Physiology. — *"A Contribution regarding the Shifting of Radio-active Equilibria under the Influence of Fluorescein"*. By Prof. H. ZWAARDEMAKER.

(Communicated in the meeting of Sept. 29, 1917).

In an earlier paper [1]) I demonstrated that the diffusible potassium in the circulating fluid of surviving organs of cold-blooded animals could be replaced by the ions of other radio active elements. This should then be done in approximately aequiradio-active quanta. It deserves notice that with summer-frogs the dosage may be much smaller than with winter-frogs, which accounts for the difficulties workers have encountered in summertime with this laboratory animal, especially in quantitative studies. In the past months we, my co-workers and myself, succeeded in determining the ratios for the Kroneckered ventricle. We first lowered the calcium content of the artificial circulating fluids, and when this turned out unsuccessful, it was raised. An amount of 250 mgrms $CaCl_2$ (without water of crystallization) per Liter proved efficient.

It will be advisable to use distilled water in vitro. The osmotic pressure was maintained by $6\frac{1}{2}$—7 grammes of sodium chloride and the reaction was made slightly alkaline by adding 200 mgrs. $NaHCO_3$. This solution is prepared the day before and can be kept (not too long though) in bottles of ordinary glass. Examined on potassium it must not contain more than 1 mgrm per Liter.

In order to ascertain whether the heart is normal, first a normal RINGER's mixture is perfused. In winter this mixture should preferably contain ± 100 mgrms of KCl per Liter. It now appears that in summer-time we have to go down to 20—50 mgrms, else the hearts will refuse to beat. If by chance the usual RINGER's mixture of the laboratory should have been used for the preliminary experiment, the hearts will perform a few beats, then stop, in order to resume pulsation for a short time, when measures have been taken to wash off the superfluous potassium. By adding a large amount of calcium to the fluid, the toxicity of potassium may be lowered.

With 250 mgrms $CaCl_2$ per Liter the summer doses range:

[1]) Proceedings Royal Society Vol. XIX p. 633.

for potassium chloride from 20 to 50 mgr. p. Liter
„ rubidium chloride from 30 to 70 „ „ „
„ a uranium salt from 0,6 to 5 „ „ „
„ thorium nitrate from 2 to 10 „ „ „

Fixing the typical winter-doses for the four salts at 100, 150, 25 and 50 mgrms per Liter, an appropriate correspondiug summer dosis will be 50, 70, 1—5, and 1—10. In summer, however, the individual differences are larger and the hearts are more sensitive to the toxic effect of too massive a dosis and to the absence of the radio-active element when the dosis is small, so that the proper concentration for each salt is more difficult to find.

Approximate Metaldosis.
(mgrms)

	In Winter.	In Summer.
K.	53	20
Rb.	105	34
U.	12	24
Th.	24	5

Radio-equivalent.

	In Winter.	In Summer.
K.	0.000045	0.000018
Rb.	0.000020	0.000007
U.	0.000040	0.000008
Th.	0.000030	0.000006

It seems to me that for the Rana esculenta a somewhat smaller quantum of radio-active element will generally suffice than for the Rana temporaria, but on the other hand its toxic dosis is also smaller. What factors determine the quantity for every type of animal we have not yet been able to decide. My impression is that the temperature, provided it be maintained, the exposure to the sun on the preceding days and the nutritive condition are of some influence here. Dr. S. DE BOER will discuss the summerdosage in detail in a special article in the "Archives Néerlandaises".

In an earlier volume of these Proceedings (Vol. XIX p. 1043) we

pointed out that a notable antagonism exists between potassium and uranium in such a sense that they mutually neutralize each other when present in amounts of a certain ratio. I have demonstrated that the coinciding calcium exerts an influence here, which naturally led us to suppose that this it was that caused the summer-equilibria and the winter-equilibria to be so differently located. Our supposition proved to be right. To destroy the effect of both the uranium and the potassium a relatively much smaller amount of uranium is required in the circulating fluid in summer than in winter, although the same amount of potassium is taken in either season, so that the fluid is getting somewhat like the potassium-free circulating fluid just described, which is used also in the intermediate experiments. The equilibria looked for are always reached with a potassium-free fluid preceding.

