THE PROPERTIES AND EFFECTS OF OSMIUM TETROXIDE AS A TISSUE FIXATIVE WITH SPECIAL REFERENCE TO ITS USE FOR ELECTRON MICROSCOPY

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It is remarkable that a compound as obscure as osmium tetroxide should have been found to be a good fixative relatively early in the history of histology. Baker (3) reports the earliest description of its use to have been presented in 1865 by Max Schultze in a study of the marine protozoan, *Noctiluca*. Subsequent expansion in the utilization of this compound for histological purposes is well known and it is now one of the essential components of classical fixatives which differentiate mitochondria, Golgi, fat, myelin, etc. The use of osmium tetroxide alone in fixation has been less extensive, due to its slow penetration and its interference with various classical staining methods.

Although nuclei are reportedly not well preserved by osmium tetroxide, it is generally agreed that OsO_4 is an excellent fixative of cytoplasmic structures, since it preserves the outlines and form of structures seen in living material, and does not cause a visible coagulation. Little is known, however, concerning the chemical action of OsO_4. Such studies as those of Hardy (14), Monckeberg and Bethe (18), Fischer (13), Mann (16), Hofmann (15), Berg (5), and Baker (3), have provided only rough indications of the reactions of various fixatives, including osmium tetroxide with proteins and fats.

That more precise chemical studies of fixation have not been made is due partly to the difficulty involved in assessing the quality of fixation. What criteria can be used to decide when a cell is well fixed? The visibility of a structure after fixation that was not apparent before, may simply mean that fixation has altered its refractive index relative to its surroundings. Hence, to label all such structures artefacts, is ill-considered. Probably the optimum requirements to be made are that the cell should not detectably change its shape under the action of the fixative and that its cytoplasm should not,

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under high resolution, show discontinuities and lacunae of irregular size and angular form. Such structures with associated surfaces, and density differences, if present in the ground substance of the living cell, would most probably scatter light in dark field microscopy, whereas under such examination the ground substance is normally quite clear and nonrefractile.

Osmium tetroxide has recently come to be used in the preparation of tissue cells and other biological objects for electron microscopy. Here, even more than for light microscopy, it is essential to obtain faithful preservation of structures and to have a more extensive knowledge of the action of the fixing agent. Interest in the properties of the compound has therefore increased and has stimulated the present limited study. This report will review what is known of the compound, present evidence to demonstrate the perfection of its action as a fixative, and show its effects on tissue cells with time of action. It is hoped that the observations presented and the problems defined will elicit more critical analyses, especially of the chemical action of OsO_4 on biological materials.

Chemical Properties of OsO_4. Osmium, element 76, is a grey to white metal with an atomic weight of 190.2. Its density per unit volume is the highest of any known element. It is found associated with platinum as osmiridium in the Witwatersrand gold reef of South Africa, in the Ural mountains, and in Alaska. It is used to a limited extent commercially in hard alloys for tipping fountain pen nibs and phonograph needles, and as pivots for scientific instruments.

The tetroxide is the highest known oxide of osmium. It is formed by oxidizing the metal in solution with *aqua regia*, after which the volatile oxide is distilled off from the solution. In the solid state it consists of volatile white to yellowish crystals which melt at 41° C. The liquid boils at 131.2° C. It has a characteristic, slightly sweetish odor which can be detected to the limit of 2×10^{-5} mg per cc of air (12). The vapor is dangerous in that it attacks and fixes the moist cellular surfaces of the nose, throat, and eyes. When vapors of OsO_4 come in contact with the skin or other tissue surface, the area of contact turns dark brown to black. This is claimed by Thorpe and Whiteley to result from the formation of a hydrated dioxide of osmium, whereas Ephriam (12) states that the colored product is $Os(OH)_4$.

Osmium is known to exist in many valency states. Compounds belonging to the +2, +3, +4, +6, and +8 states have been described. The existence of osmium with a valence of +7 is in doubt, although an unstable halogen derivative of this nature has been described (10). Opinion differs as to the existence of various oxides and hydroxides. Thorpe and Whiteley (23) state that only the tetroxide and dioxide are definite compounds, although others claim the existence of OsO, Os_2O_3, $Os(OH)_3$, and $Os(OH)_4$. Of these, OsO and OsO_2 are reported to be grey to black, Os_2O_3 to be dark brown and gelatinous, $Os(OH)_3$ to be reddish brown, and $Os(OH)_4$ to be dark brown and gelatinous (12).

The value of osmium tetroxide as a fixing agent is apparently dependent upon its ability to act as a strong oxidizing agent and to affect various components of

living material differentially, as determined by their composition and reactivity. It is known to combine actively at ethylenic double bonds to form five membered rings (8, 9), and more recently it has been demonstrated to react selectively with certain double bonds in aromatic hydrocarbons (7, 2).

