Methods for Identification and Extraction of Pigment in Rat Eyes[1]

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The purpose of this paper is to describe the methods used in identifying the pigment in rat eyes and the extraction method for preparing crude pigment for quantitative comparison of the pink, ruby and black eye.

Methods and Materials

The rats used had these eye and coat color combinations: dark eyes and agouti coat, dark eyes and black coat, ruby eyes and yellow coat, pink eyes and yellow coat. Upon removal, the eyes of the black and agouti animals were observed to be black and heavily pigmented, although not necessarily equally. The ruby eyes of the yellow animals were brown and less heavily pigmented; the pink eyes of the yellows appeared entirely lacking in pigment.

Previous work assumes the type of pigment present to be melanin, but no evidence to support this was available. In oxalic acid, potassium permanganate bleaching (Humanson, 1962), a dark eye from an agouti, a ruby eye from a yellow, and a pink eye from a yellow were compared with each other and with known reactions of common pigments.

The melanin specific histological technique of carmalum and protargol staining (Sichel, 1952) was employed. The eyes, which were stored in formaldehyde prior to fixation, were of the same types used in bleaching.

For further study, a method for extraction of the pigment was desirable. An attempt to utilize the extraction method applied on cow eyes by Stein (1954) failed; and this procedure was modified. Pairs of eyes, minus their lenses and humors, were placed in 2 M HCl and refluxed for fifty hours; the acid soluble materials were decanted; and the pigment remaining was washed with 5 N NaOH and distilled water. The water was then evaporated at room temperature. The crude pigments from the dark and ruby eyes remained as similar shiny black precipitates. No precipitate remained in flasks containing pink eyes.

Results and Discussion

The oxalic acid, potassium permanganate bleaching indicated that no pigment occurred in the pink eye and that the pigments in the ruby and dark eyes both reacted in a manner similar to melanin.

Strong positive results for melanin were obtained in the protargol and carmalum slides. In addition, the slides showed that the pigment occurred in a distinct layer, that of the black eye being much thicker than that of the ruby. No pigment occurred in the pink eye from the yellow animal. The latter case differed from the observations of Dunn (1921).

Two reasons for the necessary modification of Stein's extraction method have been suggested. The rat melanin may not have been soluble in 5 N NaOH; a more acceptable explanation was that the rat eye formed "human" more readily than the cow eye when refluxed in NaOH (Stein, 1962). The modified method apparently eliminated this problem because "human" was not formed during the HCl refluxing.

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SUMMARY

Histological tests indicated that the pigment in rat eyes was melanin. A crude precipitate of melanin was obtained from dark and ruby eyes by refluxing in 2 M HCl. No pigment was found in pink eyes.

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