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Robinson D & Stirling J L. *N*-acetyl-β-glucosaminidases in human spleen. *Biochemical J.* **107**:321-7, 1968.
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The enzyme activity in the lysosome fraction of human spleen was separated into an acidic form A and a basic form B. They had very similar kinetic characteristics but could be differentiated by the relatively greater stability of the B form to heat and pH changes. [The *SCI*® indicates that this paper has been cited in over 420 publications since 1968.]

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This paper owes its origins to my earlier existence as a toxicologist. As a young worker at St. Mary's Hospital Medical School, I had been concerned with isolating metabolic glycoconjugates. When, in 1955, colleagues working on the metabolism of coumarins demonstrated the potential of their glucuronosides as fluorigenic substrates for β-glucuronidase,[1] I set about synthesising a number of other glycosides of 4-methylumbelliferone as potential substrates. They proved to be particularly sensitive and useful for identifying the presence of isoenzymes after starch-gel electrophoresis, but at that time the technique of quantitative fluorimetry was not a common laboratory facility.

De Duve's biochemical characterisation of the lysosome and the concept of lysosomal storage diseases as specific enzyme deficiencies[2] coincided with my transfer to a faculty post at Queen Elizabeth College. Here, one of our first graduate students, John Stirling, was given the task of examining whether β-hexosaminidase displayed isoenzyme forms.

He showed that two forms of the human enzyme separated electrophoretically as an acidic Hex A and a basic Hex B. We noted Hex A was destroyed by gentle heating under conditions that left Hex B unharmed. A differential assay was thus possible.

Tay-Sachs disease seemed to break the rules for lysosomal storage diseases since patients, who by nature of their storage lesion ought to lack this enzyme, exhibited normal or even elevated levels.

We were unable to locate samples from such patients, and it was Okada and O'Brien who described in another *Citation Classic*[3] that the generalised absence of Hex A was characteristic of Tay-Sachs disease and could be demonstrated by using the differential assay even in the presence of large amounts of Hex B.

I believe the paper has been often cited for two reasons. Apart from the immediate application of the assay method to diagnosing and screening for carriers of the disease, the observation of two different forms stimulated structural studies to explain the molecular basis of the genetic lesions. Our initial suggestion of differing degrees of sialylation proved to be a red herring due to the formation of artifacts by the preservative in our neuraminidase supplies. To our chagrin, this misconception was quoted much more frequently than our subsequent correct hypothesis published in *Lancet* shortly after.[4]

The now well-established two gene-two subunit concept explained how the Sandhoff variant with little or no enzyme activity could arise by mutation of the common β-unit, while Tay-Sachs patients lacked a functional α unit unique to Hex A.

The structure and function of these subunits continues to attract the attention of workers studying the cell and molecular biology of the lysosome[5] and accounts for the continued interest in this unique enzyme using the same 4-MU substrates as 30 years ago.

1. **Mead J A R, Smith J N & Williams R T.** The biosynthesis of the glucuronides of umbelliferone and 4-methylumbelliferone and their use in fluorimetric determination of β-glucuronidase. *Biochemical J.* **61**:569-74, 1955. (Cited 145 times.)
2. **Hers H G.** Inborn lysosomal diseases. *Gastroenterology* **48**:625-33, 1965. (Cited 165 times.)
3. **Okada S & O'Brien J S.** Tay-Sachs disease: generalised absence of a beta-D-N-acetylhexosaminidase component. *Science* **165**:698-700, 1969. [See also: **O'Brien J S.** Citation Classic. *Current Contents/Life Sciences* **24**(45):16, 9 November 1981.]
4. **Robinson D & Carroll M.** Tay-Sachs disease: interrelation of hexosaminidases A and B. *Lancet* 1:322-3, 1972. (Cited 20 times.)
5. **Lowden J A, Maharan D, Novak A, Lee C & Skomorowski M A.** Hexosaminidases: changing concepts of structure and function. (Callahan J W & Lowden J A, eds.) *Lysosomes and lysosomal diseases.* New York: Raven, 1981. p. 181-94.