Osmium tetroxide is neutral to litmus and is a non-electrolyte. For this reason the practice of calling it "osmic acid" is misleading and incorrect. Osmium tetroxide can be quantitatively estimated colorimetrically by heating with hydrochloric acid and thiourea (19).

Action as a fixative of Cells. Osmium tetroxide in solution or as vapor is seldom applied as a fixative to blocks of tissue which are to be sectioned, although it is a frequent component of classical fixatives used for this purpose. That it has not been used by itself, stems from the experience that it penetrates the tissue slowly, gives uneven fixation and produces sections which tend to crumble. Moreover, many stains are ineffective after osmium fixation. Some of these ills can be overcome by applying it to the tissue as a perfusate (11), but this generally is not a convenient procedure.

For isolated cells such as protozoa or tissue units, pure OsO_4 is much more satisfactory. One of the earliest and most convincing demonstrations of this came from a study made by Strangeways and Canti (22), in which the action of various fixatives was compared on cells during examination in the dark field microscope. Of all the agents tried, OsO_4 produced the least detectable change. As the reagent reached the cell, the most evident change was an increase in light scattering by the nucleus. This describes, according to these authors, the development of a "fine precipitate."

These observations are readily confirmed. Tissue culture cells examined with the phase contrast microscope and under dark field illumination exhibit an instantaneous cessation of Brownian movement in the cytoplasm when exposed to the vapors of 2 per cent OsO_4. The cytoplasm retains the transparency of the living cell. In time, there is selective action on the cytoplasmic constituents as evidenced by gradual browning of fat droplets and increased definition of membraneous structures, but, initially, the predominant action of the treatment is one of gelation.

The electron microscope image of cells fixed with OsO_4 essentially bears out these light microscope findings. Evidence of this is provided in Fig. 1, which shows a rat myoblast which was fixed with OsO_4 vapors while bathed in a hanging drop of balanced salt solution (Earle's formula of Tyrode) at pH 7.4 (for methods see 20 and 21). The cell outline is that of the living unit and shows little evidence of shrinkage or contraction. The cytoplasmic surface and content are smooth (see also Fig. 5) with no aggregates or lacunae such as accompany coarse coagulation. Small arrays of fibers apparent in the

thinner margins of the cell resemble those which may be seen in phase contrast images of similar cells. The outlines of the formed structures of the cell such as nucleus and mitochondria are smooth and do not show the angularity of distortion expected in poor fixation. The entire image is descriptive of an extremely faithful preservation of native morphology.

The superior quality of this OsO_4 fixation is emphasized by comparing it with the action of other reagents or mixtures commonly used in tissue preservation. For example, Figs. 2 and 3 show the electron microscope image of portions of cells which had been fixed with Flemming's solution.[1] It is clear from the micrograph of the shadowed material especially, that the cell has suffered considerable damage. It appears that existing condensations of fibrous elements in the cytoplasm have served as centers for the coagulation of the otherwise homogeneous matrix with the concurrent development of interfibrillar lacunae. As mentioned earlier, such variations in density and distribution of non-volatile matter could not exist in the living unit and remain undetected by dark field or phase contrast microscopy.

It has been found that the character of the image varies with the time the material is subjected to the action of OsO_4 vapors. Nothing new in the nature of morphological entities is added to the cell. Instead, certain components are removed and the remaining structures are thereby revealed with greater clarity.

