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BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

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(Text-fig. 1)

It is well known that the kidney of the adult *Lophius piscatorius* (L.) is practically aglomerular, some of the original glomeruli disappearing late in the young animal, as we have been able to confirm by the examination of fishes a few centimetres long. The kidney of *Lophius* receives only small amounts of arterial blood, its main supply being by venous blood from the caudal vein. Several attempts have been made by previous authors to investigate the secretion of *Lophius* kidney, and to these reference has been made in a paper in which the chemical composition of the blood and urine are dealt with (Brull & Nizet, 1953). We are not aware of publications on blood perfusion experiments on *Lophius* kidneys.

The aim of the present research was to study the secretion of the perfused *Lophius* kidney and its response to variations of venous pressure at the entrance to the organ, and to measure the blood and urine flows with a view to determining whether an aglomerular kidney is sensitive, and to what extent, to variations in its circulation. In a series of previous publications one of us, with his collaborators (Brull & Dor, 1940; Brull & Louis-Bar, 1950, 1953), has investigated the response of the glomerular kidney of the dog to wide variations of blood pressure. The main result of these experiments was that the blood flow through the dog's kidney in the whole animal is to a large extent independent of variations of blood pressure from 100 mm. up to 330 mm. Hg, while the urine flow responds regularly to these variations. Previous research on isolated perfused kidneys of the dog had regularly shown increases of blood flow in response to increases in perfusion pressure.

Methods

Several favourable conditions had to be realized in order to permit the organization of our experiments: a large supply of good-sized *Lophius*, brought in well alive (so allowing the gathering of sufficient amounts of heparinized blood for perfusion) and with kidneys of sufficient size to permit catheterization of the caudal vein and ureters, etc. These conditions were fulfilled

beyond our expectations at the Plymouth Laboratory; and we are much indebted to the Director and Staff of the Laboratory for the generous and efficient way in which they supplied us with *Lophius* in good condition and with any materials which proved necessary for our investigations.

As soon as the fish were brought in, they were killed by a blow on the head, and one of the branchial arteries was cannulated for bleeding into heparin. Several *Lophius* of all sizes up to 100 cm. long were bled, and the blood was kept in cold storage. As soon as more than 500 ml. of blood had been collected, a perfusion experiment was carried out, using a kidney of a large-sized fish. The blood was put in a glass bulb at variable and measured levels above the kidney. Rubber tubing and a glass cannula inserted into the renal end of the caudal vein carried the blood to the gland, while the effluent was collected in a funnel and returned to the bulb. By so doing, the perfusion was made at room temperature, except in one experiment where all the glassware was kept in ice in order that the kidney should be perfused at a temperature of about 5° C.

In Exp. 2 the blood was oxygenated and, after perfusing the kidney, reoxygenated in a large-sized spiral tube down which it flowed in a thin layer, while oxygen ran through in the opposite direction. The degree of oxygenation of the blood was not measured. In Exps. 1, 3 and 4 unreoxygenated venous blood was used.

Urine was collected from the ureter, and microdeterminations were made of total nitrogen (Kjeldahl), chlorine (St Russnyak, 1926, p. 211), and of magnesium (colorimetrically, as described by Fister, 1950).

Results

Experiment 1

Blood from several *Lophius* was collected the day before and heparinized. The perfused kidney (from L. no. 18, weighing 10·8 kg.) weighed 19 g. Perfusion was started 47 min. after the arrival of the fish. Bladder urine before experiment: 5 ml. with 414 mg. Cl/100 ml. The results are given in Table I. Perfusion pressures are in mm. of blood, blood flows in ml./g. of fresh kidney per minute, and urine flows in ml. per gram of fresh kidney per hour. Room temperature: 24·7° C.

