Helen M. Ranney

1920–2010

BIOGRAPHICAL *Memoirs*

A Biographical Memoir by
H. Franklin Bunn

NATIONAL ACADEMY OF SCIENCES

HELEN MARGARET **RANNEY**

April 12, 1920–April 5, 2010

Elected to the NAS, 1973

During the latter half of the twentieth century, Helen Ranney was, by any objective criteria, one of America's most distinguished physician scientists. Throughout a long career, her pioneering research on hemoglobin and disorders thereof was accompanied by continuous leadership in academic medicine. She was the first female chair of a department of medicine in the United States and the first female president of both the Association of American Physicians and the American Society of Hematology. She was elected to the National Academy of Sciences and to the Institute of Medicine in 1973.

By H. Franklin Bunn

1937–1960: Education and training at Columbia

Ranney was born and raised on a dairy farm in upper New York State, and in her view, she benefited greatly from early education in a one-room schoolhouse. Her upbringing imbued her with a strong sense of responsibility and with the importance of working with her own two hands, qualities that were key determinants of her later success in research.

Full appreciation of Ranney's early career entails a reckoning not only of her impressive accomplishments, but also of the obstacles she overcame. In 1941, she graduated from Barnard College, the women's affiliate of Columbia University, and hoped to enter Columbia's College of Physicians and Surgeons (P&S) but found that gender bias made access nearly impossible. Her initial application was turned down. While she bided her time working in a research laboratory, the United States entered World War II and suddenly P&S had openings for qualified women applicants. Ranney completed both her medical school and residency in internal medicine at Columbia. During this time she was strongly influenced by a cadre of brilliant biochemists who had fled Europe to

work at Columbia. She became enamored of their discipline. During her early scientific development, her most note-worthy role models included Reinhold and Ruth Benesch, Salome Gluecksohn-Waelsch, and Irving London.

After completing her fellowship in 1953, Ranney began to work on hemoglobin in what was to be a career-long inquiry spanning more than half a century. In 1949, Itano and Pauling (Pauling et al. 1949) reported the separation of normal hemoglobin (Hb A) and sickle hemoglobin (Hb S) by the cumbersome technique of moving boundary electrophoresis. At about the same time, James Neel (Neel 1949) provided conclusive evidence that Hb S was inherited in a Mendelian manner with symptomatic patients being homozygotes (SS disease) and asymptomatic parents and children of the patients being heterozygotes (AS or sickle trait). Ranney was among the first to apply the much more practical and accessible technique of paper electrophoresis to the separation of human hemoglobins in families with sickle cell disease and with other hemoglobin variants (Larson and Ranney 1953). She exploited this technique to study the mode of inheritance of hemoglobin C (Hb C), the second most commonly encountered human hemoglobin variant among African Americans. She was the first investigator to conclusively identify CC homozygotes (Ranney et al. 1953) and SC compound heterozygotes (Ranney 1954) [see illustration 2]. Of equal importance, she convincingly demonstrated that Hb S and Hb C were co-dominant alleles. This work antedated by five years the structural analyses showing that Hb S and C were mutants of human β-globin. An impressive proportion of this initial research was done by Ranney single-handedly.