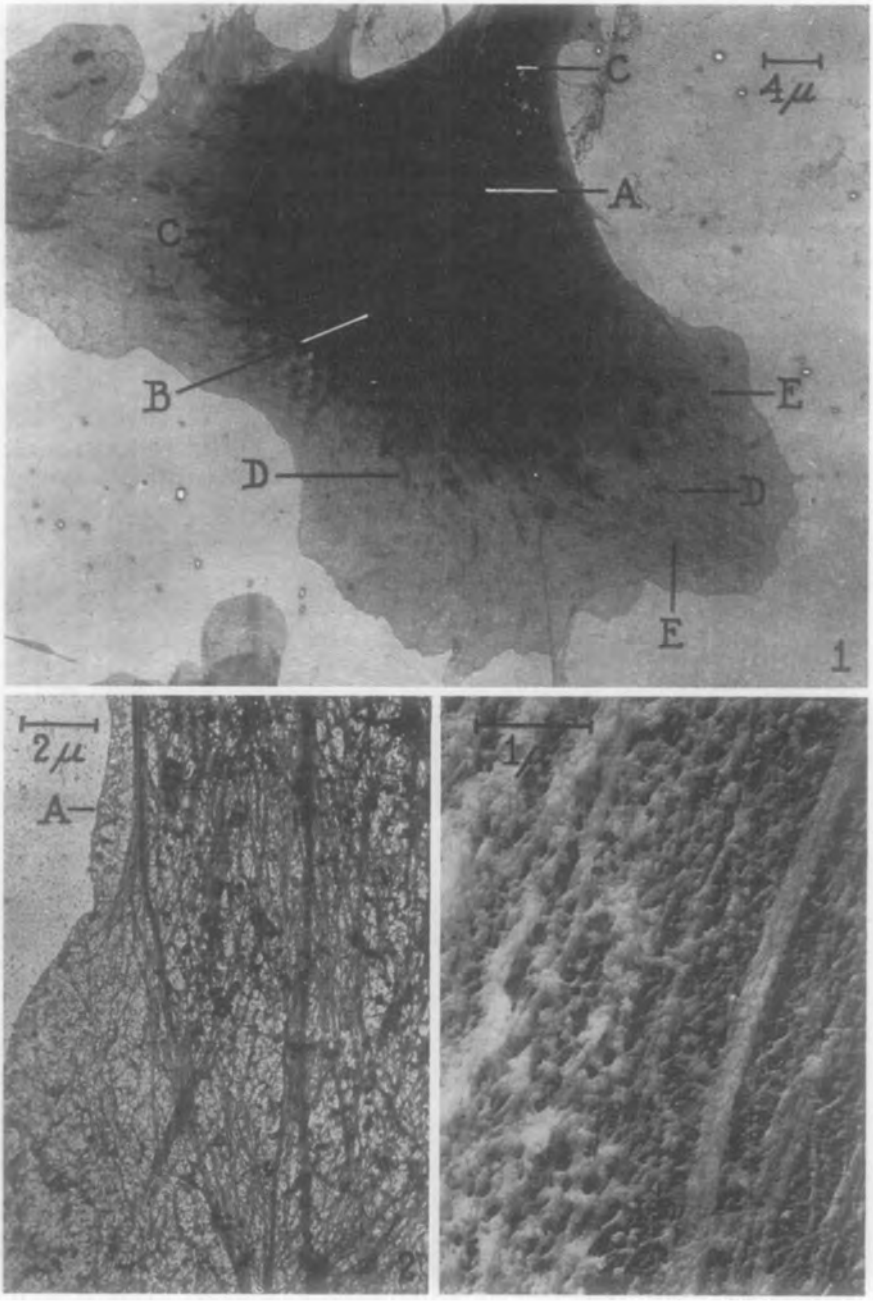
After a period of 10 minutes over vapors of OsO_4 (Figs. 4 and 5) the mitochondria (*A*), strands of the endoplasmic reticulum (*B*) and small fiber arrays (*C*) in the cytoplasmic matrix may be identified. It is evident, however,

[1] Flemming's solution (strong formula, i.e. with acetic acid) was chosen to exemplify the action of most cytological fixatives, all of which (except HCHO) are similar in causing some degree of coagulation in the cytoplasm. Unpublished studies of Dr. Bruno Schober have demonstrated that relatively coarse patterns of cytoplasmic structure are provoked by the action of all such common fixative constituents as potassium dichromate, mercuric bichloride, chromic acid, iodine, picric acid, and absolute alcohol.

Fig. 1. Electron micrograph of a fibrocyte grown in tissue culture from an explant of rat heart. The cell was fixed with vapors from 2 per cent OsO_4 for 10 minutes and, following washing and drying, was shadowed with chromium (for methods see 20 and 21). The natural outline, the absence of obvious shrinkage, and the smoothness of the cell surface define the fixation as extremely faithful. The nucleus is at *A*, the centrosome at *B*. The osmium dense lipid granules are at *C*, the mitochondria at *D*, and a small array of intracellular fibrils at *E*. The magnification, 1 800 ×, is not sufficient to depict other structures. This and all the micrographs on Plate III were taken with a Philips E.M. 100; all other figures were taken with an R.C.A. type E.M.U.

Fig. 2. Micrograph of portion of cell similar to that shown in Fig. 1 but fixed in Flemming's solution. The margin of the cell is at *A*. The dense strands and clumps and accompanying lacunae are in part a product of coagulation. Mag. 4 900 ×.

