Berson, Yalow, and the *JCI*: the agony and the ecstasy

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The isolation of insulin in 1921 by Banting, Best, Collip, and Macleod stands as one of the most dramatic stories in modern medical investigation. Only two years passed between the initial experiments in dogs to widespread human application to the awarding of the Nobel Prize in 1923. Insulin-related research has also served as a focus, at least in part, for the work of three other Nobel Prize recipients: determination of the chemical structure of insulin by Frederick Sanger in 1958; determination of the three-dimensional structures of insulin and vitamin B12 by Dorothy Hodgkin in 1964; and finally, the development of immunoassay by Solomon Berson and Rosalyn Yalow in 1959–1960, which led to a Nobel Prize for Yalow in 1977 (five years after the untimely death of Berson). The history of Yalow and Berson's discovery and its impact on the field is an illustration of the adage that every story has two sides.

It is not surprising that the 1960 article "Immunoassay of endogenous plasma insulin in man" (1) by Yalow and Berson (Figure 1) holds a record as one of the most-cited articles ever published in the *Journal of Clinical Investigation* (2,341 times at this writing). Indeed, the techniques of radioimmunoassay and immunoassay have over 84,000 and 275,000 entries in PubMed, respectively. While a skeptic might note that most of the frequently cited articles in the literature are focused on methodology, in this case, while the paper superficially appears to be only a description of a method to assay insulin, it actually marks a revolution in biology and medicine.

Immunoassays provided a method by which minute quantities of virtually any biologically interesting molecules present in blood or other fluids could be measured with sensitivity and specificity, even in the presence of hundreds of thousands of other substances. Furthermore, while the distinction between what are now known as type 1 diabetes and type 2 diabetes had been previously made by Sir Harold Himsworth (2), with this tool, Berson and Yalow clearly demonstrated that type 1 diabetes was an insulin-deficient state, whereas patients with type 2 diabetes had substantial amounts of insulin in the blood and could be classified as insulin resistant (1, 3). They later showed that obesity was also associated with hyperinsulinemia and insulin resistance (4). En route to developing the immunoassay, they showed that antibodies to insulin occurred universally in all patients treated with insulin, and they concluded that high titers of anti-insulin antibodies accounted for nearly all cases of severe insulin resistance observed at that time (3, 5). Berson and Yalow also advanced our understanding of tumor hypoglycemia by documenting inappropriate insulin secretion from islet cell tumors and absence of insulin secretion from nonislet cell tumors, laying the groundwork for later studies that would implicate insulin-like growth factors in this disorder (6). Finally, they successfully applied the technique of immunoassay to the analysis of many other hormones and substances, leading to breakthrough insights into multiple disease states (3).

In some ways Berson and Yalow seemed an unlikely team to make this important discovery. Both were from immigrant families and attended public schools. Berson bragged about the several years and 109 medical school rejections that separated his completion of college and his enrollment in New York University Medical School, where he eventually graduated near the top of his class. Yalow recalled that despite her excellent grades, she gained entrance into a graduate school in physics only under the condition that the school would have no obligation in finding her a position after graduation (7). In school she became enthralled with radioactivity, looking beyond the atomic bombing of Hiroshima to the use of radioactivity and its great peacetime potential for medicine. This led her to become the health physicist at the Veterans Administration Hospital in the Bronx. Berson, who was starting an internal medicine practice in the then-new suburbs of Long Island, took a part-time job as internist in the Radioisotope Unit at the hospital. Outside the mainstream, without the usual years of apprenticeship, Berson and Yalow embarked on research careers with applications of radioactivity to medicine as the leitmotif.

For those of us who have been frustrated by how difficult it may be at times to get an article published in the *JCI*, it may be heartening to know that Berson and Yalow sometimes shared that same problem. Indeed, at many talks, including her Nobel Prize address (3), Yalow entertained the audience by showing a reproduction of the rejection letter from *JCI* of a 1955 paper that laid the groundwork for the insulin immunoassay (Figure 2). Thus, even this illustrious team experienced the agony and ecstasy of publishing in the *JCI*. Parenthetically, the paper did finally get accepted on resubmission and was published in *JCI* in 1956, but the authors were required to substitute "insulin binding globulin" for "insulin transporting antibody" in the title (5).

