To: Study Files of Riker Experiments Nos. 0680TR0008
0680TR0010
0680TR0020
0681TR0095
0681TR0110
0680RR0018

From: E. G. Lamprecht

Subject: Fetal Rat Lens Artifact - Summary of Developments to Date

Date: November 6, 1981

A fetal rat lens artifact was incorrectly interpreted as a teratogenic change in Riker Experiment Nos. 0680TR0008 and 0680TR0010. This memo will summarize the events from the first time the change was reported to the present, including underlying factors contributing to the incorrect interpretation.

A lens change was first formally reported on December 18, 1980, (Riker Experiment No. 0680TR0008). On January 23, 1981, a similar change was reported (Riker Experiment No. 0680TR0010). The change was labeled a teratogenic effect in each of the two studies. The change was described as a developmental eye abnormality which appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus, followed by secondary aberrations of the secondary lens fibers of the fetal nucleus. All eye abnormalities were localized to the area of the embryonal lens nucleus, although a variety of morphological appearances were present within that location. The range of morphological appearances as observed under the dissecting microscope varied from a slight discoloration running through the lens, to a discoloration of part of the lens and the presence of a cleft beneath the lens epithelium. Histologically, the discolorations were attributed to the presence of lens vesicle remnants forming clefts or lens vesicle remnants surrounding the lens nucleus. Also contributing to the discolorations were primary lens fibers which appeared to have not elongated and the presence of degenerated epitrichial cells.

The laboratory continued to report the clefts at the anterior central portion and the dark colored areas at the embryonal nucleus portion of the fetal rat lenses in Riker Experiment Nos. 0680TR0020, 0681TR0095 and 0681TR0110. A noted difference between these three studies and the two mentioned in the previous paragraph was the appearance of the lens change in the control groups of these three studies and not in the first two studies.

A select set of freehand sections containing lens from each of the five studies were processed, sectioned, and stained for histopathology. Generally, the specimens chosen were from fetuses with the lens change. An effort was not made to process for histopathology a comparable number of unaffected lenses of control and compound exposed fetuses. In addition to the selective and

somewhat undisciplined approach taken to evaluate the change, the scientific literature neither discusses the observed change nor does it describe the normal morphology of the near term fetal rat lens. The literature which was available actually contributed to the incorrect interpretation. An example of this is the reference quoted in the first two studies (Hamai Y, Kuwabara T:Early cytalogic changes of Fraser cataract. An electron microscopic study. Investigative Ophthalmology 14 (7):pp 517-527, 1975) which described a developmental lens abnormality appearing in the weanling mouse lens as a cataract. The degeneration seen histologically in teratology study lenses looked similar to that portrayed in the Fraser cataract reference. However, in Riker Experiment No. 0680TR0020, it was found that the lens change in the fetal rat did not continue on to become an opacity in the weanling. Upon finding that the change seen in the fetal lens did not progress to a change in the weanling lens, it was obvious that labeling the change in Riker Experiment Nos. 0680TR0008 and 0680TR0010 as a teratogenic response was in error.

Haskell Laboratory became interested in Riker Laboratories' teratology studies because of their association with a major consumer of a member of the chemical family being studied. Two of their laboratory staff, Dr. Robert E. Staples and Dr. Chieu, were updated on the findings of Riker Experiment Nos. 0680TR0008 and 0680TR0010 (3/27/81). Haskell Laboratory elected to conduct a teratology study closely paralleling Riker Experiment No. 0681TR0110. The preliminary results of these parallel studies were exchanged (10/9/81). Differences in both the lens morphology findings and their interpretations were acknowledged and a subsequent meeting of the scientists was arranged (10/19-20/81) to clarify each laboratories' findings. At that meeting the two factors were found, in part, to account for the differences in findings between the two teratology laboratories. Riker Laboratory was more successful in bisecting the lens on freehand sections than was Haskell Laboratory, and the equipment used by Riker to illuminate the freehand lens sections enabled superior visualization of the change compared to that achieved with Haskell Laboratories' equipment. These differences in technique and equipment resulted in Riker describing a lens cleft and dark streak in fetal rat lenses of all dose groups, while Haskell reported a much lower incidence of only a lens air pocket.

Histopathology on fetal lenses was done in each laboratory and again the results differed. Riker reported a degenerative change in the embryonal nucleus, while Dr. William Kerns at Haskell reported an artifact of fixation or of freehand sectioning. Both results were reported without the benefit of available literature references describing the normal morphology of the term fetal rat lens. On 10/20/81, two individual scientists (Dr. Howard A. Hartman, Sandoz Corporation, and Alfred A. Coulombre, The National Institute of Neurological Diseases and Blindness, National Institute of Health) were queried to help resolve the differences in interpretations. Through their pooled resources, it was determined that the embryonal nucleus of the fetal rat has an anterior placement and is still opposed to the lens epithelial cells near term. In addition, the embryonal nucleus is in the process of degenerating. Haskell Laboratories' interpretation was correct. The change seen in each laboratory was an artifact.

The lens changes seen by Riker were either normal developmental changes or were artifacts caused by freehand sectioning. The cleft was a space opened up at the vestige of the lens vesicle remnant and consisted of a separation of primary lens fibers of the embryonal nucleus from the lens epithelial cells. The dark streak discoloration of the embryonal nucleus resulted from either the lens being freehand sectioned across the area of normal primary lens fiber degeneration or an artifact being created in the lens during freehand sectioning accentuating the area of normal primary lens fiber degeneration.

In summary, a fetal rat lens artifact was incorrectly interpreted as a teratogenic change in Riker Experiment Nos. 0680TR0008 and 0680TR0010. The change was an artifact created by freehand sectioning across the embryonal nucleus. Contributing to the incorrect interpretation were the lack of available literature references describing the normal morphology of the term fetal rat lens and the initial selective histopathological approach to the interpretation.

Elden G Lamprecht

E. G. Lamprecht
EGL:lam

cc: M. T. Case
E. G. Gortner