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Buphthalmia in the Rabbit: A Test for Early Diagnosis.* (30144)

R. R. FOX AND E. J. BABINO, JR.† (Introduced by N. Kaliss)
The Jackson Laboratory, Bar Harbor, Maine

Studies on buphthalmia in the rabbit indicate that a reliable technique for identification of the mutant genotype in early or prepathological stages is needed. Hanna *et al* (1) state that buphthalmia is due to a single recessive gene (*bu*) with incomplete penetrance. They also suggest that buphthalmia may be part of a primary systemic disorder. Kolker *et al*(2) report that at early ages the corneal diameter is not a reliable index of buphthalmia and that the intraocular pressures fluctuate considerably, particularly in the buphthalmic rabbits. The authors show a difference in the facility of outflow, as measured tonographically, for the two groups. They point out that their data are based only on small numbers of animals.

Unpublished observations in this laboratory on corneal diameters, and by Dr. L. B. Sheppard, Virginia Medical School, Richmond, on intraocular pressure corroborate the variability reported by Kolker *et al.* Marked strain differences among buphthalmics were also observed, strain AX having larger corneal diameters while those of strain III were considerably lower and unreliable. It was the experience of one of us (RRF) that the penetrance of buphthalmia appeared to be decreased by a dietary change made in 1959. We therefore surveyed clinical assays for nutritional deficiencies as possible diagnostic tests for buphthalmia.

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Huber and Smith(3) reported a technique for field diagnosis of bovine vit. A deficiency based on degree of cornification of the bulbar conjunctiva. Since the symptoms of vit. A deficiency in calves were similar to some of the observations made on buphthalmic rabbits it was felt that modification of this technique might provide a means for identification at preclinical stages. A procedure developed for this purpose is presented below.

Materials and methods. Animals of 3 types from The Jackson Laboratory's rabbit colony [*i.e.*, those which were (a) phenotypically buphthalmic (AX strain), (b) genetically but not phenotypically buphthalmic (III_{bu}, subline of strain III), and (c) known normals representing strain III_{mo} (another subline of strain III) and the small strains X, OS, AC, and ACEP] were used in this study. The rabbits' eyes were anesthetized with 2 drops 3% cocaine in normal saline solution (NSS) per eye. The eyelids were closed and massaged gently to insure a uniform distribution of liquid. The eyelids were held open and a probe (Fig. 1) moistened with 0.9% NaCl was placed lightly on the center of the cornea and rotated 180°. Subsequently the probe was placed lightly (*not streaked*) on a dry microscope slide that had just been removed from 95% ethyl alcohol. The slide was dried by a hot air blower for 3 minutes and stained with Giemsa (1 part stock : 20 parts distilled water) for 30 minutes, rinsed with distilled water, and dried with the blower for 20 minutes. The complete field of cells was first centered with a 3× objective and then observed under a 10× objective (Wild M-20 microscope with 15× wide field ocu-

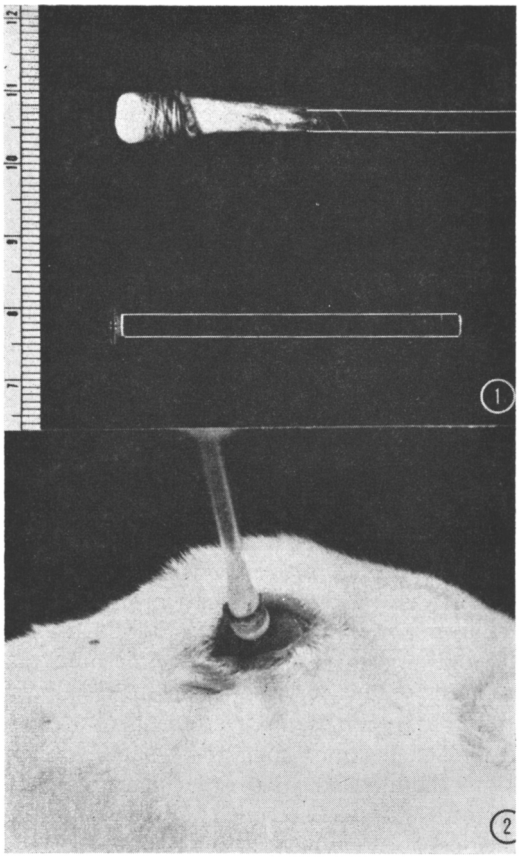

FIG. 1. (1) Probe used for removing cornified cells from intact anesthetized rabbit eye. Shows 2.5 mm glass rod flattened on one end and ground smooth and same with cotton swab attached with rubber band. (2) Position of probe during removal of cells from intact eye.

lars). The total number of cornified cells (Fig. 2) was counted and recorded, using the eye and not the animal as the unit of observation since a few cases of unilateral buphthalmia are included.

