

lower. In comparison to layered superconductors based on TaS_2 , the ternary molybdenum sulfides have higher γ , lower θ_D , and much higher T_c values.

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Electron Diffraction of Wet Proteins: Catalase

Abstract. *Electron diffraction patterns having 3500 reflections out to 2 angstroms were obtained from wet microcrystals of catalase. No diffraction was obtained if the water vapor pressure was set below 90 percent of the equilibrium value.*

Since vacuum drying usually disorders biological materials, electron diffraction with high-vacuum electron microscopes has been applied to a very limited extent in biological structure analysis. This is particularly unfortunate in cases where large crystals suitable for x-ray diffraction cannot be grown, but microcrystals of a suitable thickness for electron diffraction are available. Also, many biological structures, such as cell membranes, occur only in single layers and rarely in stacked arrays. Electron diffraction data in these cases could be of considerable aid in determining the structures. We have de-

veloped equipment that can modify any electron microscope to allow microscopy and diffraction of wet specimens. The detailed electron diffraction patterns of catalase we have obtained not only show that the hydration chamber is fully efficient, but also suggest that electron diffraction of wet biological materials is capable of giving detailed structural information. In this work we have also taken steps to minimize radiation damage.

Many attempts have already been made at constructing hydration stages for electron microscopes, some of them with considerable success [for examples,

see (1)]. Most of these stages involved the use of thin-film windows, which allowed penetration of the electron beam but acted as barriers to the gas. The disadvantages of the thin-film approach are the fragility of the windows (especially in the presence of water vapor); the long preparation times involved; the scattering of the electron beam by the windows, which raises the background level; and the contamination buildup at the windows, which aggravates the scattering problem. We have built differentially pumped hydration stages for a JEOLCO 200-kev and a Siemens IA microscope which, to a large degree, overcome the disadvantages listed above (Fig. 1). The stage consists of four collinear apertures through which the electron beam penetrates, the specimen being positioned between the inner two 75- μm apertures. A pressure gradient is maintained across the apertures by having a source of water vapor feeding the specimen and differentially pumping on the low-pressure side of the apertures. The pressure around the specimen, which was monitored by a mercury manometer, was controlled by varying the temperature of the source. The normal working temperature was 25° to 27°C, so that the measured pressure at the specimen position was 25 to 27 torr. The detailed results of testing the equipment with respect to hydration efficiency, the microscopy of water drops, and the resolution in relation to total gas pressure will be reported elsewhere.

X-ray diffraction work on catalase (2) indicated that diffraction was lost at relative humidities below 93 percent, so that the electron diffraction patterns should indicate the hydration efficiency of our chamber. We evaluated a number of recrystallization techniques for Boehringer and Mannheim ox-liver catalase for perfection in the resulting plate-like habit of the microcrystals. The microcrystals were first negatively stained with phosphotungstate (2 percent, pH 5.5) (3, 4), and the electron diffraction patterns of the dried crystals were taken. The maximum number of diffraction orders obtained from these crystals was used as the criterion for judging the crystallization procedure that gave the most ordered crystals. We chose a procedure of recrystallization from phosphate similar to that described by Sumner and Dounce (5), which gave a large number of plate crystals and a small number of prism

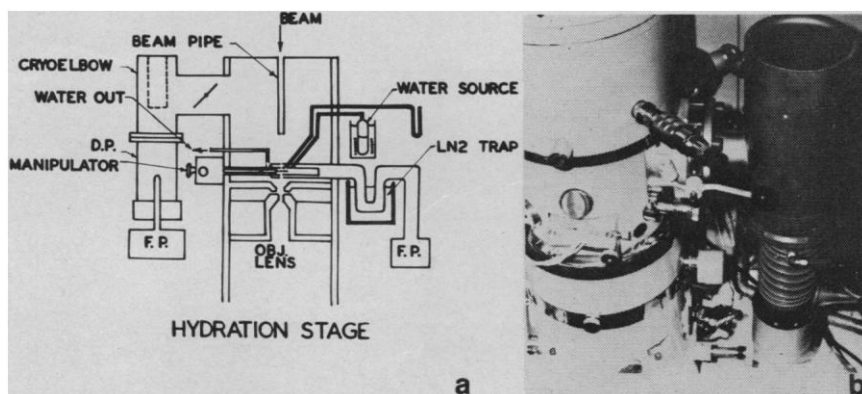


Fig. 1. (a) Schematic of the hydration stage for the JEOLCO 200-kev electron microscope; D.P., diffusion pump; LN2, liquid nitrogen; F.P., forepump; Obj. lens, objective lens. (b) External appearance of the modified JEOLCO 200-kev with hydration stage.

crystals. These preparations gave seven diffraction orders from the dried, negatively stained crystals and a large number from the wet, unstained crystals.

