Renal Function and Metabolism of Isolated Baboon Kidneys following Prolonged Bloodless Hypothermic, Hyperbaric Storage with Helium and Oxygen

J. J. W. VAN ZYL, J. A. VAN ZYL, A. LOCHNER,* M. J. WARD,**
G. S. JOHNSTON,*** C. P. RETIEF,† G. P. MURPHY ‡

From the Department of Surgery, University of Stellenbosch Faculty of Medicine, Karl Bremer Hospital, Bellville, Cape Province, South Africa and Brady Urological Institute, The Johns Hopkins Hospital, Baltimore, Maryland

WE HAVE previously described a method for prolonged isolated bloodless perfusion of baboon kidneys, exposed to oxygen or helium gases under normothermic, normobaric conditions.[10] Renal functional and metabolic assessments of these kidneys revealed depression in the renal circulation and oxidative phosphorylation processes after exposure to either gas.[10] Renal cortical circulation was, however, better during exposure to helium than during exposure to oxygen. With oxygen, more vasoconstriction and decreased renal perfusion was evident. The present report describes renal functional and metabolic measurements in isolated baboon kidneys during normothermic, normobaric bloodless perfusion, after storage for 24 hours under hypothermic (4° C.), hyperbaric (3 Atms.) conditions. During storage low flow perfusion with perfusate equilibrated with either helium or oxygen was maintained.

Material and Methods

Thirty-seven kidneys were aseptically removed from healthy conditioned male and female Chacma baboons (*Papio papio*, subspecies *Papio ursinus*) in the Stellenbosch-Hopkins primate project, Bellville, South Africa. The principles and practices of the South African Animal Welfare Society and American National Society for Medical Research were observed throughout these experiments. Animals were anesthetized with phencyclidine hydrochloride (1 mg./Kg./weight).* The renal vessels were immediately cannulated, and gently irrigated with

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* Degenerative Diseases Research Group of the C.S.I.R., University of Stellenbosch Faculty of Medicine, Karl Bremer Hospital, Bellville, Cape Province, South Africa.

** Department of Surgery, The Johns Hopkins Hospital, Baltimore, Maryland 21205.

*** Radioisotope Section, Walter Reed General Hospital, Washington, D. C. 20012.

† Department of Chemical Pathology, University of Stellenbosch Faculty of Medicine, Karl Bremer Hospital, Bellville, Cape Province, South Africa.

‡ Brady Urological Institute, The Johns Hopkins Hospital, Baltimore, Maryland 21205.

* Sernylan, Parke, Davis Co., Detroit, Michigan.

TABLE 1. *Renal Function after 24-Hrs. Oxygen*

Periods (15 min.)	Renal Arterial Pressure (mm. Hg)	Arterial pH	Arterial pCO_2	Arterial pO_2	Per Cent* Sodium Reabsorption	E_{PAH}*	E_{CR}*	C_{OSM} (ml./min.)
U_0	133.9	7.36	27.4	336.4	95.6	—	0.18	14.2
	±14.1	±0.17	±13.2	±102.3	±30		±0.34	±10.5
U_1	133.4	7.39	22.3	440.6	96.2	0.31	0.14	14.9
	±14.1	±0.22	±6.7	±209.9	±2.1	±0.46	±0.55	±14.2
U_2	133.9	7.42	20.6	449.4	96.6	0.47	0.19	18.7
	±14.1	±0.20	±8.2	±173.3	±1.7	±0.43	±0.36	±17.3
U_3	133.9	7.40	19.7	430.5	94.0	0.10	0.16	18.5
	±14.1	±0.50	±6.4	±153.5	±7.3	±0.30	±0.08	±17.3

* Per cent Sodium Reabsorption = Arterial Sodium concentration × C_{CR}/urine sodium concentration × flow rate — 100 per cent.

* E = Extraction ratio of substance = $\frac{\text{Arterial conc.} - \text{venous conc.}}{\text{Arterial concentration}}$.

dilute heparinized saline until the venous effluent was clear. The kidneys were weighed (to the nearest 0.1 Gm.) on a Harvard torsion balance before and after storage. Within an average time of 6 minutes they were placed in a specially designed sterile kidney preservation unit for 24 hours.

The preservation unit (Fig. 1) consists of a pressure vessel containing an organ container and a pneumatically driven pulsatile pumping system. The driving power and pump controls are located outside the chamber. The pressure vessel is modified from a commercial spray painting unit. The only parts requiring sterilization are the organ container and attached reservoir of stainless steel for the organ and the perfusate, the pneumatically driven silastic ventricle and the vinyl tubing and a simple one-way valve joining the reservoir to the ventricle and the cannula in the renal artery (P7, P8).

The driving force can be derived from any source of compressed gas, provided a pressure of at least 100 p.s.i.g. be maintained, for the operation of the pilot valves of the solenoid operated spool valves (E4 & P3). A pressure regulator (P1) in the supply line to the ventricle reduces the main line pressure to the driving pressure (registered on gage P2) required for the ventricle (P6). We have found that 500 mm. Hg for 2.5 seconds will provide a renal flow rate of approximately 3 ml. per stroke in kidneys weighing approximately 40 Gm. This means that the ventricle driving pressure is admitted at 500 mm. Hg above the ambient pressure in the chamber. Beyond the solenoid valve is a second gage (P4) with a six inch dial graduated in mm. Hg. The exact driving pressure applied to the ventricle is adjusted by manipulating the regulator (P1) to give a 500-mm. Hg fluctuation on this gage. The ventricle driving line (P5) enters the pressure vessel through a penetration in the lid and is attached to the underside of the ventricle.

