	27.0 (10.36)	−10.6 (3.60)	191 (77)	5.1 (0.97)	0.29 (0.030)
		Final	35.6 (0.80)	86.1 (3.60)	7.3 (3.77)	35.6 (0.80)	13.0 (2.73)	−2.4 (1.64)	100 (7)	2.4 (0.32)	0.16 (0.020)
		P	<0.05	<0.02	<0.02	<0.003	<0.005	<0.0002	<0.0001	<0.02	<0.005

P=statistical difference between initial and final observation.
Figures in parentheses refer to standard error.
MAP=Mean Arterial Pressure.
CVP=Central Venous Pressure.
NS=Not significant.

Immediate and long-term function of these hearts after transplantation has been excellent, except where affected by acute or chronic rejection. We have subsequently treated a further 70 consecutive donors with hormonal therapy (at Groote Schuur Hospital, Cape Town, and Baptist Medical Center, Oklahoma City); no donor proved unsuitable for transplantation and no recipient has died from low cardiac output failure within the first few days after operation.

Conclusions

Our findings from both the brain-dead animal and human potential organ donor suggest that, after brain death, inhibition of a common oxidative pathway for carbohydrates and fatty acids takes place in the mitochondria, which are the main site of O_2 utilisation and CO_2 production. In the absence of T_3, aerobic metabolism in the mitochondria is inhibited, and anaerobic cellular metabolism takes place.

Following T_3 therapy, the rapid increases in glucose, pyruvate, and palmitate utilisation and in CO_2 production, and the normalisation of lactate and free fatty acid metabolism, indicate reactivation of the mitochondria, resulting in aerobic energy generation. Oxidation of Krebs' cycle intermediates occurs with accompanying oxidative phosphorylation. This may be related to the non-DNA-dependent (short-term) effects of T_3, possibly mediated by increases in the calcium concentration at intracellular sites and by normalisation of ionic calcium compartmentalisation, activating key enzymes such as the pyruvate dehydrogenase complex and the adenine nucleotide translocase system; it is also known that T_3 stimulates ATPase systems of various kinds. The short period of time required following the administration of T_3 to obtain this response would suggest that DNA-linked T_3 receptors and mRNAs do not play an initial role.

The roles of corticosteroid and insulin therapy in the improvement of donor heart (or other organ) function are less clear, and have been discussed elsewhere *(11)*.

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