Fig. 3. Micrograph of a small part of the cytoplasm of a fibrocyte similarly fixed in Flemming's but here shadowed with chromium. The surface irregularities resulting from the destructive fixation are obvious. Mag. 15 000 ×.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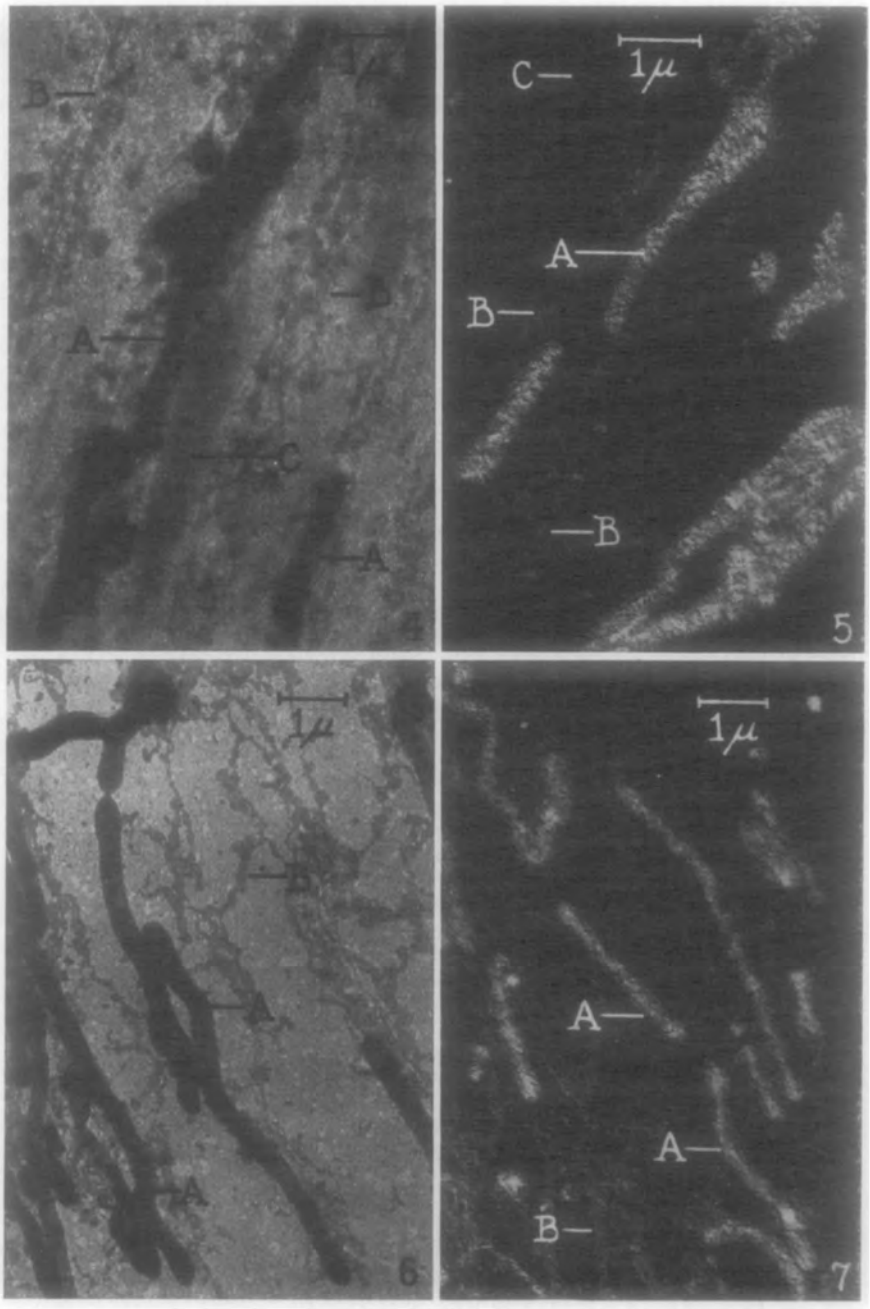
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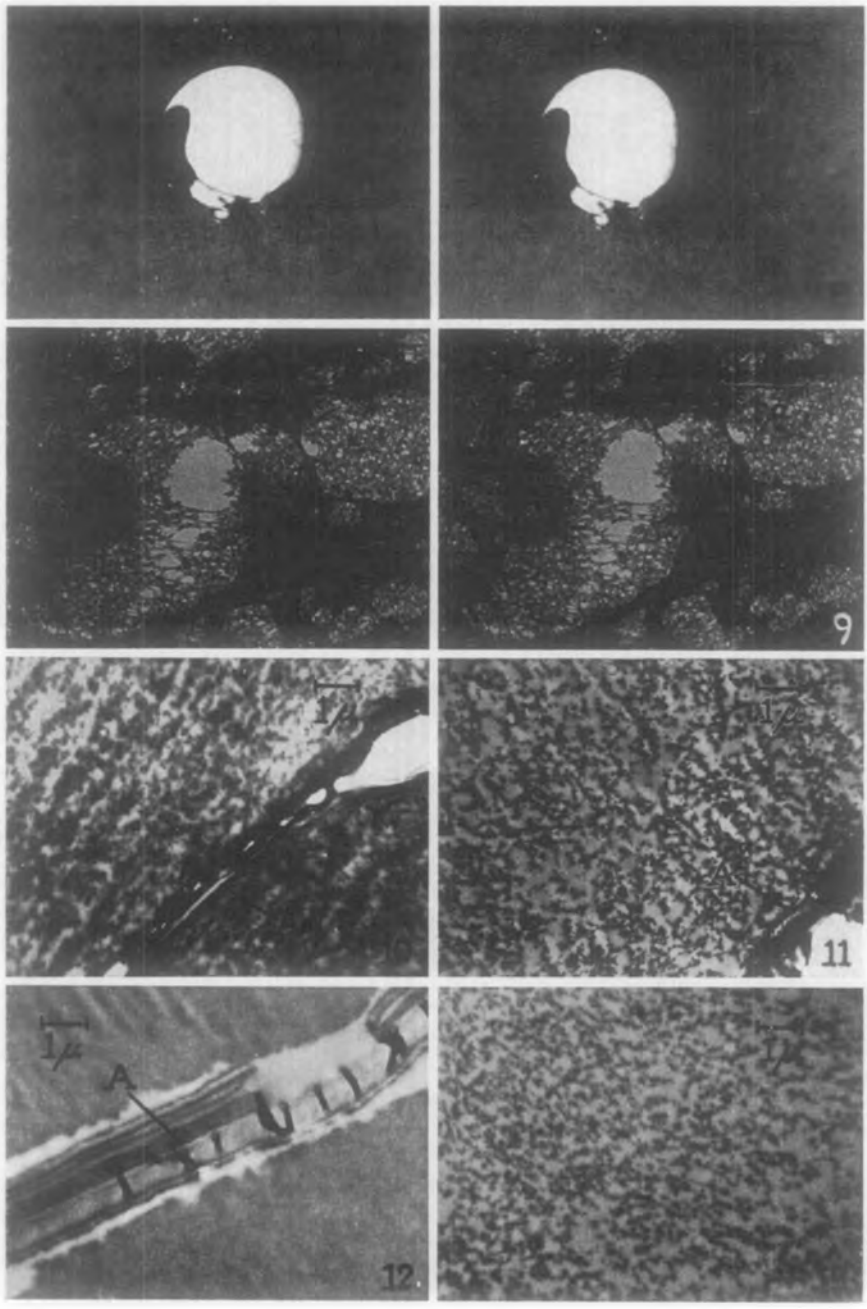
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that around the formed bodies something is present which serves to scatter electrons and blur the outlines. The limits of the mitochondria and the reticulum can be seen, but they are not sharply defined.

