EMIL FISCHER MEMORIAL LECTURE.

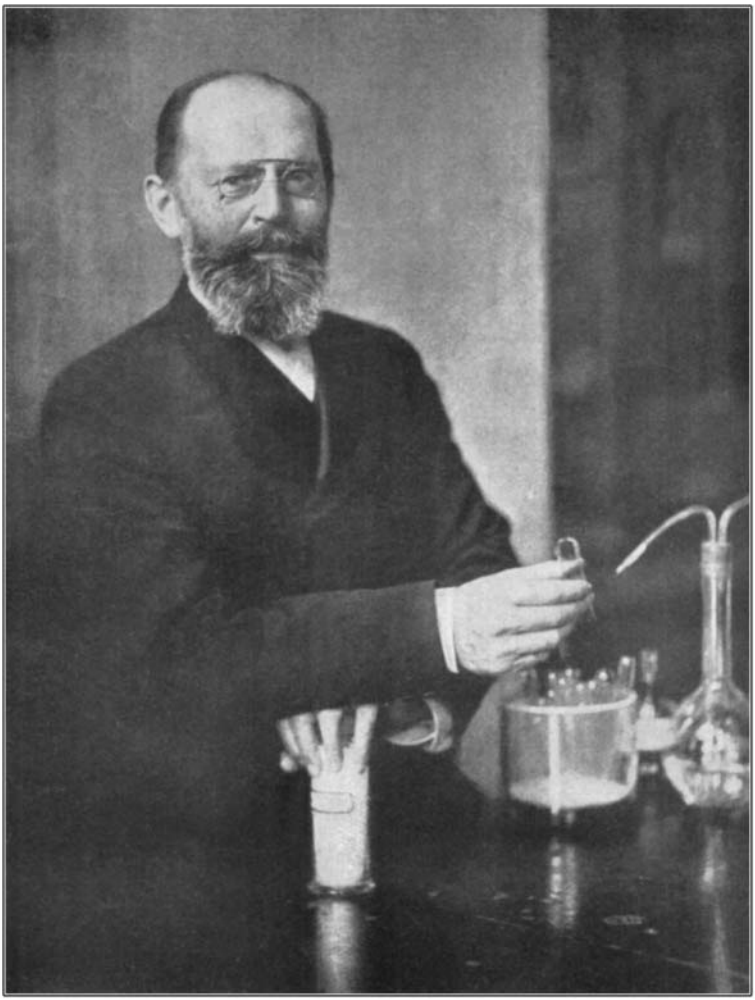
DELIVERED ON OCTOBER 28TH, 1920.

By MARTIN ONSLOW FORSTER, F.R.S., Treasurer.

To a world confused and lacerated by the bitter consequences of protracted warfare, at a time when every element of constructive and harmonising influence was most sorely needed, there came without premonition the announcement that Emil Fischer was dead. Inured as the nations had become to loss and disaster, it was probably with surprise that the scientific communities found the misfortune with which they were now confronted to be one in common with their former enemies. For the place which he occupied in our minds, first attained by his mastery of our subject, was hallowed by regard for his sterling disposition, compassion for his griefs, admiration for his outstanding power.

Emil Fischer was born on October 9th, 1852, at Euskirchen, some twenty-five miles from Cologne on the south-westerly high-road to the Eifel, about twenty miles from Bonn. Preceded by five sisters, he was the only son of Laurenz Fischer and Julie Poensgen. After leaving school at Bonn in 1869, he was apprenticed to his brother-in-law, Ernst Friedrichs, a timber merchant, but the occupation proving uncongenial, he became a pupil of Kekulé in 1871, and proceeded in the following year from Bonn to Strasbourg. Here he graduated in 1874 under von Baeyer, with whom he passed to Munich in 1875 as an assistant, becoming *Privatdozent*, and soon afterwards succeeding Volhard as *Ausserordentlicher Professor* in 1879. On the transference of Volhard to Halle, he was called to the chair of chemistry at Erlangen in 1882, whence he proceeded in 1885 to Würzburg in succession to the elder Wislicenus. He remained seven years at the Bavarian university, and on the demise of von Hofmann, in 1892, was appointed professor and director of the chemical institute in Berlin University, a post which he filled with increasing distinction until his death, which took place in the night of July 14th, 1919, at Wannsee, his country home. Such, in the barest outline, was Fischer's career—simple, straightforward, and honourable, like his nature.

Amongst those who came in contact with him, the impression left by Fischer is indelible, but the words to describe his personality



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do not readily come. Physically commanding, his authority rested on the solid foundation of natural dignity unmarred by self-assertion. The brisk, upright carriage marked the man of action; the glowing eyes revealed his attitude of constant, keen inquiry; the impatience with trivialities was one aspect of his dominating, steadfast control of essentials. With ordinary human perception, it was impossible for anyone to escape his contagious enthusiasm, and yet all the time the master did not obscure the man; for although his daily demeanour was tinged with severity, his heart when revealed was deeply kind, and, in circumstances of relaxation, joyous. The alert presence, the ardent gaze, and the resonant voice will not fade from the recollection of those to whom they became the symbols of a treasured experience.

The salient quality of his life was unswerving singleness of purpose. Rapid satisfaction of reasonable professional ambition and the fact that he was only forty when summoned to Berlin did not distract him from his chosen path. The legitimate pride occasioned by the summons, the glad recognition of increased opportunities for research, the happy anticipation of entering an unlimited scientific environment were tempered by anxiety lest the social and ceremonial demands of the capital city should make serious inroads into the real work of his life. Even his sixtieth birthday celebration was, by his express desire, an almost domestic affair. Had he sought their presence, representatives of foreign academies, captains of industry, and councillors of State would gladly have joined his less exalted admirers in their tribute to the master, but the master himself ordained otherwise; with one exception, all the participants were former students, old colleagues, or members of his household.

Although the strictness with which Fischer confined his energy to affairs of chemistry was temperamental and due primarily to his natural and cultivated taste for the science, it is probable that his condition of health was a contributory factor. In the summer of 1881 he suffered an attack of mercury poisoning, consequent on studying the action between mercuric oxide and aliphatic hydrazines; neither he nor Bösler noticed until too late that mercury diethyl is produced, although the odour did not escape von Baeyer. The direct effects of this incident lasted three months, and ten years later Fischer fell a victim to the insidious onslaught of phenylhydrazine vapour. In his own words: "Dieses allerliebste Bäschen war meine erste und dauerndste chemische Liebe. Wir haben uns 15 Jahre lang ausgezeichnet miteinander vertragen, während viele andere Menschen gleich dadurch zu Schade kamen. Aber dann brach auch bei mir das Unglück ein mit einer

chronischen und äusserst hartnäckigen Vergiftung, und es hat 12 Jahre gedauert, bis die Folgen beiseitigt waren."

It is characteristic of the man that his work is devoid of "popular" features, although common experience at every turn traverses the materials with which he dealt. The task of explaining the achievements of other great chemists, Pasteur, von Hofmann, or Sir William Perkin, for example, is relatively simple, but the fellow-citizen cannot hope to understand Fischer's chemistry of the breakfast-table until he has learned that the operations of digestion are essentially chemical transactions of a subtle nature. To the man whose sugar "melts" in tea, and who believes in a second member of the class, beet-sugar, and probably a third, namely, saccharin, how are the beauties of the sixteen stereoisomeric aldohexoses to be revealed? How is it possible to explain to a woman unversed in chemistry the family connexion between her silk dress and a scrambled egg?

In literary style his papers are uncompromisingly ascetic. The reader is quickly made aware of the subject about to be developed; in simple phrases the theme is pursued with a marked absence of verbal ornament, and the treatment, although complete, is so concise that a concluding summary of results is unnecessary. By this restraint Fischer has earned the gratitude of all chemists, for the labour of assimilating his enormous output has been reduced thereby to a minimum. Failing thorough discipline in writing abstracts of scientific memoirs, a study of the literary method adopted by Fischer offers the best possible training for the research chemist, old or young, in preparing reports of his own investigations.

By the time he had passed from Erlangen to Würzburg, Fischer's reputation had become magnetic, and from that period an increasing number of *doctorandi* sought admission to his laboratory. The aggregate of these must be several hundreds, including many nationalities, and with the passage of years he received, in addition to the customary distinctions accorded by his countrymen, the honours which it is the practice of foreign academies to offer. He became an Honorary and Foreign Member of the Chemical Society in 1892, a Foreign Member of the Royal Society in 1899, and an Honorary Member of the Royal Institution in 1904; he received the Davy Medal in 1890, the Nobel Prize for chemistry in 1902, and the Elliott Cresson Gold Medal from the Franklin Institute of Philadelphia in 1913.

Although too rarely seen in this country, he was warmly welcome when he came, and there is every ground for believing that the cordial feeling towards him which certainly existed

amongst British chemists was reciprocated, a concrete illustration of this being given by the fact that his eldest son, Hermann Fischer, passed two terms at Cambridge. Moreover, the many Englishmen who worked in his laboratory were quickly made aware of the kindly feeling towards them which he cherished, an experience adding appreciably to the benefit they derived from the association. Thus the veneration surrounding his name in this country could scarcely have been less, in depth and sincerity, than that acknowledged by his fellow-countrymen. It therefore gave lustre to the Perkin Jubilee in 1906 when, as President of the German Chemical Society, he conveyed the congratulations of that body to the veteran whilst conferring on him the Hofmann Medal.

The following year, in which he delivered the Faraday Lecture, marked another memorable occasion, postponed from 1895, when his condition of health did not permit acceptance of the invitation then given. The title of this address, namely, "Synthetical Chemistry in Relation to Biology," crystallises in a single phrase the profound significance of his own work; for, when reviewed as a chapter which is closed, this must be regarded as having established on a firm basis the fundamental, but bewildering, science of biochemistry. The assimilation of carbon dioxide and water by plants, the variety and complexity of saccharide molecules proceeding therefrom, the degradation of the proteins, the probable course of their synthesis from amino-acids, and the power of assemblage or of disruption exerted by enzymes on all these building materials of the animal and vegetable kingdoms, are subjects which Fischer not merely illuminated, but which he was the first to place in coherent arrangement and intelligible sequence. Recognition of the fact that all this was accomplished, not by revolutionary processes or tortuous theories, but by dexterous development of the thoughts and operations expanded by Liebig, von Hofmann, Pasteur, and von Baeyer, is perhaps the highest tribute which can be paid to his genius. Indeed, it offers the greatest inspiration to less gifted workers, who may thus be encouraged to perceive opportunities for discovery in adroit applications of classical principles: and pursuing methods of such simplicity may even arrive, by those devious paths which unfold themselves with delight and refreshment to an earnest inquirer, unexpectedly, as on the lifting of a curtain, directly at the threshold of a fundamental truth.

The natural curiosity which seeks to account for the biochemical trend of Emil Fischer's researches can only take refuge in predilection. There is nothing to explain it discoverable in family associations or in the tasks of his young manhood. Laurenz

Fischer, who passed the age of ninety-four, was an active and enterprising general merchant engaged in supplying the requirements of the peasant farmers inhabiting the Eifel; associated with his brothers, he was also concerned in a spinning mill and a brick-works close to his native town, and in the foundation of a brewery at Dortmund. Thus there existed every inducement for his only son to pursue a business career, and Ernst Friedrichs was quite positive in declaring his conviction that no good would come of Emil when the young apprentice relinquished an opportunity which appeared to him so full of promise. During this abortive attempt to transform him into a timber merchant, Fischer had been occupied with experiments in a home-made laboratory, and contemplated devoting himself to a study of physics; but his father, attracted by the handsome returns which the Rhenish chemical factories were already beginning to show, finally persuaded him to become a chemist.

His prepossession in favour of biochemistry was destined to resist another contrary influence. The doctorate thesis dealt with fluorescein and the phthalein of orcin, whilst his work on rosaniline in the Munich days would almost certainly have confirmed a less resolute mind in the pursuit of colour chemistry, then in the early flush of active growth. Moreover, this direction would have been a natural sequence to the powerful influence of von Baeyer, at that time immersed in the problems of indigotin synthesis. Indeed, it is a noticeable feature in the development of his character that Fischer's work detached itself so completely from household authority and so rapidly from early chemical environment. The only conspicuous mark of the training which he owed to von Baeyer was manipulative skill of the very highest order; this was revealed by results achieved with uninviting materials, frequently used in very small quantities, by the inception and adaptation of ingenious experimental devices, and by the rarity of occasion to revise earlier conclusions of his own. Fortunate he undeniably was, but the good fortune was thoroughly well earned by ceaseless industry. He was fortunate, for example, in the fact that nothing resembling the Walden inversion, that *bête noire* of optical activity, disturbed the aldohexose configuration. Imagine the confusion which would have arisen if gluconic and mannonic acids, instead of being smoothly interconvertible when heated with quinoline, had given mixtures of idonic, gulonic, galactonic, talonic, altronic, and allonic acids. Phenylhydrazine was a tremendous *coup*; but the preparation was according to plan, although its remarkable properties were fortuitous, a happy illustration of "to him that hath shall be given."

Nevertheless, that mysterious counterpoise of destiny, from which the greatest and the humblest cannot escape, plunged him into profound sorrow at the meridian of his career. In 1888 he had married Agnes Gerlach, but the happy union was cut short by her death in 1895. Thereafter his three boys were tended by Frln. Margarete Barth, who administered his household for the remaining twenty-four years of his life with watchful solicitude.

Early Work.

