Studies on the Viability of the Cadaver Kidney: Influence of Ischemia and Perfusing Solutions on Succinic Dehydrogenase Activity in Homogenates

With increasing emphasis on the use of cadaver kidneys for transplantation, techniques shall be required to determine the quality of the organ selected for transplantation. Although very good or very poor kidneys can be suspected and separated on clinical grounds, there is a vast intermediate group. In the latter group there is irreversible damage present in some of the kidneys. In order to be able to sort out these kidneys before transplantation, it was thought that such damage could be ascertained by biochemical means.

The determination of succinic dehydrogenase (SDHG) seemed to fulfill this requirement. SDHG is firmly attached to the mitochondrial structure[1]. A decrease in activity should therefore mean an irreversible damage of a vital part of the cell, viz. the energy producing center, the mitochondria. Utilizing a modification[2] of the POTTER[3] method for the manometric determination of SDHG, small amounts of tissue could be studied, and quantitative results obtained within 1 h after sampling. The present report exemplifies the use of this method on the rabbit kidney after various periods of warm ischemia. Since most medical centers produce core cooling and cleansing of the intravascular contents of the kidney by perfusion, the effects of such perfusion with different solutions were also investigated.

Material and methods. 54 rabbits of a white native strain, averaging 2.5 kg, fed a commercial food, (Ewos pellets, Södertälje, Sweden) comprised the animal material used for the study. They were randomly divided in 2 groups, perfused and non-perfused. The rabbits were killed with a single injection of pentobarbital (100 mg/kg) (Mebumal-natrium, 60 mg/ml, for veterinary use, ACO, Apotekarnas central organisation, Sweden) into an ear vein. Immediate death was always induced. The kidneys were either removed immediately after death (0 ischemia), or left in situ 2, 4 or 6 h (2, 4, 6 h warm ischemia) and then removed. Upon removal, a biopsy was taken (averaging 20–50 mg) from the cortical area.

Alternately, the kidneys were removed at zero time, or after 1 h of warm ischemia, and perfused with either saline or Dextran-40 (5% Rheomacrodex in 5% glucose, Pharmacia, Uppsala, Sweden). The kidneys were perfused by inserting a teflon catheter into the renal artery: 250 ml of perfusing fluid at 4 °C, under 100 cm of water pressure for 30 min produced the desired effect. Perfused kidneys were not biopsied until completion of the perfusion. The renal cortical biopsies obtained were weighed on a torsion balance and then homogenized in 0.25 *M* sucrose 1:100, in melting ice with a Ten Broeck homogenizer.

SDHG activity was then measured manometrically with the Warburg technique[4] utilizing a modification[2] of the method described by POTTER[3]. Briefly, aliquots of the homogenate were incubated in duplicate flasks with succinate and co-factors in optimal concentrations at 37 °C. After temperature equilibration for 6 min a rectilinear oxygen uptake was registered for 30 min. Oxygen uptake was proportional to time and enzyme concentration, allowing quantitation of SDHG. The error of the method was analyzed and found to be 9.2%. The measure of statistical significance was analyzed using the standard Student's *t*-test.

The effect of prolonged warm ischemia. With increasing periods of warm ischemia (0, 2, 4, 6 h) without perfusion there was a progressive reduction in SDHG activity (Figure 1). If zero time in this experiment is expressed as 100%, the SDHG activity at 2 h was 80%, at 4 h 58% and at 6 h 27%. All differences were statistically significant at the 5% level.

The effect of perfusing solutions. Accepting zero ischemia without perfusion as the basis for comparison, the kidneys perfused with Dextran-40 at zero ischemia were 68% of control and 27% of control if normal saline was the perfusate (Figure 2). When the period of warm ischemia was extended 1 h before beginning the perfusion, oxygen utilization fell to 47% with Dextran-40 and 13% with saline (Figure 3). The results are summarized in the Table.

Discussion. Objective methods now available to determine the suitability of a kidney for transplantation include: tetrazolium dye as recommended by TERASAKI[5] and utilized also by KHASTAGIR[6] and MAGINN[7]; the measure of enzymes in the perfusate as suggested by BELZER[8]; the

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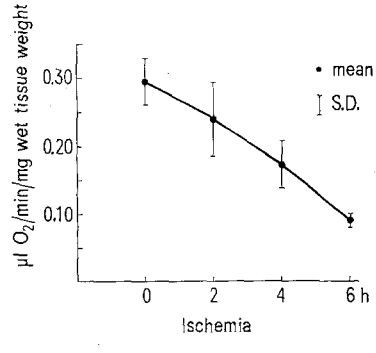

Fig. 1. Succinic dehydrogenase activity of the rabbit kidney after increasing periods of warm ischemia at 37° C.

