PRELIMINARY REPORT ON THE TOXIC EFFECT OF URANIUM TO **PARAMECIUM CAUDATUM**

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Very little work has been done on the influence of uranium on microorganisms. Stoklasa and Penkava (1) have reported an increase in bacterial metabolism at 0.00001 M. A very considerable amount of work has been done on the pathological effects of uranium to the convoluted tubules of the kidney, which is summarized by Mac Nider (2) in his Harvey Lecture. A single dose of 2 to 4 mg. per kg. in the dog produces edema and necrosis of the proximal convoluted tubules, some glomerular fibrosis, with albuminuria, granular casts, decreased PSP excretion and nitrogen retention. Recovery in the case of young animals may result in normal tubule cells which are sensitive to a second dose of uranium, or flattened, syncytial tubule cells which are resistant to a second dose of uranium. Changes in liver epithelium (3) include cloudy swelling, edema, vacuolation, necrosis and lipoid accumulation. Holman (4) has demonstrated the production of necrotizing arteritis of the large vessels in hypo- or hyperproteinemic dogs by 5 mg. per kg. of uranium nitrate. Govaerts (5) administered 7 mg. of uranium nitrate to rabbits daily until death and noted an increased permeability of capillary endothelium with the production of an edema fluid averaging 0.2% protein. Jones et al. (6) reported the specific localization of a uranium dose in liver (20%) but not bile and in kidney (23%) and its excretion in the urine of rabbits. Localization of kidney damage in the tubules of the dog kidney was demonstrated by Bobey et al. (7) who showed that after uranium administration, diodrast clearance falls to the level of inulin clearance. Bucciardi and Biscegli (8) observed the vacuolization of embryonic chick heart tissue when the culture was poisoned by uranium. Gough (9) observed the rapid loss of mitochondria from rabbit renal epithelium following uranium acetate

i. v. injection, the mitochondrial filaments being replaced by large scattered spherical bodies.

Experimental

Equal parts of a culture of **Paramecium caudatum** in 0.2% wheat chaff infusion at pH 7 and dilutions of uranyl acetate solution at pH 7 were mixed and observation was made of the times (t) at which half of the paramecia were dead. The results are shown graphically in Fig. 1, in which the logarithm of the concentration (c) of uranyl acetate in moles per liter is plotted against the logarithm of the death time. It will be noted that the slope of the left side of the curve approximates unity and that the curve breaks suddenly to approach an asymptote at 0.000037 moles per liter.

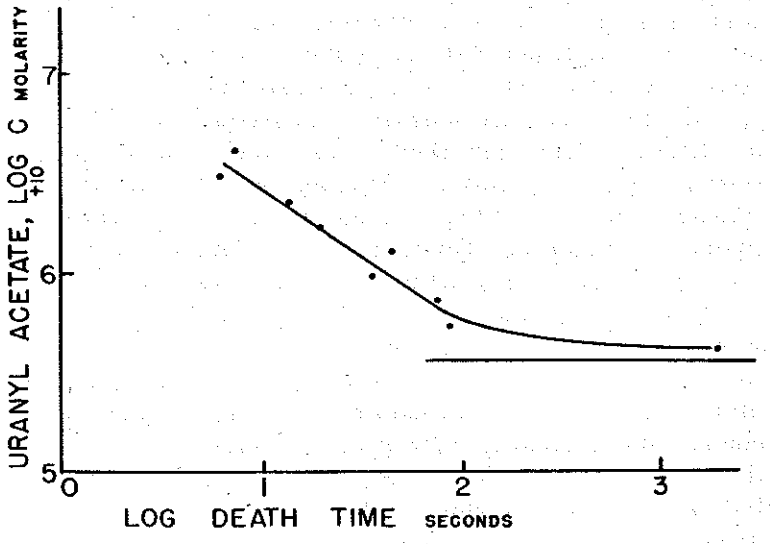

Fig. 1. Toxicity Curve of Uranyl Acetate to **Paramecium caudatum.** Full Log Plot.

These are the general criteria of a bimolecular reaction which progresses to a degree of partial completion represented by the coefficient alpha in the equation.

$$t = \frac{2.303}{(c-b)k} \log \frac{c-\alpha b}{c(1-\alpha)} \qquad (1)$$

which is derived from the general differential equation for the rate of a bimolecular reaction

$$\frac{dx}{dt} = k(c-x)(b-x) \qquad (2)$$

on integration

$$t = \frac{2.303}{(c-b)k} \log \frac{(c-x)b}{(b-x)c} \qquad (3)$$

in the special case where x, the extent to which the reaction has progressed, equals αb.

c is the initial concentration of toxicant, molarity,
b is the concentration of some reactant in the paramecia,
t is the time when half the paramecia are dead, seconds,
k is the reaction velocity constant.

