

XI

A HISTORY OF
THE ROCKEFELLER
INSTITUTE

1901 - 1953

ORIGINS AND GROWTH

George W. Corner

THE ROCKEFELLER INSTITUTE PRESS

NEW YORK CITY · 1964

comments on Avery

protein nature, available in pure form through the work of Kunitz in Princeton. Because the resulting values checked only roughly with those obtained by other methods, he put much effort into improvements of the centrifuge mechanism, intended to regulate its speed and temperature. It was at the time the only instrument in the world equipped to operate at zero centigrade. With it Rothen measured the molecular weights of a number of biologically important proteins, notably the well-defined crystalline enzyme ribonuclease which he studied in 1940. At that time protein chemists accepted a hypothesis of T. Svedberg of Uppsala that the giant molecules of the heaviest proteins are made up of "building stones" of uniform molecular weight, at first assumed to be about 35,000, then 17,000. When Rothen found the molecular weight of ribonuclease to be about 13,000, a figure which did not fit the hypothesis, Svedberg's assumption was rendered improbable.

Summarizing in 1943 his results and those of others, Rothen remarked that although the ultracentrifuge had been of great value in establishing the fact that proteins and related substances have definite molecular weights, it still did not measure these weights to a higher accuracy than about 10 per cent. Wishing to check his own method on a stable, reproducible protein, he chose apoferritin (the iron-free fraction of heme discovered by Granick). His values of the molecular weights of apoferritin from man, horse, and dog agreed with each other within a range of possible error of little more than 1 per cent. The residual error appeared to result from variations or uncertain measurement of the temperature of the rotor, which at a speed of 60,000 or more revolutions per minute was difficult to control. Even this degree of precision, however, served an important purpose. In 1944 Rothen was asked to test a nucleic acid having a molecular weight of about 500,000, which, as Avery and his associates had discovered (Chapter 18), possessed the power of transforming one type of pneumococcus into another. Rothen found that the transforming property accompanied the nucleic acid as it was sedimented in the centrifuge; in other words, the nucleic acid was itself the active agent in bacterial transformation, not some hypothetical contaminant.

The sedimentation rate of a protein, for which still greater precision of the ultracentrifuge was urgently needed, is not the only quantitative measurement required for calculating molecular weight. Two other fac-

CHAPTER EIGHTEEN

The Hospital, 1935-1953

Bacterial transformations; virus diseases; virus pneumonias; biology of viruses. Rheumatic fever; the anemias; muscular dystrophy; cirrhosis of the liver. Nephrosis and nephritis; fat metabolism; endocrine diseases. Trend toward basic investigations.

RUFUS COLE was still head of the Hospital of The Rockefeller Institute during the first two years of Gasser's directorship. When in 1937 Cole retired to a quiet life of scholarship in his country home, Thomas M. Rivers succeeded him as director of the hospital, retaining also the leadership of his own laboratory of virus research.¹ The next few years were to see major shifts in the personnel and interests of the hospital staff, as older members retired and their successors brought in new programs or new methods of attacking old problems. Cole's retirement, for example, was roughly coincident with a radical change in the Institute's study of acute respiratory diseases. Lobar pneumonia, to which he had devoted his own research career since the hospital opened in 1910, was waning in frequency and importance as a clinical problem. Oswald T. Avery still was leading the investigation of the chemistry of the pneumococcus, which he had begun long ago at Cole's suggestion; but he and his colleagues, studying the capsular polysaccharides, had gone far beyond the practical aim of finding means to control this one organism, and had built up a great structure of fundamental immunochemistry.

Among the incidental results of their comprehensive work was a discovery of considerable diagnostic value, concerning a peculiar protein that appears in the blood during the acute phase of certain infectious diseases. In 1930 W. S. Tillett and Thomas Francis, Jr., Cole's senior clinical associate on the hospital staff, observed that blood serum from a patient suffering with lobar pneumonia in the acute stage contains a substance which forms a precipitate with a dilute solution of one of the

complex sugars, designated C-polysaccharide, found by Avery and his co-workers in the cell body of the pneumococcus. A few years later, Avery and T. J. Abernethy found the unknown substance to be a protein. With Colin M. MacLeod of the hospital's resident staff, Avery then isolated it in a relatively pure state, so that its immunological properties could be studied. In 1947 Maclyn McCarty, National Research Council Fellow (later a Member of The Rockefeller Institute), succeeded in crystallizing the C-reactive protein; and in 1954 H. F. Wood, McCarty, and R. J. Slater published evidence that it may be a β -globulin. The C-reactive protein has been found useful as an index of the progress of certain acute bacterial infections, especially rheumatic fever and tuberculosis; in tuberculosis its disappearance from the blood is a good indication that the infection has become inactive.

