THE EFFECT OF HYPOTHERMIA UPON RESPIRATION AND ANAEROBIC GLYCOLYSIS OF DOG KIDNEY

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ABSTRACT

Respiration and anaerobic glycolysis were determined in dog kidney cortex and medulla. At 37 C cortical respiration was 9.09 μl of O_2 per milligram of tissue per hour with an anaerobic glycolysis of 1.45 μl of CO_2 per milligram of tissue per hour. Medullary respiration was 1.41 μl of O_2 per milligram per hour whereas glycolysis was 5.44 μl of CO_2 per milligram per hour.

Hypothermia effectively decreased respiration and glycolysis. At 27 C respiration in the cortex was reduced to one-third, at 17 C to one-sixth of normal, and at 7 C approached zero. The effects of hypothermia for 4 hr at each of these temperatures were reversible. Hypothermia significantly reduced the degree of permanent damage to respiration produced by prolonged interruption of the renal blood flow.

Local hypothermia has been used during recent years in operations requiring temporary interruption of renal blood flow. Renal function, during and after cooling, has been the subject of experimental study both in vivo and in the isolated specimen (1–3). In addition to functional disturbance following renal ischemia, the pathologic anatomic changes in the parenchyma have been studied (4). With local cooling, renal function is depressed only temporarily and parenchymal damage is insignificant after interruption of renal circulation for much longer periods than those which would produce grave damage at normothermia. The maximal time limit for clamping of the arterial flow to the human kidney with normothermia is suggested as 30 minutes (5), but transient functional disturbances have been noted after only 15 minutes (6). The majority of authors have used surface cooling of the kidney both for animal experiments and in clinical work. In experiments with dogs, Semb, Krog, and Johansen (7) have used the perfusion technique and studied variations in blood flow and kidney metabolism. They were able to confirm Levy's observation (8) that in dog kidneys perfused with blood, the oxygen consumption was only 15 per cent of normal at kidney temperature of 20 C. Oxygen consumption determined manometrically in tissue slices of various renal zones (9–11) paralleled oxygen tension measured by gas analysis in corresponding renal layers (12–15). Lactic acid production under aerobic and anaerobic conditions (9–11) has been related to renal function by Ullrich, Kramer and Boylan (16).

To our knowledge, no efforts have been made to demonstrate the effect of

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TABLE 1. *Mean and standard deviation of values obtained for respiration and anaerobic glycolysis in dog kidney cortex and medulla with normothermia*

	Respiration: Q_{O_2}	Anaerobic Glycolysis: $Q_{CO_2}^{Argon}$
Cortex	9.09 ± 1.03	1.45 ± 0.49
Medulla	1.41 ± 0.54	5.44 ± 0.74

hypothermia upon the oxygen consumption and glycolysis of the tissue slices from various strata of the kidney. Experiments were, therefore, designed to investigate the effect of hypothermia upon: (i) Renal metabolism, specifically, the respiration and anaerobic glycolysis, (ii) survival of renal cortex and medulla, and (iii) the reversibility of the changes in metabolism.

MATERIALS AND METHODS

Kidney tissue was obtained by heminephrectomy or nephrectomy, performed under aseptic conditions on anesthetized mongrel dogs. The corticomedullary junction was readily definable on gross examination and tissue blocks from cortex and medulla were sampled separately. Tissue slices were prepared and introduced into Warburg reaction flasks which then were mounted on manometers and placed on the Warburg apparatus. After perfusion and equilibration the slices were allowed to respire or glycolyze, with constant shaker speed and temperature. The details of the method have been described previously (16). The temperature in the thermobath was regulated by a sensitive thermostat to ± 0.1 C. Normal values for $Q_{O_2}^{O_2}$ [1] and $Q_{CO_2}^{Arg}$ [2] were determined at 37 C and the effect of cooling was measured at 27 C, 17 C, and 7 C. In some experiments, after the determination of the metabolism at reduced temperature, $Q_{O_2}^{O_2}$ and $Q_{CO^2}^{Arg}$ were again measured.

