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BLOOD AND URINE CONSTITUENTS OF *LOPHIUS PISCATORIUS* L.

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(Text-fig. 1)

The comparative physiology of glomerular and aglomerular kidneys has provided much valuable information which cannot be ignored in discussion of renal functions. Our knowledge, however, of the exact inorganic and organic metabolism of fishes, particularly aglomerular fishes, is still insufficient. *Lophius piscatorius* L. is the only aglomerular fish whose size permits thorough investigation of such problems. This is the reason why our research on the kidney of *Lophius* starts with an attempt to extend our knowledge in that field.

The habits of angler-fish observed in the Plymouth Aquarium have been described by Wilson (1937).

During a visit to the Plymouth Laboratory in July 1952, when a number of *Lophius* were available, we were able to attempt kidney perfusion. We also took the opportunity of analysing many urine and blood samples from these fish. The samples were taken either on board ship from freshly caught fish, or in the laboratory from fish which had just arrived or spent one night in the aquarium. Others were taken during perfusion experiments.

Nitrogen Constituents

Plasma proteins were found, by the Kjeldahl method, to be 39 g./l. in sample L28. A detailed cataphoretic analysis of the plasma of L48 and L49, made in Liège by Dr A. Nizet using Antweiler's microelectrophoretic apparatus, gave the following results: albumin, 6·7%; globulin 1, 14·3%; globulin 2, 46·1%; globulin 3, 32·9% (see Fig. 1).

It thus seems, subject to further confirmation, that the plasma of *Lophius* is not only poor in proteins, but that globulins are largely predominant, which is in keeping with a low osmotic pressure. According to Florkin (1944) the colloid osmotic pressure of teleostean fishes is usually below 20 cm. of water.

Figures in the literature for non-protein nitrogen constituents are very scarce. Denis (1913) gives the following data:

	mg. N in 100 ml.
Total non-protein N	40
Urea	8
Ammonia	3·6
Uric acid	0·9
Creatine and creatinine	5·0

In a pool of heparinized blood samples kept for 24 hr. in cold storage and centrifuged after being used in a perfusion experiment (L 18), we found in the trichloracetic filtrate:

	mg. N in 100 ml.		mg. N in 100 ml.
Total non-protein nitrogen (Kjeldahl)	*21*	Uric acid (Folin-tungstic filtrate)	0·17
Urea (urease in Conway box)	0·85	Creatine	0·21
Ammonia (Hoppe-Seyler, 1930)	0·32	Creatinine	0·28
Trimethylamine + trimethylamine oxide (Hoppe-Seyler, 1930)	11·34		
Sum of analysed constituents	*13·17* = 63 % of the total		

In a similar pool (perfusion L 28) we found *total non-protein nitrogen* to be *30* mg. N in 100 ml., of which urea was 1·5.

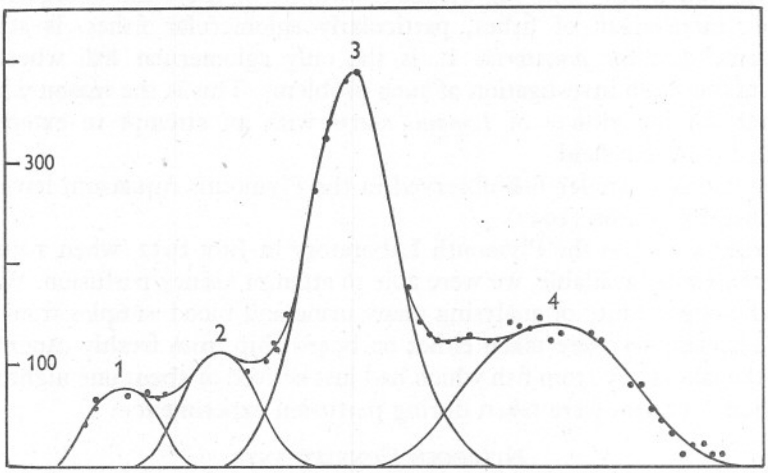

Fig. 1. Microelectrophoresis of *Lophius* plasma (Antweiler apparatus).

In a fresh sample of blood immediately centrifuged and deproteinized with trichloracetic acid (L 48 and 49 mixed), we found 16 mg. of total non-protein nitrogen and only traces of allantoin.

The haemoglobin content of *Lophius* blood is low, the cell volume being only about 17 %. We did not study the oxygen-carrying power of the haemoglobin, nor its absorption curve.

