BIOLOGY

THE BEHAVIOUR OF BLOOD CHLORIDE DURING OSMO-REGULATION IN THE SAWAHCRAB (PARATELPHUSA CONVEKA)

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RINGKASAN.

PARATELPHUSA CONVEXA, JG. MULA^2 ADALAH CHEWAN laut dan sekarang hidup diair tawar dan djelas menggambarkan kemampuannja untuk memelihara komposisi darahnja dalam berbagai matjam keadaan sekitarnja (osmoregulasi).

Bila ketam sawah itu dibiarkan didalam air suling, maka mula^2 kadar chlorida didalam darahnja menurun tetapi kadar chlorida tersebut tjepat kembali seperti semula. Kedjadian ini mungkin disebabkan karena pelepasan chlorida didalam chewan itu.

Pelepasan chlorida itu mungkin disebabkan oleh berbagai faktor, misalnja konsentrasi total daripada sekitarnja, konsentrasi chlorida, atau konsentrasi daripada ion^2 lainnja.

Banjak dari kemungkinan^2 itu telah ditjoba dengan memakai tjampuran^2 garam buatan, dan kadar chlorida dalam darah telah ditentukan setjara kwantitatip (micro Volhard). Ternjata bahwa turunnja Chlorida darah dalam air suling itu tidak hanja disebabkan karena kekurangan kadar chlorida. Faktor^2 jang sesuai sudah diselidiki dan hubungan kwantitatip antara kadar chlorida darah dan pelepasan chlorida telah dianalisa.

I. PROBLEM.

THIS INVESTIGATION HAS THE PURPOSE TO DETERMINE how the blood chloride behaves in hypotonic solutions. This blood chloride behaviour was investigated by Zwicky (1954) for the larvae of Drosophila melanogaster, which by exposure to distilled water shows an initial drop subsequent recovery of the blood chloride level. Similar results have been obtained when exposing the sawahcrab in glass distilled water. This recovery must be due to a release of chloride within the animal, and the factors releasing this chloride are the main object of this investigation.

The chloride release can be imagined to be induced by a variety of factors, such as the total concentration of the environment, the chloride concentration or the concentration of any other ion.

The following investigations are concentrated on the behaviour of the blood chloride in various hypotonic salt mixtures.

II. MATERIAL AND METHOD.

ALL EXPERIMENTS WERE PERFORMED ON THE SAWAH-crab (Paratelphusa convexa), taken from the sawahs of the Tjihampelas area in Bandung. The crabs are kept in groups of 15 individuals in aquaria filled with sawahwater which is replaced every day. They are fed on ordinary grass. Since the age of the crabs is unknown, samples of about the same size and weight are used for the experiments (7—8 grams).

Before doing the experiment with different salt solutions, the crabs are transferred to a medium which is chemically similar to sawahwater. This so-called artificial sawahwater is a salt mixture consisting of the most common ions found in ordinary sawahwater.

The composition of the artificial sawahwater is as follows :

	mM/L	g/100ml.
Na Cl	0,5	0,41
K Cl	0,7	0,45
Na H CO_3	0,6	0,50
Ca $(No_3)_2$	0,6	0,82

After 3 days in this salt mixture the crabs can be used for experiments with other salt solutions e.g.

1. salt-free sugarwater (mannose)
2. chloride-free artificial sawahwater
3. calcium-free artificial sawahwater
4. potassium-free artifical sawahwater
5. K and Cl'-free artificial sawahwater.

The compositions of the different salt mixture are given in the following table :

	Complete art. sawahw. g/100 ml.	—Cl' g/100 ml.	—Ca' g/100 ml.	—K' g/100 ml.	—K' and —Cl' g/100 ml.
NaCl	0,41	—	0,41	0,76	—
KCI	0,45	—	0,45	—	—
Na HCO_3	0,50	1,10	0,50	—	1,10
Ca $(NO_3)_2$	0,82	0,82	—	0,82	0,82
K HCO_3	—	0,60	—	—	—
$Ca_{11}H_{22}O_{11}$	—	4,42	1,80	2,05	6,50
PH	6,50	—	—	—	—

These stock solutions have to be diluted 100x before use. The total concentration and the PH of these different salt mixtures must be made similar, by adding some nitric acid to add just the PH and substituting ions with sugar.

Each experiment has been done with one individual crab at a time. It is kept in a glass beaker filled with a salt mixture which is changed every 15 minutes, i.e. after each titration.

