The Use of OsO_4 as Fixative for Feulgen-Stained Preparations. By Maria A. Rudzinska.* (*From The Rockefeller Institute for Medical Research.*)‡

In the course of an investigation of structural differences between young and old organisms in the protozoan *Tokophrya infusionum* (2–6), it became necessary to study the macronucleus by histochemical procedures (1). The macronucleus of *Tokophrya* is a conspicuous body (5 to 20 μ in diameter) which contains numerous refractile granules ($\sim$0.5 μ in diameter) clearly visible in the living organism (Fig. 3). Because of their staining properties these granules are called chromatin bodies. In electron micrographs of thinly sectioned, OsO_4-fixed specimens, the chromatin bodies appear as dense, oval profiles (Fig. 5) similar in shape and distribution to those seen in the living organism. It appears therefore that in this case, OsO_4 fixation insures the faithful preservation of a characteristic intranuclear structure that can be clearly observed *in vivo*. The finding deserves some attention in view of the opinion frequently expressed that OsO_4 is not a satisfactory fixative for nuclear structures in general.

Previous studies have shown that the macronucleus contains Feulgen-positive material (11), but the intranuclear location of the DNA responsible for this

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staining reaction could not be studied in detail, because the nuclear structures seen *in vivo* are usually altered greatly by Carnoy's fixative. The latter is, in recent years, one of the common fixatives for cytophotometric measurements of the Feulgen reaction (12). OsO_4, the fixative of choice for electron microscopy, has apparently not yet been used in histochemistry for the Feulgen nuclear staining.

Histochemical information on the macronucleus is derived therefore from Carnoy-fixed material, whereas information on the fine structure of the organelle is obtained from OsO_4-fixed specimens. The result is that structural details cannot be convincingly interpreted in histochemical terms. It is evident that a satisfactory correlation of morphological and histochemical observations requires the use of a common fixative for these two different technical approaches. For this reason it was decided to try the OsO_4 as a fixative for the Feulgen nuclear staining and to compare the results of this procedure with those obtained in the current histochemical technique that combines fixation in Carnoy's mixture with staining by Feulgen's reagent.

For this purpose cultures of *Tokophrya* were grown in hanging drops on coverslips. Since the adult *Tokophrya* is a sessile organism and attached firmly to the substrate (coverslip) the cultures may be easily fixed and stained on coverslips and thereafter examined in whole mount preparations. In the study herein reported, cultures prepared in hanging drops were divided into two groups; one was fixed with Carnoy's fluid (3 parts of absolute alcohol and 1 part of glacial acetic acid) for 10 minutes, the other with vapors of 2 per cent OsO_4 for 30 minutes. Both groups after the necessary washings were hydrolized together for 10 minutes and thereafter taken through the same staining and dehydration procedures.

The examination made after mounting disclosed that, although the intensity of the nuclear stain appeared to be the same in both groups[1] there were considerable and striking differences between the preparations fixed in OsO_4 and those fixed in Carnoy's fluid. The differences proved that OsO_4 fixation has many advantages over Carnoy's fixative for Feulgen's nuclear staining. First of all, it was found that after Carnoy's fixative, the Feulgen-stained macronucleus shows considerable shrinkage demonstrated by a large clear zone between it and the cytoplasm (Fig. 2). It contains a few irregular masses of deeply stained material. They may represent chromatin bodies clumped together by the shrinkage of the macronucleus (Fig. 2 A). In the preparations fixed with OsO_4 the macronucleus does not show shrinkage and retains its size and shape (Fig. 1). The chromatin bodies remain unchanged and can be clearly seen as separate granules (Figs. 1 A and 4). Their shape and distribution resembles those in the living organism (Fig. 3). OsO_4 fixation insures, in addition, a satisfactory preservation of the body shape, of the tentacles, and of the cytoplasmic organelles, whereas specimens fixed in Carnoy's mixture show body shrinkage, contraction or disappearance of the tentacles, and poor preservation of cytoplasmic detail.

