ALLOXAN DIABETES
A DISCOVERY, ALBEIT A MINOR ONE

N.G.B. McLetchie, Retired Pathologist, Gilford, New Hampshire, US

The remarkable discovery that a single injection of alloxan can produce diabetes mellitus in laboratory animals was made in 1942, in Glasgow, by John Shaw Dunn and Norman McLetchie. Alloxan, a simple nitrogenous organic compound, had made a long journey to get to Glasgow – it belongs to the very origins of systematic organic chemistry – from Germany. It was discovered by the fathers of this science, Frederick Wöhler and Justin J. Liebig, beginning with the synthesis of urea in 1828, then of uric acid and the naming of some 13 derivatives of uric acid, including alloxan. The name 'alloxan,' given by Wöhler and Liebig, is recorded as being derived from a combination of allantoin (a product of uric acid among others excreted by the fetus into the allantois) and 'oxalsaüre' (oxaluric acid derived from oxalic acid and urea, found in the urine).

Alloxan was originally obtained by the action of dilute nitric acid on uric acid. Unlike its parent, uric acid, which presents as a stable crystalline compound insoluble in water, alloxan presents as brownish-red crystals with great avidity for water and is very unstable with a half life of a few minutes in water at room temperature, and less at body temperature. It is also reported to be capable of developing explosive properties when stored as crystals. The name alloxan has survived although Wöhler was aware that in 1880 Brugnatelli, working and publishing in Italy, had identified the compound, naming it (in translation from the Greek) erythric acid after the red staining it caused to fingers. Liebig would go on to introduce improved oxidants for the synthesis of alloxan from uric acid. He also investigated the possibility of a physiological role for it. Alloxan's extreme ability made its reported finding in body fluids a matter of doubt and ideas of physiological action unlikely. In the period 1945–50 important work was done on the chemistry and synthesis of alloxan and its derivatives, and their possible diabetogenicity by refugees from Germany working in Israel and Switzerland in continuation of earlier work done in Germany.

Alloxan is a strong oxidising agent. Its reduction product is dialuric acid. Alloxan and dialuric acid form a compound, alloxantin, which in water can dissociate into alloxan and dialuric acid. Claims were made, and later disputed, that dialuric acid and alloxantin were diabetogenic. In any case, since dialuric acid can be oxidised to alloxan, it could be assumed that *in vivo* both compounds reverted to alloxan. In recent years it has been confirmed that the compounds are indeed diabetogenic. Moreover, *in vitro*, tested against cultures of the pancreas, alloxan and the products mentioned above produce necrosis of beta cells, while the alpha and delta cells and pancreatic exocrine cells are unaffected. This provides a striking demonstration of the high selectivity of the cytotoxic action of alloxan and its derivatives.

Early work investigated further the synthesis and testing of N-alkyl substitutes of alloxan, such as methylalloxan, and this has continued into a series of N-alkyl substitutes of alloxan such as ethyl-, propyl- and butylalloxan. Some are diabetogenic on injection, others are not; their clinical effects fall off as the chain of substitution lengthens. Somewhat startling is the fact that all are toxic to beta cells *in vitro* whilst alpha, delta and exocrine cells are spared. Among other things, this raises issues of differences derived from pharmacologic delivery to the target site.[1, 2]

In 1937, Jacobs reported that injections of alloxan in rabbits produced a transitory hyperglycaemia followed by a profound hypoglycaemia.[1] This was quite unknown to Shaw Dunn at the time of the discovery of alloxan diabetes. As to how often this fatal toxicity misled investigators is a matter of conjecture. Also, given the fatal hypoglycaemia, it has to be remembered that many general poisons often produced fluctuation of blood sugar and frequently ended in hypoglycaemia; this was commonly attributed to liver damage. However, until 1942, alloxan remained strictly in the domain of chemistry.