The effect of cooling on renal survival after interruption of the circulation was investigated by determining respiration and anaerobic glycolysis on paired samples from kidney tissue, one stored in physiologic saline at 37 C and the other refrigerated at 7 C in saline.

RESULTS

Normal values. The mean and standard deviations of values obtained for respiration and anerobic glycolysis in 19 observations on dog kidney cortex and medulla at 37 C are shown in Table 1. Respiration of cortical tissue was 9.09 μl of oxygen consumed per milligram of dry weight of tissue per hour, whereas in tissue from the medulla oxygen consumption only was 1.41. At body temperature cortical oxygen requirement is then seven times that of medulla.

Effect of hypothermia. As revealed in Figure 1 and Table 2 respiration and

[1] $Q_{O_2}^{O_2}$ is an expression for tissue respiration in terms of oxygen consumption and is defined as: Microliters of oxygen per milligram dry weight of tissue per hour.

[2] $Q_{CO_2}^{Arg}$ is an expression of anerobic glycolysis in terms of CO_2 displacement by lactic acid produced and is defined as microliters of CO_2 produced per milligram dry weight of tissue per hour.

TABLE 2. *Effects of hypothermia on respiration and anaerobic glycolysis*

	Respiration: $Q_{O_2}^{O_2}$				Anaerobic Glycolysis: $Q_{CO_2}^{Argon}$			
	7°	17°	27°	37°	7°	17°	27°	37°
Cortex	0.61	1.66	2.88	9.09	0.35	0.23	0.25	1.45
Medulla	0.00	0.45	1.16	1.41	0.67	0.61	1.13	5.44

TABLE 3. *Reversibility of the cooling effect*

	Cooling 7°	Rewarming 37°
Cortex: $Q_{O_2}^{O_2}$	0.44	8.03
Cortex: $Q_{CO_2}^{Arg}$	0.17	0.85
Medulla: $Q_{O_2}^{O_2}$	0.21	0.85
Medulla: $Q_{CO_2}^{Arg}$	0.00	4.36

anaerobic glycolysis are influenced markedly by temperature. Each value is the mean of eight observations. At 27 C respiration is reduced to one-third, at 17 C to one-sixth, and at 7 C is approaching zero. In the medulla, where anaerobic glycolysis is high, cooling to 27 C diminishes anaerobic glycolysis to one-fifth.

Reversibility of cooling effect. A return to normal of respiration and glycolysis in both cortex and medulla following a 1-hr period of reduced metabolism at 7 C is demonstrated in Table 3. This was observed in four sets of experiments.

Survival of renal tissue. Interruption of blood flow to the kidney for 4 hr without hypothermia caused a 75 per cent reduction in respiration of the renal cortex ($Q_{O_2}^{O_2} = 2.25$). However, cortical respiration remained unchanged when the kidney was cooled to 7 C and the blood supply was interrupted. Interruption of the blood supply for 24 hr at 27 C lowers cortical respiration to almost zero, while 24 hr at 7 C reduces it to one-half. All determinations of Q-values were made at 37 C by rewarming the tissue at the end of the 24-hr period.

DISCUSSION

The high oxygen consumption of renal cortical tissue which correlates well with the high oxygen tension found in this region has been considered by Ullrich, Kramer and Boylan (16) to be mainly due to the net transport of filtered sodium and hence to be only partly reproducible by in vitro experiments.

With experimental conditions kept constant, reproducible data were obtained which are believed to be within a physiologic range and it appears unimportant to the authors whether or not these data represent a maximum or minimum consumption of oxygen by surviving renal tissue.

