Non-Utilization of Succinate and Metabolism of Lactate as Non-Invasive Criteria of Viability in the Isolated Perfused Canine Heart at 4°C

The existence of a discrepancy between the consumption of oxygen and the disappearance of exogenous glucose during the maintenance of the isolated perfused heart is well-established at normal temperatures[1]. Recent findings[2] in the canine heart at 4 °C have revealed a similar picture; while the average oxygen consumption is 6 mmoles/heart/24 h, the average glucose disappearance is only 0.2 mmoles/heart/24 h. In an attempt to discover exogenous substances other than glucose which would compete for oxygen more effectively than endogenous sources of energy, the sodium salts of various organic acids have been introduced into the perfusates[2]. When sodium succinate was added, the results were not only unexpected, but, taken in conjunction with earlier studies[3,4], indicated that the simultaneous study of succinate and lactate levels in perfusates could give important and unambiguous information regarding the state of viability of the organ. Some preliminary results are given here.

Methods. 1. *Perfusion.* Dog hearts (120–135 g wet wt.) were set up as described[3,5–7]. The technique involved hypothermic (4 °C) perfusion in a closed-circuit system incorporating a filter (pore size 8 μm). Gas exchange with 97% oxygen/3% carbon dioxide occurred at the surface of the liquid; the perfusion pressure was 30 cm of water. The perfusate (1.67 l.) consisted of 1.5 l. of Krebs salt solution[3] containing 1.8% Dextran 70 (Pharmacia) and 1.5% (w/v) glucose, to which 170 ml of an iso-osmotic (300 mosmolar) aqueous solution of sodium succinate and lactate had been added to give final concentrations of 5.2–5.4 m*M* and 8.5–9.1 m*M* respectively. The lactate was a partially-racemised sample of biological origin (BDH Chemicals Ltd.); measurement of optical rotation showed that 66% of the salt was in the L-form. Aliquots (5–10 ml) of the perfusate were removed at various times for analysis.

2. *Determination of sodium succinate.* Perfusates were diluted 1:11 with water; succinate concentrations were measured enzymically[8]. Ethyl butyrate (1 ml) was used instead of ethyl acetate (4 ml) to extract the formazan produced by the reduction of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride; the response curve was linear between 20 and 50 nanomoles of succinate per tube.

3. *Determination of sodium lactate.* The concentration of sodium lactate was obtained enzymically by measuring NAD^+ reduction[9]. Perfusates (0.5 ml) were chilled to 0 °C before adding 1.2 *M* perchloric acid (0.25 ml). After thorough mixing, precipitated protein was spun down, and the supernatants were diluted 20-fold with 0.4 *M* perchloric acid before analysis. The results were referred to a standard curve, as the relationship between absorbance and amount of lactate was not rectilinear. Both optical isomers responded to the enzyme preparation (2 mg/ml LDH, obtained from Boehringer und Söhne, Mannheim GMBH, West Germany).

Results. The Table shows the behaviour of a small group of 3 viable hearts and one non-viable organ towards added succinate and lactate. Under these conditions, well-preserved organs generally show a tendency to deteriorate on the 4th day; this is reflected both in the slight drop in the succinate level and in the attenuated rate of lactate disappearance. Observations within the first 18 h of perfusion, such as an increasing vascular resistance of the coronary system and the peculiar feel of the tissue[3,6,7], indicated that the remaining heart was not viable; the succinate concentration fell significantly within the first 24 h of perfusion. On the 2nd and 3rd days succinate disappearance reached a maximum value of 7.3–7.7 μmoles/g wet wt./24 h. In further unpublished experiments with other non-viable organs, rates of disappearance have not exceeded 6 μmoles/g wet wt./24 h.

Discussion. Earlier work[2–4] established that the efficacy of potassium retention by canine hearts under these conditions was linked to viability; losses of potassium ions from non-viable organs were significantly greater than in well-preserved organs. Introduction of the uncoupling agent dinitro-*ortho*-cresol during perfusion accelerated the rate of loss of potassium[3]. The inferences were that the preservation of permeability barriers was linked with tissue viability, and that potassium retention is an energy-dependent process. Viable hearts have failed

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Levels of exogenous lactate and succinate in the perfusates of isolated canine hearts

Time of perfusion (h)	Viable organs[a]		Non-viable organ	
	Average lactate concentration (m*M*)	Average succinate concentration (m*M*)	Lactate concentration (m*M*)	Succinate concentration (m*M*)
$^3/_4$–$1^1/_4$	8.7 ± 0.20	5.3 ± 0.10	9.1	5.3
24	7.5 ± 0.07	5.3 ± 0.08	8.0	5.0
48	6.1 ± 0.18	5.3 ± 0.05	7.3	4.4
72	4.6 ± 0.24	5.2 ± 0.06	7.4	3.8
96	3.6 ± 0.28	5.0 ± 0.02	7.5	3.4

[a] Averages and standard deviations from 3 hearts.

to utilize succinate, despite the fact that the substrate concentration greatly exceeded 0.15 mM, the K_m value[10] for beef-heart succinoxidase at 20 °C. If, as in the case of fumarase[11], the K_m decreases with temperature, and if species differences are not significant, the affinity of the enzyme complex for succinate might be expected to be of an even higher order at 4 °C.

