OBITUARY NOTICE.

HENRY DRYSDALE DAKIN.

1880—1952.

H. D. DAKIN, an organic chemist who through his experimental and theoretical work contributed effectively to the establishment of biochemistry as an independent discipline, was born on March 12th, 1880, in London, whence his father, Thomas Burns Dakin, a merchant, soon moved to Leeds. Here he received all his early training; after attending Leeds Modern School, he worked for a brief period in the laboratory of Fairley, the City Analyst, and in 1898 he entered Yorkshire College, now the University of Leeds, to study chemistry under Julius B. Cohen. While still an undergraduate, he collaborated with that eminent chemist in an investigation of the halogenation of aromatic compounds under the influence of the aluminium–mercury couple. This led to systematic work on the constitution of the di-, tri-, and tetra-chlorotoluenes. In addition, Dakin's interest in analytical chemistry, no doubt stimulated by his experiences in the City laboratory, led him at the same time to study the use of phosphate in the determination of various metals and the applications of persulphate to the determination of nitrogen, manganese, and chromium.

In 1901 Dakin was granted the baccalaureate in Science of Victoria University, Manchester, of which Yorkshire College was then an affiliate. In the following year he was awarded an 1851 Exhibition scholarship which enabled him to work in other laboratories. The first of these was the Jenner Institute, in London, the title of which was soon changed to the Lister Institute of Preventive Medicine. Here he joined S. G. Hedin in a study of the proteolytic action of an enzyme present in kidney cells, and then began an independent investigation of the differential activity of lipase towards the respective stereoisomeric components of esters of racemic mandelic acid. In this work, which had an immediate effect on the theory of enzyme action, he showed (*J. Physiol.*, 1904, **30**, 253) that esters of the dextrorotatory acid are more readily hydrolysed by the enzyme than are those of the lævorotatory form. This led him to advance the view, now universally accepted, that the primary step in the process is the formation of a transitory compound of enzyme and substrate.

During 1904 Dakin worked in Heidelberg, where he collaborated with Albrecht Kossel in studies of protamines; these culminated in the discovery of arginase (*Z. physiol. Chem.*, 1904, **41**, 321). On his return to England he rejoined the staff of the Lister Institute, resuming his investigations of the action of lipase on mandelic esters and analogous compounds. In these (*J. Physiol.*, 1905, **32**, 199) he demonstrated the dependence of sensitivity to the enzyme on the configuration of the acyl group rather than on the direction of the optical rotation of the intact ester. He also observed selective enzymic hydrolysis of esters in which the potential asymmetry resided in the alkyl group, a confirmation of his previous conclusion that the enzyme combines with the intact esters before their hydrolytic breakdown. He also carried out a synthesis of epinephrine and allied bases, and related their physiological activity to chemical structure (*Proc. Roy. Soc.*, 1905, *B*, **76**, 491, 498).

In 1905 Dakin accepted an invitation from Christian A. Herter, a founder and benefactor of the *Journal of Biological Chemistry*, to work in a private laboratory which Herter had established in New York. In this environment Dakin was not only enabled but encouraged to devote his entire time to investigations of his own choice. His first publication after his arrival (*J. Biol. Chem.*, 1906, **1**, 171) concerned the oxidation of amino-acids in neutral solution by hydrogen peroxide in the presence of traces of ferrous salts, whereby deamination and decarboxylation occurred with formation of aldehydes (and acids) containing one carbon atom less. This reaction proved to be general, except in the case of glycine, which yielded notable proportions of glyoxylic acid. During the following years the action of this almost physiologically mild method of oxidation, which had previously been successfully employed by Fenton for the oxidation of sugars, was applied to a wide variety of organic substances of metabolic interest. Unlike glycine, sarcosine and glycollic acid were found to yield no glyoxylic acid; however, this was formed in traces from betaine and hippuric acid and readily from creatine and creatinine. Benzoates, studied in collaboration with Herter's daughter Mary, were shown (*ibid.*, 1907, **3**, 419) to become hydroxylated, primarily to salicylic and *p*-hydroxybenzoic acids, and secondarily to catecholcarboxylic acids.