MUIR'S PATHOLOGY
The Institute of Pathology at the University and Western Infirmary, Glasgow, was dominated during his professional lifetime by Sir Robert Muir. An Edinburgh honours graduate in medicine, he trained there under Greenfield to become pathologist to Edinburgh Royal Infirmary. After scarcely a year as professor at Dundee, he moved to Glasgow in 1899. A procession of assistants, almost all local honours medical graduates with doctoral theses in hand, moved to occupy chairs in pathology, some in bacteriology, a few in clinical subjects. And so, the very epitome of a scholar and a gentleman, adorned with the academic distinctions of his time – Ph.D., M.D., D.Sc., F.R.S., a knighthood to boot – Muir became the father-image of pathology in Britain. Sir Robert retired at age 72, in 1936, to be succeeded by John Shaw Dunn. It is said that Muir stayed on until he was 72, not uncommon in those times, because there was no pension scheme.

Looking back, some would say that he had stayed on too long, and that Muir's magic had ended in 1931 with the transfer of his assistant, Daniel Cappell, to the chair at Dundee. Along with academic freedom and a very liberal attitude, Muir's latter years had produced an extreme institutional aestheticism. There were never pep talks, departmental meetings or bulletin boards. Now things seemed frozen in time. Before World War I, clinical bacteriology, clinical chemistry and haematology had been moved over to premises in the hospital generally referred to by the pathology staff as 'the other side' (rather than the clinical laboratory). Muir's protégé, Carl Browning, went to Ehrlich's laboratory, and headed up the Division of Chemotherapy. Via a chair in London during World War I, Browning came back to Glasgow at the end of the war as the first professor of bacteriology. Browning commanded the entire top floor of the Institute for his work on chemotherapy, and also directed the clinical bacteriology on the 'other side'; he enjoyed the standing as one of the country's leading scientists.

In time, senior posts were created to direct haematology and clinical chemistry in the hospital. Somehow, the work became very much separated from the mainstream of pathology, to the extent that, when research work in pathology included the above fields, it commonly ended up with the investigator acquiring the knowledge and equipment as best could be and carrying out the work in the pathology department. The problem, at least in part, rested on the fact that each ward system had its own clinical side room for urinalysis and the complete blood counts of the time. Calls for special tests demanded that the house doctor obtain the specimen and take it down to the clinical laboratory which, in this way, was divorced from direct contact with the wards.

JOHN SHAW DUNN

Addressed as Shaw Dunn (JSD) by his fellows, a Glasgow M.A. and honours medical graduate (Figure 1), he trained under Muir to finally be appointed Director of the Clinical Laboratory. Throughout World War I, JSD served as a pathologist – first in France, then, under Sir Joseph Barcroft, in experimental pathology at Porton's division of gas warfare. Returning from military service, he successively occupied chairs of pathology at Birmingham, Manchester and the Royal Infirmary, Glasgow, which had the 'junior' chair of pathology. With Muir's retiral in 1936 JSD, now 53, moved over from the Royal to the Western. He had arrived in the country at the very summit of classical pathology with credentials which eventually claimed an obituary, including bibliography and portrait, in the *Journal of Pathological Bacteriology* of some 16 pages, the largest by far ever in the journal; it was compiled by Muir and Browning.[3] JSD was very much at home and happy when he attended autopsies which always started at 10·00 a.m. on weekdays; this was still at a time when clinical chiefs attended with their entourage. The memory of Samson Gemmel of the Royal

was still around. He had, by history and clinical examination, diagnosed a ball thrombus in the left atrium that was proven by autopsy. Furthermore, it was rumoured that T.K. Munro, the regius professor of physic had repeated the trick when JSD arrived at the Western and his clinical diagnosis was again proven by autopsy. Even though the golden years of morbid anatomy were slipping away, JSD was the ultimate master of the autopsy. His very expertise at gradually working up the history and findings to the grand finale – a sharp slice right on target to unfold the bisected organ on a platter for all to inspect the causal lesion.

In their long and laudatory obituary Muir and Browning admit that at formal lectures JSD's delivery was poor, as he seemed to talk into his collar, and they also point out that he was extremely reticent. He was also given to very tortuous argument. I was appointed to the staff by JSD at the outbreak of war in 1939, and was for some years the only one appointed by him; I was cultivated by him as his protégé (Figure 2). Being young, I saw things in the best of light so what I write is largely in retrospect and from a long distance away. For thinking humankind there is an eternal conflict between expediency and morality sharply focused by the soldier in the jungle: should I abandon my sick and wounded comrade and move on, hoping to save at least myself, or should I stay and probably die with him? JSD tended to an extreme morality, much of which seemed harsh, and he was given to unnecessary judgements. His reticence was received badly, serving only to isolate him in an institute where it had been very much established you in your small corner and I in mine.

