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Climbing activity in frogs and the effect of highly diluted succussed thyroxine

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Summary
The experiments investigate the influence of extremely dilute thyroxine (T_4) in special 'homœopathic' preparation (dilution T_4.30x) on the spontaneous tendency of juvenile frogs to leave the water and climb on land. Climbing activity was suppressed by dilution T_4.30x, with statistical significance both in comparison to the effect of the 'potentized' preparation of the solvent (dilution H_2O.30x) as well as in comparison to the control observations before the start of the treatment. Finally, in the search for optimal treatment duration, it was shown that exposure to the dilutions for even a few minutes sufficed to cause significant effects.

KEY WORDS: Homœopathic effect, Diluted hormone, Thyroxine, Amphibia, Activity, High dilution.

Introduction

A number of experiments have been performed on extremely diluted succussed substances (homœopathic preparations) up to now, apart from human studies,[1] primarily with mice and rats.[2,3] To explain the observed effects, even from dilutions beyond Avogadro's value, a memory or information function has been postulated to occur in succussed solvents. This might be based on special configurations of the solvent as well as on extremely weak electromagnetic fields.[3,4]

Our choice of an amphibian model is based on experiments on the influence of homœopathically prepared thyroxine on metamorphosis.[3] The model introduced in this paper depends on the following facts:

—Thyroxine applied in classical pharmacological concentrations may provoke hyperactivity in vertebrates.

—Physiological lack of thyroxine, e.g. due to thyroidectomy, causes inertness.[5–7]

This has led to speculation that homœopathically prepared, highly diluted thyroxine may influence the spontaneous locomotor activity, in particular the spontaneous tendency of juvenile frogs just at the end of metamorphosis to leave the water and climb on land.

Methods

Animals

Rana temporaria juveniles were taken from a highland lake (1600 m above sea level). For different types of experiments, animals were taken at the two-legged stage (about stage 31 according to Gosner's staging table),[8] at the four-legged, tailed stage (about stage 42) or at the four-legged, tailless stage of the juvenile frog (about stage 46).

Laboratory

All experiments were performed indoors at a site associated with the Ludwig Boltzmann Institut für Homöopathie in Graz, Austria.

Preparation of test solutions

The test solutions T_4.30x and H_2O.30x were prepared by the firm Dr Peithner, Vienna. The stock solution of thyroxine (Sigma) had a concentration of $1:10^4$. It was diluted in stages of 1:10 and succussed at every stage according to homœopathic standards.[9] The result is an aqueous solution of thyroxine $1:10^{30}$ parts by weight, called dilution T_4.30x. Water was prepared in an analogous way (dilution H_2O.30x).

Solution coding

The solutions for the experiments (two independent batches) were coded by Prof Dr G. Fach-

bach (Department for Animal Morphology and Histology of the Institute of Zoology, University of Graz). After the experiments the protocols were given to G. Fachbach and the codes of the testing solutions were announced.

Check for contamination
The contents of tetraiodothyronine (T_4), triiodothyronine (T_3) and thyroid-stimulating hormone (TSH) of the two treatments were checked by radioimmunoassay technique (Dr Habil. G. Passath, 1st Medical Clinic of the University of Graz). The iodine content was checked by catalytic reaction (Prof. Dr G. Knapp, Meßstelle für Spurenanalyse, Graz). Contamination with the substances mentioned above was excluded up to the accuracy of the measurement.

Treatment
Dilution T_4.30x as well as the control H_2O.30x were always applied by putting a few drops of the respective dilution into the water of the basins.

Observation of climbing activity
White plastic alimentary basins (l × w × h: 34 × 22 × 14.5 cm Firma Miraplast, Austria) were each filled with 0.5 l of water (depth of water: 1 cm). The water consisted of 90% tap water and 10% lake water. The juvenile frogs spontaneously climbing out of the water and up the walls of the basins were counted. In some cases, photos and a video were prepared as documentation.

Observation A
Tadpoles were taken out of the lake at the two-legged stage (observation A1) and at the four-legged stage (observation A2), respectively. They were all put into the water in the basins containing the dilutions T_4.30x and H_2O.30x, respectively, and after one and two days the number of animals that had climbed out of the water was counted.

Observation B
The climbing activity of juveniles in the course of a few minutes was investigated by comparing always two groups of animals in two different basins. All the animals that had climbed up the walls to a certain height were put back into the water before measurement, then both basins stump of the tail) out of the water was counted 10 seconds, 1, 2, 3 and 4 minutes after last shaking the basins. This experiment was repeated 2–5 times within 10–40 minutes. During the pauses between observations the basins were covered to prevent the juveniles from climbing out.