There are no figures in the literature of the amount of allantoin, trimethylamine and trimethylamineoxide in the blood of *Lophius*. In the pool of blood analysed, we found only negligible amounts of urea (much less than was accepted by Denis in 1913). There is very little ammonia, 0·32 (mg. N/100 ml.), as compared with the 3·6 of Denis. We have not yet tried to find whether this ammonia is preformed or not. Creatine and creatinine are not as concentrated as might be expected from the urine figures: 0·26 and 0·28 in plasma, and 51·2 and 4·3 in urine (Table II); therefore, they are highly concentrated by the kidney. Trimethylamine and its oxide comprise 55% of the non-protein nitrogen in the plasma; they are much less concentrated by the kidney than creatin, up to only 28·4 mg. per 100 ml. urine, less than three times the concentration in the blood. Allantoin is practically absent from blood and urine (Table II). We did not have a sufficient volume of well-preserved blood and

TABLE I. NITROGEN CONSTITUENTS OF *LOPHIUS* URINE (MG. N/100 ML.)

		Total non-protein N	Urea	NH_3	Tri-methyl-amine. Total oxide	Uric acid	Creatine	Creati-nine	Amino-acids
Denis (1913)	Composite sample of bladder urine, *c.* 1 hr. after death	83	12	1·2	—	0·1	14	0·7	—
Grollman (1929)	Woods Hole	180	1·2	1·0	—	0·8	50·2	7·3	25·8
	Caught in Atlantic	373	0·7	1·3	—	0·4	27·9	4·1	14·3
	Caught in Atlantic	337	0·6	1·0	—	0·2	156·0	8·0	18·0
	From aquarium	56	2·7	0·7	—	tr.	12·9	0·9	4·5
	From aquarium	—	2·7	1·3	—	—	23·0	1·5	8·0
Our determinations	Pool of bladder urines from 20 *Lophius*	148·4	1·6	19·2	28·4	0·03	51·2	4·3	4·5

Notes

According to Grollman, trimethylamine oxide constitutes the greater part of the remaining nitrogen.
According to Denis (1922), the percentage of amino-acids in urine and in blood is of the same magnitude.
Our determinations were from *Lophius* catheterized on arrival at the laboratory or after one night in the aquarium. The methods used were as for plasma, except that amino-acids were determined by a modification of Folin & Wu's method after elimination of NH_3 by permutit.

TABLE II. NITROGEN CONSTITUENTS OF *LOPHIUS* URINE

Bladder urines from individuals, conditions as in Table I.

No.	Total non-protein N	Urea	NH_3	No.	Total non-protein N	Allantoin
L 3	21	2·5	—	L 19	245	—
L 10	82	—	—	L 20	280	—
L 12	54	2·3	2·2	L 21	89	—
L 13	290	—	—	L 22	194	—
L 14	346	5·3	2·1	L 23	140	—
L 16	140	—	—	L 27	56	0·13
L 17	91	—	—	L 36	75	0·15
L 18	89	—	—	L 37	50	—

Total non-protein N in bladder urines of individuals catheterized on deck: LE, 255; LC, 210; LK, 243.

urine samples for amino-acid determinations; according to Grollman (1929), their concentration would be of the same magnitude in urine and plasma. There still remains in plasma 37% of undetermined non-protein nitrogen, while in urine, where we found 4·5 amino-acid nitrogen, we failed to identify 30% of the non-protein nitrogen. There is still the possibility that an important nitrogen constituent of plasma and urine is as yet unidentified.

Mineral Constituents

In the following table we have condensed the figures for blood of Marshall (1930) and of Smith (1929), translated into milliequivalents per 100 ml. We reproduce only the limits of variations.

Marshall: Cl, 16·8–22·8.
Smith: Cl, 16·6–20·9; PO_4, 3·2–6·7; SO_4, 1·2–3·7; Na, 18·6–21·8; K, 6·9–9·9; Ca, 2·1–3·9; Mg, 1·0–1·4.

Table III shows the figures obtained in Plymouth in July 1952. Methods used were: Cl, St Russnyak (1926, p. 211); PO_4, Briggs (1922); Na and K, flame photometer; Ca, oxalic precipitation; Mg, colorimeter according to Harold Fister (*Manual of Standard Proced. for Spectr. Chem.*), a few gravimetric controls being made.

