

3. Rowe, W. P., Capps, W. I., J. Exp. Med., 1961, v113, 831.
4. Molomut, N., Padnos, M., Smith, L. W., J. Nat. Cancer Inst., 1965, v34, 403.
5. Bueker, E. D., Schenkein, I., Ann. N. Y. Acad. Sci., 1964, v118, 183.
6. Metcalf, D., Brit. J. Cancer, 1956, v10, 442.
7. Angeletti, P. U., Salvi, M. L., Capani, F., Frati, L., Biochim. Biophys. Acta, 1965, v111, 344.

Received June 5, 1967. P.S.E.B.M., 1967, v126.

Buphthalmia in the Rabbit: Effect of Age and Repeated Testing on the Cornified Cell Count Diagnostic Technique.* (32405)

E. J. BABINO, JR. AND R. R. FOX (Introduced by N. Kaliss)

The Jackson Laboratory, Bar Harbor, Maine

Buphthalmia in the rabbit is believed to be an autosomal recessive mutation (*bu*) (1) with incomplete penetrance (2). It is of great interest because of its resemblance to congenital glaucoma in humans. Fox and Babino (3) reported a technique for the early identification of buphthalmic rabbits (*bu/bu*) based on the degree of corneal cornification. Their procedure involved the enumeration of cells removed from the intact anesthetized rabbit eye. From their data they concluded that in the normal (+/+) eye an increase in the number of cornified cells was observed at puberty. The number then declined and stabilized. They also reported that testing the same eye at one-week intervals resulted in an increasing number of cells counted with each successive test. Both of these observations, however, were based on limited data. The present study was made to determine more accurately the effect during the first year of age and the effect of repeated testing on the cornified cell counts, using the normal (+/+) rabbit eye.

Materials and methods. One hundred forty-two normal (+/+) rabbits from The Jackson Laboratory rabbit colony were used. These were all from Strain III, a partially inbred population of New Zealand White origin. Cell counts were made by the procedure of Fox and Babino (3). In brief, this procedure consists of swabbing the anesthetized eye to remove the cornified cells, placing

these cells on a clean glass slide, staining the cells with Giemsa, and counting the number and type of cells present in a given microscopic field. The individual eye was used as the unit of observation. Only healthy appearing eyes were used. Two studies were made. In the first study on the effect of age on the cornified cell count, observations were made on 262 eyes at 24 different ages ranging from 22 to 360 days of age. Each eye was assayed only once. In the second study on the effect of repeated testing on the cornified cell count the 22 eyes used were assayed 4 times with one week between each assay. The repeated measurements were made during a stable prepuberal period, from 50-80 days of age [see Fig. 1 and (3)].

Results and discussion. The mean number of cornified cells per eye at ages up to one year is shown in Fig. 1. The overall mean was 22.4. This compares well with the mean of 20.6 previously reported (3). The picture in general is one of stability from 35 to 100 days of age, an unstable period of higher counts at puberty, followed by subsequent stability with the exception of one eye with a count of 100 at 245 days. The cause of this high count is unknown. Beginning infection of the lacrimal duct or other factors might be involved.

The effect of repeated measurements on the cornified cell count is shown in Table I. From the data and the statistical analysis it may be seen clearly that repeated testing at one-week intervals has no observable effect on the cornified cell count when taken during this stable prepuberal period.

* This investigation was supported by USPHS Research Grants HD-01496 from Nat. Inst. of Child Health & Human Development, and FR-00251 from Division of Research Facilities & Resources.

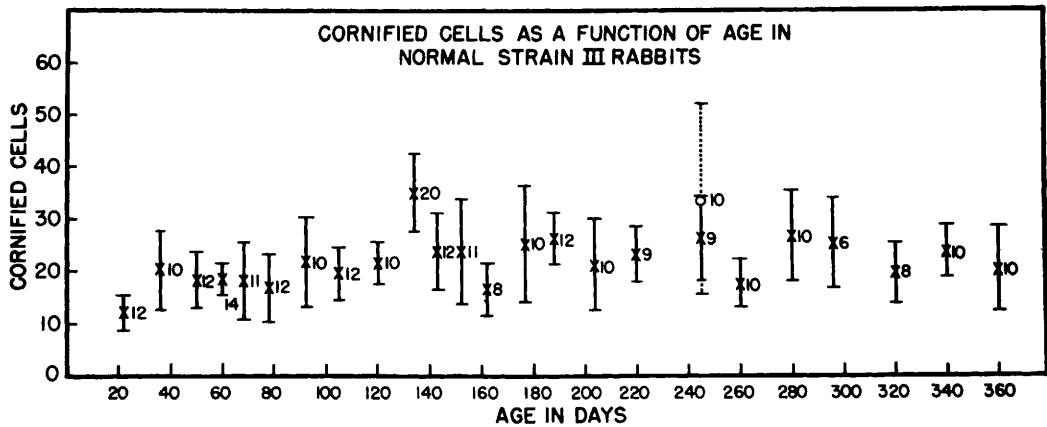


FIG. 1. Number of cornified cells as a function of age in normal strain III rabbits. Graph gives mean (X), 95% confidence interval, and number of eyes examined at each age.

TABLE I. Effect of Repeated Testing at Weekly Intervals on Number of Cornified Cells per Microscopic Field, 150 $\times$ from the Normal Rabbit Eye.

Parameter	1st wk	2nd wk	3rd wk	4th wk
n	20*	22	22	22
$\bar{X}$ †	13.3	10.6	13.3	13.2
95% confidence interval	(9.3-17.2)	(8.0-13.1)	(7.6-18.9)	(9.8-16.6)

* 2 slides were lost.

† Analysis of variance showed no significant difference due to treatment at the .05 level of significance.

It is suggested, therefore, that for diagnostic work at least 2 counts be made on each eye at a one-week interval and if the two values differ greatly that a third be also made.

Summary. With the exception of the puberal period, age has little effect on degree of cornification of the rabbit eye. Also the cornified cell count is not changed by repeated testing

at one-week intervals during the prepuberal period from 50 to 80 days of age.

1. Nachtsheim, H., *Erbpathologie des Kaninchens: Der Erbartz*, 1937, v4, 25 and 50.

2. Hanna, B. L., Sawin, P. B., Sheppard, L. B., *Genetics*, 1962, v47, 519.

3. Fox, R. R., Babino, E. J., Jr., *Proc. Soc. Exp. Biol. & Med.*, 1965, v119, 229.

Received June 7, 1967. P.S.E.B.M., 1967, v126.