**Historical Insight: Paul Ehrlich's dictum of *horror autotoxicus* and the changing orientation of the field inhibited acceptance of the reality of autoimmune disease.**

# Autoimmunity *versus horror autotoxicus*: The struggle for recognition

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*"... 1955–1965 [was] the decade marked by the question, 'Does autoimmunity exist?' ..."*

*N. R. Rose and I R. Mackay*[1]

Karl Landsteiner (1868–1943) in his Vienna laboratory in the early 1900s. (Courtesy University of Wisconsin Middleton Library.)

Why is it that certain indisputable facts in science may be so effaced from the collective memory that they must be discovered anew, even many decades later? Was Paul Ehrlich's demonstration in 1892 of the passive transfer of antibody from mother to fetus and neonate[2] so unclear—or Clemens von Pirquet's explanation of the pathogenesis of immune complex disease in 1910[3] so incomplete—that full acceptance of the phenomena had to await a repeat of the experiments 50–60 years later?

I shall point out in this offering how, in 1904, Julius Donath and Karl Landsteiner demonstrated clearly and beyond challenge that paroxysmal cold hemoglobinuria (PKH) is an autoimmune disease[4]. Over the ensuing years, other more-or-less convincing demonstrations suggested that autoimmune reactions might be responsible for sympathetic ophthalmia, for ocular inflammation due to lens antigens, for some hemolytic anemias and for certain encephalitides. Yet Rose and Mackay could say, fairly, that the question of the very existence of autoimmune disease was still open in the 1960s. We shall now explore the several bases for this curious delay in the acceptance of a clearly demonstrated fact. It involved, in part, the overarching influence of a generalization made by the famous Paul Ehrlich in 1901, which caused many investigators to disregard data that argued otherwise. It followed also from the suggestion by Ludwik Fleck that acceptance of a fact in science may depend less upon its truth than upon the willingness of the leaders in the field (whom he called the *Denkkollektiv*) to acknowledge it![5]

## The origin of *horror autotoxicus*

Fast on the heels of Jules Bordet's demonstration in 1898 of the phenomenon of immune hemolysis[6], Paul Ehrlich assigned his assistant Julius Morgenroth to extend these studies. In a series of six reports[7], they described the hemolytic antibodies that result when animals are injected with the blood of unrelated species. They followed this by attempting to immunize animals with the blood of their own species, and even with the animal's own blood. Whereas they obtained *iso*-antibodies in many instances, they failed in every attempt to elicit the formation of *auto*-antibodies. This led Ehrlich to postulate the existence of what he termed *horror autotoxicus*, the unwillingness of the organism to endanger itself by the formation of toxic autoantibodies. Indeed, Ehrlich would say that, "It would be dysteleologic in the highest degree, if under these circumstances self-poisons of the parenchyma—autotoxins—were formed"[8].

When it was called to Ehrlich's attention that Metalnikoff and others had demonstrated in animals the formation of antibodies against their own sperm[9], Ehrlich did not recant. He argued that these were not "autocytotoxins within our meaning", since they did not act to destroy spermatozoa in their normal *in vivo* location, that is, they did not cause disease[10]. Here was the true meaning of *horror autotoxicus*: not that antibodies against self cannot be formed but that they are prevented "by certain contrivances" from exerting any destructive action[11]. In spite of the interesting implications of Ehrlich's "certain contrivances" for modern studies on immunoregulation and tolerance induction, Ehrlich's absolute dictum that autoimmune disease cannot occur would resound throughout the decades and prevent full acceptance of a growing reality.

