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Further Test for the Identity of "Dysoptic" with "Blind" in Mice

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Further Test for the Identity of *"Dysoptic"* with "Blind" in Mice[1]

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Abstract. The embryos of two mutant stocks of mice, "Dysoptic" and "Blind", were studied. Both mutations are dominant, and lethal when homozygous. The homozygous embryos of each individual stock as well as those of "Dysoptic" by "Blind" matings die at the same stage of development and show a similar pattern of retarded growth. It appears that the mutations are identical.

In 1962 I reported a breeding test for the identity of the mutation called "Dysoptic" with that of "Blind" in mice. Both mutations were dominant and lethal when homozygous. The breeding test indicated their identity but the results were not quite significant at the 5% level (1). Now I have undertaken a study of the embryos to determine if the death of the embryos occurred at the same time and in the same way in the two stocks.

The "Blind" stock was given to me by Dr. Ernst W. Caspari, in whose laboratory at Wesleyan University, Middletown, Connecticut, the mutation had first occurred. Vankin (2) reported that the homozygous embryos died at the beginning of the eighth day of intrauterine life. He fount that the later process of ectodermal differential growth, which normally gives rise to neural folds and neural tube, did not take place in lethals. He postulated that the failure might be due to a lack of a mesodermal layer underlying the embryonic ectoderm (3).

The "Dysoptic" stock was sent to me by Dr. A. G. Searle, in whose laboratory at the Radiobiological Research Unit at Harwell, Didcot, Berkshire, England, the mutation had occurred spontaneously. The embryology of the homozygote had not been investigated.

The heterozygotes of both stocks show corneal opacity, lens opacity, or microphthalmia. These manifestations may be unilateral or bilateral (4). The unilateral condition is less frequent in the "Dysoptic" stock than in the "Blind".

For this study 54 pregnant females were sacrificed, and 363 embryos were observed. The presence of a vaginal plug was used as an indication of the time of mating, and embryos were taken at intervals from six to fifteen days after mating. For embryos from six to nine days old, the uterus of the female was removed and the two intact horns were fixed in Bouin's solution, embedded in Paraplast, serially sectioned at 10 microns, and stained with Hematoxylin and Eosin. Measurements were

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taken across the embroyo in the region that would become head to rump. For embryos of ten days or older, the uterus was opened and the embryos observed and measured whole.

Vankin reported that in normal by normal matings, there were 4-5% resorbed embryos. I found in "Blind" by normal matings two resorbed embryos and 53 normals, and in "Dysoptic" by normal matings three resorbed or dying embryos and 75 normals.

In "Blind" by "Dysoptic" matings, I found three completely resorbed and 22 lethal embroyos, and 52 normals. In "Dysoptic" by Dysoptic matings, I found 6 fully resorbed and 16 lethal embryos, and 54 normals. In "Blind" by "Blind" matings, I found 4 completely resorbed and 17 lethal embryos, and 56 normals. These results are near the expected one-fourth homozygous lethals.

Table 1.

Mating	No. of females	Normals	Crown-Rump	Lethals	Average Width	Implantation Sites
Blind x Dysoptic						
6 days	2	10	.07mm			2
7 days	4	17	.21mm	7	.07mm	1
8 days	5	21	.70mm	9	.15mm	
9 days	1	4	1.80mm	6	.16mm	
Dysoptic x Dysoptic						
6 days	4	21	.11mm	4	.06mm	2
7 days	3	9	.14mm	6	.07mm	1
8 days	4	19	.67mm	5	.12mm	1
9 days	1	5	1.56mm	1	.14mm	2
Blind x Blind						
6 days	1	10	.09mm			
7 days	5	25	.22mm	6	.08mm	3
8 days	4	16	.69mm	8	.19mm	1
9 days	1	5	1.74mm	3	.18mm	
Dysoptic x Normal						
6 days	2	14	.08mm			
7 days	3	14	.15mm			
8 days	3	12	.66mm			
9 days	1	7	1.59mm	1	.17mm	1
10 days	1	11	4.0 mm			
12 days	1	6	8.35mm			1
13 days	1	11	11.5 mm			
Blind x Normal						
8 days	2	7	.72mm			
9 days	1	12	2.0 mm			
11 days	1	9	6.2 mm			
13 days	1	5	11.3 mm			1
15 days	2	20	15.6 mm			1

In those embryos which I have designated as completely resorbed, the wall of the uterus was thickened, the path of implantation of the embryo could be seen, but only vestiges of cells, blood and macrophages were present. These embryos probably died at about the trophoblast stage.