This observation is clarified by comparing Figs. 4 and 5 with Figs. 6 and 7. These latter micrographs show portions of identical types of cells fixed 16 hours over vapors of OsO_4. Here the mitochondria and the reticular elements are sharply defined; no fibrous condensations are evident. In the shadowed preparation (Fig. 7) the formed elements stand up as discrete bodies with shadow-casting margins. By comparison, the surface of the briefly fixed, dry cell in Fig. 5 is smooth.

It appears then, that during the period called long fixation, an amorphous matrix (and its fibrous condensations) is removed from around the formed bodies leaving them clean and available for more detailed study. This in

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Fig. 4. Electron micrograph of a portion of a cultured rabbit endothelial cell fixed for 10 minutes in vapors of OsO_4 all the while bathed in balanced salt solution. Following fixation it was washed in H_2O for 2 minutes and then transferred to the usual grids for electron microscopy. Mitochondria are indicated at *A*, strands of endoplasmic reticulum at *B*, and a small fibrous condensation of the cytoplasmic matrix at *C*. Diffuse margins of images of formed structures define the presence of electron-scattering material around these structures. Mag. 9000 ×.

Fig. 5. Portion of cell of type similar to that shown in Fig. 4 except that here the cell was shadowed with chromium, and this is the negative image. *A* marks a mitochondrion, *B* some element of the reticulum, and *C* a faint fiber array. Mag. 10500 ×.

Fig. 6. Portion of another rabbit endothelial cell fixed for 16 hours in vapors of OsO_4 while bathed in balanced salt solution. In contrast to their images in Figs. 4 and 5, the margins of mitochondria (*A*) and endoplasmic reticulum (*B*) are here sharply defined. This indicates that during the extended fixation, the electron-scattering, more or less homogeneous matrix has been removed. Mag. 9000 ×.

Fig. 7. Portion of similar cell treated the same as that in Fig. 6 except that here it was shadowed with chromium to bring out the elevation of the margins of the formed structures, which in the briefly fixed cell (Fig. 5) is concealed by the matrix. Mag. 9000 ×.

Fig. 8. Stereoscopic pair of micrographs of a film of bovine albumin fixed in vapors of OsO_4. The film was formed by flooding a 10 per cent solution of the protein over large, 1 × 2 cm pieces of celloidin-coated copper mesh and thereafter fixing for 2 hours. Following a washing in water, the films were processed by Anderson's technique for the preservation of three-dimensional structure. An apparently structureless gel is evident. Mag. 11500 ×.

Fig. 9. Stereoscopic pair of micrographs of albumin film similarly prepared except that in this case the protein was fixed in Flemming's solution. A coarse pattern of coagulation resulted. Mag. 11500 ×.

