The scientific writings of Tom Bothwell and his contribution to iron metabolism

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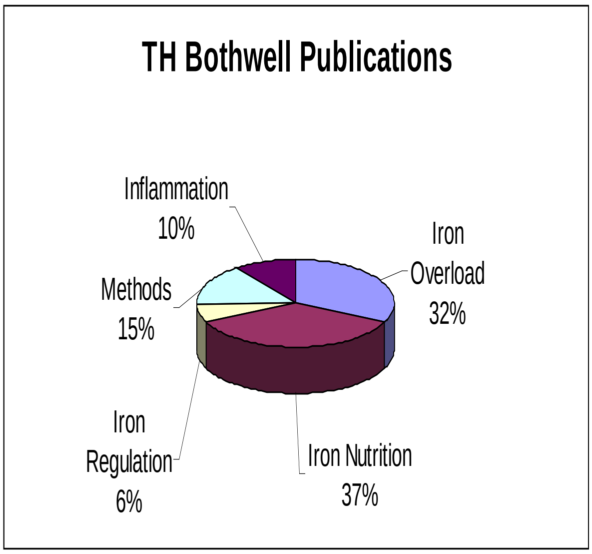
The world of iron metabolism has been taken over by molecular biology. The astounding progress over the past ten years would be nowhere had it not had a solid foundation built on years of careful research and observation. One of the pioneers in laying this foundation was Thomas Hamilton Bothwell. He entered the field just as a new technology was becoming available: radioactivity. His first paper[(1)], which set him off on a career in iron research, was not merely a case report on a patient with haemochromatosis but 'radioiron studies in a case of haemochromatosis' and was published in no lesser forum than the prestigious *Journal of Laboratory and Clinical Medicine*. It was fortuitous that this young patient presented with cardiac failure and so came to the attention of the budding cardiologist. Just as the researchers of today are exploiting technologies evolved from the polymerase chain reaction, so Bothwell used radioiron to delve into the mysteries of iron absorption, iron loss and 'internal iron exchange' – how iron is recycled and put to use again and again. Bothwell has produced over 300 publications, the vast majority on aspects of iron metabolism, and in most of his original articles, radioiron has been the essential research tool.

It was not surprising that Bothwell's early research on iron overload, both haemochromatosis and the intriguing dietary siderosis in Africans, brought him to the attention of another great iron pioneer – Clem Finch, in Seattle. Their meeting, at a sidewalk café in Paris in 1954, led to a life-long friendship and research collaboration. Undoubtedly their seminal contribution to iron metabolism was the demonstration that iron absorption is related inversely to the size of iron stores and directly to the rate of erythropoiesis. This was reported in an article published in 1958 [(21)], again in *J Lab Clin Med*, which went on to be a Citation Classic. In a commentary published in *Current Contents* in 1986 [(213)], Bothwell described this fundamental discovery in the following modest terms:

> "The article has probably been frequently cited because it has provided a framework for future studies. Any explanation of the control of iron absorption at a molecular level would have to be compatible with the observed effects of storage iron status and erythropoietic activity. Thus far, no really satisfactory explanation has been forthcoming."

The first 'satisfactory explanation' of this phenomenon, which had been confirmed again and again, came nearly 50 years later with the discovery of the iron regulatory hormone, hepcidin.[1] It is indeed ironic that the discoverers of this peptide neglected to acknowledge the article on which their work is based, probably because it describes a fundamental truth which is now basic iron lore. Besides, this is now the age of computer searching and so many things prior to 1970 have been 'forgotten'. This ties in with another basic contribution by Bothwell and Finch. They were the first to produce a truly comprehensive iron text book – *Iron Metabolism* – first in 1962 [(42)] with a revised edition called *Iron Metabolism in Man*, with Cook and Charlton, in 1979 [(164)]. Although now out of print, *Iron Metabolism in Man* remains essential reading for anyone wanting to make his mark in iron metabolism, particularly molecular biologists!



