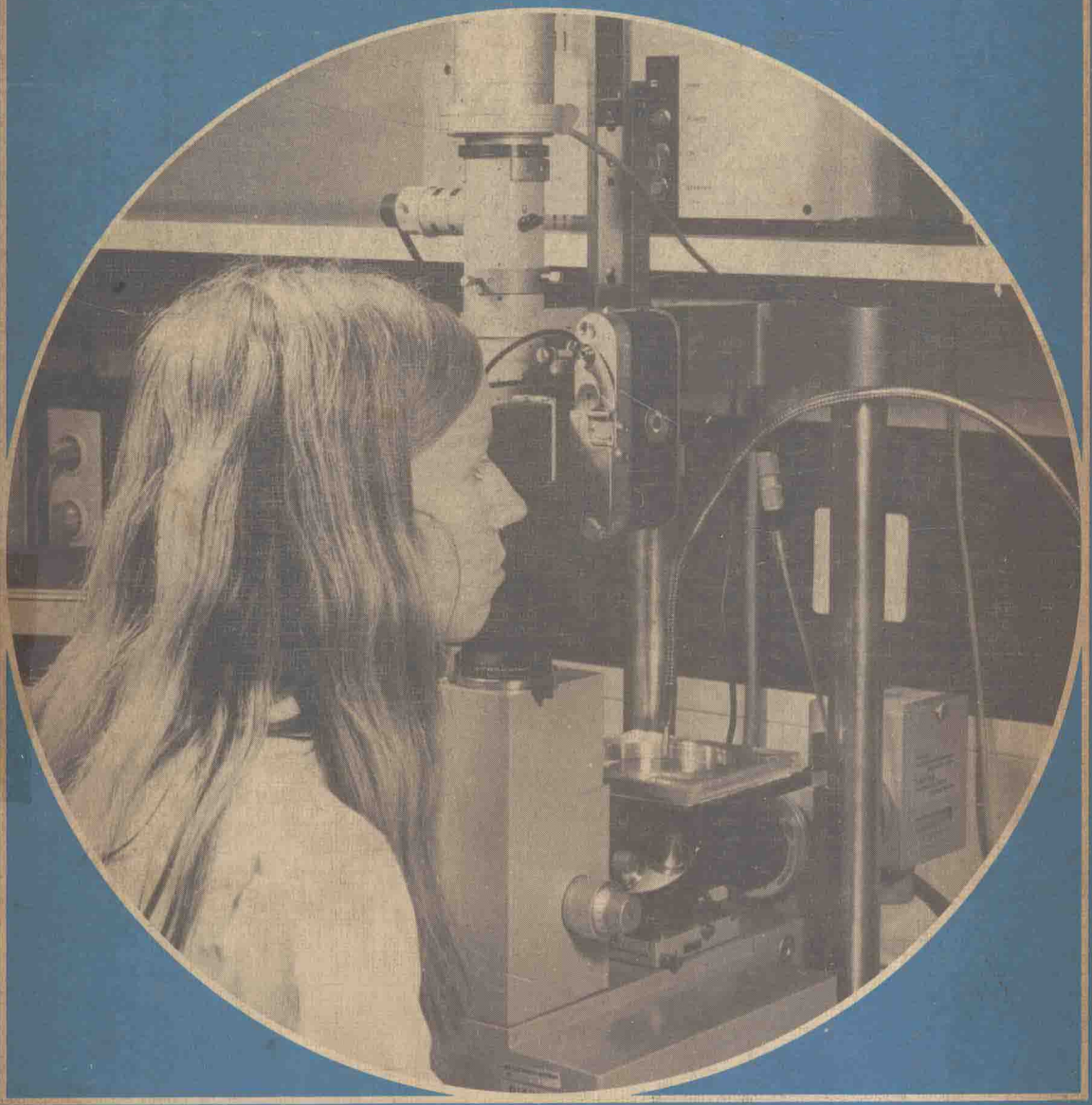
Ciba Foundation Symposium 73 (new series)

Trends in Enzyme Histochemistry and Cytochemistry



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Preface

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This symposium, planned by the Ciba Foundation at the suggestion of Dr Peter Stoward, has ensured that a number of specialists took a hard look at the current state of the art of enzyme histochemistry and cytochemistry. Of course, a rather severe process of selection of topics for discussion was necessary, as a limited time was available; nonetheless, several major aspects have been covered in depth.