FIGURE 1
John Shaw Dunn, *c.* **1941.**

JSD often told me that he wished that he had stayed in Manchester. He had done notable work there on uric acid and experimental acute reno-tubular necrosis and the related altered renal function. Manchester seemed to have the right mix of work and facilities.

Above all, JSD was a rabbit man and took great pride in his knowledge and thorough clinical evaluation of rabbits: the administration of intravenous injections, retrieving of blood samples for analysis, passing of stomach tubes, taking of temperatures rectally, etc. He saw no future in assistants who did not do animal work; his enthusiasm was highlighted by a well-appointed animal room on the roof of the Institute, which previously had lain empty for years except for a lone monkey living out life after having been injected with exudate from a chancre. Things at the Royal had not worked out for JSD and his prospects at the Western seemed gloomier. The fact is, the more so looking back, JSD was the victim of a progressive malady which first entailed an exaggeration of personal traits, especially his reticence and conflict over suppressed ideas on what should be. For the gloomy Shaw Dunn, there seemed only one way for the Institute to go: down. When recognition suddenly seemed to come with alloxan, things soon distilled into enigmas worse than Elsinore to end in tragic death.

HAROLD H. SHEEHAN

The narrative working up to the revelations regarding alloxan has to be briefly interrupted to deal with Professor Sheehan (HS); he trained under JSD in Manchester in the 1920s. At the request of Carl Browning (via JSD) HS was given the task of studying the effects of the dye styryl quinoline 90 in rabbits which Browning, in his work on the chemotherapy of trypanosomiasis in mice, had found to be peculiarly toxic. HS's study was filed in a report to Browning. At the time HS reported to JSD that some rabbits injected with the dye had developed fluctuating blood sugars and islet necrosis – such findings have never since been substantiated. In JSD's obituary Muir and Browning dismiss the study of the 1920s in a footnote. Unfortunately, in the first publication on islet necrosis by alloxan JSD decided to include the supposed parallel action of styryl quinoline. Accordingly, HS was included as a co-author.[4] This detracted from the magic of alloxan and, apart from misunderstanding, reflects JSD's illness. Eventually, HS came to Glasgow as Director of Research at the Royal Maternity Hospital. Throughout World War II, HS served in the Royal Army Medical Corps and was called away as a lieutenant colonel in the reserve. After the war, he was appointed to the Holt Chair of Pathology at Liverpool.

While in Glasgow, HS became interested in post-partum pituitary necrosis, which later became known as Sheehan's syndrome (instead of Simmonds's disease). As a medical student, I attended a lecture by Sheehan. To find examples of post-partum pituitary necrosis, HS declared that one

FIGURE 2
Norman McLetchie, 1942.

should send out postcards to women who, from hospital records, had experienced great blood loss and shock during child birth; then, call on those who did not reply! As a free spirit, Irish wit and raconteur, Sheehan then acted out the encountering, at her home, of a woman with the condition, a picture of general misery and continual complaints about cold from drafts at which HS turned up his collar, crouched and warmed his hands over an imaginary kitchen fire and jerked his head around to see that doors and windows were closed. Then he fell back, staring eyes and twitching interspersed with a fine rhythmic tremor. All of this was relieved by a hot cup of tea laced with sugar lumps, ostensibly to relieve hypoglycaemia. In 1942, I encountered an example of pituitary necrosis following the very stormy birth of triplets to a woman who died six years after delivery with a clinical history mirroring HS's acting. I sent the histological material to HS. We published the case under Simmonds's disease with a review of the literature on post-partum pituitary necrosis, including the production of hypoglycaemia and hypothermia.[5] HS's lecture and enthusiasm would serve me well when JSD turned to alloxan.