Fig. 10. Micrograph of thin section through pith impregnated with globulin and thereafter fixed in 2 per cent OsO_4 in Tyrode. There is obviously some coagulation in the gel structure but it has not resulted in the dense particle formation evident in Fig. 11 after Flemming fixation. *A* marks the wall of a pith cell. Mag. 5625 ×.

Fig. 11. Micrograph of thin section through identically prepared globulin-pith, but fixed with Flemming's solution. A dense precipitate is evident. *A* marks the wall of a pith cell. Mag. 5625 ×.

Fig. 12. Micrograph of thin section of albumin-impregnated pith fixed in 2 per cent OsO_4 in Tyrode. The gel that was formed by the fixative is seen to be remarkably homogeneous and free of evidence of coagulation. *A* marks the pith cell wall. Mag. 5625 ×.

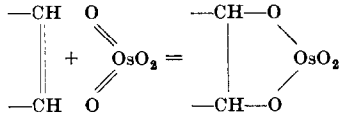
Fig. 13. Micrograph of thin section of albumin-impregnated pith fixed in Flemming's solution. Pattern of coarse coagulation is clearly evident. Mag. 5625 ×.

the vernacular of the cytologist, would be differentiation by extraction. What remains of the cell may be thought of as a membrane skeleton. Presumably the materials of the matrix are decomposed to a state which allows them to diffuse out of the cells. In support of this, it has been noted that as fixation is extended in time, the small quantity of balanced salt solution surrounding the cells acquires the brownish tint of compounds containing lower oxides of osmium. The increased fragility and relative emptiness which cultured cells show after these long fixations is probably analogous to the over-fixation effects noted by histologists (17).

The time taken to obtain this extraction effect with OsO_4 differs somewhat for different tissue. In general, cells from adult tissue explants require a longer fixation than cells from embryonic tissue. The most obvious assumption to make in this regard, is that the original hydration of the cell (higher in embryonic cells than in adult cells) determines the difference.

Chemistry of fixation. Since the cell is chemically so heterogeneous, little can be learned of the chemical nature of OsO_4 fixation from observations of its action on cells. Perhaps, therefore, the most useful alternative is to examine the action of the fixative on various relatively well-known homogeneous materials similar to or identical with cell components.

In the case of lipids, it has been assumed for many years that the reaction was a simple oxidation whereby the cell's lipids were oxidized and black osmium dioxide was deposited at the site of the reaction. Baker is more specific. He states that the OsO_4 reacts with olein which, as an unsaturated compound, reduces the tetroxide to a blackened lower oxide or hydroxide. Saturated fatty acids and OsO_4 do not react. As mentioned above, the basis for this distinction appears to reside in the particular tendency of OsO_4 to combine at ethylenic double bonds and form unstable osmium esters (8, 9).



It appears, however, that the reagent may find other chemical configurations with which to react (2). Thus the initial, rapid combination with unsaturated fatty acids may be followed by various other slower oxidation processes, as at aromatic double bonds, to give the varying degrees of osmium densities evident in both light and electron microscope images.

Less still is known of the action of OsO_4 with proteins. Hardy (14) fixed membranes of various colloidal solutions including egg white and gelatin

in: 1 per cent OsO_4 solution, OsO_4 vapors, corrosive sublimate, formalin, and dichromate, in an attempt to study fixation on physical systems. He reported that osmium vapors produced the finest fixation of any of the reagents tested. Monckeberg and Bethe (18) found that when they mixed solutions of egg white and OsO_4, no precipitation occurred, although films of egg white were fixed by osmium vapors in so far as they were no longer soluble after this action. However, no change in the films was evident microscopically.

Studies of the addition of OsO_4 to protein solution have been made by several investigators (13, 6, 3). In each instance, however, the protein preparations were far from pure, and no data were given as to concentrations or quantities of the reactant. For these reasons, it has been considered of some value to repeat and extend such observations in order to see in what respects if any, the behavior of proteins under OsO_4 fixation duplicated the behavior of cells in so far as the latter can be observed.

Solutions of 2 per cent OsO_4 and various concentrations of bovine serum albumin, bovine serum globulin, and bovine fibrinogen[1] were mixed in constant proportions of two parts protein solution to one part OsO_4. Observations were made on the rate of gelation and, where feasible, photometric determinations of opacity were recorded (see Table I). All mixtures were made in tubes of uniform size or in 1 cm square cuvettes of the Beckman spectrophotometer. It can be seen from Table I that OsO_4 brought about gelation of all three proteins when sufficient protein was present. As the concentration of protein was reduced, the gelation time was prolonged until, at the lower concentrations no gel formed. The lowest concentration of protein which produced a gel under the conditions of the experiment was different for each, as were also the speeds of the reactions and the characteristics of the gels.