At higher values of c, αb and b are small in comparison to c, and the equation (1) reduces to

$$t = \frac{2.303}{ck} \log \frac{1}{1-\alpha} \qquad (4)$$

the equation of a straight line in which the variables are c and $\frac{1}{t}$

The slope of the line $\frac{d\frac{1}{t}}{dc} = \frac{k}{2.303 \log \frac{1}{1-\alpha}} = 413$

and the c intercept when $\frac{1}{t} = 0$, and $t = \infty$, is $\alpha b = 0.000037$.

The data are replotted in Fig. 2., where the concentration of uranyl acetate is plotted against the reciprocal of the death time. The reasonable fit of the points to a straight line is a further confirmation of the theory that the toxicity represents a chemical combination between uranyl acetate and some constituent of the paramecia.

In order to test the binding of uranium to paramecia, 3 liters of paramecium culture were treated with sufficient uranyl acetate at pH7 to make an 0.00005 M solution (21 mg. per l.). The mixture was allowed to stand for 1 hour when all the paramecia were dead. The dead paramecia, amounting to a volume of 0.2 ml., were concentrated by centrifugation, spread on a 4 cm. filter paper, and dried by suction. Measurement with a Geiger counter indicated an estimated 0.5 mg. of uranyl acetate bound by 0.2 ml. of dead paramecia, as compared to 0.004 mg. of uranium acetate originally present in 0.2 ml. of the liquid, or a 100 fold enrichment. This enrichment of uranium by living organisms may be a significant factor in the accumulation of small amounts of uran-

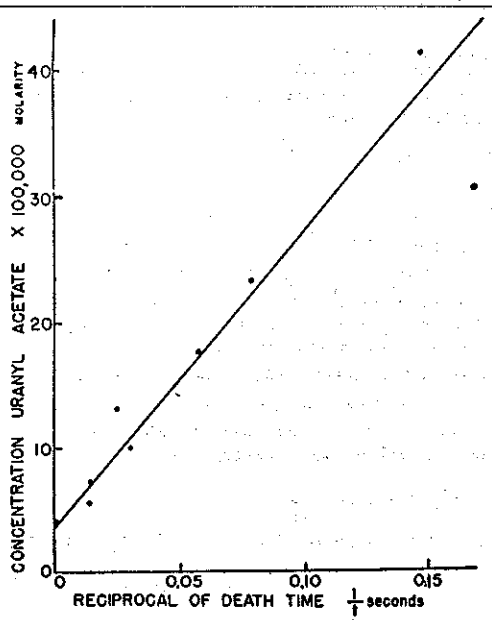

Fig. 2. Toxicity Curve of Uranyl Acetate to **Paramecium caudatum.** c vs. $\frac{1}{t}$ plot

ium in sedimentary shales. In the case of paramecia, it corresponds to a concentration of 0.006 M or 0.25% of uranyl acetate in the bodies of the paramecia. Using the figure 0.0065 micrograms of nitrogen per paramecium* and the measured dimensions of the paramecia, approximately 200 x 40 x 40 microns, it can be estimated that the concentration of nitrogen in paramecia is approximately 2% corresponding to approximately 12% of protein. Assuming a molecular weight of 34,000 for the protein, this corresponds to a concentration of 0.003 M protein in the paramecia, which is of the same order of magnitude as the estimated concentration of uranium in the bodies of the paramecia, 0.006 gm. atoms per liter.

Under the conditions of the experiment, concentration and pH, it would appear that uranyl ion no longer exists in the solution. Dittrick (10) has shown that an 0.1 N solution

*Nitrogen analysis of paramecia was supplied through the courtesy of Dr. K. K. Krueger and Mrs. Elaine Nelson Ordal of this laboratory.

of uranyl nitrate is 3.6% hydrolyzed. Gomez (11) states that below 0.01 M, some of the uranium in uranyl nitrate is present in anionic form. Britton (12) gives a titration curve for uranyl nitrate, which shows complete conversion to uranium hydroxide at pH 6.7, but failure to form sodium diuranate until pH 10.5 is reached. In view of these facts, it would appear that the uranium is present as uranium hydroxide at pH 7, and that, due to the low concentration, this substance stays in solution and is the toxicant to paramecia. The ratio of two uranium atoms per protein molecule suggests the possibility of compound formation, but uranium hydroxide at 0.0005 M is not an effective precipitant for blood, either laked or unlaked, so that it is more probable that the uranium hydroxide is adsorbed to the gross protein of the dead paramecia.