1. Early portrait.

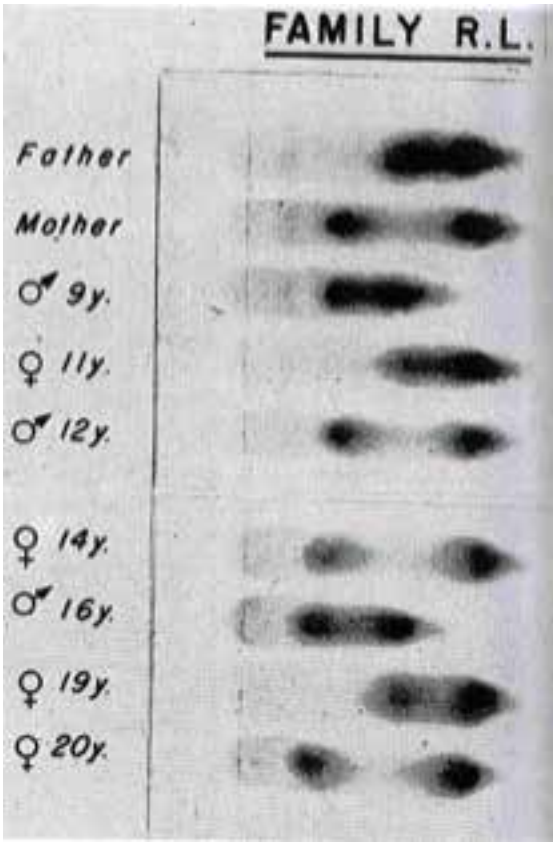
During this time, Gluecksohn-Waelsch was a leading experimental geneticist at Columbia and an important mentor to Ranney. Together they studied inbred strains of mice, each of which had a uniform pattern on hemoglobin electrophoresis: either a sharp single band or a diffuse smudge. Cross breeding experiments revealed that, like the human variants mentioned above, the “single” and the “diffuse” hemoglobins are co-dominant alleles (Gluecksohn-Waelsch et al. 1957; Ranney et al. 1960).

In those days, physiology trumped biochemistry as the fashionable discipline for clinical investigators. Despite a remarkable record of research contributions early in her career, this mind-set, and perhaps her gender, retarded Ranney’s entry into the American Society

for Clinical Investigation. Nevertheless, when finally elected, she was still among the first cadre of women to receive this accolade.

1960–1970: Albert Einstein College of Medicine

In 1960, Irving London left Columbia to become chairman of the department of medicine at the newly developing Albert Einstein College of Medicine. Helen Ranney was one of London's initial appointees. She became chief of the department's hematology division and soon thereafter founded a cross-disciplinary heredity clinic in Van Etten Hospital, part of the Bronx Municiple Hospital Center and the primary teaching hospital at Einstein. She was one of the first to appreciate how human genetics influenced so strongly and pervasively every area of clinical medicine. During her ten year tenure, Einstein became one of the nation's leading centers of research in hematology and genetics.



2. Paper electrophoresis of hemoglobin of a family showing the inheritance pattern of Hb SC disease. The spot at the far right is normal Hb A. The spot in the middle is Hb S and the spot in the left is Hb C. The father has sickle trait (AS). The mother has C trait (AC). Two of the seven children (9 year-old and 16 year-old boys) have SC disease.

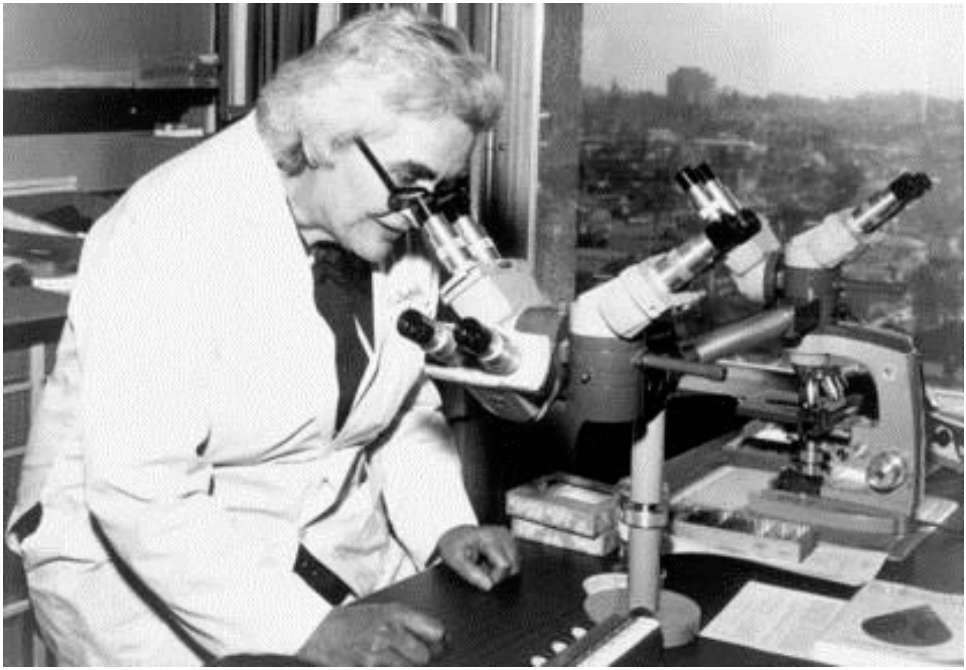
Ranney's initial research focused on the functional properties of isolated hemoglobin subunits. By that time others had shown that hemoglobin is a tetrameric protein composed of two pairs of subunits, α and β, that differ in primary structure. In collaboration with Ruth and Reinhold Benesch, Ranney discovered that the isolated β subunits of hemoglobin (Hb H) have high affinity for oxygen and lack cooperativity, thus differing markedly from native tetrameric human