In order to collect samples of the blood the last walking leg of the left hand side is cleaned first and then cut with scissors. The blood which is pressed out by the animal can be easily sucked with a capillary pipette. After collecting the necessary amount of blood, the wound is smeared with some vaseline before putting the animal back in the salt mixture. By doing this the direct contact between the injured spot and the salt mixture is avoided.

The blood chloride was analysed according to the method of Volhardmicrotitration. The principle is precipitation of proteins with nitric acid, precipitation of chlorides with a known amount of silver nitrate and the titration of the excess of silver ions with K- thiocyanate.

The blood drop is put on a slide which is covered with a thin layer of paraffine. To this drop is added an excess of nitric acid (white precipition), an accurate amount of silver nitrate and a trace of ammonium-sulfate as indicator.

During the titration this drop is stirred with a dust free air jet. The titration is done until he drop shows an orange colour. When his endpoint is reached the titration must be continued with strong stirring until the orange colour does not disappear.

Before starting the real experiment the pipettes must be calibrated first. For this calibration two determinations are needed:

1. with glass distilled water.
2. with a known chloride standard solution, which must be in the neighbourhood of the blood chloride concentration.

From these two values the factor F can be calculated by the formula :

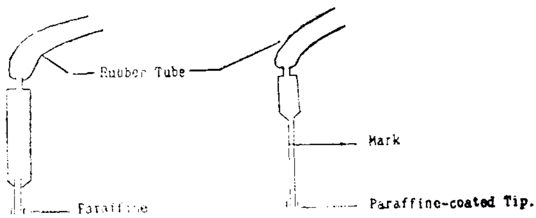
$$(Cl') = F \times (C-T)$$

Cl' = The known chloride standard solution.

(C-T) = difference which is proportional to the concentration of the chloride.

F = factor, which is characteristic for the blood and silver nitrate. It depends on the set of pipettes.

The convenience of this formula is that units of any scale can be used; no calibration in mm^3 is required. This calibration is only for a special set of pipettes, which are drawn out of capillaries. Two kinds of capillaries are used :



more accurate type for blood and silver nitrate.

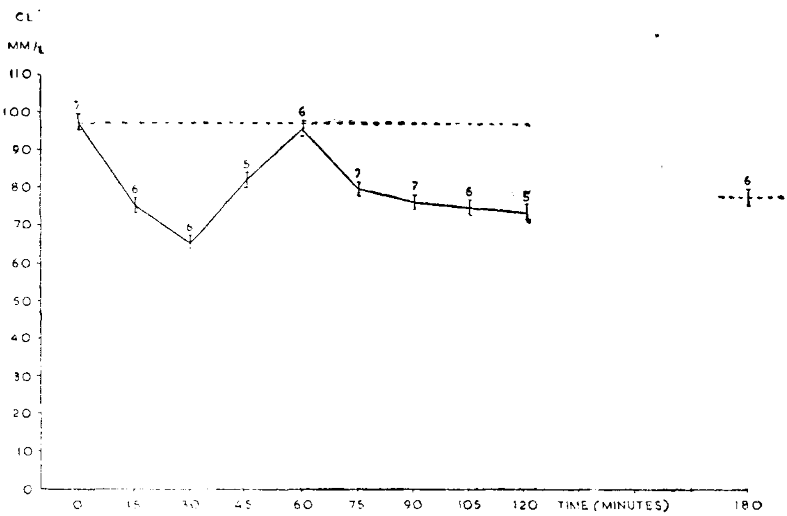
less accurate type used for nitric acid and ferric ammonium-Sulfate.

III. RESULTS.

(See diagrams).

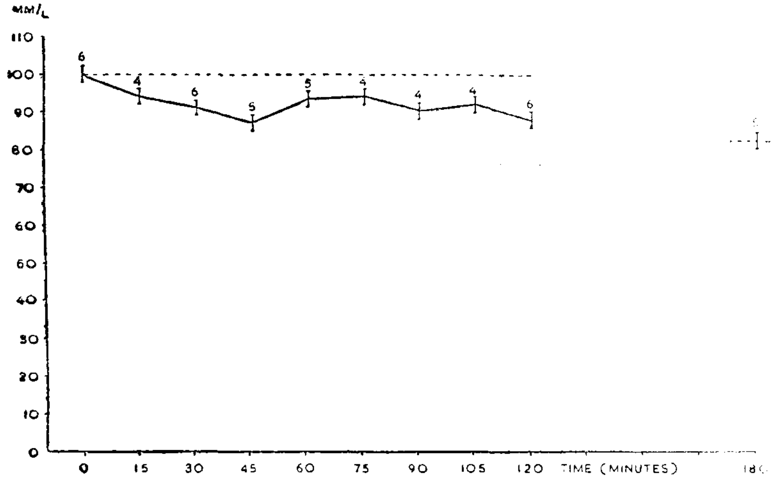
a. *Effect of glass-distilled water* (using one crab at each experiment).

