STUDIES ON EXPERIMENTAL HYPERTENSION

X. The Oxygen Consumption of the Ischemic Kidney

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The mechanism of development of experimental hypertension due to renal ischemia has now been the subject of considerable investigation (1). A humoral origin of the phenomenon appears the most likely, but its exact nature has not yet been elucidated. Studies have been carried out, with the purpose of demonstrating the hypothetical effective substance of renal origin or the chemical or physical change in the blood responsible for the phenomenon, but a pressor substance has not yet been isolated from the blood, although a vasoconstrictor effect of venous blood from an ischemic kidney has been demonstrated (2). Most of the studies have dealt with renin, a pressor substance isolated by extraction of the kidney itself, but because it was originally obtained from normal kidneys (3), its significance in hypertension has not yet been determined, even though it has now been shown (4) that renin differs significantly from all previously known pressor substances.

It has been shown that hypertension does not result when both kidneys are removed and that it develops only when one or both kidneys are being poorly irrigated with blood. There is now definite indication that there may be a significant disturbance of some non-excretory functions of the kidney in dogs with hypertension due to renal ischemia, even when there is no accompanying effect on renal excretory function (5). There is therefore a probability that some such disturbance is responsible for the elaboration of the hypothetical pressor substance.

Any alteration of respiration in the ischemic tissues might furnish a clue

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to the problem of the supposed humoral factor. These studies have been made *in vitro* of the respiration of renal tissue by the method of Warburg.

Experimental Method

The experiments were performed on the rabbit. Pickering and Prinzmetal (6) demonstrated that in this animal also renal ischemia produces hypertension.

Under ether anesthesia, unilateral renal ischemia was produced in rabbits by means of a silver clamp. The device used was a smaller silver clip similar to that used by Pickering and Prinzmetal (6). The blood pressure was not determined. From 3 hours to 6 days after the operation, the rabbits were killed by a blow on the head and both kidneys removed. In every experiment the normal kidney served as the control. The effect of the arterial constriction on the renal tissue was also determined by histological examination of both kidneys.

The respiration of the kidney was studied by Warburg's method (7) on various samples of the same tissue. All the values given in Tables II and III are the average of 2 to 4 separate determinations on slices of tissue from different parts of the kidneys. The slices of tissue weighed from 4 to 6 mg. (Weighed on the torsion balance.) One slice was placed in the Warburg cup; to this was added 0.3 cc. of phosphate buffer solution, pH = 7.2, without Ringer's solution, and, in the inset, 0.2 cc. of 0.7 per cent NaOH. From other studies (8–13) it seems that the optimum pH for the oxygen consumption of renal tissue is around 7.2. Either Ringer-bicarbonate or the Ringer-phosphate solution (14–17) can be used. The temperature was kept carefully controlled at 36.2°C. The oxygen consumed was measured by the manometric method, after 30 minutes of immersion of the glass vessel in the constant temperature bath.

The values of oxygen consumption per hour and per gram of normal renal tissue, obtained according to the formula,

$$Q_{O_2} = \frac{\text{cubic millimeters of oxygen consumed}}{\text{milligrams of tissue} \times \text{hours}}$$

are known to be different in various kinds of animals. In Table I are given some normal values obtained from recent publications on the subject. In all the tests Warburg's technique was employed, but the fluid medium, the temperature of the bath and the preparation of the tissue were not the same in all.

Table I shows the differences of Q_{O_2}, not only between species, but also between different members of the same species. All authors agree that the normal kidney in different animals of the same species can show very large variations in respiration values (*cf.* also Chuda, 26).

In different samples of the same kidney (normal rabbit), we found reasonably close values of Q_{O_2}. The following is an example:

Rabbit, normal: Temperature of bath, 36.2°C.

$Q_{O_2} = 7.1$	$Q_{O_2} = 8.2$
" $= 6.9$	" $= 8.8$
	" $= 7.8$

The values of Q_{O_2} are usually assumed to be the same for both normal kidneys. In order to test this assumption (because a great difference between Q_{O_2} values of left and

right kidneys could invalidate our results on the ischemic kidney), we determined the Q_{O_2} of both kidneys in six normal rabbits.

The results obtained are summarized in Table II, where it is shown that there is considerable variation of the value for each kidney in different

TABLE I

Animal	Q_{O_2}	Author
Frog	1.3– 3.64	Schmitt, Kerr, Bueker, 1935 (18)
Rat	21.0	Warburg, 1924 (7)
"	13.3–20.6	Krebs, 1933 (19)
"	20.1	Kisch, 1934 (20) average 41 kidneys
"	11.9–25.3	McCord, 1934 (21)
Guinea pig	15.7	Kisch, 1934 (20) average 12 kidneys
" "	14.0–22.0	Dognon, Levin, Nachmansohn, 1936 (22)
Rabbit	9.1– 9.5	Leibowitz, 1931 (23)
"	7.0	Okabe, 1934 (10) cortex, average 6 kidneys
"	5.7	Okabe, 1934 (10) medulla, average 6 kidneys
"	6.8–12.7	Nomura, 1937 (12) medullar tissue
"	9.7–14.1	Nomura, 1937 (12) cortical tissue
"	5.9– 6.1	Sarre, Eger, 1939 (24)
"	8.0–12.0	Kalckar, 1939 (25)
Ox	9.8	Kisch, 1934 (20) average 4 kidneys

TABLE II

Rabbit	Left kidney	Right kidney
79	4.3	5.1
80	7.3	10.2
81	10.8	9.8
83	4.8	7.2
84	13.8	15.0
85	7.7	6.1
Average.....	8.1 ± 3.7 S.D.	8.9 ± 3.6 S.D.

