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Authors

Mufson, MA
Starr, A

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ABNORMAL MYELIN AND LEUKOPENIA IN THE WABBLER-LETHAL MOUSE

A Suspected Case of Pleiotropism

MAURICE A. MUFSON AND ARNOLD STARR*

RECENT studies^{3,7} attribute wab-
bler-lethal, a neuromuscular mu-
tant in inbred mice, to a single recessive
gene, which in the homozygous form
(*wlw*) produces a characteristic lethal
infantile ataxia. The ataxic gait first
becomes manifest at age 12 days, and
progresses in severity until the animal
dies; usually between 28 and 32 days of
age. Non-wabblers sibs, normal (*Ww*)
and heterozygous (*Ww*) animals, do
not exhibit neuromuscular symptoms
and hence are outwardly indistinguish-
able. The wabblers-lethal gene is ex-
pressed in the homozygous form, the
mating of heterozygous individuals
yielding wabblers-lethals (*wlw*) in ap-
proximately a 1:4 ratio.

Extensive investigations on the cen-
tral nervous system of the wabblers-
lethal^{7,8} are being pursued in order to
clarify the nature of the central nervous
system myelin peculiarities and its rela-
tionship to human demyelinating dis-
eases. In the present study a search for
pleiotropic effects in other organ sys-
tems was undertaken in the hope that
the elucidation of additional defects
might eventually assist in the under-
standing of the mechanism of gene ac-
tion in the wabblers-lethal mouse.

Materials and Methods

The progeny of mating pairs hetero-
zygous for the wabblers-lethal gene
(*Ww*) were used in these studies. The
mating pairs of established genotype
were obtained from M. M. Dickie of the
Roscoe B. Jackson Memorial Labora-
tory, Bar Harbor, Maine. The animals

were housed in small animal cages in
temperature and humidity controlled
rooms. Purina Lab Chow and drinking
water were provided *ad libitum*. The
progeny were kept with their mothers
during the period of experimental ob-
servation.

Growth

Total body weight was used as an
index of growth. At ages 7, 16, 20, 28
and 32 days each animal was weighed
on a laboratory beam balance (accuracy
 ± 0.01 grams).

Peripheral Blood

At ages 7, 16, 20, 24, 28 and 32 days,
peripheral blood samples were obtained
from the progeny by the following meth-
od: The animals were warmed under a
50 watt light bulb for not more than
three minutes. The tail was severed
with a sharp razor, and the first drop
of blood discarded. Two to three drops
of blood were collected in calibrated red
blood cell pipettes, and total cells
counted using standard hematological
methods. To ascertain the effect of re-
peated bleedings on the peripheral blood
picture, tail blood samples were obtained
from 32 day old non-wabblers siblings
not previously bled.

Differential white blood cell counts
(200 cells counted) were done on
Wright-stained peripheral blood smears
from progeny at 28 days of age.

Autopsies

Routine autopsies were periodically
performed on wabblers-lethals and non-
wabblers sibs. Tissue specimens for his-

*Department of Anatomy, New York University College of Medicine, New York 16, New York. The authors are greatly indebted to Dr. Pinckney J. Harman of the Seton Hall College of Medicine and Dentistry for guidance and encouragement during the course of the study and in preparation of the manuscript. Thanks are due to Margaret M. Dickie of the R. B. Jackson Memorial Laboratory, Bar Harbor, Maine, who supplied the experimental animals, and to the administrators of the R. B. Jackson Memorial laboratory for making available facilities to complete part of this work.

tological study were taken from heart, lung, skeletal muscle, liver, kidney, spleen, lymph node, bone marrow, adrenals, pituitary, thymus, and gonads. The tissues were fixed, embedded in paraffin, and stained with hematoxylin and eosin.

The data were evaluated statistically using analysis of variance for disproportionate subclass numbers and unequal means, thereby taking into account the variations between litters¹⁰.

Observations

Growth

The total body weight in grams of wabbl-lethals and non-wabbl-sibs at ages 7, 16, 20, 24, 28 and 32 days are presented in Table I. Wabbl-lethals gain weight at a normal rate up to 16 days of age, from 4.23 grams at seven days to 5.16 grams at 16 days. After 16 days of age and until death between 28 and 32 days body weight remains relatively constant, the growth curve describing essentially a plateau (Figure 15).

