THE METABOLISM OF THE ISCHEMIC KIDNEY

I. The Respiration and the Oxidase Activity of the Ischemic Kidney

By SIGWIN B. RASKA, Ph.D.

(From the Department of Physiology, University of Toronto, Toronto)

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Many of the chemical processes of the kidney are governed by enzymic systems and any change in the enzymic equilibrium may be of physiological importance. There is the possibility that changes may be found in such systems in the ischemic kidney. In view of the marked pressor effects of some amines and the hypertension resulting from renal ischemia produced experimentally, a study, in the ischemic kidney, of respiration and of the oxidative enzymes acting on amines and amino acids, appeared to be a useful contribution to the problem of hypertension. The present paper reports the results obtained in a comparative study of the activities of these oxidative enzymes in the kidneys of dogs and rabbits with experimental renal hypertension.

Methods

Operative Technique.—In studies on dogs, the clamping procedure of Goldblatt (1) was used. Two types of operation were performed, (1) unilateral renal ischemia was produced by partial constriction of the left renal artery, (2) both renal arteries were partially constricted. With unilateral renal arterial constriction the clamp was always put on the left artery.

Unilateral renal ischemia was produced in adult rabbits by partly constricting the left renal artery by Drury's method (2). A wire of 0.85 mm. diameter was placed alongside the left artery. The tie with cotton thread was made in such a way that, without damaging the wall of the artery, the lumen of the artery was partly constricted. The wire was then withdrawn. The right kidney was left intact.

In dogs and rabbits the operations were done aseptically under sodium isoamyl ethyl barbiturate (sodium amytal, Lilly) or ether anesthesia. All tests were made with the animals under basal conditions. Young dogs of 9 to 13 kilos in weight were used. All rabbits used were males, and controls were selected so as to be of the same weight and age as the corresponding experimental animals.

In order to make sure that none of the observed effects arose from the operative technique, some of the control animals were put through all the operative procedures with the sole exception of the final clamping of the renal artery. No differences arising from these causes were observed. Both control and experimental animals were fasted for 24 hours before they were killed.

Blood Pressure Measurements.—All blood pressure determinations were made on the unanesthetized animals. The blood pressure readings for the last 3 to 5 days before the animals were used for the experiments were averaged. The blood pressure of the

dogs was measured by direct intra-arterial puncture of the femoral artery with a hypodermic needle No. 20, connected to a mercury manometer. In the initial experiments with rabbits the blood pressure was determined by using an ordinary clinical sphygmomanometer. The cuff was placed around the lower abdomen and the abdominal aorta was used for auscultation. In the later experiments the blood pressure was measured by Grant and Rothschild's capsule (3) on the central artery of the ear.

Estimation of Enzymic Activity.—The tissue slice technique and the manometric method of Warburg (4) were employed. The measurements were made at 37.5°C. and pH 7.4 in an atmosphere of oxygen. When extracts of tissue were used air was substituted for oxygen. The inner cup of the vessels contained 0.2 ml. N NaOH and filter paper according to the technique of Dixon and Elliot (5). Ammonia estimations on the fluids in the manometric vessels at the termination of the experiments were made by the method of Parnas *et al.* (6). All the reagents used were tested regularly to see that they were ammonia-free. Oxidase activity was determined by measuring the increased oxygen consumption and ammonia formation on the addition of the substrates tyramine, isoamylamine, *l*-epinephrine, histamine, *dl*-alanine, *l*-aspartic acid, *dl*- and *l*-dihydroxyphenylalanine (dopa). The *l*-epinephrine was obtained from the Connaught Laboratories and the *dl*- and *l*-dopa from Hoffmann-La Roche Inc., and the other substances from the Eastman Kodak Company. When cyanide was added to the medium the NaOH in the inner cup contained the same concentration of free HCN as the experimental fluid (Krebs, 7). The substrate and cyanide solutions were adjusted so as not to affect the pH of the medium. The amines were used as their hydrochlorides and the amino acids were neutralized before being added. Only kidneys which did not show any gross signs of necrosis were used for the experiments.