Avery retired in 1943, ceasing to work in the laboratory three or four years later.² His last years of service were marked by a great discovery that was to link together some of the basic phenomena of immunity and heredity. The story of the transforming factor begins in 1928, when a British pathologist, Fred Griffith, reported that when he inoculated mice with a mixture of a harmless strain of living pneumococci and the dead remains of a virulent strain, the mice, to his astonishment, died from infection with live organisms of the virulent type. Since he could not believe that the killed bacteria had come to life, he had to assume that something in their dead bodies had transformed the living harmless strain into the virulent one. This discovery naturally excited Avery's interest, because he and an assistant, Martin H. Dawson, had observed similar changes from non-virulence to virulence in a strain of pneumococci, induced by passage through animals or by growth on certain culture media. He therefore asked Dawson to look into Griffith's transformation. Dawson confirmed the finding; and in 1931, working at Columbia University, he and a visitor from Peking Union Medical College, Richard H. P. Sia, succeeded in causing dead pneumococci to transform living organisms, as in Griffith's experiment, but in laboratory glassware instead of in a mouse. In 1932 J. L. Alloway of Avery's group carried the feat a step further by using as transforming agent, not whole dead cells, but a cell-free extract made from them. Evidently the transforming agent was a chemical substance.

Avery himself now entered the investigation, working with MacLeod

and, later, with McCarty. Growing large amounts of the virulent Type III pneumococcus, the investigators extracted and systematically broke apart the chemical constituents of the organisms, testing the transforming power of each fraction, until in 1944 they arrived at a practically pure substance possessing the transforming power in very high concentration. This proved, surprisingly, to be a nucleic acid of a type which Levene and Jacobs had first identified years before at The Rockefeller Institute. It was deoxyribonucleic acid (DNA).

Because this substance was obtained by conventional methods of chemical analysis, Avery and McCarty could not exclude the possibility that its transforming action might be due to a small amount of protein contaminant. They knew that an enzyme, DNase, would destroy the DNA without affecting the proteins, and McCarty, venturing into the difficult field of enzyme chemistry, succeeded in preparing from beef pancreas a quantity of DNase good enough for preliminary experiments. Treated with this, the substance lost its transforming power. To clinch the matter, the Institute's great expert on the purification of enzymes, Kunitz, in 1950 prepared a highly purified crystalline DNase which confirmed McCarty's earlier results. The demonstration that a nucleic acid was the effective agent in inducing a heritable change in a living organism was unexpected, since nucleic acids had generally been thought to be chemically undifferentiated and rather inert, biologically; the general traditions of physiological chemistry, moreover, suggested that any such effect could be exerted only by proteins. Since that time, however, many investigators (among them Alfred E. Mirsky, whose work has been discussed in Chapter 14) have shown that DNA exists in chromosomes of higher animals and is a constant and characteristic ingredient of the genes. Thus the work of Avery's group on bacterial transformation points to a striking similarity of the chemical mechanism of heredity throughout the biological scale from bacteria to mammals.³

The more clinical and practical part of Cole's program, including the study of lobar pneumonia at the bedside, the investigation of immunity to the various types of pneumococci, and the effort to improve the antisera against them, was in the hands of a group of young men, including Thomas Francis, Jr. (later professor at the University of Michigan and a leader in poliomyelitis control), Kenneth Goodner (now professor at Jefferson Medical College), Theodore J. Abernethy of Wash-

similar nature, including bacterial hypersensitivity and allergic nephritis. When the antibiotic drugs became available, he promptly tried them, studying sulfanilamide with Smadel in 1938-1939 and penicillin with R. F. Watson and Sidney Rothbard in 1944. These workers were among the first to find that certain antibiotics were capable of destroying hemolytic streptococci in infected tissues and body cavities.