Table III. Minerals in Heparinized Plasma of *Lophius* (m.equiv./100 ml.) Plymouth, July 1952

Nature of sample	Cl	PO_4	Na	Mg	Ca	K	Δ
L 1+2 kept 15 hr. in aquarium under nembut. anaesthesia. Plasma separated after 5 days preservation of blood in cold storage	13·2	—	—	—	—	—	—
L 14 at end of kidney perfusion for 100 min.	14·0	—	—	—	—	—	—
Pool from L 16, 17, 18, separated after kidney perfusion 4 hr. after arrival	16·2	2·3	20·8	—	0·72	—	—
Pool from L 19, 20, 21, 22; 23, 24, 25, 26, 27, 28, 29 (2 days) kept in cold storage and after use for perfusion experiment, lasting 3.30 hr.	16·9	—	—	0·40	—	—	—
Pool from L 33, 34, 35, 36, 37, 38, 39, 40 all well alive 4 hr. after arrival and kidney perfusion	16·1	—	—	—	—	—	—
Pool from L 46, 47, 48 well alive—kept 24 hr. and after kidney perfusion 3 hr.	16·2	—	—	—	—	—	—
Plasma from L 48+L 49 (on arrival)	—	1·8	18	0·50	0·55	0·51	0·84
Plasma from L 30 and 31, arrived in good condition, bled and separated at once	19·6	—	21·7	0·58	—	—	—
Plasma L 32, arrived in good condition, bled and separated at once	19·0	—	21·3	—	—	—	—
Plasmas separated on board off Plymouth:							
L A	15·3	—	18·5	—	—	—	—
L D	15·3	—	—	—	—	—	—
L H	15·6	—	—	—	—	—	—
L E, F, +G	—	1·8	19·3	—	0·67	—	—
L	17·2	—	18·5	—	—	—	—

In one sample of pericardial fluid we found 17·9 m.equiv. chloride.

Our figure for Δ of the plasma, 0·84° C., is the same as the figure given by Smith (1929) at Woods Hole, while Bottazzi (1897) found 0·86 at Naples. In urine we confirm the figures of Smith, who found from 0·66 to 0·85. We found figures varying between 0·60 and 0·88 (Table V). We confirm the view of Marshall (1930) that urine of marine teleosts is either iso- or hypotonic to the plasma, never hypertonic.

Hypotonicity does not mean that certain, inorganic constituents are not highly concentrated by the kidney (Table IV). Mg comes first. Our figures in plasma are about 0·5 m.equiv. per 100 ml. In urine the concentration varies from 22·6 up to 34·5 m.equiv., for *Lophius* catheterized on arrival. On board ship we found similar concentrations, except that two samples contained less, 14·6 and 15·8 respectively.

TABLE IV. MINERALS IN URINE OF *LOPHIUS PISCATORIUS* (M.EQUIV./100 ML.)

Author	Cl	PO_4	SO_4	Na	Mg	Ca	K	Remarks
Denis (1913)	18	0·3	—	—	—	—	—	—
Sulze (1922)	2·1	—	0·7	—	2·5	0·36	—	Naples. Mixtures of urines from several *Lophius*
	2·3	—	1·4	—	3·2	0·42	—	
Grollman (1929)	5·6	0·8	—	—	3·8	0·2	—	Atlantic
	18·8	0·8	—	—	10·8	0·3	—	Aquarium
Smith (1929)	14·1	0·29	—	—	6·3	0·93	0·19	Aquarium (highest and lowest figures)
	20·9	1·47	—	—	10·0	1·52	0·76	—
Marshall (1930)	7·2	—	—	—	—	—	—	Aquarium (highest and lowest figures)
	22·9	—	—	—	—	—	—	
Smith (1929)	14·8	0·6	—	—	5·7	1·18	0·19	Starved in Aquarium
	20·9	0·7	—	—	6·3	1·38	0·27	
	16·1	0·28	—	—	6·4	0·93	0·19	Fed in Aquarium
	20·7	1·47	—	—	10·0	1·52	0·76	

So far as we know, Mg is the constituent of the urine for which the *Lophius* kidney shows its greatest power of concentration. Ca has been shown to be only slightly concentrated, as in man. But as one of us showed (Brull, 1930) there are three different physico-chemical forms of Ca in plasma, one of which is not excreted in urine (bound with proteins), one to a very small extent (ionized Ca), and one very fast and highly concentrated (complex Ca molecules with organic acids); therefore total Ca figures give no idea of renal concentration. The same is true for phosphates; the average plasma figure is below 2 m.equiv. %. In urine, we found from 0·15 to 9·0, which means deconcentration or concentration; but with biological methods and with the isotope ^{32}P, we demonstrated in our laboratory in Liège (Brull, 1927; Govaerts, 1947) that what is considered as inorganic phosphates in the plasma of the dog exists in at least two different physico-chemical forms, one of which only is excreted. It is possible that this phenomenon is interspecific. Potassium figures in plasma are about 0·50 m.equiv./100 ml., and the kidney seems to excrete very little potassium since concentrations in urine are lower than in plasma.