The discipline of histochemistry and cytochemistry is concerned with the chemical composition and behaviour of biological materials in relation to their original structure, be it at the tissue (i.e. histological), cellular (i.e. cytological), or cell organelle (i.e. submicroscopic morphological) level. To some extent controlled fragmentation of tissues and cells, followed by separation of the component cells or organelles by centrifugation, can be used for such studies. The separated fractions can be analysed by conventional biochemical techniques since these separation procedures generally provide enough material for such analysis. With modern developments in centrifuging techniques (e.g. zonal centrifuging with small rotors) it is possible to obtain very good separations of the component structures in tissue biopsy samples weighing only a few milligrams. Moreover, biochemical studies of isolated cell populations (e.g. isolated hepatocytes) are now producing much valuable biochemical data with respect to cell to cell variations in enzymic and other biological activities. The chemical data obtained in these ways, however, although revealing much about the molecular composition of certain types of cells and organelles, have not contributed much to our knowledge of the variations in biological activity and chemical composition between individual cells (or cell parts) within organized

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tissue structures. The problem of identifying and quantifying biological and chemical differences between cells in organized tissues remains a problem for microscopic techniques. One approach that has not been extensively used is microdissection of small pieces of tissue or individual cells from their surroundings, followed by microchemical estimation of the isolated samples. Such techniques (e.g. as practised particularly by the work of Glick, Lowry and Morrison) give quantitative information about the distribution of enzyme activity or biochemical substrates within different regions of a tissue that is heterogeneous in cellular type and in the distribution of biological activities. A second approach, which was the subject of this symposium, is microscopic histochemistry and cytochemistry.

In the latter approach, microscopic methods are used to provide data on individual cells or cell components preserved in an organized tissue framework or matrix. Histochemistry and cytochemistry are related closely in this way to histology, cytology and pathology, and, in fact, form a disciplinary bridge between these subjects and biochemistry.

The major experimental objective in histochemistry and cytochemistry is to develop 'staining' (or other visualization) methods for tissues and cells such that observation and measurement of the visualized object under the microscope (using optical absorption, fluorescence or electron-scatter techniques, for example) reveal an aspect of its molecular composition in relation to associated structures. This approach dates back in principle to Raspail's 'Essai de Chimie Microscopique Appliqué à la Physiologie' which appeared in 1830. Although over the subsequent 100 years some (very limited) success was achieved in combining microscopic and chemical approaches to cellular studies, the lack of relevant theory and of appropriate techniques inhibited most approaches. In consequence, morphology and biochemistry developed along largely independent lines.

Around 1930 Raspail's approach was taken up more successfully not only by qualitative studies of the microscopic images of stained cells, but also by quantitative studies of cellular components *in situ*. In particular, the groups of Caspersson and Pollister began to use the microscope as a quantitative measuring instrument. Since their early work there has been a progressive refinement in technology, with the recent introduction of microprocessing being a major event. Automated and computer-directed microscopes and flow instruments are now operating that can apply pattern recognition techniques, morphometry and cytophotometry to naturally present (or chemically produced) variables in cells and tissues.

Such rapid technological developments over the last few years emphasize the need to define precisely the chemical mechanisms of the histochemical and

cytochemical methods being used, and to ensure that the staining or visualization methods should localize the component in question at its original site within the cell or tissue. It is also desirable that the staining intensity (or other visualization procedure) should reflect in a reproducible manner the amount or activity of the components under study. It is probable, however, that compromise will have to be made on these aims *in toto* since, in these objectives, the histochemist/cytochemist is virtually aiming at the impossible: to study chemical components accurately within a complex intracellular matrix without significantly altering the structural relationships and interrelated biological activities. This makes such quantitative studies liable to criticism from the points of view of specificity, topology or quantitative reliability, or all three. In fact, histochemistry has for many years been a largely qualitative discipline. This is in marked contrast to biochemistry, where the emphasis has been mostly on measurement. There were at least two reasons for this. Firstly, the chemistry behind the often complex chromogenic histochemical reactions was poorly understood, and secondly, equipment for measuring histochemical end-products inside tissue sections was not readily available. In recent years, much of this has changed. More is known about the intricacies of histochemical reactions, and suitable measuring equipment can now be obtained commercially. Thus, one can now place a stained section in such equipment and obtain precise values for the intensity of staining in any desired region of the section. However, it is important not to confuse the concepts of precision and accuracy. Precise results obtained from a sample lie closely together, with only a small spread of values. They can, however, be far from the 'true result' in terms of biological activity and relevance. Although modern instrumentation can give us precise results, are they accurate? They can only be so if the histochemical methods used are valid.

It was this concern over validity that in part led to the present symposium.

