

sodium and chloride concentrations after the mercurial should not exceed those of plasma. However, we have demonstrated that the urinary sodium concentration can increase to levels significantly higher than those of plasma (Fig. 1). A possible cause for this overshoot phenomenon could be the release of anti-diuretic hormone by the mercurial and the additive effects of these 2 substances could explain this overshoot phenomenon. Pack (12) and Kuschinsky (13) have shown that following the injection of mercury into rats the concentration of oxytocic and antidiuretic principles of the pituitary gland are reduced. If the release of antidiuretic hormone were the cause of the overshoot shown in Fig. 1 this overshoot should not occur if a unilateral diuresis is produced by injecting the mercurial into one renal artery. This overshoot can occur under the above experimental conditions and it must be concluded that this phenomenon is due to some direct action of the mercurial on the kidney. Another possible explanation could be that the mercurial sensitized the kidney to circulating antidiuretic principle. The experiments on rats reported here do not support this explanation (Table III). The above findings make it rather difficult to explain the effects of mercurials as being on either the proximal or distal convoluted tubule. The possibility of more than one site of renal action of mercurials must be considered.

Summary. 1. The mercurial diuretics increase sodium and chloride concentration in the urine when these ions are hypotonic to plasma. The mercurial induced increase in

the urinary sodium and chloride concentrations can exceed those of plasma by a significant amount. 2. When urinary sodium and chloride concentrations are above those of plasma the mercurials reduce the concentration of these ions significantly. The ability of the kidney to produce hypertonic urine is not inhibited by mercurial diuretics. 3. Pituitrin induced antidiuresis and hypertonicity of the urine are not abolished by maximally effective doses of mercurial diuretics.

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Mating Types in *Stylonychia putrina*. (1957)

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Although conjugation in ciliated Protozoa was long extensively studied, especially by Maupas (1), mating types were first discovered by Sonneborn (2). It is now known that mating types exist in *Paramecium aurelia* (2,3), *P. bursaria* (4-6), *P. caudatum* (7-11), *P.*

multimicronucleatum (11), *P. trichium* (12), *P. calkinsi* (12-14), and *Euplotes patella* (15). In several of these species, a number of varieties have been recognized, each with its distinctive mating types; and the number of interbreeding mating types within a variety

is 2 in some cases, 4, 6, or 8 in others. The present paper reports 5 mating types in *Stylonychia putrina* Stokes, like *Euplotes* a hypotrichous Ciliate.

Material and methods. The clones of *Stylonychia putrina* used in the experiments reported here were collected at Lake Arrowhead in Southern California on August 30, 1949; September 25, 1949; June 27, 1950; and September 24, 1950. In all, 41 clones were isolated and established. The culture medium and methods were essentially those described by Sonneborn and Dippell (16).

Mating conditions and behavior. In testing for mating type, the clones were mixed during the first half of the forenoon. Pairing occurred only if the animals were numerous in the mixtures. The tendency to form pairs appeared to be stronger immediately succeeding food exhaustion. For this reason it was found advisable to add a drop of culture medium to each mixture, as made, as Kimball (15) did with *Euplotes patella*. If the animals were numerous, this amount of food was soon used up and pairing followed. When different mating types were mixed there was no immediate agglutinative reaction such as is found in *Paramecium*, but sometime later the animals gathered closely together in the bottom of the depression slide. Their normal jerking movements were accelerated; they appeared to be greatly excited. When pairing began, two animals faced each other and touched peristomes. After a time they became attached and twisted around till they lay side by side. Grell (17) observed similar prepairing behavior in *Stylonychia mytilus*.

In these experiments, pair formation usually began soon after noon and ceased around 5 or 6 o'clock in the evening. Occasionally pairing would begin later and would continue into the night. This statement is not intended to imply diurnal periodicity. This question requires further study. Pairs usually remained attached for about 24 hours but there was considerable variation in this.

