Bimal Kumar Bachhawat (1925–1996)

Dr Bimal Kumar Bachhawat was born on 27 July 1925, in Calcutta (now Kolkata), West Bengal. He earned the BSc and MSc degrees from Calcutta University, after which he joined Jadavpur University where he worked on antibiotics in the Department of Food Technology under the supervision of Professor A.N. Bose.

From 1949 to 1957, he studied and worked in the USA, first at the Food and Drug Administration (FDA) where he was a research trainee, and then at the University of Illinois, Urbana Champaign where he obtained the PhD degree under the supervision of Professor Vestling in 1953. Subsequently, he worked with Dr M.J. Coon, and they discovered the enzyme HMGCoA lyase—the key enzyme in the function of ketone bodies.

On returning to India, Bachhawat joined Christian Medical College, Vellore, where he started a neurochemistry laboratory. His research turned to the study of amino acid and inorganic sulphate metabolism, isolation and characterization of glycosaminoglycan and its role in the developing brain, which led to the discovery of the molecular cause of the inherited disease ‘metachromatic leucodystrophy’.

In 1976, Bachhawat moved to Kolkata, as Director of the Indian Institute of Chemical Biology, and in 1985, to Delhi University as Head of the Department of Biochemistry, and then as Dean of the Faculty of Interdisciplinary and Applied Sciences, from where he retired in 1990. He authored over 150 research articles, edited several books, and was on the editorial board of the *Indian Journal of Biochemistry and Biophysics*.

Professor Bachhawat was awarded the Padma Bhushan in 1990. He had been elected to the Fellowship of the Indian National Science Academy in 1973, and served as a Council Member (1975–77) and as Vice President (1987–88). He was also a Fellow of the Indian Academy of Science, Bangalore, and the National Academy of Sciences, Allahabad. Among the several honours and prizes that he received were the Shanti Swaroop Bhatnagar Prize (1962), the J.C. Bose Award (1980), the R.D. Birla Award (1986), Bhatnagar Fellow (1990), and Outstanding Teacher Award (1993).

Professor Bachhawat was a distinguished biochemist and neurochemist as well as an outstanding teacher. In a career that spanned over four decades, he mentored a large number of students (more than 40 PhD students). His contributions were both academic and institutional. Apart from being an outstanding scientist, Professor Bachhawat was a great builder of institutions and a science manager *par excellence*. He set up the first internationally recognized centre of neurochemistry and glycochemistry in Vellore and created one of the best departments of biochemistry in India, at the University of Delhi South Campus. As Director of the Indian Institute of Chemical Biology, Kolkata, he transformed this institution into one of the finest laboratories in biological sciences in India. Professor Bachhawat was an extremely popular teacher and was ever generous with young scientists with words of encouragement and unconditional help. Up to the very last day of his life (23 September 1996), Dr Bachhawat was deeply involved in science and its development in India.

DEBI P. SARKAR
Department of Biochemistry
University of Delhi, South Campus, New Delhi

A Cerebroside Sulphotransferase Deficiency in a Human Disorder of Myelin

By BIMAL K. BACHHAWAT, JAMES AUSTIN AND DONALD ARMSTRONG

Division of Neurology, University of Oregon Medical School, Portland, Oreg., U.S.A.
and Department of Neurology, Neurosurgery and Neurochemistry, Christian Medical College and Hospital, Vellore, India

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An unusual human disease begins during myelination. Children develop this neurological disorder (globoid leucodystrophy) at about 4 months of age. They usually die before the age of 2 years with a widespread degeneration of white matter. There is a marked decrease in the concentration of cerebroside

sulphates (sulphatides) both in white matter (Austin, 1963 a,b; Svennerholm, 1963) and in cerebral cortex (Svennerholm, 1963) of patients with globoid leucodystrophy. On the other hand, concentrations of neutral cerebrosides are proportionately elevated in white matter (Austin, 1963c) of patients with this disorder.