THE CRUSH SYNDROME

By the autumn of 1940, bombs rained down on London in air raids preparatory to Hitler's plans for the invasion of England. Out of this came the 'Crush Syndrome', apparently new in Britain, though it was documented in the German archives of World War I. A common history was of the victim buried in debris for some hours, characteristically with a heavy wooden beam pressing

on an impaled thigh. Rescued and revived by little more than cups of hot tea and seemingly recovered, the victim would suddenly collapse; by this time the involved thigh was grossly swollen. Death could come in a few days from renal shutdown with extreme oliguria and its attendant uraemia. In the autumn of 1942, Dr E.G.L. Bywaters came to a meeting of the Pathological Society in Glasgow to describe the investigation that took place during World War II in London by a team under the Medical Research Council. As well as a complete account and illustration of the renal pathology, there was a detailed description of the crushed thigh with the release into the circulation of myoglobin, haemoglobin, lactic acid, creatinine, phosphate and potassium and also graphs of the sequential estimation of sodium, potassium, chlorine and urea. In addition, there was quantitative analysis of the volume expansion of the thigh including the dead muscle and its total myoglobin release. Nothing like it had been seen before in the field of applied medical chemistry in Glasgow, or possibly in the entire country.

Bywaters emphasised the precipitation in the distal renal tubules of myoglobin in an acid urine occasioned by metabolic acidosis. This blockage contributed greatly to the gross interstitial renal oedema by raising intrarenal pressure. Other factors were also mentioned, such as vasospasm and shunting of cortical renal blood flow into the medulla. Emphasis was also made that myoglobin was a much smaller molecule than haemoglobin so that its sojourn in the renal tubules was shorter than the blockage in mismatched blood transfusion. Bywaters also dwelt on other problems: cardiac arrest from hyperkalaemia, fluid balance, supply of energy by a stomach tube without raising the nitrogenous burden: the Hammersmith cocktail.

All of this was delivered before even the dawn of the revolution in clinical pathology and at the very feet of an authority on acute reno-tubular injury: John Shaw Dunn. Indeed, in 1941 he had published, with colleagues, descriptive articles on the kidneys of the Crush Syndrome victims based on findings in London.[6] The value of such observations suffered from the fact that by the time of the death of the victim, more or less all of the myoglobin deposit had been washed out of the kidneys so that any purely morbid description lost this emphasis. The Bywaters team rectified this by experimental work on rabbits. A great impetus came from the London work. Indeed, after the war, renal shutdown and reno-tubular necrosis became something to be read up on. There were many varieties of this syndrome; for myself I would be constantly on the look out for collector's pieces and was mindful that what was found at autopsy could be very different from the clinical diagnosis. Also, the Bywaters team created an urgency to develop renal dialysis. My vivid memory of Bywaters's lecture at Glasgow is reinforced by his memoir.[7]

ALLOXAN DIABETES

With the tour de force by Bywaters and his group it might be expected that JSD took a bow at least in that direction, and the more so since clinical chemistry had passed from his grasp, apparently even his command, and animal work seemed a thing of the past. But now he very much took up the Bywaters's gauntlet, beginning with having me try out intravenous haemoglobin solutions on rabbits and then the same altered to haematins: nothing of note could be reported. Now, briefing me in his office, he questioned that derivatives of nitrogenous breakdown might come from the damaged muscle and, like uric acid, be renotubular-toxic, his seminal work at Manchester. Then suddenly he swung round in his revolving stool and plucked a thin chemical almanac from his bookshelf, thumbing through it he handed it to me and stated, 'There, try that: *alloxan*.'

Once samples of alloxan arrived I moved rabbits into JSD's laboratory. Coming in, JSD had a fine whistle from almost closed teeth, a sign he was in a good mood. He was to stand over me and see how well I gave the rabbits intravenous injections via marginal ear veins. The next morning I checked on the rabbits and found that some were dead; the survivors were put on top of a smooth bench but did not skid around (which was their unusual wont); they had staring eyes and a general unresponsiveness to handling, and also exhibited a fine tremor. The rectal temperature was low. JSD looked startled when I declared that perhaps the animals had hypoglycaemia; he was still more startled when I said that perhaps they had pituitary necrosis. I suppose from Sheehan's teaching and my interest in endocrines, the cause of stupor foremost on my mind was hypoglycaemia; also, to boot, a cause of this, identifiable by a microscopist, was pituitary necrosis. Following my unlikely and tangential assertion, JSD told me to go ahead, take blood samples and be sure to examine the kidneys; this included the living rabbits which were sacrificed. I made routine examinations including the endocrine glands. In a few days, I returned to inform JSD: 'Sir, not pituitary necrosis; necrosis of the islets of Langerhans.' A wide-eyed JSD inspected the sections. The initial severe hypoglycaemia came to be attributed to release by the damaged beta cells of the islets of their store of insulin. Once over the acute phase, diabetes ensues (generally within 24 hours) due to loss of beta cells and, hence, of insulin production. It was November 1942. Before Christmas, we had diabetic rats, also some rabbits surviving without insulin for a few days and having diabetic stigmata.