Fischer entered the field of organic chemical research at the opening of its brightest epoch. Largely in consequence of Kekulé's theory of benzene structure, the ground was ready for systematic cultivation: the advent of peace encouraged a willing and increasing band of workers to prepare themselves for fruitful labour; together they stood at the threshold of the imposing structure which was destined to arise during the next forty years.

The act of reading, in 1920, the paper on phenylhydrazine communicated from the Strasbourg laboratory in 1875, engenders the sensation of contemplating the modest source of a mighty river. In 1871, Strecker (with Römer) had treated benzenediazonium nitrate with excess of hydrogen potassium sulphite, obtaining a salt, which he represented by the formula

$$C_6H_5\cdot NH(:NH)\cdot SO_3K,H_2O,$$

and giving to diazotised aniline the expression since associated with his name. In 1875 Fischer showed that two salts arise from the diazotised base and neutral potassium sulphite, namely,

$C_6H_5\cdot N_2\cdot SO_3K$	and	$C_6H_5\cdot N_2H_2\cdot SO_3K + H_2O$
Potassium benzenediazonium sulphite.		Potassium phenylhydrazino-sulphonate.

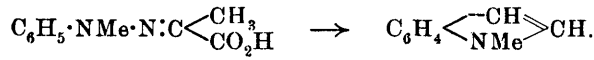
The latter was identical with Strecker's, and Fischer carried the inquiry to another stage by acting on it with benzoyl chloride, producing a substance (dibenzoylphenylhydrazine) which gave benzoic acid and phenylhydrazine hydrochloride when heated with hydrochloric acid; in this manner, phenylhydrazine itself was first obtained as an oil, which ultimately became solid. Later in the same year he simplified the preparation of the base by adding excess of sodium sulphite to benzenediazonium chloride, completing the reduction by means of zinc dust and hydrolysing the phenylhydrazinosulphonate with hot hydrochloric acid. Extending the process to diazotised sulphanilic acid, he prepared phenylhydrazinesulphonic acid, which had been actually obtained by Strecker and Römer (1871), because in this case they had heated the initial product with hydrochloric acid to remove excess of alkali sulphite.

Owing to the readiness with which hydrogen replaces the nitroso-group in secondary nitrosoamines, attempts by other chemists to prepare aliphatic hydrazines had failed. Fischer succeeded in reducing nitrosodimethylamine to dimethylhydrazine, and, having prepared diethylhydrazine and phenylethylhydrazine, he represented the primary hydrazine derivative by the formula

$$C_6H_5{\cdot}NH{\cdot}NH_2.$$

At the beginning of 1876 he described ethylhydrazine, which arose from hydrolysing diethylsemicarbazide, and expressed the intention of applying this process to the production of hydrazine itself. Throughout this and the succeeding year the simpler changes undergone by phenylhydrazine were studied, and it is noteworthy that at this period Fischer preferred the Kekulé formula for diazonium salts; he had regenerated potassium phenylhydrazinosulphonate from phenylhydrazine and potassium pyrosulphate, and, converting it by oxidation into the diazonium sulphite, was so impressed by the close experimental relationship between the two substances as to consider this an obstacle to the Strecker formula.

A survey of these early papers indicates the curious fact that although the power of phenylhydrazine to combine with aldehydes was quickly observed by Fischer, he does not appear to have recognised its tremendous value as a general agent for the carbonyl group until nearly ten years after discovering the base. This is probably due to the distraction offered by rosaniline (see below) and minor investigations, by his translation from Munich to Erlangen, and by the work on caffeine (1881), which was destined to lead him to the classification of purine derivatives. It is true that the action of acetaldehyde, benzaldehyde, and furfural had been noted, but it was probably the formation of the sparingly soluble and beautifully crystalline phenylhydrazone of pyruvic acid (1883) which revived the interest in his original discovery. The outstanding feature of 1884, an extraordinarily fruitful year, is the application of phenylhydrazine to carbonyl compounds in general, and the sugar group in particular. In that year, also, he explained the first transformation of a phenylhydrazone into an indole,



Pyruvic acid phenylmethylhydrazone. 1-Methylindole.

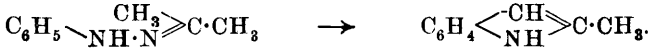
induced by the action of hydrochloric acid, and he re-assembled the evidence supporting his constitutional formula for phenylhydrazine, Erlenmeyer having advocated the alternative expression $C_6H_5{\cdot}NH_2{:}NH$.

Another interesting point in the early history of the phenylhydrazones is that the name for these important products of condensation does not appear to have been introduced by Fischer until 1888, when he represented them by the general expression $>C{:}N{\cdot}NHR$ instead of $>C<^{NH}_{NR}$; although many attempts, some of which are quite recent, have been made to resuscitate the cycloid representation, Fischer did not take part in the discussions, and the balance of evidence in favour of his formula preponderates.

Even forty years ago, when the boundaries of organic chemistry were comparatively limited, the discovery of an entirely new class of highly reactive compounds by a novice must have been recognised as a portent; but the hydrazines were not the only subject with which Fischer's name came to be associated in the text-books with respect to work accomplished before he was twenty-five years of age. While in Munich he began collaboration with Otto Fischer, and in 1876 the cousins published their first joint paper on the rosaniline bases, obtaining from diazotised leucaniline the hydrocarbon, $C_{20}H_{18}$, melting at 58°. These experiments were made with the commercial product, and, on repeating them with the dye prepared from purified *p*-toluidine and aniline, they showed in 1878 that the corresponding leuco-base gives triphenylmethane, $C_{19}H_{16}$, melting at 93°, as recorded by Kekulé, Franchimont, and Hemilian. In view of its production from *p*-toluidine, the dye was named, somewhat unfortunately, "pararosaniline," and they proceeded to regenerate it from triphenylmethane by reducing Hemilian's trinitro-derivative and oxidising the paraleucaniline thus obtained. They concluded (1) that the rosanilines producible from aniline and the toluidines are homologues, of which the simplest, pararosaniline, has the composition $C_{19}H_{17}N_3$, whilst commercial fuchsine is a mixture, of which the principal constituent has the formula $C_{20}H_{19}N_3$, assigned by Hofmann, and (2) that the parent hydrocarbon of the whole group is triphenylmethane, of which, or of its homologues, the various leucanilines are triamino-derivatives.

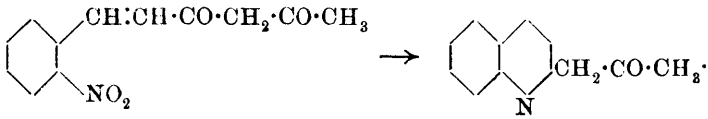
Before proceeding to review the special branches with which Fischer's name is most conspicuously linked, it will be convenient here to notice a few developments of his earlier experiments which are not appropriately included in the following sections. For example, the conversion of suitable phenylhydrazones into derivatives of indole was extended in 1886, when it was found that anhydrous zinc chloride so greatly facilitates the removal of ammonia that the condensation products of primary and secondary arylhydrazines with all saturated ketones or ketonic acids con-

taining the methyl or methylene group adjacent to carbonyl can be transformed into the corresponding indole derivatives by loss of ammonia; as an illustration, acetonephenylhydrazone gives 2-methylindole,

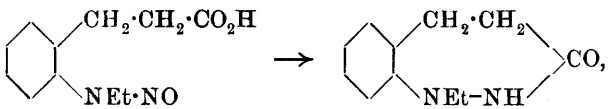


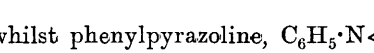
A similar change proceeds with phenylhydrazones of aldehydes containing the methylene group next to the aldo-nucleus, and dimethylindole, methylethyl-, phenylmethyl-, and phenylscatole, together with indolecarboxylic, dimethylindoleacetic, and dimethylindolecarboxylic acids, were prepared.

Other examples of ring-formation were brought to light by Fischer. In 1883 he found that benzoylacetone is produced by hydrolysing benzoylacetic ester, and, utilising this reaction to prepare *o*-nitrocinnamylacetone, he reduced this to acetonylquinoline,



Furthermore, nitrosoethylaminohydrocinnamic acid gave ethylhydrocarbazostyril,



whilst phenylpyrazoline,  , was obtained in 1887 from the phenylhydrazone of acrolein by the action of dilute sulphuric acid.

Carbohydrates, Glucosides, and Depsides.

In the year 1886, chemists recognised two aldohexoses (glucose and galactose), two ketohexoses (fructose and sorbose), and one aldopentose (arabinose); three hexobioses (sucrose, lactose, and maltose) were also known to be definite individuals, and one hexotriose (raffinose). The general structure of glucose and galactose as that of straight-chain pentahydroxyaldehydes, and of fructose as a pentahydroxyketone, also unbranched, had been determined by the work of Kiliani, who relegated to its proper position as a tetrahydroxyaldehyde the pentose, arabinose, erroneously classified by its discoverer as an isomeride of glucose. In these few lines may

be summarised the exact knowledge of crystalline carbohydrates at the time when Emil Fischer approached the subject, and a true measure of the character and magnitude of his constructive achievement may be gained by comparing the foregoing synopsis with the modern classification of the hexose group as represented by projection formulæ.

From that classification it is seen that amongst the sixteen optically active aldohexoses theoretically realisable, twelve have been either synthesised or configurated, or both, by Fischer and his collaborators. His work on ribose made it possible to include *d*-allose and *d*-altrose in the list, of which there thus remain only the *l*-forms of these two aldoses unknown. It involves also the statement that six amongst the eight possible *dl*-aldohexoses have been realised, whence it follows that *l*- and *dl*-allose with *l*- and *dl*-altrose are the only missing members of the twenty-four optical isomerides comprised in the category.

Highly as this admirable web of theory and practice must be valued, its description is not a complete estimate of his finished contribution to aldohexose chemistry. The foregoing computation of possibilities takes no account of the oxide rings now accepted amongst members of this class. His discovery of γ-methylglucoside, and the consequent recognition of cyclic relations distinct from that occurring in α- and β-glucose, have opened the way to a multitude of contingent isomerides, those of *d*-glucose alone numbering ten. Thus Fischer not only elaborated his own sugar chemistry, but he added to this the foundation of a new carbohydrate classification, the development of which will continue to inspire the prosecution of inquiry by generations following his epoch.

The instrument which enabled Fischer to bend his experimental deftness and his theoretical penetration to the purpose of elaborating so delicate a structure was phenylhydrazine. In 1884 he found that with this agent glucose and fructose yield phenylglucosazone, $C_{18}H_{22}O_4N_4$, whilst an isomeride arises from galactose; under similar conditions, maltose and lactose resemble the hexoses, forming individual isomeric osazones, $C_{24}H_{32}O_9N_4$, whilst sucrose, at first indifferent, gradually undergoes partial hydrolysis, generating phenylglucosazone from the products of inversion. Closer study showed that this reaction has the peculiarity of presenting a fully hydrogenised compound, phenylhydrazine, in the light of an oxidising (dehydrogenising) agent. The first product is the phenylhydrazone, which, owing to structural difference between glucose and fructose, is not the same from both sugars; a second molecule

of phenylhydrazine now removes hydrogen from the two phenylhydrazones, yielding two structurally different phenylhydrazones of glucosone, which then undergo condensation with a third molecule of phenylhydrazine to produce the osazone,

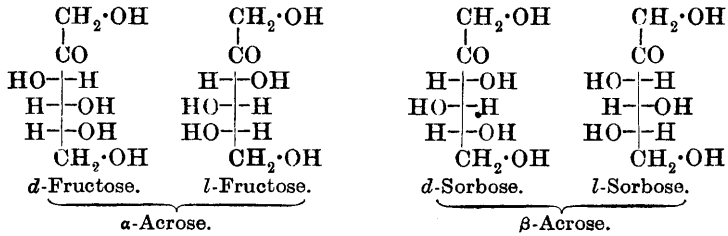
$$HO{\cdot}CH_2{\cdot}[CH{\cdot}OH]_3{\cdot}C(:N{\cdot}NHPh){\cdot}CH:N{\cdot}NHPh,$$

common to both.

The extension of this reaction to all aldoses and ketoses, the formation of phenylhydrazides from sugar-acids, and the analogous application of substituted phenylhydrazines, have rendered invaluable service in the identification and isolation of carbohydrates in general, because although the latter, when crystalline, are quite definite in purified form, they are amongst the most difficult materials to manipulate, on account of their tendency to remain as a syrup in mixtures.

Even more important, though less immediately obvious, has been the operation of this process in a synthetical direction. The aspiration artificially to produce grape-sugar is coeval with organic chemistry itself. Liebig had indicated the fascination of this problem, but the first practical step was taken in 1861 by Butlerow, whose methylenitan was a sweet, pale yellow syrup responding to common tests for glucose, but optically inactive and unfermentable by yeast. He polymerised trioxymethylene with hot lime-water, and twenty-five years later Loew, having conveniently modified Hofmann's method of preparing formaldehyde, subjected this compound to the action of cold lime-water, thus producing a syrup which he called formose. In 1888, by means of phenylhydrazine, Fischer showed that formose is a mixture of at least three aldehydic or ketonic polyhydric alcohols, of which one has the composition $C_6H_{12}O_6$, and yields a normal osazone, $C_{18}H_{22}O_4N_4$, also producible from methylenitan.

