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## Literature Cited

- Alvarez, J. 1950. Claves para la determinación de especies en los peces de las aguas continentales Mexicanas. Sec. de Marina, Dirección General de Pesca e Industrias Conexas.
- de Buen, F. 1942-1943. Los peces de agua dulce de la familia Goodeidae. Bol. Biol. Lab. Univ., Univ. Puebla, México, Año II, Núm. 3, pp. 111-148.
- Hubbs, C. L. and C. L. Turner. 1939. Studies of the fishes of the order Cyprinodontes. Part XVI. A revision of the Goodeidae. Misc. Publ. Mus. Zool., Univ. Michigan, No. 42, pp. 1-80.
- Meek, S. E. 1902. A contribution to the ichthyology of Mexico. Field Col. Mus. Publ., No. 65, Zool. Ser. 3:63-128.
- , 1904. The fresh-water fishes of Mexico north of the Isthmus of Tehuantepec. *ibid.*, Publ. No. 93, Zool. Ser. 5:1-252.
- Turner, C. L., G. Mendoza, and R. Reiter. 1962. Development and comparative morphology of the gonopodium of Goodeid fishes. Proc. Iowa Acad. Sci. 69:571-586.

## The Antagonistic Effect of Strontium Ions for Anesthetization of *Paramecium caudatum* with Nickel Ions<sup>1</sup>

CLAIR G. RAUSCH

*Abstract.* The purpose of this investigation was (1) to compute the effects of strontium ion antagonism and the observed biological effect of the strontium ion for anesthesia of *Paramecium caudatum* with the nickel ion, (2) to compare the prolonged effect of the strontium ion antagonism for nickel ions upon the vitality of *P. caudatum* for control groups using the fission rate as an index of vitality.

The experimental data showed a biological antagonism of the strontium ions for the nickel ions in anesthetization of *P. caudatum*. Further, the vitality study showed that the presence of strontium ions reduced the inhibitory effect of nickel ions for fission of *P. caudatum*.

The effects of salts of metals upon protozoans have been an object of investigation for many years. Studies made recently in the area of ion antagonism have been concerned with the disparity between the observed experimental results and the theoretically predicted results. Experimental evidence to show ion antagonism between the strontium and nickel ions has not been presented.

### REVIEW OF THE LITERATURE

The action of various salts and salt antagonisms upon the ciliary action of *Paramecium caudatum* (Ehrenberg) has been

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studied by many; the period from 1900 to 1920 was one of great activity. Wichterman (1953) gives a complete review of the research literature prior to 1952.

Von Gelei, as cited by de Puytorac, Andrivon, and Serre (1963), was the first to state that the nickel ion paralyzed the ciliary apparatus of paramecia, without immediately killing the animal. He used varying concentrations of nickel chloride, nickel sulfate, and nickel nitrate; observing the effect upon the beating of the cilia and the rhythm of the contractile vacuole. More recently, Bovee (1958) described the use of nickel sulfate as the ideal anesthetic agent for protozoa.

Grebecki and Kuznicki (1955) stated that the cation toxicity of chlorides is of the order: Hg > Cu > Ni > Cd > Pb > Zn > Co > Mn > Ba > NH<sub>4</sub> > Sr > K > Ca > Mg > Na > Li and that the toxic action of anions is due chiefly to pH changes.

Dryl (1961) induced ciliary reversal in *P. caudatum* by simultaneous action of barium and calcium ions.

Jahn (1962) analyzed ciliary reversal in terms of the Gibbs-Donnan ratio. He proposed that a given bond angle produces a specific ionic effect. This introduced a new concept of stereochemistry concerning the biological effects of ions.

Several investigators have studied the effect of nickel sulfate upon the rate of reproduction of *P. caudatum*. A recent study is that of Puytorac et al. who found that the fission rate was reduced for approximately 20 generations after the paramecia were immobilized by three grams per liter of nickel sulfate.

#### MATERIALS AND METHODS

The paramecia used in the experiment were collected from a small pond within the city limits of Charles City, Iowa. With the aid of several texts, Jahn (1949), Kudo (1957), and Wichterman (1953), the organism was identified as *Paramecium caudatum* (Ehrb.).