Alloxan was given intravenously in aqueous solutions to rabbits and intraperitoneally to our first group of Wistar rats. Curved glass needles with ends 'softened' by heat had been drawn in the laboratory with entrance to the peritoneal cavity by no more than a nick. While surviving rabbits appeared listless and lost weight, in contrast, the rats behaved like juvenile diabetics, eating voraciously,

exhibiting polydypsia, polyuria, glycosuria and hyperglycaemia. Some months later, JSD would take some of the edge off the drama of suddenly suggesting alloxan. Apparently, in his work on uric acid at Manchester, he had tried out alloxan which had resulted in the death of the rabbits. His original silence was presumably part of his common reticence or perhaps to set a challenge to me (his pupil). In the first publication on alloxan, JSD refers to the episodes in Manchester when rabbits were given alloxan with fatal results.[4]

BETA CELL IDENTIFICATION

Munger has given a history of the development of histological stains for the separation of alpha and beta cells, and later other cells in the islets.[8] In our time, histological production of distinct granules was not accomplished, at least in Glasgow, though Gomori and others in the US may have been more successful with beta cells. At best, beta cells exhibited a somewhat granular cytoplasm while the cytoplasm of alpha cells was diffusely coloured by the counterstain. More importantly, in rabbits and rodents the islets at the tail of the pancreas islets may consist entirely of beta cells or characteristically of a core of beta cells more or less surrounded by a mantle of alpha cells. Therefore, in beta cell necrosis, in sections from the tail, the islets usually show total necrosis or a core of necrotic cells with a conspicuous corona of viable cells, the hallmark of alloxan's selective action. Later, the islets at the tail either disappear or are small, and consist only of alpha cells. Corresponding changes take place at other levels of the pancreas but without any striking distribution. Looking back as an early investigator, the prevalent view that the cells in the islets at the tail of the pancreas were either identifiable alpha or beta cells acted as a tyranny. It would have been better to refer to alpha and beta cell-lines, and so to entertain the concepts of precursor cells, immature or poorly granulated cells, and mature but degranulated cells. Hence, with the technical limitations of the time, the identification of the beta cell as the target of alloxan could well rest on the distinct pattern of effects and distribution of the insulin. This would have saved much time until technical innovations came along, the more so at Glasgow where early public disclosure and publication was decided.

While the electron microscope was to add much to the separation of cellular entities in the islets and to verify the selective action of alloxan on beta cells, it was to take some 25 years before routine histological methods were sufficiently developed to illustrate alpha and beta cells, each with distinctive granules in one field under the light microscope and separated tinctorially. The improvements involved fixation in glutaraldehyde, embedding in plastic, modern microtomes and glass knives.[9]

COMMUNICATIONS

JSD was on government committees in London relating to the Beveridge Report for the implementation of a national medical service to come after the war. At such meetings, before the turn of 1942, JSD informed some of his peers of the discovery. At that time, endocrinological research in London took a premier place. For example, the editor and five members of the board of the new *Journal of Endocrinology* were fellows of the Royal Society. By any standards, the discovery that selective necrosis of the islets of Langerhans produced insulin dependent diabetes (IDD) was startling, and the more so in medical scientific circles in London. JSD seemed very upset by the close questioning he got on visits there. Whatever the reason, JSD decided there and then to communicate the discovery to scientific societies and to go to press.