Chlorides in plasma range between 13·2 and 19·6 m.equiv. %; in the literature the variations mentioned are a little wider: 18·6–22·8; in urine, figures published range from 2·1 to 20·9. This might mean that chlorides may be diluted, never concentrated. Our figures for bladder urine range from 0·33 to 19·4, which would have the same significance. But our kidney perfusion experiments have shown that the kidney may slightly concentrate chloride when the plasma concentration is raised. Under normal conditions, this either does not occur or is exceptional.

For sodium in the plasma, figures in the literature range from 18·6 to 21·8. We found the following limits: 18·5 and 21·7; according to Smith (1929)

TABLE V. MINERALS IN *LOPHIUS* BLADDER URINE. (M.EQUIV./100 ML.)

No.	Treatment	Condition	Cl	PO_4	SO_4	Na	Mg	Ca	K	Δ
L 3	Aq. 28 hr.	—	16·2	0·15	—	—	—	—	—	—
L 8	Arr.	—	18·1	—	—	—	—	—	—	—
L 10	Aq. 18 hr.	—	18·0	—	—	—	—	—	—	—
L 13	Arr.	D.	10·8	—	—	—	—	—	—	—
L 14	Arr.	H.	6·2	9·0	—	0·8	—	—	—	—
L 12	Arr.	D.	16·9	0·9	—	—	—	2·0	—	—
L 13	Arr.	D.	10·9	1·0	—	—	—	—	—	—
L 15	Arr. 4 hr.	—	17·0	—	—	—	—	—	—	—
L 16	Arr.	H.	18·4	0·1	—	—	—	1·2	—	—
L 17	Arr.	H.	18·8	0·16	—	—	—	—	—	—
L 18	Arr.	D.	11·6	—	—	—	—	—	—	—
L 19	Arr.	D.	11·7	—	—	—	—	—	—	—
L 20	Arr.	D.	11·1	—	—	—	—	—	—	—
L 21	Arr.	D.	18·7	—	—	—	—	—	—	—
L 22	Arr.	D.	18·1	—	—	—	—	—	—	—
L 23	Aq. 17 hr.	H.	16·9	—	—	—	—	—	—	—
L 24	Aq. 17 hr.	H.	18·8	—	—	—	—	—	—	—
L 25	Aq. 15 hr.	D.	18·0	—	—	—	—	—	—	—
L 27	Arr.	H.	16·4	—	9·3	—	31·0	—	—	0·68
L 33	Arr.	H.	17·0	—	—	—	—	—	—	—
L 38	Arr.	H.	—	—	7·6	—	30·5	—	—	—
L 43	Arr.	H.	13·3	—	—	—	—	—	—	—
L 14	Arr.	H.	4·0	9·0	—	0·8	—	—	—	—
L 32	Arr.	H.	19·4	—	—	—	32·4	—	—	0·74
L 47	Arr.	H.	16·2	—	—	—	—	—	—	—
Pool of bladder urines										
L 3–L 23	—	—	14·7	0·7	8·6	2·3	22·6	1·6	0·2	—
L 31	—	—	—	—	—	—	—	—	—	0·77
L 34	—	—	—	—	—	—	34·0	—	—	—
L 36	—	—	—	—	—	—	31·8	—	—	0·77
L 37	—	—	—	—	—	—	30·6	—	—	—
L 38	—	—	—	—	—	—	30·6	—	—	0·70
L 39	—	—	—	—	—	—	—	—	—	0·84
L 40	—	—	—	—	—	—	30·6	—	—	—
Catheterized on board										
L A	—	—	0·33	—	—	—	—	—	—	—
L D	—	—	7·2	—	—	—	32·5	—	—	—
L E	—	—	7·0	—	—	1·1	14·6	1·0	—	—
L F	—	—	14·8	—	—	1·5	—	—	—	—
L K	—	—	5·1	—	—	—	27·5	—	—	0·60
L I	—	—	7·5	—	—	0·4	19·0	—	0·17	0·88
L G	—	—	3·0	—	—	—	15·8	—	—	—

