EARLY ELECTRON MICROSCOPES AND THE NEED FOR NEW PREPARATION TECHNIQUES

The great potential value of electron microscopy for biological research was often stressed and quickly understood in the middle 1930s, at the time when the first laboratory models of transmission electron microscopes were being built by Ruska, von Borries (1, 2), and Marton (3, 4). The reasons were clear: the new instruments were expected to have a resolving power 40–50 times higher than the best light microscopes then available. It was also understood from the beginning that a new technology was needed for preparing biological specimens for electron microscopy, definitely more refined and in some respects quite different from the traditional histological technology then in use for light microscopy.

The limited penetration power of electrons, and the ease with which they are scattered by any atom required that specimens of unusual thinness—for the thinking and experience of those times—be examined in a relatively high vacuum (~10⁻⁴ torr). The microtomes then in use for light microscopy could not cut tissue sections thinner than 1 μ, while the desirable specimen thickness for electron microscopy was estimated at ~0.1 μ or less. In addition, since the specimens were examined in vacuo, they had to withstand the removal of their volatile components, primarily the removal of water, without collapse or deterioration of their structure.

THE AVAILABLE OPTIONS

Two choices were available for bringing specimens within the acceptable range of thickness for electron microscopy: comminution or microtomy. In the first alternative, large structures were fragmented or dispersed by a variety of mechanical means, and
from the ensuing debris those objects were selected which proved to be small enough and interesting enough for examination. With this procedure, a study of structural relations within cells and tissues was evidently impossible. The second alternative, i.e. microtomy, had the appeal of preserving exactly these relations, thereby making them available for study. It also had the backing of a technology which was already well developed for the needs of light microscopy, although it was far from being refined enough for direct extension to electron microscopy. Yet a complete “undisturbed” picture of cellular organization at the level of resolution of the electron microscope was so attractive a goal, and the usefulness of microtomy in light microscopy was so well established, that most of the early efforts in working out preparation procedures for electron microscopy were directed toward refinements in microtomy. Such refinements were, however, hard to come, and so—for a while at least—microtomy absorbed a lot of work, while yielding practically no new information.

Two main choices were also available for coping with the other problem, i.e., the collapse and deterioration of structure during drying in vacuo. One was to rely simply on the inherent hardness of some specimens and to dry them in air or in vacuo before examination, assuming that their molecules are linked into structures rigid enough to withstand the surface tension generated during drying. The other choice was to support the specimen with a rigid embedding matrix which could in fact perform its supportive function not only during microscopy, but also during the preceding step already considered, i.e., microtomy.

EMBEDDING AND MICROTOMY

The use of a supporting matrix during microscopy was not favored, however, for it was expected to reduce contrast in the image as a result of the small difference in average electron scattering between the specimen and the embedding material. Electron scattering depends on atomic number, and the elemental composition of biological specimens is rather similar to that of available embedding matrices: both have a high content of atoms of low number. Hence, it was deemed advisable to avoid the use of an embedding matrix altogether or to remove it before microscopy (if introduced for the sake of microtomy), otherwise contrast in the embedded specimen would be too low to give a useful image (5). Some ingenious procedures were tried such as embedding in high glycols (Carbowax) to provide—a solid supporting matrix which was subsequently removed from the sections by solubilization in water. The sections were finally freeze-dried before examination (5). Freeze-drying was at the time in rather widespread favor (6) in electron microscopy.

The solutions initially worked out for microtomy proper consisted in a reduction—by a variety of mechanical devices—of the rate of advance of the specimen towards the knife in the microtomes then available for light microscopy (5); or in devices (7) or maneuvers (5) that produced wedge-shaped sections, since it was assumed that their tapering edge would be thin enough for electron microscopy. Another favored formula was the use of a special microtome provided with a blade rotating at high speed while the specimen was advancing slowly against the knife (9, 10). The ratio of the specimen to blade movements could be adjusted so as to give sections as thin as ~0.1 μ (10).