Gelation proceeded most slowly in the case of albumin. From an initial viscosity of that of the protein solution, the mixture became more and more viscous until it gelled. The pH of the mixture shifted from 7.4 to 6.4 during gel formation. The OsO_4-protein mixture blackened progressively, but showed no evidence of light scattering. It was found necessary to use light of 900 mμ wave length in order to obtain a relatively high percentage transmission up to gelation time. Opaqueness such as can be developed by coagulating the protein with alcohol clearly affected the transmission of this wave length more than the darkening due to reduction of OsO_4 (Table I).

[1] Bovine plasma albumin (Armour) 97 per cent albumin. Bovine gamma globulin (Armour) Fraction II from bovine plasma. Bovine fibrinogen (Armour) Fraction I from bovine plasma. 40 to 50 per cent Na citrate ca. 70 per cent clottable N.

TABLE I

Reaction of OsO_4 and plasma proteins.

	Final protein concentration in gm per cent	Gelation time	Opacity (expressed in per cent transmission at 900 mμ)		Characteristics of gel	Subsequent behaviour
			Sol	Gel		
Albumin (in water)	20	12 min.	90	84	Clear, dark brown, firm.	Eventually liquified after several weeks.
	16	40 min.	90	74.5	Clear, dark brown, less firm.	»
	12	12 hrs.	92	(79) (not quite a gel)	Clear, dark brown, soft.	»
	8	None	93	(86)	—	
Globulin (in .85 per cent NaCl)	10	90 sec.	80	3	White, turning to brown, soft.	Gel persists
	8	120 »	Opaque		»	»
	6	Never gelled.	Developed a flocculent precipitate		White to brown.	—
	4	»	»		»	—
Fibrinogen (in Tyrode solution)	2.1	10 sec.	Opaque		White, quickly turning brown to black, soft.	Becomes fluid after several days.
	1.7	12 »	»		»	»
	1.3	20 »	»		»	»
	.85	120 »	»		Very soft.	Fluid after 24 hours.
	.43	Never gelled	—		—	—
	Control 15 gm. per cent albumin plus 50–60 per cent ethyl alcohol	Gelation with development of opacity	97 (15 mg. per cent protein alone)	3	Firm, opalescent to opaque.	

In each case 1 cc of 2 per cent OsO_4 was added to 2 cc of appropriate concentrations of protein solution contained in test tubes of uniform diameter. Gelation was determined by turning the tube sidewise to see if the mixture flowed. Opacity determinations were made with the Beckman spectrophotometer where feasible. The sol measurements were taken one minute after the osmium-protein solutions were mixed. The osmium solution was made up in .85 per cent NaCl for use with serum globulin and fibrinogen, while with serum albumin it was dissolved in water.

The reaction leading to gel formation was more rapid in the case of globulin and the product was in each case opaque. This doubtless defines some degree of coagulation.

With fibrinogen, the gelation was almost instantaneous at the higher concentrations, and as in the case of globulin, the gels were uniformly opaque. They did not appear under macroscopic examination to have the fibrous character of fibrin clots.

Although not indicated in Table I, it was also found that if the amount of protein was kept constant and the concentration of OsO_4 diminished, a lower limit was reached at or beyond which gelation would not take place. This is interpreted as indicating a possible stoichiometric relation in that part of the protein-OsO_4 reaction leading to gel formation.

The reaction of these protein solutions with OsO_4 clearly paralleled in at least two respects the apparent response or reaction of the cells to OsO_4. They initially gelled and subsequently, under the continued action of OsO_4, they (albumin and fibrinogen) decomposed and returned to the liquid state. In the case of albumin and globulin, the concentrations giving gels are not vastly different from the over-all concentration of proteins in the cell. These simularities prompted a further examination of the protein gels with the aim of further elucidating the nature and quality of fixation achieved with OsO_4.

The general appearance of the gels in itself provides some information in this regard. For example, those developed with albumin remain clear, indicating an extremely fine micellar or even unimolecular bonding. Globulin and fibrinogen on the other hand, become opaque, demonstrating a coarser unit structure.

In a further analysis of their form, 10 per cent solutions of bovine globulin and albumin were flooded over large (1×2 cm) pieces of celloidin-coated copper mesh to form protein films. Some of these were then fixed for 2 hours in vapors of OsO_4 and others, for purposes of comparison, in Flemming's solution. After washing in H_2O, they were processed for stereoscopic electron microscopy according to the method of Anderson (1). The results with albumin are shown in Figs. 8 and 9. The difference in the smoothness of the fixation of the protein with the two fixatives is striking. Three dimensional structure may be seen in both preparations, but in the case of the film fixed in Flemming's, there has resulted an extremely coarse coagulation. By contrast, the gel formed by the action of OsO_4 vapors on the albumin film is remarkably homogeneous. Similar differences were obtained with films of 10 per cent globulin, though here the OsO_4-fixed film was not so smooth and structureless as that obtained with albumin. It is readily seen that the

differences are analogous to those shown between cells fixed in OsO_4 and more destructive reagents.

