Physiology. — *"About the influence of radio-active elements on the development"*. By Prof. A. J P. VAN DEN BROEK. (Communicated by Prof. H. ZWAARDEMAKER).

(Communicated at the meeting of November 29, 1919).

One of the elements, composing the living protoplasma, potassium, is radio-active. The investigations of ZWAARDEMAKER and his pupils about the signification of potassium in the organism have proved that, chiefly by streaming experiments of the isolated frog-heart, potassium can be substituted by an aequiradio-active quantity of any other radio-active element.

On account of this ZWAARDEMAKER concludes[1]): "die Radioaktivität und keine andere Eigenschaft der sich gegenseitig vertretenden Atome erfullt die fur die Automatie notwendige Bedingung" (l.c. pag. 49). Next to this substitute ZWAARDEMAKER has fixed the attention on a second fact, viz. an antagonism between different groups of radio-active elements.

The antagonism is expressed in the following scheme:

		Uranium
Potassium	→	Thorium
Rubidium	→	Radium
(Cäsium)	←	Ionium
		Niton (Emanation)

The uranium substitutes the potassium in certain experiments; but the elements together neutralize each others' effect.

These investigations raise the question if it were possible to substitute the potassium during the development by another radio-active element. I tried to obtain an answer on this question by experimental investigation. I will give a short account of the experiments taken and of the results which I obtained. The experiments were taken with frog-eggs and carried out in the following way.

After the fecundation (in the laboratory) the egglump was parted

[1]) H ZWAARDEMAKER, Die Bedeutung des Kaliums im Organismus. Pflügers' Archiv Bd. 173.

immediately in equal quantities, these are placed in liquids containing potassium or in which different quantities of uranium-salt had been dissolved. There is practically no potassium found in the Utrecht water, thus, as much care as possible was taken, to bring up the uranium-tadpoles, with food containing no potassium, while the tadpoles that had been put in the liquid containing potassium, got as much ordinary (animal) food, as was possible. Rice, boiled in distilled water was given as food without potassium. In the first year the uranium-tadpoles were brought up in glass-bowls; to prevent the dissolving of potassium from the glass, the tadpoles were brought up in quartz bowls in the second and third years of the experiments.

I. A first series of experiments consisted in adding to the water a certain quantity of uranium-nitrate $UO_2(NO_3)_2$.

The potassium which was present in the eggs was compensated by giving 4½ mgr. uranium-nitrate pro liter; moreover another 12,5, 25 mgr. (altogether 17 and 29½ mgr.) and 50 mgr. pro liter.

At the same time as the tadpoles in these liquids, others were brought up in ordinary water, with piscidine[1]) and rice.

The eggs were laid on April 14th. On May 29th the piscidine-tadpoles are long 11—12 m.m.[2]) and have hind-limbs; on June 6th there are tadpoles of 14.2 m.m., which have hind- and front limbs; then metamorphosis and tailreduction regularly follow. On June 26th only a few tadpoles of 8—12 m.m. remain, these are not yet metamorphosed.

The rice-tadpoles develop far more slowly, and it now appears that at the same time the stage of development of the tadpoles differs considerably. On June 26th I found tadpoles of 6—12 m.m.; then the development slowly continues; on August 6th I found the first complete metamorphosis, (the tail has disappeared) the length being 15 m.m. On October 10th another tail-reduction takes place. Some tadpoles do not metamorphose, they become quite big animals, viz. 16—17 m.m., with only short hind-limbs.

As to the uranium-tadpoles they remain backwards and develop far more slowly, which are the most striking characteristics. The following table informs us about the size.

From this table it appears, that the development takes place considerably more slowly than with the animals under control, also the sizes are smaller. Although the differences seem little, the tad-

[1]) Piscidine is a preparation containing dried and powdered fish.

[2]) In these and all following measurements the length must be considered as taken from the top of the head to the beginning of the tail.

	$4^1/_2$ m.gr. uranium-nitrate pro L.	17 m.gr. uranium-nitrate pro L.	$29^1/_2$ m.gr. uranium-nitrate pro L.
June 26th	5.5—10 m.m.	5— 9.8 m.m.	5 —7.5 m.m.
July 10th	6 —11 m.m.	5—10 m.m.	4.5—9 m.m.
July 30th	7 —11 m.m.	6— 9.5 m.m.	5 —10 . m.m.
August 14th	9.5 m.m.		
August 24th	10.5—11.5 m.m.		
September 3	12 m.m.) front-limbs beginning of tail-reduction	10.2 m.m.) front-limbs beginning of tail-reduction	
September 14th	11 m.m.)	8.4 m.m.)	8.5—9.6 m.m. (frontl.)
November 27th	10 m.m.	11 m.m.	8—11.8 m.m.

poles are all the smaller in proportion as the quantity uranium-nitrate is greater. The following gives a more detailed account.

a. $4^1/_2$ mgr. uranyl-nitrate pro Liter.