These results stimulated studies of the oxidation of simple aliphatic compounds in the

animal body. Dakin showed that whereas the administration of acetate did not result in oxaluria, oxalic acid was excreted after the ingestion of glycollic acid or ethylene glycol. Glyoxylic acid, which also yields oxalic acid *in vivo*, was found not to be a precursor of allantoin.

His observation that acetone is an end-product of the oxidation of leucine by an excess of hydrogen peroxide led Dakin to his classical work (*ibid.*, 1903, **4**, 71, 91, 221, 227, 419; **5**, 173, 303) on the β-oxidation of fatty acids *in vitro* and *in vivo*. This elegant and exhaustive investigation supplied a satisfactory chemical explanation of the results secured four years earlier by Knoop in his study of the metabolic fate of terminally phenylated fatty acids. In the course of this work Dakin showed that the methyl ketones acetone and acetophenone, formed by decarboxylation of β-keto-acids, are degraded to acetic and benzoic acids in the living animal as well as by hydrogen peroxide *in vitro*. The question whether the primary products of the biochemical oxidation of fatty acids are β-hydroxy- or β-keto-acids was left open; Dakin showed, however, that when a β-hydroxy-acid is excreted it always appears as a lævorotatory form.

Incidentally to his experiments on the oxidation of organic compounds by hydrogen peroxide, Dakin introduced (*ibid.*, 1908, **4**, 235) the use of *p*-nitrophenylhydrazine for the characterization of aldehydes and ketones. He then discovered (*Proc.*, 1909, 194) a remarkable general reaction of aromatic aldehydes and ketones containing a phenolic hydroxyl group in the *ortho*- or *para*-position: for example, salicylaldehyde, when treated in faintly alkaline solution with hydrogen peroxide, breaks down into catechol and formate; *p*-hydroxyacetophenone similarly treated yields quinol and acetate.

A study of the metabolic fate of γ-phenylvaleric acid and its β-hydroxy-derivative, which gave rise to the excretion of hippuric acid, led Dakin to advance (*J. Biol. Chem.*, 1909, **6**, 221) the hypothesis that the catabolism of fatty acids involves the successive removal of two carbon groups at a time. The fact that benzoic and phenylacetic acids, the ultimate degradation products of ω-phenylated fatty acids containing respectively odd and even numbers of carbon atoms in the side chain, are excreted in the form of their glycine derivatives was correlated with the observation that conjugation with glycine diminishes the toxicity of phenylpropionic acid (*ibid.*, 1908—09, **5**, 173, 303; 1909, **6**, 203). Introduction of a hydroxyl group into the β-position of phenylpropionic acid had the same effect. Administration of β-hydroxy-β-phenylpropionic acid and of phenylglyceric acid led to the excretion of hippuric acid; on the other hand, racemic phenylalanine and phenyl-β-alanine were found to reappear in the urine in the form of their ureides without undergoing oxidative degradation (*ibid.*, 1909, **6**, 235).

In collaboration with A. J. Wakeman, Dakin demonstrated (*ibid.*, 1909, **6**, 373; 1910, **8**, 105) the presence in liver of enzymes which promote the reversible oxidation of β-hydroxybutyric acid to acetoacetic acid.

At about the same time, having shown (*Amer. Chem. J.*, 1910, **44**, 45) that optically active hydantoins (though not hydantoic acids) are rapidly racemised in dilute alkali, Dakin and Lafayette B. Mendel of Yale University demonstrated the optical inactivity of urinary allantoin, and suggested that this was due to enolisation (*J. Biol. Chem.*, 1910, **7**, 153).

He then threw some light on the chemical nature of alcaptonuria (*ibid.*, 1910, **8**, 11, 25; 1911, **9**, 151). Racemic tyrosine when administered to normal animals was found to be partly excreted in the form of its ureide and hydantoin, and its methyl ether yielded some *p*-methoxyphenylacetic acid. As tyrosine methyl ether and *p*-tolylpyruvic acid, neither of which is capable of forming a *p*-quinonoid derivative, were found to yield acetoacetic acid on perfusion through a surviving liver, Wakeman and Dakin (*ibid.*, 1911, **9**, 139) concluded that ring-opening is involved in the normal catabolism of tyrosine. An alcaptonuric subject was observed to be able to effect the complete oxidation of *p*-tolyl- and *p*-methoxyphenyl-alanine; the primary abnormality in alcaptonuria was therefore thought to consist in the formation of homogentisic acid rather than a failure to oxidise it.