naerobic glycolysis

naerobic Glycolysis: $Q_{CO_2}^{Argon}$

	17°	27°	37°
	0.23	0.25	1.45
	0.61	1.13	5.44

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Rewarming 37°
8.03
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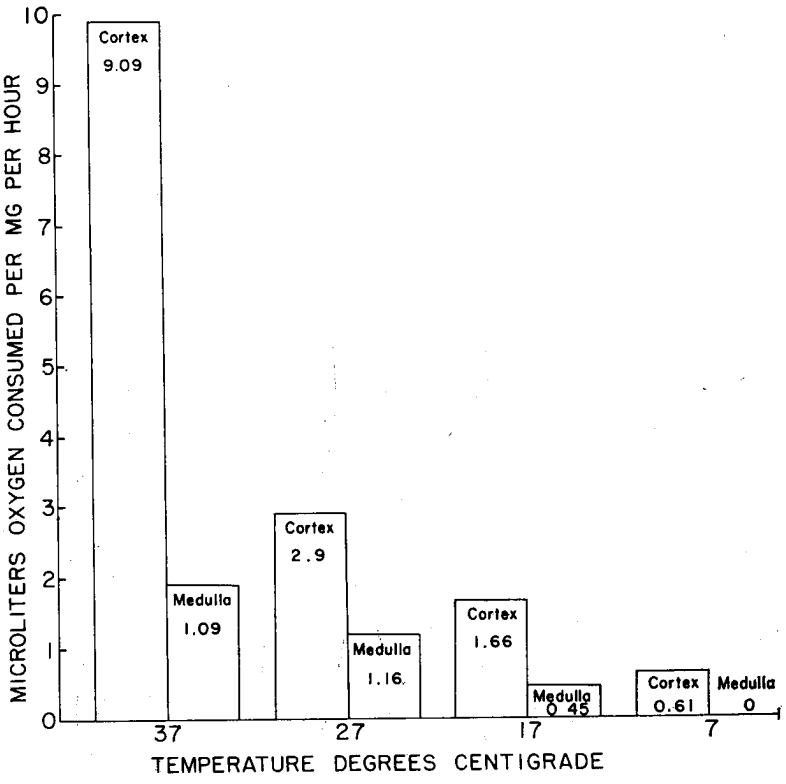

FIG. 1

TABLE 4. *Survival of renal tissue after interruption of blood circulation*

	4 hr		24 hr	
	37°	7°	27°	7°
Cortex respiration	2.25	9.09	0.15	4.91
Medulla respiration			0.00	0.45

This high cortical respiration stands in contrast to the low oxygen consumption by medullary tissue, which is known to have also a low oxygen tension.

The ability to glycolize anaerobically seems to be just reversed in the two regions considered, a unique phenomenon as compared with observations made in other organs. On the basis of the counter current system and plasma-skimming mechanism, oxygen is believed to be short-circuited into and across the parenchyma in the intramedullary area of the cortex producing a low hematocrit and oxygen-poor blood to the medulla (18). Thus, cells from the medulla

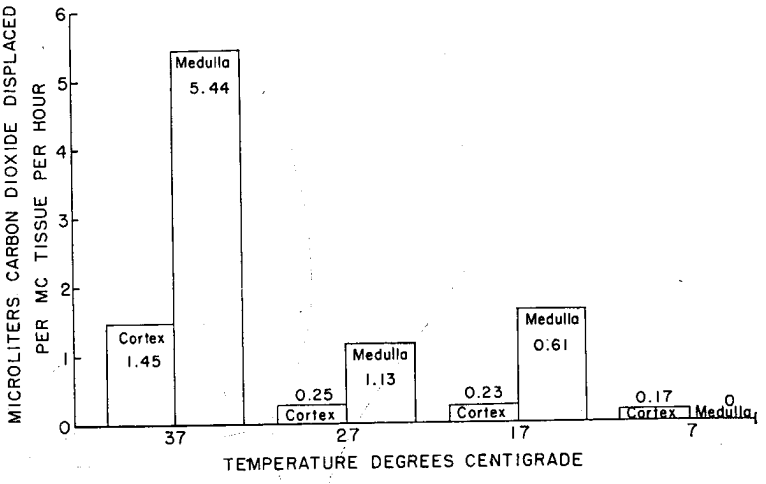

FIG. 2

are able to obtain their energy through anaerobic glycolysis, a property quite important in a region where oxygen tension may become critical.

Aerobic glycolysis in inner renal medulla as found by Dickens and Weil-Malherbe (9) could not be verified by Warburg (11) who finds aerobic fermentation confined to cancerous tissue only.