## Paroxysmal cold hemoglobinuria

This fairly rare disease is characterized by the acute intravascular destruction of red cells with a resulting hemoglobinuria, after exposure of the patient to the cold. The pathogenesis of the disease was unknown until 1904, when Viennese clinician Julius Donath suggested that the cause of the disease is due to the formation of autoantibodies against the patient's own erythrocytes[12]. To further support his thesis, Donath enlisted the aid of Karl Landsteiner, with whom he had previously collaborated on other studies. Landsteiner was already a well-established investigator, who had earlier discovered the ABO blood group system of man[13] (for which he received the 1930 Nobel Prize in Physiology or Medicine). With Donath providing the patients and Landsteiner the laboratory know-how, they attacked the problem together.

The investigations proved to be both simple and conclusive because the principle feature of the disease could be reproduced *in vitro*[14]. Blood from the affected patients was collected in oxalate, the plasma was separated and the red cells washed. Mixing the two at room temperature had no effect, but mixing them in the cold and then rewarming the mixture resulted in massive hemolysis. When the washed cells alone were subjected to the same temperature change, nothing happened, which indicated that it was something in the plasma that was responsible for the hemolytic event. When the plasma alone was cooled, rewarmed and then added to the patient's washed

erythrocytes, again nothing transpired, which suggested that the two reagents must interact in the cold before rewarming. However, when the plasma was heated to 56 °C before use in the above tests, no hemolysis occurred, which indicated that some plasma participant in the reaction is thermolabile. This labile activity, however, could be replaced by any active (that is, unheated) serum or plasma from normal individuals, which suggested that whatever the thermolabile factor is, it is not peculiar to this disease. These experiments were followed up by testing patients' plasma against the red cells of normal controls, and patients' red cells against the serum or plasma of normal controls, using the same protocol as before. The result was clear: some thermostable substance in the plasma of the patient could lyse any human erythrocytes, whereas the red cells of the patient could not be destroyed by the plasma of control individuals.

The only interpretation possible was that three components are involved in the process: red cells, a thermolabile factor and a thermostable factor. Only the thermostable factor appeared to be abnormal and to exist only in the patients' blood because the other two factors (red cells and unheated serum) could be furnished by normal controls. This finding was elegantly confirmed as follows: the patient's plasma was cooled in the presence of a large amount of washed, normal human red cells, which were then centrifuged and set aside. New cells were then added and the mixture warmed. No hemolysis resulted, which indicated that the active substance had been removed specifically by the initial treatment. However, the cells used for the absorption could then be lysed in the warm on the addition of an unheated serum, which showed that they had indeed interacted with (absorbed) the active substance. When a control was run under the condition where the initial mixture was maintained in the warm, it was found that no absorption of the active substance had taken place. Here was an interaction of active substance with erythrocyte that could occur only in the cold!

It was clear to the investigators that the hemolysis that takes place in the blood of the hemoglobinuric patient follows the general rule for immune hemolysis that had been laid down by Bordet and confirmed by Ehrlich and Morgenroth. The interaction of an antibody to erythrocytes with its target cell, followed by a dissolution of the cell, is mediated by the thermolabile substance that Bordet called alexine and Ehrlich called complement. Here was an incontrovertible demonstration of the apparently spontaneous formation of a toxic autoantibody, although admittedly a strange one in that it only attached to the target cell in the cold, whereas complement only acts when the sensitized cells are rewarmed. The pathogenesis was now clear, although the etiology remained an open question; most cases appear to be associated with a pre-existing syphilis infection. *Horror autotoxicus* had just received an apparently lethal challenge.

## The contemporary response

Donath and Landsteiner, and Landsteiner alone, would publish further on PKH[15] but their view of its autoimmune pathogenesis would remain unchanged. This view was widely acknowledged from many quarters to be a demonstration of autoimmune disease, not least from the Ehrlich camp. Ehrlich himself, in his 1906 review of recent advances in immunology prepared for the English translation of his *Collected Works*, referred to the Donath-Landsteiner finding of "hemolytic autoamboceptors"[16]. Again, Ehrlich's leading immunological disciple, Hans Sachs, would say in an extensive review of the field: "Donath and Landsteiner have produced information of the highest interest, that in the serum of this disease [PKH] an amboceptor is present that acts upon its own red cells"[17].

