BLOOD PERFUSION OF THE KIDNEY OF
LOPHIUS PISCATORIUS L.

III. ACTION OF CO₂, CYANIDE AND FLUORIDE

By L. Brull and Y. Cuypers
with the collaboration of L. Wilsens

From the Plymouth Laboratory, and Institut de Clinique et Policlinique Médicales, Liège, Belgium

(Text-figs. 1 and 2)

Proceeding with our studies on urine secretion of the agglomerular kidney of Lophius piscatorius L., in the Plymouth Laboratory (Brull & Nizet, 1953; Brull, Nizet & Verney, 1953; Brull & Cuypers, 1954a, 1954b) and using the method of blood perfusion we described previously, we attempted to study the influence of CO₂, and of cyanide and fluoride on water secretion and on Mg concentration, which seems to be the most characteristic function of that kidney.

METHODS

Two experiments were carried out with CO₂, three other experiments with cyanide, and two attempts were made with fluoride, the first one on a preparation used for cyanide, after recovery from that intoxication (Expt. G).

A sufficiently large kidney was perfused with heparinized blood from a pool obtained from several Loplius, a pool which was large enough to wash the preparation after removal of the modified or toxic blood, and to replace it by a fresh supply.

The drops of urine were registered, and after a constant or an increased urine flow was noticed, either blood in equilibrium with a different gas mixture replaced the previous one, or the toxic substance was added, dissolved in a small amount of Ringer and neutralized.

Whenever blood was removed, the circulation was stopped for a very short time, which, with practice, could be reduced to 15 sec. As soon as the effect of the toxic substance or of the modified blood was clearly established, the preparation was washed out with fresh blood, and then new blood from the same pool was put in.

Rough determinations of magnesium were made on one drop of plasma or diluted urine, with the 'Tüpfelmethode' of Feigl (1931), using titane yellow as reactive. The margin of error in these determinations is ± 20%.
Effect of CO₂

In a previous publication (Brull & Cuypers, 1954a), we have shown that the blood of *Lophius piscatorius* contains between 1.87 and 2.35 g of haemoglobin. Because of this low content, and of the fact that ferricyanide coagulates this kind of blood, determinations of the O₂ content are inaccurate in arterial blood, and practically impossible in venous blood, from which no measurable volume of oxygen is liberated in the Van Slyke apparatus.

Yet there remains no doubt that the kidneys of *Lophius*, receiving venous blood only, live on a very low oxygen tension. If the blood is oxygenated when perfusing these kidneys, there is no change in the order of magnitude of urinary volume or concentration (Brull, et al. 1953). We concluded therefore that the oxygen requirement of the *Lophius* kidney is very light.

Owing to the fact that in our previous perfusion experiments the artificial blood circulation was in contact with air or with oxygen, there resulted a progressive loss of CO₂ and a rise of pH. These alterations were not accompanied, even in the long run, by a parallel change in the water output or in the concentration capacity for Mg, except for a slow decrease of urine volume when the preparation becomes too old. Thus, the secretion is not affected by enriching the blood with O₂ and impoverishing it in CO₂.

It seemed interesting to find out whether an increase in CO₂ would have an influence, especially as it is well known that the Bohr effect is of high magnitude in the blood of marine teleosts (Florkin, 1944, p. 86). This was soon confirmed by the aspect of the blood when equilibrated with a mixture of 5% CO₂ and 95% O₂; it was much darker than any venous blood we had previously seen from *Lophius*.

Fig. 1 (Expt. H and I) illustrates the results of two experiments during which kidneys were successively perfused (1) with blood in equilibrium with air; (2) with blood in equilibrium with 5% CO₂ and 95% O₂; (3) with the same as in the first.

The perfusion pressure was constantly kept at 200 mm water in the first perfusion, and at 300 mm in the second, as we know from our previous work (Brull & Cuypers, 1954b) that these figures are above the optimum required to produce maximum urine output.

It is obvious that high CO₂ tension produces two effects: (1) an important reduction in the flow of urine, which is totally reversible, and (2) a vasoconstriction which is not reversible after 1 hr.

Since the urine flow recovers after removal of the CO₂-enriched blood, while the blood flow remains low, one may conclude that the two phenomena are independent of each other. This is not surprising, when considering that the blood flows, even reduced by the CO₂, are still above the optimum flows, according to our previous experiments.

Now, which is the factor responsible for the reduction in the urine
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output: the drop of available oxygen, the CO₃ tension itself, or the drop of pH? The question remains open.

The following experiments may shed some light on the problem.