Fischer himself has stated that the directive influence on his work amongst carbohydrates was the discovery of α- and β-acrose. In 1887, associated with Tafel, he obtained from acrolein dibromide and baryta a syrup which yielded two osazones, isomeric with one another and with phenylglucosazone. These were called α- and β-phenylacrosazone, corresponding to the two synthetical sugars α- and β-acrose, having the composition $C_6H_{12}O_6$. The former sugar he subsequently identified with *dl*-fructose, whilst β-acrose, which he suggested resembled sorbose, has been since recognised as the *dl*-form of that ketose (E. Schmitz, 1913). Thus was accomplished the first definite synthesis, in their *dl*-modifications, of the naturally occurring sugars, fructose and sorbose,

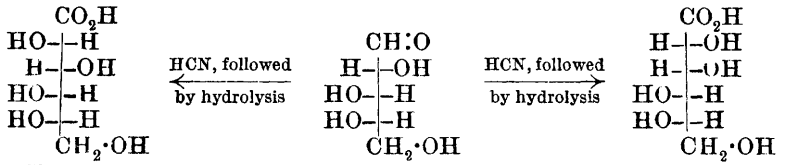


Great as was the advance represented by the acrosazones, the following step might well have daunted a less tenacious investigator. The acroses are optically inactive, and although reducible by sodium amalgam to hexahydric alcohols, of which α-acritol appeared, and was afterwards shown, to be identical with *dl*-mannitol, only 0·2 gram of α-acritol was obtainable from 1 kilogram of glycerol. Use had to be made, therefore, of another reaction, also very tedious experimentally, arising from the study of phenylglucosazone. When this compound is treated with hydrochloric acid, it is hydrolysed completely, leaving the tetrahydroxyketonic aldehyde, glucosone; on reducing this product incompletely with zinc dust in acetic acid, the aldehydic group is hydrogenised selectively before the ketonic, yielding fructose. By applying this process to α-acrosazone, in combination at subsequent stages with Pasteur's methods of separating optical antipodes, the passage from inactive synthetic α-acrose to sugars identical in all respects with *d*-glucose, *d*-fructose, and *d*-mannose was ultimately effected in the following sequence: α-acrose ⟶ *dl*-glucosazone ⟶ *dl*-glucosone ⟶ *dl*-fructose (⟶ *l*-fructose) ⟶ *dl*-mannitol ⟶ *dl*-mannose ⟶ *dl*-mannonic acid ⟶ *d*-mannonic acid (⟶ *d*-gluconic acid ⟶ *d*-glucose) ⟶ *d*-mannose ⟶ *d*-glucosazone ⟶ *d*-fructose.

One step in the above series, namely, the conversion of *d*-mannonic acid into *d*-gluconic acid, deserves attention, because the principle underlying it has been, in Fischer's hands, one of the most fruitful devices in the construction and configuration of new sugars, especially in association with the cyanohydrin synthesis and the reduction of polyhydroxylactones to the aldoses themselves. In 1890 he found that gluconic and mannonic acids are interconvertible, an equilibrium mixture of the two being produced when either is heated with quinoline at 140°. This was a far-reaching discovery, because it afterwards appeared that when any monobasic sugar-acid is heated with quinoline or pyridine, the configuration of the carbon atom adjacent to the carboxylic group becomes inverted or epimerised; gluconic and mannonic acids are consequently said to be epimeric. It was this principle, followed by reducing the lactones of the resulting acids, which enabled

Fischer to produce the artificial sugars *l*-glucose, *d*-talose, and *d*-idose through *l*-mannonic, *d*-galactonic, and *d*-gulonic acids, respectively, and the aldopentoses *l*-ribose and *d*-lyxose through *l*-arabonic and *l*-xylonic acids, respectively.

The cyanohydrin reaction, although actually devised by Kiliani (1885), was widely applied by Fischer, who regarded it as a great advance in the study of the sugar group. A typical example of its application is the conversion (1891) of *l*-arabinose, the dextrorotatory pentose derived from cherry-gum, into *l*-mannonic and *l*-gluconic acids,



l-Gluconic acid. *l*-Arabinose. *l*-Mannonic acid.

This opened a way to the artificial *l*-glucose and *l*-mannose, whilst a similar transformation of *d*-xylose (at that time known as *l*-xylose), the pentose from wood-gum and oat straw, gave *l*-gulose along with *l*-idose. The method was found to be capable of wide extension, and is limited only by the diminishing amount of material available for each succeeding step; thus, the following series were realised by Fischer:

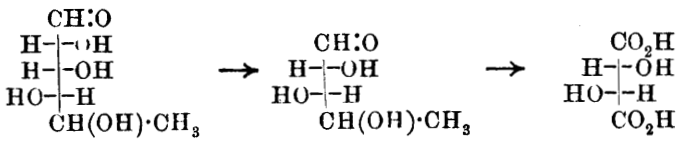
d-Glucose → {α-Glucoheptose, β-Glucoheptose} → {α-Gluco-octose, β-Gluco-octose} → Gluconononose.*

d-Mannose →	Mannoheptose →	Manno-octose →	Mannononose
$C_6H_{12}O_6$	$C_7H_{14}O_7$	$C_8H_{16}O_8$	$C_9H_{18}O_9$
Rhamnose →	α-Rhamnohexose →	Rhamnoheptose →	Rhamno-octose
$C_6H_{12}O_5$	$C_7H_{14}O_6$	$C_8H_{16}O_7$	$C_9H_{18}O_8$

In 1891 an attack was made on the complex problem of configuration, the system of which it has been necessary to assume hitherto in order to give a coherent synopsis within reasonable limits of space. Fischer's procedure was based on the requirements of van't Hoff's theory, from which it follows that the pentahydroxyaldehyde of an unbranched carbon chain, in which five carbon atoms are each associated with one hydroxyl group, should appear in sixteen stereoisomeric forms, eight of these being enantiomorphs of the remainder. The projection formulæ of the sixteen possible aldohexoses are then assembled in conjunction with the eight possible aldopentoses, thus indicating the trihydroxyglutaric acids

* Subsequently extended to glucodecose by Philippe (1911).

derivable from the latter, the tetrahydroxyadipic acids corresponding with the former, and the structurally symmetrical pentitols and hexitols. Recalling that (1) the cyanohydrin synthesis relates glucose to arabinose, and gulose to xylose, (2) the trihydroxyglutaric acid from xylose is internally compensated, whilst the isomeride from arabinose is optically active, and that (3) saccharic acid arises from both glucose and gulose, but not from any other hexose, it is possible by a process of considered elimination to limit the representation of *d*- and *l*-saccharic acids to two configurations, which are enantiomorphous, leaving the final selection arbitrary. The choice made by Fischer was a happy one, for he was able finally to confirm it in 1896. Having previously (1894) established the configuration of the three carbon atoms proceeding from the aldehydic group in rhamnose, he degraded the sugar by Wohl's method to the corresponding methyltetrose; oxidation of this yielded *d*-tartaric acid, the configuration of which is thus determined:

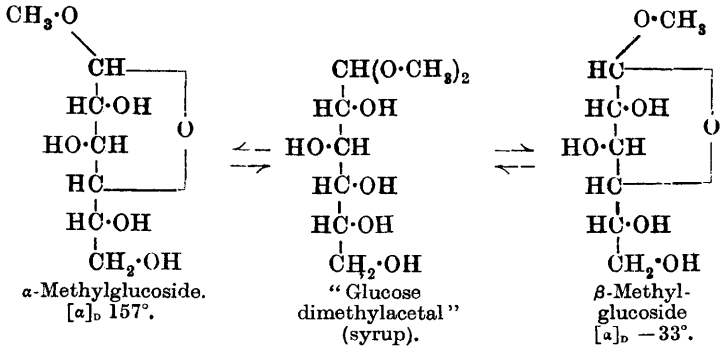


Rhamnose. Methyltetrose. *d*-Tartaric acid.

Just as the internally compensated mucic acid yields, by equally probable disruptions, *dl*-tartaric acid, so saccharic acid, and hence glucose, yield *d*-tartaric acid, thus providing the keystone for Fischer's configurational system.

Although it still simplifies discussion of the aldohexoses to represent them as containing a free aldehydic group, many years have elapsed since this view of their structure was modified. Amongst the earliest known compounds of organic origin are the glucosides, for example, amygdalin, indican, salicin, and myronic acid, owing their class-name to the production of glucose on hydrolysis, which led to their being regarded as ethereal derivatives of this or a related sugar. In 1893 Fischer found that under the influence of hydrogen chloride, glucose combines with methyl alcohol to form the simplest of the glucosides, which he called methylglucoside, and the discovery ultimately elucidated the structure of the sugar-like polysaccharides and of glucose itself. He soon showed this method of synthesis to be general with aldoses and ketoses for the one part, and applicable to the alcohols in which they are soluble, for the other; somewhat similar methods lead from acetone and benzaldehyde to the *iso*propylidene and benzylidene compounds, respectively, products which have been applied to the preparation

of mono-, di-, and tri-methyl derivatives of glucose (Irvine and Scott, 1913). Whilst Fischer indicated the probability of isomerism following the asymmetry of the methoxylated carbon atom, the second methylglucoside was brought to light by van Ekenstein (1894). The mechanism of glucoside formation, in which it seemed convenient to make an arbitrary selection of alternative formulæ for the α- and β-modifications, was then tentatively represented as follows:

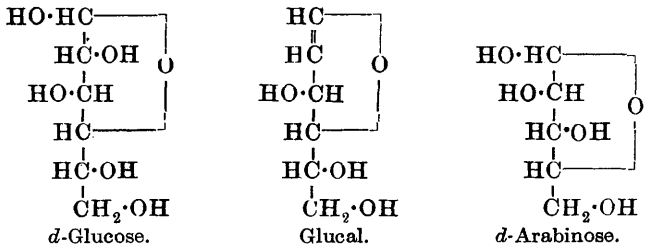


This proposal was in accord with Tollens' γ-oxide (preferably butylene oxide) formula for glucose (1883), and offered a substantial basis for the highly probable explanations, by E. F. Armstrong and by Lowry, of the long observed mutarotation of that sugar. The isolation of glucose in a second form was achieved by Tanret (1896), and it was subsequently accepted that α-glucose, with $[\alpha]_D$ 110°, and β-glucose, with $[\alpha]_D$ 19°, pass, on dissolution in water, to an equilibrium mixture having $[\alpha]_D$ 52·5°. Simon's view (1901) of α- and β-glucose as lower homologues of α- and β-methylglucoside, respectively, has been confirmed by E. F. Armstrong (1903), who correlated each methylglucoside with its parent glucose through the agency of an appropriate enzyme.

There the question remained during the next ten years, when the seed of revolution was sown by Fischer himself. In 1914, J. U. Nef, who had been occupied with a protracted survey of the oxidation undergone by sugars in alkaline solution, challenged the foregoing conception of methylglucoside isomerism by claiming this to be structural in place of steric, and representing the β-form of methylglucoside and of penta-acetylglucose as propylene oxides instead of butylene oxides. Strong arguments against this disturbing proposal were brought forward by Fischer, who simultaneously (1914) described a third methylglucoside. The syrupy companion of α- and β-methylglucoside, hitherto assumed to be the dimethylacetal, was distilled under 0·2 mm. pressure, and

found to be isomeric with them; it resembled them in stability towards alkali and Fehling's solution, but revealed a profound contrast in its behaviour towards acids, which hydrolyse it with extraordinary readiness. Moreover, whilst α-methylglucoside is hydrolysed by maltase (not by emulsin) and β-methylglucoside is hydrolysed by emulsin (not by maltase), γ-methylglucoside is indifferent to both. Fischer conceded the probability of the new glucoside owing its individuality to another form of oxide ring, and was inclined to regard it as a mixture of two or more such isomerides. Both possibilities have been supported by Irvine, Fyffe, and Hogg (1915) from a study of tetramethyl-γ-glucoside and tetramethyl-γ-glucose, in which emphasis is laid on the readiness with which it undergoes condensation with acetone and on the instantaneous oxidation of the new methylglucoside by cold alkaline potassium permanganate, an agent which has no effect on the α- and β-methylglucoside.

This remarkable behaviour towards permanganate invites comparison between the derivatives of γ-glucose and glucal, obtained as the triacetyl derivative on reducing β-acetylbromoglucose (Fischer and Zach, 1913). The conversion of triacetylglucal through the dibromide and the related bromohexose into phenylglucosazone, and the oxidation by ozone to triacetyl-*d*-arabinose (posthumous publication with Bergmann and Schotte, 1920), indicate the first and second carbon atoms as participants in the double linkage of glucal, the relationship of which to *d*-glucose and *d*-arabinose is consequently represented as follows:



d-Glucose. Glucal. *d*-Arabinose.

Glucal is thus a derivative of dihydrofuran, and gains piquancy from having beguiled Fischer into using rare words of enthusiasm when describing its production, which, he declared, "vom Standpunkt der Strukturchemie betrachtet, einer der merkwürdigsten Vorgänge ist, die man bisher in der Zuckergruppe kennen gelernt hat. Sie beweist von neuen, welch wunderbarer Stoff der Traubenzucker ist."

Although Fischer did not commit himself to the particular form of oxide represented by γ-glucose, which has not yet been isolated,

a point has been reached which indicates an ethylene oxide-ring structure for this compound. Before passing to the next branch of the subject, it should be mentioned that the experiments of Böeseken (1913) on the conductivity of boric acid as influenced by polyhydroxy-compounds have thrown some doubt on the accepted configuration of the α- and β-methylglucoside, in which, according to his deductions, the relative positions of the terminal methoxy-group and hydrogen atom should be inverted, and the corresponding rearrangement made in α- and β-glucose.