In two different groups of experiments, juveniles pretreated with dilution T_4.30x or H_2O.30x since the two-legged stage (observation B1) and juveniles pretreated since the four-legged, tailed stage (observation B2) were used.

Observation C
Activity was observed as in observation B. The juveniles had not been pretreated. In a first step which can be taken as zero-time-control, climing activity was measured without any treatment. In a second step, the frogs were treated with dilution T_4.30x and H_2O.30x. Climbing activity was observed as above. All experiments were begun with animals in the juvenile stage just after metamorphosis.

For some of the following experiments the time between both steps was two days (observation C1); for other experiments it was 10–20 seconds (observation C2).

Further conditions
Animals were fed with cooked greens (lettuce) ad libitum during pre-treatment; they were not fed while their climbing activity was being observed. Most observations were made about midday. Only very little indirect light was used. Room temperature was kept constant. Any external shocks were avoided.

A number of small sub-experiments was performed. Two basins were always used for treatment with dilution T_4.30x and H_2O.30x respectively. Each basin contained 4 to 13 animals. In each experiment, however, the number of animals per basin was the same, usually 13. The positions of the basins were changed regularly to avoid any influence of spatial factors (e.g. gradient changes in light or temperature). If an animal had reached the edge of the basin, it was put back using a disposable wooden stick.

Evaluation of data
Observation A: The cumulative frequency of climbing activity

$$F_{act} = n_{cl} \times 100/(n_{cl} + n_{noncl})$$

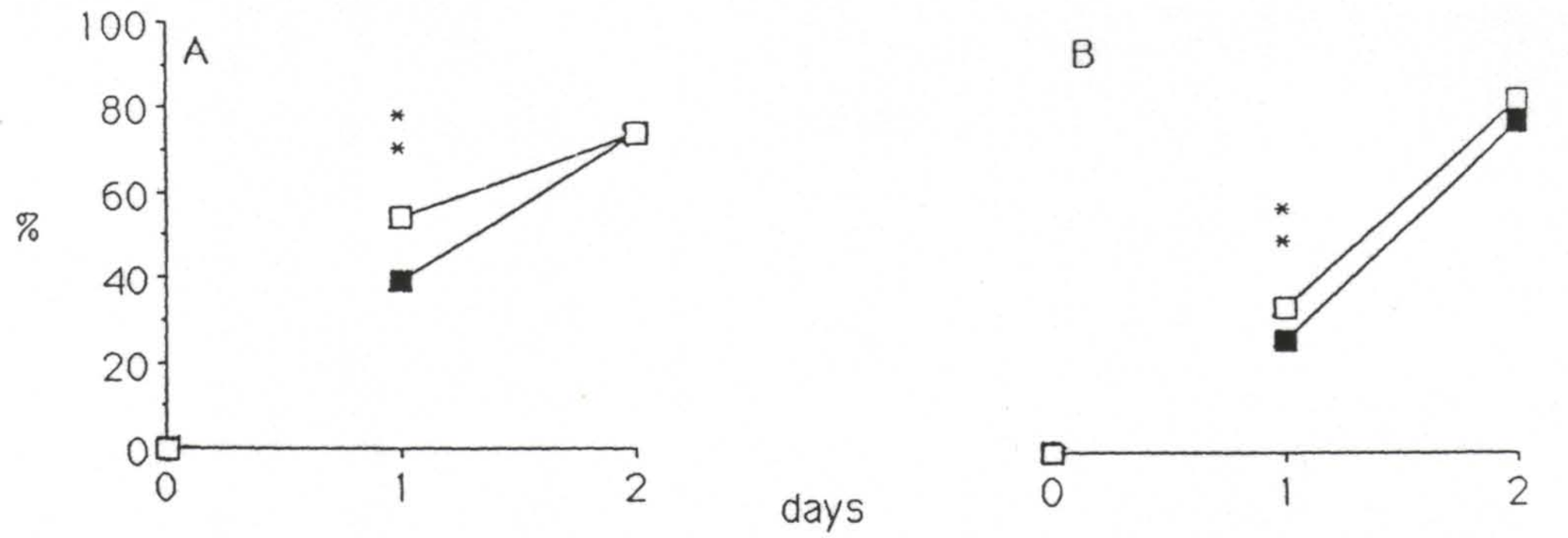

Figure 1: The climbing activity (F_{act}) of juvenile frogs treated from the two-legged stage (A) and of animals treated from the four-legged, tailed stage (B). Ordinate: cumulative frequency of climbers. Abscissa: the number of days of duration of the experiment. Black squares, animals treated with dilution T_4.30x; white squares, animals treated with dilution H_2O.30x: + $P<0.05$; ++ $P<0.01$. For more details, see text.