After 1946, when Swift retired from active direction of the group, until his death in 1953, he and G. E. Murphy attempted to produce rheumatic fever in animals. They came nearer, perhaps, to this goal than previous experimenters had come. By repeatedly inoculating the skin of rabbits with hemolytic streptococci, they produced localized heart lesions that resembled the "Aschoff bodies" characteristically found in the heart muscle of patients who die of heart failure following rheumatic fever.

Maclyn McCarty, who succeeded Swift as leader of the investigation, had come to the hospital staff in 1941, a few years after taking his medical degree at Johns Hopkins. As a member, at first, of Avery's group, he had taken part in the investigations, described earlier in this chapter, on the "transforming factor" of the pneumococcus, and on the "C-reactive protein." Later, with H. C. Anderson and H. F. Wood, he showed that the appearance of C-reactive protein in acute rheumatic fever can be used as a measure of the activity of the disease process. Anderson and McCarty, moreover, using a special form of C-polysaccharide from pneumococci as a test reagent, found that rabbits inoculated with an acute infectious disease, or with active bacterial products, such as typhoid vaccine, develop in their serum an acute-phase protein completely analogous to the C-reactive protein in man. This observation provides a laboratory model of the phenomenon, laying the groundwork for subsequent investigations. McCarty and his associates also investigated numerous other biological and immunochemical products of streptococci, trying to discover substances that might help to explain the disease picture in rheumatic fever or to suggest methods of treatment and prevention. S. D. Elliott of London, following up experiments begun while a visiting Fellow at The Rockefeller Institute, succeeded in purifying both a protein-splitting enzyme and its inactive precursor, the first crystalline products to be obtained from hemolytic streptococci; and he inves-

CHAPTER NINETEEN

The Chemistry of Heredity, Virulence, and Immunity; Antibiotics

Outcome of Avery's study of the pneumococcus; the polysaccharide antigens of pneumonia and dysentery organisms; antibiotics from soil bacilli; induced enzymes; experimental tuberculosis, improved culture methods; chemistry of bacterial transformation, relation of transforming factors to genes. Administrative personnel of the Institute; growth of the staff. Increasing endowment.

RUFUS COLE built better than he knew when in 1910 he began the study of immunity in lobar pneumonia. Few investigations undertaken at The Rockefeller Institute have been pursued continuously for so long a time, or with such fruitful results. We have seen how the effort to produce serums against pneumonia led to intensive investigation of the chemistry of the pneumococcus by Avery and his associates. This in turn opened up unforeseen lines of research in the chemistry of immunity and of heredity, which a half century later are still being followed at the Institute. Our narrative of the hospital's scientific achievements, therefore, comes to a most fitting conclusion with the work of Goebel, Dubos, and Hotchkiss, begun in association with Avery and carried on independently after his retirement.

Walther F. Goebel, who took his Ph.D. degree in chemistry at the University of Illinois in 1923, came to The Rockefeller Institute the next year as an assistant in Avery's laboratory. Joining Avery and Heidelberger in their study of the specific antigens in the capsular polysaccharides of the pneumococcus, Goebel shared in the discovery (discussed in Chapter 10) that these are complex sugarlike substances, which in their native state are probably combined with the proteins of the bacterial

cell. Later he carried the analysis further by splitting the huge polysaccharide molecule into its component simpler sugars and identifying them. Subsequently, he found that simple sugars could be experimentally linked with proteins to form antigenic compounds; that is, an animal injected with such a compound would develop antibodies against it. This led to the establishment of an important principle: the immunological specificity of a carbohydrate depends upon its precise molecular structure. Following this up, Avery and Goebel were able, by combining ordinary albumin with the specific polysaccharide extracted from the pneumococcus, to create an artificial antigen that would elicit antibodies not only against itself, but also against virulent pneumococci.

This is the stage the investigation had reached in 1929. Obviously, the next step in the effort to understand nature's chemical processes by imitating them in the laboratory was to replace the specific polysaccharides of the pneumococcus by counterparts synthetically produced. Goebel therefore began a thorough study of the type-specific capsular carbohydrates of the pneumococcus and also of another well-known organism, the Friedländer bacillus. He found that several of these substances contain glucuronic acid, and suspected that this sugar may be important in determining their immunological specificity.