The pressure vessel fits into a standard household refrigerator while the connecting tubes, leading to the control system, are led through an opening in the top of the refrigerator. The cooling capacity of the refrigerator is not sufficient to reduce the temperature rapidly enough. The pressure vessel is therefore filled with tap water to the level of the organ container and blocks of ice are added so that the whole unit is cooled down to just above freezing point. The refrigerator can maintain this temperature without difficulty and unmelted ice is

(3 atm.) 4° C. Exposure (9 Animals)

ERCF* (ml./min.)	C_{CR} (ml./min.)	C_{urea} (ml./min.)	T^cH_2O*	Urine Flow (ml./min.)	Renal Flow (ml./min./ Gm.)	Renal Oxygen Consumption (microl. O_2 ml./min./Gm.)
—	15.6	12.9	−0.18	13.8	2.04	0.30
	±15.3	±10.3	±1.5	±10.5	±0.31	±0.60
43.7	16.9	15	−0.41	15.4	2.10	0.65
±46.4	±18.7	±14.8	±0.98	±14.4	±0.38	±0.60
60.6	17.7	18.9	−0.39	17.9	2.0	0.50
±49.3	±16.0	±18.0	±0.0	±16.8	±0.0	±0.60
40.5	12.7	18.5	−0.47	17.8	2.0	0.50
±42.4	±9.0	±18.1	±0.87	±16.6	±0.0	±0.40

* ERCF = Renal electromagnetic arterial flow × E_{PAH} = effective renal cortical flow (ml./min.).
* T^cH_2O = tubular reabsorption of solute free water = C_{OSM} − V where V is urine flow rate.

regularly found in the water after a preservation period of 24 hours.

The gas mixtures utilized for storage were 100% oxygen, 100% helium, 50% oxygen and helium and 95% helium and 5% oxygen. A 5% invert sugar replacement solution * to which 50 ml. of 6% clinical dextran in saline per liter was added was mostly used as the perfusate. The ionic concentrations in the resulting mixture in mEq. per liter were:

Sodium:	103
Potassium:	15
Chloride:	118

An 8.6% solution of $NaHCO_3$ in water was periodically added to the perfusate to maintain physiological ranges of pH. Other solutions were also used, including a physiological solution containing bicarbonate instead of lactate ** also with added dextran. Where utilized, other solutions are specifically mentioned in the text. Following decompression, all kidneys were placed on an isolated bloodless kidney perfusion system at normothermic (37° C.) and normobaric conditions,[10, 13] the perfusate being equilibrated with pure oxygen. Renal functional evaluation and testing were completed, as previously described.[10] During a 60-minute perfusion arterial, venous and urine samples were obtained for determination of the following data:

Para-amino-hippuric acid clearance (C_{PAH})
Creatinine clearance (GFR)
Sodium (reabsorption) (%)
Potassium content
Chloride content
Urea clearance (GFR)
Osmolar clearance
Free water excretion (see Table 1)
Effective renal cortical flow (ERCF) (see Table 1)

Other measurements serially obtained included arterial and venous pCO_2, pO_2 and pH. Arterial flow was monitored by an electromagnetic probe.* [10] Renal oxygen consumption was serially determined during the perfusion in the manner discussed previously.[10, 13]

Immediately after completion of these renal functional tests, the kidneys were immersed in ice-cold saline for subsequent

* Baxter Laboratories, Chicago, Ill., and Keatings Pharmaceuticals, Ltd., Johannesburg, South Africa.

** Plasmalyte B.—Baxter Laboratories, Chicago, Ill., and Keatings Pharmaceuticals Ltd., Johannesburg, South Africa.

* Statham Instruments Inc.—Model 4100—Los Angeles, California.

TABLE 2. *Renal Function after 24-Hr.*

Periods (15 min.)	Renal Arterial Pressure (mm. Hg)	Arterial pH	Arterial pCO_2	Arterial pO_2	Per Cent* Sodium Re-Absorption	E_{PAH}*	E_{CR}*	C_{OSM} (ml./min.)
U_0	137.5	7.32	25.7	475	96.3	—	0.32	19.9
	±6.1	±0.20	± 8.0	±41.8	±3.2		±0.34	±8.5
U_1	137.5	7.41	26.5	468.	97.8	0.31	0.16	18.8
	±6.1	±0.10	±12.4	±89.8	±0.79	±0.41	±0.22	±12.9
U_2	137.5	7.45	32.8	430.8	97.0	0.23	0.35	15.9
	±6.1	±0.20	±23.1	±45.3	±1.8	±0.42	±0.59	±14.0
U_3	137.5	7.54	26.7	475	96.7	0.02	0.14	15.1
	±6.1	±0.14	±14.7	±90.8	±1.6	±0.0	±0.21	±16.4

*Symbols explained at bottom of Table 1.

metabolic studies. After separation of the cortex and medulla, tissue slices and mitochondria were prepared as previously described.[7-10] The *in vitro* assessments included oxygen and glucose uptake, $C^{14}O_2$, lactate and pyruvate production by tissue slices, as well as the mitochondrial phosphorylation-oxidation (P-O) ratio.[7] Metabolic data were calculated on the basis of the initial dry weights of the slices. The values obtained were compared with the metabolic data obtained from 4 unpreserved, unperfused kidneys. Statistical analysis on all data was performed with the aid of a desk top computer.**

Results

A. Following 24-hour Storage with Hyperbaric, Hypothermic Bloodless Perfusion, Exposed to 100% Oxygen

Table 1 itemizes the alterations in nine kidneys. Arterial pressure was readily maintained at 133.0 mm. Hg, without marked alterations in pCO_2, pO_2 or pH. The per cent sodium reabsorption was within a normal range (94 to 96.6%). Extractions of PAH, and creatinine were similar to those of kidneys which were perfused without storage.[10-13] GFR, as measured by creatinine or urea clearance, was 43% of the value obtained from intact animals.[10, 13] Close agreement of the urea and creatinine filtration rates was evident. Renal perfusion flow rates and oxygen consumption were reduced. Osmotic diuresis, with little tubular reabsorption of solute free water, was observed. The renal cortical circulation (ERCF) varied from 40.5 to 10.6 ml./minute. Extractions of PAH were reduced at the end of the one-hour perfusion period. Oxygen preserved kidneys had a net weight gain of 22.3% during the preservation period.