One of the basic characteristics of iron is its reactivity which explains why it can be so dangerous on one hand but essential to life on the other. This property mirrors the two major themes of Tom Bothwell's work; iron overload and iron nutrition. Bothwell's writings on iron have covered almost every aspect of iron metabolism and there is a great deal of overlap in the broad categories shown in the adjacent diagram. So, aspects of iron overload were used to define iron absorption, iron nutrition and overload have a lot to do with the regulation of iron metabolism and the methods he devised and refined transcend all the categories. In each of these fields Bothwell contributed greatly to our understanding of iron metabolism and laid the foundation for the spectacular advances that molecular biology has made in recent years. For the purposes of this review I will concentrate on the three areas that gave rise to his greatest number of publications.

Iron Overload

It was the havoc which iron overload wreaks that first kindled Bothwell's enthusiasm for iron. From this time on he produced a steady flow of papers dealing with the two major causes of iron overload seen in Africa – haemochromatosis and dietary iron overload. He has been the author of over 30 reviews dealing with iron overload more than two thirds of which concern haemochromatosis itself. He has contributed the chapter on haemochromatosis in three major medical text books and in successive editions [161,187,189]. African iron overload, variously called Bantu siderosis, dietary iron overload or haemosiderosis depending on your vintage, attracted his attention and, between 1960 and 1975, a steady stream of articles appeared, each dissecting out aspects of the pathophysiology and, for the first time, noting the intriguing link between iron overload, vitamin C deficiency and osteoporosis [68,72,73]. That this condition is causally linked to the consumption of large amounts of low-alcohol traditional beer containing massive amounts of bioavailable iron is one of those facts that most University of the Witwatersrand medical graduates seem to know [54]. While the observation, from his laboratory in 1981, that African iron overload was a fast disappearing condition [166] seemed to put an end to this line of research, the careful observations of an American medical missionary started a whole new line of enquiry. Victor Gordeuk and his co-workers have shown that there is probably a genetic susceptibility to the condition in people of African decent and that it is still common in rural southern Africa. [2]

In 1965 Bothwell demonstrated that the distribution of storage iron in haemochromatosis and dietary iron overload is quite different [60]. In the former, iron is found in hepatocytes while in the latter it is mainly in the reticuloendothelial system (RES). Bothwell extended this observation further in a paper published in 1976, again in *J Lab Clin Med* [136], which pointed out that, in haemochromatosis, the RES is devoid of iron which suggested that very different mechanisms of iron loading must be responsible. A 'satisfactory explanation' for this has only recently been discovered. Mutations of the gene coding for ferroportin, the protein responsible for the export of iron from cells and the target of the hormone hepcidin, cause iron to be trapped in the RES, as in African iron overload. This novel form of iron overload has been dubbed 'Ferroportin Disease'.[3] In other forms of haemochromatosis, where there is a breakdown of the control of ferroportin by hepcidin, iron pours out of the RES causing the phenomenon noted by Bothwell in 1976. Recently, it has been suggested that a novel ferroportin mutation, unique to Africans, may contribute to African iron overload. [4] If this can be confirmed it would neatly round off the story.