This comparative study shows clearly that OsO_4 has definite advantages over the Carnoy's fluid as a preparatory fixative for the Feulgen reaction. It seems somewhat surprising and difficult to understand why OsO_4 known as a very

[1] No spectrophotometric measurements were taken.

good cytological fixative (7) has not been used so far in the Feulgen procedure. This might be explained by the complexity of the reaction and the precautions it requires. As is known, the Feulgen reaction uses a mild acid hydrolysis for splitting guanine and adenine from desoxyribonucleic acid, and liberating aldehyde functions which stain intensively reddish purple when treated with leuco-fuchsin. It is understood that in such a procedure every step is important. Feulgen himself was much concerned about the kind of fixative (8). He warned against the use of mixtures containing chromic acid because it might destroy the nuclear material. He was also against the use of formaldehyde because it might give a positive color reaction. He recommended sublimate–acetic fixative in spite of the knowledge that it produces swelling and vacuolization of the chromatin. Bauer (9) and Hillary (10), however, studied the influence of various fixatives on the Feulgen reaction and came to the conclusion that practically every fixative can be used if the tissues are thereafter properly washed. In this connection it is even more interesting to note that Bauer (9) was strongly against the use of Carnoy's fluid describing it as the least desirable fixative for the Feulgen reaction because it changes the structure of the chromatin. He recommended moreover Flemming's fluid without glacial acetic acid. One of the three components of the Flemming's fluid is OsO_4 (1 per cent chromic acid, 30 parts; 2 per cent OsO_4, 8 parts; glacial acetic acid, 2 parts).

The finding that OsO_4 is a good fixative for the Feulgen nuclear staining is of special importance in view of the fact that electron microscopy relies on this fixative. With a common fixative, it becomes possible to compare directly electron micrographs with photomicrographs of well preserved, Feulgen-stained material, and to interpret in histochemical terms structures seen in the electron microscope.

SUMMARY

OsO_4 has many advantages over Carnoy's fixative mixture for the Feulgen nuclear staining in the protozoan *Tokophrya infusionum.*

While Carnoy's fluid used prior to the Feulgen reaction produces shrinkage of the macronucleus and coarse clumping of its chromatin bodies, OsO_4 preserves faithfully the size and shape of the macronucleus and its chromatin material.

This finding seems to be of special importance in view of the fact that electron microscopy relies on OsO_4 fixation. The satisfactory preservation of structured detail in Feulgen-stained preparations is of importance for the correlation of histochemical and morphological information.

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EXPLANATION OF PLATE 128

FIG. 1. Photomicrograph of young *Tokophrya infusionum* fixed with vapors of 2 per cent OsO_4 and stained with Feulgen's reagent. The shape of the body is pyriform as in the living organism. The macronucleus (*m*) does not show shrinkage. The cytoplasm seems to be well preserved. The tentacles (*t*) retain their length. × 2350.

FIG. 1 A. Underexposed photomicrograph from the same negative as in Fig. 1 to show the macronucleus only. × 2350.