In this experiment the loss of chloride content and subsequent release is clearly demonstrated. The blood chloride reaches a minimum value of 64,7 mM/L after 30 minutes and a maximum value of 95,2 mM/L after 60 minutes. Then the blood chloride stabilizes its value at about 75,0 mM/L. It is shown that the rapid drop and recovery of the blood chloride is just a temporary effect.



A. EFFECT OF GLASS DISTILLED WATER (USING ONE CRAB AT EACH EXPERIMENT).

Diagram a



D. EFFECT OF CALCIUM-FREE ARTIFICIAL SAWAHWATER.

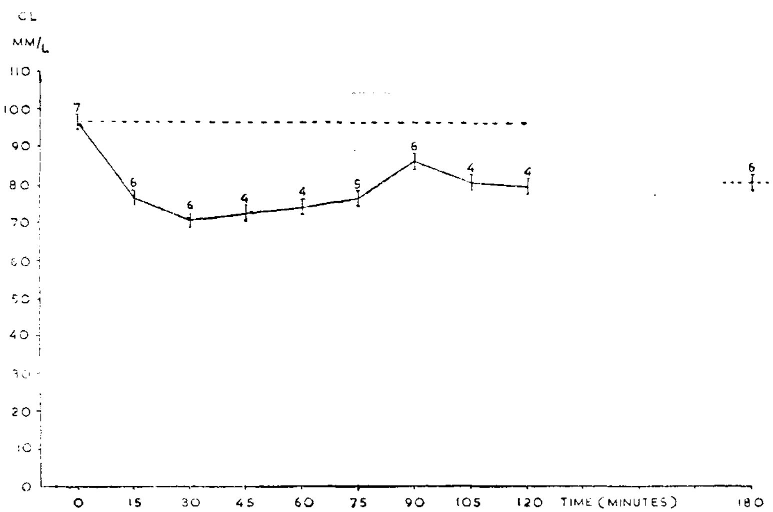
Diagram d

e. *Effect of potassium-free artificial sawahwater.*

By exposing the animals in potassium-free salt mixture the blood chloride shows a significant drop of 20 mM/L after 15 minutes. Then it reaches a minimum value of 70,2 mM/L after 30 minutes, i.e. a drop of 26,5 mML. After 45 minutes the cloride content slightly goes up, reaching a maximum value of 85,8 mM/L at 90 minutes. Then it stays fairly constant at a value of about 79,0 mM/L.

According to the T — test the difference between the minimum and the maximum value is significant (p 0,01).

Although not like in glass distilled water, experiments with potassium-free artificial sawahwater does give an initial drop and recovery of the chloride content in the blood.