In conformity with his observations on artificial glucosides, Fischer represented the disaccharides as hexosides composed of the hexoses into which they are resolved by acids or enzymes, one hexose molecule playing the part of the methyl group in the less complex derivatives. Thus maltose and lactose were represented (1893) by the structural formula

$$HO\cdot CH_2\cdot CH(OH)\cdot \underbrace{CH\cdot[CH\cdot OH]_2\cdot CH\cdot O}_{\text{O}}\cdot CH_2\cdot[CH\cdot OH]_4\cdot CH{:}O,$$

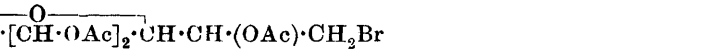
of which the left-hand portion stands for glucose in maltose and for galactose in lactose, whilst the remainder is glucose in both. Moreover, the relationship to the methylglucosides was shown (1894) by their behaviour towards emulsin, which hydrolyses lactose (glucose-β-galactoside) and is without action on maltose (glucose-α-glucoside). Structurally, this agrees with the oxidation of lactose to the monobasic lactobionic acid, $C_{12}H_{22}O_{12}$, and of maltose to the isomeric maltobionic acid (1889), which on hydrolysis yield galactose and glucose, respectively, associated in each case with gluconic acid; moreover, lactosecarboxylic and maltosecarboxylic acids, obtained from the respective disaccharide by the cyanohydrin reaction, yield the above-mentioned hexoses in company with α-glucoheptonic acid when hydrolysed.

Sucrose (cane-sugar), being devoid of aldehydic or ketonic attributes, is at once a fructoside and a glucoside. Fischer's formula (1893), based on that conception, remained unchallenged until his isolation of γ-methylglucoside, when he drew attention to the similar behaviour of these two substances towards acids. Whilst it is assured that the glucose residue has the same type of oxide ring as that of the α- and β-glucosides, his inference that the fructose component is in the γ-form (that is, an ethylene oxide) is supported by Haworth and Law (1916), who adduced new arguments in favour of representing sucrose by a formula in accordance therewith, subsequently confirming (1919) Fischer's original representation of maltose, in which the free aldehydic group is modified to the now conventional butylene oxide. By application of the methylation process, with which the St. Andrews laboratory has

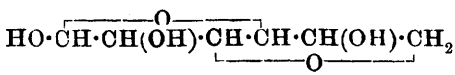
been so conspicuously associated, Haworth (1918) was led to refer Fischer's modified lactose formula (1893) to melibiose.

With so constructive a mind and an armoury of synthetical method so full of weapons, the magnetic problem of elaborating polysaccharide molecules was not likely to be neglected by Fischer. The individuality of *iso*maltose, produced by the action of cold fuming hydrochloric acid on glucose (1890 and 1895), was actively criticised; indeed, it was shown later by E. F. Armstrong that although *iso*maltose is formed in this process, it is accompanied by maltose. Subsequent attempts, however, were based on unassailable foundations. The first of these, in association with E. F. Armstrong (1902), depends on the action of β-acetylchloroglucose on the sodium derivative of galactose, and of β-acetylchlorogalactose on the sodium derivatives of glucose and galactose, the resulting disaccharides forming osazones. The galactosidoglucose closely resembled melibiose in its behaviour towards enzymes and the less delicate chemical agents, but the glucosidogalactose was distinct from lactose; all three resembled the β-glucosides in being hydrolysed by emulsin. From β-acetylbromoglucose and silver carbonate there was produced the octa-acetyl derivative of a disaccharide called *iso*trehalose (Fischer and Delbrück, 1909) from its close resemblance to the carbohydrate which is found in many fungi, and which appears to play in these the part of sucrose in chlorophyllaceous and starch-bearing plants; application of this method to acetylbromolactose gave distinct evidence of a tetrasaccharide being formed (1910), but this compound was not defined.

The group of acetyl–halogen derivatives, typical members of which are involved in the reactions just described, was destined to perform important service in Fischer's later work. When the action of hydrogen bromide on penta-acetylglucose is protracted beyond the period required for conversion into tetra-acetylbromoglucose, a second acetoxy-group is displaced by bromine, and β-triacetyldibromoglucose arises (Fischer and E. F. Armstrong, 1902). Exchange of one halogen atom for the methoxy-group leads to triacetylmethylglucoside bromohydrin, converted by warm baryta into the anhydromethylglucoside, $C_7H_{12}O_5$, which is hydrolysed by acids to anhydroglucose (Fischer and Zach, 1912):



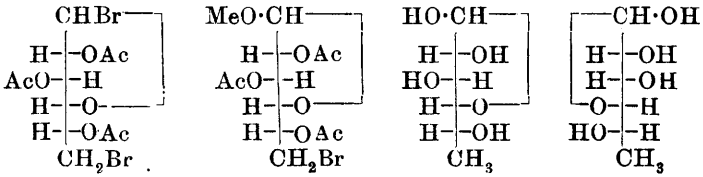
Triacetyldibromoglucose.



Anhydroglucose.

This is crystalline, produces colour in Schiff's reagent, and forms crystalline derivatives with phenylhydrazine, whilst reduction with sodium amalgam gives anhydrosorbitol; anhydrogluconic acid follows oxidation by bromine.

The result of reducing triacetylmethylglucoside bromohydrin is noteworthy also, because it enabled Fischer to elucidate the configuration of the undetermined carbon atom in rhamnose (1912). With Zach, he found that replacement of bromine by hydrogen gave a triacetylmethylglucoside, leading to a methylpentose on complete hydrolysis, proving that the bromine atom in triacetylmethylglucoside bromohydrin occupies the terminal position in the chain. It was further discovered that the new methylpentose is the optical antipode of *iso*rhamnose, which is related to *d*-glucose as is rhamnose to *l*-mannose; thus rhamnose itself is *l*-rhamnose:



Triacetyldibromoglucose. | Triacetylmethylglucoside bromohydrin. | *d-iso*Rhamnose. (*iso*Rhodeose.) | *l*-Rhamnose. (Rhamnose.)

Another remarkable application of acetylbromoglucose led to the synthesis of mandelonitrile-glucoside, which had been obtained by Fischer (1895) as a crystalline, lævorotatory, non-reducing product of hydrolysing with yeast-maltase the disaccharide group in amygdalin without severing the connexion between mandelonitrile and the residual hexose; subsequently, two isomerides were isolated, namely, sambunigrin from the leaves of *Sambucus niger* by Bourquelot and Danjou (1905), and prulaurasin from *Prunus laurocerasus* by Hérissey (1906). Caldwell and Courtauld (1907) recognised all three as β-glucosides, Fischer's being *l*-mandelonitrile-β-glucoside; they regarded sambunigrin as *d*-mandelonitrile-β-glucoside and prulaurasin as the *dl*-form. This forecast was confirmed by the synthesis (Fischer and Bergmann, 1917), which followed the action of silver carbonate on acetylbromoglucose in molten ethyl *dl*-mandelate, the product giving *dl*-mandelamide-glucoside with methyl-alcoholic ammonia; dehydration following resolution gives the mandelonitrile-glucoside in both active forms. It is to be regretted that Fischer himself did not survive to extend this process to the synthesis of amygdalin, which, in 1895, he believed to be "ein Derivat der Maltose oder einer ganz ähnlich construirten Diglucose." One of his last papers, however, describes

the synthesis of glycollonitrile-glucoside (1919), the simplest of the cyanogenetic glucosides, immediately following that of linamarin (from flax), the glucoside of acetonecyanhydrin.

Recalling the manifold parts played by the substituted amino-group in animal and vegetable metabolism, it is a singular fact that so few amino-derivatives of a glucose type have been encountered amongst the products. It was natural, therefore, that glucosamine, isolated from lobster shells by Ledderhose (1878), should attract Fischer's attention, because its empirical relationship to glucose is expressed by a simple interchange of hydroxy- and amino-groups; the actual connexion, however, is elusive, for whilst indirect processes of replacement (Irvine and Hynd) lead variously to *d*-glucose (1912) and *d*-mannose (1914), thus pointing to a Walden inversion, the direct action of nitrous acid involves dehydration in addition to the normal exchange.

The component of lobster shells which yields glucosamine having been called chitin, the sugar-like product, $C_6H_{10}O_5$, from the base and nitrous acid was called chitose; this was oxidised to chitonic acid, $C_6H_{10}O_6$, by Fischer and Tiemann (1894). By the same change they related glucosamine itself to chitamic acid, this being synthesised from *d*-arabinose (1903) by Fischer and Leuchs, who then reduced it to *d*-glucosamine; later in the same year Fischer and Andreae connected chitose and chitonic acid experimentally with hydroxymethylpyromucic acid, $\begin{matrix}CH{-}CH\\ \|\quad\quad\|\\ HO{\cdot}CH_2{\cdot}C{\cdot}O{\cdot}CH{\cdot}CO_2H'\end{matrix}$ to which is also related chitaric acid, the product from chitamic (glucosamic) and nitrous acids. Thus chitose, whether regarded as an aldehyde or a butylene oxide, is derived from tetrahydrofuran.

The triacetylmethylglucoside bromohydrin already mentioned was utilised by Fischer in relation to glucosamine. With Zach (1911) he found that ammonia converts it into a β-methylglucoside, in which the amino-group has replaced hydroxyl; but hydrolysis, instead of producing glucosamine, led to an isomeride. It has to be admitted that this branch of sugar chemistry retains a somewhat perplexing aspect, to which the loose nomenclature involved in such expressions as "aminoglucose," "aminofructose," and "methylglucosamine" contributes. This is all the more regrettable in view of the great biochemical interest attaching to glucosamine as a connecting link between carbohydrates and amino-acids.

Before closing this chapter, there remains to be described one of the most remarkable achievements in a series unsurpassed by any organic chemist, namely, the synthesis of tannin. In 1908 Fischer required the chloride of chloroacetyltyrosine for the synthesis of glycyltyrosylglycine, and, being embarrassed by the presence of the

phenolic group, protected this, prior to treatment with phosphorus pentachloride, by substituting the methylcarbonato-group for hydrogen. Immediately applying this device to phenolcarboxylic acids, he prepared *p*-hydroxyhippuric acid (the isomeride of salicyluric acid) with galloyl-*p*-hydroxybenzoic and *p*-hydroxybenzoyl-*p*-hydroxybenzoic acids. Anhydrides analogous to the last-named were rapidly multiplied, and were named "depsides" by Fischer and Freudenberg (1910), owing to the resemblance which many display towards the tannins; in parallel with the polypeptides and polysaccharides, such compounds may be classified as didepsides, tridepsides, etc., typified by the formulæ

$$HO\cdot C_6H_4\cdot CO\cdot O\cdot C_6H_4\cdot CO\cdot O\cdot C_6H_4\cdot CO_2H$$

and $(HO\cdot C_6H_4\cdot CO\cdot O)_2\cdot C_6H_3\cdot CO_2H$. The only recognised natural sources of the depsides are the lichens, of which the best known constituent is lecanoric acid, the depside of orsellinic acid, and in conjunction with his son Hermann, Fischer synthesised lecanoric acid (1903), and represented it as the *p*-ester,

$$HO\cdot C\begin{matrix}\diagup CH{-}{-}CMe\diagdown\\ \diagdown CH{:}C(OH)\diagup\end{matrix}C\cdot CO\cdot O\cdot C\begin{matrix}\diagup CH{-}{-}CMe\diagdown\\ \diagdown CH{:}C(OH)\diagup\end{matrix}C\cdot CO_2H,$$

of which evernic acid is the *p*-methyl ether.

The history of the tannins dates from the eighteenth century, but from the standpoint of this review the earliest year of importance is 1852, when Strecker deduced the formula $C_{27}H_{22}O_{17}$ for gallotannic acid or gall-nut tannin, which he regarded as a compound of grape-sugar and gallic acid in the molecular proportion 1:3. For half a century there prevailed a conflict of opinion as to the presence of a glucose residue, the production of sugar on hydrolysis being denied by several chemists, and the proportions in which it was obtained by the followers of Strecker varying much amongst themselves. In consequence of this uncertainty, Schiff's view (1871) of tannin as consisting principally of digallic acid preponderated until recently. The conductivity measurements by Walden (1897), however, paved a way for the unquestionable differentiation of the two materials by Fischer, who synthesised digallic acid in 1908 and found it to be crystalline, although astringent; moreover, in 1912, having adopted a method of purifying the principal constituent of Chinese tannin and of producing specimens having constant optical activity, he and Freudenberg proceeded to show that when hydrolysed with sulphuric acid it yields 7 to 8 per cent. of glucose, an amount which they regarded as probably too low in view of the extended period occupied in completing the operation. They then expressed the opinion that the principal constituent of tannin is not a glucoside, but a sugar

ester comparable with pentabenzoylglucose, in which the acyl group is that of digallic acid. Expressed by the formula

$$C_6H_7O_6[C_6H_2(OH)_3{\cdot}CO{\cdot}O{\cdot}C_6H_2(OH)_2{\cdot}CO]_5,$$

such a compound having the molecular weight 1700 would yield 10·6 per cent. of glucose and 100 per cent. of gallic acid on hydrolysis.

At the time of making this very penetrating speculation, they synthesised pentagalloylglucose, which they found to be a tannin, not identical with gall-nut tannin, but resembling it closely in taste, solubility, amorphism, optical activity, and feeble acidity; moreover, it precipitates gelatin and alkaloids, becomes gelatinous with arsenic acid, and develops colour with ferric chloride. In the course of this investigation they prepared hepta(tribenzoylgalloyl)-*p*-iodophenylmaltosazone, a freak molecule of gigantic dimensions (M.W. 4021), vastly exceeding those of any other synthetic product.

