THE EFFECT OF TWO TRANQUILIZERS AND A RELATED COMPOUND ON THE RATE OF FISSION AND THE SURVIVAL OF PARAMECIUM CAUDATUM

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THE EFFECT OF TWO TRANQUILIZERS AND A RELATED COMPOUND ON THE RATE OF FISSION AND THE SURVIVAL OF PARAMECIUM CAUDATUM

by

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CHAPTER I

INTRODUCTION

Tranquilizers have been widely used in India for at least five centuries for a variety of ailments including snakebite, dysentery, cholera, fevers, insomnia and insanity.\(^1\) In contrast to this, tranquilizers of known pharmacological structure, have been used in the United States since 1954. During this last decade however, the use and understanding of tranquilizers has increased rapidly until at present this class of drugs occupy a prominent position in the fields of medicine, pharmacology and biology.

It appears that most investigations with these compounds have been conducted on higher animals, in which these agents are capable of exerting a unique type of selective central nervous system depression, differing from most sedatives which act by producing a general central nervous system depression. Little is known of their action on microorganisms.

It was the purpose of this investigation to study the effect of two water soluble, organic compounds known as

tranquilizers (Thorazine®, Atarax®), and a closely related compound (Phenergan®) on the rate of fission and the survival of the protozoan Paramecium caudatum.
CHAPTER II

HISTORY

The effect of tranquilizers and related compounds on protozoans has received little attention, although the effect of such compounds on vertebrates, especially mammals, is under active investigation.

Paramecia have long been one of the favored protozoans for research work. They are visible under a low powered microscope, have a rapid rate of reproduction, thrive in a great variety of media, are easy to culture and possess to some extent greater complexity than many other unicellular organisms, which undoubtedly partially accounts for their popularity.

DePuytorac, Andrivon and Serre,1 studying the cytonarcotic action of nickel salts (nickel chloride, sulfate and nitrate) on Paramecium caudatum found that the time necessary to render ciliates motionless is proportional to the nickel-ion concentration of the solution, but that in any case there is a minimum time for narcosis; that mating types have very different sensitivity to nickel salts; and that after narcosis

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about twenty generations must pass before the rate of multiplication is again normal.

Peters, in 1904, made an extensive study of the effect of calcium chloride, potassium chloride and chloroform on the division rate of several protozoans including P. caudatum, and found that the rate of fission can be controlled to a great extent by varying the concentration and the time of exposure of the organism to the compounds used.

P. caudatum showed typical "avoiding reaction" toward pH changes, and to many other chemotactic stimuli, such as sodium chloride, calcium chloride and magnesium chloride according to Dryl. He noted a peculiar motor response which lasted four to five seconds and sometimes longer and consisted mainly of rotations around the transverse axis of the body, instead of the "avoiding reaction," when organisms were subjected to a 40 millimolar potassium chloride solution.

Observing paramecia with an electron microscope, Pitelka and Parducz, at the University of California, noted that

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1A. W. Peters, "Division in Protozoa," Journal of the American Academy of Arts and Sciences, XXXIX (April, 1904), 461.


nickel sulfate paralyzes the somatic cilia and the contractile vacuoles. Immersion of the organisms for two to five minutes in 0.005 - 0.01 per cent nickel sulfate, followed by washing and suspension in tap water or culture medium, leaves some individuals unaffected, others suffer temporary paralysis only, and some suffer paralysis accompanied by loss of cilia over the anterior one-fourth to one-third of the body with subsequent full recovery, or more extensive deciliation, bleb formation and death.

Yusa,¹ also using an electron microscope, observed that P. caudatum, after being induced to discharge their trichocysts by electric shock, are able to regenerate fifty per cent of their normal complement of trichocysts within four to six hours.

Discussing the recovery of paramecia after immobilization by X-irradiation, Wichterman² states that many immobilized specimens may remain seemingly dead through a twenty-four hour period only to recover later. Immobilized paramecia settle to the bottom of the culture medium the same as the dead ones. They do not ingest food and show active cyclosis.


and ciliary movements are not apparent. Recovery begins by ciliary activity, and then there occurs a gradual and slow gliding of the organism on the bottom of the dish without the characteristic spiral swimming which occurs normally and later after major recovery.

