The Effect of Temperature and Anoxia of Rat-Kidney Slices on their Subsequent Respiration

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1. Rat-kidney slices were kept, aerobically and anaerobically, at 4°, 20° and 38°, for 0.5–24 hr. The oxygen consumption was then measured at 38°. 2. After 6 hr. of storage at 4° the rate of oxygen consumption was the same as controls, but after 12 and 24 hr. of storage the oxygen consumption fell by 22 and 32% respectively; there was no difference between the slices kept aerobically and anaerobically for periods of 2–24 hr. at 4°. 3. Anaerobic storage at 20° for 2 hr. did not affect the subsequent rate of oxygen consumption, but after 4 and 6 hr. the anaerobically stored tissue showed loss of oxygen consumption. Aerobic storage had no effect for 6 hr. 4. There was a marked fall in the capacity to consume oxygen after 0.5 hr. at 38° without oxygen. 5. Kidney slices stored at 4° lost nitrogen. The presence of oxygen did not affect this nitrogen loss.

Lowering the temperature is an effective means of preserving tissues and is used both in laboratory studies and in clinical practice. During organ transplantation, the temperature of the organ during the period of ischaemia probably determines its survival, for short periods without circulation at 38° produce marked changes. Semb (1956) found that clamping a single kidney pedicle for 10–20 min. caused a mild elevation of the blood urea concentration and that 50 min. of occlusion produced a severe degree of uraemia. Eisendrath & Strauss (1910) subjected rabbit kidneys to 15 min. of ischaemia and noted histological evidence of damage 4 weeks later. Mitchell & Woodruff (1957) were able to prolong the period of renal ischaemia in sheep by lowering the organ temperature to 10–20°. All the animals were alive 4 weeks after a 2 hr. period of ischaemia at this temperature, but if the kidneys were kept above 25° there were no survivors. Stueber, Kovacs, Koletsky & Persky (1958) maintained the kidneys of dogs for a 6 hr. period of ischaemia at 10° with survival of all animals.

Wilhelmi, Russell, Engel & Long (1945) noted that increasing the period of ischaemia of the liver *in vivo* in anaesthetized dogs resulted in their capacity to consume oxygen *in vitro* becoming progressively less.

Our aim has been to determine how the oxygen consumption of rat-kidney slices at 38° is affected by prior anoxia and periods at lower temperatures.

METHODS

Preparation of tissues. In each experiment two adult male Wistar rats weighing 250–350 g. were killed by stunning and

cervical dislocation. The four kidneys were removed and placed in glucose-salt medium (Robinson, 1949) at 4°. The kidneys were sliced by hand and 40–50 mg. was placed in each of six Warburg flasks containing 3.0 ml. of Robinson's medium. The centre well contained 0.2 ml. of 20% (w/v) KOH and filter paper. Approx. 1 hr. was required to slice and weigh the slices. The flasks were then connected to manometers.

Design of experiments. The O₂ consumption of two of the flasks, which served as controls, was determined immediately. They were flushed with O₂ for 15 min. at 20 ± 2° and equilibrated at 38° for 10 min. The O₂ uptake was measured during six intervals of 10 min. The mean O₂ consumption during the hour was expressed in $\mu\text{l./mg. initial wet wt./hr.}$

In each experiment the remaining four flasks were treated as aerobic or anaerobic pairs, being kept at the same time and temperature. The aerobic pair of flasks were flushed with 100% O₂ for 15 min. and shaken at 130 oscillations/min. for a period that varied from 0.5 to 24 hr. in different experiments. The anaerobic pair of flasks were flushed with N₂ for 25 min. and shaken under the same conditions as the aerobic pair. At the end of the aerobic or anaerobic period the O₂ consumption was determined, in O₂, as for the uncubated control flasks.

Nitrogen loss from slices. Kidney slices were prepared and stored at 4° as described above. At the end of the period of storage at 4° (with shaking) the slices were carefully separated from the medium and the N was determined separately on the slices and on the medium, by digestion and distillation in a Markham apparatus.

Gas phase. The experiments given in detail were made with commercial 'O₂-free N₂' (British Oxygen Co.) said to contain less than 0.001% (v/v) of O₂. A single cylinder of 'O₂-free N₂' when examined by mass spectroscopy was found to have less than 0.005% (v/v) of O₂. The gas was bubbled through alkaline pyrogallol as a further precaution. A parallel series of experiments was made with commercial

'N₂' (British Oxygen Co.) without bubbling through pyrogallol. This 'N₂' was said to contain less than 0.5% (v/v) of O₂. The results of the two series were similar.

Statistical method. Since the control value varied between experiments, each of the test values was adjusted by using the control mean as a concomitant variable. This involved calculating adjusted test means by the formula:

$$Y(\text{adjusted}) = Y(\text{unadjusted}) - b(x - \bar{x})$$

where 'Y (unadjusted)' is the observed test mean and 'Y (adjusted)' is an estimate of the test mean that would have been obtained with kidney slices whose control mean was the grand mean of all control values; *b* is the average within-groups linear regression coefficient, *x* is the control mean and $\bar{x}$ is the grand mean of all the controls.

Fisher's *P* values were obtained by an analysis of variance as given by Bailey (1959).

Fig. 1. Oxygen consumption of rat-kidney slices after incubation for various periods at (a) 4°, (b) 20° and (c) 38°. The gas phase during storage was either O₂ (●—●) or N₂ (▲---▲). At the end of the storage period O₂ consumption was measured with O₂ as gas phase at 38°. The determination is given in the Methods section. The plotted results are the means of adjusted control and test values.