Tissue slices were cut from freshly dissected kidneys with a thickness usually between 0.3 and 0.4 mm., and with a total dry weight of tissue used varying from 10 to 25 mg. Scar tissue was avoided in preparing the slices. Slices of kidney cortex were taken from the central part as well as from the poles and suspended either in Warburg's bicarbonate-Ringer solution or in Krebs' phosphate-salts solution. The latter was used mainly in the study of the metabolism of the amines and amino acids. With tissue slices a final concentration of amines of M/100 and of amino acids of M/30 was used and the experiments carried on for a period of from 1 to 2 hours.

In the experiments with tissue slices where tyramine or isoamylamine were used as substrates, cyanide (M^{-3}) was added to inhibit the normal cell respiration. The symbols Q_{O_2} and Q_{NH_3} are used for the rate of reaction and mean cubic millimeters per hour per milligram of dried tissue.

The tissue extracts were prepared in a manner similar to that described by Blaschko, Richter, and Schlossmann (8) and Holtz, Heise, and Lüdke (9). 12.5 gm. of kidney tissue (cortex and medulla) from dogs were lightly ground for 20 minutes with 18 gm. of purified sand. After transfer to a graduated test tube 25 ml. of M/20 phosphate buffer, pH 7.4, were added and then extracted on a mechanical shaker for 30 minutes. After centrifuging for 5 minutes at low speed, 15 ml. of the supernatant fine suspension was dialysed for 7 hours in a cellophane sausage casing against phosphate-Locke solution (phosphate buffer 0.022 M; pH 7.4; glucose 0.08 per cent). To the dialysed sus-

pension was now added phosphate buffer, pH 7.4, to make the solution M/20 with respect to phosphate after diluting with distilled water to 25 ml. 1 ml. of this extract gave a dry weight of 40 ± 5 mg.

The extract was prepared from rabbit kidneys in the same way as from dog kidneys as described above. 3 gm. of kidney tissue (cortex and medulla) were lightly ground for 20 minutes with 5 gm. of purified sand, and then 10 ml. M/20 phosphate buffer, pH 7.4, were added. After extracting and centrifuging, 7 ml. of the supernatant were dialysed against phosphate-Locke solution of pH 7.4. To the dialysate were added phosphate buffer, pH 7.4, to make the solution M/20 phosphate after diluting to 15 ml. with distilled water. 1 ml. of this extract was found to contain 30 ± 5 mg. dry weight.

These extracts showed little residual respiration. In some experiments undialysed extracts were used. In these the velocity of oxygen uptake in the presence of the amines and amino acids was found to be 15 to 20 per cent greater than that of the dialysed extracts. In all experiments with *dl*-alanine, *dl*- and *l*-dopa undialysed extracts were used. Each vessel of the Warburg apparatus contained 1.8 ml. enzyme solution, 0.2 ml. M/4 amine hydrochloride, or 0.2 ml. M/3 amino acid. With *l*-epinephrine, isoamylamine, and tyramine as substrates, the oxidations were carried out in the presence of M^{-3} cyanide to prevent autoxidation or oxidation of the more labile substrates by the cytochrome oxidase system. In such cases the vessels contained 1.8 ml. enzyme solution, 0.2 ml. amine hydrochloride, and 0.1 ml. M/50 HCN. In those experiments dealing with tyramine where the aldehyde formed was to be estimated M/20 semicarbazide was also added to act as a fixative so that the effects of the aldehyde oxidase and mutase could be eliminated. The experiments with dog kidney extracts were carried on for a period of 90 minutes, except where *l*-dopa was the substrate when the period was 60 minutes. With rabbit kidney extracts the period of the experiment was 60 minutes. The aldehydes formed during the oxidation of the amines were isolated from the reaction products as the 2,4-dinitrophenylhydrazones. The amounts of ammonia are expressed in μl. gas to make easier comparison with volumes of oxygen consumed (17 mg. NH_3 = 22.400 μl.).

FINDINGS

The Enzymic Activity of Normal and Ischemic Kidneys.—A comparative study of the enzymic activity in normal and ischemic dog and rabbit kidneys has been made and the results are presented as follows:—

1. Table I represents a summary of all the data obtained by the slice technique with normal dogs.

2. Table II represents a summary of all the data obtained by the slice technique with dogs with renal ischemia.

3. The activity of some oxidative enzyme systems in kidneys from normal dogs is recorded in Table III.

4. The findings in dogs with unilateral constriction of the renal artery. In some of the animals, as indicated, there was a slight to moderate constriction and in the others there was a moderate to severe constriction. The normal and ischemic kidneys from the same dog were compared (Table IV).