T_4.30x or H_2O.30x) in a 4-field crosstable using the chi-square test.

Observations B and C: In the observations including successive repetitions of the experiments, the numbers of climbing frogs were added up for each basin at five different times (10 seconds, 1, 2, 3 and 4 minutes) after the start of the experiment. Again the cumulative frequency of activity was compared using the chi-square test. In a further step, to get a more general view, the experiments of one type were treated as one experiment and again evaluated as above with the chi-square test. Experiments of observation C were also evaluated with the Wilcoxon test.

Further, 'survival analysis' was used to determine statistical differences between the groups. In this test,[10] cases in which the terminal event, the climbing out of the water, had not yet occurred, are taken as remaining ('surviving') cases. Thus, every experiment is described by only one P-value.

Probability values given in the text without further specification refer to chi-square tests.

Results

The influence of the dilutions T_4.30x and H_2O.30x on climbing activity, observed at 1-day intervals

Observation A: Climbing activity of pretreated animals

Observation A1: Four experiments were performed with a total of 127 animals which had been pretreated with the dilutions from the two-legged stage. The number of spontaneous climbers was counted after the first and the second day of treatment. The difference between the groups was significant on the first day but not on the second day. The F_{act} values for the animals

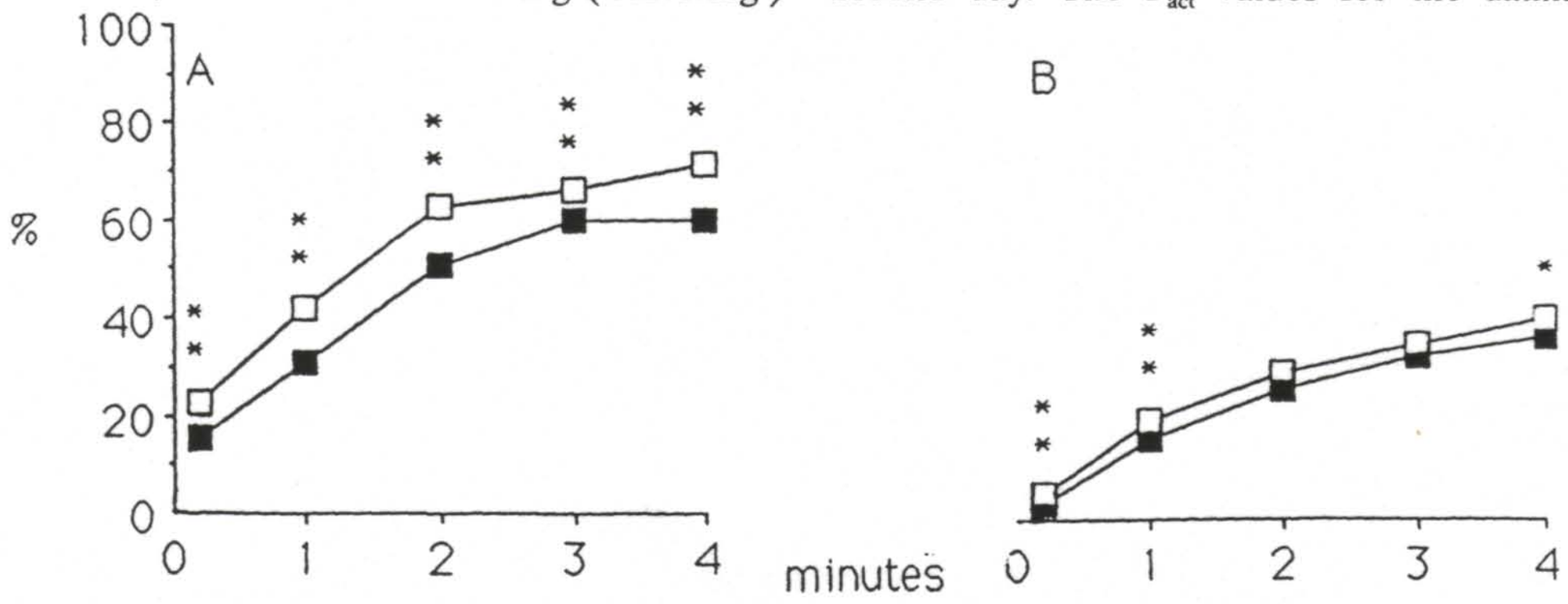

Figure 2: The climbing activity of juvenile frogs with regard to the time of pretreatment. Abscissa: the number of minutes of the duration of the experiment. A, treatment from the two-legged stage. B, from the four-legged, tailed stage. For more details. See Figure 1 and text.