In early 1943, JSD travelled to Dundee to give an account of experimental islet-cell necrosis and diabetes before the Scottish Society for Experimental Medicine. He repeated the lecture in his own department, and an edited version was published in June 1943.[11] While dealing mainly with islet-cell necrosis, this early work briefly refers to rabbits with glycosuvia and rats with full-blown diabetes mellitus. American authors were later to claim priority. In March, at a meeting of the Pathological Society in London, I gave our co-written paper on alloxan diabetes in the rat[12] (on behalf of Shaw Dunn). The term 'alloxan diabetes' was coined by the authors and first used at the meeting in London. The account covered the original series treated by intraperitoneal injections with descriptions from islet-cell necrosis to disappearance of beta cells, including the characteristic distribution of beta cells, also found in the few mice tested. Despite the fact that acute events, especially from the tail of the pancreas, lent themselves to striking black and white photomicrographs, there was an almost acrimonious exchange with a biochemist who claimed that alloxan acted primarily on the liver. Eventually, Sir Robert Muir, now in his 80th year, arose and *ex cathedra* ended the debate: '*no doubt a discovery, albeit a minor one*'. The rats recovered from the initial hypoglycaemia without assistance of glucose injections and without evidence in the acute stages of liver or kidney damage to routine histological investigations. Treated rats lived up to six weeks with all the signs and common laboratory stigmata of IDD without giving insulin, longer if the series had not been ended to start using only subcutaneous injections. The publication on alloxan diabetes in the rat did not appear until September and did not include my intraperitoneal work; it was confined to the results of the subcutaneous injections.[11]

While it is generally accepted that the discovery of the action of alloxan was one of serendipity, perhaps the ultimate in serendipity, JSD very much did not want it to be so. Rather, he wanted to present it as a continuum of his original work on the kidney and to be associated

with a team working under his direction. I can well understand this for someone who championed experimental animal work: animal work had now championed him. The first publication on the rabbit dealt with the initial stage of islet-cell necrosis by intravenous alloxan along with a summary of the action of styryl quinoline already referred to.[4] An article followed on islet-cell necrosis where, in addition to alloxan, some of the rabbits also got intravenous haemoglobin solutions.[13] The idea was that alloxan might potentiate reno-tubular accumulation of blood pigment. Indeed, as with some of the rabbits in the tests, higher doses of alloxan than those necessary to produce diabetes can produce acute reno-tubular lesions, and while added intravenous haemoglobin injections may produce iron-pigment deposits at the site of tubular injury, this in the end only served to detract from the magic of alloxan.

In early July 1943, JSD gave a complete account of the Glasgow work on the rabbit at a meeting of the Pathological Society in Manchester.[14] This included the histological separation of alpha and beta cells with the latter as the precise target of alloxan, and keeping alive diabetic rabbits. By this time, I was in the Royal Army Medical Corps but attended the meeting to give a microscopic demonstration in the student laboratory of the histologic staining of the endocrine glands in rabbits, rats and in humans, including the islets and characteristic distribution of the beta cells in the tail of the pancreas in rabbits, rats and mice. Hughes, Ware and Young referred to the minutes of this meeting in their account of the confirmation of the Glasgow finding of alloxan diabetes in the rabbit.[15] After I had left for the Medical Corps in the late spring of 1943, JSD was assisted by Dr Edward Duffy. By the autumn, JSD's malady had increased to cause much concern. By December he was on leave of absence. His tragic death followed in June 1944, aged 61. The report, given in Manchester in June 1943, was published in 1944.[16]

GLOBAL RESEARCH
Medical officers from the Forces, including American colleagues, were present at the delivering of the papers on alloxan at Dundee and London. News quickly went across the Atlantic, and an editorial on alloxan appeared in *JAMA* on 3 July 1943, including reference to the first publication by Shaw Dunn, Sheehan and McLetchie. By 31 July, Gomori of the University of Chicago, who was the leading authority on identification of islet-cells in the US, along with colleagues had produced a letter to *JAMA* announcing the production of alloxan diabetes in rabbits and dogs, and the failure of alloxan to alter the blood sugars in a patient with islet-cell carcinoma of the pancreas and in two other cancer patients.[17] Within weeks, publications followed from the US on alloxan diabetes in dogs, rabbits and rats.[18–20]

Classical pathology approaches gave way to electron microscopy, histochemistry, application of isotopes and immunohistochemistry and radioimmune assay, allowing measurement of minute quantities of hormonal output. A very important advance involved inhibiting the digestive enzymes of the exocrine pancreas, in this way avoiding autodigestion. Fine slices of pancreas including exocrine and islet tissue could be grown together in culture, since electron microscopy could now confidently separate mature alpha and beta cells and the elusive delta cells, this allowed of the ultimate demonstration of the exquisite specificity of alloxan and studies between alloxan, its derivatives and N-alkyl substitutes (as already alluded to, including streptozotocin). This cut out the tedium and uncertainty of isolating islets or beta cells.