A motility inhibitor should be reversible and non-lethal, even when in contact with the organism for prolonged periods of time. Eys and Warnock,¹ using ten quaternary ammonium compounds as motility inhibitors on P. caudatum concluded that the time between application and effect varies with the concentration of the solution. Using a low power microscope they observed that motility in one given organism is not an all or none phenomenon, but that one can approximate between normal movement and immobility in degrees of 75, 50 and 25 per cent of normal. The effect of these methonium drugs gave an extremely sharp end point. After six days of no motility, dilution will result in active growth for P. caudatum.

Anesthetics are known to decrease metabolism in the higher animals. Numerous workers have investigated the effects of various anesthetics on P. caudatum. Leichsenring²


studied the oxygen consumption (a standard measure of metabolism rate) of *P. caudatum* in varying concentrations of several anesthetics including ether, chloroform, nitrous oxide and ethylene. Ether and chloroform decreased the oxygen consumption, while nitrous oxide and ethylene had little or no effect on the amount of oxygen used. At the end of one hour of chloroform anesthesia, and two hours of ether anesthesia, the paramecia were returned to fresh medium in which, after a twenty-four hour time lapse, most of the organisms from chloroform were dead. The organisms from ether, after a twenty-four hour period, had returned to almost normal oxygen consumption. When the organisms were removed from chloroform anesthesia, after thirty minutes, they also returned to almost normal oxygen consumption in twenty-four hours.

Early work in 1869 by Richardson, as cited by Bills, generalized that the toxicity of the alcohols increases roughly with the increase in molecular weight and that the narcotic effect increases more rapidly than the toxicity. This has been confirmed by numerous later investigators. Bills found that paramecia, in a solution without food, live longer with alcohol than without it, and that starving cultures can even be restored to prosperity by the addition of suitable amounts

of any alcohol. Exposure of paramecia to weak ethyl alcohol increases their susceptibility to a stronger dose of ethyl alcohol, and to five other alcohols.

Depending on the concentration, alcohols have varied effects on the biological processes of protozoa. Non-toxic concentrations of ethyl alcohol accelerated the rate of division of protists. In media devoid of nutrition, survival of P. caudatum was prolonged by adding ethyl alcohol, thus substantiating previous work. Higher concentrations of alcohol and of other narcotics gave rise to conspicuous toxic symptoms, such as slowing of movement, reduced rate of formation of food vacuoles, and slower pulsation of contractile vacuoles. Dryl, of Poland, left paramecia in the several concentrations of alcohol for twenty-four hours, counting the dead and the living at the end of the period. His concentrations did not cause complete disintegration of the cells. He concluded that the toxic and chemotactic effects of alcohols increased with the molecular weight of the compound.

Goldschmied and Herman, cited by Dryl, noted that P. caudatum in 1, 2 or 3 per cent solutions of ethyl alcohol

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2. Bills, op. cit.

3. Dryl, op. cit.

4. Ibid.
gather, after a few minutes, on the periphery of the drop and they hypothesized that this action was due to the evaporation of ethonol on the periphery and consequent relative diminution of concentration as compared to the central area of the drop. This hypothesis was later confirmed by Dryl.

Many ideas have been advanced and later discarded on exactly how narcotics produce their effect. Regardless of how the effect is derived, it is generally agreed that narcotics are general central nervous system depressants. The word, narcotic, is derived from the Greek work, vaptkau, meaning "I am stupefied." Paramecia have frequently been the subject of experimentation to determine what effect narcotics have on a single cell.

It has been determined that higher concentrations of a narcotic are required to depress unicellular organisms than are necessary to depress multicellular organisms.¹

Using the narcotics, ethyl carbamate and chloral hydrate, Burt² in 1945 was able to inhibit the division rate of Colpoda steinii down to zero.

Conducting an investigation to determine the effect


of kinetin (6-furfuryl Aminopurine) and indole-3-acetic acid on the multiplication rate of *P. caudatum*, McManus and Sullivan\(^1\) and other workers cited in their report, obtained indefinite results. All found a day to day variation of increase and decrease in the division rate of paramecia and were uncertain as to the cause.

Wang\(^2\) studied the responses of paramecia to the toxins of cigarettes. The following cigarette components were bubbled or soaked in twenty ml. of water: cigarette smoke, unburned tobacco, the ash of the entire cigarette, and the ash of the cigarette paper only. Each resulting solution in higher concentrations proved to be lethal and was accompanied by bizarre types of deformation and irregular protrusions.