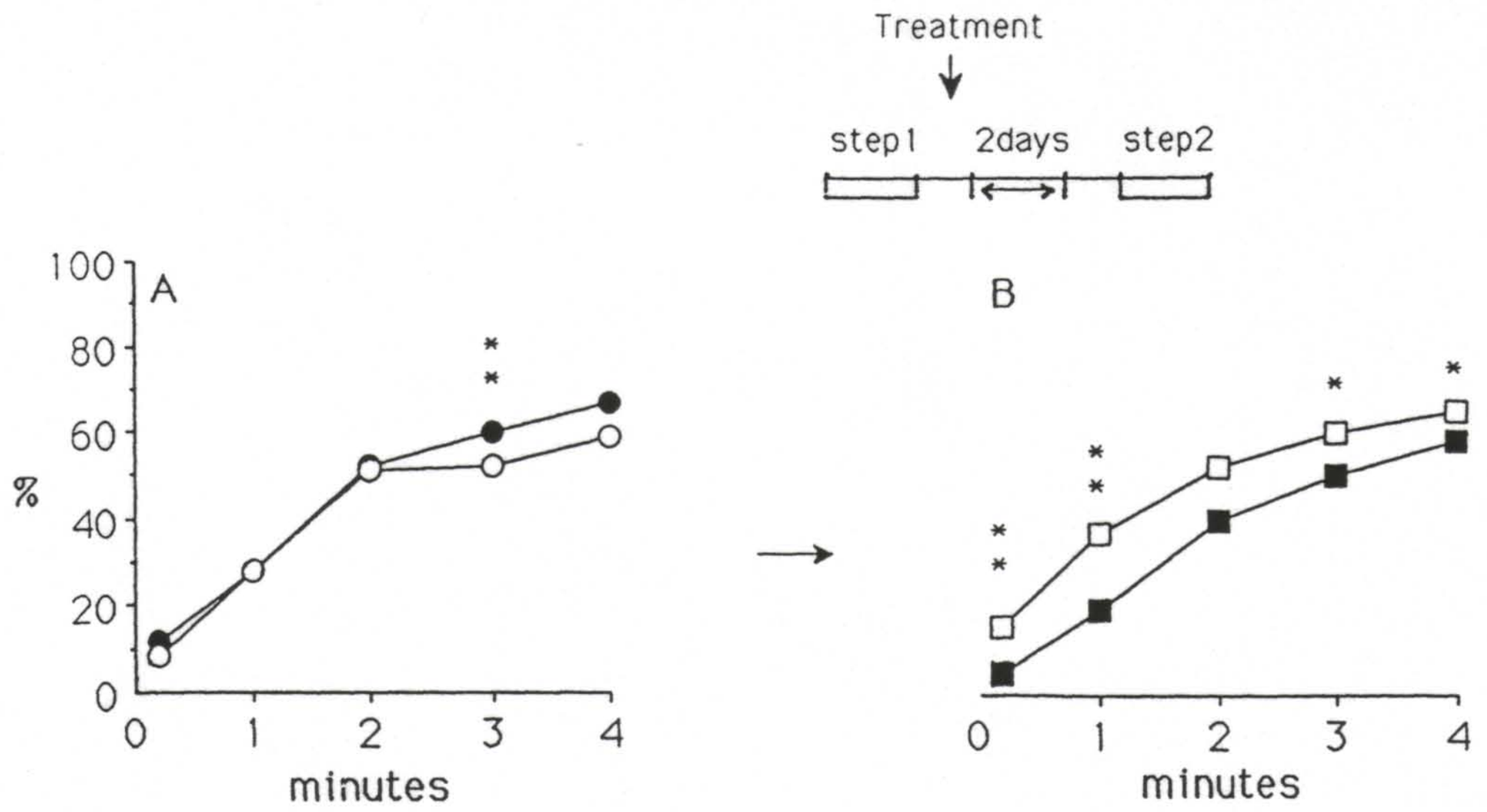

Figure 3: The climbing activity of juvenile frogs before (circles) and two days after treatment with the dilutions T_4.30x or H_2O.30x (squares). Ordinate: Cumulative frequency of climbers. Abscissa: the number of minutes of duration of the experiment. A, step 1: climbing activity before treatment: cumulative frequency of climbers assigned for later treatment with dilution T_4.30x (black circles) and with dilution H_2O.30x (white circles). B, step 2: after treatment with the dilutions. For more details, see Figure 1 and text.

treated with dilution T_4.30x are below those of the control group. (Fig. 1A).