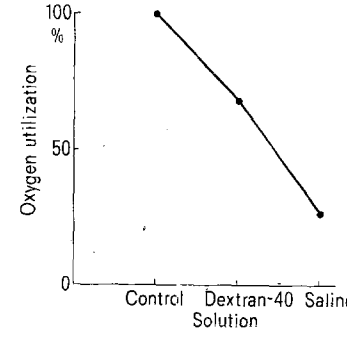

Fig. 2. Succinic dehydrogenase activity of the rabbit kidney after perfusion with Dextran-40 or normal saline.

Comparison of SDHG activity of rabbit cadaver kidneys with and without perfusion after varying periods of warm ischemia

	Non-perfused				Perfused			
					Rheomacrodex		Saline	
Ischemia time (h)	0	2	4	6	0	1	0	1
Mean	0.295	0.240	0.174	0.089	0.201	0.139	0.081	0.038
Number	6	10	6	6	9	6	5	6
Standard deviation (Sd. Dv.)	0.037	0.054	0.035	0.010	0.031	0.010	0.013	0.014
Standard error of Mean (S.E.M.)	0.015	0.017	0.014	0.001	0.010	0.004	0.005	0.006
Relative percentages	100	80	58	27	68	47	27	13

The results are expressed as µl/min/mg. S.D. and S.E.M. are included.

measure of pH by COUCH[9] and DMOCHOWSKI[10]; a method for measuring oxygen tension and consumption in the Spinner flask cell culture, as suggested by COHEN[11]; the oxygen electrode of BAUTISTA[12]; and, the measure of SDHG activity by LANNON[13].

In the present study utilizing rabbit cadaver kidneys at various ischemia times with and without perfusion, SDHG activity was used as an index of viability. Sufficient precision was obtained utilizing small amounts of tissue so as to be practicable and yield quantitative results within a brief period. The present work revealed SDHG activity to decrease with increasing periods of warm ischemia. This is, thus, in accordance with previous reports of a decreased viability and therefore a decreased suitability for transplantation[14–22]. Furthermore, perfusion with Dextran-40 or saline also decreased SDHG activity. The reason for the latter is not known and will be the subject for further studies where the SDHG method seems of value for the testing of optimal perfusion conditions.

Conclusions. 1. Prolonged warm ischemia decreases the SDHG activity of the kidney. 2. Perfusing solutions further decrease the SDHG activity, normal saline more than Rheomacrodex. 3. The measurement of SDHG activity in kidney homogenates appears to be useful in determining cadaver kidney viability.

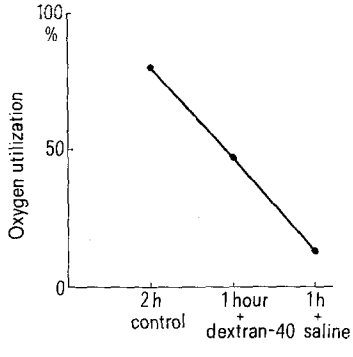

Fig. 3. Succinic dehydrogenase activity of the rabbit kidney after warm ischemia and perfusion with Dextran-40 or saline.

Zusammenfassung. Succinase-Dehydrogenase-Aktivität, als praktischer Index für die Lebensfähigkeit von Nierenrinden-Homogenaten wurde in der Warburgapparatur mit der Mikrowaage gemessen. Verlängerte Wärme-Ischämie vermindert die Fähigkeit der Sauerstoffverwertung und setzt die Lebensfähigkeit herab.

M. D. KERSTEIN[23] and P. BJÖRNTORP[24]

First Surgical Department and First Medical Department, University of Göteborg, Göteborg (Sweden); and Department of Surgery, Yale University School of Medicine and Veterans Administration Hospital, West Spring Street, West Haven (Connecticut 06516, USA), 15 January 1973.

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Lipid Peroxidation in Dietary Liver Necrosis

Dietary liver necrosis in the rat[1,2] constitutes a suitable experimental model for the study of the pathogenesis of non-toxic cellular necrosis in vivo. The condition is produced about 28 to 30 days after feeding the rat a diet deficient in vitamin E and selenium. Supplementation of the diet with either one or both factors completely prevents the onset of necrosis. A prenecrotic period, lasting about 3 weeks, preceeds the development of

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