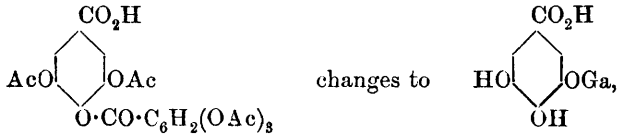
Valuable as the use of methylcarbonato-derivatives had proved, it did not suffice to perfect the aim in view, namely, to synthesise the main principle of Chinese tannin. This was accomplished in 1918, following the observation that the corresponding acetyl compounds are superior to the methylcarbonato-derivatives for depside production. In making this advance, Fischer explained that the acetylated phenolcarboxylic acids would certainly have been used much earlier had not he been misled by the statements of previous workers as to the difficulty of removing the acetyl group, which actually proceeds quite smoothly. The chloride of penta-acetyl-*m*-gallic acid, unlike the methylcarbonato-derivative, is crystalline, and with β-glucose yields the compound,

$$C_6H_7O_6[C_6H_2(OAc)_3{\cdot}CO{\cdot}O{\cdot}C_6H_2(OAc)_2{\cdot}CO]_5,$$

which is de-acetylated by cold aqueous sodium hydroxide at 0°, giving penta(*m*-digalloyl)-β-glucose (Fischer and Bergmann, 1918). The resemblance between this artificial tannin and the principal constituent of Chinese tannin is much closer than that offered by pentagalloylglucose; the two materials are, in fact, indistinguishable, excepting with respect to optical activity, and the same remark applies to penta(*m*-digalloyl)-α-glucose, the recorded $[\alpha]_D$ in water being 43·8° and 42·3° for the derivatives of α- and β-glucose, respectively, and 70° for the purified Chinese tannin. The correspondence between the potassium salts (1919) is even closer, those from Chinese tannin, pentadigalloyl-α-glucose, and pentadigalloyl-β-glucose having $[\alpha]_D$ 46·3°, 56·6°, and 33·7° respectively.

It is therefore justifiable to claim that gallotannic acid or gall-

nut tannin has been synthesised. Incidentally to this remarkable conclusion, it was observed that when acylated *p*-digallic derivatives are hydrolysed, even by the most delicate methods, the galloyl nucleus is transferred from the para- to a meta-hydroxy-group; for instance, under the influence of alkalis, ammonia, or mineral acids,



and hence penta-acetyl-*p*-digallic acid yields *m*-digallic acid.

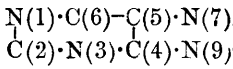
Thus was brought to a conclusion, in the closing months of his darkened life, that illustrious chapter of chemistry with which Emil Fischer first drew on himself the admiration of his contemporaries. It represents the fruit of thirty-five years' unremitted labour and the ripening of an intellectual and experimental technique but rarely developed in the history of scientific endeavour. Begun in the vigorous days of his early manhood, when domestic happiness suffused his professional activity and adorned the promise of a brilliant career, it supported him in his first tragedy, stirred to the utmost the deep resources of his mind, and finally solaced those concluding years in which he was compelled to witness the pillars of Prussianism crumbling at the feet of his disillusioned and bewildered countrymen.

Purine Derivatives.

Some of the most notable figures in chemical history have devoted themselves to the problems which cluster round uric acid and its allies. Scheele, Bergmann, Fourcroy, Prout, Liebig, Mitscherlich, Wöhler, von Baeyer, Strecker, Stenhouse, and Gerhardt are found in the list of names connected with the subject, and to a high place of honour in this galaxy Fischer is most assuredly entitled. Between 1881, when he resolved caffeine into methylcarbamide and dimethylalloxan, and 1914, the year in which he synthesised a nucleotide in the form of theophylline-*d*-glucosidephosphoric acid, the literature was enriched by a succession of systematic observations which reached a climax in 1898, when he derived purine from uric acid by means of indirect deoxidation.

Although uric acid was discovered in 1776, fifty-eight years elapsed before its composition was established, and four years later, in 1838, Liebig and Wöhler published their comprehensive survey of its oxidation products; the true nature and significance of these

were revealed by von Baeyer's experiments, described in 1863 and 1864, thus preparing the ground for the now accepted constitutional formula proposed by Medicus in 1875. This was confirmed in 1888 by Behrend and Roosen, whose synthesis of uric acid, unlike its predecessors, was achieved by definite steps, a feature also of that by Fischer and Ach in 1895. The modern system of notation, according to which the diureides are classified as derivatives of purine and referred to the bicyclic nucleus,

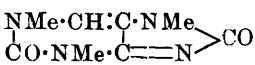


was promulgated by Fischer in 1897, thirteen years after his introduction of the name (*purum*: *uricum*) in connexion with the methyl derivative. The relationship

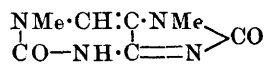
$C_5H_4N_4O_3$	$C_5H_4N_4O_2$	$C_5H_4N_4O$	$C_5H_4N_4$
Uric acid. (2 : 6 : 8-*Trioxypurine.*)	Xanthine. (2 : 6-*Dioxypurine.*)	Hypoxanthine. (6-*Oxypurine.*)	Purine.

was thus consolidated, the catalogue of purine derivatives contributed by Fischer and his collaborators prior to 1900 embracing upwards of 130 individuals.

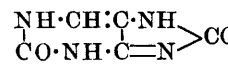
The readiness with which caffeine lends itself to experimental treatment led Fischer to open attack on the problem of uric acid by first elucidating the constitution of that base, and a preliminary communication thereon appeared in 1881. His introductory work dealt also with xanthine, theobromine, and guanine. By oxidising aqueous caffeine with chlorine, Rochleder had obtained amalic acid (tetramethylalloxantin), which Fischer showed to be preceded by chlorocaffeine, and to arise from the action of hydrochloric acid on dimethylalloxan; the latter substance and methylcarbamide were found to be the principal products of oxidising caffeine in the manner indicated, and it was proved convenient to prepare chlorocaffeine by acting with the halogen in absence of water. Similarly, theobromine was oxidised to methylcarbamide and methylalloxan, showing that when caffeine is produced by methylating theobromine, the entrant methyl group becomes attached to the alloxan ring. Next, improving the preparation of xanthine from guanine, Fischer oxidised it to alloxan and carbamide, and by acting on the lead derivative with methyl iodide obtained theobromine; on the basis of these and subsidiary experiments, he assigned the following constitutional formulæ (see, however, later):



Caffeine.



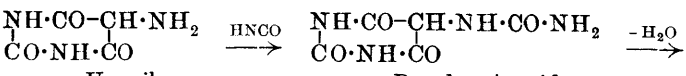
Theobromine.



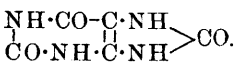
Xanthine.

It is noteworthy that even at this period (1882) the products interested him from the standpoint of relation between chemical constitution and physiological action; moreover, Dr. Ludwig Knorr is mentioned as his assistant.

In 1864 Strecker stated that Rheineck had reduced uric acid to xanthine and sarcine (hypoxanthine) by means of sodium amalgam, but he never mentioned the subject again, and did not include the observation in his "Lehrbuch." Fischer found that uric acid is not changed by sodium amalgam, and likewise that, contrary to the statement of Kossel, hypoxanthine is not converted into xanthine by nitric acid. Thus in 1884, uric acid, xanthine, and hypoxanthine, so similar in origin, behaviour, and composition, had no direct experimental connexion with one another. In that year Fischer studied methyluric acid in the hope of establishing the foregoing relationship with respect to the methyl derivatives of xanthine and hypoxanthine. To the existing compound, which gives carbamide and methylalloxan on oxidation, he added an isomeride, which yields methylcarbamide and alloxan when oxidised, and is converted by phosphorus pentachloride into trichloromethylpurine, from which methyluric acid can be regenerated. Complementary also to the dimethyluric acid, which gives methylcarbamide and methylalloxan when oxidised, he discovered an isomeric dimethyl derivative yielding cholestrophan (dimethylparabanic acid). It was thus established that, in addition to the ring which appears in the form of alloxan (mesoxalylcarbamide), uric acid contains two imino-groups associated with one another as in carbamide itself. The methylation of uric acid was then carried to the trimethyl derivative, apparently isomeric with hydroxycaffeine (see later), and to tetramethyluric acid, isomeric with methoxycaffeine, but having all the methyl groups attached to nitrogen, thus indicating the presence of four imino-groups in uric acid, as required by the structural formula previously advanced by Medicus. This was further confirmed in 1895, when Fischer and L. Ach added the final step in the transformation,



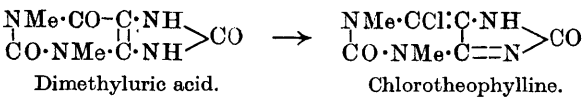
Uramil. Pseudo-uric acid.



Uric acid.

Then, applying this process to dimethyl*pseudo*uric acid, they produced a third dimethyluric acid, convertible by phosphorus penta-

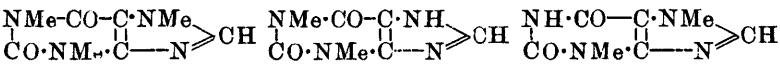
chloride into chlorotheophylline, from which theophylline arises on exchanging halogen for hydrogen:



Dimethyluric acid. Chlorotheophylline.

This completed the synthesis of theophylline, and, incidentally, of caffeine.

Then arose a situation which is rarely encountered in the course of Fischer's investigations. He was compelled by his own experiments to revise the structural formulæ which he had assigned to xanthine and its derivatives, caffeine, theobromine, and theophylline. In 1895 he had produced a fourth dimethyluric acid by exchanging halogen for hydroxyl in bromotheobromine, and the simplicity of this procedure did not harmonise with the profound structural rearrangement apparently involved. A more rigid examination of hydroxycaffeine, believed to be isomeric with trimethyluric acid, showed it to be identical with that substance, and by varying the conditions of methylation, tetramethyluric acid was obtained from it. Moreover, hydroxycaffeine was produced without difficulty from the corresponding trimethyl*pseudo*uric acid, and finally by direct methylation of uric acid itself. In consequence of these discoveries, Fischer modified his previous expressions and assigned the following in 1897:

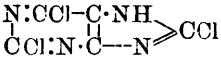


Caffeine. Theophylline. Theobromine.

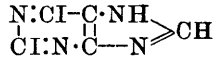
During his investigation of methyluric acid in 1884, Fischer had found that phosphorus pentachloride in presence of the oxychloride easily replaces two, and finally all three, oxygen atoms by chlorine, producing trichloromethylpurine. In 1897, assisted by L. Ach, he converted uric acid by the same process into 8-oxy-2:6-dichloropurine, reducible to 8-oxypurine isomeric with hypoxanthine, and convertible by ammonia into 6-amino-8-oxy-2-chloropurine; this was reduced to 6-amino-8-oxypurine isomeric with guanine, from which nitrous acid produced 6:8-dioxypurine isomeric with xanthine. The complete replacement of oxygen by chlorine was much more difficult, but by heating 8-oxy-2:6-dichloropurine with seventy parts of phosphorus oxychloride at 150°, Fischer obtained trichloropurine, a basic substance giving the 7- and 9-methyltrichloropurines on methylation, and providing him with material for completing the synthesis of hypoxanthine

(6-oxypurine), xanthine (2:6-dioxypurine), adenine (6-amino-purine), and guanine (2-amino-6-oxypurine).

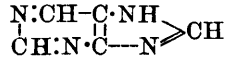
The goal towards which Fischer first directed himself in 1884 was now reached. Purine, the parent of the group, and on which the nomenclature and notation of the whole series had been based, lay open to isolation; the accomplished facts were described in 1898. Trichloropurine was reduced in two stages, the first depending on the action of hydrogen and phosphonium iodides at 0° and producing 2:6-di-iodopurine, from which purine itself was obtained by the action of zinc on a boiling aqueous solution:



2:6:8-Trichloropurine.



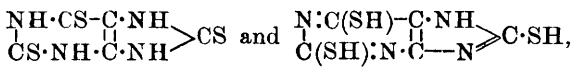
2:6-Di-iodopurine.



Purine.

Purine was thus found to be a definite entity, amphoteric in character and harmonising completely with its position in the series.

Attention has been drawn to the difficulty with which uric acid is converted into trichloropurine, and to the fact that only one halogen atom in this compound is exchanged for oxygen by alkali hydroxides. Alkali sulphides, however, rapidly displace all three, producing trithiopurine (1898), and of the two tautomeric formulæ,



Fischer preferred the latter. Another by-path was pursued in 1900, when he prepared 9-phenyluric acid from the corresponding phenyl*pseudo*uric acid, which results from the action of phenylcarbimide on uramil; this was followed by 9-phenylpurine.

The series of six dimethyluric and four trimethyluric acids was completed with assistance from F. Ach by the preparation of 1:9-dimethyl- and 1:7:9-trimethyluric acids, but the monomethyl derivatives presented a curious anomaly. According to the lactame formula for uric acid, there should be four methyluric acids, in which the alkyl group occupies the position 1, 3, 7, or 9 respectively, but the existence of two additional ones was claimed. Of these six methyluric acids, Fischer and F. Ach (1899) showed that three appear to have the alkyl group attached to the same nitrogen atom, numbered 3 in the purine nucleus, giving the same methylallantoin (on oxidation) and tetramethyluric acid. The mystery remained unsolved until 1916, when an independent examination of the materials by Biilmann and Bjerrum, Biltz and Heyn, showed that the supposed difference is due to the contamination of

3-methyluric acid with a varying proportion of the 9-methyluric acid.

