Meetings

High-Resolution Autoradiography of Diffusible Substances

General interest in the use of and the recognized need for appropriate and reliable techniques in autoradiography led to the organization of an international conference which was held at the University of Chicago, Center for Continuing Education, 2–4 June 1968. Approximately 250 scientists attended the meeting.

The conference covered problems related to autoradiography as well as problems related to tissue preparation. Freezing and thawing, freeze-drying, low-temperature tissue cutting, and maintenance of enzymatic activity were discussed in detail. It was probably not by chance that this conference—the first of its kind—was initiated and organized by pharmacologists, for they are concerned with the localization of drugs in tissue cells and subcellular components. Most drugs and hormones are not incorporated into larger molecules but interact by reversible binding or even remain unbound; it is for this reason that, to date, only few creditable studies and little information about drug localization have been reported. Although centrifugal fractionation may be useful for bound substances, the use of liquid phases and disruption of cell structure may lead inevitably to translocation artifacts. Autoradiography has the advantage of making visible simultaneously the tissue structure and the site of the radioactively labeled compounds. This potential, however, can be utilized only if, during histologic tissue preparation as well as during tissue mounting, diffusion and loss of the label are avoided. It is mainly because of this obstacle that the potential of autoradiography has not been fully realized and, alas, a considerable number of artifactual and misleading data have been published. Increasing awareness of the difficulties in the technique have led many investigators to make serious efforts to develop more valid procedures. Many methods proposed for the study of diffusible compounds have been unsuitable since they did not, in fact, exclude the possibilities of translocation and extraction of the labeled compound. Frequently, technical steps were retained in spite of the obvious potential to produce artifacts; furthermore, histologic quality and autoradiographic resolution often remained unsatisfactory.

It was the purpose of the international conference on high-resolution autoradiography to (i) critically appraise the present state of the art; (ii) elucidate the deleterious effects of the various technical steps used in the histologic preparation of the autoradiogram such as fixation, clearing, embedding, cutting, and thawing; and (iii) provide guidelines for future development of techniques suitable for light-microscope as well as electron-microscope autoradiography, that is, to set stringent prerequisites which should be observed in the development and evaluation of new methods and the data derived from them.

Chauncey D. Leake (San Francisco) discussed the pioneering work of Hamilton, Soley, and Eichorn in 1940 on deposition of radioactive iodine in human thyroid tissue, in which he participated. L. J. Roth (Chicago) outlined his own efforts to utilize autoradiography in pharmacology and to promote the development of valid techniques. The mystique of the term "fixative" in tissue preparation was attacked and the less misleading expression "denaturing solvent" was proposed since liquid fixation, frequently assumed to be necessary as well as innocuous, is known to be associated with loss of exogenously applied compounds and of micro- and macromolecular tissue constituents. In this connection T. Peters, Jr. (New York) discussed artificial binding of amino acids during fixation with formaldehyde or glutaraldehyde. Autoradiography with compounds known to be incorporated into RNA, DNA, or protein, as was outlined by W. Maurer (West Germany), has numerous pitfalls. Tissue self-absorption and variations in the incorporation rate may give misleading results especially in the quantitation of such autoradiograms. S. R. Pelc (London) reviewed his laboratory experiments and discussed autoradiographic data and resolution. He used the technique for soluble compounds worked out by T. C. Appleton (London).

An autoradiogram may be invalidated not only by positive artifacts, that is, silver grain appearance at the wrong place, but also by negative chemography, a phenomenon studied by A. W. Rogers (Oxford) and P. N. John (Ujjain, India). Disappearance or fading of the latent image was observed most extensively with procedures which involved mounting of undried tissue or emulsion, which, as S. Holt (London) remarked, may be related to enzymatic digestion of the gelatin in the photographic emulsion. Various methods were reported for the localization of labeled inhibitors at motor end plates (P. Waser, Zurich), the metabolism of iodide ion in the thyroid (N. J. Nadler, Montreal), and the localization of iodine-125 hippuran in the kidney (R. P. Wedeen, New York). H. Eckert (Basle) reviewed various technical approaches to the localization of drugs at light- and electron-microscopic resolution. Several papers dealt with the localization of labeled steroid hormones such as testosterone (H. Levi, Copenhagen), aldosterone (R. Bogoroch, San Francisco), and estradiol (E. V. Jensen *et al.*, Chicago). The value of a dual technical approach in combining centrifugal fractionation and high-resolution autoradiography was demonstrated for the establishment of a two-step mechanism for the interaction of estradiol with the uterus of the rat. The method of dry mounting of freeze-dried sections was presented by W. E. Stumpf (Chicago), and its efficacy was demonstrated. Dry mounting of freeze-dried sections excludes all known sources of diffusion, such as liquid fixation, embedding, clearing, thawing, and wet-tissue and wet-film mounting. The method is based on low-temperature tissue preparation with cryostatic sectioning at −60°C or below and freeze-drying at dry ice temperatures. D. A. Brown *et al.* (London), using the same technique, reported on the localization of the extracellular space indicators ^{3}H inulin, ^{3}H mannitol, and ^{35}S-sulfate. The use of freeze-dried sections for quantitative biochemical analysis—as developed by O. Lowry—was demonstrated in two papers presented by G. M. Lehrer (New York) and

A. M. Burt (Nashville). Plant tissue was found more difficult to prepare than animal tissue. The prevention of virus redistribution in plant cells during the preparation for autoradiography was discussed by D. E. Schlegel (Berkeley) and W. G. Langenberg (Lincoln). The superiority of section freeze-substitution in the preservation of certain enzyme reactions compared to classical fixation and embedding techniques was demonstrated by J. P. Chang (Houston). Although this method has been used for the tissue preparation in autoradiography, the application of an organic solvent, albeit at low temperature, may obviate its applicability in the autoradiography of diffusible compounds. The implications of freezing and thawing for maintaining tissue structure and viability were discussed in two papers by H. T. Meryman (Bethesda) and B. F. Trump (Durham). It was emphasized that ice crystal disruption and cryosmosis probably cannot be eliminated but may be minimized by rapid freezing and maintenance of low or ultralow temperatures during tissue preparation. Preliminary data on the utilization of low-temperature tissue cutting for electron-microscopic autoradiography were shown by T. C. Appleton (London) with a cryostat microtome and by A. K. Christensen (Stanford) with a freezing microtome. The subcellular morphology in the presented photomicrograms appeared different from those obtained by classical fixation and embedding procedures. Considerable improvement of technique will be needed, however, before judgments on authenticity can be made and classical pictures are challenged. Electron-microscope autoradiography of diffusible compounds will have to await the perfection of low-temperature tissue preparation and, as S. Ullberg (Stockholm) pointed out, the present situation is such that it is still not realizable but promising, as he stated 4 years ago at the conference on Isotopes in Pharmacology held at the University of Chicago, Center for Continuing Education, June 1964. That such hope is justified was eloquently demonstrated by M. M. Salpeter (Ithaca) who provided models for a quantitative approach to the evaluation of resolution and sensitivity based on progress made in EMAR with classical preparative techniques.

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