Results. The mean number of cornified cells per eye is shown in Table I. Table II shows the t values for cornified cell incidence in comparing control and buphthalmic animals. There were no significant differences ($P = .05$) between any of the control populations. This was also true when comparisons were made of those eyes which were phenotypically buphthalmic and those which were genetically buphthalmic (*i.e.,* progeny from a mating between 2 buphthalmics) but lacking the phenotypic manifestations of the character (*i.e.,* clouding, bulging, an increased corneal diameter of the eye). It should be noted, however, that this latter category is based on only 4 eyes. On the other hand, in the III_{bu} subline where genetically determined buphthalmics should be present, high cornified cell counts were found in animals which appeared phenotypically normal. Such animals could presumably be considered buphthalmics. This was not observed in normal strains where buphthalmics have not been recorded. Study of the genetic segregation of the *bu* gene based on cell cornification is in progress. In all cases where buphthalmics (either genetically determined or phenotypically recognizable) were compared with normals the buphthalmics had approximately a 3-fold greater cornified cell count which was statistically significant ($P<.001$). The high variance observed in the AX strain (adult and pooled) in the total buphthalmic population is due to the exceptionally high counts for the eyes of one 9-month-old doe phenotypically buphthalmic at one month of age. It was decided not to separate these two observations from the rest since their deletion would not change the mean from that of the young AX strain buphthalmics and would only reduce the variance. The resultant increase in precision was not necessary since the differences between buphthalmics and normals were already significant ($P<.001$).

Fig. 3 shows the degree of cornification as a function of age in normal strain III rabbits.

Discussion. Of major significance are the following: 1. Our diagnostic test shows that both phenotypically buphthalmic eyes and normal appearing (*bubu*) animals have a high degree of cornification. 2. In normal unrelated strains or sublines where buphthalmia has never appeared a high degree of cornification was not observed. 3. Among the siblings of phenotypically buphthalmic animals there are normal appearing rabbits also with a high degree of cornification. If these are genetically buphthalmic without the usual phenotypic manifestations, this would account in part at least for the deficiency of