With Wakeman, Dakin studied (*ibid.*, 1911, **9**, 327) the then obscure biochemical relation between urea and ammonia; as perfusion of urea through a surviving liver gave rise to no ammonia at the expense of urea, it was concluded that the postulated conversion *in vivo* of ammonium carbonate into urea was irreversible.

In a series of studies (*ibid.*, 1911, **9**, 139; 1912, **10**, 499; **13**, 513; 1913, **14**, 321) of the intermediary metabolism of amino-acids, Dakin, partly with Wakeman, extended Embden's observations according to which these compounds fell into two classes, those which are biochemically converted into glucose and acetoacetic acid respectively. It is noteworthy that he suggested the close metabolic relation, now firmly established through studies made with the aid of isotopes, of proline to ornithine and glutamic acid.

In 1913, Dakin was joined by the late Harold W. Dudley, also one of the impressive number

of brilliant students of J. B. Cohen who became leaders in the field of biochemistry. This collaboration, though it lasted only two years, was a singularly fruitful one. Their first joint publication (*ibid.*, 1913, **14**, 423) recorded the discovery of an enzyme, widely distributed among mammalian tissues, which converts methylglyoxal and phenylglyoxal into lævorotatory lactic and mandelic acid respectively. This enzyme, glyoxalase, stable to alkali but inhibited and inactivated by acid, was shown to be present also in fish, oysters, and toads, but not in green plants or (except for traces) in yeast. Red blood cells were found to be especially rich in the enzyme. On the other hand, pancreas not only yielded no glyoxalase but contained a specific, thermolabile antiglyoxalase; abdominal lymph glands contained neither the enzyme nor the inhibitor. The enzymic reaction appeared to be general for α-keto-aldehydes; for example, glyoxalase was found readily to convert glyoxal into glycollic acid. Glyoxalase was shown to differ from Neuberg's keto-aldehyde mutase, which had no effect on glyoxal, and from the aldehyde mutase of Parnas, which was not inhibited by pancreas extract. Incidentally, pancreatic antiglyoxalase was found to have no effect on the production of acetoacetate from butyric acid or tyrosine by perfusion through the liver (*ibid.*, 1913, **15**, 463; 1914, **16**, 505, 515; *Biochem. Z.*, 1914, **59**, 193).

In studies stimulated by the idea that α-keto-aldehydes may represent a cardinal phase in the metabolism of carbohydrates and amino-acids, Dakin and Dudley (*Proc.*, 1913, 156, 192; *J. Biol. Chem.*, 1913, **14**, 555; **15**, 127) discovered that when lactic acid and alanine are kept in dilute solution at 37° in the presence of *p*-nitrophenylhydrazine, the *p*-nitrophenylosazone of methylglyoxal is deposited. Analogous reactions were observed with glycollic, glyceric, and mandelic acids; also with glycine, leucine, and other amino-acids. The chemical mechanism of this remarkable reaction (Dakin, *Biochem. J.*, 1916, **10**, 313) is still obscure, and its significance in intermediate metabolism has yet to be established. By elaborate and painstaking studies, Dakin and Dudley conclusively demonstrated the metabolic formation of optically active amino-acids from glyoxals.

An outcome of Dakin's earlier work on the racemisation of hydantoins had been a preliminary study (*J. Biol. Chem.*, 1912, **13**, 357) of the action of alkali on gelatin. His theoretical prediction that the non-terminal amino-acid groups in peptide chains would lose their rotatory power was confirmed; of the individual amino-acids isolated from a hydrolysate of alkali-treated gelatin, some were optically unchanged, some partly racemic, and some optically inactive. In a laborious investigation of " racemised " casein, Dakin and Dudley (*ibid.*, 1913, **15**, 263, 271) showed that of the eleven component amino-acids isolated one, namely proline, retained its full activity and must therefore have been present in only terminal positions in the protein molecule. Of the remainder, three (alanine, valine, and leucine) were partly racemic and must therefore have been represented among the terminal units; the others were completely inactive. " Racemised " gelatin, on the other hand, yielded fully active glutamic acid, lysine, and proline, partly racemic alanine, and inactive leucine; all other amino-acids isolated were completely racemic. Apart from its altered optical activity, the alkali-treated casein closely resembled native casein in physical properties; it was, on the other hand, entirely unaffected by pepsin, trypsin, and erepsin, and when fed to a dog was excreted unchanged in the fæces.