Other of Ehrlich's adherents would refer similarly to the Donath-Landsteiner finding, although in reviewing all of these discussions, one gets the impression that the authors believe PKH is an aberrant exception to the rule and that *horror autotoxicus* still reigns. Perhaps the best indication of the firm persistence of belief in Ehrlich's *horror autotoxicus* is the story that Noel Rose tells of his teacher Ernest Witebsky[18]. Witebsky was "second generation" Ehrlich, being the student of Ehrlich's assistant, Hans Sachs. Despite a series of reports that were suggestive of autoimmune diseases (such as sympathetic ophthalmia[19], phacoanaphylaxis[20], acquired hemolytic anemias[21], "allergic" encephalomyelits[22], thrombocytopenic purpura[23] and aspermatogenesis[24]), Witebsky continued to believe in the impossibility of autoimmune disease. He would say, at the celebration in 1954 of the Ehrlich centennial: "The validity of the law (sic!) of horror autotoxicus certainly should be evident to everyone interested in the field of blood transfusion and blood disease. Autoantibodies—namely, antibodies directed against the receptors of the same individual—are not formed"[25].

So certain was Witebsky of this that initially he refused to believe the implications of his and Rose's discovery of thyroid autoantibodies associated with experimental thyroiditis. Witebsky insisted on withholding the report for over 3 years while they searched for the undoubted experimental error that had produced these data in such contravention of Ehrlich's dogma. Finally, Witebsky saw the light and the results were published[26].

## The changing preoccupations of the discipline[27]

Immunology had its origins as a purely medical science. The discoveries of Louis Pasteur and Robert Koch, and the phagocytic theory of Ilya Metchnikoff, were aimed at understanding the prevention of disease. The work of Emil Behring and Shibasaburo Kitasato and of Paul Ehrlich with antitoxins was aimed at curing disease with passive serotherapy. Even the studies of immune hemolysis and complement fixation were ultimately employed to diagnose disease. When anaphylaxis and serum sickness were discovered during the early 1900s, it was still in the context of disease, although these phenomena appeared somehow to be unrelated (or unrelatable) to the protective aspects of the immune response.

Then, about the time of the First World War, immunology ran out of easy successes. Those diseases amenable to preventive vaccine administration had mostly been dealt with and those diseases amenable to serotherapy (generally those due to exotoxins) had been attacked. New successes would be hard to come by and the field turned toward more biological and even biochemical pursuits. These were led by Landsteiner with his studies on serological specificity using chemical haptens[28]. Organic chemist Michael Heidelberger worked on the chemistry of pneumococcal polysaccharides and trained immunochemists[29], William Boyd worked on plant agglutinins and the nature of antigenicity[30] and David Pressman and Elvin Kabat worked on the structure and thermodynamics of antibodies and antigens[31]. These and other texts and monographs were chemically oriented[32]. Even the theories advanced to explain antibody formation were purely chemical[33] and paid scant attention to disease or to such biological aspects of antibody formation as the booster response or the basis for affinity maturation.

In this environment, who among the leaders of immunology would pay attention to the implications of the pathogenesis of PKH or to reports on experimental encephalitis, sympathetic ophthalmia, hemolytic anemia or aspermatogenesis[19–24]? Even studies such as those of Hans Zinsser or Arnold Rich on allergic responses to bacteria[34] or

of Dienes and Shoenheit or Simon and Rackemann on models of delayed hypersensitivity to protein antigens[35] were only of interest to bacteriologists and pathologists. Indeed, most of these reports were published in "outside" journals, out of the ken of most immunologists of the day.

Only when startling new observations from biology and medicine began, in the 1940s to 1960s, to challenge the ruling immunochemical paradigm did the field start to change radically. These came from a variety of sources. They included the work of the Medawar team on antibody-independent tissue graft rejection[36], the observation on chimerism in cattle that led to the concept of immunological tolerance[37], the observations on immunodeficiency diseases[38] that led (in part) to the work on the role of the thymus and bursa of Fabricius and, finally, Burnet's emphasis on the role of cellular dynamics in his clonal selection theory of antibody formation[39].