5. The findings in dogs with bilateral renal arterial constriction. In some of the animals there was a moderate to severe constriction of both renal arteries and in others the right renal artery was slightly constricted and the left renal artery moderately to severely constricted. The data for both ischemic kidneys are given in Table V.

6. The results in rabbits with unilateral constriction of the renal artery. The ischemic kidney was compared with the normal kidney from the same rabbit and also with kidneys from normal rabbits (Table VI).

No significant difference in enzyme activity was found between the left and right kidneys of normal animals.

TABLE I

Normal Dogs

No.	No substrate	*dl*-Alanine	*l*-Aspartic	Isoamylamine*	Tyramine*	
	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{NH_3}
1	16.8	29.4	27.7	8.3	14.3	23.5
2	15.0	26.9	31.0	12.7	15.8	25.4
3	18.3	30.0	26.2	7.4	13.0	22.0
4	17.2	27.1	24.3	9.9	17.0	30.2
5	15.5	31.8	25.7	10.3	15.7	24.9
6	19.1	26.0	25.0	12.9	12.5	23.3
7	17.8	27.8	24.4	9.9	14.1	26.1
8	16.5	25.6	27.0	11.0	18.0	32.7
9	16.1	27.9				
10	16.6	30.3				
Average..	16.9	28.3	26.4	10.3	15.0	26.0

* When measuring the activity of amine oxidase the normal tissue respiration was depressed by adding KCN to 10^{-3} M concentration.

The average figures obtained for the oxygen uptake and ammonia formation in the presence of amines and amino acids, of the kidney extracts from normal animals of the same species, and of similar size and age, agreed fairly closely and thus indicated that the procedure followed, including the preparation of the extract and determination of the consumed oxygen and formed ammonia, gives fairly reproducible results. All the figures given in the tables are the average of at least three determinations.

The results reported in Tables I and II show that there was a significant decrease in the Q_{O_2} of the tissue slices from these ischemic kidneys. Further, the oxidizing power of the kidney slices for the amino acids and amines was decreased to approximately the same extent as that of the extracts prepared from the ischemic kidney (Tables IV and V). The average differences in Q_{O_2}