The present findings suggest a reason for this distinctive change in proportions between cerebrosides and their sulphated conjugates in globoid leucodystrophy. The evidence suggests that in globoid leucodystrophy a metabolic block may be interposed between cerebroside and cerebroside sulphate (Austin, 1963b). This block appears to involve an enzyme that normally helps to conjugate cerebroside with sulphate to form cerebroside sulphate. This enzyme system, cerebroside sulphotransferase, was demonstrated in animals by Balasubramanian & Bachhawat (1965 a,b) and by McKhann, Levy & Ho (1965). The sulphate donor in the system is ‘active sulphate’ (adenosine 3′-phosphate 5′-sulphotophosphate). The reaction (in greatly oversimplified form) is as follows:

\[
\text{Cerebroside} + \text{adenosine 3′-phosphate 5′-sulphotransferase} \rightarrow \text{Cerebroside sulphotransferase} + \text{adrenosine 3′-phosphate 5′-phosphate}
\]

Evidence presented below indicates that (a) this enzyme activity does occur in normal control children, but (b) there is a characteristic deficiency of cerebroside sulphotransferase activity in post-mortem tissues of children with globoid leucodystrophy. A deficiency of this enzyme in vivo would imply a deficiency of myelin sulphatides that could lead to defective myelination.

**Materials and methods.** Enzyme activities in two unrelated globoid-leucodystrophy patients were contrasted with those in five relevant controls. All samples were assayed simultaneously. Controls were carefully selected so that they and the leucodystrophy patients were similar in terms of age at death, cause of death, interval between death and autopsy and length of time frozen (in the same freezer).

For example, globoid-leucodystrophy patient no. 1 was 1 year 7 months old at death. Tissues were frozen at –20°C within 8 hr. of death and were assayed 1 month later. Globoid-leucodystrophy patient no. 2 was 1 year 11 months old at death. Tissues were frozen within 2 hr. of death and were assayed 5 months later. Three normal control children (patient nos. 5-7) ranged from 1 year 4 month to 2 years 1 month old at death. Tissues were frozen within 5–18 hr. of death and were assayed 1–21 months later. One abnormal control child had a slightly different kind of myelin breakdown (metachromatic leucodystrophy). This child (patient no. 3) died at 6 years 11 months of age. Tissues were frozen 1 hr. later and were assayed 11 months later. A comparable normal control (patient no. 4) died at 6 years 10 months of age. Tissues were frozen 8 hr. later and were assayed 27 months later.

Methods of assay for cerebroside sulphotransferase activity were essentially as cited by Balasubramanian & Bachhawat (1965b). However, 0–16 μ-lit. of tris, and the glutathione concentration was increased to 0.1m (V. R. Bhandari & B. K. Bachhawat, unpublished work). Incubations were for 2 hr. at 38°C. Each reaction mixture contained 180 000 counts/min. of carrier-free adenosine 3′-phosphate 5′-[35S] sulphotophosphate prepared as described by Balasubramanian & Bachhawat (1964).

All cerebroside sulphotransferase assays were performed with whole homogenates as the enzyme source. The quantity of tissue used for assay was approx. 0.150 g. wet wt. Kidneys comprised 50% cortex and 50% medulla. After extraction and preliminary fractionations, 35S-labelled conjugates were separated by thin-layer chromatography on silica gel G (Austin, 1963c). One solvent system was chloroform–methanol–water (28:13:2, by vol.). The 35S-labelled products were shown by radioautoagraphy on Royal Blue film after periods of 4–60 days in comparison with spots of known sulphated standards. Control hydrolytic enzymes and proteins were assayed and expressed in specific activities as described by Austin, Armstrong & Shearer (1965).

**Results.** The results in Table 1 indicate that globoid-leucodystrophy patients have low cerebroside sulphotransferase activity. Globoid-leucodystrophy patient no. 2 had no detectable cerebroside sulphotransferase activity in white matter. In globoid-leucodystrophy patient no. 1, the cerebroside sulphotransferase activity of white matter was one-tenth that of the lowest control. In globoid-leucodystrophy patient no. 2, the cerebroside sulphotransferase activity of cerebral cortex was one-fifth that of the lowest control. Kidneys from globoid-leucodystrophy patients were also low in cerebroside sulphotransferase activity. Radioautographs demonstrated two well-labelled reaction products (Rf 0.60 and 0.56). These corresponded to those of reference standards of cholesterol sulphate and kerasin sulphate. Dihexose sulphatides were not detectable.