All of this activity was accompanied, during the 1950s, 1960s and 1970s, by the appearance of a new generation of immunologists, now trained in genetics, biology and a variety of medical subspecialties. Here was a group that participated in, and even led, the post-war biomedical explosion and who were not dismayed by the challenges implicit in the suggestion that autoimmune diseases exist. For the first time, the study of autoimmunity and autoimmune disease joined the mainstream of immunology and shared fully in the practical and conceptual successes that the new immunology engendered.




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7. Ehrlich, P. & Morgenroth, J. *Berlin klin.Wochenschr.* **36**, 6–9 & 481–486 (1899); **37**, 453–458 & 681–687 (1900); **38**, 251–257 & 569–574 (1901). (Translations: Ehrlich, P. in *The Collected Papers of Paul Ehrlich* Vol. II, Pergamon, New York, 1957.)
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9. Metalnikoff, S. *Ann. Inst. Pasteur* **14**, 577–589 (1900).
10. Ehrlich, P. & Morgenroth, J. *Berlin klin.Wochenschr.* **38,** 251 & 255 (1901).
11. For an excellent exposition of the meaning of horror autotoxicus, see the monograph by Dietlinde Goltz, Horror Autotoxicus: Ein Beitrag zur Geschichte und Theorie der Autoimmunpathologie im Spiegel eines vielzitierten Begriffes (Thesis, Univerity of Münster, 1980).
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15. For example, Donath J. & Landsteiner, K., *Z. klin. Med.* **58**, 173–189 (1906); *Zentralbl. Bakteriol. Parasitenk.* **45,** 205–213 (1908); *Zentralbl. Immunitätsforsch.* **18**, 701–704 (1913); Landsteiner, K. *Handbuch der Biochemie*, Vol. 1, 492–494 (Jena, Fischer, 1910).
16. Ehrlich, *Collected Studies*, note 8, p. 581.
17. *Erq.Allq.Pathol.* **11**, 515–644 (1907), p. 565.
18. Rose, N. R. Personal communication (1986).
19. Elschnig, A. *Albrecht von Graefes Arch. Ophthalmol.* **76**,509–546 (1910); Collins, R. C. *Am J. Ophthalmol.* **36**, 150–162 (1953).
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25. Witebsky, E. *Ann. NY Acad. Sci.* **59**, 168–181 (1954), p. 173.
26. Witebsky, E. & Rose, N. R. *J. Immunol.* **76**, 408–414 (1956); Rose, N. R. & Witebsky, E. *J. Immunol.* **76**, 417–427 (1956).Almost simultaneously, Roitt *et al.* published similar findings in human Hashimoto's disease [*Lancet* **2**, 820–822 (1956)].
27. I have expanded elsewhere on the conceptual changes that occurred during immunology's first century in Silverstein, A. M. *Cell. Immunol.* **132**, 515–531 (1991).
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37. The concept of immunological tolerance was implicit in Ray Owen's report on chimerism [Owen, R. D. *Science* **102**, 400–401 (1945)], made explicit in F. M. Burnet & F. Fenner's *The Production of Antibodies* 2nd edn (Macmillan, New York, 1949), and proved experimentally by R. E. Billingham, L. Brent, & P. B. Medawar [*Nature* **172**, 603–606 (1953)]. The demonstration earned for Burnet and Medawar the Nobel Prize in Physiology or Medicine for 1960.
38. For example, Bergsma, D. & Good, R. A. (eds) *Immunological Deficiency Diseases in Man* (National Foundation, New York, 1968).
39. Burnet, F. M. *The Clonal Selection Theory of Antibody Formation* (Cambridge University Press, London, 1959).