TABLE II

Hypertensive Dogs

No.	Pre-operated Blood pressure	Post-operated Blood pressure	Duration of renal ischemia	Right kidney: No substrate	Right kidney: *dl*-Alanine	Right kidney: *l*-Aspartic acid	Right kidney: Isoamylamine*	Right kidney: Tyramine*		Left kidney: No substrate	Left kidney: *dl*-Alanine	Left kidney: *l*-Aspartic acid	Left kidney: Isoamylamine*	Left kidney: Tyramine*		Remarks
				Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{NH_3}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{O_2}	Q_{NH_3}	
	mm. Hg	*mm. Hg*	*days*													
1	134	130	4	15.2	26.3	25.9	10.0	14.3	23.1	9.1	15.8	14.0	6.8	7.7	10.3	Moderate to severe constriction of the left renal artery
2	140	165	18	16.7	29.3	24.3	12.4	12.7	24.8	7.2	10.9	13.2	5.1	4.7	3.4	" "
3	121	186	21	19.8	27.2	26.8	10.7	10.4		6.2	13.3	12.0	4.8	3.4	6.5	" "
4	136	178	66	18.0	28.8	27.7	8.5	15.9	27.4	8.5	11.4	11.4	4.0	5.0	7.9	" "
5	120	174	130	17.5	26.0	25.2	13.5	16.3	21.5	5.7	10.0	16.6	3.2	6.9	6.2	" "
8	118	155	64	16.7	27.4	24.8	11.1	12.3	22.2	7.8			5.2	5.5	14.6	" "
27	126	178	72	15.6	26.3	29.8	9.7	15.0	25.4	6.0	14.1	14.6		2.0		" "
29‡	126	165	109	23.4	33.8	32.1	15.9	18.9	35.7	3.2	4.5					" "
Average...	128	167	54	17.9	28.1	27.1	11.5	14.5	25.7	7.3	12.6	13.6	4.8	5.5	8.1	
14	122	154	14	15.7	26.9	25.0	9.9	17.0		13.2	23.8	19.1	6.0	12.1		Slight to moderate constriction of left renal artery
15	134	194	22	16.2	29.2	26.8	11.5	15.8		12.8	15.4	16.9	7.7	10.2		" "
18	125	164	76	17.3	28.4	24.3	10.7	11.9		7.0	14.9	15.9	4.2	8.0		" "
19	128	148	39	17.1	28.8	26.4	9.1	13.7		10.6	20.2	18.2	7.4	8.7		" "
23	128	176	136	18.8	29.5	27.8	13.7	16.9		6.8	14.2		4.2	5.5		" "
28	140	172	11	15.8	30.2		13.2			7.6	12.0		5.1	4.8		" "
Average...	129	168	50	16.8	28.8	26.0	11.3	15.6		9.7	16.7	17.5	5.8	8.2		
9	117	183	120	6.5	12.0					4.7	13.6					Renal arteries on both sides moderate to severe constricted. Right renal artery was first constricted; left renal artery 11 to 14 days later
11	125	190	29	8.0	14.4					6.9	10.4					" "
12	121	215	88	6.8	12.7		5.1	4.5		8.8	8.2		6.1	5.0		" "
13	130	204	44	7.1	13.3		7.2	6.0		7.3	15.8		3.9	6.2		" "
25	134	208	74	7.7			4.4	3.9		9.3			5.6	8.2		" "
26‡	143	220	118	8.0			5.8			2.5			1.7			" "
Average...	125	200	71	7.3	13.1		5.6	4.8		7.2	11.4		5.2	6.5		

* When measuring the activity of amine oxidase the normal tissue respiration was depressed by adding KCN to 10^{-3} M concentration.

‡ Left kidneys markedly atrophied, not included in the average.

were (value for the normal animal first, ischemic kidney of the hypertensive animal second): 16.9, 7.3. The average increases in oxygen consumption (Q_{O_2}) due to the presence of the amino acids or amines were (normal animal

first, ischemic kidney of the hypertensive animal second): *dl*-alanine 28.3, 12.6; *l*-aspartic acid 26.4, 13.6; isoamylamine 10.3, 4.8; tyramine 15.0, 5.5; the corresponding average increases in ammonia formation (Q_{NH_3}) with tyramine were 26.0, 8.1. The values obtained for the unconstricted kidney of the hypertensive animal agreed closely with those obtained from normal animals. The results previously referred to are for kidneys, the renal arteries of which were subjected to a moderate to marked constriction. Also shown in Table II are the results obtained with a slight to moderate constriction. These show the

TABLE III

Oxidase Activity of Kidney Tissue Extracts of Normal Dogs

Substrate	No.	O_2	NH_3	Substrate	No.	O_2	NH_3
Tyramine	4	188	362	*l*-Epinephrine	9	124	
"	7	174	438	"	10	116	
"	8	225	314	"	12	108	
"	9	181	282	"	13	132	
"	10	198	248				
"	11	165	378	Average		120	
Average		188	337	*l*-Dopa	10	124	
				"	11	99	
Isoamylamine	4	135	234	"	12	87	
"	7	181	388	"	13	118	
"	10	151	276				
"	11	160	264	Average		107	
"	12	184	398				
"	13	146	320	*dl*-Alanine	4	118	265
				"	8	133	204
Average		159	313	"	10	141	330
				"	11	111	196
				Average		126	248

Figures reported are the increase in oxygen consumption and ammonia formation due to added substrate over the control sample, containing no added substrate. Oxygen consumption and ammonia formation in μl. over a period of 90 minutes.

same decrease in oxidizing power, although the decrease may be less than in the markedly constricted kidney. In the same table are the results obtained from animals in which both renal arteries were constricted. A similar decrease in the oxidizing power of both kidneys was observed.