hemoglobin (Benesch et al. 1961). Subsequently she and Robin Briehl found that isolated α subunits have very similar functional properties (Ranney et al. 1965). These early observations led to a decades-long inquiry into the mechanism underlying subunit cooperativity in hemoglobin.

During this period, Helen Ranney and Hermann Lehmann, who worked in Cambridge, England, were the leading investigators of genetic variants of human globin. It was clear that the predominant hemoglobin in red cells of the human fetus was Hb F, a tetramer composed of two pairs of α globin subunits and two pairs of γ globin subunits ($\alpha_2\gamma_2$). At the time of birth there is a gradual switch from γ globin to β globin resulting in the formation of adult Hb A ($\alpha_2\ \gamma_2$). Ranney (Ranney et al. 1962) reported one of the first human α globin mutants. It bound to γ globin during fetal life and to β globin after birth, thus providing biochemical evidence that Hb F and Hb A share the same α subunit, either normal or mutant. It was also known at that time that a minor component in human red cells, designated Hb A2, comprised about two percent of the total hemoglobin. She and Alan Jacobs were the first to identify and characterize genetic variants of Hb A2 (Ranney et al. 1963; Ranney et al. 1969). During the interval between these two reports it became clear that Hb A2 was composed of two α globin subunits and two other globin subunits, designated δ, that are expressed by erythroid cells at very low levels, but with a high degree of structural homology with β globin, differing in only 10 out of 146 residues.

In addition to maintaining a high level of innovative research, Ranney was one of the most highly regarded clinicians both at P&S and at Einstein. Her astute, common-sense approach to patient care was an important factor in attracting a superb group of medical residents to enter her hematology fellowship program.

Synergy between her biochemical expertise and her clinical acumen enabled her to identify both common and rare hemoglobin variants and to establish the genetic basis for their inheritance. Some of the novel variants that her group discovered were encountered in patients who had anemia owing to premature destruction of red blood cells. They were shown to be heterozygous for mutant β-globin subunits that lowered the solubility of hemoglobin, leading to the formation of precipitate in the red cell cytosol (Ranney et al. 1967; Ranney et al. 1968a). Occasional patients who came to medical attention because they were cyanotic (having skin with a dusky blue hue) were found to have a mutation in a globin subunit at the site of binding to the heme iron. Ranney completed a thorough examination of the functional properties of these M hemoglobins, including oxygen



3. Examining a blood film.

equilibria and kinetic measurements of the binding of heme ligands, demonstrating that the heme iron in the mutant subunit is in oxidized to Fe^{3+} (Ranney et al. 1968b; Udem et al. 1970) She and Max Perutz (Perutz et al. 1972) reported the structural analysis of Hb M (Milwaukee) by x-ray diffraction, thereby elucidating the mechanism by which the heme iron is stabilized in the ferric state.