B. Following 24-hour Hyperbaric, Hypothermic Bloodless Perfusion, Exposed to 100% Helium

Table 2 summarizes the renal functional results obtained in six kidneys. Renal arterial flow rates were higher, at similar blood pressure ranges, than in the kidneys preserved with oxygen. pCO_2, pO_2 and pH values showed little change. The per cent tubular reabsorption of sodium and the glomerular filtration rate were similar to those of the oxygen preserved kidneys, but the renal oxygen consumption was higher. The renal cortical circulation and extraction of PAH was more reduced at the end of the one-hour test perfusion period. Osmotic diuresis with grossly reduced net tubular reabsorption of solute free water was also observed in both oxygen and helium preserved kidneys. In the helium

** Programma 101—Olivetti, Cape Town, South Africa.

Helium (3 atm.) 4° C. Exposure (6 Animals)

ERCF* (ml./min.)	C_{CR} (ml./min.)	C_{urea} (ml./min.)	T^cH_2O*	Urine Flow (ml./min.)	Renal Flow (ml./min./ Gm.)	Renal Oxygen Consumption (microl. O_2 ml./min./Gm.)
—	18.5	20	+0.26	17.8	2.50	0.85
	±10.2	± 8.5	±0.28	±9.3	±0.43	±0.57
25.2	22.1	18.1	−0.28	17.7	2.50	0.97
±34.2	±13.7	±11.9	±0.36	±12.2	±0.43	±1.25
59.6	18.6	15.6	+0.03	15.8	2.50	0.46
±7.8	±15.7	±13.1	±0.15	±12.9	±0.43	±0.41
2.6	16.8	14.7	−0.03	15.2	2.50	1.28
±2.9	±17.9	±15.9	±0.85	±14.3	±0.43	±0.72

preservation group the average weight gain was only 5.8 per cent.

C. Following 24-hour Hyperbaric, Hypothermic Bloodless Perfusion, Exposed to 50% Oxygen and 50% Helium

The renal functional results obtained in nine kidneys are shown in Table 3. A striking increase in renal arterial perfusion pressure without an increase in renal flow rates was apparent. Renal resistance was therefore increased. Arterial pCO_2, pO_2 and pH levels were similar to those of the previous groups. The renal vasoconstriction decreased the glomerular filtration rate and was associated with a reduction in the extraction of PAH and decreased renal cortical flow (ERCF). Urine flow rates and osmolar clearances were reduced. The per cent tubular reabsorption of sodium was generally reduced. Some net tubular reabsorption of solute free water was present. Renal oxygen consumption was increased. These kidneys had an average weight gain of 11.6 per cent.

D. Following 24-hour Hyperbaric, Hypothermic Bloodless Perfusion, Exposed to 95% Helium and 5% Oxygen

The renal alterations observed in seven kidneys are itemized in Table 4. Renal arterial perfusion pressures were markedly elevated, without an increase in flow rate. Thus, renal resistance was increased and vasoconstriction was still present. The pCO_2 levels were elevated and arterial pH levels were generally in the alkalotic range. Renal oxygen consumption was higher than that of oxygen preserved kidneys. A marked decrease in renal cortical flow and E_{PAH} was noted. Glomerular filtration rates were maintained. Osmotic diuresis and increased urine flow rates occurred. Little tubular reabsorption of solute free water was observed. The per cent tubular reabsorption of sodium was normal. An average gain in weight of 13.8% was noted in this group.

E. Following 48 to 72-hour Hyperbaric, Hypothermic Bloodless Perfusion Exposed to 50% Helium and 50% Oxygen

Two kidneys were preserved for 48 and 72 hours, respectively. Renal vasoconstriction was observed with arterial flow rates of 0.8 to 2.0 ml./minute/Gm. Renal oxygen consumption was reduced to an average of 0.53 and 0.60 microliters of oxygen/ml./minute/Gm. of renal tissue after 48 hours and 72 hours, respectively. The per cent tubular sodium reabsorption in both kidneys was above 95. Extraction of PAH averaged 0.20 at 48 hours and 0.19 at 72 hours. This corresponded to renal cortical flows (ERCF) of 13 ml./minute and 23 ml./minute. A solute diuresis with little net

TABLE 3. *Renal Function after 24 Hr. 50% Helium*

Periods (15 min.)	Renal Arterial Pressure (mm. Hg)	Arterial pH	Arterial pCO_2	Arterial pO_2	Per Cent* Sodium Reabsorption	E_{PAH}*	E_{CR}*
	169	7.39	22.0	440	94.7	—	—
U_0	±5.7	±0.12	±8.0	±113	±1.4		
	171	7.42	23	350	91.2	0.32	0.26
U_1	±5.9	±0.14	±8.0	±143	±7.4	±0.33	±0.17
	170	7.45	22	439	94.1	0.11	0.14
U_2	±5.8	±0.25	±7.0	±142	±3.8	±0.24	±0.20
	168	7.48	21	460	95.5	0.14	0.11
U_3	±5.7	±0.13	±8.0	±7.4	±2.8	±0.21	±0.20

* Symbols explained at bottom of Table 1.

