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# THE GOLDEN AGES OF HISTOLOGICAL TECHNIQUE*

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## Summary

The growth of our knowledge of normal and pathological histology has been accelerated by two periods of spectacular technical developments. The first, at the end of the nineteenth century, produced most of the dye staining methods for sections that are now in use. The second, dating from the 1950's to the present time, has seen the application of electron microscopy, histochemistry and immunohistology to histopathological problems. The combination of the older dye staining methods with the more recent non-dye staining techniques has overcome many technical restraints and offers new dimensions of sensitivity and precision to histopathology.

Scientific advances have been greatly dependent upon technical methodology and new knowledge has always followed the development of more advanced apparatus and more sophisticated laboratory techniques. These considerations have been true in the case of histopathology and it may be worthwhile to look at the two golden ages of histological technique during which spectacular progress has been made. One of these was in the past, but we are in the second at the present time.

In the first half of the 19th century the knowledge of the histological structure of animal tissues was severely limited by the ***quality of the microscope,*** the ***thickness of tissue sections*** and the ***lack of satisfactory dye staining*** of tissues. Compound microscopes were then in use, but microscope lenses were of simple type which were not corrected for chromatic or spherical aberration and their resolution was poor. The fact that sections were thick and the precise staining of tissue components was not possible did not matter at that time, as it was not possible to observe the details of histological structure with the microscopes then available. By the middle of the century an achromatic objective had been developed by Lister, the father of Joseph Lister of antiseptic fame, and in the later decades the appearance and performance of compound monocular microscopes did not substantially differ from those of today. The stage was not set for the detailed examination of tissues and for the application of histology to the field of human pathology. Western Europe, especially Germany, was the scene of the first golden age.

## THE FIRST GOLDEN AGE OF HISTOLOGICAL TECHNIQUE (1875–1905)

The first requirement of histologists was for ***sections of tissues*** that were ***sufficiently thin*** to ***be*** satisfactorily examined by transmitted light. Early microtomes were little more than holders in which tissues could be supported by vegetable material such as pith. From these, free-hand thick sections were obtained, but German pathologists used ***freezing*** to give tissue sufficient support for section cutting and a high degree of expertise in the cutting of frozen sections was developed towards the end of the 19th century. The next development, also in Germany, was brought about by the application of the new products of the ***synthetic dye industry.*** These dyes, originally used for animal and vegetable fibres such as wool and cotton, were ideally suited to the staining of tissues and by the end of the century many of the histological staining methods that are in present use had been developed. ***Fixatives,*** combining the preservation of tissues and compatibility with subsequent staining methods, were products of this time, as were the routine stains such as the ***haematoxylins and eosin*** (Table I). All that was needed for successful histology was now available – satisfactory sections, differential dye staining of cellular components, and microscopes that were capable of observing these structures. Even though few sections from this era have survived, elegant pen and ink drawings have been published and these speak eloquently of the quality of the histological preparations. This golden age of histological technique made possible the development of

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TABLE I

THE DEVELOPMENT OF FIXATIVES AND ROUTINE STAINS

| FIXATIVES | |
|---|---|
| FLEMMING | 1884 |
| CARNOY | 1887 |
| ZENKER | 1894 |
| BOUIN | 1897 |
| KAISERLING | 1897 |
| **HAEMATOXYLIN (1876)** | |
| EHRLICH | 1886 |
| HEIDENHAIN IRON | 1896 |
| MALLORY P.T.A.H. | 1897 |
| HARRIS | 1900 |
| MAYER | 1903 |
| WEIGERT IRON | 1904 |
| **EOSIN (1876)** | |

histopathology, which was inaugurated by Virchow's book on "Cellularpathologie"(1). The gross appearances of diseased tissues were recognised in the post mortem room, their cellular structure was determined using histological sections, bacteria were observed, cultured and classified and the early stages of theoretical and practical immunology were reached. Moreover it was not just the routine haematoxylin and eosin staining that was in use but many of our ***special histological techniques*** originated from this time. The staining of bacteria in tissue sections was developed from the methods of the bacteriologist and Perls' reaction for haemosiderin is a classical example of a chemical method in histology(2). Specialised types of cells such as connective tissue fibres were differentiated by van Gieson or elastic stains and intracellular components of the nucleus and cytoplasm were also demonstrable. Much interest in the human brain, stimulated by theological, philosophical, and scientific argument, led to the appearance of neurohistological methods that are still in use today. By the turn of the century most of the staining techniques that we now use for the demonstration of tissue morphology had been developed (Table II). Whilst histological technique was very much an art it was becoming a science.