Total proteins in all samples were essentially the same and do not account for the low specific activity in globoid leucodystrophy. In common with other synthetic enzyme activities, cerebroside sulphotransferase activity in tissues does slowly decrease with time in the freezer. However, the autopsy information and duration of frozen storage do not in themselves explain the low cerebroside sulphotransferase activity in globoid leucodystrophy. Appropriate additions of samples from globoid-leucodystrophy patients to normal samples showed no evidence of any inhibition of cerebroside sulphotransferase activity.

Samples from globoid-leucodystrophy patients showed no selective deficiency of the other categories of enzyme activity assayed. Thus neither sulphatase A nor acid phosphatase activities were decreased in globoid leucodystrophy below control values (Table 1).

**Discussion.** The low cerebroside sulphotransferase activity could be more of a primary event (i.e. genetically determined), or it could be only secondary to the pathological changes in white matter associated with globoid leucodystrophy. Weighing against a simple
secondary explanation is the finding that the cerebroside sulphotransferase deficiency extends to tissues that have not obviously degenerated. For example, cerebral cortex and kidney from globoid-leucodystrophy patients each have low sulphotransferase activity, but do not show histological abnormalities that would explain the deficiency. Moreover, cerebroside sulphotransferase activity is still present in a different leucodystrophy (metachromatic leucodystrophy) in which white matter is also destroyed (Table 1). This control finding would make it difficult to attribute the low cerebroside sulphotransferase activity of globoid leucodystrophy to a simple secondary degeneration of white matter. Further, despite the obvious degeneration of white matter in globoid leucodystrophy, other transfersases were within normal limits in brain from a globoid-leucodystrophy patient in previous studies (e.g. UDP glucose–glycogen glucosyltransferase) (Austin et al. 1963).

An enzymic disorder of sulphate conjugation has not hitherto been reported. The expected result in vivo of a cerebroside sulphotransferase activity deficiency would be a deficiency of sulphatides. Because sulphatides are normally most concentrated in myelin, a deficiency of sulphatides could interfere with the proper development of myelin in this human disease.

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**Table 1. Cerebroside sulphotransferase specific activities in two globoid-leucodystrophy patients and five controls**

<table>
<thead>
<tr>
<th>Patient and diagnosis</th>
<th>Cerebral white matter</th>
<th>Cerebral cortex</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebroside sulphotransferase</td>
<td>Sulphatase A</td>
<td>Acid phosphatase</td>
</tr>
<tr>
<td>1. Globoid leucodystrophy</td>
<td>4</td>
<td>35.4</td>
<td>1.4</td>
</tr>
<tr>
<td>2. Globoid leucodystrophy</td>
<td>0</td>
<td>16.8</td>
<td>0.9</td>
</tr>
<tr>
<td>3. Metachromatic leucodystrophy*</td>
<td>40</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>4. Normal</td>
<td>69</td>
<td>32.8</td>
<td>0.5</td>
</tr>
<tr>
<td>5. Normal</td>
<td>70</td>
<td>31.8</td>
<td>0.4</td>
</tr>
<tr>
<td>6. Normal</td>
<td>368</td>
<td>28.7</td>
<td>0.3</td>
</tr>
<tr>
<td>7. Normal</td>
<td>594</td>
<td>12.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Low sulphatase A in patient no. 3 confirms the histological diagnosis of metachromatic leucodystrophy (Austin et al. 1963, 1965).
† The patient received nitrogen-mustard therapy 1 week before death. This may explain the relatively low cerebroside sulphotransferase activity.
The three decades of the late 1950s to the 1970s in the 20th century at Christian Medical College (CMC), Vellore were an epoch-making period in neurosciences. Led by the pioneer neurosurgeon Jacob Chandy, neuroscience at CMC, Vellore, was both path-breaking and pace-setting in clinical and basic sciences. It was recognized globally, and served as a model locally, inspired by the great institutions in North America where Chandy trained. Bimal Bachhawat, a young and accomplished biochemist who had worked in the finest laboratories of Carl Vestling and Minor Coon in enzymology and basic biochemistry during its golden period in the USA, was brought on board. He joined CMC in 1958 to set up a neurochemistry laboratory, an adventure that launched him from rudiments to achievements in the new and fledgling area of neurochemistry. He and his associates provided leadership and brought global recognition for their discoveries and contributions to biochemical mechanisms in rare, inherited neurological disorders in glycolipid storage, and in delineating the associated enzymatic process. Several PhD scholars including D.K. Basu, A.S. Balasubramanian, T.N. Pattabhiraman, A. Surolia and a host of others achieved eminence in their careers and became leaders.