And so alloxan took off, and from 1944–5 there were more than 50 publications, mainly outlining work done in the US. In 1991, at the World Congress of the International Diabetes Society at Washington, I was given a tome: a print-out of details of over 1,500 papers in scientific journals on alloxan for the period 1964–88 with over 80 in one year. Alloxan is for all seasons: from rabbits to rats, to mice, to goats, to monkeys, to bats, to fish (including goldfish and sharks), to amphibia, to fowl, to pregnant ewes and pregnant swine. Variation between the species lies in susceptibility. Early on, the rat emerged by far as the favourite subject. Liver and kidney damage can be produced but at a dosage adequate for IDD generally there is no such damage identifiable by routine methods of examination. Perhaps the sum of the plethora of publications says more about humankind than about alloxan.

Chemical mechanisms of the selective action of alloxan are reviewed by Cooperstein and Watson,[21] and also by Webb.[23] Early on it was recognised that alloxan inhibits the key enzyme glucokinase originally extracted from the liver and later recognised to be present in beta cells. Glucokinase is part of the signal mechanism whereby blood-glucose levels monitor insulin production. A second early and important theory on the specific toxic action of alloxan involved its reaction with SH groups and thiol groups, especially oxidation of the peptide glutathione widely present in cells. In this reaction, the product of alloxan is dialuric acid. The reaction with glutathione is held to initiate oxidation-reduction cycles via the oxidation of dialuric acid back to alloxan with the liberation of peroxides, superoxides and hydroxyl radicals, all highly cytotoxic. An important finding was that markedly raised levels of blood sugar protect against the actions of alloxan. Some of the confusing findings at the time of the discovery of alloxan's diabetogenic action and for a few years thereafter were no doubt because this inhibition had not been appreciated. Hyperglycaemia prevents, and starvation enhances, the action of alloxan. Another reason for early confusion was failure to fully appreciate the marked lability of alloxan and its (possible) deterioration in storage as crystals, and in aqueous solution.

STREPTOZOTOCIN

In 1963, Rakeitin and his colleagues reported that streptozotocin (STZ), an antibiotic, produced IDD on injection into rats and dogs. Indeed, its biological actions correspond closely to alloxan being both labile and hydrophilic. Streptozotocin is a nitrosourea derived from a methylnitrosourea from which it differs by the addition of a D-glucose profile. At the end of World War II, beginning with mustard gas, an expanding industry developed relating alkylating agents to DNA damage to act variously as mutagens, carcinogens, anti-cancer agents and cytotoxins. The nitrosoureas are potent members of this group. Like its parent nitrosourea, STZ is carcinogenic. Streptozotocin is more prone to complications than alloxan, especially liver and kidney damage. For the reasons given it never took off like alloxan.[22] Attention is next directed to STZ having a prime toxic action against DNA, and its possession of a glucose profile being central to its affinity for beta cells.

BETA CELLS AND SPECIAL GLUCOSE PORTALS

Since blood glucose levels monitor insulin production and elevated levels can block the entry of alloxan and STZ into beta cells, it has long been considered that beta cells have special portals or transporters which are exploited by diabetogenic agents. Beta cells, like all cells, take up glucose for metabolism but also have special monitoring glucose transporters. A specific surface glucose transporter (GLUT2) has now been characterised on the surface of beta cells which is exploited by alloxan and STZ.[24] As might be expected, insulinoma cells (I cells), harvested from rat tumors and cultured as clones, do not express GLUT2 as a manifestation of their tumour autonomy in escaping from normal blood glucose controls. Furthermore, bioengineering has isolated the appropriate DNA for GLUT2 and also transfected it to I cells. In this way, the investigator has I cells minus GLUT2 and I cells plus GLUT2 to test against diabetogenic agents and their chemical relatives. For practical purposes the investigator has clones of beta cells with and without the glucose transporter. Insulinoma cells can also be transfected so that they express glucokinase in excess.[25]

Using such innovations and more, *in vitro* toxicity trials have been conducted testing the relative toxicity of diabetogenic agents and related compounds against cultures of beta cells with and without GLUT2 and other crucial singularities, with results being inspected under the electron microscope. In this way, the Lenzen group, including the work of others, have drawn up the following list of generalisations for STZ with a personal communication from Lenzen that, by and large, the work on alloxan proceeds in the same direction:[24]