The word, tranquilizer is derived from the Latin word, *tranquillas*, meaning calm, quiet, or still. Some compounds referred to as tranquilizers are also ataractic drugs. The word, ataractic, comes from the Greek word, *Ataraxic*, meaning cool, impassive, not mentally disturbed, without confusion, delusions or hallucinations.

Chlorpromazine (Thorazine\(^\circ\), Largactil\(^\circ\), Megaphene\(^\circ\))

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was the first of the ataractic phenothiazine drugs to be synthesized (in 1950). The synthesis was accomplished by the Rhone-Poulenc Specia Laboratories of France.\(^1\)

Chlorpromazine reduces spontaneous motor activity and "drive" and causes quietness, detachment and indifference to the external environment, sleepiness and drowsiness without inducing hypnosis or anesthesia. Animals normally fierce and difficult to handle become peaceful and easy to handle when treated with chlorpromazine. It has been shown to increase the hypnotic effect of barbiturates and prolongs the duration of nitrous oxide and ether anesthesia in experimental animals. Chlorpromazine may be used to lower body temperature and decrease oxygen uptake. It is widely used in psychiatric medicine in the treatment of hyperactive, disturbed mental patients.\(^2\)

Chlorpromazine is used to depress vomiting associated with numerous causes such as pregnancy, radiation sickness, narcotics, cancer, leukemia, etc. It has proved to be a value in the treatment of acute alcoholism, delirium tremens, hiccups, migrane headaches and intractable pain in cancer.\(^3\)

\(^1\)Donald Blair, Modern Drugs for the Treatment of Mental Illness (Springfield, Illinois: Charles C. Thomas, 1962), p. 35.


\(^3\)Blair, op. cit., p. 38.
The sexual activity of male rats was reduced by Chlorpromazine. Male rats receiving Chlorpromazine required more time to mount and to copulate. They accomplished few mounts and fewer copulations during a given time period.¹

The offspring of albino rats, injected with Reserpine, Chlorpromazine, or Meprobamate during the gestation period, are significantly slower than controls to reach the criterion of learning.²

In all cases, rats and mice injected with Chlorpromazine one hour prior to a lethal injection of several bacterial toxins, increased the survival time over those receiving none.³

Injecting rhesus monkeys with Chlorpromazine produced an increase in the time required to react to a stimulus due to "deconditioning properties" and its general depressant effects.⁴

Promethazine (Phenergan) is a phenothiazine derivative


and until recently was referred to and used as a tranquilizer. It is a antihistaminic compound, has a hyponotic action, is an anticonvulsant and is used for preanesthetic, as well as in antihistamine therapy, where some degree of sedation is not desirable. The important clinical uses are preoperative medication and antihistamine therapy. It is no longer used as a tranquilizer, but by using Promethazine in combination with Chlorpromazine the dose of the latter can be reduced, and satisfactory clinical results can be obtained without getting some of the undesirable side effects of Chlorpromazine. The main disadvantage of Promethazine is that it produces drowsi-ness.1

Artificial hibernation may be induced by using a combination of Promethazine and Chlorpromazine together with methods for cooling the body.2

Promethazine gives protection against sea-sickness and air sickness. It also is used in the treatment of vomiting due to drugs, vomiting of pregnancy, etc. The side effects of a dry mouth and blurring of vision may accompany Promethazine therapy.3

Hydroxyzine (Atarax, Vistaril), a benzhydral derivative

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2 Lewis, op. cit., p. 313.
3 Ibid., p. 453.
and a minor tranquilizer when compared to the phenothiazine derivatives, is a potent antihistamine and has sedative and tranquilizing properties with no accompanying hypnotic effect. It has vasodilator properties, is used to cause skeletal muscle relaxation, as a local anesthetic, to treat nervous irritability, emotional stress, agitation, anxiety, tension and certain skin diseases.\textsuperscript{1}

Tranquilization of dairy bulls, from which semen is collected artificially, resulted in easier handling and more live spermatozoa per ejaculate.\textsuperscript{2}

The uses of tranquilizers are many. The immediate external effects can readily be observed, and an understanding of the mechanism of their physiological activity is rapidly developing.

\textsuperscript{1}Ibid., p. 319.