Among the early objectives discussed for the symposium were, first, to attempt to obtain agreement on the minimum number of criteria that quantitative histochemical and cytochemical techniques should meet when applied to the localization and quantitative measurement of enzymes and other components in tissues and cells; and, secondly to assess how far existing techniques meet such 'agreed criteria'.

It became clear during the symposium, however, that some participants felt that histochemical systems are so complex and, at present, so little understood in chemical terms that it would be premature to draw up hard and fast recommendations. Another view expressed was that even if firm criteria of rather general applicability could be stated, they would be so complicated and hedged with qualification and traps for the unwary, that they would be no advantage for most workers entering the field.

In the event, the participants at the symposium spent little time talking of 'agreed criteria' and devoted most time to discussing the authoritative presentations of the new and varied methodologies currently used in quantitative histochemistry and cytochemistry. In addition, some considerable time was devoted to the relationships of quantitative cytochemical techniques and concepts to other relevant disciplines.

Of course, in an evaluation of the appropriate methodology for tackling a biological problem involving the association of structure and activity, histochemistry is only one type of approach and no more. It is not exclusive of other approaches and may not even be appropriate in the particular circumstances under study. In many cases, histochemical and cytochemical techniques may be better used in conjunction with other approaches, such as traditional biochemical techniques, to obtain a clearer understanding of the functions of cells and tissues, and the mechanisms operating in disease states.

During the symposium there were repeated references to the chemical complexity of many of the staining reactions currently used, and to the importance of increasing our understanding of the underlying mechanisms of such reactions. I have no doubt that this is one of the important and more immediate goals for work in this discipline, and one which should be coupled to a more general use and availability of chemically purified reagents. At present, there are obvious deficiencies in studies of certain reactions: modern technology has allowed very precise measurements to be made but the accuracy (or biological significance) of such measurements is clouded by lack of understanding of the basic reactions and, very often, by the use of uncharacterized initial reactants.

At present histochemical and cytochemical techniques fall into two main classes, qualitative and quantitative. The qualitative staining methods are useful where the stained product can be related empirically to some cellular or physiological function. A much-used example has been the reaction for succinate dehydrogenase as a morphological marker for mitochondria. Where such a reaction is used as a qualitative pointer to mitochondrial location it has, in general, some considerable value, and for such observations it is not necessary to understand the detailed chemical kinetics of the reaction. However, when such a reaction is used to reflect mitochondrial respiratory activity the reflection obtained may be grossly distorted. As pointed out clearly in the symposium, for such quantitative data on enzyme sequences to be obtained, the enzyme to be measured must be carefully chosen to reflect metabolic flux through the sequence. Given such a choice of enzyme it is further necessary, for quantitative studies, to validate the method so that changes in enzyme activity arising from alterations in the physiology of the cell or tissue can be reliably distinguished from artifactual changes originating from technical variables.

Many papers presented at the symposium emphasized the great advances in data acquisition systems and in data processing that have occurred recently. It seems clear that quantitative histochemistry and cytochemistry are undergoing a revolutionary development and expansion through these technological innovations. What is needed, in addition, is some similar expansion in the variety of enzymes and other cell components that can be accurately measured by staining procedures. Such developments will clearly serve to strengthen the contributions that quantitative histochemistry and cytochemistry will make to our overall appreciation of cellular and tissue biology; they will also increase the use of the relevant techniques by a much larger group of investigators than at present.

The symposium allowed a group of like-minded persons to get together and discuss the current use and quantitation of histochemical and cytochemical procedures, and to critically examine present standards in the areas of study. No unequivocally clear conclusions were reached that would allow firm recommendations to be made concerning standards to be adopted in future studies. On the whole, however, the symposium gave timely opportunity for valuable interactions to occur between workers with different approaches to quantitative histochemistry and cytochemistry.

In my view, the use of quantitative histochemical and cytochemical methods in pathology will be one of the key developments during the next 10 years and will encourage important and related developments in the related disciplines of cytology and biochemistry.

The future of quantitative histochemistry and cytochemistry looks bright when viewed against the increasing awareness of the importance of precisely relating activity to structure in heterogeneous tissues and cells, and against the rapid advances in microcircuit technology that are making cheaper and more sophisticated instrumentation available.

In writing this preface to the symposium I have been much helped by notes provided by Professor van Duijn, Dr Stoward and Dr Altman. In addition, a post-symposium meeting at Brunel University between these colleagues and Professor Holt was of great assistance to me; I am most grateful to them for their advice and help.

Introduction

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This symposium was designed to examine in depth the major concepts, principles and practices of quantitative histochemistry. During the presentations or the discussions some of you are sure to say, no doubt with quiet but deep conviction, that you don't think much of the procedures, practices or techniques of Dr X or Professor Y. In such cases, I shall interrupt to ask you to justify your criticism. It is important, in my view, that we do this in order to understand the basis for criticism so that we can then decide whether it is well founded in fact or is based on hearsay or even on private prejudice.

