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CYTOCHEMICAL BIOASSAYS

TECHNIQUES AND CLINICAL APPLICATIONS

edited by
J. Chayen
and
Lucille Bitensky

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Techniques and Clinical Applications

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Preface

In recent years there has been a resurgence of interest in bioassays. Two factors have contributed to this interest. The first is the fact that, in a proportion of cases, immunoassay has yielded results that are at obvious variance with the clinical and physiological status of the patient. The second is the development of sensitive in vitro bioassays that are at least as sensitive as the equivalent radioimmunoassays, so permitting comparison between immunoactivity and bioactivity. The most sensitive of these in vitro bioassays are the cytochemical bioassays that are at least one thousand times as sensitive as the equivalent radioimmunoassays. They have the further advantage that the same apparatus, and the same expertise, can be used for the bioassay of any polypeptide hormone. The cytochemical bioassay system has also proved to be of special value in helping to elucidate the mode of action of hormones. This use of the system has disclosed the existence of immunoglobulins that can block the effect of hormones.

These bioassays, and the cytochemical bioassay system, are now being used widely both for clarifying clinical conditions, such as the role of blocking antibodies in thyroid pathology, and for more academic research. Consequently we agreed with the editors of this series that the time seemed ripe to review this rapidly expanding field. We therefore invited some of the leading investigators to describe the cytochemical bioassay relevant to their investigations and to discuss the clinical and research implications of their findings.

J. Chayen and Lucille Bitensky

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CYTOCHEMICAL BIOASSAYS

about the book . . .

This authoritative volume provides comprehensive coverage of *Cytochemical Bioassays*–a powerful, in vitro technique one thousand times more sensitive than equivalent radioimmunoassays. With contributions by 29 leading international experts, this single-source reference provides the information required for thorough understanding and effective application of this advanced methodology.

For each hormone examined, *Cytochemical Bioassays* offers complete guidlelines to the assay–from basic endocrinology to procedures and analyzing the results. Research and clinical endocrinologists, biochemists, clinical chemists, pathologists, molecular and cell biologists, and geneticists will welcome this important information as a vital aid to their work.

about the editors . . .

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General Introduction to Cytochemical Bioassays

J. Chayen and Lucille Bitensky / Kennedy Institute of Rheumatology, London, England

ORIGINS

Quantitative cytochemistry, as it is used in the cytochemical bioassays, was developed over many years as a form of truly cellular biochemistry, that is, the measurement of metabolic activity or of active moieties in individual cells within a histologically complex tissue. These developments have been discussed elsewhere (Chayen and Bitensky, 1968; Chayen, 1978a; 1980). To achieve this histological specificity, the sensitivity of measurement had to be increased so that the activity of one cell could be measured, as contrasted with the mean activity of one million cells that is used in conventional biochemistry. This was done by the use of scanning and integrating microdensitometry, which had been developed earlier (Deeley, 1955) for measuring the amount of Feulgen stain (for DNA) in individual nuclei. It is now clear that microdensitometry of individual cells yields results that are quantitatively comparable to those obtained by more conventional procedures, done on aliquots of 10^6 cells (Chayen, 1978b; Olsen et al., 1981).

The methods of quantitative cytochemistry depend on chilling the tissue, and sectioning it at low temperature, without producing any observable ice artifact. Perhaps the best validation of these techniques (Chayen and Bitensky, 1968; Chayen, 1978a) is the fact that sections, prepared by the techniques described in Chapter 3, respond to the relevant polypeptide hormone with the same sensitivity as do the segments of the target organ. Methods were then devised for

retaining the integrity of the undenatured sections during the cytochemical reaction designed for disclosing the required enzymatic or other activity (as discussed in Chapter 3). The aim of quantitative cytochemistry is to precipitate the colored reaction product in the cell in which the chemical activity resides. The section is then inspected in the microdensitometer to determine the histology and to identify the target cells; the instrument can then measure the amount of reaction product specifically in these cells.

At the same time as the methods of quantitative cytochemistry were being developed, recourse was being made to the system of nonproliferative organ maintenance culture that had been developed by Trowell (1959). Thus, for example, samples of human synovial tissue were maintained in vitro, with no apparent change either in histology or in biochemical activity (Chayen and Bitensky, 1982), in order to test the effect of anti-inflammatory agents.

These methods, both of maintenance culture and of quantitative cytochemistry, found use in many diverse applications (Pattison et al., 1979). About 1970, the late Professor John Daly suggested that, because of their sensitivity and because they could measure changes solely in the target cells, they ought to be applicable to the development of very sensitive bioassays of polypeptide hormones. At that time, his interest in adrenocorticotropic hormone (ACTH) coincided with our rheumatological interest in this hormone. The first demonstration of the feasibility of this project was given in 1971 (Chayen et al., 1971). The first cytochemical bioassay, which was for ACTH, followed shortly (Chayen et al., 1972).

THE NEED FOR SENSITIVE BIOASSAYS OF POLYPEPTIDE HORMONES

For polypeptide hormones at least, there can be no doubt that "the hormone" is a biological concept and must be measured by the biological activity which it evinces. Consequently, for many years, such hormones were detected, defined, and measured by in vivo bioassay. However, these generally proved to be too insensitive for measuring normal circulating levels in humans or in animals; the best that they could achieve was to demonstrate excessively high circulating levels. [It may be remarked that, according to some authorities, such as Orth (1977), this is the most clinically useful purpose of assays.]

The advent of radioimmunoassay, and now of the other types of immunological assay, produced profound changes in the assaying of polypeptide (and other) hormones. The much improved sensitivity over the older in vivo bioassays, made it possible, in most cases, to measure the normal circulating levels of these hormones and to define conditions in which there was excessive, or too little, secretion of the hormone. The fact that these immunoassays could be automated gained them ready acceptance in routine clinical chemistry.