Paramecia were cultivated upon a timothy hay tea and *Aerobacter aerogenes* media. (The *A. aerogenes* were furnished by Dr. Rodney A. Rogers, Drake University.) From the dense population thus established, two paramecia were isolated by use of a micropipet. Each paramecium was washed within depression slides in seven successive baths of sterile distilled water. Clones 1 and 2 were established, then cultured in depression slides supplied with nutrient media, and sealed to prevent contamination. Repeated observations at 24-hour intervals, for viability and contamination, confirmed the species purity. Clones 1 and 2 were the source of all subsequent subcultures. Large populations of the two clones were raised by transferring them

to glass containers containing one liter of the hay infusion medium. The cultures were maintained at temperatures of 25-30° C in rooms not receiving direct sunlight. Paramecia used in this study were from cultures 1-3 weeks old.

Distilled water (doubly distilled in pyrex glass), hydrated nickel chloride,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ , and the hydrated strontium chloride,  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ , (reagent grade, A. C. S.) were used to prepare stock solutions of  $8.4 \times 10^{-4}$  molality. All weight measurements were made with a chain analytical balance. Further dilutions were made by volumetric measurements from the original stock solution. The concentration of nickel and strontium chloride in the solution used in the study was  $8.4 \times 10^{-4}$  molal unless otherwise indicated.

The experimental technique for observation of anesthesia was as follows: Paramecia were transferred by pipet to a depression slide. The individual paramecium was then washed in three successive baths of distilled water, and placed within the depression of a ground glass slide with a micropipet. The pipets were calibrated, as nearly as possible, to give 30 drops to the ml. To the drop containing the paramecium was added a drop of solution containing the ions in the concentration to be tested. For low-power observation and for handling the organisms, a magnification of 10x -20x was used. For detailed observation of ciliary movement, functioning of the contractile vacuole, and bleb formation, high-dry and oil-immersion lenses were used.

Anesthesia was considered to have occurred when all forward locomotion of the organism had ceased, and the cilia were beating in an unsynchronized manner. Measurements of the time required for anesthesia were made with a stop watch, or a watch with a "sweep" second hand. The mean time for anesthesia and standard deviation were calculated for each alone and the various ion solutions.

To study the prolonged effects upon the paramecia, observations were made of the life-cycle, using isolation cultures and the fission rate as an index of vitality as suggested by Calkins (1941). The method of recording the fission rate was modified after that of Eckert and Feiler as cited by Wichterman (1953). Vitality studies were made on the fission rate for a twenty-four hour period, and for a five-day period.

The procedure consisted of isolating a paramecium and washing it three times in distilled water. The paramecium was then placed within one-tenth ml of distilled water within a glass spot plate depression. The test solution was then introduced in a one-tenth ml aliquot. After two minutes, the paramecium was removed, again washed three times in distilled water, examined

for injury, and then placed within the depression in a glass spot plate. One-tenth of hay infusion medium was added to the fluid transferred with the paramecium. When the nine depressions in the spot plate were filled, the plate was covered to prevent evaporation. The paramecia were then observed 24 hours later, when a count was made to determine the fission rate.

The prolonged study consisted of duplicating the above procedure, but at the end of each 24-hour period, one individual from each depression plate was isolated and placed within one-tenth ml of nutrient medium. The cycle was repeated every 24 hours for five-day periods. The mean rate of fission and standard deviation were calculated for each clone. Comparison of the mean rate of fission of the control organisms and of those treated with the test solutions was made to find the effect of the test solution upon the organism's vitality.

The glassware used in the experiment was initially autoclaved for ten minutes at 15 pounds pressure. Thereafter, it was rinsed three times in hot water and dried with paper toweling. The pH of the medium ranged from 6.7-7.2 for all experiments.

The average dimensions of the animals of Clone 1 were: length, 252.5 microns; width, 58.4 microns. For Clone 2: length, 217.8 microns; width, 49.5 microns. This was for 50 individuals.

#### RESULTS AND INTERPRETATION OF DATA

The paramecia of Clones 1 and 2 were experimentally tested for the time necessary for anesthetization with  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  and for  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  antagonized with an equimolar concentration of  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ . For anesthetization, those paramecia anesthetized with  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  were considered the control. Introduction of a drop of stock  $8.4 \times 10^{-4}$  molal solution into a drop of distilled water containing a paramecium lowered the actual concentration of nickel ions to  $4.2 \times 10^{-4}$  molal. The typical reaction sequence of paramecia introduced into a  $4.2 \times 10^{-4}$  molal solution of nickel ions was as follows: avoidance, loss of synchronization of ciliary beat, ineffective stroke and recovery of the cilia, reduction in rate of locomotion, swelling (probably due to reduced functioning of the contractile vacuole), anesthesia, discharge of trichocyst, bleb formation or blistering, and death.

