RECESSIVE BUPHTHALMOS IN THE RABBIT¹

BERTRAM L. HANNA,² PAUL B. SAWIN³ AND L. BENJAMIN SHEPPARD⁴

Received September 8, 1961

BUPHTHALMOS (hydrophthalmos, congenital infantile glaucoma) in rabbits has been of interest to European geneticists but has attracted little attention in the United States despite its recurrent appearance in laboratory and commercial breeding stocks. This condition is of particular interest to the field of experimental ophthalmology because of its similarity to congenital glaucoma in humans.

The earliest report of rabbit buphtalmos appears to be that of SCHLOESSER (1886), who presented the detailed histopathology of the left eye of a brown rabbit which developed an acute glaucoma following irritation of both corneas to induce traumatic cataract. Other single case reports are by PICHLER (1910), ROCHON-DUVIGNEAUD (1921) and BECKH (1935), although in the last case the buphtalmos may have been secondary to a yaws infection. VOGT (1919), reported the occurrence of buphtalmos bilaterally in three siblings purchased at nine months of age. A mating between two of these produced a litter of three, all of which developed high grade buphtalmos. NACHTSHEIM (1937) and GERI (1954, 1955) studied the inheritance of buphtalmos and concluded that it is transmitted as an autosomal recessive trait. FRANCESCHETTI (1930) noted a deficiency of affected offspring from matings of heterozygous carrier parents. GERI (1955) found 12.5 percent affected offspring from carrier matings and suggested that the deficiency results from fetal death of buphtalmic animals. MCMASTER (1960) reported a mating of two animals with bilateral buphtalmos which produced a litter of seven, only four of which were affected.

Although histologic abnormalities of the eye may be noted at birth (Figures 1-4), buphtalmos may not be diagnosed by gross examination at this time. The condition may be diagnosed at two to three weeks of age in many affected animals but appears later in others of the same family. It appears initially as a light cloudiness on the posterior aspect of the cornea, which appears bluish in color and velvety in texture. This is followed by progressive opacity and flattening of the cornea, ectasia of the eyeball, particularly in the sclero-corneal region, deepening of the anterior chamber with detachment and fragmentation of the iris mem-

¹ Supported in part by PHS grant B-772 from the National Institute of Neurological Diseases and Blindness, Public Health Service and by grants from the Alfred P. Sloan Foundation and the A. D. Williams Research Fund, Medical College of Virginia.

² Department of Biology and Genetics, Medical College of Virginia and Human Genetics Section, Clinical Investigations Branch, National Institute of Dental Research, National Institutes of Health, Bethesda 14, Maryland.

³ Hamilton Station, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

⁴ Department of Ophthalmology, Medical College of Virginia, Richmond 19, Virginia.

FIGURE 1.—Sagittal section of normal adult rabbit eye (30 $\times$) showing lens (L), iris (I), ciliary process (CP), space of Fontana (SPF), ciliary body (CB), ora serrata (OS), episcleral veins (EV), and sclera (SC). The rectangular area shows the zone of the trabecular canal and intrascleral channels.

FIGURE 2.—Sagittal section of eye of normal newborn rabbit (130 $\times$). Note open angle (A), trabecular canal (TC), episcleral veins (EV), iris pillar (IP), and space of Fontana (SPF). Cornea (C), sclera (S), ciliary body (CB), ciliary process (CP), iris (I) and detached retina (R).

FIGURE 3.—Sagittal section of adult buphthalmic rabbit eye (130 $\times$). Note open angle (A), rudimentary trabecular canal (RTC), absence of space of Fontana and iris pillar. Cells in sclera (S) suggest underdeveloped intrascleral channel. Iris (I), ciliary body (CB), cornea (C).

FIGURE 4.—Sagittal section of eye of newborn rabbit from carrier parents; selected for study because of a corneal diameter significantly greater than that found in normal newborns. (200 $\times$). Note closed angle, absence of space of Fontana, iris pillar, trabecular canals and intrascleral channels. Ciliary process (CP), ciliary body (CB), cornea (C) and sclera (S).

brane, partial atrophy of the ciliary body and glaucomatous excavation of the papilla. The protrusion from the orbital cavity may become so pronounced with increasing age that the eye cannot be closed. The corneal opacity may lead to blindness. Traumatic rupture of the cornea may occur, followed by scarring and/or vascularization. Buphthalmos may occur either unilaterally or bilaterally. NACHTSHEIM (1937) and GERI (1954, 1955) reported difficulty in breeding affected animals, which appeared to suffer from loss of general health, appetite and libido.