Notwithstanding these exercises in ingenuity, the results obtained by such techniques appear to us today as generally and profoundly disappointing. But the im-

---

1 Even freehand cutting was tried to obtain such sections (8).
pression they made on the early electron microscopists was quite different. A perusal of the literature of the period that covers the late 1930s and early 1940s shows that papers pertaining to electron microscope techniques or biological electron microscopy were amazing mixtures of highly enthusiastic texts and useless micrographs in which unidentified structures were described in great detail. It was assumed that these structures were unidentifiable on account of their novelty, but in retrospect it is clear that extensive damage to gross structures, rather than discovery of new fine structures, was responsible for the situation. In fact, the information extracted from such specimens was more limited and more fragmentary than that obtainable at the time by light microscopy. The reasons for these early failures can be traced in part to the lack of familiarity of the investigators with biological materials and histological techniques (most of them were physicists), and in part to the laborious character of the procedures used, and to the crude quality of the microtomy. The electron microscopes of the period were not at fault: they had already reached a resolving power 40-50 times higher than that of the light microscopes; their limit of resolution was at ~50 Å (11).

**FRAGMENTATION**

Studies of specimens prepared by fragmentation and drying, rather than microtomy, were advancing much faster. The choice of objects was limited primarily to protein fibers, but their investigation was proving rewarding and exciting. As far as preparation procedures went, it was—one could say—the survival of the toughest specimens, and the toughest was collagen (12), followed at a short distance by tropomyosin (13) and a few other fibrous assemblies (14). Electron microscope studies were revealing a high degree of order in the construction of these fibers, and the order was often of an unusual and unexpected character: the fibers were periodic, asymmetric structures. Such studies, carried out primarily by F. O. Schmitt and his collaborators (14), had the advantage of possible correlations with other means of inquiry—primarily low angle X-ray diffraction—and in time were to lead to the discovery of a new formula of biological structure: the production of fibers by the staggered assembly of highly asymmetric protein molecules. But exciting as they were, these investigations were contributing little to an understanding of cellular organization at the new level of possible exploration. The spin off was primarily technical and came from the finding that electron scattering at the level of certain bands or spots in such fibers could be increased by treating them with heteropolyacids, like silicotungstic, phosphomolybdic, and chloroplatinic acid (14). It was the discovery of staining equivalents for electron microscopy: heavy atoms, or complexes containing heavy atoms, could be used to increase the "density" of specific sites in a given structure. In time, this finding was going to affect substantially the development of preparation procedures; it finally led to the general use of heavy metal salts as means of enhancing contrast, and thereby rendered unnecessary the removal of embedding matrices before microscopy. Marton (11) had already called the attention of electron microscopists to another possibly useful "stain," namely osmium tetroxide, considered at the time as primarily a fixative.

**ALBERT CLAUDE'S FIRST CONTACT WITH ELECTRON MICROSCOPY**

This was the state of biological electron microscopy in the middle 1940s when Albert Claude, who at the time was associated with The Rockefeller Institute for Medical Research, began to take an interest in the new technique as a result of his studies on
subcellular components and on the Rous sarcoma virus. He had isolated and purified the virus and he had partially characterized it as a "ribose nucleoprotein" in the late 1930s (17). In connection with this work, he had also discovered that normal tissues contain particulate "phospholipid-ribonucleoprotein complexes" of comparable size (18). At the beginning he called these complexes "small particles," and later on changed their name to "microsomes" (19). The virus particles as well as the microsomes were of "submicroscopic" dimensions, by reference to the resolving power of the light microscope; hence, they were natural objects for an investigation by electron microscopy. Besides, Claude had been working on the isolation of a series of cell fractions from tissue homogenates by differential centrifugation, and had studied, among others, a mitochondrial fraction isolated from a rat lymphosarcoma (20). These mitochondria were of "microscopical" dimensions, i.e. they measured more than 0.2 μ in diameter, but electron microscopy could be used to explore their internal structure.