Assays of insulin in blood prior to RIA

The ability of insulin to lower blood glucose levels was the key to its isolation and purification. The first insulin assays assessed the fall in blood glucose following injection of an extract of tissue or serum into normal or depancreatized animals or, subsequently, animals that had undergone adrenalectomy or hypophysectomy to increase their sensitivity to insulin (8). However, the method was not nearly sensitive enough (1,000 μU/ml) to measure the low levels (10–20 μU/ml) in

Nonstandard abbreviations used: NSILA, nonsuppressible insulin-like activity.

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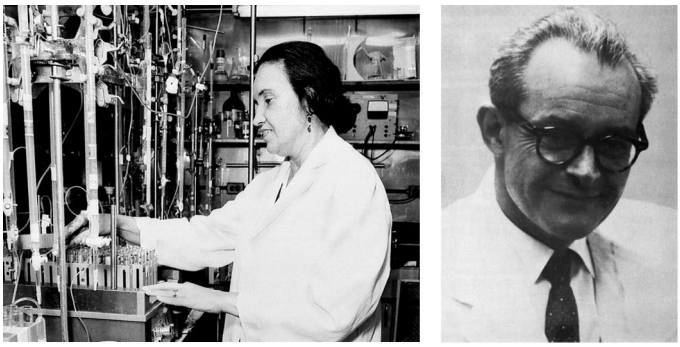


Figure 1
Rosalyn Yalow (left; image courtesy of the National Library of Medicine) and Solomon Berson (right).

blood (for reference, 25 μU/ml = 1 ng/ml = 160 pM). Indeed, one study that attempted to use this approach involved extraction of 100 ml of plasma to allow assay of insulin levels in a fasting dog (9).

Subsequently, in vitro bioassays were developed using rat diaphragm muscle and then epididymal fat pads (10, 11). These assays depended on measurements of either glucose uptake (initially measured by glucose depletion from the medium and later by use of radioisotopes) or glycogen synthesis. Like the in vivo assays, the muscle assay was also not very sensitive, and both assays were subject to the effects of other insulin-like molecules in serum as well as a large number of specific and nonspecific effects. At times it appeared that almost anything in serum at a high enough concentration could exert some insulin-like effect in these assays. In addition, there was a problem referred to as the dilution effect, i.e., detection of insulin added to serum varied depending on the dilution of serum used in the assays (12). The immunoassay method conquered all of these problems. It had high degrees of specificity and sensitivity, no dilution artifact, and the ability to assay a large number of samples using a very small amount of blood (1).

The RIA's modest origins

The creation of the RIA started with investigations concerning the metabolism of ^{131}I-labeled insulin in nondiabetic and diabetic subjects (5). Berson and Yalow observed that, contrary to their expectation, radioactive insulin disappeared more slowly from the plasma of patients who had previously been treated with insulin than from the plasma of subjects never treated with insulin (5). Immunologists of the mid-1950s did not believe that insulin was immunogenic — hence the *JCI* rejection (Figure 2). However, Berson and Yalow eventually proved that the retarded rate of insulin disappearance was due to the binding of labeled insulin to anti-insulin antibodies present in the serum of insulin-treated diabetics (5). Initially they used labeled and unlabeled insulin to examine the characteristics of the antibodies. For the immunoassay, they recognized that antibodies could also be used to examine the hormone and further, that competition between unlabeled insulin in a sample and the ^{131}I- or ^{125}I-labeled insulin for binding to sites on the anti-insulin antibodies could provide the basis of a sensitive and specific assay of the hormone (1, 3).

