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Note

Kinetic Interference Upon Embryo Development

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Biomphalaria glabrata (molúsco, gastrópodo), em diferentes estágios de desenvolvimento foi tratado com D_2O a 1% e 0,1 v/v em H_2O (~5 vezes a abundância natural). Além de uma alta mortalidade em relação ao controle, observa-se uma velocidade de desenvolvimento extremamente baixa. Surpreendentemente, este animal pode ser mantido por ~1/3 da sua vida em dimensões reduzidas. O retorno às condições normais leva a uma retomada do desenvolvimento atingindo o estado de adulto após um intervalo de tempo muito maior do que o observado no controle. O real papel dos isótopos na natureza é questionado.

Biomphalaria glabrata(mollusc, gastropod), in different development stages were treated with D_2O at 1% and 0.1 v/v in H_2O (~5 times natural abundance). Besides a high mortality rate in relation to the controls an amazing slow rate of development was observed. Surprisingly, this animal may be kept for ~1/3 of its life in reduced dimensions. Recovering is however possible. Upon return to normal conditions they resume development reaching adult stage in a much longer period of time as compared to the controls. The actual role of isotopes in nature is discussed

Keywords: Biomphalaria glabrata, *deuterium, environment, isotope, toxicology*

Introduction

Isotope substitution has been a quite common technique to investigate chemical reactions. More recently[1-4] however, this same technique has shown a special interest for studies concerning activity of drugs[4], complex mechanisms such as taste of living beings, growth and transforming activities of microorganisms, such as *Streptococcus pneumoniae*, cell survival[3] and temperature shift, effect over some disease such us Fanconi's anemia, and its effect upon human carcinoma.

As it is well known, isotope effect upon a chemical reaction[9] is quite unpredictable specially when we consider chemical reactions among high molecular weight compounds are considered. Obviously, more unpredictable effects can be expected when dealing with living organisms where the modified molecules take part of an enormous number of reactions in concert. In this paper we deal with the effect of isotope substitution upon the process of growth of an animal from its embryo phase. For the purpose we have chosen as our subject *Biomphalaria glabrata* (mollusc, gastropod) in different developmental stages (Phase **I**, close to 130 blastomers; **II**, *Veliger*, 5 days after first cleavage; **III**, already formed *embryo*, ready to hatch out, and **IV**, already ecloded, two days old).

Materials and Method

Biomphalaria glabrata, from the State of Minas Gerais, Brazil was used in the present research. Adults were raised in laboratory for ten years. *Biomphalaria glabrata* is a simultaneous hermaphrodite and reproduce either by selfing or cross-fertilization. In the present research embryos and adults were used. The adults were raised according to our laboratory routine[5,6,7] and the embryos were kept in Petri dishes and filtered water, at room temperature.

Embryos at different stages of development were treated in water containing 0.1% and 1% D_2O. Controls were kept in plain water. The experiment were performed using adults, and embryos which were classified according Camey[5] as follows: phase **I**, 130 blastomers, one day after the eggmass was laid; phase **II**, Veliger, five days after the eggmass was laid; phase **III**, embryos ten days old embryos and finally, phase **IV**, pos-hatching, young snails one or two days old. In all cases controls were used under the same conditions. Experiments were carried out with mutant albino and wild type.

The eggmasses of phases **I-III** were kept in mini Petri dishes (2 x 2 cm), containing two eggmasses each. For each plate the number of eggs and hatching ratio were verified.

In all experiments the subjects were treated with two differents solutions of D_2O (Aldrich, 99% D_2O), in water, at convenient concentrations of 0.1 and 1% (v/v). The animals in phase **IV**, post-hatching numbered 18 young snails were kept only at 0.1% (v/v) D_2O in H_2O. All of them were kept in the same dish containing D_2O at 0.1% in water (v/v), fed with dry ground lettuce leaves.

Results and Conclusions

Although all experiments were carried out with albino and wild type (pigmented), we will present the results considering only the wild type since the response to the tests were identical for both strains. The results that follow are summarized in Table 1.

As it can be seen from our data, the content of D_2O in the environment is critical for different phases. From them we notice that younger animals (phases I and II) are more resistant than the older ones, as far as the D_2O effects are concerned. Although a similar behavior was observed when those animals were tested with nicotine sulfate[6] and copper sulfate[7] that was not the case with caffeine[8]; in this case younger animals were more susceptible to its effect than the older ones.