The primary defect responsible for the development of buphthalmos is not known, but various experiments (SMITH 1944; GREAVES and PERKINS 1951; AURICCHIO and WISTRAND 1959; McMASTER 1960) suggest an abnormality of the drainage mechanism which results in an inability to maintain normal fluid relationships within the eye. This view is further supported by preliminary histologic findings, which demonstrate an absence of the space of Fontana, the iris pillars and either total absence or a rudimentary development of the trabecular canals and intrascleral channels (Figures 3, 4). (See also BABEL 1944.)

Buphthalmic rabbits have occurred since 1946 in the New Zealand white stocks maintained at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. These stocks have been bred for the maintenance of genetic traits other than buphthalmos. The relationships among the affected animals in one small segment of the stock are shown in Figure 5. Affected rabbits also occur in the New Zealand white breeding colony at the National Institutes of Health where, despite stringent selection against buphthalmos, the frequency of occurrence continues to increase. Attempts to breed affected animals at Bar Harbor have generally failed, but several such matings have been successfully made at the N.I.H. The litters from such matings are sometimes deserted, trampled or destroyed by the blind mother. Of the litters which have survived until diagnosis may be made, some have contained all affected and others only a few affected animals.

In the Bar Harbor stock both a deficiency of affected offspring from carrier mat-

FIGURE 5.—Relationships among buphthalmic animals of the Ax, III and IIIc lines, New Zealand white stock at Hamilton Station.

ings and the occurrence of normal offspring from buphthalmic by buphthalmic matings have been observed. In addition an excessive number of carrier matings have produced no buphthalmic offspring. The occurrence of buphthalmos in litters appears to be sporadic in repeated matings of the same carrier parents. The data which follow were taken from the breeding and litter records at the Hamilton Station, Jackson Memorial Laboratory, in an attempt to ascertain those variables which might affect the penetrance of the buphthalmic condition in this stock.

Mode of inheritance: It may be seen from Figure 5 that all of the affected animals in this breeding line trace their ancestry to a single female. Most of these animals are connected through both the paternal and maternal ancestry. The fact that some animals do not appear to derive two alleles from the common pool does not invalidate a hypothesis of autosomal recessive inheritance, since the condition is known to have occurred in other stocks introduced to the colony over the years and in other breeder's colonies.

A preliminary analysis of the distribution of buphthalmic animals among the offspring of 16 independently ascertained carrier matings was performed in early 1957, using the method of HALDANE (1932). This method requires ascertainment through affected individuals, so only litters in which buphthalmos occurred were included in the analysis. The probability of buphthalmic offspring estimated from these data was 0.262 with a standard error of 0.054. This probability does not deviate significantly from the 0.25 buphthalmic offspring expected if the trait were autosomal and recessive. A total of 31 independently ascertained sibships now available for analysis gives a probability of 0.103 ± 0.014 . This low probability suggests that buphthalmos is either not expressing itself in some individuals homozygous for the allele, or is lethal in some proportion of affected individuals. The average litter size in the earlier sample was 5.6 but was 7.1 in the later sample.

Expression of buphthalmos: Buphthalmos may occur bilaterally and unilaterally within the same litter. Among 25 affected animals for which this information was recorded, 17 (68%) had bilateral occurrence and eight (32%) had a single eye affected (three right, five left). Only two litters had more than one animal unilaterally affected; in each of these both were left eyes. The occurrence of unilateral or bilateral buphthalmos does not appear to be related to sex ($\chi^2_{(1)} = 0.1, P > .3$).

Birth sex ratio: The sex distributions among the offspring of 65 matings of 27 pairs of known carrier parents are given in Table 1. Thirty-one of these matings produced at least one buphthalmic offspring; 34 matings of the same parents produced no buphthalmics. The proportion of live-born males among the buphthalmic offspring (43.2%) is not significantly smaller than that among the normal littermates (45.2%) ($\chi^2_{(1)} = 0.1$) or that among those offspring born in normal litters to the same parents (47.1%) ($\chi^2_{(1)} = 0.4$).