The actual kinetics of the death, with the asymptote at approximately 0.00004 M uranium, a level which is less than 1% of the measured final concentration of uranium on the dead paramecia, probably involves a material that is more critical for survival and present in lower concentration than the gross protein of paramecia.

Bright contrast phase photomicrographs of the changes at death and postmortem are shown in Plate I.

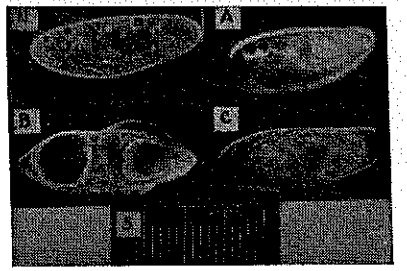

Plate I. Death of **Paramecium caudatum** by 0.0005 M Uranium at pH 7.0.

Paramecium N: Normal.
Paramecium A: 2 minutes postmortem, one contractile vacuole and several food vacuoles enlarged.
Paramecium B: 60 minutes post mortem, both contractile vacuoles dilated and clear, with two localized clear blisters.
Paramecium C: 80 minutes postmortem, showing contraction of granular material leaving clear zone at posterior end.
S: micron scale, lines are 10 microns apart.

The earliest change is an increase in the size of the vacuoles, which is comparable to the vacuolation of liver epithelium cells (3) and of embryonic chick heart cells (8) under the influence of uranium as described by previous workers. The swelling of the dead organisms, amounting to an approximately 100% increase in volume, is probably due to partial protein autolysis, which, with an intact bounding membrane leads to osmotic entry of water into the cell. The formation of clear blisters and retraction of granular cytoplasm suggests the possibility that gelation or coagulation of the cytoplasm has taken place. Except for the enlarged contractile vacuoles, internal boundaries, such as gullet, food vacuoles, and nucleus have become unresolvably mingled in the coagulated cytoplasmic background.

Summary

The death of **Paramecium caudatum** under the influence of uranium at pH 7 shows the properties of a bimolecular chemical reaction to partial completion. The limiting toxic concentration, 0.00004 M, is much smaller than the estimated protein concentration of paramecia, 0.003 M, so that it would appear that the death is caused by the chemical reaction of uranium with something more specific than the gross total protein of the paramecium. Phase photomicrographs show the fundamental cytological changes to be vacuolation, coagulation and swelling, with the loss of internal bounding membranes except those of the dilated contractile vacuoles, but with the retention of the external bounding membrane intact, so that swelling leads to the formation of clear blisters.

BIBLIOGRAPHY

1. J. Stoklasa and J. Penkava, Biochem. Zeitsch., **194**, 15 (1928).
2. Wm. DeB. MacNider, Harvey Lecture, 1928-9, p. 82; Am. J. Med. Sci., **178,** 449 (1929).
3. Wm. DeB. MacNider, Proc. Soc. Exp. Biol. and Med. **30,** 238 (1932).
4. Russell L. Holman, Amer. Jour. Path., **17,** 359 (1941).

5. P. Govaerts, Bull. Acad. Roy. Med. Belgique, **8,** 33 (1928).
6. H. D. Jones, R. Goslin, K. D. Crane, and G. B. Johnston, Amer. Jour. Physiol., **105,** 693 (1933).
7. M. E. Bobey, L. P. Longley, R. Dickes, J. W. Price and J. M. Hayman, Jr., Amer. Jour. Physiol., **139,** 155 (1943)
8. G. Bucciardi and V. Bisceglie, Arch. Internat. Physiol., **31,** 272 (1929).
9. J. Gough, Jour. Path. and Bact., **34,** 423 (1931).
10. C. Dittrick, Z. physik. Chem., **29, 465 (1899); in J. W.** Mellor, **Comprehensive Treatise on Inorganic and Theoretical Chemistry,** XII, p. 122.
11. L. Gomez, Anal. fis. quim., **17,** 24 (1919); in J. W. Mellor, **Comprehensive Treatise on Inorganic and Theoretical Chemistry,** XII, p. 122.
12. H.T.S. Britton, J. Chem. Soc., **127,** 2148 (1925).