CHAPTER III

MATERIALS AND METHODS

In order to determine the effect of two different tranquilizers and a related compound on the rate of fission and the survival of *P. caudatum*, an aquatic protozoan, three compounds which are water soluble were selected. An effort also was made to select individual compounds which are widely used and which would represent the major tranquilizer groups.¹

The Rauwolfia alkaloids make up the first major group. This group consists of four closely related derivatives, of the Asian shrub *Rauwolfia surpentina* and other rauwolfia species, which possess similar properties including that of being sparingly soluble in water: thus no representative of this group could be used in this investigation.

The Phenothiazine-derivatives, the second major group, are divided into two subgroups. The Amino-propyl side chain subgroup consists of seventeen synthetic derivatives including Chloropromazine Hydroxide (Thorazine®, Largactil®, Megaphen®), or 2-chloro-10-(3-dimethylaminopropyl) phenothiazine hydrochloride, which is very soluble in water and was one of the

¹Wilson and Grisvold, *loc. cit.*
tranquilizers used in this investigation.

The Amino-ethyl side chain is another subgroup of synthetic phenothiazine derivatives consisting of five compounds which are very soluble in water. Promethazine hydrochloride or Phenergan hydrochloride (Phenergan®), was selected to represent this subgroup of closely related compounds.

The Diphenylmethane derivatives make up the third major group. Consisting of seven synthetic derivatives, this group includes Hydroxyzine hydrochloride (Atarax®), vistaril®, which is very soluble in water and was the second tranquilizer chosen.

The three compounds used will subsequently be referred to as Thorazine®, Phenergan®, and Atarax®.

A culture of P. caudatum was obtained from The Southern Biological Supply Company, McKenzie, Tennessee. Several clones were established to alleviate, insofar as possible, the problem of individual variation of paramecia. To establish a clone, a single paramecium was isolated with a micropipette and washed through nine spot plate depressions of distilled water. A micropipette was made by heating a piece of glass tubing, five mm. in diameter, then drawing until the inside diameter of the tip approximated two hundred microns. The pyrex depression plates used had nine depressions, each depression having a capacity of one ml. and a diameter of twenty-two mm. Distilled water from the Glenwood-Inglewood
Company, Minneapolis, Minnesota, was used. When removed from the ninth depression, the paramecium was placed in a depression containing one ml. of culture medium to facilitate fission. The young clone was transferred to a test tube of culture medium when approximately twenty individuals were present, and later to a larger container.

All clones and cultures of paramecia were cultured in dark, moist chambers at a constant temperature of twenty-seven degrees Centigrade as recommended by Beale, Sonneborn, and others.

Paramecia from one clone (clone "C") were used throughout the investigation. Limited studies were made to determine the variation of tranquilizer and related compound effects on individuals of different clones.

A hay infusion medium was prepared, in a manner similar to the method used by Wichterman, by boiling for twenty minutes six grams of dry timothy hay in one liter of spring water. This hay broth was autoclaved for fifteen minutes at fifteen pounds of pressure, cooled to room temperature,

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inoculated with one cc. of broth containing Aerobacter aerogenes and allowed to ripen twenty-four hours before using. Fresh medium was prepared for each series of tests. The bacterial culture was kindly furnished by the biology department at Drake University, Des Moines, Iowa. Wichterman\(^1\) and others used Aerobacter aerogenes as a food source for paramecia, although other kinds of bacteria have been used.\(^2\) Glaser and Coria\(^3\) found that \(P. \text{caudatum}\) would not grow under sterile conditions without the presence of some other organism to serve as a food source.

Preliminary experimentation was conducted with numerous concentrations of the compounds to determine a feasible range of concentrations of these chemicals to use in this investigation.

Using hay infusion medium as the diluting solution, the three compounds were diluted from an original concentration of 25 mg./cc. to the following concentrations: 0.1 mg./cc., 0.01 mg./cc., 0.001 mg./cc., 0.0001 mg./cc., 0.00001 mg./cc. and 0.000001 mg./cc. Dilution was accomplished by means of


\(^2\)Ralph Wichterman, \textit{The Biology of Paramecium}, p. 100.

a two cc. hypodermic syringe and one ml. pipettes. Ten milliliters of each concentration was prepared.

To avoid any possible deterioration of the compounds due to age, the dilutions were made just prior to each series of tests, and were kept in tightly capped dropping bottles.