PHASE OF CONSOLIDATION (1905–1950) This period of spectacular progress in histology was followed by a time in which there was slower, but steady, development. One of the most important advances was the use of ***paraffin sections.*** These replaced frozen sections for most purposes and the ability to preserve paraffin blocks and prepare permanent stained sections were particularly valuable in the growing field of diagnostic histopathology. Additional dye-staining methods continued to appear, but at a slower rate, and these included the first methods for reticulin fibres in the 1930's modified from the original Bielschowsky silver impregnation method for neurofibrils(3); the trichrome methods also appeared at about the same time. Towards the end of this period there were important events that were to foreshadow the second golden age, such as the first systematic interest in chemical methods for the demonstration of tissue components. Lison(4), working in France, brought forward the science of ***histochemistry*** by the publication of "Histochimie animale" in 1936 and Gomori described histochemical techniques for enzymes such as phosphatases shortly after this; generously Gomori(5) wrote that Lison deserved to

TABLE II

THE DEVELOPMENT OF SOME SPECIAL STAINING METHODS

| | |
|---|---|
| PERLS' (Haemosiderin) | 1867 |
| GOLGI (Nerve cells and processes) | 1875 |
| ZIEHL-NEELSEN (AFB) | 1882 |
| GRAM (Bacteria) | 1884 |
| VAN GIESON (Fibres) | 1889 |
| KULTSCHITSKY (Myelin) | 1890 |
| ROMANOWSKY (Blood) | 1891 |
| SOUTHGATE'S MUCICARMINE | 1896 |
| SUDAN (Lipid) | 1896 |
| WEIGERT (Elastic fibres) | 1898 |
| METHYL GREEN (DNA) PYRONIN (RNA) | 1902 |
| BIELSCHOWSKY (Neurofibrils) | 1904 |

be called "the founder of histochemistry, who cleared away much pseudo-scientific rubbish from the path of advancement". Additionally, in the 1930's the first prototypes of the electron microscope were being built in Germany and England but the second world war engulfed first Europe and then S.E. Asia, deferring the major progress in histology that was just about to take place.

## THE SECOND GOLDEN AGE (1950 – PRESENT TIME)

The tremendous advances at the end of the nineteenth century were almost wholly based on the sectional method and dye staining, but by the middle of the twentieth century progress was of a different kind. There were advances in tissue sections, such as semi-thin sections from resin-embedded tissue which produced a 1 pm slice of a cell, and sections of hard tissues such as bone in the undecalcified state. But the recent changes can be divided into three main groups – morphological, analytical and functional. ***Electron microscopy*** is the obvious morphological advance and conventional transmission electron microscopy (T.E.M.) now plays an essential part in histological methodology. Scanning electron microscopy (S.E.M.) is of great interest to the biologist but is of lesser interest to histopathologists, though there seems little doubt that the combination of these two methods, scanning transmission electron microscopy (S.T.E.M.) will play an increasingly important role in the future. The analysis of tissue components can be undertaken by the electron probe micro-analyser which provides details of the ultrastructural morphology and chemical composition. The ultra-microtome has overcome the restraints of section thickness and the electron microscope has broken through the limits of resolution and depth of focus set by the light microscope. The old staining methods for intracellular organelles have now passed into obscurity, though they will be mourned by few histologists as they were not easy to carry out and were difficult to interpret.

The ***functional developments*** in the second golden. age in histology have a special attraction to pathologists as we increasingly study cellular functional abnormalities in relation to structural changes. The wheel has now almost completely turned in comparison with the developments of the last century, because the new histological methods are usually not dye staining techniques and they may not use sections. ***Autoradiography*** has given new knowledge, but has been overshadowed by the development of histochemistry and immunohistology. The emergence of the ***cryostat refrigerated microtome*** in the 1950's brought ***histochemistry*** within reach of all histology laboratories, but histochemical techniques do not wholly consist of methods for enzymes. Many other tissue components can now be demonstrated by chemical reactions with a well understood scientific basis which are truly histochemical. The structure of intracellular components, visualised by electron microscopy, has been complimented by the new knowledge of chemical function that has emerged from histochemistry. In the same way that electron microscopy is almost free from technical restraints there are unlimited lengths to which histochemistry can go, but there are still some disadvantages in histochemical methods. The need for fresh tissue, the soft colours of the final reaction products, and the difficulties with permanent preparations all leave something to be desired, and for histopathologists it may be inadequate to demonstrate chemical end groups or to classify into types of compounds. We need to be able to identify specific active compounds, and ***immunohistology*** is capable of achieving this.

