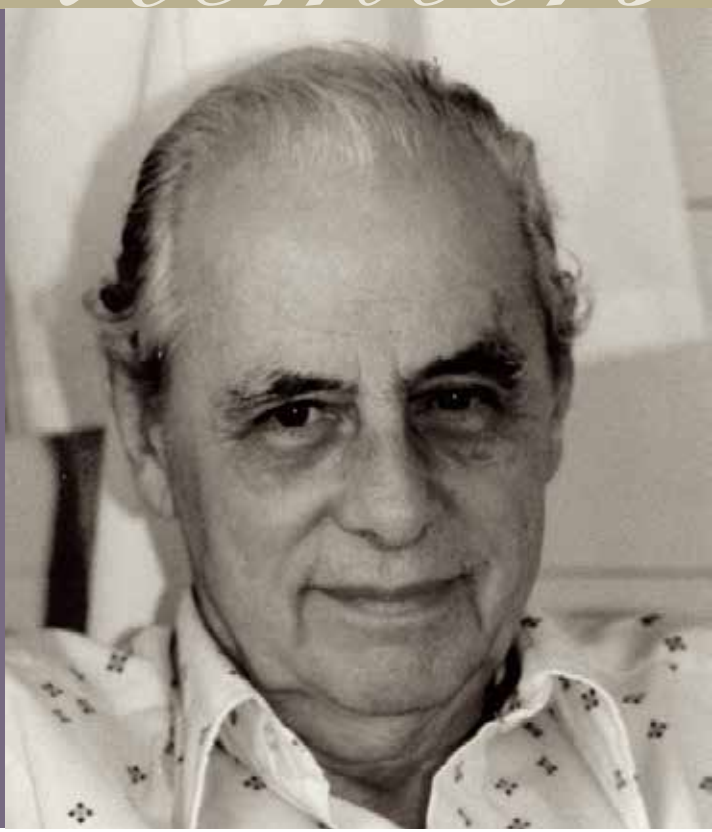
G. Gilbert Ashwell

1916–2014

BIOGRAPHICAL *Memoirs*

A Biographical Memoir by
John A. Hanover
and William B. Jakoby

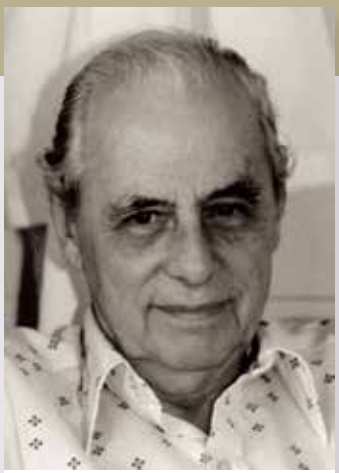


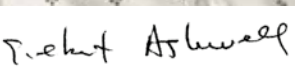
NATIONAL ACADEMY OF SCIENCES

G. GILBERT **ASHWELL**

July 16, 1916–June 27, 2014

Elected to the NAS, 1979





By John A. Hanover and William B. Jakoby

Through experiments that provided an early clue to understanding cell-ligand recognition, biochemist G. Gilbert Ashwell advanced the field that would become known as glycobiology—the study of saccharides, or sugars, which are a key component of all life forms. Ashwell's research ranged from carbohydrate chemistry to metabolism and enzymology. His milestone work included, with Anatol Morell, identification of probably the first known receptor and, with Toshisuke Kawasaki, discovery of an avian hepatic binding protein—both findings becoming vital to subsequent biological and medical advances.

Ashwell earned a B.S. in chemistry in 1938 and an M.S. in 1941 from the University of Illinois. He worked for the Merck chemical company until 1944, then took an M.D. degree at Columbia University in 1948 but never practiced medicine, instead beginning a lifelong career in research, first in the Columbia laboratory of Zacharias Dische. Joining the Public Health Service in 1950, he began work at the NIH's National Institute of Arthritis and Metabolic Diseases, where he spent the rest of his professional life. He became chief of the Institute's Laboratory of Biochemistry and Metabolism in 1967, a position he held until being named the NIH's first Institute Scholar in 1984.