So Claude was ready to start his career in electron microscopy with a set of projects well suited for this type of investigation. But, in addition, he was entering the field with the point of view of a cytologist who had had already a long period of experience with animal cells in tissues and in culture. He knew and respected the cytological literature of the light microscope era and his intent was to use it as a foundation for future developments, rather than to disregard it. He had a clear understanding of what was essential and what was questionable in histological techniques, and was willing to experiment extensively using these techniques as a starting base. Finally, he believed in gentle, careful handling of biological specimens, and had an innate feeling for biological structure, which he expected to be, at the newly explorable level (or at any level), a "wonder of creation." His results, hence also his micrographs, had to measure up to these expectations.

ELECTRON MICROSCOPY OF ISOLATED MITOCHONDRIA

Claude's first piece of work in electron microscopy was a study of isolated mitochondria (21). He used the fraction obtained from the rat lymphosarcoma because he assumed it to be free of "secretion granules," and thus more homogeneous than equivalent fractions isolated from other sources. Technically the investigation followed the usual line then used in studying tissue fragments, but it dealt with a selected class of particles, and included a rather detailed study of the effects of a series of fixatives and of various extraction procedures on the morphology of the particles. Claude found strong suggestive evidence for the existence of a mitochondrial limiting membrane and for the presence of small particles (~100 nm in diameter) within the mitochondria. The limiting membrane, about which Claude continued to collect evidence in subsequent studies (22), turned out to correspond to the now familiar two mitochondrial membranes (inner and outer), whereas his small intramitochondrial particles may represent the intramitochondrial granules of later studies, or may have resulted from incomplete, uneven extraction of the mitochondrial matrix.

The work on mitochondria was carried out in collaboration with Ernest Fullam, using an early RCA microscope that belonged to an industrial laboratory (Inter-

---

2 This discussion does not cover early work carried out in virology. For pertinent reviews, references 15 and 16 could be consulted.
3 Formaldehyde, potassium dichromate, osmium tetroxide, and a few fixative mixtures recommended in histological technology for the fixation of mitochondria.
chemical Laboratory, New York). It was Claude’s only published venture in the electron microscopy of cell fractions. Although continuously interested in the morphology and the intracellular origin of the microsomes, the fraction he had recently isolated and characterized (18, 19), he did not publish a full paper on their morphology. The article on mitochondria (21) included, however, an electron micrograph of isolated microsomes which was primarily intended to demonstrate that they are different from mitochondria. Apparently at the time, Claude was interested in obtaining evidence on the existence and morphology of microsomes in situ, i.e. in intact cells, before proceeding further. At this juncture, his knowledge of the cytological literature was acting as a restraining factor. He could not identify satisfactorily the intracellular origin of the microsomes and he felt that he needed more evidence before deciding whether the microsomes were truly newly discovered structures or fragments of already known cytoplasmic components, like mitochondria, for instance (18, 20).

**ELECTRON MICROSCOPY OF CULTURED CELLS**

Since in the work on isolated mitochondria he had already encountered difficulties on account of the excessive thickness of the specimens, and since advances in microtomy were slow to come, Claude started searching for adequate specimens of a different type, specimens which could be processed for electron microscopy without the need of embedding and sectioning, cells—for instance—flat enough and thin enough to let most of the electron beam pass through. With a new and distinguished collaborator—Keith Porter, who had in the meantime joined the same laboratory4 at The Rockefeller Institute—and with Fullam he began to investigate the periphery of thinly spread fibroblasts grown in vitro from chick embryo explants (23). The cells were grown on glass coverslip covered with a thin plastic film (Formvar), they were fixed with a variety of reagents in an attempt to study systematically the effects of different types of chemical fixation, and a neat technique was developed to transfer the fixed cells, together with their supporting film, from the glass coverslips to the grids of metal wire used as specimen holders in electron microscopy. The specimens fixed in osmium tetroxide vapors or solutions gave truly remarkable images. The other fixatives (chromic acid, acid formaldehyde, Flemming’s mixture) caused coarse precipitation artifacts. In the thin peripheral layer of the cytoplasm of OsO₄-fixed cells, Porter, Claude, and Fullam (23) found a “lace-like reticulum” with strands consisting of “vesicle-like bodies... ranging in size from 100 to 150 nm” (Fig. 1). The “ground substance” around the reticulum appeared to be made up of particles ~100 nm in diameter. Claude was convinced by the results of his recent experiments with centrifuged hepatocytes of *Amphiuma* (24) that the microsomes were small, distinct particles which could be sedimented in a discreet layer within the cell. With this in mind, the fine particles of the ground substance, rather than the strings of vesicles of the “lace-like reticulum,” appeared to him and to his collaborators as the most likely intracellular equivalents of the microsomes. As far as the reticulum was concerned, they wondered whether it was not the animal cell equivalent of the “kinoplasm” of plant cells, at that time a fashionable but now forgotten entity.

