Technical methods

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References


Letters to the Editor

Absorption spectra of pigments in vertebrate and non-vertebrate muscle

I enjoyed reading the article by Dr Rinsler in the March issue of the Journal. However, there is one historical inaccuracy which I would like to correct.

I refer to the work of Dr Charles MacMunn, who established the differing absorption spectra of pigments in vertebrate and non-vertebrate muscle in various oxidant states. Dr Rinsler refers to this work as distinguishing the spectrum of myoglobin in muscle from that of circulating haemoglobin. Although it is probable that Dr MacMunn did observe such changes, the pigment he described as “myohaematin” was not myoglobin, but what we would now call cytochromes, in particular reduced cytochrome c.

The description of this work is clearly indicated in the book by David Keilin, published posthumously in 1966.

In fact, Dr MacMunn’s clear descriptions of the cytochromes were vehemently denied by Hoppe-Seyler, who attributed MacMunn’s findings to bad technique. MacMunn’s work preceded the “reductase-oxidase” dispute in the early 20th century—a dispute only finally solved by the rediscovery in the 1920s by David Keilin of MacMunn’s original observations.

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Dr Rinsler replies as follows:
Dr Boulton correctly points out in his letter of 7th April an inexactitude in my very brief note of CA MacMunn’s work on pigments.1 MacMunn described a four-handed spectrum of a material, widely distributed in animal tissues, which behaved as a respiratory pigment. He was unable to isolate this material but called it histohaematin.2 It was this material that Keilin called cytochrome in 1925.

MacMunn observed similar spectra in mammalian muscle which he distinguished from haemoglobin and attributed to another unisolated material which he called myohaematin. The lack of pure material led to confusion with myoglobin and the controversy with Hoppe-Seyler. Keilin himself states the “Fischer’s opinion that MacMunn’s findings were correct was based upon an erroneous identification of myohaematin with myoglobin and on the false belief that what MacMunn had demonstrated was the existence of myoglobin as distinct from blood haemoglobin”.3 Anyone who wishes to have a greater understanding of these issues will enjoy reading the account by Florkin and Stotz of the story of “histohaematin” and “cytochrome”.4

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Comparison of the Dicopac with the conventional Schilling test

The Dicopac is a variant of the Schilling test based upon the simultaneous administration of free $^{57}$Co cyanocobalamin (Cn-Cbl) and $^{58}$Co Cn-Cbl combined with human intrinsic factor. Several groups have shown that the ratio, $^{57}$Co:$^{58}$Co, does not always distinguish patients with pernicious anaemia from those without.1-3 We have experienced similar difficulties in this laboratory and had to perform conventional Schilling tests to clarify the diagnosis in 13 patients ultimately shown to have pernicious anaemia by typical results on the conventional test, megaloblastic anaemia responsive to cobalamin and a low serum cobalamin. The conventional Schilling tests were performed in two parts each using the appropriate capsule provided for the Dicopac test (Radiochemical Centre, Amersham); for part I the $^{54}$Co Cn-Cbl capsule was given and for part II the $^{57}$Co Cn-Cbl capsule containing intrinsic factor was given.

The Figure shows our data expressed as the $^{57}$Co:$^{58}$Co ratio and is presented so that the results obtained by the Dicopac method and by the conventional Schilling test can be compared on a patient basis. In all instances the ratios

References

were higher with the conventional Schilling test than with the Dicopac (p < 0.001 by paired t test; \( t = 5.0 \) with 12 degrees of freedom). This difference in ratio could be ascribed to the \( ^{58}\text{Co} \) excretion being higher in the Dicopac method than in the conventional Schilling test, the respective means being 4.3 and 1.9\% (p < 0.001 by paired \( t \) test). There was no difference in \( ^{57}\text{Co} \) excretion in the Dicopac and conventional Schilling method, the respective means being 8.5 and 9.5\% (p > 0.05 by paired \( t \) test).

The most likely explanation for this phenomenon is that the \( ^{57}\text{Co} \) Cn-Cbl attached to intrinsic factor exchanges in the patient's gut with the free \( ^{58}\text{Co} \) Cn-Cbl when both capsules are given together in the Dicopac method; Knudsen and Hippe consider that the dissociation constant for the Cn-Cbl intrinsic factor complex is low enough for such a reaction to occur.

The Radiochemical Centre draw attention to discrepancies between the Dicopac and the Schilling test in their instructions which state “typically, for cases of pernicious anaemia, the cobalt-58 value will be slightly higher than the excretion in the first part of the Schilling test.” However, our results show that the \( ^{58}\text{Co} \) excretions in the Dicopac method are often considerably higher than the \( ^{58}\text{Co} \) excretions in the first part of the Schilling test. This large discrepancy effectively invalidates the Dicopac technique in our pernicious anaemia patients since it brought their \( ^{57}\text{Co}:^{58}\text{Co} \) ratios unacceptably close to those which are regarded as normal.

We wish to thank Mrs L Warne for typing this paper.

References

Book reviews

The ability to diagnose systemic disease on the basis of cutaneous changes poses a continual challenge to both internal physicians and dermatologists. Since Irwin Braverman wrote his classical monograph on this subject our understanding of disease processes has been magnified by spectacular advances particularly in the field of immunopathology and this has helped to clarify the relationship between internal diseases and their cutaneous manifestations. In this recent addition to the literature Jeffrey Callen has marshalled an impressive array of dermatological experts who deal individually with the pathogenesis, clinical features, and treatment of multi-system diseases. With large numbers of contributors there is always the risk of repetition but the author was clearly aware of this problem and the result is a comprehensive and authoritative text which can be used as a standard reference work by any practicing physician. For the dermatologist the information contained in this volume is obligatory reading but the book is clinically orientated and contains no detailed pathology.

F Wilson Jones


This comprehensive atlas of renal biopsy pathology has been published in the same basic format as the AFIP tumour fascicles, but is rather longer—covering some 385 pages. The book is pleasant to handle and it contains a large number of black and white illustrations of light and electron microscopy. In addition there are occasional pages of immunofluorescence pictures in colour. Anyone new to the subject would gain the impression that the light microscopy was unimportant and that electron microscopy was required for an accurate diagnosis in every case. In fact almost all cases can be accurately diagnosed by light microscopy provided one strives to attain high technical standards of slide preparation.

I personally liked the layout of the book, consisting as it does of a series of illustrative case histories. The danger is of course that the reader may find it
Comparison of the Dicopac with the conventional Schilling test.

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