Iron Nutrition

While the mechanisms of iron absorption and iron overload occupied the young researcher, it was the broader problem of iron nutrition that was to dominate Tom Bothwell's research in later years. International recognition of his standing in iron nutrition dates back to 1963 when he was appointed to the Expert WHO Committee on Nutritional Anaemia. Since then he has been an active member of the International Nutritional Anaemia Consultative Group (INACG) and latterly he has worked closely with SUSTAIN, an organisation devoted to fostering iron fortification. His first paper on the absorption of iron from food appeared in 1971. He made use of a novel technology to measure the small amounts of radioiron which appear in red cells following the absorption of a meal containing tracer amounts of ^{55}Fe and ^{59}Fe. The technique which made this possible was the simultaneous assay of ^{55}Fe and ^{59}Fe by liquid scintillation counting. This method allowed the comparison of radioiron absorption, in the same subject, from two different food sources and was exploited by Bothwell in numerous studies which defined the bioavailability of iron in different foods. Perhaps his greatest contribution to this field was his careful and systematic discovery of substances in food which either inhibit or promote iron absorption. While others in this field tended to concentrate on iron absorption from 'typical meals', Bothwell turned his attention to the effect of individual food substances on iron absorption. This in turn, led to a greater understanding of the dose dependent effects of compounds such as ascorbic acid and polyphenols on iron absorption. A number of seminal articles stand out. The papers in which the promotive effect of vitamin C is dissected out [(113,146,200)] contributed to policy changes in infant nutrition. The serendipitous observation that tea drastically inhibits iron absorption [(132)], from which followed a series of papers in which the inhibitory effects of polyphenols and phytates were described [(195,199)] and the frequently cited work showing that ascorbic acid prevents the dose-dependent inhibitory effects of these compounds on non-heme iron absorption [(270)]. From this work came the realisation that it is the mix of these inhibitors and promoters in the diet that determines the iron nutritional status of different populations. People, mostly in the developing world, living on a diet based mainly on cereals, rich in inhibitors and with few promoters, have poor iron nutrition. In the western world where the diet is rich in promoters and cereals have been refined, iron deficiency is relatively uncommon. The obvious sequel to this line of reasoning was to explore how the diet could be modified, or how iron could be modified in some way, to escape the effects of these ubiquitous inhibitors. This brought Bothwell squarely into the field of iron fortification. It soon became apparent that forms of iron that were well absorbed caused unacceptable organoleptic changes while iron that did not spoil food was not absorbed. This is the central dilemma of iron fortification and nowhere is this more clearly enunciated than in an INACG monograph [(147)], of which he was the major author, and in a subsequent review [(256)], both of which laid out a strategy for the fortification of food with iron. In 1977, Bothwell turned his attention to the use of a chelate of iron – iron EDTA – which seemed to be able to escape the effects of inhibitors in food. This was confirmed in a series of papers from his laboratory [(182,284,305)] which clearly showed that, in the setting of cereal foods, the absorption of iron was more than two times greater than from ferrous sulphate, the accepted standard. This was taken further by applying this technology to a double blind fortification trial using iron EDTA to fortify curry powder [(247,248)]. The results were spectacular. In two short years it was possible to reduce the prevalence of iron deficiency anaemia in women in the targeted group from 20% to 5%. Moreover, fortification did not cause an increase in iron stores in subjects who were already iron replete – a result which might have been anticipated from Bothwell's seminal work of the 1950s [21]. Even though EDTA was already regarded as a safe food additive, undisputed acceptance of iron EDTA, seen as an 'unnatural' compound, was slow in coming. It is now widely used in fortification programmes in Latin America and China.

Iron methods

Tom Bothwell was always quick to apply novel methods to the study of iron metabolism. It is not surprising therefore that he was in the forefront of the development of new methods to measure every step of the passage of iron round the body. His early work in which he used radioiron to explore ferrokinetics [20,26,168], the term used to describe the movement of iron in, out and within the body, laid the foundation of our understanding of iron regulation and external and internal iron exchange. His methods for measurement iron in serum [17] and in tissues [80,172] are used in laboratories throughout the world. Recognition of his role came in the form of membership of the Iron Panel of the International Committee for Standardization in Haematology [96, 109, 156, 157, 206, 211, 272] and in the appointment of his laboratory as a reference laboratory for the measurement of iron in serum and tissues, and ferritin in serum.

Perhaps the greatest contribution that Tom Bothwell made to iron metabolism and to science itself was in the style of his writing. There are no better examples of clear, concise and unambiguous scientific writing. In so many of the articles of which he was co-author the fact that he had edited them, and often rewritten them, shone through. He set about writing in a thoroughly systematic fashion. First graphs and tables, then a narrative description of the results, then the discussion and finally the easy bits: methods, introduction and the summary. In the days before word processors, Tom Bothwell developed his own method of 'cut and paste'. The first draft was handwritten double spaced and then typed out. Then, equipped with sticky tape and a pair of scissors, usually in the left hand, he would set about the text. Cutting a piece out here and inserting a new handwritten section there, all stuck together with sticky tape, while the manuscript grew in length and coiled up on the floor. Every Research Fellow knew that Tom would take his final, if sorry, draft and*shatter it to bits – and then remould it nearer to the heart's desire.*

References

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