With a similar purpose in mind, small, 1–2 mm cubes of pith were impregnated under vacuum with 35[1] per cent solutions of albumin and 15 per cent globulin, and thereafter fixed 4 hours in OsO_4 or in Flemming's. Following a period of washing, the blocks were taken up through alcohols and imbedded in *n*-butyl-methacrylate. Thin sections of these were cut and studied with the electron microscope. The results, as shown in Figs. 10–13, are more or less as expected. Even though subjected to alcohol and methacrylate treatments, the albumin gel is too finely constructed to show any component units at 15000 diameters. Blocks fixed in Flemming's solution but otherwise treated identically show pronounced evidence of coagulation. The globulin, as predictable from the gels formed in test tubes, is not as finely dispersed after OsO_4, but the result is somewhat better than with Flemming's solution.

From these simple experiments it is seen that OsO_4 reacts with certain plasma proteins to form gels which are remarkably free from evidence of coarse coagulation. From this and aforementioned evidence, it is probable that it has a similar effect on proteins and lipoproteins in the cell, and thus brings about their relatively faithful fixation. This process may be non-specific, such as that produced by alkalinization and acidification in experiments reported by Barbu and Machebœuf (4), but it may also be a specific action of OsO_4. Electron micrographs indicate that the effect of OsO_4 differs significantly from that of Flemming's solution, or for that matter, from most cytological fixatives.

Lack of precise knowledge of the structure of proteins, and some uncertainty as to their purity, are of course barriers to study of the chemistry of their interactions. It is worth noting, however, from Table I that fibrinogen and globulin react with OsO_4 to form gels at lower concentrations and at much more rapid rates than with albumin. An examination of the amino acid content of these proteins reveals that differences in the amount of tryptophane roughly parallel the differences in reactivity of the proteins with OsO_4 to form gels. In view of the configuration of the tryptophane molecule and the availability of double bond positions for OsO_4 interaction, it seems reasonable to assign a role in the gelation phenomenon to this particular component. It is not unlikely that the OsO_4 interacts with the complex protein molecules in a number of different ways. The progress of the reaction

[1] 7.5 per cent albumin infiltrated into pith, fixed in OsO_4, sectioned, and examined in the electron microscope, was found to be similar to 35 per cent albumin in being smoothly fixed.

in the case of albumin was reflected in viscosity changes over relatively long periods, the mixtures becoming progressively more viscous until they gelled. Even after gel formation, the reaction appears to continue, resulting finally in a reversal of the gelation. This is interpreted as indicating that the excess OsO_4 further acts on the protein to bring about a lysis of the gel micelles.

From present data, it is reasonable to propose that in osmium tetroxide fixation, there occurs an initial partial denaturation of proteins with a concurrent gelation which renders insoluble many components of the cell. This may be followed by a further denaturation and a final hydrolysis of certain proteins producing soluble end products which are capable of being washed out of the cell after long fixation. It may be supposed that the gelation phenomenon is produced by a linking together of proteins and lipoproteins by way of terminal groups and side chains capable of reacting with OsO_4. A two-step system was postulated by Berg (5), who believed that OsO_4 caused a gelation in the cell by binding together amino groups of the protein, and that with more prolonged treatment the osmium tetroxide caused the oxidation of the compound previously formed, during which time osmium tetroxide was reduced to the black, lower oxide.

The preceding discussion, if nothing else, emphasizes the lack of precise chemical knowledge concerning the nature of the combinations which form between osmium tetroxide and the proteins and other cell constituents. It is to be hoped that these reactions and others between osmium tetroxide and amino groups, nucleic acids, and lipids will come to be thoroughly investigated and better understood, for at least in electron microscopy, a great deal of the ultimate interpretation of the images of osmium-fixed material will depend on the availability of such information.

SUMMARY

Osmium tetroxide has been demonstrated to produce excellent fixation of the cytoplasm of cells, particularly for those to be examined in the electron microscope. The action of osmium tetroxide on various tissue constituents differs in its rate and nature. The reaction with lipids is probably by way of an affinity of osmium tetroxide for double bond configurations, thus forming unstable osmium esters which decompose to deposit osmium oxides or hydroxides at the site of activity.

Little is known of the reaction of osmium tetroxide with proteins, but in preliminary studies it has been shown to produce quickly an initial gelation

of protein solutions which, in the case of albumin, show no well-defined structure in the electron microscope. Coagulation images are, however, evident after other fixing agents. It is postulated that by virtue of its tetrahedral configuration, OsO_4 may form polymer-like structures with proteins establishing linkages at double bond containing groups. This initial gelation may then be followed by a further oxidation and the production of soluble end products, which after long fixation wash out of the cell.

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