Occurrence of buphthalmic litters: Table 2 gives the distribution of litters born to proven carrier parents according to litter size (s) and the number of affected individuals (r) per litter. For each litter size from $s = 1$ to $s = 9$ the exact

TABLE 1

The sex distribution among the offspring of 65 matings of 27 known carrier pairs

A. Buphthalmic litters (31)							
Live-born							
	Buphthalmic		Normal		Stillborn		
	Male	Female	Male	Female	Male	Female	Unsexed
Number	19	25	85	103	6	5	2
Proportion	.4318	.5682	.4521	.5479	.5454	.4546	

B. Normal litters (34)					
Live-born			Stillborn		
	Male	Female	Male	Female	Unsexed
Number	106	119	11	27	3
Proportion	.4711	.5289	.2895	.7105	...

TABLE 2

Buphthalmos in rabbits. Numbers of litters of size s having r affected

	s=1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
r=															
0	1	.	5	3	3	4	4	5	3	1	.	1	1	2	.
1	.	1	2	2	3	3	2	3	2	.	3	1	1	.	1
2	.	.	.	.	1	1	1	.	1	.	.	1	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Totals $n_s =$	1	1	7	5	7	9	7	8	7	1	3	3	2	2	1

s=	Probability that, given n_s litters, the observed number of unaffected litters could occur by chance	Probability that, given n_s litters, the observed array of litters could occur by chance
1	.7500	.7500
2	.4375	.3750
3	.0938	.0500
4	.1480	.0564
5	.1583	.0305
6	.0475	.0002
7	.0072	.0100
8	.0004	.00001
9	.0108	.00002

probability that the observed number of unaffected litters could occur by chance has been calculated $[P = \binom{n_s}{u} q^u p^{(n_s-u)}$, where u is the number of affected litters among n_s litters of size s , p is the probability of obtaining a normal offspring from a mating and $q = 1 - p$]. These values indicate that for litters of five or less there is no excess of unaffected litters. For litters of six or more there is an excess of unaffected litters significant at the 0.05 level. The exact probability that the observed distributions or arrays of affected and normal litters could occur by chance has also been calculated for each litter size from one to

nine $[P = \binom{n_s}{a_1, a_2, \dots, a_s} \phi_1^{a_1} \phi_2^{a_2} \dots \phi_s^{a_s}]$, where a_r is the number of litters of size s containing $r = 1, 2, \dots, s$ affected and $\phi_r = \binom{s}{r} p^{s-r} q^r$. The probability values indicate that for litters larger than five there is a deficiency of litters having many affected individuals. These conditions appear to hold also for litters of size greater than nine, but the small numbers of these litters available for examination makes conclusions unreliable. The observations suggest that the fetal death of buphthalmic rabbits may be affected by *in utero* crowding in large litters, with the chance of survival to birth decreasing with an increase in litter size.

It should be noted that these conclusions are based on live-birth frequencies. The actual number of litters of given size conceived is unknown; fetal death and resorption may lead some of these to be smaller at the time of birth. No attempt was made to determine the effect of litter size on the sex ratio, since the number of animals in each class was too small for reliable testing.

Stillbirth frequencies: Of 251 offspring produced by matings of carrier females with noncarrier males, 25 (9.6%) were stillborn (term). Of these, 11 were female and ten were male. Forty-one (15.6%) of 263 offspring in normal litters born to carrier parents were stillborn (27 female, 11 male and three unsexed). In the buphthalmic litters born to these parents only 5.4 percent (13 of 243) were stillborn (six males, five females and two unsexed). A test for heterogeneity of live-born–stillborn frequencies among these samples gives a $\chi^2_{(2)}$ value of 14.2 ($P < .01$); large deviations from the expected values occur in the two stillborn classes for carrier matings (Table 3). A contingency test of the live-born–stillborn ratios in the two types of litters born to carrier parents gives $\chi^2_{(1)} = 12.8$ ($P < .01$). To test the hypothesis that the increased number of stillborn offspring occurring in litters in which buphthalmos does not occur may result from fetal

TABLE 3

Offspring produced by carrier females mated with normal and carrier males classified as (I) live-born and stillborn and (II), live-born and combined buphthalmic and stillborn.