Besides the above mentioned effect of D_2O upon the animals, embryos in phase **I** and **II**, survived but their development was considerably slowed down. In these cases egg hatching out was possible only mechanically. Animals of the other phase eventually died as the result of the treatment.

It is interesting to notice that, following our treatment, we were able to keep embryos encapsulated for 26 days, living normally, when we decided to ecclode the eggs mechanically. Normally an embryo stay encapsulated for no more than 10 days before to ecclode. The animals thus mechanically obtained managed to survive; however, all them had an extremely slow development; their dimensions were kept surprisingly diminute regarding weight and size as compared to the normal under control. We did not observe any anomaly in their shell in spite of their dimensions of about ~1/4 to ~1/6 of the controls at the same age.

Table 1.

Phase 1.

Plate-No.	Total of Eggmasses	Total of Eggs	Eggs Viable	Eggs Non-Viable
1-1%	2	72	72	-
2-0.1%	2	66	66	-
3-0.1%	2	80	80	-
4-0.1%	2	62	62	-

Phase 2.

Plate-No.	Total of Eggmasses	Total of Eggs	Eggs Viable	Eggs Non-viable
1-1%	2	82	45	37
2-1%	2	90	23	67
3-0.1%	2	82	29	53
4-0.1%	2	97	51	46
5-0.1%	2	78	70	8

Phase 3.

Plate-No.	Total of Eggmasses	Total of Eggs	Eggs Viable	Eggs Non-viable
1-1%	2	61	60	1
2-1%	2	67	66	1
3-0.1%	2	68	65	3
4-0.1%	2	59	56	3
5-0.1%	2	68	64	4

Phase 4.

Plate No.	Total of Animals
A - 1%	20
B - 0.1%	20

When those animals thus described were submitted to normal feeding conditions including water with natural isotope abundance of deuterium, they approximately recover regular dimensions. After 9 months their shell diameter reached ~15 mm as compared to the controls with ~20 mm on the average. This recovering process showed itself feasible even when the animals were kept for about 1/3-1/4 of his life before returning to normal condition. Finally, after recovering the animal initiated normal reproduction.

For almost half a century stable-isotope has found usage[1] in human metabolic studies without documented significant adverse effect. Side-effects with acute deuterium dosing are reported transitory with no demonstrated evidence of a permanent deleterious action. The threshold of D toxicity has been defined in animals and is far in excess of concentrations used in human studies. For that reason it is thought that D may have additional beneficial pharmacological applications. In fact, recently there are reports concerning the effect of D_2O retarding the growth of human carcinomas[2] inoculated in mice after ingestion of water containing ~30 atom % D_2O.

Also, PtK2 cells[3] grown in presence of D_2O remained non-proliferating; however, they were able to divide after recovery in a non-deuterated medium. On the other hand, when they were incubated for 8 weeks in 75% D_2O mitotic activity was not resumed, as reported recently. Besides, it is reported that mice ingesting 30 to 50% D_2O have developed a dose-dependent depression[4] of formed peripheral blood elements in 4 to 9 days. In this case, despite pancytopenia in the peripheral blood, it was observed that bone marrow cellularity and morphology remained normal. Although platelet and neutrophil concentrations may return to normal within 48 to 72 h upon substitution of D_2O with tap water, a prolonged deuteration has proven toxic in animal models.

The above mentioned studies however deal with a rather high content of D_2O. Consequently follows the idea that much lower % of deuterium in a medium might turn out to be harmless. According to our studies, differents phases of an animal development can behave in a quite distinct manner. An embryo may be more resistant than an adult, when we consider the effect of deuteration. As a consequence, unpredictable effects upon their development are observed.

Natural abundance of deuterium is ~ 0.02% relative to hydrogen; however, working with values only five times higher, it is possible to retard the hatching out of an embryo for a rather long period of time. Further, the animal thus grown may be kept for almost 1/4-1/3 of its live in suprisingly reduced dimensions and later it is possible to recover its development to an almost normal size without apparent anomalies and without affect its reproduction.

For those reasons we are then tempted to ask what is indeed the role isotopes are playing upon the living organisms on Earth. Is deuterium a subtle mechanism allowing nature to fine tune kinetics processes of life?

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