Born to John and Alice Clarke Ashwell on July 16, 1916, in Jersey City, New Jersey, George Gilbert "Gil" Ashwell came of college age during the Depression and was admitted to Worcester Polytechnic Institute on a scholarship. He was attracted by the chemistry courses at this engineering school but found that his interests were far broader. He wanted greater exposure to the liberal arts, and he made the track team, starring in the 440-yard race. Transferring to the University of Illinois at Urbana-Champaign, he graduated in 1938 with a B.S. in chemistry and appears to have minored in philosophy.

That interest in philosophy led to his arranging for further study at the Sorbonne. It did not, however quite work out that way, as during the ocean voyage he met and fell for Edna Fleischmann, a fellow American passenger and graduate of Hunter College who was returning to Europe for another year as a medical student in Vienna. Gil changed his plan and enrolled in Vienna. That worked out fairly well until November, when Edna was no longer welcome at the university. They both left for Switzerland on the day of Kristallnacht. Edna continued her medical education in Basel, and Gil studied philosophy there at Erasmus University. Edna did get her degree, and in 1941, just before the United States entered World War II, they returned home.

[Gil] did have the distinction of being involved in the first clinical (and successful) use of penicillin for the treatment of a patient in the United States.

He began work as a chemist at the Merck research laboratories, assigned to the isolation of penicillin. At this stage of his career he was not a major participant in this complex project, but he did have the distinction of being involved in the first clinical (and successful) use of penicillin for the treatment of a patient in the United States. He lyophilized the small amount of penicillin that was available and arranged for it to be sent to the Grace-New Haven Hospital for the treatment of a severely ill patient. Of interest is that one of the three interns involved in administering the new drug was Herbert Tabor. Tabor also collected the patient's urine and sent it back to Merck, where Gil reclaimed the precious antibiotic that had been excreted. Gil and Herb, subsequently long-term colleagues at the National Institutes of Health (NIH) in Bethesda, Maryland, did not meet on that occasion, nor did they even know of the coincidence until they had a brief chat in a Bethesda grocery store four decades later.

Gil was encouraged by Merck to obtain additional training and served as a graduate assistant in organic chemistry for a year at Harvard. Then he was back at Merck, working on the isolation of streptomycin until 1944, when he enrolled in the Columbia University College of Physicians and Surgeons, graduating with an M.D. in 1948. He never interned, however, whereas Edna had a long career as a pediatrician for the Health Department of the District of Columbia.

Gil, instead, followed his interest in research by spending time in the laboratory of Zacharias Dische in Columbia's Biochemistry Department, and upon graduation accepted an appointment as a research assistant with him. Dische was interested in the metabolism

of carbohydrates, particularly focusing at that time on the Pasteur effect and the patterns of glycolysis and respiration. Dische's laboratory contributed significantly to developing analytical methods for carbohydrates.

Gil's initial effort was to study the effect of intracellular ions on glucose metabolism in nucleated red blood cells (Ashwell and Dische, 1950). That led to hands-on knowledge of the methods for analyzing carbohydrates, a number of which had been devised by Dische. Interest in and development of carbohydrate analysis became a constant throughout his work.

Gil met the obligation for universal military service by accepting a commission in the Public Health Service at the NIH, in Bethesda, where he remained throughout his career. The impact of atomic energy was being vigorously explored when he arrived at the NIH in 1950 because of the far-reaching health effects of radiation that had become evident after the use of nuclear weapons against Japan late in the war. This was where he started, but within weeks after his arrival in Bethesda Gil was hospitalized, at the age of 34, with a myocardial infarction. After a short convalescence, he initiated a rich scientific program that lasted until his death on June 27, 2014. His first publication from the NIAMD presented preliminary work using extracts of a radio sensitive tissue, mouse spleen, in which he examined the effects of glucose utilization under aerobic and anaerobic metabolism following X-radiation (Ashwell and Hickman, 1952). It also began his collaboration with Jean Hickman, who was initially a laboratory technician. Gil's background in Dische's laboratory made him a natural choice for investigating the metabolism of sugars, and that was how he began.

