residing in polluted and nonpolluted areas, the children residing in polluted areas had lower lung function during periods of heavy pollution. Experimental inhalation of dust deposited in polluted areas of Kawasaki City led to increased airway resistance in healthy humans. This is reversed by bronchodilator drugs. While classical bronchial asthma occurs predominantly in the autumn, about the same time of year that so-called "Tokyo-Yokohama Asthma" occurs, the highest number of bronchial disorders of children occurs in the spring at the same time of the highest levels of air pollution.

McKerrow (Pneumoconiosis Research Unit, Llandough Hospital, Wales) investigated the causes of chronic respiratory disease. Epidemiological studies have revealed an increase in morbidity due to chronic bronchitis in urban populations as compared with rural populations; this increase is accompanied by a lower ventilatory capacity. The prevalence of persistent cough and sputum was related to the number of cigarettes smoked. In men aged 55 to 64 a reduction in mean ventilatory capacity as related to the number of cigarettes smoked was also demonstrated. In laboratory studies, normal subjects exposed to coal dust for periods of 4 hours, 19 mg per cubic meter of air, showed an increase in respiratory resistance, but values had returned over half way to normal 1 hour after exposure. A 3-year prospective study of the ventilatory capacity in a group of ex-miners with pneumoconiosis and chronic bronchitis showed an annual cyclical change; the lowest and highest values were found early in February and August, respectively.

Studies of respiratory diseases and air pollution in Los Angeles were made by Oelsner, Wehrle *et al.* (Infectious Diseases Laboratory, University of Southern California). The prevalence of asthma was compared with cases in New Orleans in order to detect similarities, however none existed. School absenteeism and symptoms and signs of acute respiratory illnesses were studied in various groups. Work is still under way and a large number of relevant variables have been examined. No clearcut relationship to air pollution has been observed as yet.

Weill *et al.* (Tulane University School of Medicine) and Horton and McCaldin (Public Health Service, Division of Air Pollution) collaborated on further studies of asthma in New Orleans. Previous outbreaks of asthma have been related to point sources in city dumps in which underground combustion occurs. An increase in gaseous atmospheric pollution occurred on 5 October 1962, followed by an outbreak of asthma between the 7th and 10th of October. Extracts of the smoke from the burning dumps were analyzed by intradermal and scratch tests both on persons who had asthma during the outbreak and on students. The students who had a history of asthma or hay fever had more positive skin reactions than those without. Subjects who had current symptoms had a higher percentage of positive reactions than those whose symptoms were not active, and the persons who had episodes of asthma in the outbreak had a larger proportion of reactions than among the students.

The papers and discussion at the conference will be published in the near future in the *Archives of Environmental Health.*

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Electron Microscopy: Proteins in Experimental Pathology

Among the many sessions of the 47th annual meeting of the Federation of American Societies for Experimental Biology in Atlantic City (18 April) was a symposium on Electron Microscopy of Proteins in Experimental Pathology which was sponsored jointly by the Intersociety Committee for Research Potential in Pathology, Inc., and the American Society for Experimental Pathology.

In discussions on electron microscopy of the fibrinogen molecule and the fibrin clot, C. E. Hall (Massachusetts Institute of Technology) focused attention on changes that take place as fibrinogen molecules polymerize. Some years ago, Hall and Slayter proposed a model for fibrinogen, which consisted essentially of 3 beads, about 65 Å in diameter, that were held together by very thin strands; the length of the molecule was about 230 Å. More recent electron micrographs have indicated that at *p*H well above the isoelectric point, the average molecule of bovine fibrinogen appears to increase in length. A minimum length of approximately 230 Å was observed at the isoelectric point (*p*H 5.6) while at *p*H 11.9 the length averaged about 400 Å. The proposed model appears to be consistent with molecular weight data obtained from other sources. In a brief consideration of intermediate polymers of fibrinogen, Hall pointed out that polymerization appears to be end-to-end. Electron micrographs of fibrin filaments in clots have revealed that, in addition to the axial period of about 230 Å, there are intermediate bands. The fine structure of soluble fibrin closely resembles that of polymerizing fibrinogen, that is, there are units indistinguishable from fibrinogen molecules as well as end-to-end aggregates like those of intermediate fibrin polymers. While recent x-ray diffraction work by other researchers has confirmed the presence of the major periodicities in fibrinogen and fibrin as observed by electron microscopy, the relative intensities of diffraction orders cannot as yet be explained on the basis of electron microscopy; the x-ray findings may indicate the presence of substructure in the units observed by Hall.

S. J. Singer (Univ. of California, San Diego) discussed the development and present status of ferritin-antibody conjugates as stains for electron microscopy. The method, conceived and first developed by him and his co-workers, depends upon the chemical coupling of the ferritin (highly electron-scattering) molecule with antibody. At first, Singer used *m*-xylene diisocyanate as coupling reagent, and showed that such conjugates were only partly linked by covalent bonds. Use of another coupling reagent, toluene 2,4-diisocyanate, results in ferritin-antibody conjugates that are linked only covalently. Methods and controls necessary to check activities and specificities of ferritin-antibody conjugates were presented, and sources of error that have often been overlooked were pointed out. When an antigen to be reacted with conjugated antibody is in solution or in colloidal dispersion, the localization of the tagged antibodies can be quite specific. This is evident from electron microscopy of, for example, particles of tobacco mosaic virus or of influenza virus after contact with specific, conjugated antibodies. In spite of encouraging and significant results obtained in a number of laboratories, the application of the technique to the localization of material in sectioned cells is still encumbered by many unresolved problems—non-specific adsorption of specific conjugates, or of

unconjugated ferritin, or of inactive conjugates, and incomplete penetration into the depths of cells. Techniques whereby thin sections, suitable for electron microscopy, can be stained directly with antibody conjugates remain to be developed. In the methods hitherto used, antibody conjugates were applied to suspended cells or to thick sections; this was followed by preparation for electron microscopy. Singer demonstrated some encouraging results obtained by modifying current techniques for processing tissue and by use of a new embedding process.