The non-wabbl-sib gains approximately 0.6 grams per day during this period, from 4.41 grams at seven days to 13.90 grams at 32 days. Non-wabbl-sib animals not used for bleeding studies (controls) weigh 13.38 grams at 32 days of age.

Peripheral Blood

The peripheral blood picture of the wabbl-lethal gives evidence of a moderate leukopenia as early as seven days of age, before growth abnormalities have become manifest, which progresses till their death usually between 28 and 32 days (Figure 16). Application of analysis of variance to these data demonstrates that at ages 7, 16, and 24 days the differences in the white blood cell count between wabbl-lethals and non-wabbl-sibs are significant at

AGE VARIATION

Figure 15

The age variation of total body weight, white blood cells, and red blood cells of wabbl-lethal and non-wabbl-sib mice.

the 0.05 level, and at age 20 days at the 0.01 level (Table I).

Table II shows representative total white blood cell counts for seven litters at ages seven and 20 days.

At age 20 days the white blood cell count of the wabbl-lethal has a marked peak between 2000 and 4000 cells per cmm., while the counts of the non-wabbl-sib are distributed symmetrically centering mainly at 5000 to 7000 cells per cmm. At age seven days this sharp

TABLE I. The age variations of total body weight, white blood cells and red blood cells of wabbl-lethal and non-wabbl-sib mice

Age (Days)	Total body weight (grams)		White blood cells (per cmm.)		Red blood cells (Millions per cmm.)	
	Wabbl-lethal	Non-wabbl-sib	Wabbl-lethal	Non-wabbl-sib	Wabbl-lethal	Non-wabbl-sib
7	4.23 (7)	4.41 (7)	6616 (7)*	7715 (7)	—	—
16	5.61 (8)	5.95 (8)	5306 (4)*	10513 (4)	7.21 (4)	6.80 (4)
20	5.71 (8)**	7.07 (8)	3608 (7)**	6494 (7)	7.83 (7)	7.33 (7)
24	5.79 (8)**	8.83 (8)	3219 (5)*	6305 (5)	7.84 (5)	7.27 (5)
28	5.76 (4)**	10.31 (4)	3497 (4)	7026 (4)	8.34 (4)	7.67 (4)
32	—	13.90 (3)	—	7548 (3)	—	6.06 (3)
32	Control	13.38 (3)	—	7215 (3)	—	6.46 (3)

The figures in parenthesis indicate the number of litters examined.

* P less than 0.05.

** P less than 0.01.

distinction is less marked, although the difference in mean total white blood cells between wabblor-lethals and non-wabblor sibs is statistically significant (Table I).

At age 28 days, differential counts on Wright-stained smears suggest that the leukopenia is referable to a lymphopenia (Table III).

The total red cell counts of wabblor-lethals and non-wabblor sibs are shown in Table I. No significant differences in total numbers of red blood cells in the peripheral blood have been detected. Examination of peripheral blood smears did not reveal any red cell abnormalities.

Repeated bleedings apparently do not alter the total numbers of cellular elements in the peripheral blood as the determinations at 32 days on control animals, not previously bled, were similar to the non-wabblor sibs who were bled several times.

TABLE II. Total white blood cells (per cmm.) of wabblor-lethal and non-wabblor sib mice within litters, ages 7 and 20 days*

Litter	Age 7 days		Age 20 days	
	Wabblor-lethal	Non-wabblor sib	Wabblor-lethal	Non-wabblor sib
I	5661	5439	4440	5661
	7437	5661		
		9102		
		5106		
		8769		
II	7558	8880	3219	7569
	7104	10101		
	4773	8769		
		8214		
		7992		
III	6549	7770	2775	4440
		4884		
		8436		
		10878		
		6438		
IV	7992	6438	3330	3774
		9657		
		9879		
		8214		
		7659		
V	9768	6438	5439	3108
	4773	7326		
	7104	9102		
		6660		
		7770		
VI	6660	6216	6105	5550
		9102		
		7548		
		7104		
		5883		
VII	5328	7104	2331	6660
	4440	5883		
		9435		
		4218		
		10212		

*The frequency distributions of these data are presented in Figure 16.