The carrier matings are divided to show the outcome in litters in which buphthalmos does not (A) and does occur (B)

Sire		Offspring			
		Classification I		Classification II	
		Live-born	Stillborn	Normal live-born	Buphthalmic and stillborn
Normal	Observed	226	25	226	25
	Expected	224.8	26.2	209.9	41.1
Carrier (A)	Observed	222	41	222	41
	Expected	235.5	27.5	219.9	43.1
Carrier (B)	Observed	230	13	185	58
	Expected	217.6	25.4	203.2	39.8
		$\chi^2_{(2)} = 14.2$		$\chi^2_{(2)} = 17.6$	

loss of affected animals, a test of the heterogeneity of normal live-born and grouped buphthalmic and stillborn offspring among the samples was made. Test of the heterogeneity among the three offspring groups gives $\chi^2_{(2)} = 17.6$; a deficiency in the observed number of stillborn offspring in normal matings is seen (Table 3). Test of the heterogeneity between affected and unaffected litters from carrier parents gives $\chi^2_{(1)} = 4.98$ ($.05 > P > .02$). These probabilities suggest that there may be an increased late fetal mortality in litters born to carrier parents, perhaps resulting from a greater mortality among buphthalmic offspring. A test of the distribution by sex of 74 stillborn offspring in these three types of matings shows no significant deviation from the expected sex distribution ($\chi^2_{(2)} = 5.4$, $P > .05$).

Maternal age and birth order effects: The relative frequency of occurrence of buphthalmos at different maternal ages and the relationship of litter birth order to the occurrence of buphthalmos were examined. No effect of either birth order or maternal age on the occurrence of buphthalmos can be demonstrated (Figures 5 and 6, Table 4).

Birth weight: Birth weight was recorded for all offspring produced by known carrier females. Table 5 gives mean weights, standard errors and standard deviations for both sexes for matings with carrier and noncarrier males. Comparison was made of the variation in weight of the various groups using the F test for comparison of variances. The variation in weight of females is the same among normal litter mates, siblings (not litter mates) and females from non-carrier crosses, but that of the buphthalmic females is significantly greater than that of any other female class. The distribution of weights in the buphthalmic

FIGURE 6.—Relationship of the frequency of litter birth to order of birth for normal and buphthalmic litters.

FIGURE 7.—Relationship of the frequency of litter birth to the age of the dam for normal and buphthalmic litters

TABLE 4

Chi-square tests of the occurrence of buphthalmos in (A) relation to order of litter birth and (B) according to age of the mother

A. Birth order	Observed number	Expected number	$\chi^2_{(4)} = 2.1$ $P > 0.7$
1st	15	12.43	
2nd	6	6.69	
3rd	5	4.14	
4th and 5th	2	3.51	
6th, 7th and 8th	1	2.23	
B. Age of mother	Observed number	Expected number	$\chi^2_{(4)} = 2.6$ $P > 0.5$
6-9 mo.	10	9.24	
10-13 mo.	8	6.69	
14-17 mo.	6	5.10	
18-25 mo.	3	4.14	
28-34 mo.	2	3.83	

TABLE 5

Statistics on birth weight in matings of 27 carrier females: matings with carrier and noncarrier males

A. Carrier matings						
Buphthalmic litters						
	Buphthalmic		Non-buphthalmic		Normal litters	
	Males	Females	Males	Females	Males	Females
Number of offspring	20	25	88	109	99	113
Mean weight (grams)	54.85	58.16	54.11	51.18	57.67	53.74
	± 2.13	± 3.15	± 1.29	± 1.12	± 1.26	± 1.12
Standard deviation	9.52	15.74	12.10	11.74	12.53	11.89
B. Noncarrier matings						
	Males	Females				
Number of offspring	107	115				
Mean weight (grams)	57.87	55.52				
	± 1.05	± 1.13				
Standard deviation	10.85	12.09				

females is skewed to the right. The mean weights of males in the various classes do not differ significantly, although lower in both buphthalmic males and their normal litter mates. The mean weight of buphthalmic females is significantly greater than that of their normal female litter mates but not greater than that of either the nonlitter mates or females from matings with noncarrier males. The mean weight of the normal female litter mates is significantly less than that of either of the other two normal classes.