It is well documented that the electron beam at current densities normally used in the microscope is able to cause damage in a crystal. As a very rough rule, a biological specimen can withstand 10^{-3} coulomb/cm² before appreciable damage is noted (6). In our work we maintained the exposure for viewing and photographing well below this level.

By using the phosphor screen as an electron collector (collection efficiency roughly 50 percent) we were able to continuously monitor the current density and estimate the dose given to the specimen. So that we could scan the specimen and photograph any parts of interest at current densities below 10^{-3} coulomb/cm², a channel plate image intensifier was used for the scanning process.

Figure 2 shows an electron diffraction pattern of an unstained and unfixed wet plate crystal of catalase. More than 3500 reflections are present, extending out to 2 Å. The patterns are similar, in general, to those obtained by x-ray diffraction of protein crystals. After a beam exposure of 2×10^{-3} coulomb/cm², and the resulting radiation damage, only the first two orders remained visible. About 90 percent of the thin crystals gave patterns of varying resolution when the vapor pressure of water was above 90 percent of the equilibrium value, and none diffracted when the pressure was less than that, which agrees with Longley's results (2). The same symmetry and unit cell dimensions were obtained with wet crystals fixed with glutaraldehyde.

The low-angle data showed systematic absences of $l = 2n + 1$ for 00 l and $h = 2n + 1$ for $h00$, which tentatively indicates two screw axes and an orthorhombic space group of $P2_1-2_1$ with cell dimensions $a = 73$ Å and $c = 184$ Å. This unit cell is identical in symmetry and similar in size to that found for the plate crystals by shadow replication (4, 7) and negative staining (3, 4), but it is quite different from the unit cell of the prism habit, which has been shown by x-ray diffraction (2, 8) to have trigonal symmetry. In addition, the difference between the two habits is shown by shrinkage on drying. The platelike crystals contract isotropically 2 to 5 percent when they are dried, while the

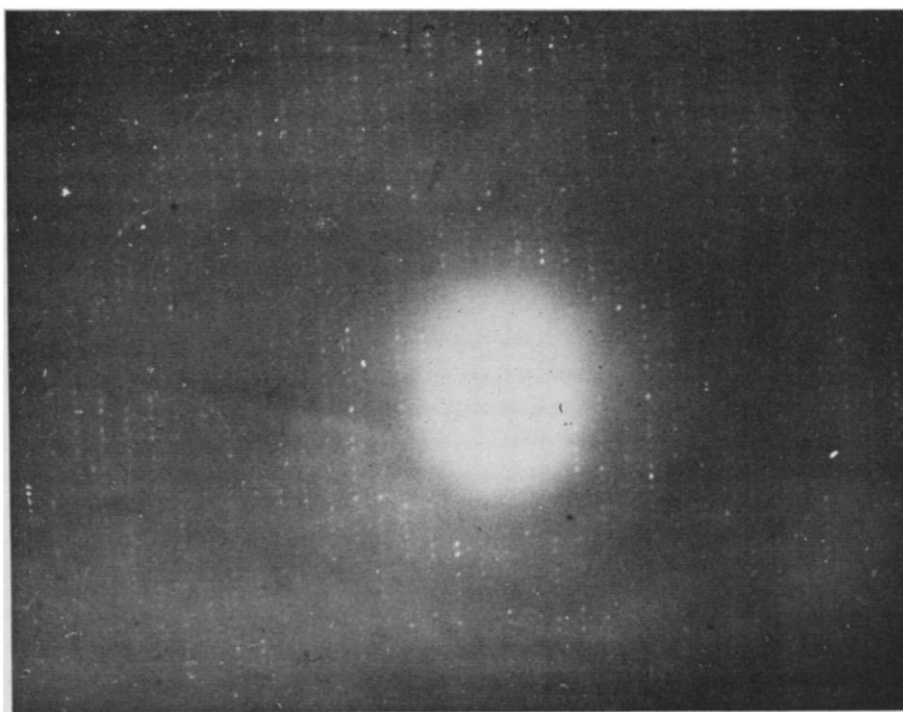


Fig. 2. Diffraction pattern of wet, unfixed, and unstained catalase crystal taken in the hydration stage (26 torr, 200 kev) showing the a and c axes. The photograph has been dodged to show the higher orders.

prisms contract anisotropically 10 to 25 percent.

Several workers have attempted to overcome drying disorder by fixing biological crystals and by embedding them in resin (9), but our work shows that detailed diffraction patterns can be obtained directly from the wet, unfixed crystals. In addition, our results open up the possibility of using the trigonal prism habit in order to calculate the structure of catalase from the corrected electron diffraction intensities by using phases obtained by the Fourier transform of images produced by selected reflections (10). This is more direct than previous methods, such as basing the synthesis on optical diffraction intensities from images of the negatively stained catalase crystals (11), or enhancing the images of the stained catalase molecule by various superimposition techniques (12), or calculating the catalase structure three-dimensionally from images of the negatively stained crystals (13).