The essence and greatness of the discovery

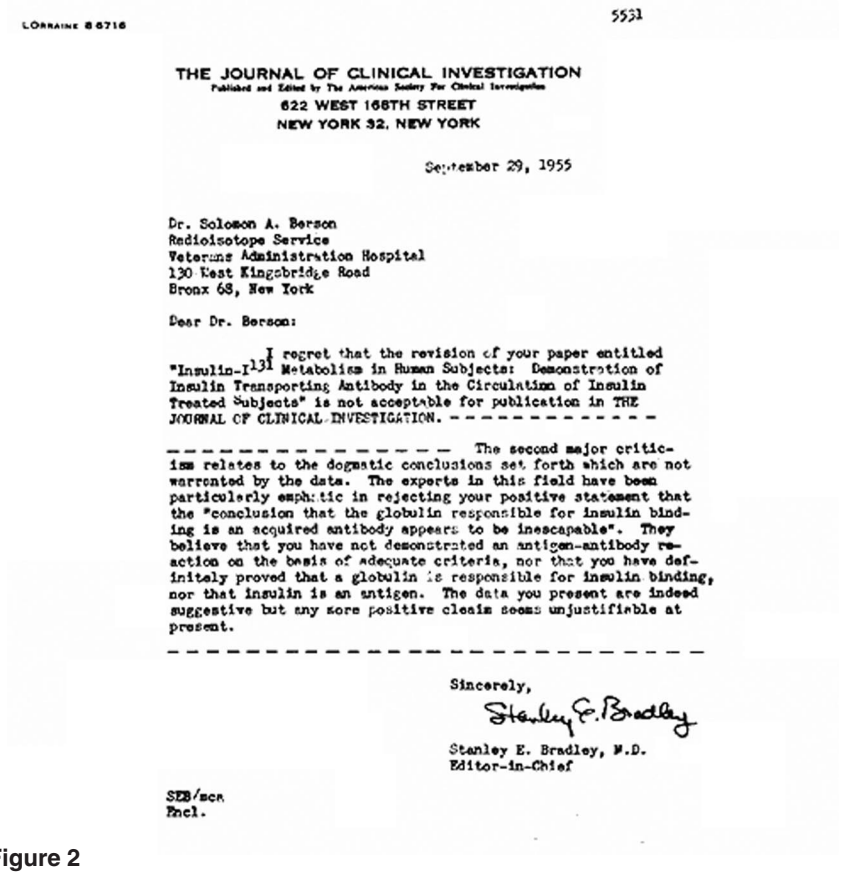
The creation of the RIA and the use of radioisotopic methods for detection of soluble antigen-antibody complexes for small molecules introduced a revolution in biomedical research (3). It not only clarified our understanding of diabetes and the physiology of glucose homeostasis, but also provided important new insights into immunology and eventually had an impact on virtually every field of biomedical investigation. RIA technology made it possible to actually measure insulin (and other hormone) levels and thus to scientifically define many physiological and pathophysiological states. In cases of diabetes and insulin resistance, this included states in which the circulating insulin levels were increased while glucose levels were normal or minimally abnormal, such as obesity, gestational diabetes, acromegaly, and Cushing disease (3, 4).

Downsides of the discovery of RIA: the snowplow effect

As with all revolutionary aspects of science, the introduction of new thoughts and technologies has many effects, most of which are positive but some of which may be inadvertently negative, as when a snowplow clears a path after a big storm but, at the same time, buries parked cars and blocks driveways and side roads. Similarly, Berson and Yalow's achievements moved the field giant steps forward, but in the wake of their success, research in several areas was actually significantly impeded.

Defining the components of insulin-like activity in blood. The early observation that serum contains 200–400 μU/ml of total insulin-like bioactivity conflicted with results of the RIA, which detected 10–20 μU/ml of immunoreactive insulin. Further, anti-insulin antiserum could only block a small portion of the insulin bioactivity of serum. These observations led to a major controversy in the field. Harry Antoniades explained the controversy by postulating that circulating insulin existed in two forms — one free to act on glucose metabolism, which could be inhibited by anti-insulin serum, and the other a bound form that was not reactive with insulin antibodies (13). Another group, led by Nagib Samaan, also proposed two forms of circulating insulin, which they referred to as typical and atypical, depending on whether their action on fat pads was inhibited or not inhibited by insulin antiserum (14). The third group, led by Rudi Froesch in Switzerland, also using a similar bioassay, referred to the two forms of insulin as suppressible and nonsuppressible insulin-like activity (NSILA) (15). Froesch's group further noted that NSILA itself was heterogeneous, with a low molecular weight form (6,000–7,000 Da) and a high molecular weight form (70,000 to 150,000 Da).

