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 oxidate 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 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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References


2 MacMunn CA. Researches on myohaematin and the histohaematinis Philos Trans R Soc Lond 1886;177:267-98.


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 57Co cyanocobalamin (Cn-Cbl) and 57Co Cn-Cbl combined with human intrinsic factor. Several groups have shown that the ratio, 57Co:57Co, 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 57Co Cn-Cbl capsule was given and for part II the 57Co Cn-Cbl capsule containing intrinsic factor was given.

The Figure shows our data expressed as the 57Co:57Co 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


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Comparison of $^{57}\text{Co}:^{58}\text{Co}$ ratios when the Dicopac method is used and when the Dicopac reagents are used to perform conventional Schilling tests. The line of equality is shown.

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 invalidated 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.

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