Carbohydrate enzymology

Gil's study of sugar metabolism using spleen extracts led to his demonstrating the accumulation of xylulose 5-phosphate from ribose 5-phosphate (Ashwell and Hickman, 1954) Accompanying the careful chemical identification of the product, a characteristic of his work, was his first attempt to at least partially purify and characterize an enzyme, which was the epimerase catalyzing the interconversion.

The importance of these findings lay in their timeliness. In 1955 the pentoses were central to active research on the alternative oxidative pathway of glucose utilization by which glucose 6-phosphate is oxidized to CO_2, resulting in the harvest of a large energy yield in nicotinamide adenine dinucleotide phosphate (NADPH). Groups led

by Ephraim Racker at the New York Public Health Research Institute and Bernard Horecker at the NIH were actively attempting to clarify the cyclic set of reactions, the "pentose phosphate shunt," that takes an intermediate of glucose metabolism, ribulose 5-phosphate, and produces carbohydrate structures bearing 3-, 4-, 5-, 6-, and 7-carbons. Gil's observations of the epimerization of the two pentose phosphates produced the vital findings that enabled researchers active in the field to prove xylulose 5-phosphate to be the substrate of transketolase, an enzyme, along with transaldolase, which is a key feature of this important metabolic cycle (Srere, et al., 1955; Horecker, et al., 1956).

One pentose led to another. Combining newly acquired skill in handling enzymes with his background in sugar chemistry, Gil chose to attack the several problems involving pentose synthesis, which in turn led him to the enzymes participating in the degradative mechanism of other sugars, including the uronic acids. Both L-xylulose, the sugar excreted by people with pentosuria, and the vitamin ascorbic acid were known to arise through oxidation of glucose to D-glucuronic acid. For L-xylulose formation, the next steps included oxidation to L-gulonic acid before eventual loss of CO_2 to form L-xylulose. Although beta-3-keto-L-gulonic acid had been postulated as an intermediate prior to decarboxylation, it had not been established as such. Working with Julian Kanfer and John Burns (Burns, et al., 1959), Gil settled the fact of this labile intermediate not only through isolation and chemical characterization of the compound, but also by characterizing a partially purified hog kidney NADP-linked dehydrogenase that catalyzed its production.

Interestingly, the specificity of this enzyme was sufficiently broad as to require its listing as a generic ß-L-hydroxy acid dehydrogenase, since it accommodates all of the hexonic, pentonic, and tetronic acids with a levo configuration at the ß-carbon—and that included acetoacetate (Smiley and Ashwell, 1961). Gil and his colleagues answered previous questions assuming the spontaneous decarboxylation of ß-3-keto-L-gulonate by identifying the decarboxylase that catalyzes its conversion to L-xylulose in mammalian kidney (Ashwell, et al., 1961), as well as by that enzyme's partial purification from mammalian liver. Once the 3-ketogulonate was available, it could be evaluated as a precursor of ascorbate. It is not, however, a direct precursor. Rather, L-gulonate is converted to L-gulonolactone, which is oxidized to 2-keto-L-gulonic acid before decarboxylation to ascorbic acid (Smiley and Ashwell, 1961). One catabolic fate of ascorbate was delineated in vitro as leading to the formation of two pentanoic acids, L-lyxonic and L-xylonic acids.