As seen in Tables III and IV, the average increases in oxygen consumption (μl.) of the extracts from the kidneys of normal dogs (Table III) and hypertensive dogs (Table IV) were (normal animal first): tyramine 188, 111; isoamylamine 159, 98; *l*-epinephrine 120, 60; histamine —, 45; *l*-dopa 107, 44; *dl*-alanine 126, 68. The corresponding values for ammonia formation (μl.) with

TABLE IV

Comparison of the Oxidase Activity on Various Amine and Amino Acids of Kidney Tissue Extracts from the Normal and Ischemic Kidney of Dogs with Unilateral Constriction

Substrate	No.	Pre-operated Blood pressure	Post-operated Blood pressure	Duration of renal ischemia	Right kidney		Left kidney	
					O_2	NH_3	O_2	NH_3
				days				
Tyramine	1	134	130	4	196	415	165	202
"	2	140	165	18	176	356	121	102
"	3	121	186	21	227	445	93	122
"	4	136	178	66	199	390	114	88
"	5	120	174	130	153	124	99	115
"	7	126	195	109	210	401	83	77
"	8	118	155	64	175	394	102	166
Average		128	169	59	191	361	111	124
Isoamylamine	1	134	130	4	159	332	126	186
"	2	140	165	18	188	303	108	148
"	3	121	186	21	174	211	88	82
"	4	136	178	66	192	386	103	110
"	5	120	174	130	124	254	88	162
"	7	126	195	109	158	338	66	136
"	8	118	155	64	176	422	104	160
Average		128	169	59	167	321	98	140
l-Epinephrine	2	140	165	18	112		65	
"	3	121	186	21	96		43	
"	4	136	178	66	127		49	
"	5	120	174	130	134		84	
Average		129	176	59	117		60	
Histamine	2	140	165	18	125		58	
"	3	121	186	21	101		67	
"	4	136	178	66	83		25	
"	5	120	174	130	89		29	
Average		129	176	59	99		45	
l-Dopa	1	134	130	4	116		71	
"	2	140	165	18	92		59	
"	3	121	186	21	107		20	
"	4	136	178	66	101		33	
"	6	130	174	124	74		29	
Average		132	167	47	98		44	

TABLE IV—*Concluded*

Substrate	No.	Pre-operated Blood pressure	Post-operated Blood pressure	Duration of renal ischemia	Right kidney		Left kidney	
					O_2	NH_3	O_2	NH_3
				days				
dl-Alanine	1	134	130	4	136	210	83	120
"	2	140	165	18	116	275	68	115
"	3	121	186	21	121	200	50	84
"	4	136	178	66	98	160	61	101
"	6	130	174	124	130	302	78	78
Average		132	167	47	120	229	68	100
Tyramine	14	122	154	14	152		143	
"	15	134	184	22	177		185	
"	17	117	152	25	163		180	
"	18	125	164	76	196		123	
"	19	128	148	59	181		108	
"	20	132	165	132	173		131	
Average		126	161	55	174		145	
Isoamylamine	14	122	154	14	170		160	
"	15	134	184	122	131		152	
"	17	117	152	25	154		120	
"	20	132	165	132	188		78	
"	21	128	156	62	183		81	
Average		127	162	71	165		118	

Figures reported are the increase in oxygen consumption and ammonia formation due to added substrate over the control sample, containing no added substrate. Oxygen consumption and ammonia formation in μl. over a period of 90 minutes. In the last two series (tyramine and isoamylamine), the constriction was slight to moderate, moderate to severe in all the other series.

added substrate were: tyramine 337, 124; isoamylamine 313, 140. Hence there were significant decreases in oxygen consumption of the extracts from the ischemic kidneys with all the substrates and the ammonia formation was decreased to a corresponding extent. With slight constriction of the artery maintained over a shorter period, the decrease in oxygen consumption was less. These results were for moderate to severe constriction of the left renal artery. With slight to moderate constriction the findings were similar, but in general the decrease was not quite so great. As with tissue slices, the results obtained with the unconstricted kidney of the hypertensive animal agreed closely with the results from the normal animals. Hence, the difference between normal and constricted kidney was even more marked where the results from the two kidneys were compared in the same animal. Such a comparison is only justi-

fied, however, in these short periods of partial constriction where the values as shown remain normal.