The mean time for anesthesia was but slightly different for Clones 1 and 2. (See Table 1.) The concentration of nickel ions was highly toxic to the paramecia. Death usually resulted within a few minutes.

Paramecia introduced into a  $4.2 \times 10^{-4}$  molal solution of  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$  reacted in a manner typical for strontium ions. Described as "recoil" by Eisenberg-Hamburg (1930), this reaction was manifested by the test organism swimming backwards for

Table 1. The time required for anesthesia of *Paramecium caudatum* with  $4.2 \times 10^{-4}$  molal solutions of nickel and strontium chloride.

Solution	Calculated mean time of anesthesia	Observed mean time of anesthesia (in seconds)	Standard deviation (in seconds)
NiCl <sub>2</sub> .6H <sub>2</sub> O		Clone 1 39.7	16.4
NiCl <sub>2</sub> .6H <sub>2</sub> O		Clone 2 40.5	29.1
NiCl <sub>2</sub> .6H <sub>2</sub> O + SrCl <sub>2</sub> .6H <sub>2</sub> O	50.5 seconds	Clone 1 84.2	33.7
NiCl <sub>2</sub> .6H <sub>2</sub> O + SrCl <sub>2</sub> .6H <sub>2</sub> O	51.3 seconds	Clone 2 74.8	23.5
For fifty individuals			

a few seconds, probably indicating ciliary reversal. The concentration was not toxic to the organism, nor did it have any discernible anesthetic effect.

*Paramecia* in a drop of distilled water, to which a drop of  $8.4 \times 10^{-4}$  molal NiCl<sub>2</sub>.6H<sub>2</sub>O and SrCl<sub>2</sub>.6H<sub>2</sub>O was added, exhibited a response unique for that solution. The sequence was as follows: avoidance, a to and fro movement (apparently a backward sweep with the cilia moving the organism forward, followed by a forward sweep of the cilia moving the organism backward. The net effect was a jerky motion of the organism with little forward movement.), slight swelling (probably due to reduction in the rate of the contractile vacuole activity), and anesthesia. At that concentration of nickel and strontium ions, blebs were rarely formed. The concentration was not lethal for most of the organisms during the observation period.

To calculate the theoretical time for anesthesia with  $4.2 \times 10^{-4}$  molal solution of nickel ions antagonized by equimolar strontium ions, use was made of the Debye-Huckel expression, as cited by Daniels and Alberty (1955).

$$-\ln \gamma_i = \frac{e^3 z_i^2}{(DKT)^{3/2}} \sqrt{\frac{2\pi N \mu}{1000}}$$

where  $\gamma_i$  = activity coefficient of ion species  $i$ .

$i$  = charge on ions species  $i$ .

$e$  = charge of an electron =  $4.803 \times 10^{-10}$  electrostatic units.

$D$  = dielectric constant of the solution = 78.56 for water at 298 K.

$N$  = Avogadro's number =  $6.023 \times 10^{23}$ .

$k$  = gas constant per molecule =  $R/N$  =  $1.3805 \times 10^{-16}$  erg degree<sup>-1</sup> molecule<sup>-1</sup>.

$\mu$  = ionic strength =  $\frac{1}{2}(c_1z_1^2 + c_2z_2^2 + c_3z_3^2 + \dots)$ , the summation being taken over all ions in the solution, where  $c_i$  is the concentration of ion species  $i$  in moles per liter.

$T$  = absolute temperature.

Introducing the mean activity coefficient  $i$ , and putting in numerical values for water at 25°C for an electrolyte containing three kinds of ions, the equation becomes  $-\log \gamma = 0.509 \sum z_i^2 c_i$ .