Another aspect of my chairmanship, and one for which I offer no excuse, is that I am largely ignorant of the finer details of the experimental procedures of quantitative histochemistry. My own areas of interest are free radical biochemistry and cancer, so I hope to be educated here in a vigorously developing and important area of scientific enquiry. For a specialized meeting to have such an uninformed chairman has some clear advantages and some disadvantages: the advantages are that I have no histochemical axe—or knife—to grind and I shall be looking at the problems that are raised from a different direction, scientifically, to most of you. The disadvantage is that I will probably be asking very naive questions—but this may be a blessing in disguise if it helps the non-specialist to understand the main points at issue. On this particular point may I ask you to avoid the use of jargon or technical phrases peculiar to you or your group: even if you don't confuse our foreign visitors you will certainly confuse the chairman.

Having stressed that my own scientific expertise is not in the particular area of this symposium, let me hasten to add that I have long recognized the

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tremendous contribution that histochemistry and cytochemistry can make to biochemistry in general and to my own area of study—tissue injury—in particular. The application of histochemical techniques directs much-needed biochemical attention to the variability of cellular activities in organized tissues, and to the dangers inherent in working with homogenized suspensions obtained from complex cellular varieties. Even nowadays, in my opinion, some biochemists are not sufficiently aware of the complex cellular structure of tissues such as lung or liver, or of the spectrum of biological activities of which cells of a similar type are capable, depending on their precise tissue location. Three examples based on my own experience will indicate my interest in this area.

When the amino acid analogue ethionine is given to a rat in an acute dose it produces a fatty liver; chronic dosing produces profound biochemical changes in the liver tissues of the treated animals. Ethionine is also a rather weak carcinogen and I was interested (Slater & Sawyer 1966, Slater & Delaney 1971) in studying the profound changes that occur in liver ATP, liver protein synthesis, liver nucleotide levels, bile flow and so on. However, how can such changes that occur after chronic dosing be interpreted? By that time, the whole architecture and cellular make-up of the liver has changed. Ethionine produces hyperplasia of the bile duct and the proportions of the cells that make up the complex liver tissue change markedly (Rubin *et al.* 1961) so that one ends up looking at a tissue with a completely different cellular structure. This is an example of biochemical changes being associated with a change in tissue organization, and in such cases analysis of homogenates or tissue fractions is difficult if not impossible to interpret.

A similar example, which I discussed here at the symposium on lysosomes in 1963, is the increase in the activity of lysosomal enzymes in the rat mammary gland during early mammary involution. Before 1960 or so, about 16 enzymes had been studied in the various stages of the lactation cycle in the rat. Most of them reached a peak during lactation that coincided with the peak milk production and then fell rapidly during mammary involution. In about 1960 to 1962 a few components—lysosomal enzymes—were found that actually increased in activity in involution, as discussed at the 1963 symposium (Slater *et al.* 1963). It turned out in retrospect that this increase is due to a migration into the mammary gland of a completely different cell type so that in early mammary involution we were analysing a different set of cells to those we were analysing in late lactation. This is an example of biochemical changes being associated with changes in cell type, and these data depended greatly on the application of histochemical procedures for their elucidation.

As a final example of this point, I can mention some recent studies that we have done with the hepatotoxic agent carbon tetrachloride, which requires

metabolism in the liver by cytochrome *P*-450 to a free radical product that is chemically very active. This free radical product (probably the trichloromethyl radical: Slater 1979) destroys some of the parenchymal cell cytochrome *P*-450. However, this destruction is not uniformly spread throughout the lobule but is concentrated very much in the cells of the centrilobular areas. The *P*-450 in these particular groups of cells is almost completely destroyed whereas the *P*-450 in the periportal region is untouched (J. Chayen, J. Johnston, K. Cheeseman and T.F. Slater, unpublished). In this particular case, which was studied using microdensitometry, we found that a biochemical change, which had been studied earlier by gross analysis of microsomal fractions, was actually located within a particular group of cells of the same type in the same organ.

I have described these three examples to explain why, although I am not a specialist in the area of histochemistry and cytochemistry, I have long been sympathetic to it and have valued very much its contributions to biochemistry in general.

The critical use of histochemical and cytochemical procedures raises many questions relating to precision, reproducibility, specificity and validity. Quantitative histochemistry is an area of some considerable controversy, and this symposium is an important opportunity to raise, discuss and evaluate many issues of current interest.

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