Although for many years displaced from his experimental attention, the purines became involved in Fischer's synthesis of nucleotides. It has already been seen that acetylbromoglucose is a valuable agent in elaborating glucosides, and in 1914, assisted by B. Helferich, he brought this compound into action with the silver derivative of numerous oxypurines, producing the *d*-glucosides of theophylline, theobromine, adenine, hypoxanthine, and guanine; the outstanding feature of these compounds is the readiness with which they are hydrolysed, thus being distinguished from derivatives of glucosamine. Finally, by adding a cold mixture of phosphoryl chloride and pyridine to a solution of theophylline-*d*-glucoside in pyridine, he prepared, later in the same year, theophylline-*d*-glucosidephosphoric acid, the first synthetic nucleotide.

Amino-acids, Polypeptides, and Proteins.

In view of their extent and the far-reaching biochemical conclusions which have been based on them, the labours of Emil Fischer in the region of proteins will make the same appeal to the imagination and evoke the same delight in craftsmanship as his activities amongst carbohydrates. Owing to the much greater complexity of the subject, however, the intrinsic results may at first appear less complete; but the relative success is equally remarkable, because his treatment of this branch reveals the skilful manipulation of sensitive materials, the deft application of experimental indications, and the constructive diligence in synthetic achievement already so admirably displayed by his earlier work.

The amino-acids bear to the proteins a relationship recalling that of a hexose to a polysaccharide. Accordingly, it was with those materials that Fischer began, in 1899, experiments which were destined to reveal the chemical nature of the proteins themselves, and to furnish material which indicates, at least, the manner in which lifeless protein may ultimately be synthesised. It had then been recognised that nine amino-acids, three diamino-acids, and cystine were obtainable by hydrolytic or enzymic disruption of protein molecules; the synthesis of glycine, alanine, α-aminovaleric acid, leucine, aspartic acid, glutamic acid, phenylalanine, and tyrosine, all in their *dl*-forms, had been accomplished either by Strecker's method or by substituting the amino-group for halogen in the respective α-chloro- and α-bromo-acids, but serine, although discovered in 1865, was not synthesised until 1902 (Fischer

and Leuchs). The resolution of these products into their optically active components had been limited by their amphoteric nature, and was easy only in the case of aspartic acid, Piutti having shown, in 1887, that asparagine is resolved by simple crystallisation from water.

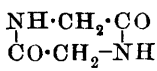
By suppressing the basic aspect of the amino-acids, and thus encouraging their capacity to form recrystallisable salts with the natural alkaloids, strychnine and brucine, Fischer and his collaborators first resolved into their antipodal components the *dl*-forms of alanine, α-aminobutyric acid, leucine, α-amino-*n*-caproic acid, phenylalanine, tyrosine, aspartic and glutamic acids, valine, serine, *iso*serine, and proline. The device by which this was effected consists in benzoylating (1899), formylating (1905), and, in the case of serine, *p*-nitrobenzoylating (1906) the amino-group, resolving the *dl*-acylamino-acid by recrystallising its salts with strychnine or brucine, and hydrolysing separately the antipodal benzoyl, formyl, or *p*-nitrobenzoyl derivative of the *d*- and *l*-amino-acid. In this manner were accumulated, in greater quantity and variety, optically active units, which thus became available as building materials for the construction of polypeptides approaching the peptones in physical characteristics.

The foregoing acyl derivatives, in common with others depending on the reactivity of the amino-group with phenylcarbimide and benzenesulphonyl chloride, are useful for identification as well as isolation of their parent compounds, but some still better for the former purpose followed from combination with β-naphthalene-sulphonyl chloride (1902); the resulting derivatives are formed in good yield, are sparingly soluble, and crystallise sufficiently well to facilitate the recognition of hydroxyamino-acids and even poly-peptides themselves, in addition to the compounds from which the latter are built. Thus, to a minor extent, β-naphthalenesulphonyl chloride assumes the part played by phenylhydrazine in the sugar group, affording an instrument for isolating freely soluble and elusive substances.

Early attempts to explain the structure of proteins had been hampered by experimental obstacles to the separation of amino-acids produced in such complex mixture by hydrolysis; with the exception of tyrosine and cystine, which are sparingly soluble in water, the major portion of the mixture remains as a syrup after the principal constituent has crystallised. It was thus a practical advance of the first magnitude which Fischer made as the result of his inquiry (1900) into the esters which, owing to the suppression of the carboxylic function, have the properties of aliphatic amines; this feature had been recognised in 1883 by Curtius, whose process

for isolating the esters was modified by Fischer and applied by him to separating the amino-acids in a complex mixture through fractional distillation. The procedure offers considerable experimental difficulty, but it is the only one which has been really successful, and its purpose has now been achieved with a large number of elaborate molecules. Edestin, elastin, fibrin, globin, gluten, keratin, and the albumins of egg, serum, and milk are examples of the zeal with which Abderhalden has pursued this line of inquiry, whilst Osborne and his collaborators, by similar methods, have ascertained the component amino-acids of amandin, excelsin, gliadin, glycinin, hordein, phaseolin, and zein. Particularly to Fischer himself is due the resolution of the fibroin produced by silkworms and spiders, incidentally emphasising the remarkable biological fact that there is only slight chemical difference between the synthetic products of two creatures whose diet is so vastly divergent. He showed (1907) that the silk of the Madagascar spider gives, per cent.: glycine 35·1, *d*-alanine 23·4, *l*-leucine 1·7, *l*-tyrosine 8·2, proline 3·7, *d*-glutamic acid 6·1, diamino-acids (calculated as arginine) 5·2, ammonia 1·1, and fatty acids 0·6; thus the principal difference is the large proportion of glutamic acid, which has not been derived from ordinary silk, and the absence of serine.

Some indication of the nature and variety of the chemical units from which the proteins are constructed having been given, attention must now be directed towards the notable attempts made by Fischer artificially to elaborate protein molecules from their component elements. It is a noteworthy feature of the proteins that, in spite of certain basic properties and the prompt appearance of the amino-group on hydrolysis, the amount of nitrogen liberated by nitrous acid is trifling compared with the percentage of that element in the original material. This fact, in conjunction with the early recognition of hippuric acid as benzoylglycine, gives a clue to the manner in which amino-acid molecules are associated in the natural products now under consideration, and several observations by the earlier investigators confirm it. The simplest of these is the production of a bimolecular anhydride of glycine by the auto-condensation of the ethyl ester (Curtius, 1888). Two such anhydrides are conceivable,



Diketopiperazine.

and $NH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CO_2H$

Glycylglycine.

according to the proportion of water eliminated, and Curtius's anhydride belongs to the former class. In 1900 Fischer obtained

analogous products from the esters of α-aminobutyric, α-amino*iso*-caproic (leucine), and α-amino-*n*-caproic acids, and classified them as diketopiperazines, in consequence of an observation by Mylius (1884), who prepared the anhydride of sarcosine. Realisation of the alternative type, glycylglycine, came in 1901, when, in conjunction with Fourneau, Fischer hydrolysed diketopiperazine without detaching the two glycine molecules, and thereafter began that astonishing series of elaborations which culminated (1907) in the synthesis of an octadecapeptide,

$$NH_2\cdot CH(C_4H_9)\cdot CO\cdot[NH\cdot CH_2\cdot CO]_3\cdot NH\cdot CH(C_4H_9)\cdot CO\cdot [NH\cdot CH_2\cdot CO]_3\cdot NH\cdot CH(C_4H_9)\cdot CO\cdot[NH\cdot CH_2\cdot CO]_8\cdot NH\cdot CH_2\cdot CO_2H,$$

composed of glycine (15) and *l*-leucine (3) molecules, and having the molecular weight 1213. The name "polypeptide" was adopted to emphasise the similarity to peptones displayed by the properties of the new class, whilst recalling the manner in which polysaccharides are compounded of simple carbohydrate molecules.

Subsequently to the process just reviewed, namely, arrested hydrolysis of a cyclic anhydride, two general methods were employed by Fischer to effect these imposing syntheses. Briefly stated, they depend on elongating the amino-acid chain at the basic and acidic terminals respectively. By the former mechanism a chloro- or bromo-acyl chloride, acting on the amino-acids (or polypeptides already synthesised therefrom), produces a halogen derivative, which only needs treatment with ammonia to become converted into the corresponding amino-compound, for instance, glycylalanine:

$$CH_2Cl\cdot COCl + NH_2\cdot CHMe\cdot CO_2H \longrightarrow CH_2Cl\cdot CO\cdot NH\cdot CHMe\cdot CO_2H.$$

In this manner, the glycyl, alanyl, α-aminobutyryl, leucyl, phenylglycyl, phenylalanyl, and prolyl (α-pyrrolidinecarboxylic) groups were introduced (1903—1905).

The complementary process arose from the discovery (1904) that chlorides of halogenated acylamino-acids could be prepared by the action of phosphorus pentachloride on the acid dissolved in acetyl chloride; when such acyl chlorides act on the ester of an amino-acid or a polypeptide, the product only requires to be hydrolysed and treated with ammonia in order to yield the higher polypeptide corresponding to its component molecules:

$$\underset{\text{Bromo}\textit{iso}\text{capronylglycyl chloride.}}{C_4H_9\cdot CHBr\cdot CO\cdot NH\cdot CH_2\cdot COCl} + \underset{\text{Glycylglycine ester.}}{NH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et}$$

$$\longrightarrow C_4H_9\cdot CHBr\cdot CO\cdot NH\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et$$

$$\longrightarrow \underset{\text{Leucyldiglycylglycine.}}{C_4H_9\cdot CH(NH_2)\cdot CO\cdot[NH\cdot CH_2\cdot CO]_2\cdot NH\cdot CH_2\cdot CO_2H.}$$

Although this process is elastic, there presented itself an experimental obstacle to great extension, namely, the solubility of many such acyl chlorides in acetyl chloride, and the consequent difficulty in separating them from solution without decomposition. This was overcome by preparing the acyl chlorides of the amino-acids or of the polypeptides themselves; these chlorides, having the general formula $R{\cdot}CH(NH_3Cl){\cdot}COCl$, are also ammonium chlorides, and are generally not readily soluble in acetyl chloride. As they act smoothly on the esters of amino-acids and polypeptides, the device has been a most fruitful one, and particularly useful in application to the *d*- and *l*-amino-acids, with consequent synthesis of optically active polypeptides. Straightforward as these reactions appear in description, they represent a very remarkable feat experimentally, the rigid exclusion of water being necessary throughout.

Another advantage offered by this process is the facility with which it can be applied to elaborating polypeptides of diverse units, such as glycyl-*d*-alanylglycyl-*l*-tyrosine (1908), isomeric with the tetrapeptide which Fischer obtained in the previous year from silk hydrolysis, and herein lies the connecting link between these notable synthetic operations and the peptones arising from incomplete disruption of protein molecules.

The far-reaching consequence of the method provided by Fischer to separate the components of an amino-acid mixture has already been indicated, but the esters thus isolated were, until 1902, those of amino-acids only, unassociated with polypeptides. In that year, however, assisted by Bergell, he produced from silk fibroin, by successive hydrolysis with hydrochloric acid, trypsin and baryta, a dipeptide which appeared to be glycyl-*d*-alanine, although it could not be identified with the synthetic product; but in 1906, with Abderhalden, he obtained from the same source a methyldiketopiperazine, $\begin{matrix}NH\text{---}CH_2{\cdot}CO\\ |\qquad\quad |\\ CO{\cdot}CHMe{\cdot}NH\end{matrix}$, identical with that producible from glycine and *d*-alanine, thus indicating that glycyl-*d*-alanine is amongst the degradation products of silk fibroin. Then followed the recognition of glycyl-*d*-tyrosine (silk fibroin), glycyl-*l*-leucine and *d*-alanyl-*l*-leucine (elastin), *l*-leucyl-*d*-glutamic acid (gliadin), and glycyl-*d*-alanylglycyl-*l*-tyrosine (silk fibroin).

It is obvious that the field of investigation opened by the foregoing experiments is limited only by material considerations, and an interesting calculation of the possibilities presenting themselves was made by Fischer in 1916. According to this, the octadecapeptide has 816 possible isomerides, whilst a polypeptide comprising 30 amino-acid molecules, of which 5 are glycine, 4 alanine,

3 leucine, 3 lysine, 2 tyrosine, 2 phenylalanine, and 13 various other descriptions, has a number of possible isomerides reaching $1{\cdot}28 \times 10^{27}$. In these computations it is assumed that the mechanism of linking the amino-acid groups is limited to that of glycylglycine, but further complexity would arise from alternative junctions, such as that of diketopiperazine, the possibility of which was not excluded by Fischer. Moreover, he recognised that hydroxyamino-acids, such as tyrosine, serine, and hydroxyproline, may participate in the linkages peculiar to esters and ethers. Although the aggregate number of synthetic polypeptides must be well in excess of 200, this only serves to illuminate the gulf which still separates the chemical investigator from his goal. That Fischer appreciated this baffling factor to the fullest extent appears not only from his frequent references thereto, but also from the nature of his later synthetic operations. Following the octadecapeptide, these were directed more particularly to the association of optically active *Bausteine*, which were varied amongst themselves as much as possible with a view to synthesising those peptide fragments which possess the natural configuration, a property to be revealed by zymolysis. Drechsel's iodogorgonic acid from coral (1896), having been found identical with 2 : 5-iodotyrosine, was linked with glycine (1908), whilst *d*-valine, α-aminostearic acid, β-aminobutyric acid, α-methyl*iso*serine, *l*-histidine, and *l*-proline were introduced also in the latter year, followed by *l*-cystine and *l*-phenylalanine; polypeptides containing *d*-tryptophan, *iso*serine, lysine, arginine, asparagine, *d*-glutamic acid and aspartic acid as components have since been prepared.

