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Citation Classics

Rondle, Charles J M & Morgan, Walter T J. The determination of glucosamine and galactosamine. *Biochemical Journal* **61**:586-9, 1955.

This paper describes an updating of the earlier method of Elson and Morgan for the determination of hexosamines by treatment with acetyl acetone followed by addition of Ehrlich's reagent. Emphasis is placed on the importance of using purified reagents and rigidly controlled conditions. Details are given of the accuracy likely to be obtained in any test or series of tests. Evidence is presented on the stability of the coloured substances produced. [The *SCI*® indicates that this paper was cited 604 times in the period 1961-1975.]

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"I am astonished but delighted to learn that this paper has been cited so frequently. Perhaps some readers would like to know how it came to be written. As a raw Cambridge graduate I was lucky to join Walter Morgan's team as a Ph.D. student. At that time (1951) we were all working on the isolation and chemical composition of 'human' blood group substances obtained from one source or another–from hog mucin to human ovarian cysts. It had been apparent for many years that hexosamines were important constituents of blood group substances. At the Lister Institute of Preventive Medicine [London, England] they were detected and measured in acid hydrolysates of material by methods based on that introduced by Elson and Morgan in 1933. My first acquaintance with a 'modification' was in the use of a colourimeter made, in true Lister fashion, from a bell transformer, a discarded cigar box and a flying spot galvanometer which had to be used in a dark room. I would add for the benefit of younger workers that even in those not too distant days it was customary to purify or repurify nearly all reagents–an A.R. grade was frequently unavailable. Reference sugars were often prepared from natural sources or certainly by careful recrystallisation of commercial material. Good samples were carefully hoarded and exchanged for others among trusted colleagues. One consequence of this is shown in the paper; we claimed that glucosamine and galactosamine gave the same amount of colour, others have denied this. All I can say is that with the samples available to me results were within 4%. We investigated the stability of the colour formed not so much from chemical interest but when performing many tests daily it was necessary to pace 'setting up' and 'reading' times.

"The paper really came into being with the arrival of a 'proper' spectrophotometer. It was decided that a thorough investigation of the Elson-Morgan method should be made and thousands of tests were done. The suggestion that the results should be examined statistically was met with mild concern by my senior colleague who felt that the results should be obvious and not require mathematical investigation or manipulation. However, investigated they were, but not manipulated! I think the effort was worthwhile because it showed the test to test variation to be expected in the method described. With hindsight I feel that the variation could have been lessened by the use of alkaline buffer rather than unbuffered sodium carbonate and indeed some later workers have done this.

"Why it has been so often cited I do not know. Perhaps because it reviews the earlier literature, gives precise instructions for a test which works and indicates the sort of accuracy to be expected from it. Possibly, too, it appeared at a time when interest in hexosamines was increasing, an interest which has been maintained to the present day.

"I can only say it was a pleasure to do the work, and a privilege to have behind me the support and guidance of my distinguished senior colleague."