On June 26th and July 10th the measurements are as mentioned; the biggest tadpoles only have an indication of hind limbs. On July 30th the biggest tadpoles have hind limbs which do not lie any more straight along the tail, but which are abducted. On August 14th it was the first time that front limbs broke through with one tadpole of 9.5 m m ; on Sept 3rd. and Sept. 14th the first tail-reductions were observed. On November 27th the last tadpole of 10 mm. with small hind limbs, showing signs of diminishing vitality, was killed and fixed.

b. 17 mgr. uranyl nitrate pro Liter.

From April 14th till July 10th the growth makes very little progress, the maximum length only being 10 mm ; tiny little points of hind limbs are present, and it is not before July 30th that one tadpole has hind limbs in abduction; on September 3rd. the first tail-reduction is observed. On November 27th the last living tadpole has a length of 11 mm.

c. $29^1/_2$ mgr. uranyl-nitrate pro Liter.

The growth still goes more slowly than the preceding ones. On July 30th I see tadpoles with points of hind limbs The beginning of tail-reduction was observed for the first time on September 7th; after that regularly; the size of the tadpoles then being 9,6—11,5 mm. On November 27th the last two tadpoles of 8.5 and 11 mm. were fixed.

II. In different periods of the development tadpoles were taken out of the uranium solutions and put into ordinary water. It then appeared that almost immediately the development took place much more quickly than with the tadpoles that remained in the uranium-solution; it must be well understood that the piscidine-tadpoles grew more quickly than the rice-tadpoles.

III. A number of tadpoles being in a young stage of development (on April 30th) were put into a liquid, containing a quantity of uranium-salt and an aequiradio-active quantity of potassium. In this liquid the tadpoles grew almost as quickly as the animals under control; on July 15th I already found one tadpole with front- and hind limbs; several other tadpoles soon get them as well; the smallest ones are 8—9 m.m. So these tadpoles grew more quickly than the uranium-tadpoles.

It may be said, that as a general result of the experiments made, tadpoles in a medium containing a radio-active substance antagonistic to potassium, grow and metamorphose less quickly than in a medium only containing potassium. The food given to the tadpoles, is not the cause of this lingering, for in regard to the tadpoles in ordinary water, fed with rice, the uranium-tadpoles also remain backward in development.

The first question that can be put is, if the tadpoles have taken uranium next to or instead of potassium.

This question cannot yet be affirmed. From an investigation voluntarily undertaken by Prof. RINGER, it appeared that no uranium could be demonstrated in the tadpoles.