Substituting in this equation the molality of  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O} = 4.2 \times 10^{-4}$ ,  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O} = 4.2 \times 10^{-4}$ , charge of nickel ion = 2, charge of strontium ion = 2, charge of chloride ion = 1 the equation becomes:

$$-\log \gamma = (0.509) (2) (2) (1).$$

$$\sqrt{\frac{1}{2} [(4.2 \times 10^{-4} \times 4) + (4.2 \times 10^{-4} \times 4) + (8.4 \times 10^{-4} \times 2)]}$$

$$-\log \gamma = 0.1022012$$

$$\gamma = 0.79$$

Thus, 79% of the nickel ions are active in the solution. Using this figure, one may calculate the theoretical time for anesthesia, e. g., letting 40.5 seconds represent the time necessary for anesthesia in a solution containing nickel ions at 100% activity, a solution containing 79% of the nickel ions active should theoretically require 51.4 seconds to anesthetize the organism. (see Table 1.)

Isolation cultures of Clones 1 and 2 were made. The fission rate of the control organism, maintained on hay-infusion medium, was used as an index of vitality. The mean rate of fission and the mean rate of reproduction of the control organism were used as the standard in comparison with the test organisms. (See Tables 2, 3.) Clones 1 and 2, treated with a two-minute immersion in  $4.2 \times 10^{-4}$  molal solution of the test ions, then washed and transferred to hay-infusion medium, gave the results as presented in Table 4.

Data obtained from anesthetization of *P. caudatum* with nickel ions antagonized by equimolal strontium ions, showed a biologically inhibitory effect exhibited by the strontium ions for nickel ions.

Although extreme care was taken to be accurate in all measurements, it must be noted that even the slightest variation in the drop size would greatly alter the concentration of ions in solution. Variation in drop size could result from chipping of the aperture of the glass pipette, shaking of the operator's hand, temperature differentials, differences in solution concentration, differences in vapor and barometric pressure of the air, angle at

Table 2. The 24-hour fission rate of *Paramecium caudatum* treated for two minutes with nickel and strontium chloride solutions.

Concentration of solution	Time immersed in solution	Clone	Mean X	S. D.
Control		Clone 1	1.86	1.09
		Clone 2	1.69	1.27
4.2 x 10 <sup>-4</sup> molal NiCl <sub>2</sub> .6H <sub>2</sub> O	2 min.	Clone 1	0.79	0.39
		Clone 2	0.72	0.46
4.2 x 10 <sup>-4</sup> molal NiCl <sub>2</sub> .6H <sub>2</sub> O + SrCl <sub>2</sub> .6H <sub>2</sub> O	2 min.	Clone 1	1.35	0.74
		Clone 2	1.20	0.78

For fifty individuals

Table 3. The 24 hour population growth of *Paramecium caudatum* treated with nickel and strontium chloride solutions.

Concentration of solution	Time immersed in solution		Range	Mean X	S. D.
Control		Clone 1	1-10	3.55	2.18
		Clone 2	1-9	3.58	2.53
4.2 x 10 <sup>-4</sup> molal NiCl <sub>2</sub> .6H <sub>2</sub> O	2 min.	Clone 1	1-4	1.40	0.67
		Clone 2	1-6	1.44	0.91
4.2 x 10 <sup>-4</sup> molal NiCl <sub>2</sub> .6H <sub>2</sub> O + SrCl <sub>2</sub> .6H <sub>2</sub> O	2 min.	Clone 1	1-8	2.62	1.69
		Clone 2	1-8	2.41	1.55

For fifty individuals

Table 4. Reproduction and fission rate of *Paramecium caudatum*, treated for two minutes in 4.2 x 10<sup>-4</sup> molal solutions of nickel and nickel anatagonized by strontium ions, as compared with the control organism.

Reproduction rate			
	Control	Ni <sup>++</sup>	Ni <sup>++</sup> /Sr <sup>++</sup>
Clone 1	100%	39%	74%
Clone 2	100%	40%	67%
Fission rate			
	Control	Ni <sup>++</sup>	Ni <sup>++</sup> /Sr <sup>++</sup>
Clone 1	100%	42%	73%
Clone 2	100%	43%	71%

For fifty individuals

which the pipet was inclined, etc., making the volumetric measurements of drops the least accurate measurement of this experiment.

The physical differences of the test organisms vary to some degree. Clone 2 showed a rather large S. D. for anesthesia with nickel ions. (See Table 1.) Two individuals from Clone 2 digressed markedly from the mean. One was in the 198-202 sec

onds range, and one was in the 103-107 seconds range. In Clone 1, for anesthesia with nickel ions antagonized by strontium ions, four individuals were within the 208-292 seconds range.