While these new developments can be applied directly to structures expected to give diffraction patterns consistent with kinematic scattering of electrons (for example, cell membranes), the resolution of protein structures must await further work. The problems involved in obtaining the complete three-

dimensional data, the difficulties of interpreting intensity variations due to thickness variations, and the effects of crystal bending and dynamic scattering must be evaluated. The technical problems of determining the best phasing method and finding a low-intensity recording technique for the inelastically filtered diffraction patterns must be resolved.

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Coral Chronometers: Seasonal Growth Bands in Reef Corals

Abstract. *Autoradiographs and x-radiographs have been made of vertical sections through the centers of reef corals from Eniwetok. Radioactivity bands in the coral structure are caused by strontium-90 and are related to specific series of nuclear tests, thus making possible calculation of long-term growth rates. These data indicate that the cyclic variations in radial density revealed by x-radiography are annual.*

Growth rates and growth patterns of corals are of interest for several reasons. Wells (1) and Runcorn (2) have interpreted growth bands in fossil corals as indicators of past variations in the length of day, year, and lunar month. Chave *et al.* (3) have summarized the results of various investigations of the growth rates of contemporary reef corals. Other workers have commented on apparently annual bands in coral growth patterns (4), but their nature, origin, and periodicity have not been conclusively characterized or calibrated.

Bonham (5) has reported on the use of autoradiography in combination with optical thin-section photography to measure the growth rate of a giant clam, *Tridacna gigas*, which was sampled at Bikini Atoll and which contained fallout radioactivity from nuclear tests. He observed variations in shell translucence which he identified as annual and tentatively ascribed to seasonal variations in the water temperature.

We have used autoradiography to study the growth rates and x-radiography to identify structural density variations in a variety of reef corals listed

in Table 1. Samples 1 to 5 from Eniwetok Atoll (also the site of nuclear tests, 1948 to 1958) were subjected to both types of radiography. Samples 6 and 7 (from Fanning Island and Fort Lauderdale, Florida, respectively) were x-radiographed only; these samples were included in the study in order to ensure that the observed growth bands were not peculiar to the Eniwetok environment.

We cut a vertical slice less than 2 cm thick through the center of each coral specimen. For autoradiography, no-screen x-ray film was placed in direct contact with the samples; lead backscatter plates or fluorescing intensifier screens, or both, were placed behind the films to increase exposure speeds. After 40 days, the autoradiograph of sample 1 showed four radioactivity bands, that of sample 2 showed two bands, and that of sample 3 showed one definite band and a possible second band. Radioactivity bands for samples 2 and 3 were near the base (origin) of the coral, an indication that the earlier test series occurred prior to the inception of growth. Samples 4 and 5 showed no discernible concentrations of radioactivity, thus indicating total ages younger than the elapsed time since the most recent nuclear test series.

Nuclear weapons test series at Eniwetok were conducted during April–May 1948, April–May 1951, October–November 1952, May 1954, May–July 1956, and May–July 1958 (6). In each radioactivity-containing specimen the

Table 1. Growth rates and dimensions of reef corals.

Sample No.	Species	Date collected	Location*	Average linear distance, origin to surface† (cm)	Average upward growth rate based on 1958 radioactivity band (cm/year)	Average upward growth rate based on density band count (cm/year)
1	<i>Favia speciosa</i>	Feb. 1971	Eniwetok (Chinimi)	18 ± 0.5	0.46 ± 0.02‡	0.46 ± 0.02
2	<i>Goniastrea parvistella</i>	June 1971	Eniwetok (Bogen)	16 ± 1	1.25 ± 0.05	1.25 ± 0.05
3	<i>Goniastrea retiformis</i>	June 1971	Eniwetok (Chinimi)	10 ± 0.5	0.78 ± 0.03	
4	<i>Porites lutea</i>	June 1971	Eniwetok (Chinimi)	15 ± 1	> 1.2	1.35 ± 0.05
5	<i>Psammocora tobianensis</i>	June 1971	Eniwetok (Chinimi)	26 ± 0.5	> 2.0	2.9 ± 0.1
6	<i>Platygyra rustica</i>	Aug. 1971	Fanning Island (Suez Pond)	22 ± 1		2.2 ± 0.1
7	<i>Montastrea annularis</i>	Jan. 1970	Florida (Fort Lauderdale)	14 ± 2		1.7 ± 0.3

* All samples were taken from water depths ranging from 2 to 5 m; Eniwetok samples were all from the reef flat toward the lagoon, except for sample 2, which was taken from a pinnacle reef top. † Samples 1 through 4, 6, and 7 are approximately hemispherical; measurements are the average of those along several different radial transects; error limits represent the range of the extreme values determined. Sample 5 is a branching coral; the measurement is along the longitudinal axis of the stalks x-rayed, with error limits representing the difference between adjacent stalks. ‡ Growth rate between 1952 and 1958 radioactivity bands equals 0.47 ± 0.02 cm/year.