Arguing that the immunoassay was both sensitive and specific, Berson and Yalow took a strong stand against the relevance of the observations and interpretations of these investigators (12). They pointed out that there was an exact correlation between the low blood levels of immunoreactive insulin in type 1 diabetes and the development of hyperglycemia whereas there was little correlation between the levels of atypical insulin, bound insulin, or NSILA and metabolic status. As a result of the importance of the discovery of the RIA and the strong reputation of Berson and Yalow, this entire field of research on other insulin-like molecules in serum went into a temporary, but almost total, state of suspension, and the career momentum of some of the investigators working on atypical and bound insulin dissipated. It was not until the mid-1970s



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September 29, 1955

Dr. Solomon A. Berson
Radioisotope Service
Veterans Administration Hospital
130 West Kingsbridge Road
Bronx 68, New York

Dear Dr. Berson:

I regret that the revision of your paper entitled "Insulin-I^{131} Metabolism in Human Subjects: Demonstration of Insulin Transporting Antibody in the Circulation of Insulin Treated Subjects" is not acceptable for publication in THE JOURNAL OF CLINICAL INVESTIGATION. - - - - - - - - - - - - -

- - - - - - - - - - - - - - - The second major criticism relates to the dogmatic conclusions set forth which are not warranted by the data. The experts in this field have been particularly emphatic in rejecting your positive statement that the "conclusion that the globulin responsible for insulin binding is an acquired antibody appears to be inescapable". They believe that you have not demonstrated an antigen-antibody reaction on the basis of adequate criteria, nor that you have definitely proved that a globulin is responsible for insulin binding, nor that insulin is an antigen. The data you present are indeed suggestive but any more positive claim seems unjustifiable at present.

- -

Sincerely,

Stanley E. Bradley

Stanley E. Bradley, M.D.
Editor-in-Chief

SEB/mcm
Encl.

Figure 2
Copy of a 1955 *JCI* rejection letter to Berson and Yalow.

that this area of research reemerged, when Froesch and his colleagues were able to successfully purify and sequence two insulin-like molecules, IGF-1 and IGF-2, from serum (16) and Klara Megyesi and her colleagues were able to demonstrate separate receptors for these hormones on cell membranes (6). Now it is apparent that these insulin-like growth factors do have both bound and free forms and that their effects are primarily on growth rather than glucose metabolism, which accounts for many of the previously controversial observations.

Insulin autoimmunity. The demonstration of anti-insulin antibodies in insulin-treated patients was so central to Berson and Yalow's work that they convinced themselves and others in the field that antibodies to insulin only appear in patients who have previously been treated with insulin. They considered autoimmunity to insulin at most a theoretical possibility and postulated that insulin induces immune tolerance. Using more sensitive approaches to antibody detection, those in the field have come to recognize that patients can also develop autoantibodies to insulin without any prior treatment in association with both type 1 diabetes (17) and an autoimmune form of hypoglycemia (18). Indeed, a test detecting autoantibodies to insulin has become standard in the assessment of individuals at risk for type 1 diabetes or with early signs of the disease.

Inhibitors of insulin action and insulin resistance. Berson and Yalow recognized glucocorticoids, growth hormone, and other hormones, in addition to anti-insulin antibodies, as contributors to insulin resistance. They disparaged other postulated inhibitors of insulin action or insulin antagonists invoked by others, especially the so-called synalbumin antagonist of insulin described by Vallence-Owen, which migrated on electrophoresis with albumin instead of slow-moving globulins like anti-insulin antibodies. Now researchers recognize that many molecules can modify insulin action, including free fatty acids (which bind to serum albumin), cytokines, and even insulin itself, which when chronically elevated may desensitize the target cell (19–22). Likewise, we now recognize that extreme insulin resistance may be due to autoantibodies against the insulin receptor or genetic defects in the receptor and may occur at intracellular steps in the insulin action cascade (23, 24).

Identification of cell surface receptors for peptide hormones. Despite their brilliant work regarding immunoassay development, Berson and Yalow were slow to recognize the potential of this approach being extended to the area of membrane receptors, and their critique of existing studies slowed the development of this field. As early as 1949, William Stadie and coworkers studied how insulin might act by binding to tissues. In 1952, these investigators noted that when the diaphragm was immersed in a solution containing ^{131}I- or ^{35}S-labeled insulin, a small fraction of radioactivity remained fixed to the tissue even after repeated washing (25). Katharina Newerly and Berson, however, noted that labeled insulin binds to a wide variety of surfaces, including glass and paper, and therefore concluded that "binding of insulin by isolated rat diaphragm in vitro is not demonstrably of biological significance but is attributable to nonspecific adsorption of the proteins" (26).