TABLE 4. *Renal Function after 24 Hr. 95% Helium*

Periods (15 min.)	Renal Arterial Pressure (mm. Hg)	Arterial pH	Arterial pCO_2	Arterial pO_2	Per Cent* Sodium Reabsorption	E_{PAH}*	E_{CR}*
	190	7.64	51	228	95.1	—	0.16
U_0	±4.7	±0.21	±28	±135	±4.5		±0.37
	198	7.62	56	234	97.5	0.03	0.13
U_1	±2.5	±0.23	±27	±146	±0.22	±0.04	±0.25
	190	7.58	53	263	97.7	0.09	0.07
U_2	±2.8	±0.33	±25	±105	±0.70	±0.12	±0.08
	187	7.57	53	261	97.0	0.08	0.06
U_3	±3.5	±0.30	±27	±146	±1.4	±0.14	±0.15

solute free water reabsorption was observed in both instances.

In Vitro Renal Metabolism

A. After 24 hours' Preservation-Perfusion with Perfusate Exposed to 100% Oxygen

Table 5 summarizes the *in vitro* metabolic data on three kidneys after 24 hours storage followed by one hour oxygenated perfusion at normal temperature and pressure. Comparative values obtained from four kidneys not preserved or perfused are shown in Table 6. These values demonstrate that storage and perfusion of kidneys in the presence of an excess of oxygen, resulted in a marked decrease in cortical and medullary respiration both with and without glucose as substrate. Glucose uptake by cortex slices of the perfused kidneys was depressed, while no significant change was observed in the glucose uptake of medulla slices. $^{14}CO_2$ production was not significantly altered. Lactate production as well as the L-P ratio were also increased in the perfused renal cortex and medulla, indicating increased anaerobic glycolysis. The mitochondrial oxygen uptake and P-O ratio of oxygen exposed kidneys were depressed in both cortex and medulla mitochondria.

B. After 24 hours' Preservation-Perfusion with Perfusate Exposed to 100% Helium

The *in vitro* metabolic results obtained in six kidneys after 24 hours storage and one hour oxygenated perfusion, are summarized in Table 7. Two different perfusion solu-

50% Oxygen (3 atm.) 4°C. Exposure (9 animals)

C_{OSM} (ml./min.)	ERCF* (ml./min.)	C_{CR} (ml./min.)	T^cH_2O*	Urine Flow (ml./min.)	Renal Flow (ml./min./ Gm.)	Renal Oxygen Consumption (microl. O_2 ml./min./Gm.)
4.2	—	—	0.18	4.3	2.06	1.31
±6.8			±0.27	±6.5	±0.56	±0.7
5.4	37.8	5.0	1.01	5.5	2.10	0.98
±8.4	±36.8	±6.6	±0.41	±8.3	±0.40	±0.33
6.3	11.7	7.2	−0.02	6.3	2.10	1.05
±7.8	±24	±14.1		±7.8	±0.40	±0.47
7.2	15.0	7.4	0.04	7.2	2.12	0.632
±7.5	±20.7	±8.4	±0.08	±7.4	±0.48	±0.54

5% Oxygen (3 atm.) 4°C. Exposure (7 animals)

C_{OSM} (ml./min.)	ERCF* (ml./min.)	C_{CR} (ml./min.)	T^cH_2O*	Urine Flow (ml./min.)	Renal Flow (ml./min./ Gm.)	Renal Oxygen Consumption (microl. O_2 ml./min./Gm.)
9.4	—	22.7	−0.09	9.4	2.14	0.58
±4.6		±38	±0.17	±4.5	±0.70	±0.51
13.2	3.9	20.3	−0.02	12.7	2.12	0.56
±8.5	±8.9	±21	±0.23	±8.9	±0.68	±0.41
13.2	4.5	14.5	0.09	13.1	2.11	1.09
±5.4	±6.4	±6.5	±0.07	±5.4	±0.69	±0.51
13.4	6.1	13.1	0.01	13.4	2.10	0.93
±11.4	±9.1	±11.5	±0.18	±11	±0.60	±0.91

tions were utilized, viz. "Plasmalyte B" plus dextran and "GSH replacement solution" * plus dextran. It has been found that the 5% invert sugar replacement solution containing dextran can obscure the uptake of glucose by tissue slices.[10] Although a detailed study of the effects of different perfusion solutions is beyond the scope of the present series of investigations, it was necessary to consider separately the metabolic results obtained with these different solutions.

The results presented in Table 7 show that on comparison with an oxygenated preservation system (Table 5) kidneys preserved with an anoxic system responded as follows.

Using plasmalyte B and dextran as perfusion solution, 100% helium preservation resulted in no significant change in cortex and medulla oxygen uptake. $^{14}CO_2$ production and the L-P ratio were decreased in both cortex and medulla. Similarly, the oxygen uptake and P-O ratio of cortex mitochondria were depressed when compared with the oxygen-preservation study (Table 5).

An improved metabolic pattern of glucose-U-C^{14} was obtained when replacement solution with dextran was employed as substrate. Oxygen uptake, with and without substrate, was increased after helium preservation. Glucose uptake and $^{14}CO_2$ production were unchanged while the L-P ratio was decreased, indicating a reduction in anaerobic glycolysis after helium as compared with oxygen preservation. The P-O

* See Materials and Methods.

TABLE 5. *Renal Metabolism after*

Expt.	Endogenous Uptake	O_2 Uptake (Glucose as substrate)	Glucose Uptake	$^{14}CO_2$ Production
Cortex				
19	8,062	7,694	118.47	61.82
22	3,807	3,467	33.78	10.58
9	3,361	3,312	92.58	21.62
Av. ± S.E.	5,077 ±1,498	4,824 ±1,436	81.61 ±25.06	31.34 ±15.57
Medulla				
19	5,671	3,928	209.07	71.87
22	4,103	2,875	194.70	9.64
9	5,528	2,797	380.86	8.01
Av. ± S.E.	5,101 ±501	3,200 ±365	261.54 ±59.80	29.84 ±21.02

Tissue slice oxygen uptake expressed as μl/g dry wt./hr.
Mitochondrial oxygen uptake expressed as μ atoms/mg. mitochondrial protein/20 minutes.

ratio remained depressed after both preservation methods.