It is noteworthy that this paper has cited in it some relevant contributions from Bimal’s laboratory. Altogether, they represent the technical ingenuity in devising enzyme assay systems, for measurement of arylsulphatase and cerebroside sulphotransferase activities in specimens of the human brain, assay methods with isotopically labelled substrate, $^{35}$S sulphophosphate-methods designed and standardized for application to human specimens. Garrod’s ‘one gene, one enzyme hypothesis’ in inherited diseases—proposed six decades before this period—spurred enzymologists to explore genetic diseases, and the Vellore group’s investigations on neurological genetic diseases was a calculated, productive venture.

A fruitful collaboration with James Austin, neurologist at university of Oregon Medical School, USA, provided complementary outcomes of clinical findings and specimens while skilful and well-designed enzymatic assays were performed by the Vellore group. As subsequent publications reflect, the Vellore laboratory became one of the well-recognized centres for work in neurochemistry. The contributions made to the enzymology of clinical problems in inherited diseases was an impressive achievement at that time, considering the modest infrastructure, resources and sparse guidelines available then for such clinical biochemical work. It was particularly inspiring for biochemists and clinical researchers in India to realize what was possible with dedication and spirit of adventure, despite modest facilities.

These reports are early forerunners of what was to come in the application of basic knowledge to unravelling disease mechanisms in the language of molecules, biochemistry in its quintessential place at the core of medicine. For me and several contemporary biochemists, Bimal and his outstanding and inspired colleagues were beacons and eye-openers to what was possible in India, during this period.

In the tradition of good medical science, these classics on glycolipids in metachromatic leucodystrophy and globoid leucodystrophy, moved to the study of enzyme processes defective in storage disorders, such as Gaucher and Tay-Sach disease. The methods for prenatal diagnosis of such conditions followed. Much basic research continued at the Vellore laboratories on the biosynthesis, degradation and mechanisms of cerebroside-$3'$-sulphate and cell surface N-acetylneuraminic acid. What began as a pioneering adventure in bringing difficult biochemistry to clinical problem-solving, blossomed over the years to a laboratory of excellence, in glycosaminoglycan, glycolipid, cell surface receptor biochemistry ahead of its time, also to site-specific delivery of drugs. Translational medicine/research was not bandied about then, as it is at present. It was a seamless union of basic science and medicine.

This paper deserves to be called a classic for more than its scientific content. In the Vellore laboratory from where it emanated, the authors led by Bimal Bachhawat have inspired a couple of generations of biochemists for the adventure that is biochemistry in a clinical setting.

Among clinicians, they helped develop a curiosity-driven deference to research in biochemistry as a foundation discipline in the advancement of modern medicine, inclusive of cell and molecular biology, molecular genetics and immunology. When the history of Indian biochemistry will be written, the ‘Vellore Era’ in the mid-20th century and its shine will find a place of pride and admiration with Bimal Bachhawat as a harbinger of the good word that is biochemistry. This classic is a fitting component of it and a tribute to this much loved and admired pioneer and peer.

P.R. KRISHNASWAMY
Sri Devaraj Urs Academy of Higher Education and Research
Sri Devaraj Urs Medical College
Department of Genomics and Central Research Laboratory
Kolar
and
Centre for Nano Science and Engineering
Indian Institute of Science
Bengaluru
Karnataka