Occurrence of other congenital anomalies: Other congenital anomalies (hydrocephalus, prognathism, microphthalmia, herniation, limb and/or sacral abnormalities, strap ovary, flexed tail, etc.) occur within the litters produced by carrier parents. Although the incidence of all abnormalities is significantly greater in buphthalmic animals (22.0%) than in their normal litter mates (8.1%), there is no evidence that any one of these is related to the occurrence of buphthalmos.

More congenital abnormalities are found among normal offspring in litters in which buphthalmos occurs, but the frequency is not significantly greater than in litters from the same parents in which buphthalmos does not occur.

Other authors have reported the occurrence of buphthalmos in pigmented animals but, until recently, this condition has occurred only in albinos of the Bar Harbor stock. In backcrosses of pigmented with albino carriers, buphthalmos occurred only in the albino offspring. A single affected Chinchilla animal recently obtained may represent a crossover between two closely linked loci. This possible genetic relationship is under investigation.

DISCUSSION

The present data substantiate the conclusion of GERI (1954, 1955) that buphthalmos in rabbits is a semilethal condition. The occurrence of buphthalmos appears to be unrelated to the birth order of the litter and to the age of the dam. The proportion of buphthalmic animals in affected litters appears to be related to litter size, with both a deficiency of affected offspring in litters of large size and an excess of large litters in which no affected offspring occur. These observations would indicate that the death and resorption of affected embryos may be influenced by a density factor, with the probability of fetal loss being directly related to litter size.

The mean weight of all males born in litters containing buphthalmic animals is smaller than that of males born in normal litters, although not significantly so. The mean weight of buphthalmic females in these litters is, however, significantly greater than that of their normal female siblings, although not significantly greater than that of females born in normal litters. These observations, together with the observations of GERI (1954, 1955) that the rate of weight gain in buphthalmic animals is less than that of their normal siblings, suggests that the morphological abnormalities seen in buphthalmos may be secondary to a generalized systemic effect, perhaps an abnormality in the maintenance of water balance within the developing embryo. Such an abnormality, perhaps occurring earlier in females than in males, would explain the observed excess of female stillbirths from carrier matings, and would account for the greater mean weight of affected live-born females. Preliminary studies of the serums from buphthalmic and normal adult rabbits, using starch gel electrophoresis, have demonstrated no differences in protein patterns. The small amount of protein in the aqueous of the rabbit (VON SALLMANN and MOORE 1948) makes examination of the intraocular fluids of buphthalmic rabbits impossible at this time.

ALLEN, BURIAN and BRALEY (1955) proposed that congenital glaucoma may result from a generalized disturbance in the development of mesodermal structures of the eye. The occurrence of chamber angle abnormalities in patients with Marfan's syndrome, Legg-Perthes' disease, idiopathic scoliosis, slipped upper femoral epiphysis and other collagen disorders indicated to BURIAN, VON NOORDEN and PONSETI (1960) the possibility that some forms of congenital glaucoma in humans may be a manifestation of an inborn error of metabolism,

probably of mucopolysaccharide synthesis. The occurrence of other congenital anomalies of mesodermal structures in litters born to carrier matings lends support to the concept that under debilitating conditions buphthalmos is one of the more likely anomalies resulting from a genetically determined generalized systemic developmental abnormality in the rabbit. A systematic study of the embryogenesis of this abnormality may thus increase our knowledge of important physiologic relationships during fetal development.

SUMMARY

Data from rabbit litters segregating for the recessive eye abnormality buphthalmos obtained from the breeding and litter records at the Hamilton Station, Roscoe B. Jackson Memorial Laboratory, are analyzed to ascertain variables affecting the penetrance of the abnormality in this colony. Maternal age and litter birth order are shown to be unrelated to the occurrence of buphthalmos in these litters. Among litters of large size there is a deficiency of litters containing a large number of affected animals and an excess of litters containing no buphthalmic animals. There is an excess of stillborn females in those litters from carrier parents in which no buphthalmos appears. The birth weight of buphthalmic females is significantly greater than that of their normal female siblings, but the birth weights of normal and buphthalmic males do not differ. It is suggested that buphthalmos may be a frequently occurring symptom of a systemic developmental defect in rabbits.

ACKNOWLEDGMENTS

Many routine observations were made by assistants DORCAS D. CRARY and EUGENE FARRIER at the Hamilton Station.

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