Encouraged by the results obtained in the first study of cultured cells, and this time in collaboration with Keith Porter and Edward Pickels, Claude (25) then engaged in a much more elaborate project: to study cells grown in vitro from explants of

4 The Laboratory of Pathology of James B. Murphy.
chorioallantoic tumors produced in chick embryos by two different strains of Rous sarcoma virus, namely the original chicken tumor I strain (26), and the chicken tumor 10 strain isolated and maintained by Murphy and Sturm (27). In control cells, grown from buffy coats and assumed to be macrophages, the "lace-like reticulum" with its strings of small bodies and vesicles was found again clearly defined (Fig. 2). In cells infected with chicken tumor I virus, dense round particles, 67-80 nm in diameter, were detected singly, in doublets, or in small clusters, and tentatively identified as virus particles (Fig. 3). In cells infected with the chicken tumor 10 strain, similar particles were found in large two-dimensional clusters (Fig. 4).

The last two papers (23, 25) introduced a new type of specimen in electron microscopy—the thinly spread cultured cell; provided the best electron micrographs of cells and cell structures available at the time; produced the first convincing evidence for the existence of elaborate structure within the cytoplasm, below the limit of resolution of the light microscope: the "lace-like reticulum" that later on became generally known as the endoplasmic reticulum; and demonstrated for the first time by electron microscopy the presence of virus particles in infected cells. Without exaggeration, it can be said that these papers mark the beginning of the electron microscope era in cell biology. In retrospect, it is clear that the high quality of the micrographic evidence presented in these articles, the high contrast, and the sharp definition of the structures studied were due to a combined effect of fixation and staining of cellular membranes by OsO₄, and of partial extraction of the proteins of the cytoplasmic matrix by prolonged fixation and subsequent washing.

Tissue sections

Notwithstanding their many favorable features, cultured cells had their own limitations. To begin with, they represented an in vitro system quite removed from the normal conditions cells experience in situ in an intact organism. And to end with, even when spread in culture, only the periphery of the cells was suitably thin for electron microscopy; their central region remained too thick to give satisfactory images of the cell components it usually contains, i.e. the nucleus, the Golgi complex, and the centrioles. A possible solution still available was microtomy and Claude was already experimenting in this direction before the publication of the paper on chicken tumor cells. The group at the Interchemical Laboratory was developing at the time a high speed microtome (10) which was used to cut thin sections from rubber, acrylic resins, and nylon. The group was also experimenting with eutectic mixtures as embedding.
materials. Some of these mixtures were miscible with alcohol; they hardened and could be sectioned at low temperature, and they volatilized at 32°C. With such features, the then current desideratum of removing the embedding matrix before microscopy could be easily satisfied. Theoretically, the procedure had definite advantages since no solvent was needed for the extraction of the embedding material and thus damage during drying was averted. Claude became interested in this general approach and, with Fullam's collaboration, tried to obtain tissue sections from guinea pig liver fixed by perfusion with a solution of OsO₄ (28). Selected tissue blocks were embedded in an eutectic mixture of α-camphor and naphthalene and were sectioned with the high speed microtome developed by Fullam and Gessler (10). Claude followed the approach of a careful cytologist, and showed first that the specimens thus prepared gave satisfactory light microscope images to which electron micrographs of the same material could be easily related. These electron micrographs were above the standards of the day, for they showed more structural details than the corresponding light microscope pictures. The cytoplasmic matrix (or "ground substance") of the cells appeared finely granular and contained, in addition, a few strands of "filamentous material." Claude again assumed that the fine particles of the ground substance represented the microsomes and was quite reserved in his interpretation of the "filamentous material," since his fixation experiments with cultured cells had shown that many fixatives can produce coarse fibrillar precipitates in the cytoplasm. In retrospect, one can surmise that those "filaments" were probably elements of the endoplasmic reticulum, but in 1946 there was no retrospect.