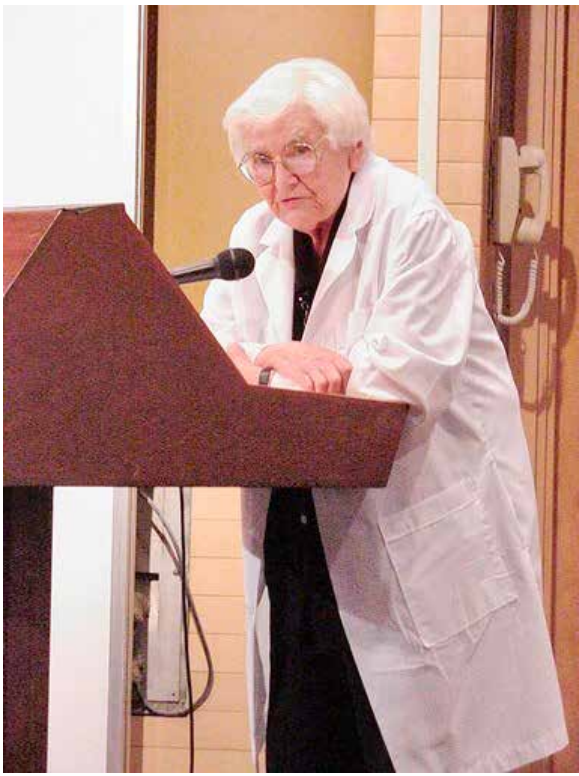
During her time at Einstein, Ranney oversaw the training of an outstanding group of investigators. She and Ronald Nagel performed the first thorough investigation of the functional properties of haptoglobin, a plasma protein that binds hemoglobin with very high affinity and specificity. They showed that haptoglobin failed to bind either to deoxyhemoglobin (Nagel et al. 1965) or to β homotetramers (Hb H) (Nagel and Ranney 1964). Thus, this interaction is exquisitely dependent on the conformation of oxyhemogobin.

The research on hemoglobin variants that had the most impact at the interface of biochemistry and medicine was Ranney's collaboration with Robert Bookchin and Ronald Nagel. Their studies provided early and critically important information on the polymerization of sickle hemoglobin. They determined the minimum concentration at which deoxy Hb S formed a gel owing to polymer formation. This provided an indirect, though quite reproducible and valid, measurement of the equilibrium solubility of deoxy Hb S. They then systematically determined the minimum gelling concentration (MGC) of mixtures of S and non-S hemoglobins. It was known that pure deoxy Hb S had a low MGC whereas that of a 50:50 mixture of Hb S and Hb A was moderately higher and that of a 50:50 mixture of Hb S and Hb F was markedly higher. Thus, polymerization of Hb S was strikingly inhibited by the γ subunit of Hb F. Early in their collaboration, Bookchin, Nagel, and Ranney (Bookchin et al. 1967) reported a novel hemoglobin variant named Hb C-Harlem in which the β subunit had not only the replacement of Glu with Val at residue 6 (Hb S), but also the replacement of Asp with Asn at position 73. They found that the MGC of deoxy Hb C-Harlem was substantially higher than that of deoxy Hb S. As this work was in progress, Hermann Lehmann had discovered in healthy individuals from Ghana a new hemoglobin variant called Hb Korle-Bu that had only the Asp to Asn substitution at position β73. Ranney and her team showed that mixtures of Hb Korle-Bu and Hb S gelled at a considerably higher concentration than mixtures of Hb A and Hb S. Taken together, these experiments provided compelling evidence that β73 is an important contact site in the polymerization of sickle hemoglobin. The discovery of Hb C-Harlem was noteworthy in two other respects. It was the first globin mutant shown to have amino acid replacements at two separate sites. Second, because of the known presence of Hb Korle-Bu in Africa, it is highly likely that Hb C-Harlem arose because of homologous crossover between two parental β-globin genes.