In this study he had Avery's cooperation in immunological matters, and the assistance, from 1929 to 1935, of Frank H. Babers, a biochemist. When Babers left for a post elsewhere, Rollin D. Hotchkiss joined the group, synthesizing various new compounds for use in the experiments. By 1935 Goebel had acquired a masterly command of the chemistry of these substances, and was ready to prepare artificial antigens containing a wide range of sugars of advancing complexity. Within a year the search was getting warmer; an artificial antigen containing a glucoside of glucuronic acid, prepared in the laboratory, reacted with the serum of horses immunized against virulent pneumococci. With one more step — the introduction of a more complex carbohydrate, cellobiuronic acid — the goal was achieved; Goebel had created, with a synthetic sugar derivative, an artificial antigen so close to that formed by living pneumococci that when injected into rabbits it protected them against infection with the highly virulent Type III organisms.

This triumph of immunochemical skill and insight gave final proof of the concept, developed by The Rockefeller Institute's workers during

popular articles, Dubos has been a lucid expositor of modern bacteriology and a critical judge of its influence upon the public health.

ROLLIN D. HOTCHKISS came to The Rockefeller Institute as a Fellow in Avery's group, immediately after taking his Ph.D. degree in chemistry at Yale. Like Avery's two other chief associates in his later years, Hotchkiss was to go on through all ranks of the Institute. In 1935-1936 he worked with Goebel, taking an active part in the program of synthesizing saccharides in order to create artificial antigens akin to the natural polysaccharides responsible for type specificity of the various strains of the pneumococcus. He and Goebel achieved the synthesis of several key derivatives of glucuronic acid, especially aldobionic acid, one of the disaccharide carbohydrates formed by the virulent Type III pneumococcus. In 1937-1938 Hotchkiss went to the Carlsberg Laboratory in Copenhagen to investigate with K. U. Linderstrøm-Lang the hydrolysis of peptide bonds in proteins.

Returning to The Rockefeller Institute, he joined Dubos, who in 1938 was beginning his search for antibacterial biological agents. The part Hotchkiss took in this influential investigation, by applying his biochemical experience and skill to the extraction and purification of tyrothricin, tyrocidin, and gramicidin, has already been recounted. Some years later, Hotchkiss also collaborated with Albert Claude, of J. B. Murphy's cancer research laboratory, in the investigation, described in Chapter 15, by which the mitochondrial granules present in all cells were proved to contain important oxidative enzymes. G. H. Hogeboom, whose cooperation Hotchkiss soon enlisted, carried on these studies to most fruitful conclusions.

In Chapter 18 we recounted the story — now a classic of genetic biochemistry — of Avery and McCarty's discovery of the transforming factor, which has the power when taken up by pneumococci of various types to convert them into the virulent Type III. Following this pioneer work, other investigators elsewhere looked for additional capsular transforming agents, and within a decade two dozen or more transformations of antigenic and other biological characteristics of bacteria were discovered. In each case the transforming agent was identified as deoxyribonucleic acid (DNA). The molecular structure of this substance, as we now know, is so complex that it can exist in an enormous number of

of Vincent Dole, studied primary atypical pneumonia. Part of Horsfall's work on interference between viruses was carried on while he was leading the Naval Research Unit. Lewis Thomas and various colleagues studied the viruses of cat and mouse pneumonias. Most of the work of Swift, Lancefield, Watson, Dole, A. T. Wilson, Rothbard, and their associates on streptococci and streptococcal diseases has also been described earlier. Lancefield, as a practical service to the medical profession, had for many years supervised the manufacture of sera for grouping and typing streptococci; demands from the armed forces now made this a very heavy task. In 1943-1945 Wilson directed, at the Naval Medical Center, Bethesda, Maryland, a laboratory for the study and identification of streptococci isolated from patients in Navy hospitals, in close collaboration with Lancefield, through his association with the Institute. The wartime work of Hoagland, Gilder, Shank, and their associates on epidemic hepatitis, recounted in Chapter 18, led to general studies on liver disease, which became a continuing interest of Labby, Henry Kunkel, and Ahrens. Because Maclyn McCarty was one of the Navy contingent, some of the work Avery and he did on the transforming factor of the pneumococcus was credited to the Navy.

