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Editorial

Several negatives make a positive

Till the first quarter of the twentieth century, a mysterious and fatal form of anaemia sometimes afflicted middle aged persons. Its prognosis was worse than that of leukaemia today; hardly anyone survived more than three years after diagnosis (1). Hence the anaemia was called pernicious. Around 1918 George Whipple started some experiments in which he rendered dogs anaemic by repeated systematic bleeding. Many dietary supplements were given to the anaemic dogs, some of which succeeded in replenishing their haemoglobin. The most potent supplement in this respect was liver, and Whipple attributed its efficacy to the liver proteins. Taking a cue from these experiments, in 1925 George Minot invited William Murphy to join him in a liver therapy trial for treatment of pernicious anaemia. The results of the rigorous trial conducted on 45 patients were most conclusive: a diet containing 120-240 g of lightly cooked beef liver daily cured pernicious anaemia. That such a dreaded disease had such a simple treatment seemed too good to be true. The study was repeated at several places in the world with the same results. Whipple, Minot and Murphy received the Nobel Prize in 1934 for their contribution to amelioration of suffering.

When William Castle (1897–1990) joined Dr. Francis Peabody's Laboratory in 1927, the Boston air was agog with excitement about the newly discovered liver therapy for pernicious anaemia. Besides, it had also been known for a long time that pernicious anaemia is associated with achlorhydria, and follows gastrectomy. With this background, Castle asked two very pertinent questions: first, how normal persons can keep pernicious anaemia away without taking half a pound of liver every day; and second, can some normal digestive process of the healthy stomach substitute for half a pound of liver. The excellent formulation of questions was the first step towards the success of Castle's experiments (2). Since Minot and Murphy had attributed the success of liver therapy to proteins, Castle chose for his experiments a similar diet. But for some reason, which is not entirely clear, he chose to give his patients 200 g of lean beef muscle instead of liver, assuming quite correctly that there is not much difference in the nutritive value of liver proteins and muscle proteins. Somewhat unexpectedly, the muscle supplement was not effective in bringing about any improvement in the anaemic status of patients. This provided justification and rationale for the next trial based on the association of pernicious anaemia with achlorhydria. This time the supplement given was 200 g of muscle which had spent 1 hour

in the stomach of a normal person and had been further incubated in the gastric juice of the normal person before being given to the patient. This altered mode of administration imparted curative properties to the muscle meal, comparable to the liver meal. The next question was whether the improvement was due to normal gastric juice alone, or due to its action on muscle. It was found that 150 mL of normal gastric juice was just as ineffective as 200 g of muscle alone. Some more permutations and combinations were also tried and the consistent conclusion from all the studies was that the administration of beef muscle and normal gastric juice *together* was important for curing pernicious anaemia. Castle explained these observations by postulating that an intrinsic factor present in normal gastric juice interacted with an extrinsic factor present in beef muscle to supply some essential participant in erythropoiesis (3). Although no evidence was available to say so, it was assumed that the intrinsic factor was an enzyme. Today we know that intrinsic factor is not an enzyme but a glycoprotein which binds the extrinsic factor and facilitates its absorption. The extrinsic factor is vitamin B_{12}, or cyanocobalamin. Our knowledge has now gone far beyond these facts but the recent advances could not have occurred without the fundamental discoveries made by Castle and his associates.

The story of the conquest of pernicious anaemia has many interesting facets. Whipple's dogs rendered anaemic by rebleeding had responded to the iron in the liver, not to liver proteins. Whipple had tried inorganic iron also but the response to it was, at best, erratic (1), which might have been due to poor absorption. The error was fortunate because it encouraged Minot and Murphy to try liver therapy for pernicious anaemia. Again, liver therapy worked in pernicious anaemia due to the high vitamin B_{12} content of liver, not due to its proteins, as was then thought. The ignorance was useful because it prompted Castle to use beef muscle. The departure was lucky because muscle contains much less vitamin B_{12} than liver. The lower vitamin B_{12} content made the presence of the intrinsic factor essential for absorption of adequate amount of the vitamin. Thus using muscle instead of liver made discovery of the intrinsic factor possible. It was really a chain of propitious errors and accidents which led to some very basic discoveries in relation to pernicious anaemia, somewhat like the way two negatives may make a positive.

An interesting aside to the story is the fact that George Minot got diabetes in 1921. He was one of the first beneficiaries of the discovery of insulin (4). Had insulin not been discovered in time, Minot might not have done his studies on liver therapy. Had those studies not been done, Castle could not have done his studies. Further, Minot & Murphy evaluated their patients'

response with the help of the reticulocyte response. This valuable tool, which made quick and conclusive evaluation of treatment possible, had been described by Krumbhaar only in 1922. Had all these events not been so well-timed, it is anybody's guess how much longer the conquest of pernicious anaemia might have had to wait.

Castle traced the history of growth in our knowledge about pernicious anaemia at length in 1980 (1). The account gives significant clues to his character and personality. He refers to himself in third person and does not make any effort to highlight his own work. He gives the background of his work and older studies in so much detail as to make it almost look as if his studies were merely a natural and inevitable sequel. And, he does not stop at his work but rather traces the developments till 1980 and points out the questions that still remained unanswered. Thus he was an objective, self-effacing person who never stopped learning and asking questions. We would like to end this tribute to William Castle during his birth centenary with what Wintrobe has termed Castle's law: "There's nothing like a fact to stop an argument" (1).

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