In Table V are given the results obtained with extracts from dogs in which both renal arteries were constricted. Here both kidneys showed a significant

TABLE V

Comparison of the Oxidase Activity on Various Amines and Amino Acids of Kidney Tissue Extracts from the Two Kidneys of Dogs with Both Renal Arteries Constricted

Substrate	No.	Pre-operated Blood pressure	Post-operated Blood pressure	Duration of renal ischemia	Right kidney		Left kidney		Remarks
					O_2	NH_3	O_2	NH_3	
				days					
Tyramine	9	117	183	120	127	111	95	90	Renal arteries on both sides moderate to severe constricted. The right renal artery was first constricted, the left renal artery 11 days later
"	11	125	190	29	105	129	82	131	
"	12	121	215	88	81	156	103	107	
"	13	130	204	44	107	145	77	80	
Average		123	198	70	105	135	89	102	
Isoamylamine	9	117	183	120	97	115	91	107	" "
"	11	125	190	29	75	154	57	49	
"	12	121	215	88	86	136	64	88	
"	13	130	204	44	114	87	75	114	
Average		123	198	70	93	125	72	89	
l-Epinephrine	11	125	190	29	57		67		" "
"	12	121	215	88	71		48		
"	13	130	204	44	45		53		
Average		125	203	54	58		56		
l-Dopa	9	117	183	120	46		39		" "
"	11	125	190	29	31		20		
"	12	121	215	88	53		34		
"	13	130	204	44	47		41		
Average		123	198	70	44		33		
dl-Alanine	9	117	183	120	87	154			" "
"	11	125	190	29	70	108	96	120	
"	12	121	215	88	102	90	65	78	
"	13	130	204	44	53	74	47	103	
Average		123	198	70	78	106	69	100	

TABLE V—*Concluded*

Substrate	No.	Pre-operated Blood pressure	Post-operated Blood pressure	Duration of renal ischemia	Right kidney		Left kidney		Remarks
					O_2	NH_3	O_2	NH_3	
				days					
Tyramine	16	118	177	29	107		74		Slight constriction of the right renal artery; 8 to 12 days later the left renal artery moderately to markedly constricted
"	22	135	189	44	111		81		
"	24*	151	220	81	143		39		
Average		126	183	37	120		77		
Isoamylamine	16	118	177	29	92		85		" "
"	22	135	189	44	124		70		
"	24*	151	220	81	104		33		
Average		126	183	37	107		77		

Figures reported are the increase in oxygen consumption and ammonia formation due to added substrate over the control sample, containing no added substrate. Oxygen consumption in μl. over a period of 90 minutes.

* Left kidney markedly atrophied, not included in the average.

decrease in oxygen consumption and ammonia formation with the different substrates. Also shown are the results obtained in animals in which moderate to severe constriction was applied to one renal artery and slight constriction to the other artery. A marked decrease in oxygen consumption was observed in the kidney with marked constriction whereas there was only a slight change in the oxygen consumption of its fellow kidney, which was only slightly constricted.

Table VI shows that the average increases in oxygen consumption of the kidneys in normal and hypertensive rabbits were (normal animal first, ischemic kidney second): tyramine 136, 88; isoamylamine 148, 79; *dl*-dopa 81, 55; *dl*-alanine 105, 70.

It is of interest to mention two observations made during this study. In three dogs with unilateral renal arterial constriction, the constrictions were made great. Approximately $3\frac{1}{2}$ months later the animals were killed. At autopsy the left renal arteries were found practically occluded, the left kidneys atrophied and necrotic. The blood pressures were within the normal range at the time the dogs were killed. The right normal kidneys were found hypertrophied, and the slices of these kidneys showed an increased oxygen consumption and an increase in the content of oxidative enzymes of about 30 to 40 per

cent compared with the average figures obtained with normal dogs of approximately the same age and size. In one dog 3 months after unilateral nephrec-

TABLE VI

Oxidase Activity of Kidney Tissue Extracts of Normal and Hypertensive Rabbits with Unilateral Constriction of Left Renal Artery