A nine depression spot plate was used for each concentration of each chemical. Four drops (0.2 ml.) of culture medium with the appropriate compound was placed in each depression and one paramecium was added, using a micropipette. These depression plates containing the paramecium were incubated as previously described. At the end of each twenty-four hour period, one paramecium was transferred from each depression to corresponding fresh medium in another plate regardless of whether any division had occurred. Observations were made frequently during the first twenty-four hour period and daily thereafter. The rate of fission, survival time, appearance of bleb formation, unusual actions and general motility were noted and recorded. The total progeny of individual paramecia in each depression was computed at the end of three days, assuming that all paramecium would have continued to divide at the same rate as the one which was transferred daily to fresh medium.
CHAPTER IV

RESULTS AND DISCUSSION

Preliminary investigation revealed that subjecting paramecia to Thorazine®, Phenergan®, or Atarax® in concentrations of 0.1 mg./cc. or more, resulted in instantaneous death. To determine the effect on the survival and the rate of fission of P. caudatum, these compounds in concentrations of 0.1, 0.01, 0.001, 0.0001, 0.00001, and 0.000001 mg./cc. were used. Preliminary studies with Thorazine® were repeated three times for the purpose of developing techniques for the study of the problem, and the investigation was repeated in its entirety three times. The results of the investigation are illustrated by citing data from the last series of studies. Percentages were computed from the results after using sixty-three individual paramecia for each of the three compounds.

After being first introduced to culture medium containing a compound, each paramecium was continually subjected to the same specific compound until the organism settled to the bottom of the depression or until the study was terminated at seventy-two hours.

Subjecting paramecia to a 0.1 mg./cc. concentration of Thorazine® resulted in an instantaneous, convulsive reaction consisting of rotations around the transverse axis of the body,
subsequently referred to as the "baton reaction." This reaction lasted only for a few seconds and was followed by settling to the bottom of the depression in ten to twenty-five seconds.

The 0.01 mg./cc. concentration of Thorazine® produced normal movements accompanied by the avoiding reaction even though no solid obstacle was present. After about five minutes an unusual movement occurred which consisted of normal gyration except that the posterior end of the paramecium moved in a yawing-fishtailing spiral. This movement can best be described as a large circular rotation of the posterior end of the paramecium while the anterior end remained fairly stable. This was accompanied by slow forward progression. This reaction subsequently will be referred to as the yaw-tailing reaction. A gradual decrease in speed was observed until motility ceased in approximately ten to fifteen minutes and the organism settled to the bottom of the depression.

A Thorazine® concentration of 0.001 mg./cc. proved to be lethal to paramecia between six and twenty-four hours of exposure. A gradual slowing of motility was observed accompanied by an occasional "baton reaction." Thorazine concentrations of 0.0001, 0.00001, and 0.000001 mg./cc. proved less toxic. Some paramecia survived for at least twenty-four hours in each of the three weakest concentrations. Table I is a summarized account of the effects of the various concentrations
### TABLE I

**THE EFFECT OF SEVERAL CONCENTRATIONS OF THORAZINE**

**ON THE SURVIVAL AND FISSION OF**

**PARAMECIUM CAUDATUM**

<table>
<thead>
<tr>
<th>mg. of Thorazine per cc. of medium</th>
<th>per cent survival after a time lapse of</th>
<th>per cent of Paramecium which have undergone fission after a time lapse of</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>0.01</td>
<td>100 100 55 0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>0.001</td>
<td>100 100 100 100 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>0.0001</td>
<td>100 100 100 100 11 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>0.00001</td>
<td>100 100 100 100 78 55 55 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>0.000001</td>
<td>100 100 100 100 100 100 100 11 11 11 11</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Control</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100</td>
<td>100 100 100 100 100 100 100 100 100 100 100 100</td>
</tr>
</tbody>
</table>
of Thorazine® on the survival and reproduction of P. caudatum as compared to the control.

The second compound used in this investigation was Phenergan®. A 0.1 mg./cc. concentration of Phenergan® resulted in an instantaneous "baton reaction" followed by settling to the bottom of the depression in approximately ten to fifteen seconds.

A Phenergan® concentration of 0.01 mg./cc. produced frantic activity accompanied by an avoiding reaction. This frantic activity was considerably slower after one minute, and during the second minute, an abrupt cessation of motility occurred and the organisms settled to the bottom of the container.