Although the simpler polypeptides are crystalline, and in that aspect bear no resemblance to the proteins, their tendency to amorphism increases with molecular weight, and aqueous solutions of the more complex ones are opalescent, yielding precipitates with ammonium sulphate, phosphotungstic acid, and tannin. Naturally, they do not respond to the colour test depending on tryptophan and tyrosine when those groups are absent, but they give the biuret reaction, and these features, in conjunction with their behaviour towards enzymes (see later), afford the strongest possible evidence in support of the protein diagnosis outlined by this chapter of Fischer's work. One passes to the next with the sensations of an explorer liberated from the perplexing entanglements of a dense forest to find himself on the shore of a limitless ocean.

Zymo-chemistry.

Since chemistry emerged from the crucible of alchemy, its association with biology has become increasingly intimate. Viewed materially, life is a process in which alternations of growth, decay, and regeneration present themselves in the light of chemical transformations, delicate, manifold, and inscrutable. Within the tissues of a living organism there proceed perpetual analyses and syntheses of which we gain an occasional glimpse, but cannot hope, by the comparative brutality of the test-tube, to stage the likeness. Nature's agents are photosynthesis and enzyme action, magic wands which transform carbon dioxide, water, and nitrogen into carbohydrates, fats, and proteins, and which, having synthesised these materials in plants, empower animals to analyse them and assimilate the products of disintegration. In the words used by Fischer during his Faraday Lecture, 1907, "the ultimate aim of biochemistry is to gain complete insight into the unending series of changes which attend plant and animal metabolism."

Having now reviewed some of his most important contributions to our knowledge of the building materials, and proceeding to consider the use which he made of the enzymes themselves, it is noteworthy that this began on a by-road of the great thoroughfare which he cut through the realm of carbohydrates. In 1889, having shown that the "seminose" produced by Reiss from vegetable ivory (*Phytelephas macrocarpa*) is identical with mannose, he isolated alcohol from the products of fermenting that sugar, and in the following year cultivated yeast in solutions of *dl*-mannose and α-acrose (*dl*-fructose); in each case, the *d*-component was devoured, and thus *l*-mannose and *l*-fructose were isolated.

In 1894, assisted by Thierfelder, Fischer made a comparative study of natural and synthetical monosaccharides in respect of their attitude towards various families of yeast, from which it followed that whilst *d*-mannose, *d*-fructose, and, in lower degree, *d*-galactose resemble *d*-glucose, the yeasts are indifferent towards *d*-talose, *l*-mannose, *l*-glucose, sorbose, *l*-arabinose, rhamnose, α-glucoheptose and α-gluco-octose. This indicated that the fermentative principle of yeast is an asymmetric agent which is capable of attacking only those molecules of which the geometrical form does not differ too widely from that of *d*-glucose. It suggested also the possibility that, by persuasive tactics, a reluctant yeast might be tempted ultimately to modify its inherited taste and to accept as nutriment a sugar with which the asymmetry of its enzyme was not originally harmonious. This possibility has not yet been definitely realised.

Pasteur's notable discovery in connexion with *Penicillium* was

thus recalled, and, on extending the inquiry to natural and artificial glucosides, Fischer found that these materials arrange themselves into distinct groups in respect of their behaviour towards air-dried yeast extract (maltase) and emulsin, the *α-d*-glucosides being hydrolysed by maltase and indifferent to emulsin; the latter, however, hydrolyses the *β-d*-glucosides, which are not attacked by maltase, both enzymes being without action on *l*-glucosides, *d*-galactosides, arabinosides, xylosides, rhamnosides, and gluco-heptosides. Maltose is hydrolysed by the yeast extract, not by emulsin, whilst lactose displays the converse behaviour. It was by observations such as these that Fischer was led to emphasise the close relationship connecting the configuration of a sugar with that of the enzyme which attacks it, and to depict the mechanism of enzyme action by the simple analogy of a lock and key.

In the same year one of the many rocks which await the unwary voyager on this particular ocean was charted by Fischer. The "invertase" of that period was precipitated by alcohol and did not hydrolyse maltose, but Fischer replaced the solid material by an aqueous extract of air-dried (Frohberg) yeast; this does hydrolyse maltose, but neither the disaccharide nor α-methyl-glucoside is affected by an aqueous extract of the fresh yeast which has not been dried, although cane-sugar is inverted. After being ground with powdered glass, the same yeast yields an extract capable of hydrolysing maltose and α-methylglucoside, but the action is much more feeble than when the glucosides are left in contact with the suspended organism which has been narcotised. In consequence of divergent observations by G. H. Morris, it was found that complications are introduced by the proportion of chloroform employed, and this led to the use of toluene in its place.

The foregoing imbroglio emphasised the importance of specifying an enzyme by some reference to its origin, because an enzyme from one source is almost invariably associated with others, differing from those which accompany it when the origin is different. Fischer showed that whilst the extract of dried yeast hydrolyses both cane-sugar and maltose, the enzyme producing the latter effect cannot be invertase, because individual yeasts which contain invertase fail to hydrolyse maltose; moreover, invertase purified by alcohol is also indifferent towards this disaccharide. The specific maltoclastic enzyme had been called glucase by other workers, and it was for this name that Fischer substituted maltase, previously used by Bourquelot.

Lactose, which is indifferent towards brewer's yeast, is fermented by the milk-sugar yeasts, *S. Kefir* and *S. Tyrocola*. It had been claimed by Beyerinck, on somewhat slender evidence, that these

contain an enzyme, lactase, capable of hydrolysing lactose prior to fermentation; this was established by Fischer, who showed that the same result, intensified, was produced by kefir granules. Other observations of this period related to trehalose and melibiose, and it appeared to follow as a general conclusion that disaccharides are not fermented as such, but only in consequence of a preliminary hydrolysis by a specific enzyme.

In 1898 Croft Hill showed that the hydrolytic action of yeast maltase is reversible, a disaccharide being produced when that enzyme acts on glucose in concentrated solution; subsequently, Emmerling regenerated amygdalin by the action of yeast maltase on a mixture of mandelonitrile-glucoside and glucose, whilst Hanriot revealed the esterifying effect of lipase. In 1902 Fischer and E. F. Armstrong subjected a mixture of glucose and galactose to the action of kefir lactase, and, having removed the unchanged monosaccharides by fermentation, found in solution a disaccharide, which they called *iso*lactose, observing that in its behaviour towards enzymes it stands midway between lactose and melibiose; moreover, the kefir lactase which links its generators has the power to separate them.

Impressed with the differences in chemical behaviour revealed by the enzymes of micro-organisms, Fischer turned attention to secretions of animal origin, and in 1896, assisted by Niebel, studied the attitude of starch, glycogen, maltose, lactose, sucrose, trehalose, amygdalin, and some artificial glucosides towards blood serum from several sources and a great variety of tissue extracts and juices. It was in the domain of proteins and polypeptides, however, that the principal use was made of these agents. In 1903, when his fruitful association with Abderhalden began, casein was subjected to protracted hydrolysis by the pancreatic enzyme, with the surprising result that proline and phenylalanine, although liberated from the protein by acid and alkaline hydrolysis, were not recognisable amongst the products. These were tyrosine, alanine, leucine, glutamic acid, aspartic acid, and a polypeptide material which, when hydrolysed by hydrochloric acid, gave an amount of the two missing amino-acids in close correspondence with the quantity obtainable from casein itself, together with alanine, leucine, glutamic acid, and aspartic acid. It was also found that edestin, hæmoglobin, egg-albumin, fibrin, and serum-globulin resemble casein in the foregoing aspect, and thus was revealed a product of hydrolysis lying between the peptones and the amino-acids.

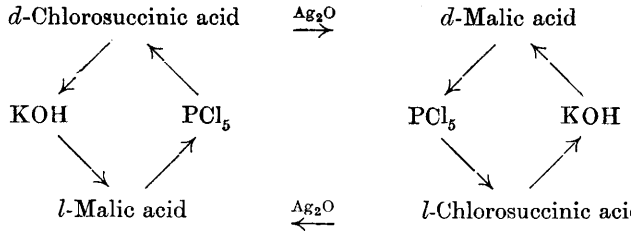
Acceptance of the relationship between proteins and amino-acids, established by the interposition of polypeptides, involves the

susceptibility of these materials to zymolysis, and, as their number grew, Fischer accumulated many studies of such action. Thus with Bergell (1903) he found that under conditions which favour the tryptolysis of naphthalenesulphoglycyl-*l*-tyrosine and carboethoxyglycyl-*dl*-leucine there is no change with glycylglycine, naphthalenesulphoglycyl-*d*-alanine, and hippuric acid; later it was shown that glycyl-*l*-tyrosine and leucylalanine may be tryptolysed, the disruption of the latter resembling that of carboethoxyglycylleucine by giving rise to active products. This branch of inquiry was greatly extended with the assistance of Abderhalden in 1905, when twenty-nine polypeptides were differentiated by their behaviour towards the tryptic enzyme, leading to conclusions based on the number, individuality, and configuration of the amino-acids involved. At the same time, it was found that glycyl-*l*-tyrosine, dialanylcystine, leucylalanine, leucylglycine, and leucylleucine are not hydrolysed by pepsin–hydrochloric acid.

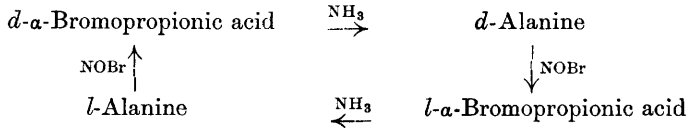
Thereafter, the development of this field has been left to other workers, of whom Abderhalden is recognised as the pioneer, and the remarkable achievements of the subsequent period are a splendid tribute to Fischer's foresight and genius in laying the foundations of a branch of science at once so complex and so fundamental. He it was, indeed, who first clothed with systematic experimental observations and established facts the dictum of Berzelius (1837) "that in living plants and animals there take place thousands of catalytic processes between tissues and fluids."

The Walden Inversion.

Prior to 1895, the conversion of an optically active substance into its enantiomorph had been accomplished only by racemisation preliminary to resolution by one of Pasteur's methods. The system of cyclic changes dating from that year, and classified as the "Walden inversion," exhibited a direct reversal of rotatory power, and offered, perhaps, the most elusive amongst the many interesting problems connected with optical activity. Walden showed that each chlorosuccinic acid will give both malic acids according to the agent selected for replacing halogen by hydroxyl; silver oxide leaves the sign unchanged, whilst potassium hydroxide produces an acid of opposite sign. As the regeneration of chlorosuccinic acid from malic acid by phosphorus pentachloride also involves a change of sign, the typical Walden inversion is represented as follows:

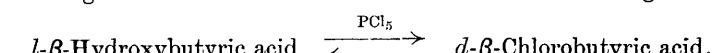


During the years 1907—1911, Fischer described numerous examples of sign-reversal arising from the study of amino-acids, for instance,

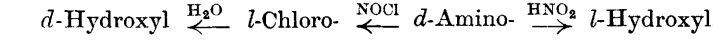


In the foregoing cycle it is a matter of indifference whether the replacement of halogen by an amino-group takes place in the acid or in the ester, but the converse change, attended by reversal of sign when alanine is transformed into α-bromopropionic acid, proceeds without reversal when alanine ester is converted into α-bromopropionic ester, the latter yielding, when hydrolysed, an acid having the same sign as the alanine employed. Other amino-acids, such as leucine, aspartic acid and phenylalanine, are distinguished from their esters in the same way, but *l*-valine (α-amino-*iso*valeric acid), whilst giving with nitrosyl bromide *d*-α-bromo*iso*-valeric acid, is regenerated by the action of ammonia on that substance. Fischer found, also, that whilst *l*-lactic acid arises from *l*-α-bromopropionic acid by action of silver oxide, this agent converts *l*-α-bromopropionylglycine into a hydroxy-compound which gives *d*-lactic acid on hydrolysis. Furthermore, although *l*-valine is produced when ammonia acts on *d*-α-bromo*iso*valeric acid, *d*-valine arises from hydrolysing the product of *d*-α-bromo*iso*-valerylglycine and ammonia.