Observation A2: Seventeen experiments were performed with a total of 368 animals pretreated since the four-legged, tailed stage. The difference between the groups was also significant on the first day but not on the second day. Again, the F_{act}-values for animals treated with dilution T_4.30x are below those of control (Fig. 1B).

The influence of the dilutions T_4.30x and H_2O.30x on the climbing activity, observed within minutes.

In these observations climbing activity F_{act} is measured at the end of the first ten seconds and of the first, second, third and fourth minutes.

Observation B: Climbing activity of pretreated animals

Observation B1: Four experiments were performed (85 animals, 5 repetitions, total: 425 cases). Animals pretreated since the two-legged stage were taken. F_{act} values for T_4.30x animals are below those for control (Fig. 2A). The difference is statistically significant according to the chi-square test as well as the survival analysis.

Observation B2: Ten experiments were performed with animals pretreated since the four-legged, tailed stage (248 animals, 5 repetitions, total: 1240 cases). Here, F_{act} values for animals treated with dilution T_4.30x are also below those for control (Fig. 2B). Evaluation with the chi-square test shows a statistically significant difference between the groups: survival analysis does not show a significance difference.

Observation C: Climbing activity before and after treatment

Observation C1: Two experiments were performed with non-pretreated juveniles (33 animals, 5 repetitions, total: 165 cases). In a first step, both groups of animals were compared before treatment. The experiment was repeated five times in immediate succession.

In Fig. 3A, the two curves give the increase of F_{act} for animals assigned for later treatment with dilution T_4.30x or dilution H_2O.30x. At the end of the third and fourth minute F_{act} values for the animals assigned for later treatment with dilution T_4.30x are above the F_{act} values for later reference ($P<0.01$). The survival analysis does not confirm this difference.

In a second step, the animals were treated with dilutions T_4.30x and H_2O.30x respectively. After an interval of two days, the experiment