Phenergan® concentrations of 0.001 and 0.0001 mg./cc. produced a gradual slowing of motility and an occasional "yaw-tail" reaction and no apparent motility after three to twenty-four hours.

The two weakest concentrations of Phenergan®, 0.00001 and 0.000001 mg./cc., resulted in the survival of some paramaecia for the seventy-two hour period, but fission was completely inhibited. A summary of the effects of the various concentrations of Phenergan® on the survival and reproduction of P. caudatum, compared to a control, is presented in Table II.

Atara® was the third compound used in this investigation.
TABLE II
THE EFFECTS OF SEVERAL CONCENTRATIONS OF PHENERGAIN® ON THE SURVIVAL AND FISSION OF PARAMECIUM CAUDATUM

<table>
<thead>
<tr>
<th>mg. of Phenergan per cc. of medium</th>
<th>per cent survival after a time lapse of 1 min. 2 min. 10 min. 15 min. 24 hrs. 48 hrs. 72 hrs.</th>
<th>per cent of Paramecia which have undergone fission after a time lapse of 24 hrs. 48 hrs. 72 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0 0 0 0 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>0.01</td>
<td>100 0 0 0 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>0.001</td>
<td>100 100 100 100 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>0.0001</td>
<td>100 100 100 100 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>0.00001</td>
<td>100 100 100 100 55 55 44</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>0.000001</td>
<td>100 100 100 100 89 78 78</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Control</td>
<td>100 100 100 100 100 100 100</td>
<td>100 100 100 100</td>
</tr>
</tbody>
</table>
The 0.1 mg./cc. concentration produced normal movement of paramecia for ten to fifteen seconds, followed by the "yaw-tail" reaction and by subsequent loss of motility in less than one minute.

Paramecia were quite active for one or two minutes, when placed in a 0.01 mg./cc. concentration of Atarax®, but their movement subsequently slowed, they demonstrated the "baton reaction," and all ceased motility in less than ten minutes.

All paramecia survived for twenty-four hours, and forty-four per cent survived for seventy-two hours in a 0.001 mg./cc. concentration of Atarax®. Eighty-nine per cent or more of the paramecia survived in the three weakest concentrations of Atarax® for the entire seventy-two hour period.

Fifty-five per cent of the paramecia underwent fission during the seventy-two hours when subjected to a 0.000001 mg./cc. Atarax® concentration. A summary of the effects of the several concentrations of Atarax® on the survival and the reproduction of P. caudatum as compared to the control are shown in Table III.

The general effect of the compounds studied on the survival and reproduction of paramecia is similar. As the concentration of the chemical compound is increased, the survival time of paramecia subjected to it, is shortened, and fission is decreased or completely inhibited.
### TABLE III

**THE EFFECTS OF SEVERAL CONCENTRATIONS OF ATARAX ON THE SURVIVAL AND FISSION OF PARAMECIUM CAUDATUM**

<table>
<thead>
<tr>
<th>mg. of Atarax per cc. of medium</th>
<th>per cent survival after a time lapse of</th>
<th>per cent of Parmecia which have undergone fission after a time lapse of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min.</td>
<td>2 min.</td>
</tr>
<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.01</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.001</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.0001</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.00001</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.000001</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Phenergan was observed to be somewhat more toxic to paramecia at all concentrations than either of the other two compounds used. Phenergan completely inhibited fission of paramecia in all six concentrations used, while some fission occurred in the 0.000001 mg./cc. concentrations of Atarax and Thorazine. The reactions of paramecia, when first subjected to the compound Phenergan, were more frantic and the cessation of motility was more abrupt.

Atarax was observed to be the least toxic of the three compounds used except that the 0.01 mg./cc. and the 0.000001 concentrations seemed to be slightly more toxic than the corresponding levels of Thorazine. The 0.0001 mg./cc. concentration of Thorazine and Phenergan proved to be one hundred percent lethal in the first twenty-four hours while all paramecia survived in the same concentration of Atarax. All other concentrations of Atarax resulted in greater survival time. Paramecia also exhibited a higher fission rate in the 0.000001 mg./cc. concentration than occurred in the same concentration of Thorazine.