In Gil's laboratory the enzymatic aspects of uronic acid metabolism in bacteria were a particularly active area for research at this time (Ashwell, et al., 1960). Among these were the analysis and isolation of enzymes catalyzing the metabolism of the alginic acids (Preiss and Ashwell, 1962)—polymers of blocks of ß-D-mannuronate and of its C-5-epimer, ß-L-glucuronate. The organism, a pseudomonad isolated by the enrichment culture technique, metabolized alginic acid into a series of unsaturated oligosaccharides, resulting in a monosaccharide identified as ketodeoxygluconaldehyde (4-deoxy-L-*erythro*-5-hexoseulos uronic acid). With *Escherichia coli* grown on polygalacturonic acids, enzymes were isolated that converted both D-galacturonic and D-glucuronic acids to 2-keto-4-deoxy glucuronic acid, the compound subsequently phosphorylated before being metabolized to pyruvate and triosephosphate (Preiss and Ashwell, 1963). A good portion of the basis of the field of bacterial uronic acid metabolism on an enzymatic level is represented by this work by Gil and colleagues. Typically, it was characterized by attention not only to the enzymology but also to basic carbohydrate chemistry in identification of products.

During this period, and particularly with Othmar Gabriel and W. A. Volk, Gil isolated a number of novel sugar nucleotides and began an investigation of the biological mechanisms involved in the formation of deoxysugars. This was the groundwork for what were to become Gil's major scientific accomplishments.

Glycobiology

In planning for a sabbatical leave in 1966, Gil wanted to gain laboratory experience in immunological methods and arranged to spend six months with Elwin Kabat at Columbia University. Although he would be without his family, who would remain behind in Bethesda, he had close friends in Manhattan. Helena Morell, by then a practicing psychotherapist, had been a "dishwasher" in Dische's laboratory when Gil was a post-doc; her husband, Anatol, was presently working in Herbert Scheinberg's laboratory at the Albert Einstein College of Medicine. The Morells were family friends from the old days, and Gil often had dinner at their home during this sabbatical period. Anatol was working with the copper-binding protein ceruloplasmin, mutation of which leads to Wilson's disease, an autosomal recessive disorder characterized by the deposition of copper in many organs.

Dinner conversations included the question of how long this protein remains in the circulation and how it was degraded. Jim Jamieson had shown that ceruloplasmin was a glycoprotein with sialic acid as the terminal sugar on an oligosaccharide chain and with

galactose in the penultimate position. Gil's background led him to consider methods for labeling the galactose with a radioactive isotope so as to follow the metabolism of the protein. Since this was not yet the day in which radioactive galactose could be purchased and applied, the approach required the use of a sialidase to remove the terminal sialic acid; after this step came oxidation of the then-exposed galactose residue of asialoceruloplasmin with galactose oxidase; this was followed by reduction of the oxidized galactose with tritiated borohydride; finally came enzymatic restitution of the terminal sialic acid. The result was a ceruloplasmin that had its penultimate sugar, galactose, radioactively labeled so that the metabolic fate of the glycoprotein could be followed.

Injected into rabbits, the labeled ceruloplasmin remained in the circulation for days. However, upon removal of the terminal sialic acid, thereby leaving galactose as the new terminal sugar, the resultant asialoceruloplasmin was cleared from the circulation within minutes and could be found in the parenchymal cells of the liver. Furthermore, the terminal galactose was shown to be the specific sugar necessary for this recognition, and thus began a new chapter in what is now known as glycobiology.

The initial observations of Gil and his friend Anatol Morell were reported in a series of papers focused on asialoceruloplasmin (Morell, et al., 1966; Morell, et al., 1968; Hickman, et al., 1970; Van Den Hamer, et al., 1970) and extended to studies on follicle-stimulating hormones (FSH) (Vaitukaitis, et al., 1971b) and human chorionic gonadotropin (hCG) (Vaitukaitis, et al., 1971a; Van Hall, et al., 1971b; Van Hall, et al., 1971a).

The recognition that a system existed by which desialylated serum glycoproteins were removed from the circulation called for investigation of the clearance mechanism, and Gil turned to biochemical tools to define that mechanism. His group developed membrane-binding assays that conclusively demonstrated the involvement of a binding protein (Pricer and Ashwell , 1971; Van Lenten and Ashwell, 1972).