C. After 24 hours' Preservation-Perfusion with Perfusate Exposed to a 50% Oxygen Mixture

Table 8 illustrates the results of *in vitro* metabolic studies on four kidneys after 24 hours storage and one hour oxygenated perfusion. On comparison with kidneys preserved in the presence of either 100% oxygen or 100% helium, these kidneys show a greater depression of cortical and medullary respiration. In contrast to results obtained after oxygen and helium preservation (Tables 5 and 7), oxygen uptake was more depressed in the cortex than in the medulla. Although glucose uptake was increased in both medulla and cortex, no increase in $^{14}CO_2$ production was observed. Medullary lactate production as well as L-P ratio were significantly increased. Cortex and medulla mitochondrial oxygen uptake and P-O ratio were depressed.

D. After 24 Hours' Preservation-Perfusion with Perfusate Exposed to 95% Helium and 5% Oxygen

The data obtained from metabolic studies on four kidneys after 24 hours preservation in a low oxygen system and one hour oxygenated perfusion, are summarized in Table 9. Oxygen uptake by both cortical and medullary slices are higher than those obtained by the previous preservation methods (Tables 5, 7, 8) and compare well

TABLE 6. *Metabolism of (U-C^{14})-glucose and Oxidative Phosphorylation*

Tissue	Endogenous** Oxygen Uptake	Oxygen Uptake with Glucose as Substrate	Glucose Uptake	$^{14}CO_2$ Production
Cortex	9,923	10,520	128.8/	15.98
	8,704	10,566	136.35	20.98
	9,995	11,258	176.37	27.18
	9,389	10,728	118.33	19.62
X ± S.E.	9,052 ±298	10,768 ±169	139.96 ±12.68	26.94 ±2.33
Medulla	7,099	—	—	—
	7,604	7,490	260.38	37.04
	6,673	7,851	369.66	22.89
	6,031	6,967	229.73	25.30
X ± S.E.	6,851 ±333	7,436 ±256	286.59 ±42.46	28.41 ±4.37

*Mean values of 4 experiments. Glycogen contents before and after incubation were done in 1 experiment.
** Tissue slice oxygen uptake expressed as μ/Gm. dry wt./hr.

*Oxygen Preservation for 24 Hours**

Pyruvate Production	Lactate Production	L-P Ratio	Mitochondrial O_2-uptake	P-O Ratio
1.40	97.65	69.75	1.20	1.94
3.19	84.52	26.50	1.19	1.29
1.93	91.53	47.43	0.53	0.39
2.17 ±0.53	91.23 ±3.79	47.89 ±12.49	0.97 ±0.22	1.21 ±0.45
2.33	91.92	39.45	2.30	1.30
3.87	184.11	47.57	1.43	0.16
2.87	266.92	93.00	—	—
3.02 ±0.45	180.98 ±50.42	60.01 ±16.66	1.87 ±0.43	0.73 ±0.57

Glucose uptake, $C^{14}O_2$, pyruvate and lactate production expressed as μ moles glucose equivalents/Gm. dry wt./hr.

with those of the unperfused control kidney (Table 6). Glucose uptake and $^{14}CO_2$ production by cortex slices were higher than those observed in control kidneys. Lactate production and the L-P ratio were increased in the medulla. However, the mitochondrial oxygen uptake as well as P-O ratio showed the same trend as obtained by the other preservation methods and remained depressed.

E. After 72 Hours' Preservation-Perfusion with Perfusate Exposed to 50% Helium and 50% Oxygen

In vitro metabolic studies were performed on one kidney after 72 hours' preservation with 50% oxygen and 50% helium and one hour oxygenated perfusion. Endogenous oxygen uptake, as well as oxygen uptake with glucose as substrate, was higher than the values obtained in kidneys preserved with the same gas mixture for 24 hours (Table 8). Glucose uptake and $^{14}CO_2$ production were higher in the cortex. Lactate production and the L-P ratio were decreased in both cortex and medulla, indicating a reduction in anaerobic glycolysis when compared with the 24-hour preserved kidneys. Mitochondrial P-O ratio, however, remained depressed.

Discussion

The use of hyperbaric oxygen as an aid in organ preservation may have limited benefit. For example, it has been shown in man [14] that peripheral vasoconstriction is present with hyperbaric oxygenation. Such a vasoconstriction could have obvious dele-

*in Control Baboon Kidney Tissue (without perfusion)**

Pyruvate Production	Lactate Production	L:P Ratio	Mitochondrial Oxygen Uptake	Phosphorylation:Oxidation Ratio
2.36	32.24	13.66	1.60	2.39
2.47	35.54	14.39	1.76	2.37
3.52	50.72	14.41	1.73	2.57
2.56	31.22	12.20	1.66	2.69
2.72 ±0.26	37.43 ±4.52	13.66 ±6.50	1.68 ±0.03	2.50 ±0.07
—	—	—	2.33	2.29
5.61	108.69	19.37	2.53	2.15
13.04	168.33	12.91	—	—
7.82	117.55	15.03	2.58	2.72
8.82 ±2.20	131.52 ±18.58	15.77 ±1.90	2.48 ±0.07	2.39 ±0.17

Mitochondrial oxygen uptake expressed as μ atoms/mg. mitochondrial protein/20 minutes.
Glucose uptake, $^{14}CO_2$, pyruvate and lactate production expressed as μ moles glucose equivalents/Gm./dry wt./hr.