Previous studies of 30 hearts have established the value of following the fate of lactate produced metabolically during perfusion[2-4]. Regardless of the state of preservation of the tissues, small quantities (20–300 µmoles) of lactate always appeared in the perfusates during the first 4–6 h. In the cases of well-preserved organs, these amounts had invariably fallen by about 40% within the next 18–20 h; conversely, lactate levels in the perfusates of deteriorating hearts rose by a factor of between two and fourteen within the same period of time[4]. These observations constitute a sensitive and early indication of the state of viability of the tissues. However, when hearts are presented with such large amounts of exogenous lactate, as in the present experiments, the position is far less clear. The fact that an organ continued to metabolise lactate at an appreciable rate for nearly 48 h, even though other criteria[3] had already shown conclusively that the heart was no longer viable, indicates that the presence of a large quantity of added lactate, possibly due to a mass-action effect, does not give information regarding the state of the tissue at a sufficiently early stage.

Although the number of hearts studied so far is small, the clear-cut nature of the results and the pressing need for non-invasive tests of viability have dictated the early presentation of these findings. In selecting the original level of succinate, the aim was to present the tissues with an amount of substrate the oxygen equivalent (30 mmoles) of which was adequate to cover the oxygen requirement of the tissue for 3–4 days. Considerations are necessarily different when a concentration appropriate for monitoring loss of viability is to be selected. The greatest fall in the succinate level yet obtained has been 0.6 mM in 24 h; not all deteriorating tissues would necessarily cause a decrease of this magnitude. Although, in other experiments, a monitoring concentration of 2 mM has been employed, 0.5–1 mM would appear to be a more suitable range if the significance of differences less than 0.6 mM are not to be masked by experimental error, especially if the interval between sampling times needs to be shortened. The phenomenon of lactate production and utilization by the isolated perfused heart and its relationship to organ viability is sufficiently well documented[3,4] to stand. In future investigations the question as to whether or not the fate of added succinate is affected by the introduction of comparatively large amounts of exogenous lactate must be given careful attention.

The problem of whether an organ is genuinely viable or not is of cardinal importance in preservation. The ideal would be to perform rapid and simple tests exclusively on samples of perfusate, thereby presenting no hazard to the organ. While orthotopic transplantation must remain the ultimate test of viability, the procedure is particularly difficult to carry out with success[12]. Detailed discussions of the criteria of viability applied in this investigation have appeared elsewhere[3,6,7].

Résumé. Dans le cœur isolé du chien, perfusé à 4 °C, le succinate de sodium ajouté n'est pas utilisé par des tissus vivants, mais il est métabolisé par le cœur qui a perdu sa viabilité. Le destin du lactate exogène ne donne pas d'informations assez claires sur la viabilité des tissus, tandis que c'est le cas du lactate produit par métabolisme.

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Inhibition of Beef Heart and Rat Brain Nucleoside-3′:5′-Monophosphate Phosphodiesterase by 3β-14-Dihydroxy-21-oxo-23-desoxo-5-β-card-20(22)-enolide 3-acetate (AY-17,605) and Structurally Related Compounds

The importance of cyclic adenosine-3′:5′-monophosphate (cyclic-AMP) as an intracellular mediator of various hormone actions is well documented[1]. Nucleoside-3′:5′-monophosphate phosphodiesterase (PDE) hydrolyzes cyclic-AMP to AMP and appears to be of relevance as a control mechanism for the intracellular levels of cyclic-AMP. Various compounds which have been demonstrated to be inhibitors of PDE have also been shown to affect, i.e. increase, hormonal actions. Recently, a member of a novel series of compounds which are isomeric to the natural cardiac glycosides has been found to exhibit properties of cardiotonic agents[2]. These isomers have the steroid nucleus attached to the α-carbon instead of the β-carbon of the α, β-unsaturated lactone (Figure). The inhibitory activities of compounds of this series on beef heart PDE and rat brain PDE have been determined in the present studies.

Materials and methods. The measurements of PDE activity were carried out essentially as described previously[3,4]. The beef heart PDE (Boehringer Mannheim) was a dialyzed preparation; the rat brain PDE was prepared as described previously[3]. The protein concentration was determined by the method of Lowry et al.[5]. The enzyme assay contained beef heart PDE (0.12 mg protein/ml) or rat brain PDE (0.095 mg protein/ml), snake venom (1 mg/ml) and bovine albumin (2 mg/ml) in 60 mM *Tris*-HCl buffer, pH 7.8 with 5 mM mercaptoethanol. The PDE was omitted in the blanks. 50 µl of the enzyme preparation were added to a glass scintillation vial containing 5 µl of the test compound in dimethyl sul-

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