Substrate	Normal rabbits		Hypertensive rabbits					
	No.	O_2	No.	Pre-operative Blood pressure	Post-operative Blood pressure	Duration of renal ischemia	Right kidney O_2	Left kidney O_2
				mm. Hg	*mm. Hg*	*days*		
Tyramine	1	132	1	90	125	23	125	85
"	2	118	2	95	90	23	138	93
"	3	145	3	85	140	16	150	74
"	4	161	4	87	105	10	110	92
"	5	127	5	90	135	19	141	100
"			6	90	174	18	133	81
Average		136		90	128	18	133	88
Isoamylamine	1	126	1	90	125	23	159	62
"	2	153	2	95	90	23	149	57
"	3	138	3	85	140	10	166	85
"	4	164	4	87	105	19	137	105
"	5	157	5	90	135	18	151	84
Average		148		89	119	19	152	79
dl-Dopa	3	75	1	90	125	23	87	67
"	4	93	3	85	140	16	71	51
"	5	69	7	87	168	44	100	47
"	6	87						
Average		81		87	144	28	86	55
dl-Alanine	4	89	7	87	168	44	108	92
"	6	101	8	102	177	36	117	62
"	7	110	9	90	130	25	121	58
"	8	118	10	90	145	46	124	69
Average		105		92	155	38	117	70

Figures reported are the increase in oxygen consumption due to added substrate over the control sample, containing no added substrate. Oxygen consumption and ammonia formation in μl. over a period of 60 minutes.

tomy the activity of amine oxidase and amino acid oxidase was tested by the slice technique and found to be about 30 per cent higher than the figures obtained with the nephrectomized kidney of the same dog. These findings are of interest, and experiments are under way to confirm these observations.

Isolation and Identification of 2,4-Dinitrophenylhydrazone of p-Hydroxyphenylacetaldehyde.—The aldehyde formed during the oxidation of tyramine by dog kidney extract preparations could be isolated and identified. The oxidations were carried out in the presence of M^{-3} cyanide and M/20 semicarbazide to prevent the aldehydes being destroyed by further oxidation or by dismutation. 30 mg. of tyramine hydrochloride were dissolved in 10 ml. phosphate buffer solution pH 7.4. To this was added 25 ml. of the extract preparation, and the volume made up with water to 75 ml. The suspension was gently shaken in an atmosphere of oxygen for 4 hours in water bath at 37.5°C. The mixture of the reaction products was diluted to 100 ml. by adding 10 per cent trichloroacetic acid and the precipitate formed was filtered off and to the clear filtrate were added 100 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in N HCl. There was immediate formation of a yellow precipitate and this was allowed to stand at room temperature overnight. The yellow crystalline precipitate was filtered, washed with a little N HCl, and then with a little water, and finally after recrystallization from benzene the following values were obtained: m.p. 182–184° (decomposed); C, 53.1; H, 3.9; N, 17.4 per cent. $C_{14}H_{12}O_5N_4$ requires C, 53.2; H, 3.8; N, 17.7 per cent. The amounts of phenylhydrazone obtained from normal and ischemic kidneys were not compared quantitatively, but it was evident from rough estimates that the amounts obtained by using extracts from ischemic kidneys were decreased.

When the ischemic kidney preparations were incubated under aerobic conditions with tyramine in the Warburg apparatus under the condition described above, the presence of free tyramine at the end of the incubation period could be established by isolation and identification of the dibenzoyl derivative of tyramine m.p. 168–170°. This compound with the dibenzoyl derivative prepared from pure tyramine showed the same melting point. Kidney extracts from normal dogs subjected to the same treatment yielded amounts of free tyramine which were very much smaller than those found when using ischemic kidney preparations.

DISCUSSION

The experiments reported constitute part of a study of the metabolism of the ischemic kidney. The conditions under which these experiments have been performed are different from those *in vivo*, but the results obtained suggest that there is a definite diminution of the tissue respiration and a marked disturbance of the enzymic equilibrium in the ischemic kidney. Holtz, and Holtz, Heise, and Lüdke demonstrated the presence of an amino acid decarboxylase system in the kidney acting under anaerobic conditions, and this was confirmed by Bing (10) and by Bing and Zucker (11) in their studies with guinea pig, cat, and human kidneys. The equilibrium between such decarboxylating systems and the oxidative systems in the ischemic kidney may be altered in such a way