By the method of Fisher (32) at the 1 per cent level, $t = 0.38$, which indicates that the difference between the means of the two sets is not statistically significant.

animals, but no systematic significant difference between the respiration of the two kidneys and hardly a significant difference between the means of the two groups.

In these experiments, Q_{O_2} was determined at 36.2°C. and referred to 1.0 gm. of fresh tissue; the values are therefore, on an average, lower than the values obtained by some authors at 37.0°C. and referred to 1.0 gm. of dried tissue. On account of the large varia-

tions of Q_{O2} for different normal kidneys, we thought that it was necessary always to compare an ischemic kidney with a normal kidney of the same animal. The criterion for the normality of the other kidney was the histological examination.

An exact estimate of the degree of renal ischemia was very difficult at the time of the operation. Comparison on the basis of histological examination of presumably ischemic and normal kidneys was of value only when there were recognizable changes in the tissue. In some instances the kidney with a clamp applied on the renal artery showed no recognizable histological changes, but did show significant reduction of Q_{O_2}. It was impossible to be certain that this was due to ischemia. For the same reason, when Q_{O_2} of a presumably ischemic kidney was as high as the control, it was impossible to be certain that ischemia existed if there were no microscopic histological changes in the kidney.

In most of the rabbits, as shown in Table III, the oxygen consumption of the ischemic renal tissue was much less than that of the control, and the average values for the two groups were significantly different. In two cases in which the value for Q_{O_2} was almost the same in both kidneys (rabbits 57, 58), the histological examination failed to reveal any indication of renal ischemia. In general, the degree of degeneration of renal parenchyma was a fairly good indicator of the probable value of Q_{O_2}. The average Q_{O_2} in the ischemic kidney was 4.2 ± 1.4 P.E., and in the normal kidney it was 9.3 ± 2.6 P.E. When the values of Q_{O_2} in rabbits 57, 58, 61 and 65, in which there was no histological evidence of renal ischemia, are omitted, the average Q_{O_2} of the ischemic kidneys is 3.9 ± 1.4 P.E., while that of the normal is 10.0 ± 2.2 P.E. The difference, in either case, is statistically significant. The lowest values correspond fairly closely to the most damaged kidneys (rabbits 48, 56, 60, 62, 63, 64). This does not mean that a parallelism between cellular respiration and histological examination can be demonstrated in every instance. Such a parallelism was denied by Wildbolz and Walthard (27) for the human diseased kidney.

In some of our observations on the rabbit, there was found a very definite diminution of the tissue respiration without any evidence of histological changes (rabbits 52, 61, 65). This may be an example of the priority of the functional changes over the morphological, or the inadequacy of present methods for the determination of cytological and histological changes due to reduction of circulation.

With a different technical procedure, it has been demonstrated (28, 29) that the oxygen consumption rises as a linear function of renal blood flow. In dogs with experimental hypertension induced by renal ischemia, Levy, Light and Blalock (30) observed a diminution of renal blood flow and of

oxygen uptake (about 43 per cent). Our results, on the rabbit, are in agreement with the findings of these authors.

If the oxygen consumption of a tissue be regarded as "a measure of the sum total of the activity of the various individual oxidase systems" (Stare

TABLE III

Rabbit	Duration	Q_{O_2}		Histological examination	
		Left kidney*	Right kidney	Left kidney*	Right kidney
47	3 hrs.	3.7	7.2	+	0
49	3 "	8.8	16.7	+	0
50	3 "	3.2	7.3	+	0
51	3 "	2.8	11.6	+	0
52	3 "	2.8	9.1	±	0
54	3 "	5.7	5.7	+	0
48	1 day	1.3	6.1	++++	0
56	1 "	3.3	9.6	++	0
57	1 "	5.4	3.4	0	0
58	1 "	4.5	5.2	0	0
59	1 "	3.3	10.6	+	0
60	1 "	1.5	12.0	+++	0
61	3 days	5.6	8.7	0	0
63	3 "	3.3	10.2	++++	±
65	3 "	4.8	9.7	0	0
35	6 "	6.4	13.5	+++++	0
62	6 "	2.4	5.2	++++	0
64	6 "	2.2	14.8	+++++	0
36	8 "	8.3	10.9	++++	0
Average Q_{O_2}.....		4.2 ± 2.1 S.D.	9.3 ± 3.5 S.D.		
Average Q_{O_2}.....		3.9 ± 2.0 S.D.	10.0 ± 3.1 S.D. (without 57, 58, 61, 65)		

By the method of Fisher (32) at the 1 per cent level, $t = 5.53$ with, and 7.22 without, rabbits 57, 58, 61 and 65. This indicates that the difference between the means of the two sets is statistically significant in both cases.

± Stasis in glomeruli and interstitial tissue, slight. + Stasis in glomeruli and interstitial tissue, moderate, and parenchymatous degeneration, slight. ++ Stasis, moderate, plus parenchymatous degeneration, moderate. +++ Stasis, moderate, plus parenchymatous degeneration, severe. ++++ Stasis, moderate, plus necrosis, patchy. +++++ Extensive necrosis.

* The clamp was always put on the left renal artery.

and Elvehjem, 31), the following significance may be attached to the results of our experiments. In renal ischemia one or more of the oxidation reduction systems must be impaired and therefore the oxidation reduction potential of the kidney is altered. Further inferences are premature. The present investigations, however, will be continued in the hope that they

may give some clue to the nature of the humoral mechanism of renal origin which is responsible for the hypertension that results from renal ischemia.

SUMMARY

The consumption of oxygen by slices of renal tissue of the rabbit was studied by means of the Warburg manometric method. The ischemic kidney of the rabbit shows a definite diminution of oxygen consumption, compared with the normal kidney.

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