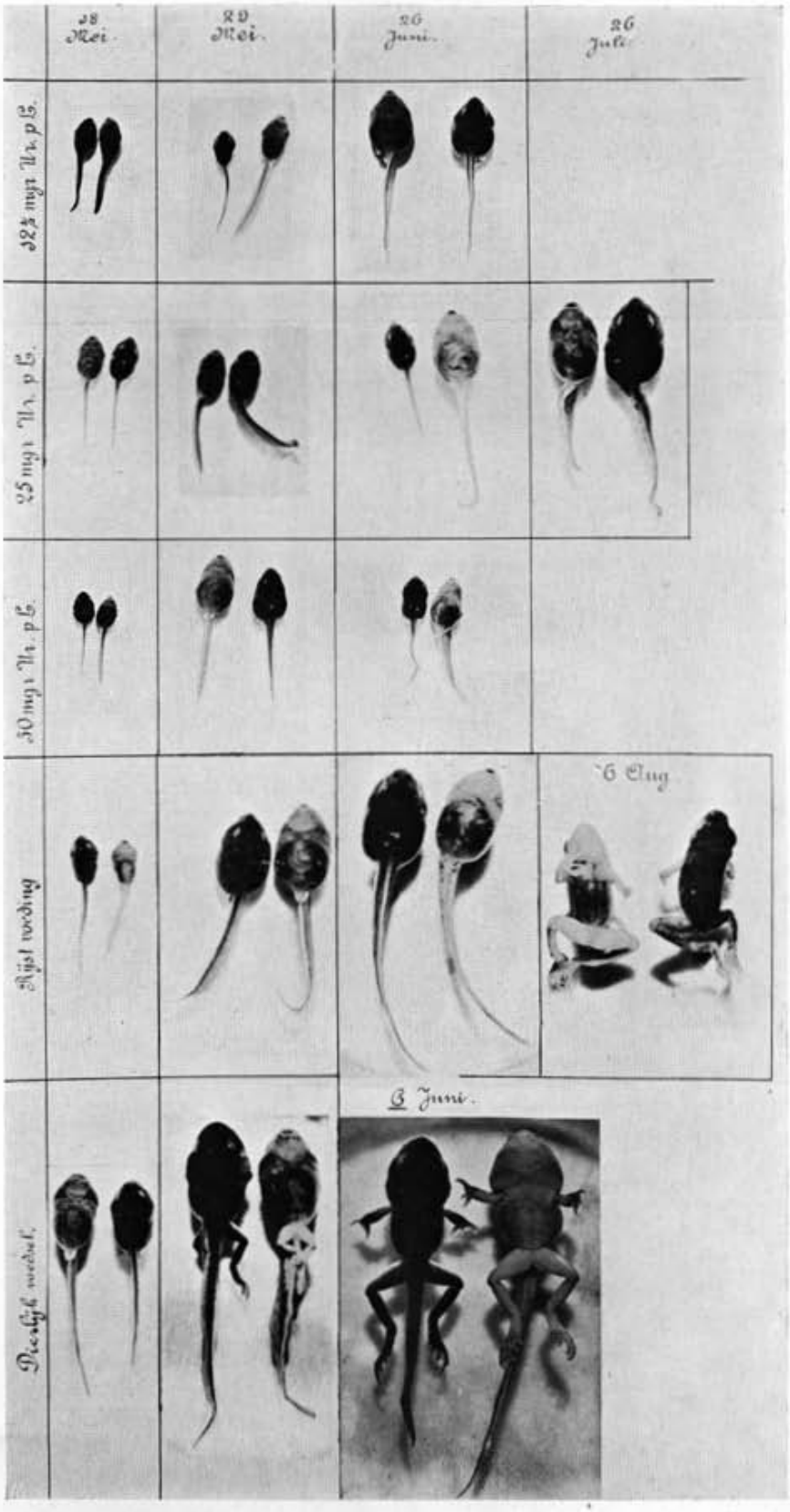
In another respect this experiment had a very important result though the percentage of potassium found in the tadpoles fed with piscidine, was in two cases 0.76 and 0.82 % from the dried tadpoles. The uranium tadpoles had evidently taken the minimal potassium quantity and even 0.91 % kalium was found. The potassium-percentage was only 0 49 % of those tadpoles, which had been in water containing 17½ mgr. $UO_2(NO_3)_2$ and 50 mgr. KCl pro Liter (being aequiradio-active quantities). It here makes the impression that the presence of ± aequiradio-active quantities of the antagonistic substances has thrown obstacles in the way of taking in potassium. Quite remarkable it is though that the concerned tadpoles should only stay little behind in growth to those, which had been normally brought up. [1])

The remaining backward in growth might be imputed to a possible poisoning caused by the uranium salt.

In 1919 I have made a single experiment on this subject. On May 6th I had five bowls containing 4 L. water each, and resp. 0, 2½, 5, 7½ and 10 mgr. uranium-salt; every single bowl contained 75 tadpoles as well; on June 19th resp. 51, 38, 24, 20 and 20 tadpoles

[1]) Prof. RINGER fixes the attention on the fact, that the chemical investigation, viz. the investigation of the radio activity of the dried tadpoles, took place with so small a quantity of the dried tadpoles, that it is desirable this investigation should be repeated, namely about the absence of uranium.

A. J. P. v. d. BROEK: "On the influence of radio-active elements on the development".



On this plate are shown the minimum- and maximum-length of tadpoles from different solutions of uraniumsalt at various data. The fourth row from above are tadpoles fed with rice, the fifth (last) row are tadpoles fed with piscidine.

were still alive in these bowls; it thus seems as if the tadpoles die sooner and in greater number, while being in higher concentration. This result does not agree with HIRSCH's [1]) results, who supposes that the quickest development takes place in the concentration, nearest to the concentration in which life is impossible. In this case one should expect a quicker development in the higher concentrations than in the lower-ones, but this has not been proved.

Moreover HIRSCH has every time extended his investigation over a very short period (7 days) which does not seem desirable, if one takes into consideration the great variability in the development of tadpoles. Although apparently a poisoning in the solutions with a greater quantity of uranium-salt does not seem impossible, the fact that many tadpoles develop, and that they live quietly on, in much stronger uranium solutions, and the failing of uranium in the body, might plead against the poisoning of the uranium-salt as a cause of the more slow development.

Microscopic investigation of series sections from some uranium-tadpoles, in comparison with normal tadpoles of the same size, has not yet shown differences in structure or in degree of development of certain organs, which should be of importance for the growth.

[1]) E. HIRSCH, Die biologische Wirkung einiger Salze Zool Jahrbücher. Band 34.