The work on sectioned hepatocytes convinced Claude that microtomy was still far from perfection, and for a while he resumed his search for procedures that could avoid it.

REPLICAS

Impressed by the extensive use of surface replicas made at the time in metallurgical electron microscopy, Claude tried to replicate fixed, dried cells assuming that uneven distribution of structures as well as of water and solids should be detectable by this procedure. The assumption proved correct, but the structural details detected by replication turned out to be less informative and less impressive than those found in thinly spread cultured cells (29). A further development along this line of work was a

The fine particles seen in the cytoplasmic matrix were either particulate glycogen or protein precipitates.

FIGURE 2 "Sector of extended macrophage" published as Fig. 1 in reference 25, Electron microscope study of chicken tumor cells. 1947. Cancer Res. 7:421. (Reprinted by permission.)

The original legend mentions that the micrograph shows "common cell constituents previously described, i.e., Golgi bodies, mitochondria and components of the ground substance." The description of the micrograph in the text states that "the open region between the center of the cell and the cell margin is occupied in part by a material of finer texture and lesser density, seemingly made up of small bodies or vesicles of rather uniform size. The latter cell constituent may correspond at least in part to particulate components of the ground substance (microsomes)." The micrograph shows very clearly the chains of vesicles of the endoplasmic reticulum. Cultured cell fixed for 24 hr in a 2% OsO₄ solution, then rinsed for 20 min in distilled water. × 8000.
project he carried out in collaboration with Sanford Palay in which the morphology of giant chromosomes was studied by using replicas of squash preparations of salivary glands of Drosophila larvae (30).

**BACK TO MICROTOMY**

Since the gain in information obtained with surface replicas turned out to be rather limited, Claude turned once more to microtomy and, with the expert help of Joseph Blum, then the instrument maker of The Rockefeller Institute, built an experimental microtome which reflected in its design the various—mostly negative—aspects of his recent experience with high speed microtomes. The new instrument had a mechanical advance system and was operated manually at low speed. The knife was fixed, and the specimen holder was mounted on a revolving wheel which brought the specimen in contact with the knife only once, during the downstroke when a section was cut; the knife was bypassed during the upstroke, a device through which damage to the surface of the block—and therefore to the next section—was avoided. Another important innovation was the addition of a liquid-filled trough mounted against the knife's edge. As they were being cut by the knife, the sections began to float on the liquid surface and thus their folding was prevented and their spreading facilitated (22). The "single pass" and the "collecting trough" were novelties in microtome design. They were soon recognized as highly useful features, and were subsequently introduced in practically all new types of microtomes built for electron microscopy.

**AN END AND A NEW BEGINNING**

In 1948, Claude gave a Harvey lecture (22) in which he reviewed his work in electron microscopy and in cell fractionation and correlated—to the extent made possible by his findings—the structure and biochemistry of the subcellular components he had isolated and studied. At this time, his long period of hesitation concerning the intracellular origin of microsomes came to an end: he concluded that the most likely intracellular source of the new particles was the "lace-like reticulum" he, Porter, and Fulham had discovered a few years before. The conclusion was later on repeatedly confirmed by other investigators. A year after his Harvey lecture, Claude left The Rockefeller Institute to become director of the Jules Bordet Institute in Brussels. The first period of his activity was coming to an end, but the large vistas into the future his work had already revealed, and the power of the techniques he had introduced or perfected were already attracting many other investigators to the field he had opened. This field was to become in a short while the cell biology of the present time.