The phenomenon of bleb formation was observed in each concentration of each compound which proved lethal in twenty-four hours or less. In some cases the blebs were larger than the paramecium. In other instances numerous small blebs appeared to cover the entire exterior surface of the paramecium. The time lapse necessary for the formation of blebs,
after paramecia were subjected to the stronger concentrations of compounds varied from a fractional part of a second to almost twenty-four hours. In all observed cases of permanent cessation of motility, blebs were present. In numerous instances, paramecia without blebs were observed resting on the bottom of the depression, but these individuals resumed activity after being disturbed by a gentle stream of medium from the tip of a micropipette. Limited attempts were made to revive paramecia which had formed blebs and which exhibited no motility. This was attempted by transferring them to distilled water for a few seconds and then to fresh medium containing no compound, but none revived. Some paramecia were removed in a similar manner, after they had formed blebs, but before motility ceased. No restoration to normal motility occurred and the blebs persisted and the organisms settled to the bottom of the depression. An understanding of the mechanism of the physiological activity of tranquilizers is progressing rapidly. It seems that lower non-toxic concentrations are selective in their effect on paramecia, allowing most physiological activities to function in a normal manner, but disrupting normal fission by complete inhibition in most cases. The inhibition of reproduction is not immediately cancelled by transferring paramecia to fresh media lacking the chemical compound. A limited study was made on paramecia which had survived from four to six days in the lower concentrations of the chemical
solutions, with no fission occurring. After being transferred to control medium, the first division occurred after four days. In some cases nine days lapsed before any division occurred.

The toxicity of Thorazine®, Phenergan® and Atarax® varied directly with the concentration of the solution. The same relationship was reported by DePuytorac, Andrivon and Serre, using nickel salts and by Bills² using alcohols.

Peters³ reported that the fission rate of paramecia could be controlled by varying the concentration of calcium chloride, potassium chloride and chloroform. The conclusions of this investigation are similar. It was shown by this investigation that the motility of paramecia varies inversely with the concentration of the chemical compound used, which is similar to that reported by Eyr and Warnock⁴ with ammonium compounds.

It may be concluded that, with respect to the effect of the compounds Thorazine®, Phenergan® and Atarax®, the survival of paramecium varies inversely with the concentration of the chemical; that Phenergan® is a more powerful compound

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¹DePuytorac, Andrivon and Serre, loc. cit.
²Bills, loc. cit.
³Peters, loc. cit.
⁴Eyr and Warnock, loc. cit.
than Thorazine and Atarax; that Atarax is the least toxic of the three compounds used; that fission of *P. caudatum* is reduced by weak concentrations of tranquilizers and related compounds and completely inhibited by stronger non-toxic concentrations.

Further investigation seems feasible to determine the variation of the effects of tranquilizers and related compounds on different clones of paramecia. It would be interesting to find how exceedingly dilute a solution might be and still influence the rate of fission. A profitable study might be made to determine if any concentration of tranquilizers or related compounds will cause immobilization without resulting in death.
CHAPTER V

SUMMARY

Tranquilizers have been used in India for at least five centuries for a variety of ailments. In contrast to this, tranquilizers of known pharmacological structure, have been used in the United States since 1954. This class of drugs occupy a prominent position in the fields of medicine, pharmacology and biology. As far as can be determined, no investigations have been conducted to study tranquilizer effects on protozoans, although the effect of such compounds on vertebrates, especially mammals, is under active study. It was the purpose of this investigation to study the effect of two water soluble, organic compounds known as tranquilizers (Thorazine® and Atarax®) and a related compound (Phenergan®) on the rate of fission and the survival of the protozoan Paramecium caudatum.

Each of the three compounds were diluted to concentrations of 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 mg/cc. using timothy hay medium as the diluting solution. Individual paramecia from one clone, were subjected to the various concentrations of each chemical compound in spot plate depressions containing 0.2 ml. of culture medium. Exposure to each concentration of compounds studied was continuous for seventy-
two hours or until the organism settled to the bottom of the depression. Paramecia were kept in dark, moist chambers at a constant temperature of twenty-seven degrees Centigrade. Survival time varied from ten seconds to seventy-two hours, depending on the compound and the concentration used. The fission of *P. caudatum* was completely inhibited in all except the 0.000001 mg./cc. concentration of Thorazine® and Atarax®, and was reduced even in such a dilute solution.

It was concluded that the survival of *P. caudatum* varies inversely with the concentration of the three compounds used and that fission is reduced by weak concentrations of tranquilizers and related compounds and completely inhibited by stronger non-toxic concentrations.
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