Other studies published jointly by Dakin and Dudley (*ibid.*, 1914, **17**, 29, 451, 275; *J.*, 1914, **105**, 2453) related to a method for the resolution of α-uramino-acids by means of their strychnine salts; a demonstration of the metabolic conversion of the " unnatural " variety of alanine into glucose; some limitations of the Kjeldahl method for the analysis of cyclic compounds containing nitrogen in the ring; and a general method for the synthesis of alkylglyoxals from γγ-diethoxyacetoacetic ester.

During 1914 Dakin published a report (*J. Biol. Chem.*, 1914, **18**, 91) on the formation of benzoylcarbinol and L-mandelic acid from phenylglyoxal through the agency of active fermenting yeast. Early in 1915 he recorded (*J.*, **107**, 434) an attempt to resolve 3-methylallantoin into optical isomers, the failure of which supported the view that enolisation, rather than the transitory formation of a bicyclic structure, is responsible for the apparent lack of asymmetry in allantoin.

When war broke out in 1914, Dakin at once returned to Britain, but finding in his own country no immediate outlet for his ambition to aid in the war effort, he joined a research group at a French military hospital at Compiégne, where a study of methods for the antiseptic treatment of wounds had been organized by the late Alexis Carrel of the Rockefeller Institute for Medical Research in New York. Here he developed the hypochlorite solution which bears his name. By the use of borate buffer, he succeeded in greatly reducing not only the instability but also the irritant action of commercial hypochlorites. His review articles on the use of anti-

septics in the treatment of infected wounds and on the history of hypochlorites (*Brit. Med. J.*, 1915, II, 318, 809) are minor classics. In a note communicated to the French Academy of Sciences in August, 1915, and soon thereafter published (*Compt. rend.*, 1915, **161**, 150), he made generally available the details of the preparation of Dakin's solution. In this note he also referred to the possibilities of sodium salts of aryl-*N*-chlorosulphonamides, which had been investigated in 1904 by his former professor, J. B. Cohen. In an extensive study of compounds of this class, the results of which were published early in 1916 by Dakin, Cohen, Daufresne, and Kenyon (*Proc. Roy. Soc.*, 1916, *B*, **89**, 232), sodio-*N*-chlorotoluene-*p*-sulphonamide (chloramine-T) was found to be the most effective member of a long series tested. The mode of action was investigated, with amino-acids as models, in the Department of Biochemistry and Pharmacology of the Medical Research Committee (*Biochem. J.*, 1916, **10**, 313, 319; 1917, **11**, 79). With one molecular equivalent of the chloro-amide the initial product proved to be a chloro-amino-acid, which subsequently broke down into an aldehyde, carbon dioxide, and ammonia; with two equivalents, a dichloro-amino-acid was first formed and this decomposed into a nitrile and carbon dioxide.

At the time of the attempted invasion of the Dardanelles, Dakin was invited by the military authorities to assist them in their attempt to combat the infections prevalent among the wounded who were being evacuated. Through his personal efforts an electrolytic unit for the preparation of ample quantities of hypochlorite solution from sea-water was installed in the *Aquitania*, which was being employed as a hospital ship. A great and immediate reduction in the incidence of infections on board resulted. When the ship resumed her normal passenger trade, this unit was retained as part of her regular equipment and remained in use throughout her years of service.

With Major E. K. Dunham of the U.S. Army Medical Service, Dakin also developed (*Brit. Med. J.*, 1917, I, 682) the use of chloramine-T for the disinfection of drinking water, and noted that this agent was more effective when the water was weakly acidified.