Over the ensuing decade, Nagel and Bookchin, who remained at Einstein, carried out a comprehensive survey of MGC on mixtures of Hb S with a large number of other hemoglobin variants having amino acid replacements at different sites on either the α or β subunits (Nagel et al. 1980). This information led to the identification of many other loci on the surface of the hemoglobin tetramer that significantly influence polymer formation. These assignments were corroborated with a high degree of fidelity by subsequent structural analyses of the sickle polymer using high resolution electron microscopy (Dykes et al. 1978) and x-ray diffraction (Wishner et al. 1975). Over subsequent decades, Einstein remained the leading center for research on sickle cell disease. Ronald Nagel and his colleagues pioneered the development of a transgenic mouse model and Robert

Bookchin and Virgilio Lew in Cambridge, England, were the leading investigators of the regulation of cations and water content in the cytosol of sickle red cells.

Ranney made major contributions to the investigation of non-enzymatic glycation of hemoglobin and its relevance to diabetes. This body of work was prefaced by a fortuitous yet highly relevant finding early in her career. One of her last research endeavors before she left Columbia was the intravenous infusion of ^{59}Fe into patients with normal hemoglobin as well as those with hemoglobin abnormalities (S trait, C trait, and β-thalassemia) and measurement of the incorporation of the iron into the hemoglobins of the circulating red cells (Ranney and Kono 1959). She found in normal humans, as well as in a parallel study in rabbits, that the incorporation of radiolabeled iron into the main hemoglobin component occurred rapidly, reflecting the approximate five day period of maturation of erythroid precursor cells and thus hemoglobin synthesis in the bone marrow. In contrast, in a minor electronegative hemoglobin component in both the human and the rabbit, the incorporation of the iron label occurred slowly over the life span of the red cell. The significance of this finding was not clear until ten years later when Samuel Rahbar, a clinical investigator from Iran, showed that patients with diabetes had a roughly two-fold elevation in a fast (electronegative) hemoglobin, later shown to correspond to Hb AIc, the most abundant minor component in human red cells (Rahbar 1968).



4. Giving a lecture.

Soon thereafter, Rahbar came to Ranney's lab at Einstein to pursue further studies of this puzzling and potentially important finding. Shortly before his arrival, Robert

Bookchin and Paul Gallop, a distinguished organic chemist at Einstein, analyzed the tryptic peptides in the β globin of Hb AIc and showed by mass spectrometric analysis that a hexose was linked to the N-terminal amino group (Bookchin and Gallop 1968). Soon thereafter Rahbar and Ranney, in collaboration with Olga Blumenfeld, showed that the β-N-terminal peptide of the minor hemoglobin component that is elevated in diabetics had the same distinguishing structural features as the Hb AIc isolated from normal individuals (Rahbar et al. 1969). During the subsequent two years, Ranney and her colleagues studied a large group of diabetic patients and found that the elevation in Hb AIc was independent of age, duration or type of diabetes, complications, and insulin therapy (Trivelli et al. 1971). A study of identical twins discordant and concordant for diabetes showed that there is no genetic contribution to the level of Hb AIc (Tattersall et al. 1975). Thus, elevations are entirely due to metabolic abnormalities. In view of the structural analysis of the Einstein group, the elevation of Hb AIc in diabetics was almost certainly due to increased levels of blood glucose. These early studies helped set the stage for the subsequent application of Hb AIc measurement world-wide in the diagnosis and longitudinal management of diabetic patients.

1973–1991 University of California, San Diego

From 1970 to 1973, Dr. Ranney was professor of medicine and chief of hematology at State University of New York at Buffalo. During this time, she continued to direct a productive research laboratory and remained highly respected and revered as a clinician and teacher. In 1972 she was awarded the Martin Luther King Jr. Medical Achievement Award in recognition of her work on hemoglobin biochemistry and genetics.