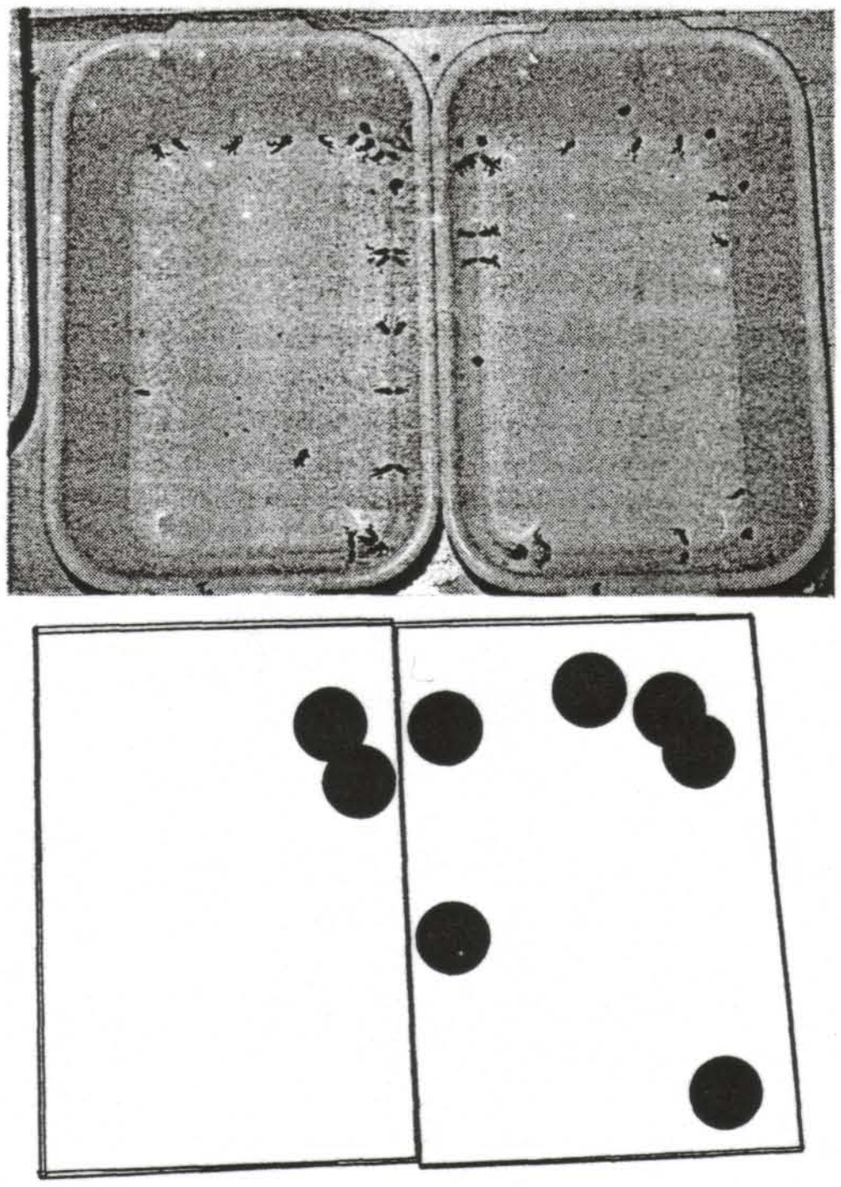

Figure 4: The climbing activity after treatment with the dilutions. Left: group treated with dilution T_4.30x, two climbers are visible. Right: group treated with dilution H_2O.30x, more than 2 climbers can be seen. For more details, see text.

was again repeated five times. In Fig. 3B (see also Fig. 4), the curves clearly show a difference: the F_{act} values for T_4.30x animals are now below the F_{act} values for control. The difference is significant with $P<0.01$ in two and with $P<0.05$ at two further time points. The survival analysis here shows a non-significant ($P = 0.06$) difference which can be considered a trend.

Observation C2: Three experiments were performed with non-pretreated juveniles (78 animals, 2 repetitions, total: 156 cases). Fig. 5A shows the situation before treatment: the curves are practically identical at all time points. Survival analysis also indicates no difference.

In the second step, dilutions T_4.30x and H_2O.30x were added and the observation was twice repeated immediately, starting 10–20 seconds after adding of the dilutions. Fig. 5B shows that the F_{act} values for T_4.30x animals are now below the reference values. The difference is statistically significant at the end of the second minute of observation. Fig. 5C shows a similar situation for the second repetition of the experiment after treatment (Chi-square test: $P<0.01$ at two time points). For the sum of both repetitions, the Wilcoxon test shows $P>0.05$ and the survival analysis shows $P = 0.04$.

Discussion

The results presented in this study refer to juvenile highland frogs at the end of metamorphosis. They show that there are differences in spontaneous climbing activity between the groups of animals treated with dilution T_4.30x as compared to groups treated with H_2O.30x. The differences indicate that T_4.30x slowed down activity significantly. It obviously suffices to add a few drops of the dilution T_4.30x to the water in the basins to induce a slowing down of the rate of spontaneous climbing activity in the amphibia.

This tendency to reduce activity is opposite to that known for thyroxine in pharmacological

