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# EDITORIAL

*Editorial opinions expressed are those of the writers.*

## 70TH ANNIVERSARY OF THE RUBINO REACTION

Seventy years ago, the Uruguayan scientist Miguel C. Rubino,[1] while working with complement fixation reactions for the diagnosis of syphilis using formolized sheep red blood cells, noted that among approximately 800 human sera tested there was one that induced rapid cell sedimentation and supernatant clarification. Since he had not observed this phenomenon previously, he determined the origin of the serum and identified it as a sample from a leprosy patient.

Rubino then devoted his time to the study of the nature of the reaction, to the standardization of the conditions under which it occurs, and to its application to a large number of human sera of different origins.[2–6] He reproduced the phenomenon in 84% of 38 other sera from leprosy patients, observing red cell sedimentation within less than an hour.[3] Only rarely did sera from nonleprosy subjects cause sedimentation of formolized sheep red cells so rapidly and these sera, in contrast to those from leprosy patients, also caused the sedimentation of natural, nonformolized red cells. He thus concluded that the reactivity of sera from leprosy patients was mainly characterized by a different behavior in the presence of formolized and natural red blood cells.[3, 5, 6] These first observations by Rubino demonstrated the specificity of the reaction, which was positive only for leprosy patients; a single exception among 300 control sera tested was serum from a patient with filariasis who, however, was suspected to be also infected with *Mycobacterium leprae*.[4]

The pioneering work of Rubino motivated the study of the reaction by several investigators. Marchoux and Caro,[7] while testing 10 sera from leprosy patients, observed 50% positivity without the occur-

[1] Rubino, M. C. Nueva reacción sorológica en la lepra. Rev. Méd. Uruguay **29** (1926) 143–155.
[2] Rubino, M. C. Nuevas reacciones sorológicas en la lepra. Rev. Soc. Arg. Biol. 2 (1926) 407–416.
[3] Rubino, M. C. Nouvelle réaction sérologique dans la lèpre. Rev. Méd. Uruguay **32** (1929) 85–116.

tected a 44% rate of positive reactions among leprosy patients with the absence of nonspecific reactions. Bier and Arnold[9] obtained only one positive reaction among 954 sera from individuals without leprosy. In 1929 Rubino[3] reported that he had tested more than 2000 sera from nonleprosy individuals without observing any positive reactions. Evidence for the absolute specificity of the reaction accumulated during the following years.[10-24] The few reports which questioned the specificity of the test by other investigators can be attributed to the fact that no countertest with natural red blood cells was performed.[25-29]

The nature of the Rubino reaction was studied extensively by Marchoux and Caro,[7] who attributed it to the presence of a specific factor in the serum of leprosy patients which was selectively adsorbed to formolized red cells. They observed that the adsorption of this specific factor required the participation of a second factor also present in the serum of normal individuals, and that formolized sheep red blood cells could completely deplete leprosy serum of the active substance. They reported an unsuccessful attempt to isolate the specific factor in leprosy serum by solubilizing the factor adsorbed to the surface of formolized sheep red blood cells.

Rubino[5] considered the reaction described by him to correspond to agglutinosedimentation of formolized red cells of the same nature as microbial agglutinations and serum flocculations. Ambrogio[13] attributed this reactivity to a principle present in positive serum that possibly acted synergistically with electrolytes causing a change in potential, increased repulsion between globules and consequent sedimentation. Benetazzo[30] considered the reaction to be related to colloidal plasma changes caused by the absolute increase of globulins. Despite the facts and conjectures about the origin of the Rubino reaction, in 1936 Bier[22] referred to the factor responsible for the Rubino reaction as an "enigmatic factor" not related to the immunologic factors known at the time.

All of the studies carried out invariably indicated that the Rubino reaction was absolutely specific, although its sensitivity was low and variable and its positivity occurred only when the diagnosis of leprosy was already implicit. These characteristics limited its diagnostic applicability, a fact probably justifying the hiatus of almost

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three decades in the study of the Rubino reaction.

In 1962, Almeida[31] demonstrated the absence of correlation between positivity of the Rubino reaction and anti-complementary activity of sera from leprosy patients. During the same year, Curban[32] published a comprehensive review of the literature in which he emphasized the importance of the test not as a diagnostic tool but rather as an unexplored field that might contribute to the understanding of the immune response in leprosy.

