Application of Perchloric Acid Technique to Protozoa

B. R. Seshachar and Eleanor Weiss Flick[1]

Department of Zoology,
University of Pennsylvania, Philadelphia

In making a study of the cytochemistry of the ciliate protozoon, *Chilodonella uncinatus,* with the more familiar tests for nucleic acids and proteins, the perchloric acid technique recently developed by Ogur and Rosen (*2*) is of considerable interest. They have reported the extraction of nucleic acids from certain plant tissues with the use of perchloric acid. Their data indicate that prolonged contact in the cold with perchloric acid extracts pentose nucleic acid (PNA) but not desoxypentose nucleic acid (DNA); while at elevated temperatures, both of these are extracted from the homogenates. This leads them to conclude that cold perchloric acid appears comparable to ribonuclease. Their technique was adapted by us to slide analysis and was applied to *Chilodonella* in this manner:

The organisms were fixed in bulk in acetic acid-alcohol (one part acetic acid to three parts 95% alcohol). This fixative is particularly useful in that it also extracts the inorganic phosphates and does not interfere with any subsequent tests by the deposition of heavy metals. The organisms were affixed to albumin-smeared slides in the usual manner. After hydration the slides were processed in 2%, 5%, and 10% perchloric acid concentrations at various temperatures and for different lengths of time. After adequate washings they were stained with Feulgen (*4*), Unna-Pappenheim mixture, methyl green (*3*), and toluidine blue. Adequate controls were maintained by processing duplicate slides under the same conditions but without added perchloric acid. The results noted are based upon visual judgments using the compound microscope.

Results of the various treatments are as follows:

In any treatment, 2% or 10% perchloric acid, in the cold or at higher temperatures, the clearing of cytoplasmic basophilia in toluidine blue preparations and the lack of pyronin staining in Unna-Pappenheim mixture would seem to indicate that PNA is removed from the cytoplasm. The extent of PNA extraction in 2% perchloric acid in the cold depends, however, on the length of contact (see Table 1).

Two percent perchloric acid used in the cold, although apparently having no effect on the Feulgen reaction and thus on DNA, would appear to depolymerize this nucleic acid progressively, as shown by the decrease in methyl green staining capacity. Such structures of the nuclear apparatus that fail to stain with methyl green are stained with pyronin in Unna-Pappenheim mixture. According to Kurnick (*1*), such a reaction indicates the presence of a lower polymer, as pyronin stains only PNA and depolymerized DNA.

Ten percent perchloric acid at 5° C removes PNA very rapidly. In addition, our preparations of *Chilodonella* show that prolonged contact with the acid decreases the Feulgen staining capacity of the nuclear apparatus. It would seem that DNA is also being extracted. The methyl green preparations are even more striking; at 72 hr the reaction is essentially negative.

[1] We wish to express our thanks to Dr. D. H. Wenrich for his interest and criticism.

TABLE 1
DETAILS OF TREATMENT WITH PERCHLORIC ACID ON *Chilodonella uncinatus*

% $HClO_4$	Temp (C)	Time (hr)	Results	
			cytoplasm	nucleus
2	5°	3, 10, 18, 24, 72	Progressively decreased basophilia, beginning at about 10 hr.	Feulgen reaction remains unchanged. Methyl green intensity decreases progressively starting at about 18 hr.
10	5°	3, 10, 18, 24, 72	No basophilia.	Feulgen intensity progressively decreases. Methyl green staining becomes less intense, until at 72 hr it is practically nil.
5–10	35°	18	No basophilia.	Feulgen as well as methyl green staining are very faint.
5–10	70°	20 min	No basophilia.	Feulgen and methyl green reactions are negative.

Five percent or 10% perchloric acid at temperatures of 35° C and above is capable of extracting DNA quite rapidly, total extraction occurring at 75° C where, within 20 min, the nuclear apparatus becomes Feulgen negative and the cytoplasm shows essentially no basophilia. However, hot water treatment of the animals reduces the staining intensity with the specific stains, Feulgen and methyl green. In particular, the methyl green reaction becomes rapidly negative. It is thus extremely important to carry adequate controls before interpreting results.

It seems possible that in *Chilodonella,* cold perchloric acid on prolonged application is able, in addition to extracting PNA, to depolymerize DNA. This depolymerization might be an essential preliminary step in the process of extraction of this nucleic acid. The extraction appears to take place to a considerable extent in 10% perchloric acid in the cold, indicating that rather rigid conditions may be necessary before a parallel between the actions of perchloric acid and ribonuclease can be established. It may be noted, however, that the nuclear apparatus of *Chilodonella* is unique in many respects, and the reactions that one finds here may not necessarily be applicable to other animal cells or even to other protozoa.

References

1. KURNICK, N. B. *Cold. Spr. Harb. Sympos. quant. Biol.,* 1947, **XII** (Discussion), 141.
2. OGUR, M. and ROSEN, G. U. *Fed. Proc. Amer. Soc. exp. Biol.,* 1949, **8**, 234.
3. POLLISTER, A. W. and LEUCHTENBERGER, C. *Proc. nat. Acad. Sci.,* Wash., 1949, **35**, 111.
4. RAFALKO, J. S. *Stain Tech.,* 1946, **21**, 3.