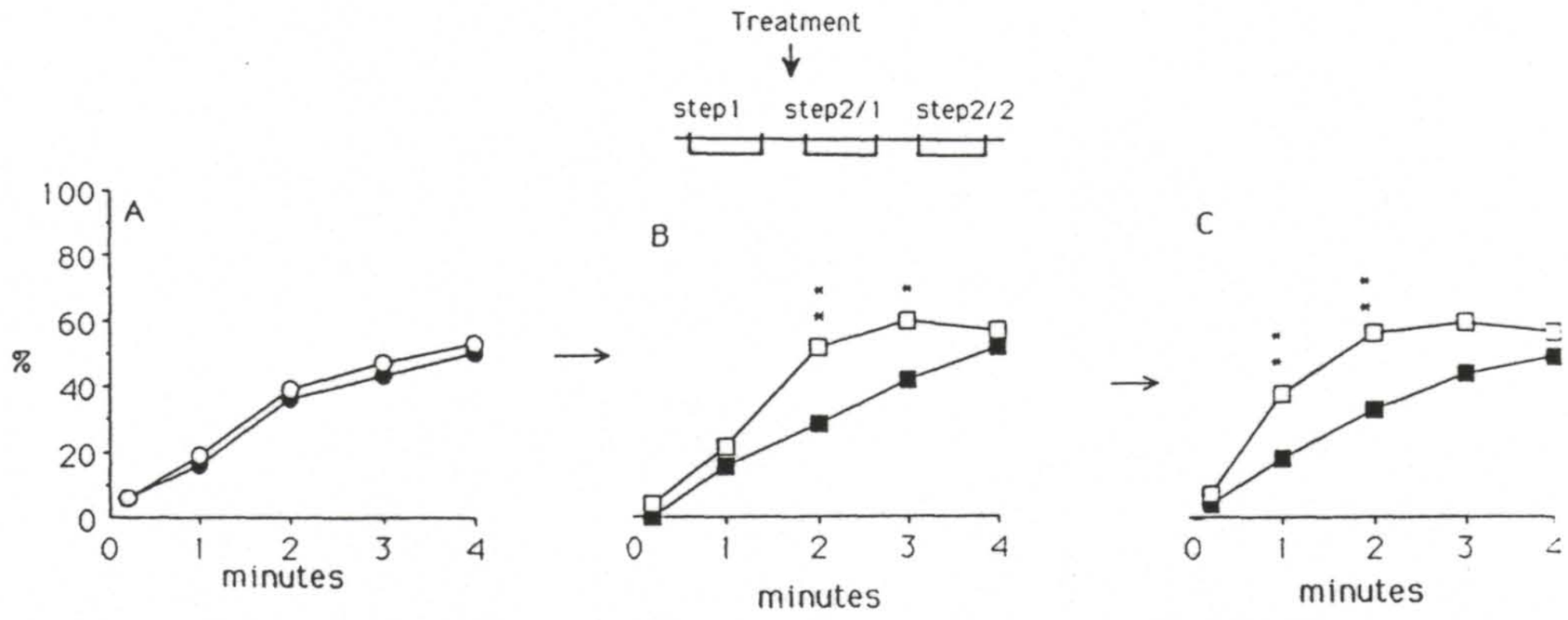

Figure 5: The climbing activity of juveniles before and minutes after treatment with the dilutions. A, step 1: before treatment; B, step 2/1: 10–20 seconds after treatment; C, step 2/2: 4–5 minutes after treatment. For more details, see Figure 1 and text

doses,[5-7] but is consistent with the results of our study on the effect of dilution T_4.30x on metamorphosis of highland frogs.[3] In that study, in two laboratories in Graz (Austria) and in one laboratory in Utrecht (Netherlands) an inhibitory effect of dilution T_4.30x compared to the analogous preparation of the solvent (dilution H_2O.30x) was proved with statistical significance. Development was slowed down by the succussed dilution T_4.30x both during transition from the 2-legged to the 4-legged tadpole as well as during transition from the 4-legged, tailed tadpole to the juvenile frog. In that study,[3] in one experiment the number of climbing frogs was counted and taken as a parameter for the end of metamorphosis from the water-living tadpole to the land-dwelling frog. There were fewer climbers in the T_4.30x group.

The results from the study on metamorphosis on the one hand and from the present study on climbing activity on the other hand show that a long-term influence of dilution T_4.30x may be superposed by a short term influence of the dilution on activity.

The physiological changes resulting from pharmacological application of thyroxine are not only due to an effect of the thyroid hormones per se, but are also the result of an interaction with the hormones of the adrenal medulla and with the sympathic nerve system.[6] It is further known that sympathicoadrenal actions occur in seconds to minutes, while the response to applied thyroid hormones occurs only after a delay of hours or days.[5,6,11] At present we have no insight into the physiological processes caused by dilution T_4.30x, but we think that a neurohormonal interconnection similar to the one pointed out above might also be involved in the effects of high dilutions on the climbing activity of juvenile frogs.

To distinguish between possible direct effects of the dilutions (key function of water clusters?) and of effects caused by radiation phenomena[4] involving the dilutions, a further study is in progress.[12] Here, the dilutions T_4.30x as well as H_2O.30x are not dropped directly into the aquaria but are kept in glass vessels which are put into the water of the aquaria to avoid any direct contact between the dilutions and the water of the aquaria or the organisms.

Finally, we wish to emphasize that the basic experiments we have introduced have to be confirmed by independent repetitions in other laboratories. Only then can our study and its implications on natural science be generally accepted.

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A personal word: The authors submit the discussion of the amphibian model to the public in the hope that it will be a challenge to scientific creativity to further explore it, but without any investigation harmful to the animals.