FREQUENCY DISTRIBUTION OF WHITE CELLS

Figure 16

Frequency distribution curves of white blood cell counts for wabblor-lethal and non-wabblor sib mice at seven and 20 days of age.

Autopsies

Histological examination of hematoxylin and eosin preparations of other tissues did not reveal any degenerative or inflammatory changes. The bone marrow and lymphoid tissue were carefully studied and no alterations from the normal pattern were observed.

Discussion and Summary

The altered pattern of growth of wabblor-lethals, characterized by an early normal development during the first two weeks of life after which no further growth occurs, has also been observed in other mutant strains of mice, e.g. Jittery², Grey-lethal and Pituitary dwarfism⁶. One explanation to account for the initial normal growth of these strains may be that substances (hormones, metabolites) obtained in utero are essential for post-natal development. Assuming that the genetic defect in these mutant mice limits their ability to manufacture these factors, catabolism during these first few weeks would not be accompanied by an adequate replacement. In consequence, the course of growth is inhibited and the animals die prematurely. In Gray-lethal and Pituitary dwarfism dysfunctions of the parathyroid and pituitary, respectively, occur and the disordered growth in these animals has been attributed to these hormonal aberrations. Moreover, the administration of normal anterior pituitary extracts to the Pituitary dwarfism strain results in the restoration of normal growth. Wabblor-lethal and Jittery were originally investigated

because of their bizarre neuro-muscular behavior but the similarity of their growth pattern to that of Grey-lethal and Pituitary dwarfism, strains of mice without neurological abnormalities but with hormonal disturbances, is striking. Routine histological preparations (hematoxylin and eosin) failed to reveal any significant alterations in normal structure. It is anticipated, nevertheless, that the application of histochemical and physiological techniques may reveal hormonal or metabolic differences which would clarify the basis of the growth and development disturbances of the wabblor-lethal mouse.

The demonstration of a statistically significant leukopenia in the wabblor-lethal mouse, an animal with an established myelin derangement, attests to the diversity of expression of the wabblor (*wl*) gene. The leukopenia persists throughout the wabblor-lethal's life and is apparently referable to a decrease in the circulating mononuclear cells. At seven days of age, one week prior to the clinical identification of the wabblor-lethal, the individual white blood cell count cannot be utilized as a means of distinguishing wabblor-lethals from non-wabblor sibs, even though statistically significant differences were demonstrated for these two groups, since the frequency distribution curves of their white blood cell counts overlap widely. Later in the course of the diseases, however, the animal's phenotype can be correlated with its white blood cell count, an observation compatible with the progressive nature of the wabblor-lethal's disorder.

Although inherited leukemias have been demonstrated in a number of inbred lines of mice⁴, the dyscrasia described in the present report is unusual in that the total white cell count is reduced and appears to be referable to a decrease in circulating lymphocytes.

Leukopenias have also been produced in mice by chronic starvation⁹, "viral-like" agents^{1,11}, and dysfunctions of the hypothalamic-pituitary axis⁵. The presence of a statistically significant leukopenia at seven days of age, at a period of normal growth, mitigates against inanition as an important factor in the etiology of the wabblor-lethal's low white blood cell count. Further, the wabblor-lethal's myelin sheath peculiarities and concomitant leukopenia may be correlated with the homozygous expression of a recessive gene and it appears un-

likely that an infectious agent is responsible for these traits. The possibility remains that the genetic constitution of these animals may render them more susceptible to certain environmental factors which might be initiating elements in their disease. The role of the hypothalamus in regulating many body functions either directly, through neural means, or indirectly, through the pituitary hormones, is well established. Myelin peculiarities can be demonstrated in the hypothalamic portions of the medial forebrain bundle and hypothalamic-tegmental tract in wabblor-lethals as early as eighteen days of age⁸. The Marchi method, used in these studies, specifically visualizes abnormal myelin and one might anticipate that other hypothalamic alterations could be demonstrated at a still earlier age if more sensitive histochemical techniques were utilized. It may be that the observed hypothalamic alterations will prove to be essential to the disturbances of growth and peripheral blood in the wabblor-lethal.