This is the first renal blood perfusion experiment carried out in *Lophius*. We do not know the pressure in the caudal vein at its entrance to the kidney in the live animal, but considering the thinness of the wall, this pressure is likely to be very low. The urine flow began with a blood pressure of 20 mm., and raising this pressure progressively from 40 to 50 mm. up to 120 mm. had no influence on the rate of urine flow. This flow is abundant, and especially so when we consider that about 40% of the kidney is lymphoid and not renal tissue: the rate of flow (per g. fresh tissue) is, indeed of the same order as that in the living dog. The urine is acid (pH 5), and colourless. The kidney does not concentrate chloride, but concentrates total non-protein nitrogen more than five times in sample 3, and three times in sample 5. Magnesium is highly

TABLE I. EXPERIMENT 1

Time (min.)	Perfusion pressure, (mm. blood)	Blood flow (ml./g./min.)	Urine No.	Urine Volume (ml.)	Urine ml./g./hr.	Urine Cl (mg./100 ml.)	Urine Mg (mg./100 ml.)	Urine N (mg./100 ml.)	
0	20	0·36	—	—	—	—	—	—	Perfusion started (venous blood)
20	—			First drop collected					pH of urine: 5
41	15	0·20	—	—	—	—	—	—	—
42	40	0·48	—	—	—	—	—	—	Pressure raised
60	40	0·52	1	2·0	0·16	594	—	—	pH of urine: 5
73	50	0·65	—	—	—	—	—	—	—
90	50	0·55	2	2·0	0·20	598	—	—	—
93	50→65	0·55; 1·10	—	—	—	—	—	—	—
98	70	—	—	—	—	—	—	—	—
118	70	—	3	2·2	0·25	596	270	113	—
150	70→120	1·10→	4	2·0	0·20	584	255	—	Pressure raised
153	120	4·60	—	—	—	—	—	—	—
180	120	—	5	2·0	0·20	—	222	70	—
188	—	—	6	1·2*	—	—	180	—	Cl in plasma 576, N 23 mg./100 ml.

* Including vol. in catheter.

TABLE II. EXPERIMENT 2

Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g.min.)	Urine No.	Urine ml./g./hr.	Urine Cl (mg./100 ml.)	Urine Mg (mg./100 ml.)	Urine N (mg./100 ml.)	
0	—	—	—	—	—	—	—	Perfusion started (oxygenated blood)
5	55	0·47	—	—	—	—	—	—
10	—	—	—	—	—	—	—	Ureter catheterized: flow starts
20	55	0·26	—	—	—	—	—	Spontaneous drop of blood flow
33	55	0·19	—	—	—	—	—	—
36	55	0·35	—	—	—	—	—	Spontaneous rise of blood flow
46	60	0·32	—	—	—	—	—	—
55	70	0·38	—	—	—	—	—	—
60	80	0·50	—	—	—	—	—	—
70	80→125	1·00	1	0·11	—	—	70	Pressure raised
75	160	1·10	—	—	—	—	—	—
85	190	1·50	—	—	—	—	—	—
108	210	1·40	—	—	—	—	—	—
130	228	—	2	0·13	595	279	—	—
135	228→340	—	—	—	—	—	—	Pressure raised
136	340	2·30	—	—	—	—	—	—
145	—	—	—	—	—	—	—	Blood sample I: Cl in plasma 595 mg., non-protein N 30 mg./100 ml.
160	360	2·40	3	0·16	—	291	—	3 ml. 5 M-NaCl added to the blood
175	—	—	4	0·14	—	—	86	—
180	385	2·50	—	—	—	—	—	—
205	380	—	5	0·14	970	384	—	Blood sample II: Cl in plasma 825 mg., non-protein N: 36 mg./100 ml.

concentrated seeing that *Lophius* plasma contains only about 6 mg./100 ml.: at the beginning of the experiment the urine Mg was about 45 times the