Note: Various papers on the amphibia studies are included in a book. *Examples on Research in Homœopathy,* edited by P. C. Endler and colleagues. This book will provide an approach to homœopathic research for both orthodox and complementary readers. Among others, F. -A. Popp, C. W. Smith, M. Righetti, B. Poitevin, E. Davenas, D. Taylor-Reilly and P. Fisher contributed.

To make publication of the book possible, further subscribers are needed. If you are ready to support our work in this way, please write for a copy to the following address: Dr P. C. Endler, Wittenbauerstr. 137, A-8042 Graz, Austria. The price of the book will be less than £15 sterling.

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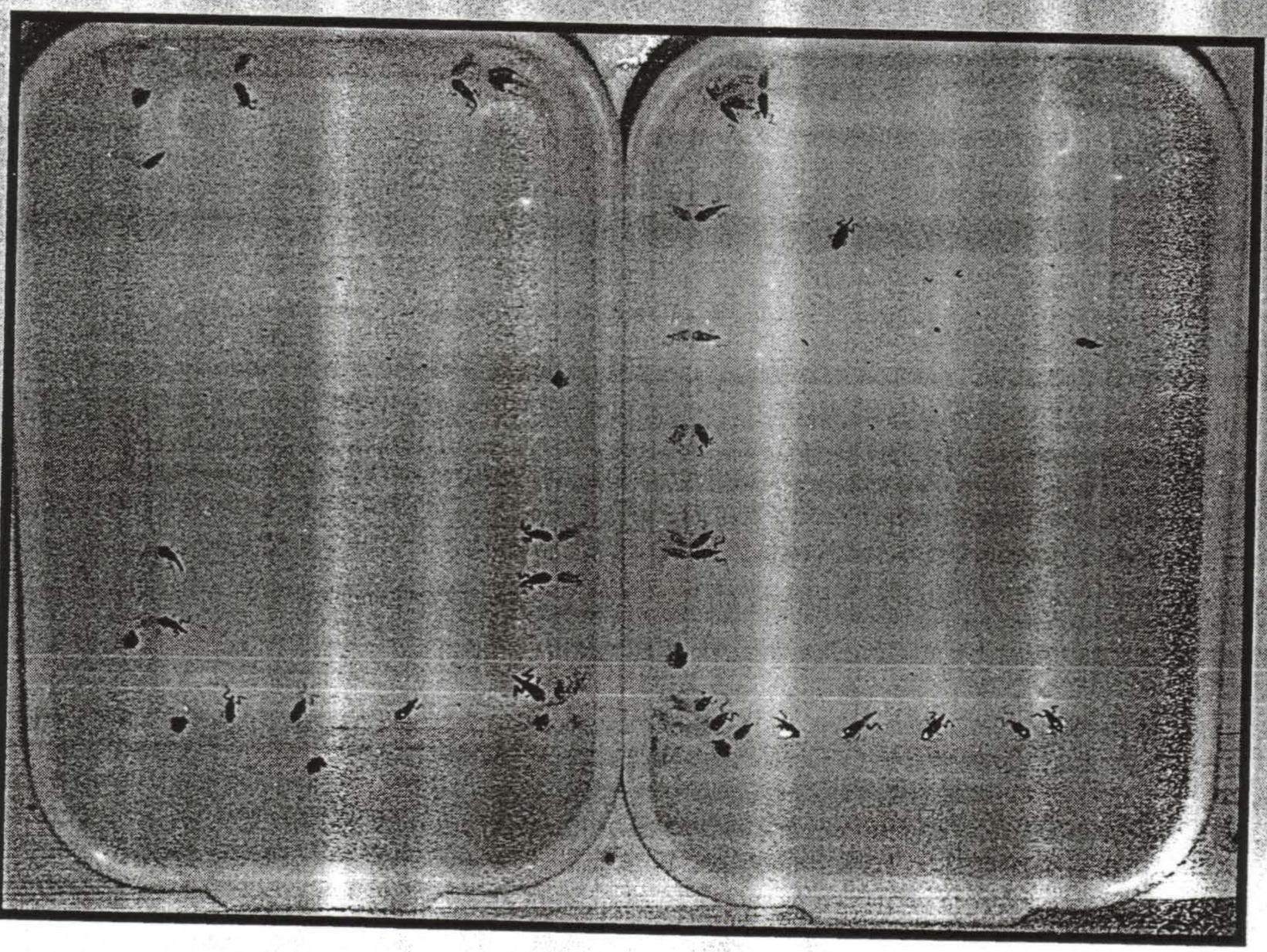

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