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The history of pyridinium oximes as nerve gas antidotes: The British contribution

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Irwin B. Wilson, working in the laboratory of David Nachmansohn at Columbia, demonstrated the ability of hydroxylamine to reactivate cholinesterase inhibited by organophosphates. Soon thereafter Wilson and Ginsburg reacted pyridine-2-aldoxime with methyl iodide to synthesize the first pyridinium aldoxime reactivator of clinical relevance, 2-PAM (pralidoxime). Independently, and at the same time, similar work was conducted in Britain at the Chemical Defence Experimental Establishment in Porton by Green leading also to the synthesis of 2-PAM and the recognition of its reactivating properties. While the American contribution is well known, the British achievements were less publicized. The present contribution attempts to shed some light on the life and work of the people who contributed to the early development of cholinesterase reactivators, the pyridinium aldoximes at Porton.

1. Introduction

The acute toxicity of organophosphorus esters is due to inhibition of the enzyme acetylcholinesterase, which metabolizes the neurotransmitter acetylcholine. The inhibition results from phosphylation (i.e. either phosphorylation or phosphonylation) of serine in the active centre of the enzyme and translates into an "endogenous acetylcholine poisoning" (Davies and Green 1959).

The therapy of poisoning of cholinesterase by organophosphorus inhibitors includes in addition to respiratory and cardiovascular support the generous use of atropine (to block muscarinic overstimulation), a benzodiazepine GABA receptor agonist such as diazepam (to control convulsions) and of a pyridinium aldoxime such as pralidoxime (to de-phosphylate and thus reactivate the acetylcholinesterase).

The history of the development of organophosphorus inhibitors of cholinesterase and of pyridinium aldoxime enzyme reactivators has been described (Petroianu 2008, 2009, 2010, 2012). The present contribution attempts to shed some light on the life and work of the people who contributed to the early development of cholinesterase reactivators in Britain at the Chemical Defence Experimental Establishment at Porton, Wiltshire: Daniel Richard Davies (1904–1973) and Albert Lawrence Green (*1929).

2. Pralidoxime (2-PAM) discovery

Irwin B Wilson (*1921) working at Columbia in the laboratory of David Nachmansohn (1899–1983), using hydroxylamine, delivered the proof of concept that cholinesterases inhibited by organophosphates can be reactivated (Wilson 1951). The work was inspired by results obtained earlier in the same laboratory by Shlomo Hestrin (1914–1962). Hestrin reported that when acetylcholinesterase was incubated with hydroxylamine in the presence of acetate, hydroxamic acid was generated. The reaction was inhibited (among others) by the organophosphate tetraethyl pyrophosphate (Hestrin 1949).

While hydroxylamine as a reactivator was far superior to water, the kinetics of the reaction were still far from useful, and the search for a reactivator of clinical relevance was pursued both in the United States and in Britain at the Chemical Defence Experimental Establishment (CDEE) at Porton. It was towards the end of 1953 that Albert Lawrence Green, a then young PhD in chemistry, joined the Chemistry section (Leslie Williams was head of chemistry) and later moved to the biochemistry section of the medical division headed by Daniel Richard (Dan) Davies at Porton. A F Childs was a senior member of the staff in the Chemistry Section at CDEE and it was he who asked Green to help with the initial synthetic work. [Figure 1 & 2].

Dr. Green remembers: "*Shortly after I arrived there they became aware of Irwin Wilson's paper reporting that certain hydroxamic acids were capable of reactivating organophosphate inhibited cholinesterase (Wilson and Meislich 1953). I was asked by Dr Childs to help Colin Stratford and Gordon Sainsbury, who were technicians in the Chemistry section, to synthesise a wider range of hydroxamic acids. It soon became clear that we were not going to get any significant improvement, and I decided to try synthesizing other types of compound containing an acidic N-OH group. Ordinary oximes were not acidic enough, but by making oximes with an adjacent electron withdrawing group such as CO or CN, I found these were much better. In particular, pyridinium oximes with a positively charged nitrogen, especially 2-PAM, which I think I first made in late 1954 or early 1955. An account of some of this earlier work on reactivation was reported by Childs, Davies, Green & Rutland (1955). John Rutland was in Dan Davies Biochemistry Section and he did most of the preliminary reactivation experiments*.