**AN ATTEMPT TO ASSESS THE IMPORTANCE OF CLAUDE'S WORK**

In introducing electron microscopy in cell research, Claude had over his contemporaries the advantage of a good knowledge of the objects of his interest. For him, all

---

**Figure 3** Chicken tumor I cell published as Fig. 3 in reference 23, Electron microscope study of chicken tumor cells. 1947. *Cancer Res.* 7:421. (Reprinted by permission.) The original legend states that the micrograph shows "the causative agent of the tumor as it occurs singly, in pairs, or in rows of 4 or 6. These bodies are approximately 70 to 85 mμ in diameter." Cultured cell fixed for 20 hr with a 2% solution of OsO₄. × 16,500.

George E. Palade *Biological Electron Microscopy* 15 d
those many granules one could stain within cells, in the vivid colors of some light microscope preparations, were as many intriguing mysteries worthwhile solving; they were far from being questionable entities hardly deserving any attention. Throughout his studies, he strived to work out a technique, or a combination of techniques, which could give—in structural terms—a full account of the cell, comparable to the full account he had achieved in cell fractionation. This search explains his continuous experimentation with such a variety of preparatory techniques and his eventual return to microtomy. The fixation, embedding, and microtomy procedures, which finally made possible the examination of all types of cells in situ, and revealed the complexity of the organization of eukaryotic cells, were worked out by other investigators in the early 1950s, but the development of their procedures relied heavily on many of Claude’s ideas and findings.

There was in the history of biology another period of comparably fast advances in instrumentation and preparatory procedures for microscopy, and in the accumulation of new findings bearing on tissue and cell structure. It occurred towards the end of the last century and it followed the introduction of lenses satisfactorily corrected for chromatic aberration which made possible a small but significant reduction of the limit of resolution in light microscopes (31). But, with the single exception of the discovery of chromosomes, the advances recorded at that time remained an isolated development which affected very little, if at all, the rest of biological sciences.

What was unique in the recent wave of advance that started in the middle 1940s was the immediate impact the new structural findings had on other biological sciences, primarily biochemistry and cell physiology. The impact was made possible by the concomitant introduction of the cell fractionation procedure which was Claude’s other major achievement. Subcellular components, already known from light microscopy or newly discovered by electron microscopy, could be isolated in mass from various tissues. Isolation was the first step in determining their biochemical composition and biological activities, which in turn was a prerequisite for understanding their function. Integrated structural-functional studies of various cell structures became possible, and with such studies modern cell biology started.

Since the middle 1940s techniques in this field have been continuously improved and refined, the mass of accumulated findings on the structure, biochemistry, and function of subcellular components has increased at an amazing rate, and our understanding of many aspects of cellular organization has progressed impressively. But spectacular as they may appear to us today, all these achievements are ultimately based on a few critical papers Claude alone or with his collaborators published in the middle 1940s. Seldom has a field owed so much to a single man.

REFERENCES
1. Ruska, E. 1934. Über Fortschritte in Bau und in der Leistung des magnetischen Elektronen mikroskopes. Z. Physik. 87:580.

Figure 4 Chicken tumor 10 cell published as Fig. 2 in reference 25, Electron microscope study of chicken tumor cells. 1947. Cancer Res. 7:421. (Reprinted by permission.) According to the original legend, this portion of the cell shows “in addition to normal constituents, patches or ‘colonies’ of the causative agent of the tumor.” Cultured cell fixed for 3 hr in a 2% OsO4 solution, then rinsed in distilled water for 15 min. X 10,700.
2. von Borries, B., and E. Ruska. 1939. Aufbau und Leistung des Siemens-Übermikroskopes. Z. Wiss. Mikrosk. 56:317.

3. Marton, L. 1934. La microscope électronique des objets biologiques. Bull. Acad. Roy. Méd. Belg. 20:439.
   Marton, L. 1934. Electron microscopy of biological objects. Nature (London). 133:911.
   Marton, L. 1935. La microscope électronique des objets biologiques. Bull. Acad. Roy. Méd. Belg. 21:606.

4. Marton, L. 1935. Le microscope électronique et ses applications. Rev. Opt. Théor. Instrum. 14:129.

5. Richards, A. G., T. F. Anderson, and R. T. Hance. 1942. A microtome sectioning technique for electron microscopy illustrated with sections of striated muscle. Proc. Soc. Exp. Biol. Med. 51:148.

