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Leblond C P & Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. NY Acad. Sci.* 55:548-73, 1952.
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Spermatids arise as plain roundish cells but follow a complex evolution to become the elongated sperm cells. Staining of the Golgi region and its products with the periodic acid-Schiff technique makes it possible to precisely define 19 developmental stages in rat spermatids. Furthermore, since the spermatids at each step are associated with other distinct cell types, they have been used to divide the cycle of the seminiferous epithelium into definite stages (14 in the rat). [The *SCI*® indicates that this paper has been cited in over 555 publications since 1955.]

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In the spring of 1949 I was visited by Yves Clermont, a candidate for graduate work in anatomy. I proposed to him the following problem:

> Fifty years ago in Paris, C. Regaud reported that the cells in the rat testis were grouped in distinctive cell associations, and the number of these associations was limited.[1] He proposed two postulates: (a) in any region of the seminiferous epithelium, the various cell associations succeed one another *in time* and the sequence repeats itself indefinitely; this sequence is the *cycle of the seminiferous epithelium*; and (b) the associations also succeed one another in space along the length of each seminiferous tubule in the same way as in time; the space sequence is the *wave of the seminiferous epithelium*. However, Regaud's work is a mixture of fact and brilliant intuitions. It has never been confirmed. Fortunately, there is now a tool that may make it possible to check whether or not his views are correct, and I would like you to use it in reexamining Regaud's postulates.

There was a slide under my microscope. I showed it to him and added:

> In 1948 R.D. Hotchkiss demonstrated that treatment of sections with periodic acid followed by the Schiff reagent results in a specific staining for carbohydrates.[2] My lab assistant, Hans Torunski, has used this procedure to stain sections from paraffin blocks including 40 rat tissues. The results are fascinating, particularly in the testis, in which seminiferous tubules display a variety of patterns. A number of seminiferous tubules have been photographed and placed in what appears to be their natural order. They will be included in a manuscript surveying the stained rat tissues.[3]

The procedure, which soon became popular and is now named the "periodic acid-Schiff technique," was particularly effective in showing the features of spermatids at various stages of development. Within a few months Clermont did a remarkable job in dividing the development of spermatids into 19 steps.[4] When the work was presented at scientific meetings, the general reaction was that the number of steps was excessive. We stuck to our guns, and today our classification of rat spermatids is still in general use.

The developmental steps of spermatids were then used as markers to follow the changes in the "cycle" postulated by Regaud. We could do so with far more accuracy than he could and were able to divide the rat cycle into 14 precise stages. This classification is also in general use today.[5] While we confirmed the validity of Regaud's first postulate, we later found that the second was not correct. The wave does not reproduce the cycle in space; it is less regular and generally more complex than the cycle.[6]

During the 50 years that followed Regaud's work, there had been little interest in the subject and his terminology had fallen into disuse. The editors of the *Annals of the New York Academy of Sciences* agreed to include a glossary at the end of the article. The terms, as defined in the glossary, are still in common use.

When this work was being done, there was little interest in the testis. We expected that, as often happens with morphological studies, our article would only be a "voice in the desert." This was not to be. Just about that time, a wide interest in the effects of radiation was developing and, since the testis was one of the most radiosensitive structures, sperm precursor cells elicited great interest. To our surprise, our 300 reprints of the article were soon gone!

1. **Regaud C.** Étude sur la structure des tubes séminifères et sur la spermatogénèse chez les mammifères (Study on the structure of seminiferous tubes and spermatogenesis in mammals). *Arch. Anat. Micr.* 4:101-56; 231-380, 1901. (Cited 85 times since 1955.)
2. **Hotchkiss R D.** A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.* 16:131-41, 1948. (Cited 870 times since 1955.)
3. **Leblond C P.** Distribution of periodic acid-reactive carbohydrates in the adult rat. *Amer. J. Anat.* 86:1-50, 1950. (Cited 210 times since 1955.)
4. **Leblond C P & Clermont Y.** Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid technique." *Amer. J. Anat.* 90:167-216, 1952. (Cited 375 times since 1955.)
5. **Parvinen M, Vihko K K & Toppari J.** Cell interactions during the seminiferous epithelial cycle. *Int. Rev. Cytol.* 104:115-51, 1986.
6. **Perey B, Clermont Y & Leblond C P.** The wave of the seminiferous epithelium in the rat. *Amer. J. Anat.* 108:47-77, 1961. (Cited 140 times.)