Fig. 1: Albert Lawrence (Bert) Green (*1929).

At Porton, Dan Davies was the administrative head of the biochemistry section of the Medical Division. He had ceased to be a laboratory worker but had a gift of being able to rapidly arrange technical help both with the biochemistry and the toxicity testing. Most importantly he was able to convince the security minded authorities in a military establishment to allow us to publish, provided we did so in an obscure way, hence the unlikely appearance of our contribution in Discussions of the Faraday Society where we were the first to disclose the reactivating properties of 2-PAM (Davies and Green 1955). Without these invaluable skills of Dan's we probably would not have been allowed to publish for several years.

*At this August 1955 Meeting of the Faraday Society in the University Laboratory of Physiology at Oxford, Wilson delivered a talk but made no mention of 2-PAM (*Fig. 3*), but after my contribution about 2-PAM he subsequently got up and said that he and Sara Ginsburg (1908–1997) had also discovered this compound and had submitted this (in July) for publication in Biochim Biophysica Acta (Wilson and Ginsburg1955).*

Both Ginsburg at Columbia and Green at Porton used the same (and apparently only practical) approach to 2-PAM synthesis by reacting pyridine-2-aldehyde (picoline-aldehyde) with hydroxylamine to yield pyridine-2-aldoxime (picoline-aldoxime) and then with methyl iodide to obtain the desired pralidoxime iodide. Wilson, Nachmansohn and Ginsburg are holders of the US Patent # 2.816.113 (1957) describing the synthesis of pralidoxime (Wilson et al. 1957; Petroianu 2012).

Dr. Green remembers: *"2-PAM had a major weakness as a practical therapeutic agent in that it was only poorly water soluble, but shortly afterwards I synthesized the methane-sulphonate salt (P2S) which was much more soluble. We never tried to patent 2-PAM, but did patent this compound" (*Fig. 4*).*

It can be concluded that pralidoxime (2-PAM) was independently synthesized in Britain by Green and in the US by

Fig. 2: A F Childs. Photo of Dr. Childs as published in Chemistry & Industry 1970 12: 383. Courtesy of John Wiley & Sons, Chichester, United Kingdom.

DISCUSSIONS OF THE FARADAY SOCIETY

No. 20, 1955

A GENERAL DISCUSSION

ON

THE PHYSICAL CHEMISTRY OF ENZYMES

A GENERAL DISCUSSION on the Physical Chemistry of Enzymes was held in the University Laboratory of Physiology, Parks Road, Oxford (by kind permission of the Vice-Chancellor) on the 10, 11 and 12th August, 1955. The President, Prof. R. G. W. Norrish, F.R.S., was in the Chair and over 250 members and visitors were present.

Fig. 3: At the August Meeting of the Faraday Society in the University Laboratory of Physiology at Oxford Wilson delivered a talk titled "Promotion of Acetycholinesterase Activity by the Anionic Site" but made no mention of 2-PAM.

Wilson & Ginsburg. Green made the first public announcement about 2-PAM in August 1955; Wilson & Ginsburg submitted their manuscript in July 1955 and the paper was published in November. Due to the severe restrictions on publication in place in the UK the American contribution is much better known.

3. Biographical notes

Anthony Francis (Tony) Childs: After Taunton's School, Southampton he went on to Lincoln College, Oxford (BA,1943) and then to the Dyson Perrins laboratory, Oxford (D. Phil, 1945). The Dyson Perrins Laboratory was the main centre for research into organic chemistry of the University. It was founded with an endowment from the heir to the Lea & Perrins Worcestershire sauce company (Knowles 2003). In 1943 Childs became a Fellow of the Royal Institute of Chemistry. Between 1945 and1948 he lectured Chemistry at the Royal Military College of Science, Shriverham. Subsequently he joined the Chemical Defence Experimental Establishment at Porton (Ministry of Supply) where he stayed until 1956 when he moved to Albright & Wilson, Birmingham as Research Director. Albright and Wilson was founded in 1856 as a United Kingdom manufacturer of