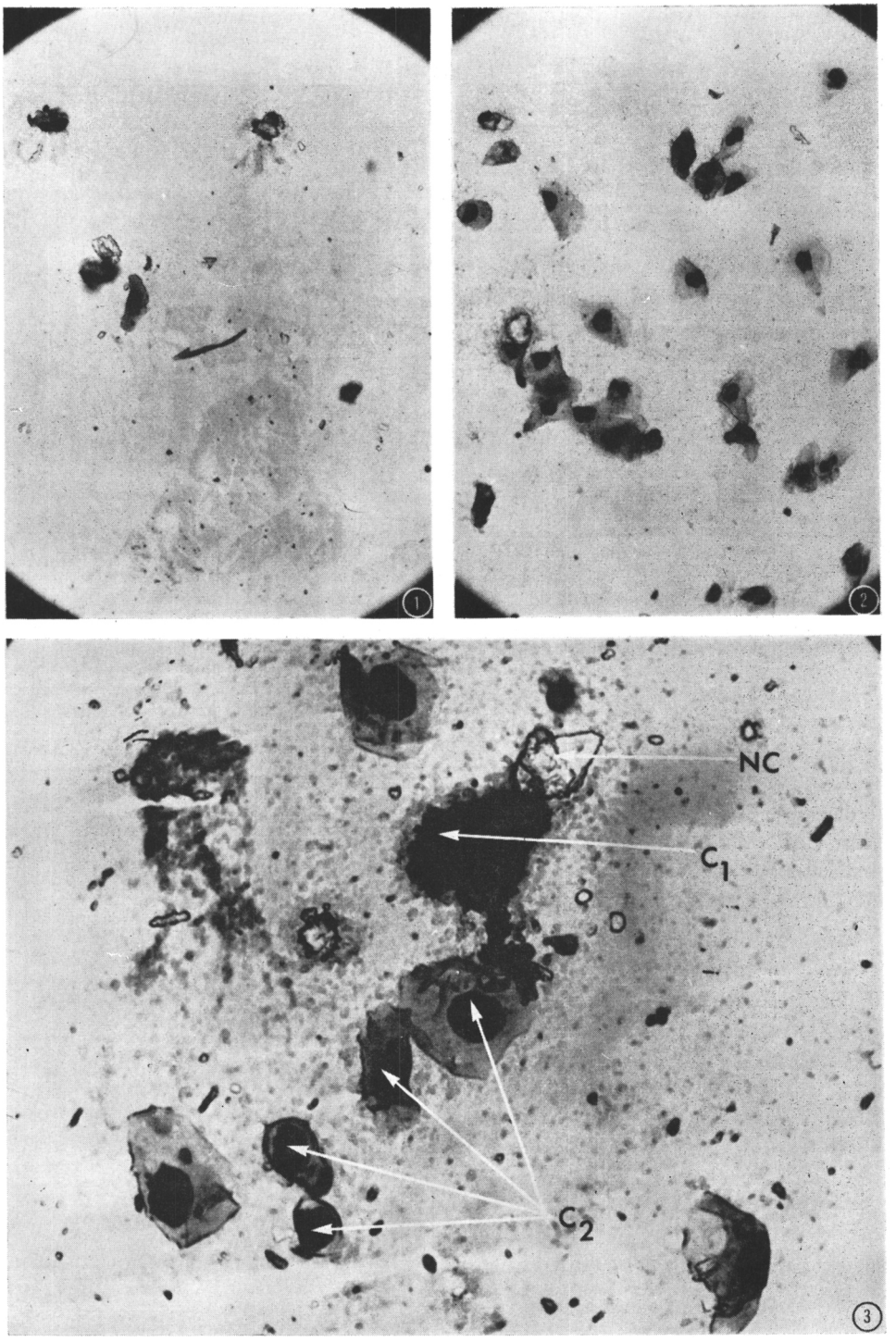

FIG. 2. (1) Representative field of cornified cells from normal rabbit. (2) Representative field of cornified cells from buphthalmic rabbit (full sib of normal in (1) and taken at same age). (3) Higher power field with typical cells labelled according to our system of classification. N.C.: Not cornified—clear unstained particles almost jelly-like in appearance. C_1: Cornified—darkly stained cells; often difficult to distinguish nucleus and cytoplasm. C_2: Cornified cells with darkly stained pycnotic nuclei and lightly stained cytoplasm; various degrees of folding over of the cytoplasm.

TABLE I. Number Cornified Cells/Microscopic Field (150×).

	Rabbit strain (origin)	Mean/eye	No. of eyes	Variance
Controls	Total	20.6	81	124
	ACEP (Dutch)	19.9	8	154
	AC (Dutch)	29.1	8	330
	X (Castle's small strain)	22.8	12	97
	OS (Rockefeller Inst. 1948)	18.7	10	121
	Pooled small strains	22.4	38	165
	III (New Zealand White Castle '32 mo subline)	18.9	43	86
Buphthalmics	Total	58	30	954
	AX* (outcross of Chinchilla strain V to strains III & X)			
	young animals	50.8	18	314
	adults	71.8	8	2661
	pooled AX strain	57.3	26	1055
	III† (New Zealand White Castle '32 bu subline)	62.8	4	394
Unilateral cases	AX strain			
	buphthalmic eye	55.5	2	60
	normal eye	36.5	2	4

* Animals which phenotypically are classified as buphthalmics based on bulging of the cornea and in some cases clouding.

† These animals are phenotypically normal but genetically buphthalmic.

buphthalmics reported by Hanna *et al*(1). 4. It is also apparent from these data based on initial counts that this phenomenon of increase in cornification in buphthalmic rabbits is not only similar to that reported for vit. A deficient cattle(3) but that there was a 3-fold increase in both abnormal groups. The inference from this and other data not reported herein of a possible relationship between buphthalmia and vit. A metabolism is under investigation.