Aq. 28 hr.: kept in aquarium for 28 hr. Arr.: on arrival at the laboratory. Arr. 4 hr.: 4 hr. after arrival. H., healthy; D., dying.

figures for urine are the same, viz. 18·6–20·9; we find lower limits of variations, from 0·4 to 2·3. Sodium is not in line with chlorine. Sulphates in plasma vary from 1·2 to 3·7 according to Smith (1929); Sulze (1922) found from 0·7 to 1·4 in urine; we found higher figures: 7·6, 8·6 and 9·3. This means a concentration of at least three times, although much less than for magnesium.

Several authors suggested that the urine secreted by *Lophius* in the aquarium is not the same as that secreted by fishes living under normal conditions. Grafflin (1931) emphasizes that skin injury in toad-fish increases the concentration of chloride in plasma and urine, while normal urine is poor in chloride and rich in nitrogen. In *Lophius*, taken in 1 hr. trawl, he finds 13·2 and 13·9 m.equiv. %. Preliminary experiments made by one of us (E.N.) tend to show that the rubbed skin of *Lophius* is more permeable, *in vitro*, to crystalloids than normal skin. Grollman (1929) finds lower figures in urine of fish caught at sea, for instance 5·6 m.equiv. against 18·8 in the aquarium, 3·8 mg. sodium against 10·8; for phosphorus the figures are the same; for calcium, 0·2 against 0·3 m.equiv./100 ml. Our figures concern plasma and urine either collected on deck as soon as the fish was brought up, or on arrival at the laboratory after having been kept in running sea water on board.

If we consider the analyses of plasmas separated on board or on arrival, and only take into account plasma separated immediately, we find for chloride the following figures: on board, 15·3, 15·3, 15·6 and 17·2; on arrival 16·1, 19·6 and 19·0. There is therefore a tendency to increase which may explain a similar increase in urine. The same appears to be true for sodium: 18·5, 18·5 and 19·3 on board against 21·7 and 21·3 on arrival.

The Δ for urine of fishes examined on board give us the lowest figure: 0·60° C. and also the highest, 0·88° C., while on arrival they vary from 0·70° up to 0·84° (Table V); for chloride, figures on board vary between 0·33 and 14·8 m.equiv./100 ml., the mean of seven figures being 6·4, while urine from bladders catheterized on arrival in the laboratory gave figures from 4·0 up to 19·6, with a mean of 15·1. For sodium and calcium our figures are insufficient in number. A pool of twenty bladder urines taken on arrival in the laboratory gave 22·6 m.equiv. of magnesium, while the average of fifteen determinations on board was 20·2. Again, on board we have two of the lowest figures: 14·6 and 15·8. More results will have to be obtained before we can really assert that within a few hours after being caught in the trawl there is an increase in the urine inorganic constituents. Our values for chloride, sodium and magnesium tend to confirm the hypothesis of an increased absorption of minerals, at least of NaCl, presumably through the skin.

SUMMARY

The blood of *Lophius piscatorius* is poor in haemoglobin, the volume of red cells being only 17%. The plasma contains less than 40 g. of protein/l., of which only 6·7% is albumin. This explains its low osmotic pressure. As is well known, it contains more crystalloids than mammalian blood, Δ (depression of freezing-point) being 0·84°, the same figure being found at Naples, Woods Hole and Plymouth. This rather high concentration is not due to organic constituents, that of total non-protein nitrogen being of the same magnitude as in mammals; it is mainly due to a high content of sodium

chloride. Chloride is at a concentration of 15·3 m.equiv./100 ml., sodium at 18·5, while in mammals they reach 9 and 15 respectively.

Total non-protein nitrogen concentrations in plasma are similar to concentrations in mammals; the main non-protein nitrogen constituents of plasma are neither urea, ammonia, uric acid or allantoin, but trimethylamine or trimethylamineoxide. Of our analysis 37% of non-protein nitrogen of plasma remain unidentified, so far as we can rely upon our chemical methods. The power of concentration of the kidney for non-protein nitrogen on the whole is not high; it varies up to fifteen times. But the degree of concentration by the kidney, small for most constituents, even for trimethylamine, seems to be very high for creatine which is the main representative of non-protein nitrogen. Of our urine analysis, 30% of non-protein nitrogen remain as yet unidentified.

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