The embyos which I have designated as lethal were greatly reduced in size compared with their litter mates and showed few if any mitotic figures; ectoderm failed to differentiate and mesoderm was lacking. The pattern was identical in the three types of matings. It appeared that a slow-down of growth began between the sixth and seventh day, but the embryo remained alive usually until the ninth day. There was little or no differentiation of parts after the sixth day. The illustrations are of eight-day-old embryos.

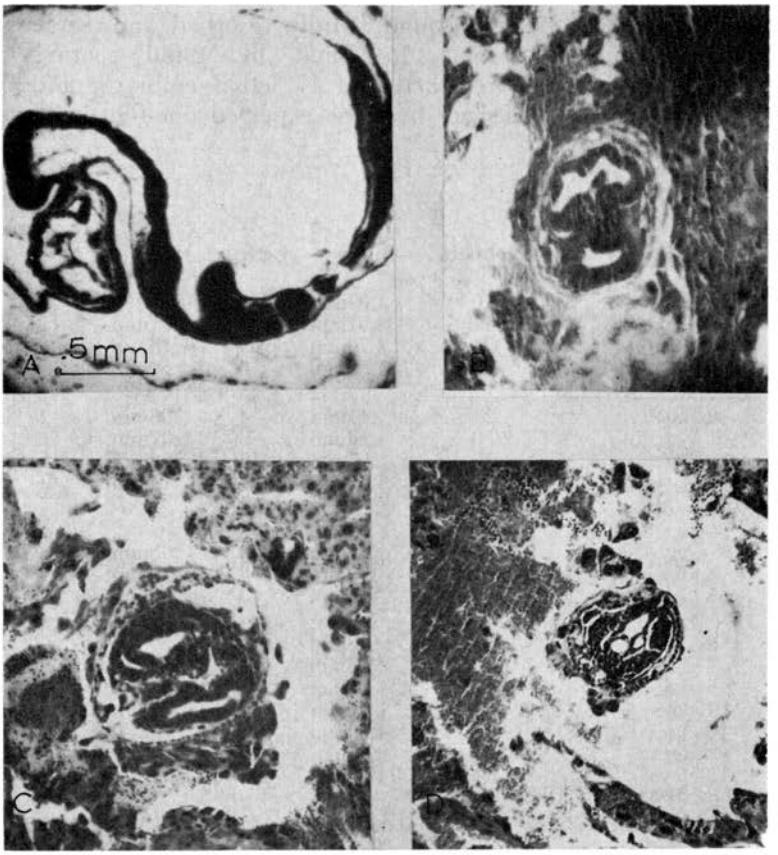

Fig. 1. A. Normal embryo, eight days old
B. Homozygous lethal embryo, eight days old, Blind x Dysoptic
C. Homozygous lethal embryo, eight days old, Dysoptic x Dysoptic
D. Homozygous lethal embryo, eight days old, Blind x Blind
All to scale shown in A.

From the study of the early embryos of the "Blind" and "Dysoptic" stocks of mice, it appears that these two spontaneous mutations are in fact identical. A study of the linkage relations would be a final test.

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A Method of Culturing Air Bacteria for Protozoa Media

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Abstract. An infusion-filtrate method for a continuous supply of air-borne bacteria is described, using either hay, soil, rice, or alfalfa for the infusion-filtrate bacterial cultures. Four cc of autoclaved infusion filtrate provided 1 cc of available bacteria each day. Three experiments each for hay, rice, soil, and alfalfa filtrate for periods of 31 and 60 days are described.

This experiment was designed to provide a continuous supply of bacteria, at a time when desired, for research with cultures of holozoic protozoa. The bacteria were supplied in concentrated and even distribution, with a continuous overlapping of the growth factors for all the bacteria over a period of time.

A method for controlling the hydrogen ion condition for amoeba cultures was described by Hopkins (1926). Hopkins and Johnson (1928) described buffer salts to adapt the amoeba to the increasing salt concentration so that a constant pH value was obtained. Needham (1937) reviewed the use of fresh sterilized hay whereby the acid condition opposes the alkaline tendancy of the culture.

Alfalfa, rice, soil, and hay-infusion filtrate were the basis for the bacterial culture media of this experiment which consisted of the following:

1. Preparation of the infusion.
2. Obtaining air-borne bacteria.
3. Preparation of the autoclaved filtrate.
4. Use of the autoclaved filtrate as a basis for the bacterial media.
5. Inoculation of the autoclaved filtrate medium with air-borne bacteria.
6. Daily requirements to maintain the bacterial cultures.