A kidney which must be rendered ischemic for a certain length of time is protected markedly by the use of hypothermia. Respiratory and glycolytic enzymes in both cortex and medulla are affected equally by hypothermia. Under in vitro conditions with the temperature at a constant level during the period of measurement, 7 C proved to be sufficient to reduce respiration to a measurable minimum. Such depression of enzymatic activity is found totally reversible up to 4 hr. Storage of renal tissue after interruption of its blood supply for 24 hr with hypothermia to 7 C seems to be detrimental to the respiratory apparatus of the renal cortex.

It is concluded that hypothermia reduces the oxygen consumption of renal cortex to one-third at 27 C, to one-sixth at 17 C, and almost to zero at 7 C.

Inasmuch as cortical respiration is approximately seven times that of the medulla, it is suggested that methods of inducing hypothermia surrounding the kidney with cooled solutions should prove efficient.

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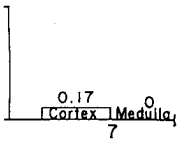
REFERENCES

1. HARVEY, R. B.: Effect of temperature on function of isolated dog kidney. Amer. J. Physiol., **197:** 181, 1959.
2. BIRKELAND, S., VOGT, A., KROG, J., AND SEMB, G.: Renal circulatory occlusion and local cooling. J. Appl. Physiol., **14:** 227, 1959.
3. HUMPHRIES, A. L., RUSSELL, R., OSTAFIN, JR., AND GOODRICH, S. M.: Successful reimplantation of canine kidney after 24 hr. storage. Surgery, **54:** 136, 1963.

4. MITCHELL, R. M.: Renal cooling and ischemia. Brit. J. Surg., **46:** 593, 1959.
5. SEMB, G.: Partial resection of the kidney, anatomical, physiological and clinical aspects. Ann. Roy. Coll. Surg. Eng., **19:** 137, 1956.
6. KERR, W. K., KYLE, V. N., KEVESKEI, A. G., AND SMYTHE, C. A.: Renal hypothermia. J. Urol., **84:** 236, 1960.
7. SEMB, G., KROG, J., AND JOHANSEN, K.: Renal metabolism and blood flow during local hypothermia, studied by means of renal perfusion in situ. Acta Chir. Scand. (Suppl.), **253:** 196, 1960.
8. LEVY, N. M.: Oxygen consumption and blood flow in the hypothermic, perfused kidney. Amer. J. Physiol., **197:** 111, 1959.
9. DICKENS, F., AND WEIL-MALHERBE, H.: Metabolism of normal and tumor tissue; note on metabolism of medulla of kidney. Biochem. J., **30:** 659, 1936.
10. GYÖRGY, P., KELLER, W., AND BREHME, TH.: Nierenstoffwechsel and Nierenentwicklung. Biochem. Z., **2000:** 356, 1928.
11. WARBURG, O.: *Weitereentwicklung der zell physiologischen methoden,* p. 499. Georg Verlag, Stuttgart, 1962.
12. ULFENDAHL, H. R.: Intrarenal oxygen tension. Acta Soc. Med. Upsal., **67:** 95, 1962.
13. AUKLAND, K., AND KROG, J.: Renal oxygen tension. Nature, **188:** 671, 1960.
14. RENNIE, D. W., REEVES, R. B., AND PAPPENHEIMER, J. R.: Oxygen tension of urine and its significance. Fed. Proc., **16:** 693, 1957.
15. LEONHARDT, K. O., AND LANDES, R. R.: Oxygen tension of the urine and renal structures. New Eng. J. Med., **269:** 115, 1963.
16. ULLRICH, K. J., KRAMER, K., AND BOYLAN, J. W.: Present knowledge of the counter-current system in the mammalian kidney. Progr. Cardiov. Dis., **3:** 395, 1961.
17. WALTON, K. N., SCHIRMER, H. K. A., AND SCOTT, W. W.: The effect of prolonged stimulalation with pilocarpine upon secretory and metabolic activity of normal dog prostate gland. Invest. Urol., **1:** 23, 1963.
18. LEVY, M. N., AND IMPERIAL, E. S.: Oxygen shunting in renal cortical and medullary capillaries. Amer. J. Physiol., **200:** 159, 1961.



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