***Fluorescent immunohistological*** methods, developed from the techniques of Coons and his colleagues(6, 7), have become widely used for the demonstration of antigens, antibodies and antigen-antibody complexes in tissues. They have also been the means of recognition of auto-antibodies in human blood. The specificity and sensitivity of the techniques is so high that we can recognise and identify specific compounds which are present in tissues in very low concentration. ***Any compound to which an antibody can be raised*** can now be ***specifically demonstrated*** in tissue sections; a quantitative assessment can often be made and its exact site can be localised in the tissue. Disadvantages of immunofluorescence – dark rooms, frozen sections, poor background detail, impermanent preparations, incompatibility with electron microscopy – have been overcome by the emergence of ***immunoenzyme techniques.*** The

labelling of antibodies with enzymes such as horse-radish peroxidase was introduced independently by Avrameas and Uriel(8) and Nakane and Pierce(9) and the immunoperoxidase method can be used with paraffin sections and an ordinary light microscope. The peroxidase, seen as a brown end-product, identifies the presence and the site of the specific antigen, and as the end-product becomes electron dense with osmium tetroxide the method is applicable to the electron microscope. Sensitivity is increased by the peroxidase – anti-peroxidase (PAP) method of Sternberger et *al.*(10) in which the antigen is visualised by a soluble complex of enzyme-anti-enzyme, thereby increasing the colour of the positive reaction. Our ability to identify by immunoenzyme techniques the presence of infective agents, specific cells and cell products such as serum proteins, hormones and enzymes is now being applied to histopathological problems (Table III). Tumour markers can also be visualised, and these help in the classification of malignant tumours.

## THE FUTURE ROLE OF HISTOPATHOLOGY TECHNIQUES

The problems of the histologist are no longer those of definition, magnification, or identification. We can visualise structures and recognise their normal and abnormal functions by histological methods that give results of a specificity and sensitivity that were unimaginable forty years ago. It is essential that the histologist accepts the non-dye staining methods of electron microscopy, histochemistry and immunohistology and uses these products of the second golden age in conjunction with the dye staining techniques of the first golden age. Some of the same new methods are also being used by the chemist and immunologist in the examination of body fluids, but histopathology will not be overtaken or superseded if we use our methodology for the purposes of identi-

TABLE III

SOME PRACTICAL APPLICATIONS OF IMMUNOHISTOLOGICAL METHODS

| TYPE OF COMPOUND | TISSUE COMPONENT | PRACTICAL APPLICATION |
|---|---|---|
| ENZYMES | Phosphatases, etc. | Prostatic and other carcinomas. |
| HORMONES | Pancreatic and G.I.,<br>Pituitary,<br>HCG,<br>Oestrogen, progesterone, etc. | Identification and predicted treatment response of various tumours. |
| SERUM PROTEINS | Immunoglobulins.<br>(Heavy chains, Light chains, etc.) | Diagnosis and classification of lymphomas |
| | Alpha-l-antitrypsin | Yolk sac tumours,<br>Hepatocellular carcinoma. |
| VIRAL ANTIGENS | Hepatitis B antigen. | Hepatitis,<br>Hepatocellular carcinoma |
| | Herpes simplex associated antigens | Various tumours. |
| TUMOUR MARKERS | Alpha-fetoprotein | Hepatocellular carcinoma,<br>Germ cell tumours. |
| | Carcinoembryonic antigen. | Carcinoma of colon, etc. |
| | Casein. | Carcinoma of breast. |
| INFECTIVEAGENTS | Fungi, etc. | Aetiology of infective diseases. |

fying the morphological and functional abnormalities of the cells in a tissue specimen. The histologist's ambition of being able to identify a cellular change that is a specific feature of malignancy is still a dream, but if this is proved to exist, we probably have the technical expertise to demonstrate it. More important at the present time is the continued application of our new methods to an ever-widening number of pathological problems. It is possible, even probable, that the use of the technical methods of the second golden age will bring as much new knowledge to histopathology as did the new methods of the last century.

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