In 1970, Almeida,[33] in a review of the serology of leprosy, referred to the factor responsible for the Rubino reaction as a gamma-globulin, and stated that there was no correspondence between the factor and the complement-fixing antibodies that recognize tubercle antigen preparations. The same author later demonstrated that the Rubino reaction was inhibited when carried out in the presence of antigen preparations of *M. leprae* or some, but not all, mycobacteria.[34, 35]

A new stage in the investigation of the Rubino reaction became possible after standardization of the criteria for the classification of the clinical forms of leprosy.[36]

Silva, *et al.*[37–39] studied 60 patients with the lepromatous clinical form and 16 with the tuberculoid form and observed that the Rubino reaction was invariably negative among patients with the tuberculoid form and positive in 50% of the patients with the lepromatous form. In a study of a group of 178 leprosy patients, Opromolla, *et al.*[40] confirmed the negativity of the reaction among tuberculoid patients and reported 9% positivity among borderline patients. For the group of lepromatous patients, they obtained 55% positive reactions for those with active disease and 6% for those considered to be clinically cured. These observations were confirmed when these investigators expanded their series, a fact that led them to propose the existence of an inverse relationship between positivity of the Rubino reaction and host resistance to *M. leprae*.[41] As these studies were being conducted, a new application of the Rubino test was suggested, i.e., as a criteria for the cure of the disease.[40] However, this application became unfeasible when positive reactions were detected among 57% of clinically cured patients.[42]

Research interest then started to converge on the relationship between the positivity of the Rubino reaction and the impaired specific immune response in leprosy (The Figure). Subsequent studies were implicitly based on the search for a correspondence

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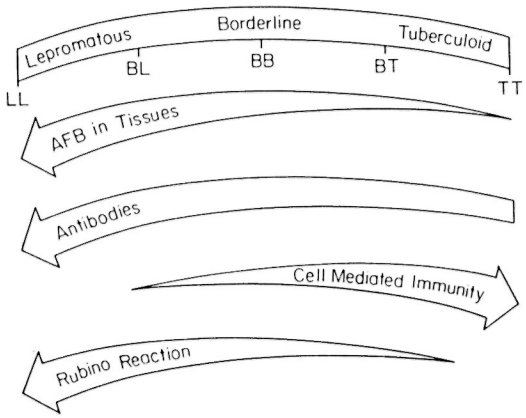


THE FIGURE. Correlation between positivity of the Rubino reaction and the clinical forms of leprosy. LL = lepromatous leprosy; BL = borderline lepromatous leprosy; BB = borderline leprosy; BT = borderline tuberculoid leprosy; TT = tuberculoid leprosy. AFB = acid-fast bacilli. Adapted from Bloom and Jacobs, 1989.[43]

between the Rubino factor and specific components of the serum of lepromatous patients. Hypergammaglobulinemia was observed in lepromatous patients and increased IgM levels were observed only in Rubino-positive sera.[37, 38] Rheumatoid factor was detected in lepromatous patients but its presence was not associated with positivity of the Rubino reaction. Anti-thyroglobulin antibodies were detected only in the serum of Rubino-positive patients.[39] A subsequent study showed that the positivity of the reaction did not correlate with changes in serum IgG or IgM levels, an observation interpreted to indicate the independence between the Rubino reaction and these immunoglobulin isotypes.[44]

The specific factor responsible for the Rubino reaction (Rubino factor) was recently identified as an immunoglobulin of isotype M.[45] The Rubino factor/IgM was isolated from a pool of sera from 20 patients with lepromatous leprosy whose IgM levels were higher than those of normal individuals but similar to those of 20 other sera from Rubino-negative patients with lepromatous leprosy. Anti-IgM antibodies block the Rubino activity of positive serum or of the purified factor. The availability of purified Rubino factor/IgM permitted the unequivocal demonstration of the requirement of a cofactor present in normal serum for the Rubino reaction.[46]

Recent interest in the mechanisms which underly the Rubino reaction is based on the expectation that they may contribute to the understanding of the cell-mediated immune unresponsiveness typical of lepromatous leprosy. However, the Rubino reaction completes 70 years as an enigma still to be understood.

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