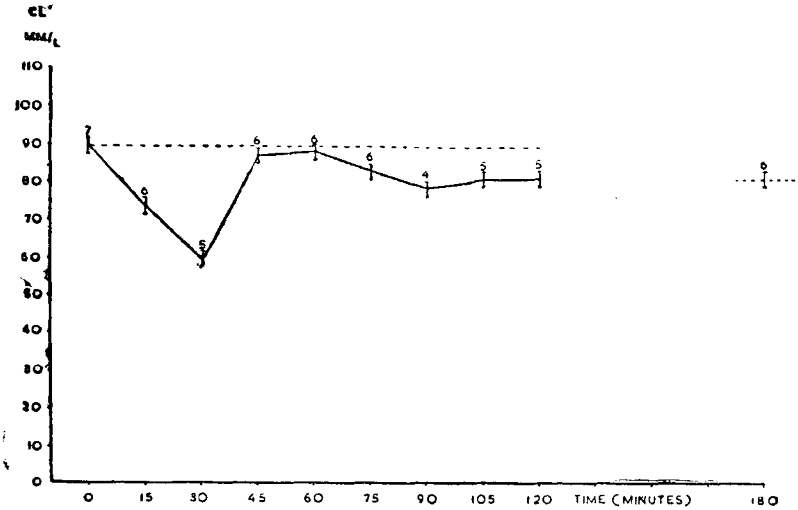


E EFFECT OF POTASSIUM-FREE ARTIFICIAL SAWAHWATER

Diagram c

f. *Effect of potassium and chloride — free artificial sawahwater.*

The normal chloride content in the blood ranges between 80,8 — 93,2 mM/L with a mean of 89,2 mM/L. After 15 minutes exposed in this salt mixture the chloride content goes down to 83,0 mM/L. After 30 minutes it reaches its minimum value of 58,7 mM/L. The blood chloride recovers at 45 minutes to a value of 86,6 mM/L, reaching a maximum value of 87,6 mM/L at 60 minutes, which is approaching its normal value. Then the chloride content stabilizes its value at about 80 mM/L.
From this result it seems that the lack of both potassium and chloride ions gives a stimulation for the release of the chloride reserve within the animal. The lack of potassium alone seems not to be a sufficient stimulation for the recovery of the chloride content in the blood.



F: EFFECT OF POTASSIUM AND CHLORIDE - FREE ARTIFICIAL SAWAHWATER.

Diagram f

IV. DISCUSSION.

IT IS CLEAR FROM THE RESULTS OBTAINED WITH GLASS-distilled water that the sawahcrab (Paratelphusa convexa) at first loses its chloride reserve within the body. In this respect the sawahcrab behaves in a similar way as the larvae of Drosophila melanogaster (Zwicky, 1954).

In the experiments with mannosa, which is kept salt free, it is established that the chloride loss and recovery are not due to a change in the total concentration of the environment. Both experiments with glass-distilled water and sugarwater show the same behaviour of the chloride content in the blood.

So it seems that the factors responsible for this behaviour of the blood chloride are the changes in the ion constituent in the artificial salt mixture. The most probable factor for the release of the chloride reserve may be the lack of chloride ions in the external medium. However, experiments with chloride-free artificial sawahwater only show a slight drop of the chloride content in the blood and no significant recovery. It is proved with this experiment that the chloride release is not merely due to the lack of chloride ions in the environment.

Another factor which may cause the recovery is the lack of calcium, ions, since these ions play an important role in the permeability of the skin. By exposing the crabs to calcium-free artificial sawahwater the blood chloride behaves more or less the same as treated with chloride-free water.

Further experiments have been attempted with potassium-free salt mixture, which gives an initial drop but a slow and insufficient-recovery. It seems that the lack of potassium ions in the environment is nat the only factor for releasing the chlaide reserve within the animal. There must be another factor which, combined with the lack of potassium ions, gives a more positive result.

Lack of chloride ions which was first imagined to cause the chloride release in the blood was tested in combination with lack of potassium. This potassium-and chloride-free salt mixture indeed gives an initial drop and subsequent recovery in the chloride content of the blood. From this result it was concluded that the lack of potassium ions has only an effect on the blood chloride when combined with other factors that help reduce the chloride level even further.

Further investigations in this direction are advisable. May be there are other factors, besides the lack of potassium and chloride, which are of more importance for the release of the chloride reserve.

Experiments with other combinations of ion deficiencies in the medium (i.e., -Ca -K, -Cl -Ca) are in preparation.

The relationship between K and Cl is not clear. Tentative expements with high K- concentration (2 and 3 times the normal concentration in the medium) did not clarify the problem, since they, too, showed a slight drop in blood chloride.

Another problem is that of the chloride "bump"; which is the factor that triggers off the release of chloride? The evidence suggests that this regulatory mechanism is set off not by external factors, but by the chloride level of the blood itself. A number of likely factors, among others, may be imagined. There may exist a threshold value of blood chloride if the blood chloride falls below this level a burst of chloride is released. Alternatively, this may be the case with respect to the chloride loss. A different mechanism might result in gradual releases of chloride resulting in "bump" proportional to the chloride level or the chloride loss. The first two cases would represent an all or none response, the last two a graded one. To test these possibilities regressions were plotted and calculated for 23 pairs of experimental values. The diagrams clearly point to a graded response, the "bumps" ranging from 3,8 to 37,3 mM/L (as measured from the preceding minimum). Correlation coefficient were calculated for the relationship between „bump" and preceding minimum on one hand and between „bump" and preceding loss on the other. The value are 0,557 and -0,685 respectively, indicating that the release of chloride is more closely connected with the absolute chloride level than with the amount of chloride lost.

VI. LITERATURE.

1). Krogh, A., 1939, Osmotic regulation in aquatic animals.

2). Zwicky, K., Osmoregulatorische Reaktionen der Larve von Drosophila melanogaster.
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