At first it was believed that this type of change is associated only with α-substitution, Fischer and Scheibler showing that the following transformations are free from reversal of configuration:

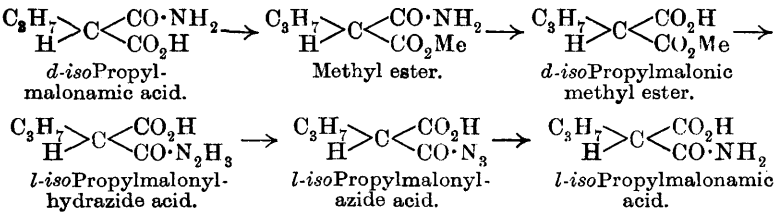


In 1911, however, they found that each β-aminobutyric acid will give both β-hydroxybutyric acids, according to the method of changing the substituents, thus:



The same year witnessed a comprehensive survey of the subject by Fischer (*Annalen*, 1911, **381**, 123), who described an ingenious model in which the central carbon atom and the movable groups have bristle-covered faces to facilitate their attachment in selected positions; thus the four substituents may be moved at the same time over the spherical surface of a central carbon atom, or they may be transferred singly to positions adjacent to those which they previously occupied. He represented substitution as preceded by the formation of an additive compound, which, on disruption, may or may not lead to a relative distribution of the substituents; thus the entrant group need not take the place of the one it dislodges, and if it assumes another position, the configuration of the product will differ from that of the original material. Viewed in this light, the Walden inversion is a normal process determined by the chemical agents employed and by the nature of the other groups attached to the asymmetric carbon atom. Simultaneously, the problem was discussed by Werner from the standpoint of supplemental valency, and criticisms by Biilmann in the following year led to a rejoinder by Fischer; it is not the purpose of this reference, however, to do more than indicate his contact with the subject, which still awaits a complete explanation.

Two important deductions from the theory of the asymmetric carbon atom received experimental confirmation at the hands of Emil Fischer by methods which the Walden inversion cannot vitiate, because the involved groups remain attached to the central nucleus throughout the changes. Assisted by F. Brauns, in 1914 he showed that optical activity disappears when two of the different groups become identical, producing ethyl*iso*propylmalonic acid (inactive) from *d*-ethyl*iso*propylmalonamic acid. He next made the following transformations:



*d-iso*Propylmalonamic acid. Methyl ester. *d-iso*Propylmalonic methyl ester.

*l-iso*Propylmalonylhydrazide acid. *l-iso*Propylmalonylazide acid. *l-iso*Propylmalonamic acid.

Since the original *d-iso*propylmalonamic acid has $[\alpha]_D$ 48·8°, and the resulting *l*-acid $[\alpha]_D$ −44·4°, agreement is sufficiently close to prove that the expectation of sign-reversal following systematic interchange of two substituents in an asymmetric system has been experimentally realised.

Technology.

Fischer's relationship to the chemical industry was intimate and beneficent. Viewed superficially, the subjects on which is founded his unrivalled reputation as an investigator do not appear to have much bearing on factory problems; but the value of a life-work cannot be estimated with accuracy unless the qualities of the worker are taken into account. It is a common observation that absorption in laboratory practice, coupled with unremitted study and theoretical reflection, tend to draw the chemical investigator so much away from practical affairs as to diminish his perception of commercial and industrial factors. Either because of his early training, or owing to his inborn love of knowledge in all its branches, Fischer was unusually free from this disability, and the reliance placed on his opinions by leaders of the German chemical industry ultimately grew into an attitude of trust which was quite exceptional. As early as 1883 he was a marked man, for in that year the chairman of the Badische factory selected him as director of research, in succession to Caro, at a salary of £5000, but the offer was not accepted. Whilst it is impossible to compute the results which might have accrued from his occupancy of the post, it is equally impossible to regret the decision which he then made. Although so tempting a proposal could not detach him from his chosen course, he remained throughout life in close communion with factory operations, becoming and continuing *persona grata* with the chemical industrial magnates and exerting a profound influence on the industry.

Probably his greatest direct contribution to technology lies in the stream of young chemists passing regularly from his laboratory to the factories, men soundly trained in the methods of systematic inquiry and in whom a love of chemistry had been made fruitful by the radiation of his galvanising personality. Nevertheless, more concrete associations with manufacture emerge from time to time. Phenylhydrazine was destined to become one of these, although its contact with industry through antipyrine, pyramidone, and tartrazine was made by other hands.

As already noticed, it might have been expected that the subject of his thesis for the doctorate, fluorescein, and the important contribution to the structure of triphenylmethane colouring matters for which he and his cousin were responsible so early in life, would have committed him definitively to the chemistry of dyes; but his interest in biochemistry rapidly became absorbingly predominant, and it was consequently in the field of synthetic drugs that his

personal connexion with chemical industry became most fruitful. Based on principles developed in his laboratory, methods were adopted in the Böhringer and Bayer factories for the manufacture of caffeine, theophylline, and theobromine, whilst the practicability of replacing atropine by a synthetical substitute may be traced to his early work on triacetonalkamine.

A very definite contribution to manufacturing practice was made in 1903, when the improvement which he effected in the production of diethylbarbituric acid, arising from his work on purines, led to that substance becoming one of the most valuable hypnotics in pharmacy under the name veronal, the manufacture being undertaken by the Merck, Bayer, and Höchst factories. His collaborator in that work, von Mering, was associated with him also in 1907, when calcium iodobehenate, or sajodin, was brought out as a tasteless preparation of iodine easily tolerated by the organism. This was followed by calcium dibromobehenate, or sabromin, as an instrument for introducing bromine, and the production of both remedies was undertaken by the Bayer and Höchst factories, the latter developing also the preparation of strontium chloroarsinobehenolate, or elarson, in connexion with which the preliminary experiments were made by Fischer and Klemperer. When war broke out he was taking part in the search for a carcinoma remedy, that path so thickly strewn with hopes deferred.

It is not difficult to imagine the demands which were made on his energy and wisdom during the five years which were destined to be the closing period of his life, and these have been delineated by A. von Weinberg. It is now known that the war could not have been continued by Germany beyond the middle of 1915 had not synthetic nitric acid begun to take the place of Chile saltpetre. The probable course of events revealed itself to Fischer in September, 1914, when he urged on the Westphalian manufacturers the need of prompt action, and was rebuked in consequence by the military authorities; but on October 1st he made a detailed report to the War Ministry with reference to the possibility of increasing the supply of ammonia from coke-ovens, and his services were in constant requisition during the growth of the synthetic nitric acid industry, which subsequently reached such enormous proportions.

Two months later the diminishing store of camphor led him to recommend the use of dimethyl- and diethyldiphenylcarbamide in powder stabilisation, these being actually adopted, and in February, 1915, he was presiding over a commission for stimulating the production of benzene and toluene by gas-stripping. Whilst the commission accomplished its original purpose before the end of that year, it remained in being to deal with such matters as the pro-

duction of heavy oil from naphthalene, the extraction of phenol and cresol from coke-oven tar, and, at the beginning of 1916, with a search for applications of the superabundance of benzene which then existed. By the end of that year, however, the Hindenburg programme had shattered the technical scheme for providing a sufficiency of this hydrocarbon, and converted plenty into famine; Fischer then demanded the demobilisation of 50,000 coal-miners.

As early as 1915 his attention was directed to the dwindling of pyrites reserves, and he became president of a gypsum and kieserite commission, charged with inquiry into means for utilising the sulphur content of those minerals; although technical difficulties prevented the application of kieserite to this purpose, the obstacles connected with gypsum were overcome and much valuable information was obtained.

At the beginning of 1916 Germany found it necessary to limit the saponification of fats, and Fischer was invited to examine more closely the alternative sources of glycerol. His first idea being to replace that substance by glycol, he recommended the construction of a factory at Essen for that purpose, but the subsequent process of Connstein and Lüdecke for producing glycerol by fermentation obviated the need for this measure. He took an active part in developing the new industry, in utilising the aldehyde-alcohol which offered itself as a by-product, and in solving the cognate problem of converting fatty acids to service as food, the result of which was ester-margarine.

It was the food shortage in all its aspects, however, which claimed his attention more and more pressingly. Interwoven with the demands of the explosives industry came the call for nitrogen fertilisers, and in January, 1917, associated with Nernst and Haber, he urged on the War Ministry the need for a foodstuffs commission to assume the task of stimulating food production on behalf of men and animals. With terrible earnestness he portrayed the secondary position occupied by patriotic heroism in relation to physiological law, the neglect of which must lead inevitably to psychological breakdown. From that time until its final session in 1918, the commission attacked with feverish energy a multitude of diverse problems, amongst which the conversion of straw into a digestible fodder for horses and cattle took a prominent place. The possibilities of wood, also, were explored from this point of view, the utilisation of leaves, rushes, and couchgrass (quitch), the germination of grain, and the preservation of vegetables. With special attention Fischer devoted himself to providing a coffee substitute, improving considerably on the knowledge which he had accumulated prior to the war, whilst attempts to augment the

supply of albuminoid esculents emerged in "mineral yeast" and the utilisation of lupines.

In spite of all these efforts, however, Fischer and his colleagues foresaw the inevitable results of increased disease, diminished capacity for work, and impaired moral resistance arising from the lamentable condition of the people. They embodied their conclusions in a memorial addressed to the heads of military and civil government in January, 1918, explaining the helplessness of science and technology to meet the situation, but their representations were unheeded.

Conclusion.

Harassed by these distractions and anxieties, tormented from time to time by bodily pain, and bowed down by the loss of his second and third sons, his inextinguishable spirit found refuge in the calm pursuit of scientific inquiry. It was during this period that his life-long work on carbohydrates and the correlation of these with depsides received many decisive additions, and in the closing months of 1918 he witnessed, in the establishment of the "Deutsche Gesellschaft zur Förderung des Chemischen Unterrichts," the launch of an enterprise very near to his heart. As the war continued, he had become gravely concerned at the diversion of young chemists to its requirements, and the consequent injury to the scientific spirit of the new generation. To assist in combating this danger and the accompanying embarrassment to teaching institutions arising from the diminished value of money, he raised a considerable fund, which, in his own words, constituted "der letzte Dienst den ich der deutschen Wissenschaft leisten kann."

In this connexion, it should be remembered also that it was largely by the inspiration and energy of Emil Fischer that the idea of establishing a research foundation independent of teaching duties ultimately took shape in the Kaiser-Wilhelm-Institut für Chemie. Associated with Nernst and Ostwald, he had invited a company, amply representing both science and industry, to discuss preliminaries in October, 1905, but it was not until March, 1908, that the "Verein Chemische Reichsanstalt" was legally registered for the purpose of advancing chemical science and technology. Although a suitable site at Dahlem was allocated by the Prussian Treasury, and many substantial donations had been made, it was not until the projected foundation of the Kaiser-Wilhelm-Gesellschaft in 1910, and the subsequent co-operation of this body with the Verein, that constructive steps could be taken. These culminated in the ceremony witnessed by the German Emperor on October

23rd, 1912, when Fischer, in the name of the Verein Chemische Reichsanstalt, as president of the executive committee, transferred the building to the president of the Kaiser-Wilhelm-Gesellschaft.

His life-work has now been reviewed, but only another generation can grasp its full significance. We shall not survive to witness the momentous consequences of its impulse, but we can perceive that Emil Fischer, in one branch of science the master, gave a new meaning to another branch, physiology, inasmuch as he placed biochemistry on an assured basis. The germ of this profound influence may be traced to a remote inquiry completely detached, as it would then have seemed in the mind of its author, from the trend of its ultimate development. The effect which was destined to be produced on physiological chemistry by the discovery of phenylhydrazine in 1875 offers but another example of the constant interplay between fact and thought. Although twelve years elapsed before that base enabled him to claim the synthesis of a natural sugar, progress thereafter was rapid and sweeping. The array of synthetical carbohydrates which had been assembled by the year 1894 provided him with the material necessary for the fundamental discovery that the specific action of an enzyme is intimately related to the configuration of the substrate. In the wealth of practical achievement which followed this discovery, the applications of its underlying principle constantly recur. The classification of the glucosides was a substantial consequence, but far more important was the utilisation of tissue extracts in the study of artificial polypeptides, showing that it is only those constructed of the amino-acids supplied by nature which yield to the attack of peptoclastic enzymes. Thus it may be claimed for Fischer that he forged and perfected a new and delicate instrument with which the investigator may solve abstruse problems in biochemistry, for when once the technique is acquired, the use of enzymes in configuration diagnosis is unapproached by ordinary chemical processes in respect of precision and rapidity.

Reflecting on the essence of life in its chemical aspect, regarding the act of living as a complex alternation of digestion, assimilation, and oxidation, the mind begins to arrange in one beautiful fabric the coloured strands from which is woven Fischer's contribution to the knowledge of the centuries. He not only regularised the most fruitful of laboratory methods for studying life processes, but he assembled more richly, and in greater variety than any other chemist, the materials on which those processes depend. Carbohydrates, glucosides, depsides, purines, and polypeptides have, during the years of his activity, been brought to our delighted vision and ranged in perspective by his control of enzymes. As

interpreted by him, we recognise amino-acids as the basis of our being. All this knowledge will give definite form to countless inquiries dealing with digestion and assimilation, and by shaping the problems connected with such changes will assist in taking that first and most important step towards solving them. Indeed, it is not unjustifiable to hope that further advances along the lines now firmly laid by his life-work may bring biochemists of a future period to a clearer view of that elusive province in which hormones and advitants (miscalled vitamines) exercise their subtle influence on the alchemy of living bodies.

Even when due allowance has been made for the storehouse of accumulated facts on which the chemists of his era were empowered to draw and for the variety of technique which was at their command, it can scarcely be claimed that in wealth of revelation and manipulative skill Emil Fischer is eclipsed by any of his predecessors. It is difficult to imagine that he can be surpassed by any of his successors; but, whether this be so or not, his achievement will remain for all time a monument of industry, a masterpiece of symmetry and a gospel of inspiration. His contemporaries, who have watched the growth of a wonderful structure with admiration and pride, may leave to posterity, in happy confidence, the office of enshrining his work in the history of their beloved science:

> "For Time shall with his ready pencil stand,
> Retouch your figures with his ripening hand,
> To future ages shall your fame convey
> And give more beauties than he takes away."