TABLE 7. *Renal Metabolic Results after Helium*

Expt.	Endogenous Oxygen Uptake	O_2-uptake (glucose as substrate)	Glucose Uptake	$^{14}CO_2$ Production
Cortex:				
Plasmalyte B & dextran:				
10	5,291	5,031	14.41	14.17
11	3,274	3,585	14.93	9.11
Av. ± S.E.	4,283 ±1,009	4,308 ±723	14.67 ±0.26	11.6 ±2.53
Replacement solution & dextran:				
12	5,862	6,168	78.27	16.38
16	8,945	9,895	—	19.76
17	9,488	1,011	135.89	40.75
18	8,045	9,119	42.58	18.74
Av. ± S.E.	8,053 ±799	8,823 ±910	64.19 ±28.16	23.91 ±5.66
Medula:				
Plasmalyte B & dextran:				
10	3,646	3,155	231.36	11.29
11	7,973	5,015	219.11	12.19
Av. ± S.E.	5,816 ±2,164	4,085 ±930	225.24 ±6.12	11.74 ±0.45
Replacement solution & dextran:				
12	5,241	6,675	222.88	12.35
16	6,183	5,389	235.14	22.11
17	10,719	9,056	263.04	30.21
18	9,743	7,733	239.59	23.60
Av. ± S.E.	7,972 ±1,333	6,938 ±884	242.66 ±8.28	22.07 ±3.69

terious effects in isolated kidney preservation and perfusion. In fact, we have previously shown that the isolated kidney suffers from vasoconstriction, and the greatest decrease occurs in cortical perfusion when exposed to oxygen under normothermic, normobaric conditions.[10, 13] Helium, an inert gas, was substituted for the oxygen and under similar conditions an improvement in renal perfusion was observed.[10, 13] Basic renal ionic performances such as salt conservation and glomerular filtration rate were not depressed even after 3 hours of isolated bloodless perfusion with the anoxic perfusate.[10, 13] Bloodless perfusion of organs or intact animals has been successfully achieved previously by others [1, 6, 11] and has advantages over blood or diluted blood mixtures. The present studies were undertaken to compare the effects of helium or oxygen under isolated conditions with a hyperbaric and hypothermic environment similar to that described by others.[5, 8, 9]

The preservation system described herein has several advantages over those previously described: (1) The compact cylinder easily fits into a household refrigeration unit, and sterility can be readily maintained. (2) Because the pump for the renal perfusion is small, it fits inside the preservation chamber obviating pumping from normobaric to hyperbaric pressures. (3) The use of a calibrated solenoid device permits the calibrated perfusion of the kidney with suitable accuracy for prolonged periods with as little as 0.2 ml./minute/Gm. of kidney tissue. This helps to reduce edema and weight gain, as documented by the observed changes in the wet weights of the kidneys.

Baboons are phylogenetically closer to man and the function of their kidneys after renal storage and perfusion should therefore be more relatable to circulatory and metabolic conditions in the human than can be expected in the dog or rat.

By testing the performance of a kidney after prolonged preservation on a well tried perfusion system, comparable functional assessment can be obtained and compared to the results obtained with fresh specimens.[2] Such a test-system provides comprehensive evaluation of renal function and metabolism. Evaluation of the effectiveness of a storage system by autotrans-

Preservation (100%) for 24 Hours

Pyruvate Production	Lactate Production	L-P Ratio	Mitochondrial O_2-Uptake	P-O Ratio
3.29	92.67	28.17	0.35	0
3.05	85.36	27.99	0.31	0.20
3.17 ±0.12	89.02 ±3.65	28.08 ±0.09	0.33	0.10
2.96	91.03	30.75	0.64	1.71
2.60	117.78	45.30	1.06	1.59
1.59	62.06	39.03	1.28	0
2.28	68.06	29.85	1.63	2.09
2.36 ±0.29	84.73 ±12.66	36.23 ±3.66	1.15 ±0.20	1.35 ±0.46
4.61	137.85	29.90	—	—
5.43	176.91	32.58	—	—
5.02 ±0.41	157.38 ±19.53	31.24 ±1.34	—	—
4.39	141.44	32.22	—	—
4.37	167.97	38.44	2.52	1.48
3.56	188.82	53.04	—	—
6.43	163.91	25.49	0.88	2.40
4.69 ±0.61	165.54 ±9.71	37.30 ±5.88	1.70 ±0.82	1.94 ±0.46

plantation only, although essential, can be technically troublesome and requires prolonged follow-up study. *In vitro* evaluation gives the opportunity to make rapid comparisons between various systems or variations in detail in a closely comparable manner.

The present study has enabled us to select a gaseous environment which is less deleterious in its renal circulatory and metabolic effects than heretofore available either in dogs or in primates. The results detailed have confirmed that hyperbaric oxygen can induce relative renal vasoconstriction and decreased perfusion. Although the capacity for handling of salt is maintained, oxygen consumption is reduced (Table 1). Renal cortical flow is reduced compared to isolated normobaric normothermic perfusion of the fresh organ with helium or oxygen exposed perfusate.[10, 13] In contrast kidneys preserved with 100% helium (Table 2) have better renal blood flow, and good tubular performance. Renal oxygen consumption is not abolished by the anoxia of hyperbaric helium, even after 24 hours, and the directly measured values (Table 2) show better oxygen uptake values than kidneys preserved with hyperbaric oxygen. Tubular reabsorption of salt is unaltered. When pure helium or mixtures with a high proportion of helium with little oxygen are used for renal preservation, a lesser gain in wet tissue weight was found than when pure oxygen was used. The decrease in edema and kidney weight should improve the chances for successful restoration of the renal circulation and function after prolonged storage. Mixtures of equal parts of helium and oxygen under hyperbaric conditions did have significant deleterious effects (Tables 3, 4). Renal perfusion pressure was elevated, with vasoconstriction and decreased flow rates. Renal oxygen consumption and tubular salt reabsorption were less impaired after one hour oxygenated perfusion when 95% helium and 5% oxygen were used during preservation than was the case with a 50% helium-oxygen mixture. In both gas mixtures renal cortical perfusion was decreased. It is unknown what agent or substance could cause such an effect. The prolonged storage period makes it unlikely that renin or angio-