While in the laboratory of the Medical Research Committee, Dakin joined the late George Barger in a study of the metabolic fate of ingested glyoxalinealdehyde and in an attempt to synthesize urocanic acid (*Biochem. J.*, 1916, **10**, 376). His important paper " On Amino-Acids," was published in 1918 (*ibid.*, **12**, 290); as it was entitled " A Report to the Medical Research Committee " it seems that the experimental work therein described was, at least in part, carried out in the same laboratory. His discovery that the monoamino-monocarboxylic acids are selectively extracted from neutral aqueous solutions by wet butanol not only contributed a valuable technique in the analytical isolation of the component amino-acids of proteins but formed the basis of essential features of some of the modern chromatographic methods for their separation. This work led to the isolation of a new amino-acid, hydroxyglutamic acid, the relation of which to glutamic acid was established by reduction. However, the precise structure of this acid, the properties of which (*ibid.*, 1919, **13**, 398) did not conform entirely with those deducible from the characteristics of optically inactive β-hydroxyglutamic acid synthesized at the time by Dakin and nine years later by Harington and Randall, is still uncertain. Indeed, some—though not all—attempts in other laboratories to confirm its very existence were unsuccessful.

After returning to New York, Dakin collaborated, across the Atlantic, with H. H. (now Sir Henry) Dale in a comparative study (*Biochem. J.*, 1919, **13**, 248) of the chemical structure and antigenic specificity of the crystalline ovalbumins of the domestic hen and of the duck. Dale's experiments indicated that these two proteins, which were apparently identical in amino-acid composition, could be distinguished from each other by anaphylactic tests; Dakin's work, carried out with the aid of his new procedure on hydrolysates of the " alkali-racemised " albumins, showed definite differences in the optical activity of the leucine, histidine, and aspartic acid.

Another important application of the use of partially miscible solvents to the separation of amino-acids was the quantitative isolation of the constituent units of gelatin (*J. Biol. Chem.*, 1920, **44**, 499) and zein (*Z. physiol. Chem.*, 1923, **130**, 159). Dakin's figures for the amino-acid composition of these proteins were the first to give an approximately complete accounting. His initial failure to isolate valine from zein led to a repetition of the work with a hydrolysate prepared by T. B. Osborne, whose previous report of the presence of valine in that protein was thereby confirmed (*J. Biol. Chem.*, 1924, **61**, 137).

During the early twenties Dakin devoted much of his time to the synthesis, resolution, and biochemistry of amino-hydroxy-acids, the action of muscle tissue on fumaric, maleic, malic, and glutamic acids, and the catabolism of fatty acids. His experimental findings (*ibid.*, 1922, **52**, 183; 1923, **56**, 43; 1924, **61**, 139) undoubtedly contributed much to current views on the carbohydrate cycle and its relation to the metabolism of fats and proteins. In 1922 he was

joined for a brief period by C. R. (now Sir Charles) Harington, with whom he studied the anomalous behaviour of α-diketones in the Strecker reaction, which was found to induce scission of the linkage between the carbonyl groups (*ibid.*, 1923, **55**, 487).

In 1926 he subjected thyroxine to chemical study and soon clarified its constitution. He submitted a paper on his results to the *Journal of Biological Chemistry*, but on learning that Harington had independently reached essentially the same conclusions he withdrew his manuscript from publication. In the same year, in collaboration with Eleanor B. Newton and the late Stanley R. Benedict of Cornell University (*Science*, 1926, **64**, 602; *J. Biol. Chem.*, 1927, **72**, 367), he identified thiasine, a constituent of blood, with ergothioneine, isolated from ergot by Tanret in 1909, the constitution of which had been established in 1911 by Barger and Ewins.

Two years later he and Randolph West, a young clinician, recorded (*J. Biol. Chem.*, 1928, **78**, 91, 745, 757) a novel reaction of α-amino-acids consisting in the conversion of these, by the action of acetic anhydride in pyridine, into carbon dioxide and the corresponding α-acetamidoalkyl methyl ketones. He then took up a study of the condensation of aromatic aldehydes with amino-acids, acylamino-acids, peptides, and proteins (*ibid.*, 1929, **82**, 439; **84**, 675), but soon abandoned this line of work in favour of a laborious attempt to isolate the hæmopoietic factor present in liver. The pursuit of this compound, which has only recently been isolated (vitamin B_{12}) and has not yet been fully characterized, was beset with insurmountable difficulties. At that time the only method for assaying the potency of preparations consisted in clinical tests on human subjects suffering from pernicious anæmia. These tests were tirelessly performed by Randolph West; however, the general application of liver therapy made it increasingly difficult to find suitable untreated patients. Secondly, the unpredecentedly high potency of the vitamin, then unrecognized, constituted a major pitfall in that any fraction which contained it as a difficultly separable contaminant had to be regarded as a concentrate. Thirdly, the precision of the clinical assay was low. Among the false leads pursued by Dakin and West were an acidic peptide which yielded hydroxyglutamic acid and hydroxyproline on hydrolysis; a tribasic acid containing the pyrrolidone ring; and a polypeptide containing a hexosamine in combination (*ibid.*, 1931, **92**, 117; 1935, **109**, 489; 1936, **115**, 771; *Proc. Soc. Exp. Biol. Med.*, 1939, **40**, 124).