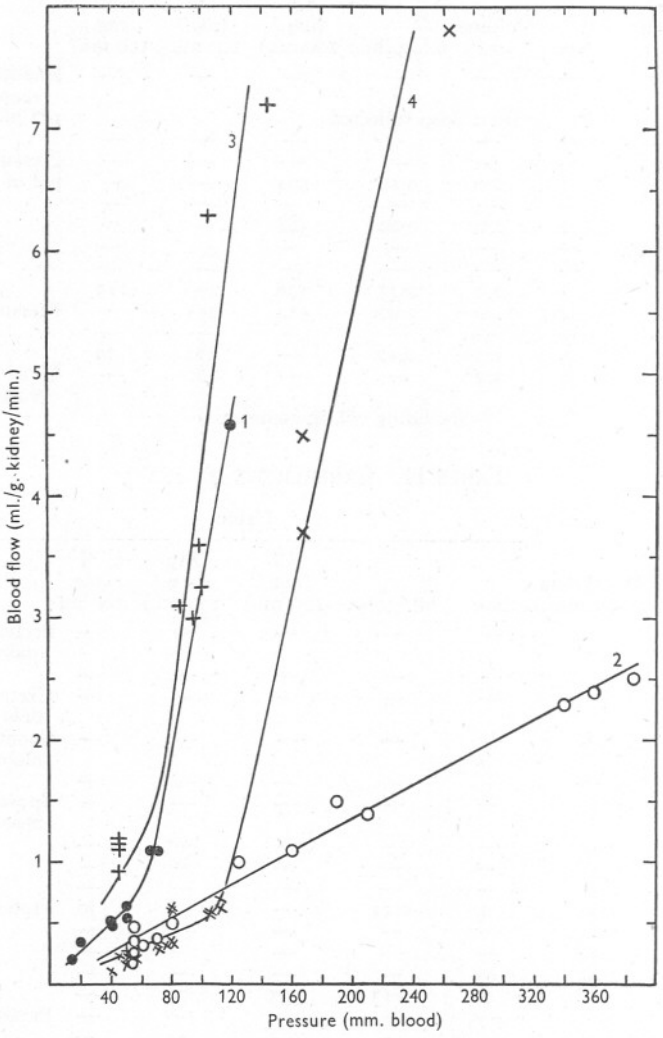

Fig. 1. The relation between blood flow and perfusion pressure in the four experiments described in the text. In Exps. 1, 3 and 4 the kidney was perfused with venous blood, in Exp. 2 with oxygenated blood. In Exp. 4 the perfusate was cooled, its inflow temperature being between 2 and 8° C (Table IV).

plasma Mg, and still 30 times at the end. Thus Mg secretion may be considered as one of the best tests for renal activity in this species. The blood flow, at perfusion pressures between 15 and 40 mm., varies from 0·2 up to 0·5 ml./g. of

fresh kidney per minute, and when the pressure is raised from 40 to 70 mm. the flow increases from 0·5 to 1·1 ml./min. With a higher pressure (120 mm.), the response is proportionally much greater, showing that the resistance of the vascular bed has diminished (Fig. 1).

Experiment 2

Blood was collected from several *Lophius* just before the perfusion, and the day before. The blood was oxygenated. Room temperature 24·2° C. The perfused kidney (from L. no. 28 weighing 7 kg.) weighed 17 g. The results of this experiment are given in Table II.

The renal blood flow shows spontaneous fluctuations, but follows the pressure more or less passively throughout (Fig. 1). The urine flow increases with perfusion pressure, but only to a very small extent: a 500% increase in pressure is associated with a 25% increase in urine flow. The kidney excretes Cl at the same concentration as it is present in the plasma until the plasma level is raised from 595 to 825 mg./100 ml.; from then on a slight concentration of Cl (970 mg./100 ml.) occurs. Nitrogen is concentrated twice, and this concentration continues throughout the experiment. Magnesium is highly concentrated, as in Exp. 1.

Experiment 3

270 ml. mixed heparinized venous non-oxygenated blood was used as the perfusate. Room temperature 21·7° C. The perfused kidney (from L. no. 36 weighing 7 kg.) weighed 12 g. Bladder urine before experiment: 20 ml. with Cl. 680 mg. and non-protein nitrogen 75 mg./100 ml. The results of this experiment are given in Table III.

The renal blood flow follows the perfusion pressure, but the variations of flow are proportionally very much greater than the variations of pressure (Fig. 1). In this experiment the urine flow is directly proportional to the perfusion pressure. Chloride in the urine is slightly concentrated from the beginning, and more so after the plasma chloride is raised. Non-protein nitrogen is high in the plasma and is not concentrated. Magnesium is again highly concentrated.

Experiment 4

550 ml. venous non-oxygenated mixed heparinized blood was used as the perfusate. The blood reservoirs were cooled in ice with sodium chloride. The kidney weighed 23 g. (from L. no. 45 weighing 10 kg. fished the previous day and kept alive in the aquarium). The results of this experiment are given in Table IV.