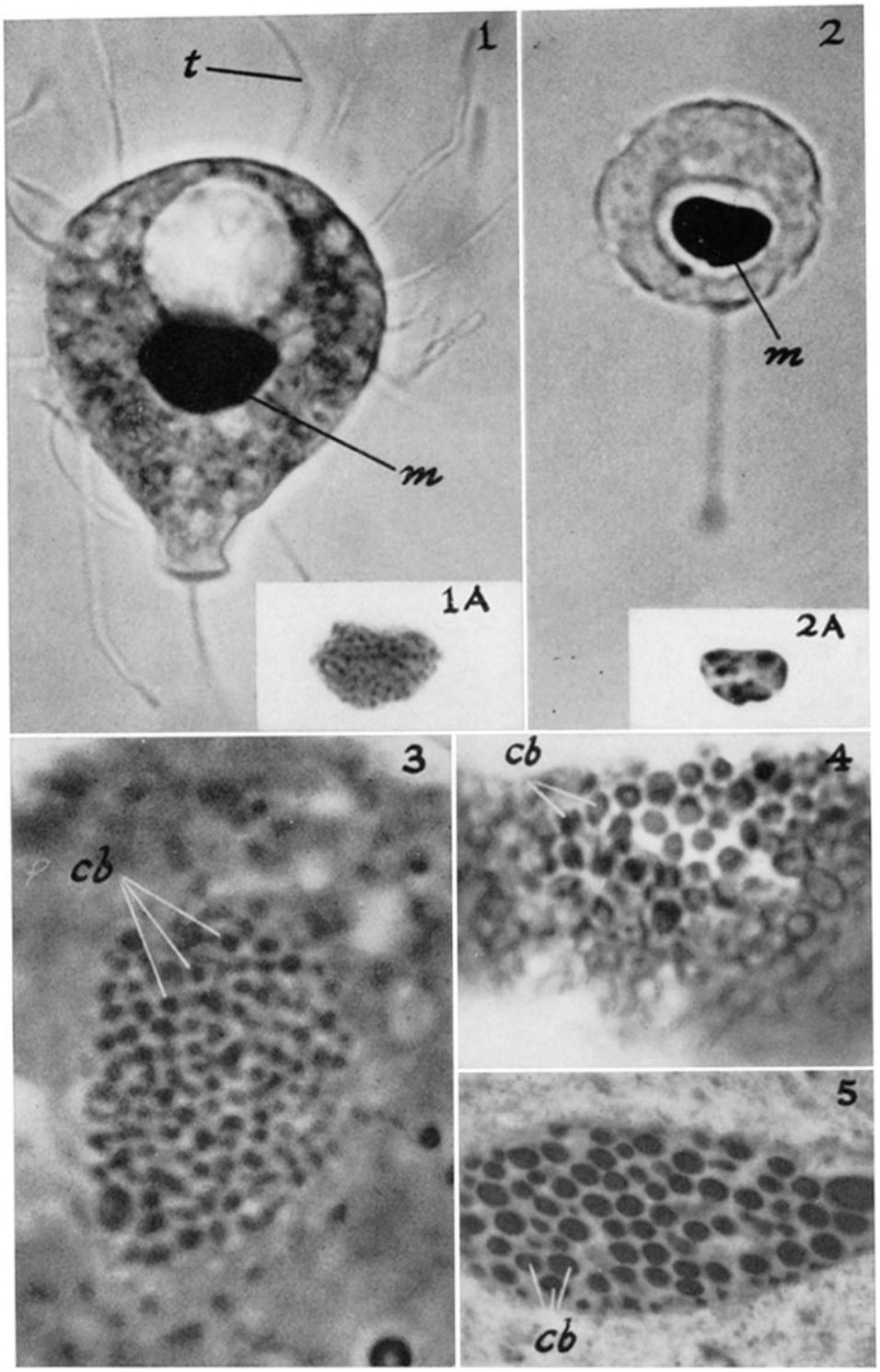
FIG. 2. Photomicrograph of young *Tokophyra* fixed in Carnoy's fluid and stained with Feulgen's reagent. The shape of the body is changed to one that is spherical. Between the macronucleus (*m*) and the cytoplasm a large clear area can be seen, indicating that the macronucleus underwent considerable shrinkage. Little detail is visible in the cytoplasm. The tentacles have contracted or disappeared. × 2350.

FIG. 2 A. Photomicrograph showing the macronucleus of Fig. 2 only. The technique used was the same as in Fig. 1 A. × 2350.

FIG. 3. Part of body of a living organism in phase contrast microscopy showing the macronucleus and its chromatin bodies (*cb*). × 3420.

FIG. 4. Photomicrograph of macronucleus fixed with vapors of 2 per cent OsO_4 and stained with Feulgen's reagent. The chromatin bodies (*cb*) are clearly visible and look like the ones seen in the living organism (Fig. 3). × 3580.

FIG. 5. Electron micrograph of section through macronucleus; *cb*, chromatin bodies. × 6000.



(Rudzinska: OsO_4 as fixative for Feulgen-stained preparations)