Again, the dominance of Berson and Yalow and their skeptical view of hormone binding to tissues put this field in limbo for over a decade. Ultimately, however, it was the scientific children and grandchildren of Berson and Yalow (including the authors of this paper) who showed that, when properly performed, radioactive ligands could be used to detect membrane receptors, thus extending the work of Berson, Yalow and Stadie to help open the new field of the study of cell surface receptors (20, 21, 23, 24).

Summary and perspective

The discovery of the RIA was one of the major accomplishments of medical research in the 20th century. Berson and Yalow were rightly recognized as giants in the field, and their article from 1960 holds a record as one of the most cited articles in the 80-year history of the *JCI*. The technique of RIA and its application to a wide variety of biological systems has led to important insights in endocrinology, immunology, cardiology, gastroenterology, nephrology, neuroscience, and many other disciplines. The work also led its discoverers and the field astray in a few places, and some scientific discoveries were in limbo for over a decade. As Berson and Yalow wrote in the Banting Award Lecture to the American Diabetes Association in 1965, "It is in the interest of attainment to a higher knowledge than we presently possess that frank and penetrat-

ing appraisal be made by interested participants and observers alike; it is to be hoped that these will have the purpose and the effect of stimulating an ever more critical approach to the problems that beset us."

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1. Yalow, R.S., and Berson, S.A. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**:1157–1175.
2. Himsworth, H.P. 1936. Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types. *Lancet.* **i**:127–130.
3. Yalow, R.S. 1978. Radioimmunoassay: a probe for the fine structure of biologic systems. (Nobel Lecture, 8 December 1977). *Science.* **200**:1236–1245.
4. Yalow, R.S., Glick, S.M., Roth, J., and Berson, S.A. 1965. Plasma insulin and growth hormone levels in obesity and diabetes. *Ann. N. Y. Acad. Sci.* **131**:357–373.
5. Berson, S.A., Yalow, R.S., Bauman, A., Rothschild, M.A., and Newerly, K. 1956. Insulin-I131 metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin treated subjects. *J. Clin. Invest.* **35**:170–190.
6. Megyesi, K., Kahn, C.R., Roth, J., and Gorden, P. 1974. Hypoglycemia in association with extrapancreatic tumors: demonstration of elevated plasma NSILA-s by a new radioreceptor assay. *J. Clin. Endocrinol. Metab.* **38**:931–934.
7. Straus, E. 1998. *Rosalyn Yalow, Nobel laureate: her life and work in medicine: a biographical memoir.* Plenum Trade. New York, New York, USA. 277 pp.
8. Gellhorn, E., Feldman, J., and Allen, A. 1941. Assay of insulin on hypophysectomized, adreno-demedullated, and hypophysectomized-adreno-demedullated rats. *Endocrinology.* **29**:137–140.
9. Anderson, E., Wherry, F.E., and Bates. R.W. 1961. Method of extraction and bio-assay of insulin in blood. *Diabetes.* **10**:298–303.
10. Groen, J., Kamminga, C.E., Willebrands, A.F., and Blickman, J.R. 1952. Evidence for the presence of insulin in blood serum; a method for an approximate determination of the insulin content of blood. *J. Clin. Invest.* **31**:97–106.
11. Renold, A.E., et al. 1960. Measurement of small quantities of insulin-like activity using rat adipose tissue. I. A proposed procedure. *J. Clin. Invest.* **39**:1487–1498.
12. Berson, S.A., and Yalow, R.S. 1965. Some current controversies in diabetes research. *Diabetes.* **14**:549–572.
13. Antoniades, H.N. 1961. Studies on the state of insulin in blood: the state and transport of insulin in blood. *Endocrinology.* **68**:7–16.
14. Samaan, N., Fraser, R., and Dempster, W.J. 1963. The "typical" and "atypical" forms of serum insulin. *Diabetes.* **12**:339–348.
15. Froesch, E.R., Buergi, H., Ramseier, E.B., Bally, P., and Labhart, A. 1963. Antibody-suppressible and nonsuppressible insulin-like activities in human serum and their physiologic significance. An insulin assay with adipose tissue of increased precision and specificity. *J. Clin. Invest.* **42**:1816–1834.
16. Zapf, J., Schoenle, E., and Froesch, E.R. 1978. Insulin-like growth factors I and II: some biological actions and receptor binding characteristics of two purified constituents of nonsuppressible insulin-like activity of human serum. *Eur. J. Biochem.* **87**:285–296.
17. Devendra, D., and Eisenbarth, G.S. 2003. 17. Immunologic endocrine disorders. *J. Allergy. Clin. Immunol.* **111**:S624–S636.
18. Hirata, Y., Tominaga, M., Ito, J.I., and Noguchi, A. 1974. Spontaneous hypoglycemia with insulin autoimmunity in Graves' disease. *Ann. Intern. Med.* **81**:214–218.
19. Boden, G., and Shulman, G.I. 2002. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur. J. Clin. Invest.* **32**(Suppl. 3):14–23.
20. Roth, J., et al. 1975. Receptors for insulin, NSILA-s, and growth hormone: applications to disease states in man. *Recent Prog. Horm. Res.* **31**:95–139.
21. Virkamaki, A., Ueki, K., and Kahn, C.R. 1999. Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. *J. Clin. Invest.* **103**:931–943.
22. Dandona, P., Aljada, A., and Bandyopadhyay, A. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* **25**:4–7.
23. Flier, J.S., Kahn, C.R., and Roth, J. 1979. Receptors, antireceptor antibodies and mechanisms of insulin resistance. *N. Engl. J. Med.* **300**:413–419.
24. Taylor, S.I., and Arioglu, E. 1998. Syndromes associated with insulin resistance and acanthosis nigricans. *J. Basic Clin. Physiol. Pharmacol.* **9**:419–439.
25. Stadie, W.C., Haugaard, N., and Vaughan, M. 1952. Studies of insulin binding with isotopically labeled insulin. *J. Biol. Chem.* **199**:729–739.
26. Newerly, K., and Berson, S.A. 1957. Lack of specificity of insulin-I 131-binding by isolated rat diaphragm. *Proc. Soc. Exp. Biol. Med.* **94**:751–755.