The blood flow is roughly proportional to the perfusion pressure up to a pressure of about 100 mm.; thereafter the variations of flow are proportionally very much greater than those of pressure (Fig. 1). After the kidney had been subjected to a pressure of 285 mm. of blood, the return of blood flow to its initial value when the pressure was lowered to its initial value (column 1, 290–300 min.) shows that the vessels had not lost their elasticity as a result of this operation. The urine flow, non-existent at the beginning, begins to be

appreciable only at a pressure of 70 mm., and at higher pressures there is no parallelism between perfusion pressure and urine flow. Nitrogen is concentrated three times at the most, no more than during perfusion at room temperature. Chloride is not concentrated. Magnesium is highly concentrated, but no more, or even less, than at room temperature.

TABLE III. EXPERIMENT 3

			Urine					
Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g./min.)	No.	ml./g./hr.	Cl (mg./ 100 ml.)	Mg (mg./ 100 ml.)	N (mg./ 100 ml.)	
0	—	—	—	—	—	—	—	Perfusion started (venous blood)
10	50	0·7	—	—	—	—	—	0·2 ml. urine in cannula
15	45	1·15	—	—	—	—	—	Spontaneous rise of blood flow
30	45	0·92	—	—	—	—	—	—
50	45	1·10	—	—	—	—	—	—
62	45	1·20	—	—	—	—	—	—
70	45→85	—	1	0·10	—	—	—	Pressure raised
73	87	3·10	—	—	—	—	—	—
108	101	3·30	—	—	—	—	—	—
115	101→140	—	2	0·20	—	345	64	Pressure raised
120	145	7·20	—	—	—	—	—	—
125	—	—	—	—	—	—	—	Blood sample I: Plasma Cl 670 mg., non-protein N 87 mg./100 ml.
140	145→105	—	3	0·29	760	321	—	2 ml. 1 M-NaCl added to the blood. Pressure lowered
146	105	6·30	—	—	—	—	—	—
152	—	—	—	—	—	—	—	Blood sample II: Plasma Cl 787 mg./ 100 ml.
165	—	—	4	0·16	—	348	—	—
167	100	3·60	—	—	—	—	—	—
200	100	—	5	0·18	894	378	—	—
210	95	3·00	—	—	—	—	—	—
220	95	—	6	0·17	—	—	—	—
235	—	—	7	0·16	—	—	80	—

DISCUSSION

Lophius kidneys, perfused with heparinized blood, retain for several hours the power of producing urine. This urine is colourless but its chemical composition shows that the gland is actively secreting.

Among the constituents investigated, magnesium was concentrated up to 50 times by the kidney and reached the same degree of concentration as is found in the bladder urine of freshly killed fish. Total non-protein nitrogen is also concentrated by the perfused kidneys, but not so much as in the living fish. Some of the separate constituents are perhaps more concentrated than the figures for the total non-protein nitrogen would suggest: the small volume of the urine samples did not enable us to answer this question. The perfused