The next chapter in the evolving story of the hepatic receptor came from work Gil carried out with Toshisuke Kawasaki. Here the receptor itself was shown to be a glycoprotein of defined structure (Kawasaki and Ashwell, 1976), one that could be inactivated and reactivated by manipulation of its own sialic acid residues (Paulson, et al., 1977). These studies also led to the identification and properties of both the mammalian and avian receptor (Ashwell and Kawasaki, 1978).

The role of the receptor in cell physiology (Pricer and Ashwell, 1976; Novogrodsky and Ashwell, 1977; Hubbard, et al., 1979) was approached by producing antibodies against the purified receptor, thereby allowing its subcellular membrane topology and turnover to be evaluated (Tanabe, et al., 1979) and the intracellular organelles involved in its endocytic uptake and catabolism of the serum glycoproteins to be defined. Gil's work in this area triggered a more general interest in cell surface receptors and their endocytic properties, which he carried out in collaboration with Richard Klausner, Joe Harford, Clifford Steer, and John Hanover. Although Gil continued his efforts on the hepatic lectin with Morell, he also began to explore the biochemical properties of other receptor systems, such as that for transferrin, and the mechanisms of iron mobilization (Klausner, et al., 1983).

The development of anion-exchange chromatographic systems that used pulsed amperometric detection for selective identification of saccharides proved vital in Gil's collaboration with Evelyn Grollman, Peretz Weiss, and William Gahl (Konig, et al., 1988; Konig, et al., 1989; Forsythe, et al., 2006; Ghosh, et al. 2014).

Gil's work on the hepatic lectin came full circle with his involvement in the development of mice lacking each of the subunits of the receptor—called knockout mice—(Braun, et al., 1996; Soukharev, et al., 2000). Carried out with Joachim Herz, John Hanover, and Brian Sauer, this research provided a tool by which the physiological role of the receptor might finally be approached. These knockout mice have been widely used since their introduction and have enabled researchers to gain an increasing understanding of the role of the receptor in mammalian physiology.

Recent findings suggest a role for the "Ashwell-Morell" receptor in regulating coagulation in sepsis (Grewal, et al., 2008; Grewal, et al., 2013). The receptor is involved in the normal clearance and maintenance of platelets (Rumjantseva, et al., 2009; Rumjantseva and Hoffmeister, 2010; Grozovsky, et al. 2010; Grozovsky, et al., 2015); uncontrolled platelet coagulopathy can lead to rapid death. Thus, the Ashwell-Morell receptor is now known to play a key role in mitigating this pathological coagulopathy and in maintaining normal platelet levels. Gil displayed his enthusiasm about these new research approaches in an interview he granted that in association with some of the early findings (2008).

Career

Throughout several administrative iterations, Gil was always associated with what is now the National Institute of Diabetes and Digestive and Kidney Diseases of the NIH. In 1959 he became head of the Section on Enzymes of the Laboratory of Biochemistry and Metabolism; he succeeded to the post of chief of the laboratory in 1967. During most of the early period Jean Hickman was his assistant; she was later able to successfully do her dissertation work for the Ph.D.—from Georgetown University—with Gil. Although he had only one other graduate student throughout his career, Joan Lunney (Johns Hopkins), there usually were two or three postdoctoral fellows in the laboratory. Only a few are named here, although the references indicate the participation of many of the others.

Despite performing departmental duties with his usual perception and effectiveness, Gil was never an enthusiastic administrator. In addition to the honor, he felt relieved when in 1984 he was promoted to the newly created designation of Institute Scholar, a post that left him free to "work at the bench."

Gil's career as an investigator was also recognized by his election to the National Academy of Sciences (1979), as well as by his receipt of the Gairdner Foundation Award (1982) for outstanding contributions to medical sciences, the Merck Prize (1984) of the American Society of Biological Chemistry, an honorary doctorate from the University of Paris (1988), the Alexander von Humboldt Foundation Senior Scientist Award (1989), and the Karl Meyer Award (with Saul Roseman) of the Society for Glycobiology (1993).

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