That ferritin is particularly suitable for studies relating to uptake of proteins by cells was demonstrated by J. B. Caulfield (Harvard Medical School). He has studied the uptake of ferritin by Ehrlich ascites tumor cells in vitro. The ferritin molecule, roughly spherical and measuring about 120 Å in diameter when hydrated, is a globular protein. However, it contains 23-percent iron in the form of ferric hydroxide micelles, which, in electron micrographs, have a characteristic and specific appearance. These are situated in the centers of the molecules and can be considered as labels of the protein, apoferritin. Caulfield found that uptake of ferritin by cells can occur in two ways. In one of these, single ferritin molecules are adsorbed on the cell surface within 5 minutes, particularly when serum albumin or horse serum are present in the incubating media. Later there is invagination of the plasma membrane and eventually formation of pinocytic vesicles. Stages in the evolution of these vesicles into larger vacuoles were inferred from serial electron micrographs which also indicated that the macromolecules are eventually concentrated into a few intracellular compartments. The second form of uptake of ferritin molecules by the cells appears to be less specific; it is connected with adsorption on extracellular debris derived from dead cells. In regions of cell surfaces close to debris a wavy plasma membrane can frequently be seen, thus indicating considerable surface activity. Large numbers of ferritin molecules enmeshed in such debris are taken up by cells. Ingestion of such aggregates may be a response of the entire cell by involving large-scale modification of cell surface activity, and resembling phagocytosis. By contrast, uptake of single ferritin molecules involves quite localized modifications of the cell surface.

The importance of checking such qualitative findings against quantitative data obtained by other methods was pointed out by H. J. P. Ryser (Harvard Medical School). In collaborative work with Caulfield, Ryser studied uptake of I^{131} serum albumin by Ehrlich ascites tumor cells in suspensions in vitro. Recently, he has extended this work to include Sarcoma 180 cells, thus combining methods of isotope analysis with cell culture methods. The I^{131} serum albumin becomes fixed to the cells in two phases: an initial, rapid, temperature-independent adsorption and a subsequent slow fixation that increases linearly with time and that can be diminished by lowering the temperature of incubation. The nature of this second phase suggests uptake by means of pinocytosis. Control experiments indicated that biosynthetic incorporation of labeled I^{131} mono- or diiodotyrosine from the medium into cell protein was unlikely during the periods of observation. In both cell systems, uptake at 37°C was of the same order of magnitude, that is, 1.2 to 1.7 $\times$ 10^5 molecules per cell in 2 hours; these values are smaller than other reported estimates. Though probably insignificant as a source of nitrogen for the cell, the ingested protein may be important as a carrier of other substances.

G. W. Richter (Cornell University Medical College) presented observations on the quaternary structure of apoferritin and on the production of ferritin and apoferritin by two cell lines derived from human cancers and growing in vitro in defined media. Using modifications of the staining procedure with uranyl nitrate, he has found that the apoferritin shell of the ferritin molecule is composed of 20 equal subunits with diameters in the neighborhood of 20 Å, and situated at the vertexes of a regular dodecahedron. This is in agreement with recent chemical findings of British investigators.

Sequential changes in fine structure of HeLa and KB cells, associated with the production of apoferritin and ferritin during the first hours after administration of iron, were demonstrated. Both types of cells synthesize ferritin within 4 hours after administration of iron, and ferritin molecules are first encountered within discrete cytoplasmic organelles. While there are several kinds of such bodies, previously termed siderosomes, those which are seen within 4 hours after administration of iron appear to be associated with production of ferritin and may play a role in the insertion of iron hydroxide micelles into apoferritin shells. However, the final assembly of the apoferritin molecule, that is, construction of the dodecahedral shell, may also take place in these organelles.

Ferritin obtained post-mortem from livers or spleens of humans, horses, and rats was fractionated by disk electrophoresis in acrylamide gels to yield five fractions termed α-, β-, γ-, δ-, and ϵ- ferritins. Fractions from each species have characteristic electrophoretic mobilities. The fractions produced by HeLa cells differ from those obtained from human livers or spleens. Only an α-ferritin was obtained from KB cells and this had the same mobility as HeLa α-ferritin, thus differing from α-ferritin in human liver or spleen. That the fractions separated by electrophoresis are indeed ferritins was shown by electron microscopy but as yet no differences in their fine structure have been discerned, presumably because the electrophoretic determinants are too small to be revealed by methods now in use. In serological studies HeLa ferritin had the same specificity as ferritin from human liver or spleen, but KB ferritin lacked an antigenic determinant.

The discussion was led by G. Majno (Harvard Medical School). The proceedings of the symposium, which was supported by U.S. Public Health Service grant 2G313, will be published in *Laboratory Investigation*.

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Root Diseases Biologically Controlled

A first of its kind, an international symposium on factors determining the behavior of plant pathogens in soils, was held on the Berkeley campus of the University of California during the week, 7 to 13 April. A principal purpose of the symposium was to bring together investigators from divergent disciplines to focus attention upon the problems of biological control of root diseases. Representatives were invited from the fields of bacteriology, mycology, nematology, virology, soil science, entomology, zoology, anatomy, physiology, biochemistry, and ecology, in addition to plant pathology. Over 300 participants from 18 countries attended.