1. the manifest selective action of STZ depends on its uptake into cells possessing GLUT2;
2. beta cells, also liver and reno-tubular cells, possess GLUT2. GLUT2 channels, when not occupied by glucose, are exploited by the diabetogens;
3. the possession of a D-glucose profile is the key to its affinity for GLUT2; alloxan also possesses a glucose profile;
4. glucokinase along with GLUT2 is part of the signalling mechanism whereby blood-glucose levels monitor insulin production; however, glucokinase is not critical to the toxic action of STZ; and
5. the cytotoxicity of STZ, as with nitrosoureas in general, depends on damage to DNA; in turn, this leads to exhaltation of DNA-repair mechanisms so that resulting energy exchanges account for the rapid cytotoxicity with disrupture of the intracellular membranes and organelles of the beta cell.

In regard to the ultimate pathway of cytotoxicity, alloxan may well differ from STZ. The theory that alloxan acts through oxidative reactions to release toxic oxygen species still holds sway.

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This year will see the 60th anniversary of the discovery at Glasgow of alloxan's action on the islets. I recall that around that time Professor Carl Browning, reminiscing on his early days in Germany, said that Ehrlich thought it inadvisable to take up research on cancer unless one was prepared to lay bare the secret of life itself. Indeed, rather than a discovery, it often looks more that Shaw

FIGURE 3
Norman McLetchie on his retiral, 1989.

Dunn, the sorcerer, had allowed his apprentice to let the genie out of the bottle. There never was anything before like alloxan and never anything quite like it since. But Rip van Winkle now awakens to a new Sleeply Hollow, where technology advances at a breathtaking rate – except for me with little breath. So there remains little doubt, that this new year or maybe a little further on, the chronicler of alloxan will no longer have to stumble along. Hopefully also there will be spin off so that the laying bare of the secrets of alloxan will be for more than just 'it was there' or that it concentrated minds to clearly separate IDD from NIDD. The discovery, 'albeit a minor one,' provided investigators with a magic wand to conjure up IDD in laboratory animals, and still does.

I did not see the Glasgow publications until after the war, except for a brief look at the first.[4] This was confined to the initial islet-cell necrosis, and there I got a surprise with the inclusion of styryl quinoloine, with alloxan taking second place in the order of presentation. This was at a time when professors of pathology were 'gods', and perhaps JSD was the last of the gods. Papers were prepared exclusively by him and the co-authors were of his choosing.

In the confusing and uncertain times which followed the war, I had more important things to think about other than alloxan. So it was long after, when I came to look at the unfolding story of alloxan, that I came to realise that the Glasgow publications seemed laboured and dwelt on the particular, individual test animals, instead of fertile generalisations as befitted the startling discovery, its circumstances and hasty revelation. The fault seemed largely to come from using small numbers of experimental animals, so exaggerating the variability of outcome which comes from, as previously mentioned, factors such as the chemical instability of alloxan, the importance of the glycaemic and general nutritional status of the test animals and also the vagaries of subcutaneous injections in rats, which were so much less precise than intraperitoneal delivery. The Glasgow Institute had the capacity to field animals in much greater numbers but the mental climate of war, the early decision of disclosure and JSD's illness and special agenda set the tone. Be that as it may, the Glasgow work on alloxan, virtually over by June 1943, uncovered all of the facts available to routine pathology of the time, except for the ability of induced hyperglycaemia to abort the action of alloxan; not least was the provision of superb photomicrography from Glasgow. When the publications of the American workers came out from late July onwards, apparently due to wartime communications, they were only then aware of the first Glasgow publication on islet-cell necrosis. So they claimed priority of discovery in regard to the beta cell as the precise target and the production of diabetes mellitus. Commonly, when the history of alloxan as a diabetogenic agent is given, the mistake is perpetuated with credit to the Glasgow workers confined to the uncovering of islet-cell necrosis. While this was the salutary discovery to me, any reasonable account of the Glasgow work has to be full of irony; indeed, there is much more than I have seen fit to describe, ' "For what is the truth" said the jesting Pilate; nor waited he for an answer.'

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