During his last ten years, Dakin undertook only minor problems which had presented themselves in the course of his earlier studies; the synthesis of β-aminovaleric and β-methylaspartic acids; the production of betaines from amino-hydroxy-acids (in the course of which he recorded his doubt that the hydroxyglutamic acid which he had isolated from protein hydrolysates was a β-hydroxy-derivative); the co-precipitation of lysine and ornithine with silver hydroxide; the synthesis of γ-hydroxyleucine; the formation of γ-methylproline in an attempted synthesis of δ-hydroxyleucine. There is little doubt that the exacting and discouraging search for the hæmopoietic factor had taxed his health, never very strong, more than he was willing to admit.

Dakin loved to work with his own hands and avoided opportunities to direct the activities of research groups. The younger biochemists who had the privilege of working under him, and of thereby assimilating his scientific ideas and learning his experimental methods, were therefore few, but included at least two who subsequently became leaders. It is characteristic of him that he personally carried out all his analytical work, including combustions. He was probably the only organic chemist who consistently employed Barger's method for the determination of molecular weight. Incidentally, his knowledge of biochemical techniques was most successfully applied, as a result of Herbert Hoover's " noble experiment," to the annual preparation of wines of admirable quality.

Dakin wrote with ease and elegance. He was the author of a Monograph on Biochemistry entitled " Oxidations and Reductions in the Animal Body " which appeared in 1912 and again in 1922, and of Physiological Oxidations (*Physiol. Reviews*, 1921, **1**, 394). He was co-author, with his kinsman E. K. Dunham, of a small " Handbook of Chemical Antiseptics " (1917).

On the other hand, his horror of public speaking kept him from scientific conventions and other occasions on which he might have become more widely known to his scientific colleagues in person. His circle of friends, all of whom were devoted to him, was not large but included men of a wide variety of professions. To them all, he was known as " Zyme."

Many honours came his way. His own University of Leeds, which had granted him the Ph.D. in 1907, conferred on him the honorary Doctorate of Laws in 1936; Yale University gave him the honorary Doctorate of Science in 1918; Heidelberg University awarded him the Doctorate in Philosophy, *honoris causa*, about 1938. At the close of the first World War, the French Government made him a Chevalier of the Legion d'Honneur. In recognition of his chemical contributions to medicine, he was awarded the Conné Medal of the New York Chemists' Club about 1933; in 1942 he received the Davy Medal of the Royal Society.

In 1901 he was admitted to the Chemical Society and, as an Associate, to the Institute of Chemistry, of which he became a Fellow in 1904. He was elected to the American Society of Biological Chemists soon after its inception, and to Fellowship in the Royal Society in 1917.

Throughout the period 1909 to 1930 he was a member of the Editorial Board of the *Journal of Biological Chemistry*, which had been generously endowed by Christian A. Herter, and for many years he managed the finances of that *Journal* with a degree of efficiency rarely found among scientists. In his later years he served on the Board of Scientific Advisers of the Merck Institute of Therapeutic Research and ultimately became a Director of Merck and Co.

After the death of Dr. Herter in 1910, Dakin had, at the desire of Mrs. Herter, continued to work in the laboratory in New York City until his temporary return to Europe to serve in war work. In 1916, he and Mrs. Herter married. Two years later, after the Armistice, they moved to an estate in rural Scarborough-on-Hudson, N.Y., where he converted an adjacent building into a commodious laboratory and scientific library. Here, with the assistance of a family retainer who served as his laboratory " Diener," he carried on his researches until the end. His marriage was an ideally happy one. His wife's death in 1951, after a long illness during which he tended her with the utmost devotion, seemed to leave him with little desire to continue alone. His own death came peacefully, in his sleep, on February 10, 1952. He had no children.

HANS T. CLARKE.