Table 1. Statistical analysis of differences between oxygen consumption before storage and after aerobic and anaerobic storage

Technical details are given in the Methods section. *P* indicates the significance of differences between tests and the control values.

Conditions of storage				No. of experiments	No. of observations	S.E.M.	Fisher's <i>P</i> value
Temp.	Time (hr.)	Aerobic	Anaerobic				
—	0					0.09	
4°	6	+		2	12	0.06	> 0.05
4	6		+	2	12	0.05	> 0.05
—	0					0.14	
4	12	+		1	6	0.16	< 0.001
4	12		+	1	6	0.18	< 0.001
—	0					0.07	
4	24	+		2	12	0.10	< 0.001
4	24		+	2	12	0.06	< 0.001
—	0					0.05	
20	4	+		4	24	0.08	> 0.05
20	4		+	4	24	0.10	> 0.01, < 0.05
—	0					0.06	
20	6	+		2	12	0.06	> 0.05
20	6		+	2	12	0.06	< 0.001
—	0					0.08	
38	0.5	+		1	6	0.08	> 0.05
38	0.5		+	1	6	0.11	< 0.001
—	0					0.09	
38	1	+		2	12	0.11	> 0.05
38	1		+	2	12	0.09	< 0.001
—	0					0.14	
38	2.5	+		1	6	0.03	> 0.05
38	2.5		+	1	6	0.09	< 0.001

Table 2. *Loss of nitrogen from kidney slices incubated at 4° under aerobic and anaerobic conditions*

Each result is the mean of two flasks. Experimental details are given in the Methods section.

Conditions of storage			Loss of N (%)
Time (hr.)	Aerobic	Anaerobic	
6	+		4.7
6		+	4.0
24	+		21.0
24		+	22.2

RESULTS

Three series of experiments, involving storage at $4 \pm 2^\circ$ (Fig. 1a), $20 \pm 2^\circ$ (Fig. 1b) and $38 \pm 0.05^\circ$ (Fig. 1c), under aerobic and anaerobic conditions, were carried out. The statistical analysis is given in Table 1. The loss of nitrogen under aerobic and anaerobic conditions at 4° is shown in Table 2.

DISCUSSION

In transplantation, restoration of tissue to normal function is of decisive importance, but there is little information as to what biochemical process determines the reversibility of damage. At the present time one is limited to studying the effect of various factors on arbitrarily selected processes, which may serve as indices of damage. For this purpose the oxygen consumption was selected for study under various conditions of temperature and oxygen deprivation.

Fig. 1(a) shows that 6 hr. of anoxia at 4° did not decrease the subsequent rate of oxygen consumption of kidney slices when compared with aerobic slices kept for the same period of time at 4° . This suggested the possibility that the system contained enough oxygen to provide for the low consumption at 4° . The following calculations make this improbable. The calculated oxygen content of the gas phase is $0.25 \mu\text{l.}$, assuming that the composition is reduced to that of 'oxygen-free nitrogen'. As the oxygen content of water at 0° is 0.05% of the gas (Umbreit, Burris & Stauffer, 1945) it may be ignored. Fuhrman & Field (1942) showed that the oxygen consumption of kidney slices at 4° is less than 10% of that at 38° . Thus 50 mg. of tissue would require $10\text{--}15 \mu\text{l.}$ of oxygen/hr. at 4° . The oxygen consumption of the incubated tissues at 4° would have removed all the oxygen in about 1 min. It is clear that the conditions for anaerobiosis in our experiments were satisfied.

Kidney slices kept for 12 hr. at 4° (Fig. 1a) had a

decreased rate of oxygen consumption when subsequently measured at 38° (82% of the control value). After 24 hr. a further decrease was noted (60% of the control value). It seems probable that these decreases were due to the period of storage at 4° , rather than anoxia, for the aerobic and anaerobic series behaved similarly. Under the conditions of our experiment the tissues were shaken during storage and this may have been a contributing factor to the decreased oxygen consumption of tissues stored longer than 6 hr. at 4° . In the absence of shaking, the slices might have withstood more prolonged storage. We found (Table 2) that there was a progressive loss of nitrogen into the medium at 4° .

Anoxia led to a decreased oxygen consumption after storage at 38° and at 20° . After 30 min. of storage at 38° there was a fall to 65% in the subsequent rate of oxygen consumption (Fig. 1c). However, if the temperature of storage was 20° the tissues could be stored for at least 2 hr. without affecting the subsequent oxygen consumption (Fig. 1b). After 4 hr. of anoxia at 20° the subsequent oxygen consumption was 90% of the control value.

The cause of the decreased oxygen consumption due to anoxia at 38° has been considered by various authors. Berthet & de Duve (1952) suggested that activation of hydrolytic enzymes led to changes in the permeability of the mitochondrial membrane. Gallagher, Judah & Rees (1956) considered that there was a loss of respiratory cofactors, either by their enzymic destruction, or by diffusion due to increased cell permeability. Weinbach (1959) observed a loss of nucleotides from mitochondria kept for 72 hr. at 4° .

The experimental results cannot be applied directly to transplantation studies in another species, but suggest that, if cooling to 4° is rapid, 4–6 hr. of storage may not produce damage to the mechanism of oxygen consumption. The much greater sensitivity to anoxia at 38° suggests that a delay in cooling from 38° to 20° may have a more profound effect than longer periods of storage at lower temperatures.

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