In mid-1943 the high command of the Navy Medical Corps, already concerned about the prevalence of scrub typhus, hepatitis, and other infectious diseases in Navy personnel in the Pacific, foresaw that impending campaigns would expose our forces to a great variety of tropical diseases, many of them not well understood, or at least unfamiliar to the Medical Corps. Rivers, promoted to Captain, was therefore sent on an extensive tour of the South Pacific islands, to determine the best location for an advance base as near as possible to the fighting lines, from which research teams could investigate diseases occurring in any part of the area. This was a novel enterprise for the armed forces. Both the Army and the Navy had on occasion sent out commissions to study specific medical problems in the field, but this was the first time that either service had attempted to organize a fully equipped research laboratory in a forward area. On the basis of Rivers's report, Guadalcanal was at first considered as the location of the unit, but the fighting front advanced so rapidly that Guam was finally chosen.

U.S. Naval Medical Research Unit No. 2 was commissioned at the Institute in June 1944, under the command of Captain Rivers. As nu-

(Notes on Chapter Twenty, continued)

casional Papers by Faculty and Friends of The Rockefeller Institute, No. 4, New York, Rockefeller Institute Press (not dated).

- 9 Nicholas Murray Butler, "Scientific research and material progress," *The Rockefeller Institute for Medical Research: Description of the Buildings; Addresses Delivered at the Opening of the Laboratories in New York City, May 11, 1906*, privately printed, 1907.
- 10 *New York Times* editorial, June 29, 1953.
- 11 The following list of investigations made at The Rockefeller Institute and deemed historically significant is compiled with as little verbal modification as possible from Arturo Castiglioni, *A History of Medicine*, translated from the Italian and edited by Edward B. Krumhaar, New York, Alfred A. Knopf, 1947; and Ralph H. Major, *A History of Medicine*, 2 vols., Springfield, Illinois, C. C. Thomas, 1954. The choice is strictly that of the above-named authors; their selection does not cover all fields of the Institute's work, and the citations, having been removed from context, are not all adequately indicative of the respective investigations. The list is presented merely to amplify statements made in the text about external estimates of the Institute's work, and should not be quoted as a balanced assessment of its most important researches.

John Auer: recognition of bronchospasm as cause of death in experimental allergy in the guinea pig. *Oswald T. Avery*: identification of polysaccharide antigens (with *A. Raymond Dochez* and others); recognition of the "transforming principle." *Alexis Carrel*: Carrel-Dakin treatment of infected wounds; development of tissue culture. *René Dubos*: discovery of gramicidin. *Francisco Duran-Reynals*: recognition of the "spreading factor." *Albert H. Ebeling* (with *Anne Carrel*): a successful experimental corneal graft. *Simon Flexner*: serum treatment of epidemic cerebrospinal meningitis; transmission of poliomyelitis to monkeys. *Christian A. Herter*: description of infantilism associated with intestinal disease. *Moses Kunitz*: crystallization of trypsin and chymotrypsin. *Rebecca C. Lancefield*: classification of streptococci. *Karl Landsteiner* and *Alexander S. Wiener*: discovery of the Rh factor. *Phoebus A. T. Levene*: studies on the chemistry of proteins. *Jacques Loeb*: studies on the colloidal behavior of proteins; application of the Donnan equilibrium to living tissues; electrolyte balance in tissue fluids. *Duncan A. MacInnes* and *Lewis G. Longworth*: advancing the knowledge of proteins through ultracentrifugation and electrophoresis. *Samuel J. Meltzer*: introduction of intratracheal anesthesia. *Leonor Michaelis*: study of reversible oxidation-reduction systems; (with *C. V. Smythe*) measurement of potentials in systems of biological interest. *Hideyo Noguchi*: demonstration of spirochete of syphilis in brains of paretics; proof of the relation of Oroya fever to verruca peruviana and cultivation of the causative organism; luetin test. *John H. Northrop*: crystallization of pepsin. *Peter K. Olitsky*: cultivation of *Bacterium (Dialister) pneumosintes*. *Louise Pearce*: contributions to experimental medicine (should read: discovery of a cure for African sleeping sickness) (with *Wade H. Brown*, *M. Heidelberger*, and *Walter A. Jacobs*). *Peyton Rous*: production of sarcoma by a virus; storage of blood for transfusion (with *J. R. Turner, Jr.*, and *Oswald H. Robert-*