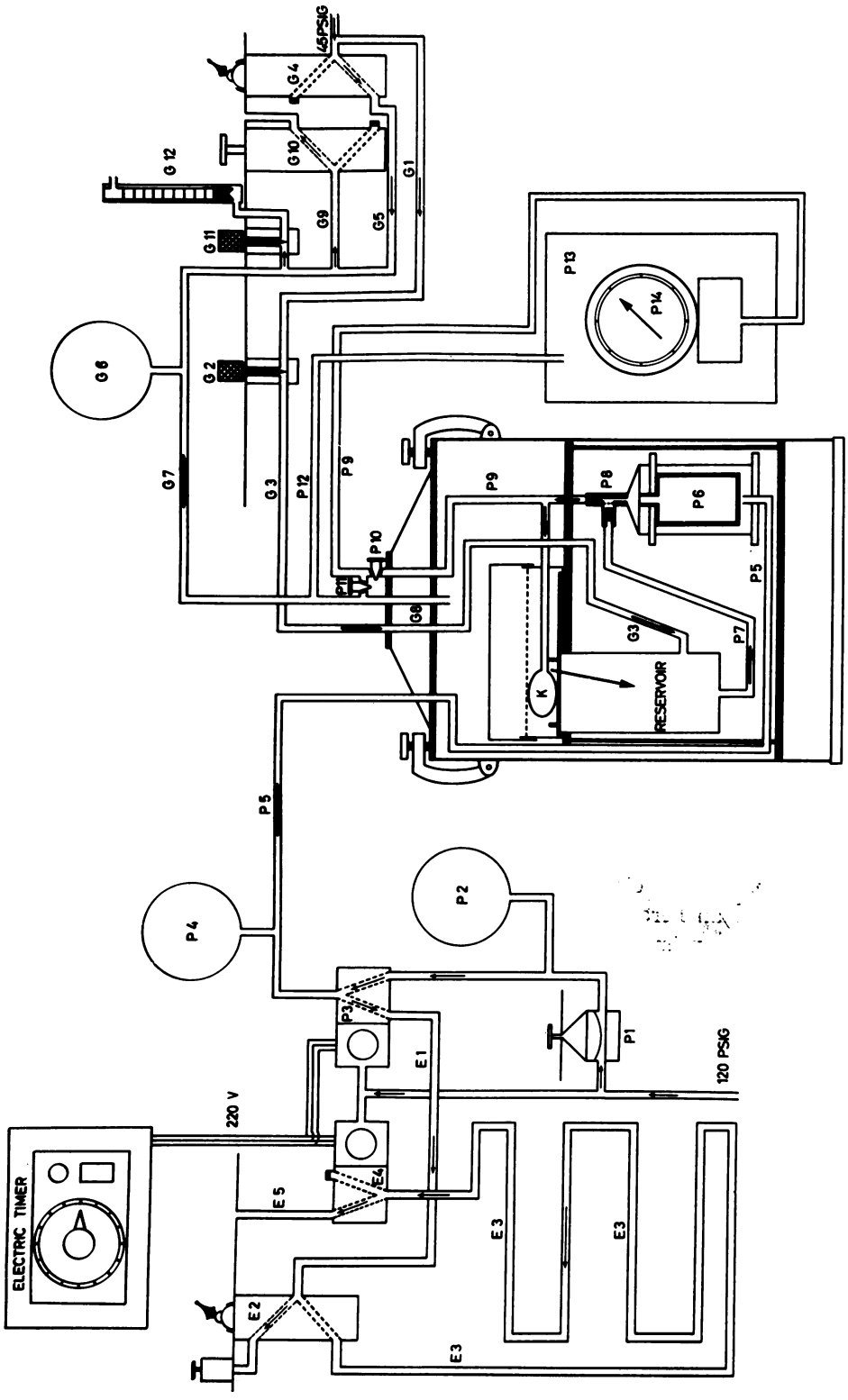

FIG. 1. Diagram of hypothermic, hyperbaric perfusion unit. See text for details.

tensin could be possible causes. The hypertension and vasoconstriction have been successfully decreased by the addition of 1% procaine to the preservation perfusate or by irrigation of the kidneys with papaverine before storage in our current studies. Such alterations indicate the importance of vasodilators in effective renal storage, although they have no detectable circulatory benefit in the acute isolated bloodless perfusion.[10, 13] It seems likely that the presence of oxygen, even in a low proportion, is directly or indirectly responsible for the intense vasoconstriction and observed cortical ischemia (Tables 3, 4).[14] Hitchcock and associates [12] have noted hypertension in baboons following reimplantation of kidneys preserved with oxygen. Such a result may well have its inception during the preservation period. The ischemia is located in the renal cortex and can functionally involve the glomeruli as well as the renal vessels and tubules.

In vitro metabolic assessment of cortex and medulla tissue slices after preservation and perfusion indicated that both oxygen and helium preservation resulted in a depression of cortical and medullary oxygen uptake with a concomitant increase in L-P ratio. The 50% oxygen and 50% helium gas mixture had the most deleterious effect on the metabolic patterns studied. The depression in oxygen uptake, measured in terms of endogenous oxygen uptake or with glucose as substrate, was greatest, while the P-O ratio was impaired to greater degree than with the other gas mixtures. On the other hand, metabolism of glucose-U-^{14}C was better maintained after preservation with 95% helium and 5% oxygen. The respiratory activity of 95% helium preserved kidneys compare well with those of the unperfused control kidney (Table 6). These observations show a similarity to the results obtained during normobaric, normothermic perfusions in which 50% helium and oxygen mixtures had a more deleterious

TABLE 8. *Renal Metabolic Results after 50% Helium 50% Oxygen Preservation for 24 hours*