Ranney's prowess and comfort in all three components of medical academe (research, teaching, and patient care) made her a compelling choice to assume the chair of the Department of Medicine at University of California, San Diego, for what became a seventeen-year tenure. Ranney and her predecessor, Eugene Braunwald, transformed the department of medicine at UCSD into one of the nation's leading centers for close and bidirectional interactions between basic biomedical research and cutting-edge clinical care. The department thrived under her leadership, even with the retrenchment in government funding during the Reagan administration. Like Braunwald, Ranney recruited top-notched residents, fellows, and junior faculty, and she placed a high priority on nurturing their careers. Unlike some division chiefs and department chairs, she had

5. Helen with Oscar Ratnoff (left) and Samuel Rapaport (right).

neither the need nor the desire to augment her power or prominence by standing on the shoulders of talented junior colleagues.

While she was at UCSD, Ranney's research lab focused primarily on two topics: the interaction of hemoglobin with the red blood cell membrane, and detailed studies of the kinetics of ligand binding to hemoglobin.

At the beginning of her career, Ranney and Frederick Klipstein showed that following water lysis of red cells, positively charged hemoglobins such as Hb A2, Hb S, and Hb C bound preferentially to the inner surface of the membrane in comparison to Hb A (Klipstein and Ranney 1960). This phenomenon was explored in detail sixteen years later when Nurith Shaklai, a biochemist from Israel, came to UCSD to work with Ranney. They identified ~10^6 sites that bound hemoglobin with high affinity [$1x10^8$ M^{-1}] (Shaklai et al. 1977). In addition, they utilized membrane-embedded fluorescent probes to confirm and amplify the earlier finding that both Hb S (Shaklai et al. 1981) and Hb C (Reiss et al. 1982) had increased affinity for the red cell membrane. Based in part on the effect of hemoglobin's charge, they concluded that the binding was primarily electrostatic and localized to the anion channel (band 3), the most abundant red cell membrane protein and one for which the N-terminal cytosolic domain has an unusually high density of negative charge. Subsequent studies in a number of laboratories have corroborated the model of electrostatic binding of hemoglobin band 3 proposed by Shaklai and Ranney. This interaction underlies changes in red cell morphology seen in red cells containing Hb S and Hb C, and it has direct bearing on underlying pathophysiology.

While at UCSD, Ranney also collaborated with Vijay S. Sharma in studies of the functional properties of normal and variant human hemoglobins, as well as animal hemoglobins, primarily utilizing stopped-flow kinetics to measure rates of association and dissociation of various ligands to heme. Subsequently, Dr. Sharma developed an independent and productive research program that continued to focus on heme proteins.

After retirement as chair of medicine at UCSD in 1986, Ranney was until 1991 a Distinguished Physician of the Veterans Administrations, the first woman ever appointed to that position. When she left the University of California in 1991, UCSD established an endowed chair in her name.

In 1991, she became a board member, advisor, and consultant to the Alliance Pharmaceutical Corporation in San Diego. Her work focused on the medical uses of fluoro-

carbons for oxygen transport. In the years both prior to and following her retirement from UCSD, she served on the visiting committee at Harvard Medical School and also was a member of the board of directors of E. R. Squibb, Inc. In 2005 she was one of the influential women physicians honored in a National Library of Medicine exhibition entitled "Changing the Face of Medicine."

Personal qualities

Dr. Ranney's legacy in academic medicine and hematology rests in no small part on the force of her personality. She was widely known for her uncanny ability to size up people and to trouble-shoot complex inter-personal and institutional problems. She jested in earnest. She once famously described a charming but rather ineffective dean as "all grease but no machinery." A hyper-ambitious junior colleague was scolded for "overselling his goods." Her magnetism was based in large measure on a no-nonsense style and avoidance of self-promotion. Once, at a plenary session at the annual meeting of the American Society of Hematology, her chair slipped and she fell over the back of the platform. She attributed her subsequent election as president of the society to widespread sympathy from those in attendance. Ranney was disarmingly modest about her research accomplishments, pointing out that "if we hadn't done it, it would have been done some years later by someone else. We're not Bach. If he had not lived, his music would never have been written."

An exemplar of the physician-scientist, Dr. Ranney achieved iconic status as a role model for medical academicians in general and for hematologists and women in particular.

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