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HIGH RESOLUTION ELECTRON MICROSCOPY OF BIOLOGICAL SPECIMENS

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Ultrastructural studies of biological systems are being pursued in a comprehensive research program in electron microscopy at the University of Chicago. The new electron microscope facility provides optimum conditions for consistent attainment of high resolution, including a central highly regulated power supply (220V AC with 0.05% voltage stability, low harmonic distortion) designed for 12 electron microscopes. Modified high resolution instruments (e.g., Siemens Elmiskop I, Hu-11) and specially designed cryo-electron microscopes have been operating on vibration-free foundations under conditions of minimum magnetic and electrical perturbations in controlled clean room environment. The research program includes:

(1) Improved instrumentation: (a) Improved point cathode sources with single-crystal filaments and new types of molybdenum gun design used routinely to provide stable coherent microbeam illumination of high brightness, small spot size, low energy spread. Point resolutions of 2.8-3Å (Fig. 1a, 1b) were consistently attained with axial illumination, using stabilized power supplies, cooling and anticontamination devices. (b) With short focal length objective lenses (f=1.8mm) of improved stability (circuitry for Elmiskop developed by H. Armbruster), we confirmed and extended the results of R. Heidenreich by recording phase contrast high resolution images of carbon atom arrays (hexagonal cells of 5Å - Fig. 1c) in ultrathin (10-20Å) single-crystal graphite specially prepared by a technique described earlier (1a). Other pauciatomic structural patterns of organic systems were also resolved under similar conditions. (c) High resolution phase contrast was also achieved by precise alignment of multi-hole or annular condenser apertures and objective phase plates of composite ultrathin single-crystal films (graphite, mica, silver) with adjustable electrical fields, or ferromagnetic thin-film apertures permitting phase shift control. In preliminary experiments, marked enhancement and resolution was noted in thin biological

specimens. It was also possible to resolve structures of 5-8Å in unstained ferritin (Fig. 4) and apoferritin molecules.

(2) Improved preparation techniques: (a) Systematic use of ultrathin carbon films (10-20Å) prepared by evaporation in ultrahigh vacuum (10^{-8} torr) on special supports. Even thinner lamellae (10Å) of single-crystal graphite and diamond have been used which are extremely smooth and stable under intense irradiation, and can be used for high resolution shadow casting of DNA with carbon. These single-crystal substrates may be ideal atomic monolayer substrates for high resolution electron microscopy. (b) Specimens were mounted without background support on asbestos filaments (Fig. 2,4) or related substrates. DNA strands were also mounted on thin single-crystal mica with regular holes (80-100ÅØ) produced by fission tracks. (c) Extending earlier work (1), wet or hydrated biological specimens were examined by using special vacuum-tight microchambers, low intensity microbeam illumination, and cryogenic devices to minimize dessication and radiation damage. (d) Thin frozen sections of native, unfixed tissues can also be examined directly after ultrathin sectioning (20-100Å) with a diamond knife (1b,c) in microtomes operating in liquid nitrogen or helium cryostats, and transferred without thawing to the low temperature electron microscope stage. Representative examples are presented in which useful resolutions of 6-15 Å have been consistently attained, corresponding, in many cases, to resolution of quarternary or tertiary structures. These include correlative investigations of biological membranes and derivatives, hemocyanin and apohemocyanin (3), multienzyme complexes (e.g., pyruvate dehydrogenase complex (2), Fraction-I protein), catalase, RNA polymerase, and DNA and RNA systems.

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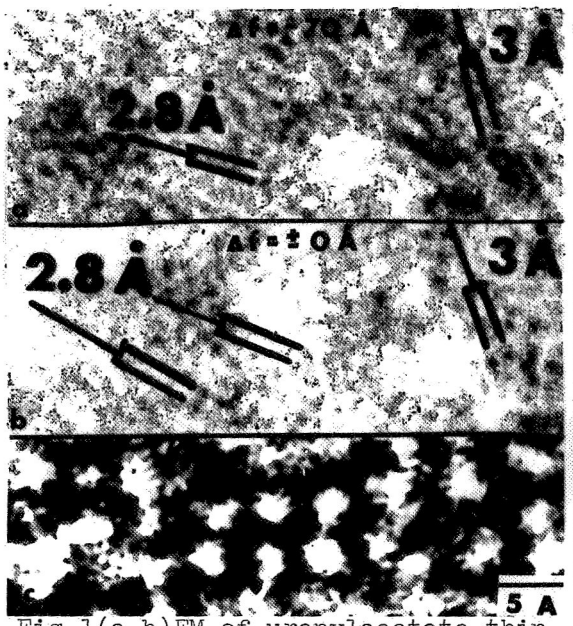