TABLE IV. EXPERIMENT 4

Time (min.)	Perfusion pressure (mm. blood)	Blood flow (ml./g./min.)	Blood T (° C) at kidney: In-flow	Blood T (° C) at kidney: Out-flow	Urine: No.	Urine: ml.	Urine: ml./g./hr.	Urine: Cl (mg./100 ml.)	Urine: Mg (mg./100 ml.)	Urine: N (mg./100 ml.)	
0	—	—	—	—	—	—	—	—	—	—	Perfusion started (venous blood)
5	40	0·11	5	15	—	—	—	—	—	—	—
25	40	—	—	—	—	—	—	—	—	—	—
35	40→46	—	5	14	—	—	—	—	—	—	Pressure raised
36	46	0·23	5	13	—	—	—	—	—	—	—
50	50	0·20	—	—	—	—	—	—	—	—	—
55	56	0·20	—	12	—	—	—	—	—	—	—
60	70	0·32	2	11	—	—	—	—	—	—	—
65	70	0·32	—	—	1	1·6	0·07	—	110	65	—
70	72	0·28	—	—	—	—	—	—	—	—	—
80	77	0·32	—	10·5	—	—	—	—	—	—	—
85	80	0·35	—	10·5	—	—	—	—	—	—	—
120	81	0·32	—	—	2	2·7	0·12	552	153	75	—
135	105	0·57	—	—	—	—	—	—	—	—	—
165	105	0·58	—	—	3	3·0	0·17	—	170	—	—
180	107	0·62	—	—	4	1·0	0·17	—	176	—	—
185	107	0·69	—	—	—	—	—	—	—	—	—
195	107→162	—	—	—	—	—	—	—	—	—	Pressure raised
200	165	—	2	9	—	—	—	—	—	—	—
205	168	—	—	—	—	—	—	—	—	—	—
215	168	3·70	—	—	—	—	—	—	—	—	—
225	160	—	—	—	5	2·4	0·14	525	170	—	Blood now looks well oxygenated
245	168	4·50	2	9	—	—	—	—	—	—	—
255	166	—	—	—	6	2·2	0·19	—	170	37	—
260	166→230	—	—	—	—	—	—	—	—	—	Pressure raised
270	265	7·80	—	—	—	—	—	—	—	—	—
280	285	—	8	8·5	—	—	—	—	—	—	—
285	—	—	—	—	7	1·8	0·15	—	170	—	—
290	285→45	—	—	—	—	—	—	—	—	—	Pressure lowered
292	45	0·20	—	—	—	—	—	—	—	32	—
300	45	0·16	—	—	8	1·2	0·19	—	196	32	—
	45→80	—	—	—	—	—	—	—	—	—	Pressure raised
305	80	0·68	—	—	—	—	—	—	—	—	—
325	80	0·60	—	—	—	—	—	—	—	—	—
330	—	—	—	—	9	1·2	0·11	508	202	—	Plasma at the end of perfusion: Cl 581 mg., non-protein N 20 mg./100 ml.

kidney excretes chloride usually at the same concentration as in plasma; but when the plasma chloride is artificially raised the concentration of chloride in the urine is higher than that in the plasma.

The activity of the kidney does not seem to be improved by oxygenation of the blood, which is not surprising seeing that venous blood is by far the main supply to such kidneys under normal conditions. Neither is this activity appreciably different at room temperature and at low temperatures close to those at which *Lophius* normally lives. Changes in perfusion pressure up to 100-150 mm. of water raised the urine flow; above 150 mm. there was no effect. This result is in marked contrast with the effects of such changes in a glomerular kidney.

The renal venous vascular net offers but little resistance to the flow of blood: at perfusion pressures between 20 and 40 mm. blood (about 1·5 to 3 mm. Hg) the flow is of the order of 0, 3 ml./g. fresh tissue/min., whereas in the dog the flow at arterial pressures of about 100 mm. Hg is of the order of 3 ml./g. fresh tissue/min. At low perfusion pressures the blood flow in *Lophius* is approximately proportional to the pressure. At higher pressures, however, the flow became proportionately greater in the three experiments in which venous blood was the perfusate, and this change in the slope of the pressure-flow relation occurred at a higher pressure in the experiment (no. 4, see Fig. 1) in which the temperature of the perfusate was lowered. In the experiment (no. 2, see Fig. 1) in which oxygenated blood was the perfusate the blood flow was approximately proportional to the pressure over a very wide range, viz. 50–385 mm. blood; and in both Exps. 2 and 4 the blood flows at a given pressure were lower than in the other two experiments. Further research will show whether the temperature and oxygen tension of the blood play a part in the tone of the kidney vessels of *Lophius* and in their responses to changes in perfusion pressure. In its blood-flow responses to changes in perfusion pressure, therefore, the kidney of *Lophius* behaves, too, in a manner which is in marked contrast with the behaviour of the dog's kidney.

Summary

Lophius kidneys perfused with the heparinized blood (venous) of the fish secrete urine in which total non-protein nitrogen is concentrated, magnesium highly concentrated, and chloride only slightly so or not at all. Oxygenation of the blood, or lowering the temperature of the perfusate from *c.* 20° to *c.* 5° C. does not appear to influence secretion. The blood flow through the kidneys increases with the perfusion pressure, the increase often becoming disproportionately large. The urine flow, on the other hand, above a certain critical level is largely independent of changes in perfusion pressure.

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