The following figure shows the winter- and the summer-equilibria between potassium and uranium. The first have been determined by Mr. T. P. FEENSTRA, the second by Dr. S. DE BOER. The character of the curves is very much alike and both decline with the larger doses more and more gently, running at last nearly parallel to the abscisse. The addition of a very large quantity of potassium salt has a disproportionately slighter effect than that of a much smaller dosis of uranium-salt. As yet only uranyl compounds were at my disposal, but no difference revealed itself here between the various salts (nitrate, acetate, sulphate).

Also the summer-equilibria between potassium and thorium and those between rubidium and uranium have been established by us. The curve of the counterbalanced concentrations runs, on the whole, like the one in the figure below.

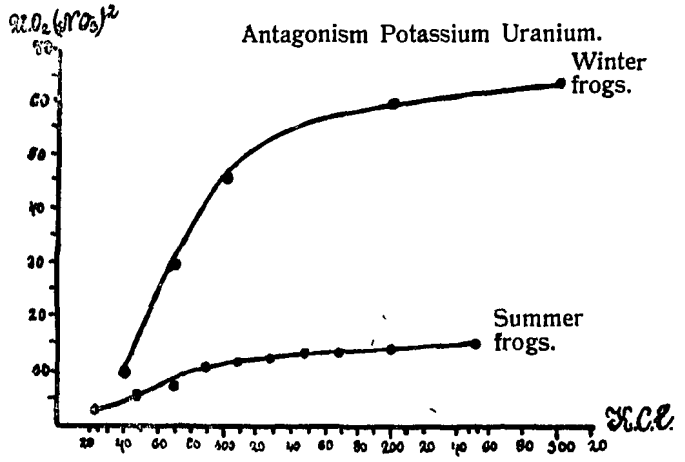

Fig. 7.

All our equilibria have been maintained for some time, 5—10 minutes at the least, and we have watched for some latent automatic pulsation that might exhibit itself, when a single contraction was elicited by a single gentle push.

Our interest was now excited to see whether by the addition of fluorescein the equilibria would be shifted, and whether this would occur in the same sense as was the case with the summer-equilibria, when compared with the winter-equilibria. The results confirmed our supposition.

I found e.g. for the equilibria:

50 mgr potassium-chloride + 5 mgr uranylnitrate = 0 (per liter);
100 mgr potassium-chloride + 15 mgr uranylnitrate = 0 (per liter), [1])

that an extra addition of 100 mgrms of fluorescein to the heart, arrested in equilibrium, made it resume its normal beats.

For the equilibrium:

50 mgrms potassium-chloride + 6 mgr uranylnitrate = 0 (per liter),

I demonstrated the same and also that radiation or an extra addition of 250 mgr of potassium chloride again caused a standstill without injuring the heart in the least, as was made out by a prolonged experiment.

The same experiment was performed for a potassium-thorium-equilibrium:

25 mgr potassium-chloride + 5 mgr thorium-nitrate = 0 (per liter).

By the addition of 100 mgrms of fluorescein the pulsations recurred and this time by an increment of 50 mgr of potassium-chloride a fresh standstill was brought about.

It will be understood that finally in all these experiments a perfusion of potassium-free fluid was administered, after which the normal RINGER's mixture made the heart recover its normal condition.

It is evident, then, that fluorescein promotes the sensibility to the radio-active elements, in the same way as the summer does, viz. the action of the heavy metals more than that of the light ones. It follows that an increment of light metal is wanted to restore the equilibrium. Mesothorium-radiation can also be taken instead of potassium. We might also put it in this way: that both the summer and the fluorescein depress the curve of the potassium-uranium equilibria (also that of the rubidium-uranium equilibria, besides that of the potassium-thorium equilibria). Calcium, on the other hand, yields a higher curve. What must be done to restore the equilibrium may be gathered from the curve itself.

[1]) Different types of animals.

It is certain, meanwhile, that neither the summer shifts nor the fluorescein shifts depend on the radio-active elements, as these are the same in summer and in winter. We are inclined to think the different reaction of the muscle cells to be the cause. But before ascribing the different behaviour to the more or less mysterious nature of the automatic rhythm it will be good to study the factors to which the adsorption of the heavy and the light metals is due. Much [1]) in the phenomena observed may perhaps be accounted for by the mutual adsorption-extrusions of the ions. The antagonism, which manifests itself after the adsorptions have brought the ions in the vicinity of the irritable substance, does not offer any ground for explanation.

[1]) For instance: standstill inside one minute, when a suitable uranium fluid is rapidly replaced by a potassium fluid, of itself also suitable; the approximately horizontal character of the equilibrium curve with the higher doses; the calcium effect.