

Morgan C R & Lazarow A. Immunoassay of insulin: two antibody system, plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes* **12**:115-26, 1963.

The authors present a detailed account of a two-step procedure for radio-immunoassay of insulin. In the first reaction, insulin forms a soluble complex with its specific antibody obtained from immunized guinea pigs. In the second reaction, this soluble complex is precipitated by an antibody to guinea pig serum obtained from immunized rabbits. Using a radioactive insulin tracer, the amount of radioactivity in the precipitate is dependent upon the concentration of insulin in the reaction mixture; i.e., with increasing concentrations of unlabeled insulin, the amount of radioactive insulin in the precipitate is decreased correspondingly. [The *SCI*[®] indicates that this paper was cited 1069 times in the period 1963-1976.]

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"It is gratifying to have developed a method that has found wide acceptance. This work was done as a part of my Ph.D. thesis. The late Arnold Lazarow, former Head of the Department of Anatomy at the University of Minnesota, was my mentor. I had completed my M.A. at the University of Nebraska. The following four years of experience as a technician in the laboratories of Dr. J.T. Syverton, then Head of Bacteriology at the University of Minnesota, served me well on this project. Thanks to the USPHS training grants program, in the reflected light of Sputnik, I resumed my graduate training.

"At the time these studies were initiated (1958), research on diabetes mellitus was inhibited by the lack of a specific, easily reproducible method of measuring large numbers of samples containing less than a nanogram of insulin.

"The ground work had been laid. Moloney & Coval (1955) reported that guinea pigs could be routinely and reliably immunized to other mammalian insulins.¹ Using a hemagglutination method, Arquilla & Stavitsky (1956) had shown the feasibility of using immunological procedures for assaying insulin.² (131). Insulin was available commercially. Skom & Talmage (1958) reported on the use of anti-human gamma globulin to precipitate the non-precipitating insulin antibodies in insulin-resistant human serum.³ With these facts in hand, Lazarow and I proceeded to develop our two-antibody method for the immunoassay of insulin. There was a manual, single channel gamma radiation counter in the laboratory. With intense anticipation we watched the rows of blinking lights to see if our efforts were to be successful.

"Other immunoassay procedures were developed in other laboratories during this time. It is apparent that such a method was in great demand. The methodology has been adapted to the immunoassay of other hormones. I attribute the acceptance of our method and the interest in our paper to the relative ease, reproducibility, and precision of the two-antibody method.

"Arnold Lazarow was pleased with this work, and he considered it an important breakthrough. It was a satisfying experience for me to work with him, and I am pleased that our work has been useful in the continuation of research on diabetes."

1. **Moloney P J & Coval M.** Antigenicity of insulin: diabetes induced by specific antibodies. *Biochemical Journal* **59**:179-85, 1955.
2. **Arquilla E R & Stavitsky A B.** The production and identification of antibodies to insulin and their use in assaying insulin. *Journal of Clinical Investigation* **35**:458-66, 1956.
3. **Skom J H & Talmage D W.** Nonprecipitating insulin antibodies. *Journal of Clinical Investigation* **37**:783-6, 1958.