6. Sjöstrand, F. 1943. Electron microscopic examination of tissues. Nature (London). 151:725.

7. von Ardenne, M. 1939. Die Keilschnittmethode, ein Weg zur Herstellung von Mikrotom- schnitten mit weniger als 10⁻³ mm Stärke für elektronenmikroskopische Zwecke. Z. Wiss. Mikrosk. 56:8.

8. Richards, A. G., and T. F. Anderson. 1942. Electron microscope studies of insect cuticle, with a discussion of the application of electron optics to this problem. J. Morphol. 71:135.

9. O'Brien, H. C., and G. M. McKinley. 1943. New microtomes and sectioning method for electron microscopy. Science (Washington). 98:455.

10. Fullam, E. F., and A. E. Gesseri. 1946. A high speed microtome for the electron microscope. Rev. Sci. Instrum. 17:23.

11. Marton, L. 1941. The electron microscope: A new tool for bacteriological research. J. Bacteriol. 41:397.

12. Schmitt, F. O., C. E. Hall, and M. A. Jakus. 1942. Electron microscope investigations of the structure of collagen. J. Cell. Comp. Physiol. 20:11.

13. Schmitt, F. O., C. E. Hall, and M. A. Jakus. 1943. In Frontiers in Cytochemistry. Biological Symposia. Jacques Cattel Press, Tempe, Ariz. 10:261.

14. Schmitt, F. O. 1944. Ultrastructure and the problem of cellular organization. Harvey Lect. 40:249.

15. Ruska, H., B. von Borries, and E. Ruska. 1939. Die Bedeutung der Ubermikroskopie für Virussforschung. Arch. Virusforschung. 1:155.

16. Luria, S. E., M. Delbrück, and T. F. Anderson. 1943. Electron microscope studies of bacterial viruses. J. Bacteriol. 46:57.

17. Claude, A. 1938. Concentration and purification of chicken tumor I agent. Science (Washington). 87:867.

18. Claude, A. 1939. Chemical composition of the tumor producing fraction of chicken tumor I. Science (Washington). 90:213.

19. Claude, A. 1941. Particulate components of cytoplasm. Cold Spring Harb. Symp. Quant. Biol. 9:263.

20. Claude, A. 1943. The constitution of protoplasm. Science (Washington). 97:451.

21. Claude, A. 1944. The constitution of mitochondria and microsomes and the distribution of nucleic acids in the cytoplasm of leukemic cell. J. Exp. Med. 80:19.

22. Claude, A., and E. F. Fullam. 1945. An electron microscope study of isolated mitochondria. Methods and preliminary results. J. Exp. Med. 81:51.

23. Porter, K. R., A. Claude, and E. F. Fullam. 1945. A study of tissue culture cells by electron microscopy. Methods and preliminary observations. J. Exp. Med. 81:233.

24. Claude, A. 1943. In Frontiers in Cytochemistry. Biological Symposia. Jacques Cattel Press, Tempe, Ariz. 10:111.

25. Claude, A., K. R. Porter, and E. G. Pickels. 1947. Electron microscope study of chicken tumor cells. Cancer Res. 7:421.

26. Rous, P. 1911. A sarcoma of the fowl transmissible by an agent separable from the tumor cells. J. Exp. Med. 13:397.

27. Murphy, J. B., and E. Sturm. 1932. Properties of the causative agent of a chicken tumor. VI. action of the associated inhibitor on mouse tumors. J. Exp. Med. 56:483.
28. Claude, A., and E. F. Fullam. 1946. The preparation of sections of guinea pig liver for electron microscopy. J. Exp. Med. 83:499.
29. Claude, A. 1949. Electron microscope studies of cells by the method of replicas. J. Exp. Med. 89:425.
30. Palay, S. L., and A. Claude. 1949. An electron microscope study of salivary gland chromosomes by the replica method. J. Exp. Med. 89:431.
31. Hughes, A. 1949. The development of microscopic observation. In A History of Cytology. Abelard-Schuman Ltd., London. 1.