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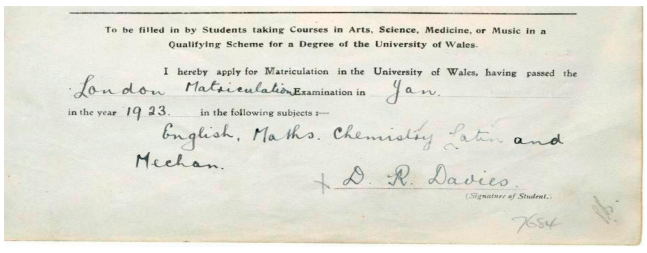
2,996,510
QUATERNISED PYRIDINE ALDOXIMES
Albert Lawrence Green, Salisbury, England, assignor to National Research Development Corporation, London, England, a British corporation
No Drawing. Filed Sept. 5, 1958, Ser. No. 759,146
4 Claims. (Cl. 260—294.8)

The present invention relates to quaternised pyridine aldoximes which may be used as therapeutic agents to counteract the inhibiting effect of various organo-phosphorus compounds on the enzyme cholinesterase in animal bodies.

Quaternised pyridine aldoximes are known to be particularly effective antidotes to these cholinesterase inhibitors as they restore the activity of cholinesterase that has been inhibited and in some instances decompose the inhibitor in the animal body.

Fig. 4: 2-PAM had a major weakness as a practical therapeutic agent in that it was only poorly water soluble, so Green shortly afterwards synthesized the methanesulphonate salt (P2S) which was much more soluble for which a US patent was obtained.


To be filled in by Students taking Courses in Arts, Science, Medicine, or Music in a Qualifying Scheme for a Degree of the University of Wales.

I hereby apply for Matriculation in the University of Wales, having passed the London Matriculation Examination in Jan.
in the year 1923. in the following subjects :—
English, Maths. Chemistry Latin and Mechan.
D. R. Davies
(Signature of Student.)

Fig. 5: Daniel Richard Davies signature on the application for admission to the University College of South Wales & Monmouthshire in Cardiff.

white phosphorus for the match industry. For much of its first 100 years of existence, phosphorus-derived chemicals formed the majority of its products. The final step in his career was to become a Consultant for the Atomic Energy Research Establishment (AERE) Harwell (1981–1985).

Daniel Richard (Dan) Davies (1904–1973) was born November 18, Penrhiwceiber, Glamorgan, Wales. His father Ivor was an elementary school teacher. After passing the matriculation exams Davies was admitted 1923 to the University College of South Wales and Monmouthshire in Cardiff (Reg # 7684) and later for an M. Sc. at the University of Wales in Bangor (Fig. 5). In 1932 he was awarded the Mrs. John Nixon Research Scholarship at the Welsh National School of Medicine in Cardiff where he investigated the phosphatases of mammalian tissues. In 1943 he became a Fellow of the Royal Institute of Chemistry and in 1947 he joined the Chemical Defence Experimental Establishment in Porton where he was promoted to the grade of Senior Principal Scientific Officer in 1955.

*Dr. Albert Lawrence (Bert) Green (*1929)* was born in East Ham, an east London suburb. At 18 he received a State Scholarship to study Chemistry at King's College London, where he obtained a 1st class honours degree and went on to obtain in 1953 a Ph.D. in organic chemistry with a thesis titled *Some studies in intramolecular acylation*. From 1953 to 1960 he worked in the chemistry and later biochemistry sections of the Chemical Defence Experimental Establishment, Porton, Wilts. Subsequently he joined the Smith, Kline & French Research Institute, Welwyn Garden City, Herts before being appointed in 1967 as Senior Lecturer in the Department of Biochemistry at the University of Strathclyde. In 1975 he was promoted to reader within the same Department. Dr. Green retired in Sept. 1993 from the University of Strathclyde.

John P Rutland worked at Porton from the late 40's to the early 70's. In the mid 50's he spent time at the Wellcome Research laboratories at Beckenham, Kent.

Leslie Thomas Douglas Williams B.Sc (Lond.) was an Associate of the Royal Institute of Chemistry (A.R.I.C) from the 1920's. In 1941 he became a Fellow of the Chemical Society (The Chemical Society and the Royal Institute of Chemistry amalgamated in 1971 to form the Royal Society of Chemistry). In 1962 he was appointed by the Queen to the Order of St. Michael and St. George. Williams occupied a number of very senior positions in research administration and was also posted overseas (Washington).

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