Conjugation was observed in certain mixtures of 26 of the 41 clones tested. In preliminary work 5 of these clones were found to conjugate with each other in all possible combinations. Since selfing was never ob-

served in any of the 5 clones either in the culture vials which were under constant observation, or in unmixed controls set up under the same conditions as the experimental mixtures, it is obvious that 5 mating types were present. On being tested the other 21 clones were found to belong to the same 5 mating types, which have been designated as mating types A, B, C, D, and E of Variety I of *Stylonychia putrina*. In one of the clones selfing was observed, a few pairs being frequently seen in the culture vial. However, when this clone was mixed with clones of the 5 mating types, there would be many pairs in mixtures with mating types A, C, D, and E, but in the mixture with a clone of type B, the number of pairs would not be significantly greater than in the unmixed control. This clone was therefore assigned to mating type B. Fifteen of the 41 clones tested failed to conjugate with any of the 5 clones given above and various attempts to get them to conjugate with each other also failed. This complete lack of reactivity on the part of over one third of the clones tested remains to be explained. They may belong to another variety, they may be sexually immature, or their lack of reactivity may be due to some other unknown condition.

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Pharmacologic Action of β -Phenylethylglucosamine.* (19958)

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The quantitative difference in activity between the optical isomers of various sympathomimetic amines has been widely investigated. The present report concerns the changes in pharmacologic activity of optically inactive β -phenylethylamine which result when it is conjugated with an optically active sugar moiety.

Materials and methods. β -Phenylethylglucosamine (PEGA) was prepared by the method of Pigman *et al.* (1). It was a white crystalline solid (M.P. 82°) which gradually darkened on standing. **Analysis.** Calculated for $C_{14}H_{19}O_5N$; N, 4.98. Found: N, 5.06. Mutarotation studies of PEGA in water were performed with standard polarimetric techniques. Freshly prepared aqueous solutions of PEGA had a specific rotation $[\alpha]_d^{20} -21^\circ$ however; upon standing at room temperature for 15 minutes, the value fell to $[\alpha]_d^{20} -15^\circ$ and remained constant over a period of 2 hours. Heating the solution for 1 hour at 50° yielded a value of $[\alpha]_d^{20} +8^\circ$ which remained constant over a period of 1 hour. Heating PEGA solutions at temperatures of above 60°C, however, very quickly hydrolyzed the glucosamine. Toxicities were calculated by the method of probits following intraperitoneal injections of aqueous solutions in groups of 20-30 white mice. The effects on carotid arterial blood pressure were determined in pentobarbitalized dogs following intravenous injections of aqueous solutions of the drugs.

Results. The toxicities of the two forms of PEGA (+8 and -15) and of β -phenylethyl-

TABLE I. Acute Toxicity Study.

Compound	LD ₅₀ (mg/kg)	LD ₅₀ moles $\times 10^3$ /kg
PE	366	1.47
PEGA (+8)	426	1.53
PEGA (-15)	535	1.89

amine hydrochloride (PE) are compared in Table I on both a milligram and molar basis. Whereas PEGA (+8) and PE were apparently not different, PEGA (-15) was definitely less toxic. The observable responses in the mice were essentially the same for all materials although PE appeared to exert its effects more rapidly than either form of PEGA. Following the injection of lethal doses of all materials the mice initially were quite aggressive† then followed a period of relative inactivity which merged into fine tremors, later coarse tremors and finally asymmetrical convulsions. Upon cessation of respiration, opening the chest cavity revealed the heart was still beating.

In their effects on the dog's blood pressure, PEGA (+8) and PE produced qualitatively and quantitatively similar pressor responses and were roughly 1/300 as potent on a weight basis as epinephrine. PEGA (-15), however, appeared to be only about $\frac{1}{3}$ - $\frac{1}{5}$ as active. Qualitatively, PEGA (-15) in low dosage ranges (up to 2 mg/kg) produced a more prolonged rise in pressure than did PEGA (+8) or PE at a dose of equal pressor height. Doses of PEGA (-15) in high dosage ranges (5 mg/kg and larger) gave responses qualitative-

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† The term 'aggressive' here denotes the fact that mice were observed to run about the cage biting one another.