that the former is in pathological excess, *i.e.* the anaerobic degradation processes may exceed the aerobic oxidation processes. Hence, pressor substances may be formed in larger amounts than normally or new ones may be made. Renin and angiotonin-like substances which may conceivably be formed at any stage of the degradation of proteins or polypeptides accumulate, owing to the reduced oxidizing power of the ischemic kidney. Further, the decarboxylation of the amino acids leads to amines (some of them pressor amines) which the ischemic kidney cannot metabolize efficiently. The results reported in Tables II, IV, V, and VI show a decrease in the oxidative systems of the ischemic kidney. However, the activity of the oxidative enzymes after slight constriction of the renal artery over a shorter period was sometimes found to be similar to that observed with normal kidneys. It is important to point out that, in ischemic kidneys where no necrosis or atrophy could be found, the respiration of the kidney tissue slices, their oxidizing power for amines and amino acids, and also the oxidizing power of the kidney extracts, were markedly reduced compared with the normal kidney. It is possible that in the first stage of renal ischemia the oxidative enzymes are still present in sufficient quantity, but their activity is reduced by lack of oxygen, and possible lack of other substances supplied by the blood and necessary for the utilization of the oxygen. In the later stage of ischemia, through pathological changes of the cells, there is probably a decrease in the enzyme content of the kidney, due either to lack of the prosthetic group or to diminished production of the enzyme protein. Levy, Light, and Blalock (12) have shown that in ischemic kidneys made so by the Goldblatt technique, the decrease in blood flow through the kidney was accompanied by a decrease in oxygen consumption of the whole organ.

Since a low Q_{O_2} has been found for the ischemic kidney tissue it seems to be a logical speculation that the functioning of the cytochrome-cytochrome oxidase system is reduced.

The results reported in Tables II, IV, and V show also a decreased ammonia formation by the tissue slices and tissue extracts of the ischemic kidneys. The formation of ammonia in the kidney is utilized for the regulation of the acid-base balance. Hence these results indicate an interference with the ability of the kidney to regulate the acid-base balance. Krebs obtained similar results with tubercular kidneys.

Examination of the results reported in Table II for the Q_{O_2} of dog cortical tissue without added substrate shows a marked diminution of the Q_{O_2} of ischemic tissue. This finding has previously been reported by Gerbi, Rubenstein, and Goldblatt (13) on rabbit tissue. Mason, Robinson, and Blalock (14), on the contrary, did not find any decrease in maximal respiratory activity of renal tissue of the hypertensive dog.

The reduced oxidizing power of the ischemic kidney tissue as demonstrated *in vitro* by the reduced respiratory activity and by the reduced oxidizing power

for amines and amino acids, may be responsible for the formation of the pressor principles of renal origin, *e.g.* of the humoral vasoconstrictor agent which appears in the blood of the renal vein and in the peripheral blood when hypertension is induced in dogs by the Goldblatt method or by the cellophane perinephritis method of Page (15), and by the method of Taylor (16).

SUMMARY

The consumption of oxygen by slices of kidney tissue of dogs made hypertensive by the Goldblatt technique was studied manometrically. The respiration of the ischemic kidney tissue was found to be much less than that of the normal kidney. Further, a marked reduction in oxidizing ability, as measured by the oxygen uptake and ammonia formation in the presence of the added amines and amino acids, tyramine, isoamylamine, *dl*-alanine, and *l*-aspartic acid, was observed.

Extracts of the kidneys were made and tested for amine oxidase, amino acid oxidase, and polyphenol oxidase activity by measuring the increased oxygen consumption and ammonia formation in the presence of the substrates listed above with the addition of *l*-epinephrine, histamine, and *dl*- and *l*-dihydroxyphenylalanine. The preparations from ischemic kidneys of dogs and rabbits showed much lower activity. Animals with varying degrees of constriction of the renal arteries and therefore varying degrees of renal ischemia were prepared and studied. The results with these animals suggested a direct relationship between the degree of renal ischemia and the decrease in oxidizing power of the tissue.

The product of the enzymic oxidation of tyramine was identified as *p*-hydroxyphenylacetaldehyde by isolation as the dinitrophenylhydrazone of *p*-hydroxyphenylacetaldehyde.

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