There is a definite tendency for repeated measurements on the same eyes to give slightly higher counts. These differences were

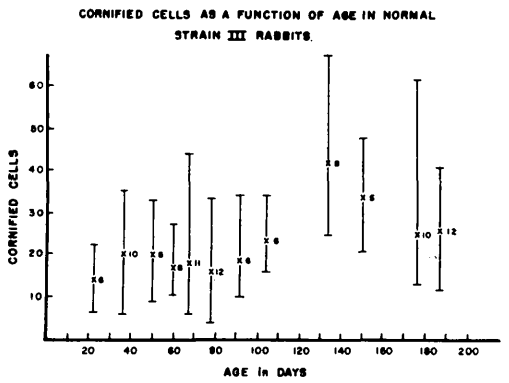

FIG. 3. Number of cornified cells as a function of age in normal strain III rabbits. Graph denotes mean (×), total range of observations, and number of eyes examined at each age.

TABLE II. Cornified Cell Incidence in Control *vs* Buphthalmic Animals.

	Degrees of freedom	Value of "t"
Between control groups		
ACEP *vs* AC	14	1.192
vs X	18	.577
vs OS	16	.204
AC *vs* OS	16	1.424
vs X	18	.900
OS *vs* X	20	.911
III_{mo} *vs* ACEP	49	.196
vs AC	49	1.545
vs X	53	1.218
vs OS	51	.054
Between buphthalmic groups		
AX* adults *vs* young	24	1.123
AX *vs* III_{bubu}†	28	.466
Unilateral (normal appearing eye *vs* buphthalmic)	2	3.333
Controls *vs* buphthalmics		
III_{mo} *vs* III_{bubu}†	45	4.390‡
Pooled small strains *vs* III_{bubu}†	40	3.990‡
III_{mo} *vs* pooled buphthalmics	71	6.627‡
Pooled small strains *vs* pooled buphthalmics	66	5.836‡
Pooled control *vs* pooled buphthalmics	109	6.448‡

* Animals which phenotypically are classified as buphthalmics based on bulging of the cornea and in some cases clouding.

† These animals are phenotypically normal but genetically buphthalmic.

‡ ($P < .001$). All other "t" values in table are not significant at the .05 level.

not significant; however, this is still under investigation and all data reported herein are based solely on initial readings. Significant differences were observed between normal and buphthalmic animals as early as 35 days of age.

It should be noted that even though the AX strain was not used as a control it contained phenotypically normal animals with cornified cell counts analogous to the other normal animals. These were not used since it has not been determined at this point whether there is an effect in the heterozygote (*Bubu*) and we did not therefore wish to confound the data. The III strain control which is known not to contain the buphthalmic gene is a different subline from that in which the buphthalmics were observed.

There appears to be an increase in number of cornified cells with age in the early weeks of life followed by a plateau 5 to 14 weeks with a subsequent increase to a peak at about 130 days. The numbers then decline and stabilize. The peak at 134 days might be due to chance variation or possibly to a metabolic upset occurring concurrently or caused by reaching the age of puberty. Two months has now been taken as the standard age for observations because of the apparent stability at this early prepuberal age.

Summary. Pathological and preclinical stages in the buphthalmic rabbit's eye can be distinguished by the degree of corneal cornification. This is determined by enumeration of cells removed from the intact anesthetized eye. Five normal strains and 2 buphthalmic strains were investigated. The buphthalmics had cell counts significantly higher than those of genetically normal animals. No significant differences were observed either between any of the control strains or between buphthalmic groups.

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Chromosome Changes in PPLO-Infected FL Human Amnion Cells.* (30145)

JØRGEN FOGH AND HELLE FOGH (Introduced by Vernon Riley)
Division of Experimental Pathology, Sloan-Kettering Institute for Cancer Research, New York, and the Sloan-Kettering Division, Cornell University Medical College, New York City

Several reported effects of mycoplasma (pleuropneumonia-like organisms, PPLO) are very similar to virus effects. These include transmissable cytopathic effects(1,2,3) and ultrastructural changes(1) in cultured mammalian cells, hemagglutination(4), and change of host cell surfaces which makes them capable of hemadsorption(5). This report extends the observations on the similar capacities of PPLO and viruses. A study of FL human amnion cells(6), following PPLO infection, has shown the mycoplasma able to affect the genetic composition of the mammalian cells.

Major changes in the chromosomes of FL cells, cultured continuously for 9 years, have not occurred since the first analysis in 1957 (10). Chromosome numbers were determined again in 1961(11), more extensively in 1962(7), and, in addition, from control preparations for the present study. Other characteristics which distinguish these transformed human cells from the normal human karyotype have also been reported(7).

Materials and methods. A strain of PPLO (3) which we isolated from tissue cultures demonstrated the characteristics of mycoplasma by agar cultivation and electron microscopy and was sensitive to aureomycin.

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