Superoxide production by phagocytic leukocytes: the scientific legacy of Bernard Babior

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It was 32 years ago that Bernard Babior, Ruby Kipnes, and I submitted a paper to the *JCI* reporting that polymorphonuclear leukocytes produce superoxide (O_2^-) during phagocytosis and that this highly reactive oxygen radical might function as a microbicidal agent. The story of how our lab came to this discovery is one of a special relationship between a student and his brilliant mentor.

We were pleasantly surprised in 1972 to hear that our paper (1) had been accepted for publication. We were even more surprised recently to learn that this report was among the *JCI*'s most frequently cited articles and was to be highlighted as part of the *Journal*'s 80th-anniversary celebration. There was a sad irony, though. Within a few weeks after Bernie (Figure 1) enthusiastically agreed to write a historical commentary on the article, a lingering illness intensified that led to his passing on June 29, 2004. He was not able to share with us his perspectives 3 decades after one of his most important discoveries. I was honored to be asked by the *JCI* to step in for Bernie to write the commentary and, in the process, to pay tribute to this wonderful, creative, and spirited investigator and man.

The story behind our article has, as many discoveries do, an unlikely origin — in this case, a growing special relationship between a student and his mentor. I was a freshman at Harvard College, majoring in biochemistry and in search of an adviser, and learned that there was a brilliant young professor of medicine and gastroenterologist at Harvard Medical School's Thorndike Laboratory at Boston City Hospital — Bernard Babior — who was also a tutor in the Biochemistry Department at the college. He agreed to take me under his wing and for the next 2 years patiently taught me the complex biochemistry of vitamin B_{12} in rigorous one-on-one tutorials and, eventually, at the bench in his laboratory (Figure 2).

Nonstandard abbreviations used: CGD, chronic granulomatous disease; HOCl, hypochlorous acid; O_2^-, superoxide.

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