Fig. 1. Upper curve. Expt. H: a kidney of 7 g is perfused with blood oxygenated with air. At \(\downarrow^1\) blood in equilibrium with carbogen replaces the air-oxygenated blood. At \(\downarrow^2\) back to air-oxygenated blood. Perfusion pressure constant at 200 mm water. Lower curve. Expt. I: a kidney of 11 g is perfused with blood oxygenated with air. At \(\downarrow^1\) blood replaced by blood in equilibrium with 5% CO₂-95% O₂. At \(\downarrow^2\) back to previous blood. Perfusion pressure constant at 300 mm water.
EFFECT OF CYANIDE

In the first experiment (Fig. 2, Expt. E), the amount of cyanide given is apparently too large, there being practically no recovery after removal of the poison.

In Expt. F (Fig. 2) recovery is better but not complete. In Expt G only 8 mg KCN were added to 100 ml. of circulating blood (1·3 M/1000). This concentration reduced the urine output to one-fifth of its former level, and there was a sharp drop in the Mg concentration in the urine, from 150 mg %, down to 30 mg. Although the washing out of the kidney may not have been complete, these figures are sufficient to show that independently of the water secretion, the mechanism of which is put out of action by cyanide, its power of Mg concentration is also affected. Not only is there a drop of Mg excretion due to the decrease or arrest of urine flow, but also a marked decrease in the concentration power of the kidney for Mg. Both phenomena are reversible when the amount of cyanide is 1·3 M/1000 KCN.

EFFECT OF FLUORIDE

In Expt. G (Fig. 2), after the urine volume and Mg concentration had come back to approximately their original level, 350 mg NaF were added (at arrow 3), the volume of the blood being about 150 ml. There is an immediate and sharp drop in urine output, right down to one drop in 10 min. after 16 min; the last drop of urine taken from the proximal end of the ureteral cannula shows that the Mg concentration has dropped from about 160 mg % down to 40 mg.

Expt. K (Fig. 2) is a second attempt with fluoride. When the urine flow is steady, 0·5 % NaF are added (65 mg in 130 ml. of blood). Urine volume steadily decreases, down to less than 50 % of the original level, after 40 min. Mg in urine drops from 160 mg down to 60 mg %. The fluorized blood is then removed, and washed out with fresh blood; this experiment, however, was stopped by a subcapsular haemorrhage in the kidney.

DISCUSSION

It is known, through Warburg’s experiments (1914), that cyanide stops or reduces some oxidative processes in the cells by blocking the iron. We shall not go into the extensive literature on the mechanism of action of HCN and of fluoride. Both chemicals reduce aerobic respiration, and fluorides especially affect the carbohydrate cycle, namely by an inhibition at the level of pyruvic acid (Peters, Rydin & Thompson, 1935; Melrose & Terner, 1953). Their effects on water secretion and Mg concentration add new evidence in favour of the concept that these kidney activities are really active processes involving energy derived from the carbohydrate cycle and oxygen consumption. Although
Fig. 2. Expts. E, F, G, K. Expt. E (lower curve): 160 mg KCN are added to 175 ml. of blood at 15 min (arrow 1). At 27 min the cyanided blood is removed; the preparation is washed with 100 ml. fresh blood (arrow 2). At 49 min this blood is removed and 150 ml. new blood put in (arrow 3). Expt. F (upper curve): at 14 min (arrow A) 20 mg KCN added to 130 ml. circulating blood. At 43 min (arrow B) the cyanided blood is removed. At 45 min circulation removed with 210 ml. fresh blood from which the first fifty ml. from the renal outflow are discarded. Expt. G: at the first arrow 8 mg KCN are added to 100 ml. circulating blood. At arrow 2, cyanided blood removed; the preparation is washed with 50 ml. fresh blood and new blood from the same pool is put in. At arrow 3, 350 mg NaF are put in. Expt. K: at the arrow, 65 mg NaF are added to 130 ml. circulating blood.
there is much evidence that these kidneys require relatively little oxygen, they do require aerobic processes. It is possible that by greatly lowering the oxyphoretic power of the blood, due to an important Bohr effect, CO$_2$ acts on the kidney by bringing the oxygen tension down below the minimum requirement.

**SUMMARY**

Kidneys of *Lophius piscatorius* L., perfused with heparinized blood, respond to a high tension of CO$_2$ in the blood by a drop in urine flow and in their capacity for concentrating Mg. At the same time, but independently, CO$_2$ reduces the blood flow. Both cyanide and fluoride may stop or reduce water secretion and Mg concentration, according to the concentration used, showing that both these activities are dependent on aerobic processes and presumably on the integrity of the carbohydrate cycle.

**REFERENCES**