These digressions may be due to experimental error or to individual variation. A limited number of tests were run on paramecia immediately after they had undergone fission. To the drop of medium containing the organisms, a drop of the test ion solution was added. Often there would be a variation in the time for anesthesia to occur, the most extreme variation being nine minutes for a pair of organisms within a  $4.2 \times 10^{-4}$  molal nickel and strontium solution.

With those variations in mind, it is interesting to note that the anesthetic effects of the ion solutions upon the clones were very similar. With the larger volumes of medium and test solutions utilized in the vitality studies, a more quantitative measurement was obtained. The results of the vitality studies show a more normal standard deviation.

According to de Puytorac et al., the metallic salt toxicity is a relation between the cation, lipids and proteins, with the nickel ion being one of the more toxic cations. With weak doses of the nickel ion, the lesions are reversible. This takes about 20 generations to complete. In the vitality series of this experiment, the paramecia were not observed for a long enough period to confirm their observation. Paramecia treated with nickel ions antagonized by strontium ions had recovered the normal rate of fission at the end of five days. However it should be pointed out that the fission rate of the two clones employed in the experiment was slightly lower than that of the paramecia observed by other investors as cited by Calkins and Summers (1941).

Oliphant (1942) demonstrated that ciliary reversal occurs immediately with all monovalent alkaline earth ions, with  $Ba^{++}$  and  $Mn^{++}$ , but not with other non-toxic divalent cations ( $Sr^{++}$  and  $Mg^{++}$ ). Eisenberg-Hamburg (1930) stated that in solutions non-fatal to *P. caudatum*, there is a reaction of recoil specific for  $SrCl_2$ .

It was observed that the normal movements of the paramecia were interrupted for 10-20 seconds, immediately after contact with strontium ions. In this study, it was further noted that *P. caudatum* usually exhibited an avoidance reaction which often consisted of swimming backwards, indicating ciliary reversal.

Jahn (1962) raised the question of the role of the divalent ion within the cell membrane, and gave two possible explanations, one morphological and one physiological: (1) to maintain the mechanical structure of the cell membrane, and (2) to provide an electron conductor for reactions in the cell membrane. One

might conjecture that the more active strontium ion replaces the calcium ion within the cell membrane, but is not as readily replaced by the nickel ion, thereby decreasing the toxic effect of the nickel ion. Confirmation of this hypothesis must await future studies.

#### Literature Cited

- Bovee, E. C. 1958. Nickel Sulfate as an Anesthetic for Protozoans. | *Turtax News*, 36:78.
- Calkins, G. N. and F. N. Summers (ed.). 1941. *Protozoa in Biological Research*. Columbia University Press, New York.
- Daniels, F. and R. A. Alberty. 1955. *Physical Chemistry*. John Wiley and Sons, New York.
- Dryl, S. 1961. The Ciliary Reversal in *Paramecium caudatum* Induced by Simultaneous Action of Barium and Calcium Ions. *J. Protozool.*, 8:16.
- Eisenberg-Hamburg, E. 1930. L'influence des Sels de Strontium sur les Mouvements du *Paramecium caudatum*. . . *Acta Biol. Exptl.*, 4: 261-277.
- Grebecki, A. and L. Kuznicki. 1955. The Relation of *Paramecium caudatum* to the Chemical Properties of its Medium and the Protective Effect of Aggregation against Inorganic Substances. *Trans. A. Pigon., Fol. Biol.*, 3:127-157.
- Jahn, T. L. 1962. The Mechanism of Ciliary Movement. . . *J. Cell. Comp. Physiol.*, 60: 217-228.
- and F. F. Jahn. 1949. *How to Know the Protozoa*. Wm. C. Brown Co., Dubuque, Iowa.
- Kudo, R. R. 1947. *Protozoology*. Third ed. C. C. Thomas, Springfield, Illinois.
- Oliphant, J. F. 1942. Reversal of Ciliary Action in *Paramecium* Induced by Chemicals. *Physiol. Zool.*, 15:443-452.
- Puytorac, P. de, C. Andrivon et F. Serre. 1963. Sur l'Action Cytonarcotique des Sels de Nickel chez *Paramecium caudatum* Ehrb. *J. Protozool.*, 10:10-19.
- Wichterman, R. 1953. *The Biology of Paramecium*. Blakiston, New York.