Fig.1(a,b)EM of uranylacetate thin film test specimen showing point resolution of 2.8-3Å in focal series, 70Å steps,100kV.(c)High resolution phase image of array of hexagonal cells carbon atoms in ultrathin lamella of single-crystal graphite.El. opt:300,000x;Elmiskopla,Obj.f=1.8mm, focal steps 30Å;80kV.Point cathode.

Fig.2.Hemocyanin molecules(Busycon) attached to asbestos filaments in different orientations, stained with uranylformate,showing structural details of 6 to 8Å (arrows). Compare with typical 7.3Å crystalline lattice period of asbestos filament. Point cathode, 80kV. Liquid-nitrogen cold stage.

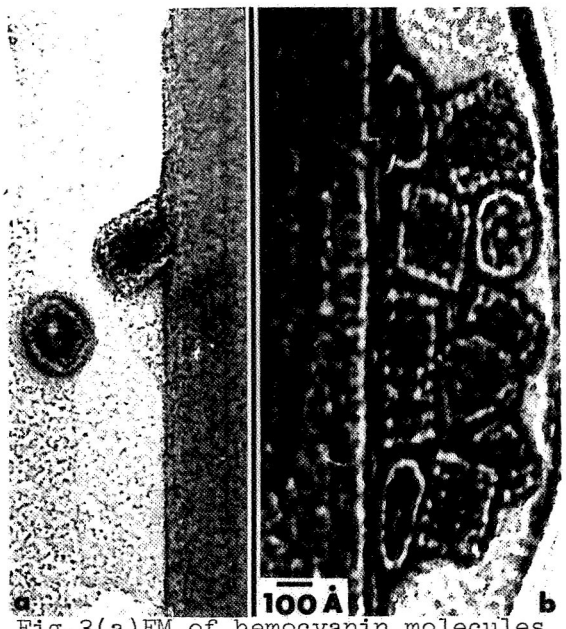

Fig.3(a)EM of hemocyanin molecules in uranylform.film on asbestos, taken with 50μ standard objective apert. (b) Phase contrast electron micrograph of adjacent area showing enhanced contrast and resolution of fine structure.Multi-hole phase plates. All other recording and reproduction conditions in (a,b) are identical. 80kV.

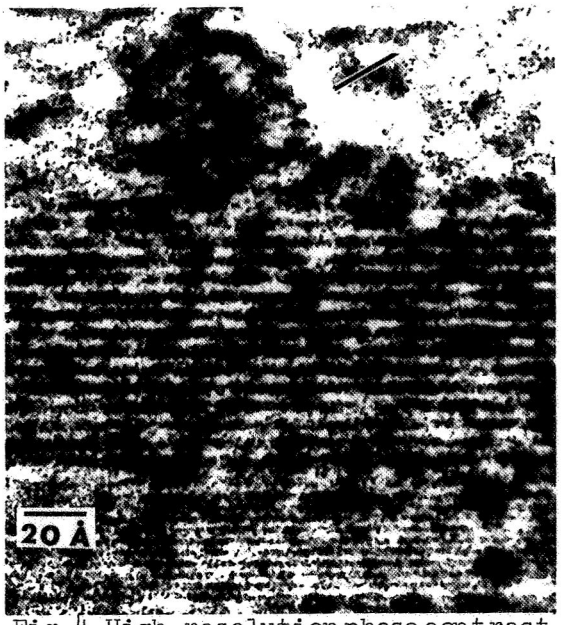

Fig.4.High resolution phase contrast electron micrograph of unstained asbestos filament exhibiting distinct 7.3Å and 3.7Å(central) lattice period. Compare with attached unstained ferritin molecule showing structural details of 5to8Å in outer protein shell(arrow).Obj:f=1.8mm;thin-film ferromagnetic phase plate. 80kV.