Expt.	Endogenous Oxygen Uptake	O_2-uptake (glucose as substrate)	Glucose Uptake	$^{14}CO_2$ Production	Pyruvate Production	Lactate Production	L-P Ratio	Mitochondrial O_2-Uptake	P-O Ratio
Cortex									
24	2,026	2,676	143.36	12.34	1.38	77.19	55.93	—	—
25	671	1,065	122.37	4.11	0.79	47.44	60.05	0.42	0
26	1,562	2,536	160.14	9.83	2.07	83.78	40.47	0.67	0.44
27	587	700	82.82	2.32	1.42	31.49	22.18	0.48	0.11
Av. ± S.E.	1,212±350	1,744±504	127.17±16.68	7.15±2.36	1.42±0.26	59.98±12.35	44.66±8.60	0.53±0.07	0.18±0.13
Medula									
24	3,039	2,571	416.71	40.71	3.24	305.74	94.36	—	—
25	3,067	4,309	251.77	21.25	1.27	198.17	156.04	0.63	0
26	3,165	3,416	239.47	9.14	3.07	184.08	59.96	1.30	0.35
27	3,033	3,859	377.74	11.88	3.79	276.47	70.57	0.95	0.41
Av. ± S.E.	3,076±31	3,536±371	321.42±44.55	20.75±7.14	2.84±0.55	241.12±29.61	95.23±21.51	0.96±0.19	0.25±0.13

TABLE 9. *Renal Metabolic Results after 95% Helium 5% Oxygen Preservation for 24 Hours*

	Endogenous Oxygen Uptake	O_2-uptake (glucose as substrate)	Glucose Uptake	$^{14}CO_2$ Production	Pyruvate Production	Lactate Production	L-P Ratio	Mitochondrial O_2-Uptake	P-O Ratio
Cortex									
34	8,336	12,338	376.05	71.26	2.98	92.07	30.90	1.29	1.10
35	11,130	12,129	219.91	47.21	3.87	64.28	16.61	1.47	0.46
36	12,940	18,877	178.39	84.38	7.28	45.20	6.21	1.25	0.11
37	11,803	20,622	646.65	143.28	5.50	58.60	10.63	1.19	0.11
Av. ± S.E.	11,052±979	15,992±2,198	380.25±94.46	86.53±20.42	4.81±0.95	65.04±9.86	16.09±5.38	1.30±0.05	0.45±0.23
Medulla									
34	7,392	6,264	377.83	35.39	6.37	206.46	32.41	1.30	0.69
35	7,320	7,068	231.35	24.58	5.65	217.26	38.45	—	—
36	8,953	8,289	245.04	29.27	6.45	212.15	32.89	1.73	0.59
37	6,732	9,234	486.01	55.07	4.85	203.83	42.03	1.94	0.15
Av. ± S.E.	7,599±475	7,714±615	335.06±60.19	36.08±6.71	5.83±0.37	209.93±3.00	36.45±2.31	1.66±0.19	0.48±0.16

effect on kidney function than 95% helium and 5% oxygen.

All preservation technic resulted in an increase of the L-P ratio, indicating increased anaerobic glycolysis. Glucose uptake was not generally increased. The increased lactate production may thus be attributed to either increased breakdown of glycogen stores, or lactate accumulation in the kidney during the preservation or perfusion period, with subsequent leakage into the medium during incubation.

Uncoupling of oxidative phosphorylation and depression of mitochondrial oxygen uptake are present to some degree in all preserved kidneys. The increase in glycolysis might therefore represent a compensatory mechanism to supplement the decreased oxidative phosphorylation with an increase in anaerobic phosphorylation. However, the exact mechanism by which these preservation and perfusion technic depresses oxidative phosphorylation remains to be elucidated. Possibilities include the absence of, or damage to, an obligatory enzyme or the presence of an inhibitory factor. Changes in mitochondrial ion transport mechanisms or membrane permeability also have to be considered. Other instances of uncoupling of oxidative phosphorylation have been induced by ultrafiltrates of uremic serum.[4] Similar changes occurred in guinea pig kidney mitochondria during experimental heart failure.[3] These changes in renal transport may be dependent on co-enzyme A (Co A) alterations and suggest that significant changes can occur within the renal tubular cellular enzymatic processes during various states of stress, including renal preservation and oxygen toxicity.

The fact remains that helium preserved kidneys function as well as oxygen stored ones in many respects. These changes are in some way relatable to the degree of anoxia. With helium after 24 or more hours storage, renal oxygen consumption is still present and tubular reabsorption of sodium

is maintained. The renal cortical vasoconstriction observed with normobaric, normothermic oxygen bloodless perfusion [10, 13] is accentuated with hyperbaric, hypothermic perfusion preservation with a 50% helium-oxygen mixture. In terms of the various *in vitro* postpreservation and perfusion renal metabolic tests, good function is detectable after 95% helium and 5% oxygen exposure. The renal hypertension and cortical ischemia observed with helium and oxygen mixtures after prolonged preservation can be altered with vasodilating agents.

Thus a new, and suitable method for storage of primate kidneys, using hyperbaric hypothermic conditions and perfusion with perfusate exposed to helium gas has been devised and tested.

Summary and Conclusion

(1) A method has been developed for storage of primate kidneys under hyperbaric, hypothermic conditions and perfusion with perfusate exposed to helium gas mixtures.

(2) Renal circulatory and functional benefits not seen after oxygen preservation are evident during oxygenated perfusion subsequent to anoxic (helium) preservation.

(3) Fifty per cent helium and oxygen gas mixtures are of little renal functional and metabolic benefit.

(4) Renal vasoconstriction present with hyperbaric oxygen is also seen to some degree after 24 hours storage with 95% or 50% helium gases.

(5) Renal *in vitro* metabolism of glucose-U-^{14}C was best maintained after preservation with 95% helium and 5% oxygen. Uncoupling of oxidative phosphorylation and depression in mitochondrial uptake were present to some degree in all preserved kidneys.

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