The lack of statistically significant alterations in the red blood cell counts suggest that the leukopenia is not secondary to an abnormality of the hematopoietic system as a whole, but rather to a relatively specific cellular defect. In view of this it also appears likely that the leukopenia is a primary disorder, characteristic of the pathological genetics of the wabblor-lethal mouse.

The findings suggest that the wabblor-gene is pleiotropic in action, exhibiting effects on such diverse targets as the myelin sheaths and the white blood cells. The mechanism of action of the wabblor-lethal gene in producing these manifold effects on two apparently unrelated tissues remains to be elucidated.

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TABLE III. Differential white blood cell count in wabblor-lethals and non-wabblor sibs: Age 28 days

	Lymphocytes	Neutrophils	Immature granulocytes	Monocytes	Eosinophils	WBC count Average
Wabblor-lethals						
Percent	38.5	59.5	1.0	0.5	0.5	—
Total	1540	2380	40	20	20	4000
Non-wabblor sibs						
Percent	69.0	28.5	0.5	1.0	1.0	—
Total	6210	2565	45	90	90	9000

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PLANT BREEDING AND CYTOGENETICS

IN spite of a wealth of material, textbooks and reference works that seek to combine the findings of cytogenetics with the principles of plant breeding are virtually non-existent. Professor Elliott has set out to fill this gap in our bookshelves, and on the whole has succeeded admirably.* He is well equipped by training and experience for this task, having spent his graduate days at the University of California and Iowa State College. For a number of years Professor Elliott taught plant breeding to both undergraduate and graduate students at Washington State College where he was a member of the Agronomy Department before transferring to the Farm Crops Department of Michigan State University in 1957.

A rough idea of the content and coverage of the book can be gained by a glance at the titles of the 13 chapters. The first chapter discusses "The nature of crop varieties", then follow five chapters primarily concerned with cytogenetics. Their titles are: "The chromosome mechanism in heredity" (Chap. 2); "The significance of recombination in plant breeding" (Chap. 3); "The role of mutations in plant breeding" (Chap. 4); "Polyploidy and plant breeding" (Chap. 5); "Chromosome substitution and aneuploid analyses" (Chap. 6). Chapters 7 to 12 deal almost exclusively with plant breeding as evident from the titles that follow: "The function of introduction in plant breeding" (Chap. 7); "Hybridization and selection of self-fertilized crops" (Chap. 8); "The improvement of cross-fertilized crop plants by hybridization" (Chap. 9); "Breeding for disease resistance" (Chap. 10); "Testing plant materials before release as varieties" (Chap. 11); "Handling plant materials after release as varieties" (Chap. 12).

There is a final chapter on "Organization for plant improvement" (Chap. 13). This chapter outlines the functions of those government bodies set up to administer funds allocated to

publicly supported institutions for plant improvement both in this country and abroad.

Professor Elliott is frank to admit that solutions to all of our plant breeding problems are not included within the covers of this book, since in his words it is neither a "manual nor a cookbook." However, he has culled the essentials from a great deal of scattered material, and organized it into an integrated picture of plant breeding and cytogenetic phenomena, rather than depending exclusively upon one group of crops (*e.g.* cereal crops). As a result, the reader learns something about crops as widely separated phylogenetically as pineapples and guayule.

Chapter 4 ("The role of mutations in plant breeding") written in collaboration with R. A. Nilan is an excellent up-to-date account of this increasingly important subject. The authors are concerned principally with mutation-inducing radiations and chemical mutagens. Their discussion will be of much value to the practical plant breeder because it is stripped of the excess technical verbiage that makes so many papers in radiation genetics tedious and difficult reading.

To cope with the alarmingly large array of diseases, insect pests and problems of adaptability to specific environments, the plant breeder should have at hand a representative cross section of the genotypic variability within the species, and of species that may be expected to exchange genes with those that are to be improved. In a chapter entitled, "The function of introduction in plant breeding," Professor Elliott has given serious attention to this problem. After outlining the main centers of origin of our important crop plants, the remainder of the discussion is occupied with the function and organization of those agencies in this country

(Continued on Page 242)

**Plant Breeding and Cytogenetics*. Fred C. Elliott, 1-395, f 1-1 to 9-13, McGraw-Hill Publications, New York, 1958. \$8.50.