Mouse Leukemia

XV. Resistance to Spontaneous Cases in Hybrids Induced by Milk

E. C. MacDowell*

(Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N.Y.)

In the first generation of a cross between strain STOLI, which has a very low incidence of spontaneous leukemia, and strain C58, which has a very high incidence, the disease has been found to occur more frequently if the mother comes from the STOLI strain, provided that her age is 18 weeks or more (1). An experiment, which was carried out before the age factor was recognized and which by chance included no young STOLI mothers or foster nurses, indicated that STOLI foster nurses reduced the incidence of leukemia in hybrids from C58 mothers and STOLI fathers to equal that in the reciprocal hybrids from STOLI mothers and C58 fathers. The present experiment was designed to confirm the transmission of this resistance factor by milk and to determine directly the influence of the age of the nurse.

STOLI females were used exclusively for both mothers and foster nurses, and the critical class consisted of F_1 females (STOLI/C58) from young mothers fostered by old nurses (Y-O). The chief control class consisted of similar F_1 females nursed by young foster nurses (Y-Y). Secondary control classes included F_1 females from old mothers, with old foster nurses (O-O) and with young nurses (O-Y). The classes Y-Y and O-O repeated a previously reported experiment, from which experiment the data are used for comparison and, as indicated in one chart, are combined with the new data to increase numbers.

MATERIALS AND METHODS

On April 8, 1947, males of the leukemic strain C58 were mated with young and old STOLI females. The old females (103), on this date 201–232 days old, came from the 66th–69th generations of brother-by-sister inbreeding; they had all produced offspring by their brothers up to a maximum of seven litters, but, since they had been separated from their mates for at least 23 days, none was pregnant at this time. While mated with their brothers, 44 of these females had produced and nursed the females (124) which served as young mothers; these, on the above date, were 63–81 days old and had been separated from males since birth.

The C58 males (54, from 76th to 78th generations, 83–108 days old) were assigned alternately, according to age (pedigree no.) to a box of old and a box of young females. Mating plugs and blood signs of pregnancy were recorded, and, beginning on the day before expected parturition, each pregnancy was closely watched, so that the young could be removed for foster-nursing by the other age group before they received any of their own mother's milk. Litters found after nursing had begun were used for the control classes Y-Y and O-O. Only female young were used. These were nursed in groups of four or five; when a litter contained less than four females, the number was made up with males, or with females from another litter born the same day and distinguished by slightly docked tails. On the 28th day the females were weaned, marked, and assigned to permanent boxes, with five to the same class in each box and the boxes arranged on the shelves so that every fourth box was of the same class.

The number of mice finally included in each class is given in Table 1, with the number of mothers and foster nurses represented as well as the average age (weighted) when they gave birth to the experimental animals or started to nurse them.

As mothers, the old and young STOLI mice produced litters of virtually the same size (averages, 6.9 and 6.5), with nearly the same number failing to survive parturition (averages, per litter, 0.22 and 0.26). As foster nurses, the old females lost slightly fewer nurslings, whether their own or from young mothers (4.5 per cent and 4.4 per

* Assisted by M. J. Taylor.

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cent, compared with 6.8 per cent and 7.7 per cent). Between weaning and 6 months there were no deaths. A spot test of body weight after 9 months (seven boxes from each of the four classes) indicated that the box-average weights of mice from old mothers tended to range above those from young mothers, but no real difference appeared between mice with young and old foster nurses.

All animals remained in excellent health until terminal weakening. The length of life ranged from 261 to 1150 days. In about half the cases, gross autopsies permitted clear diagnoses of "leukemic" or "negative for leukemia"—which term, for purposes of these and the preceding experiments in this series, includes related conditions. All cases uncertain in gross autopsy were diagnosed with the aid of histological and cytological observations of sections of representative tissues. However, even with these observations, certain cases presented difficulties which seem to be unavoidable. This is a familiar experience, but should not be overlooked. An early phase of leukemia may be present when death is due to another cause; again, inflammatory reactions may so closely resemble leukemic disease that diagnosis becomes a matter of opinion. Our early associate, Dr. M. N. Richter of University Hospital, New York University, rendered highly valued assistance by reviewing all doubtful cases. Borderline cases introduce a minor element of uncertainty to the totals, which should be considered estimates rather than absolute values.

RESULTS

Length of life.—Table 2 gives absolute numbers of mice in the four experimental classes, diagnosed

TABLE 1

SUMMARY OF STOLI/C58 FEMALES INCLUDED IN FINAL RESULTS

The number of STOLI mothers represented is given with their weighted average age at birth of offspring. The number of STOLI foster nurses and their ages when nursing started are also indicated.

Mother-nurse	Mice diagnosed	Mothers				Foster nurses			
		No.	Age Av. (days)		Range	No.	Age Av. (days)		Range
Y–Y	120	48	87.6		74–105	37	87.7		75–105
Y–O	108	37	84.5		68–102	33	276.3		253–301
O–O	82	33	280.7		265–299	33	279.0		266–300
O–Y	83	33	272.1		253–300	25	83.1		72–101

TABLE 2

NUMBERS OF MICE CLASSIFIED BY LENGTH OF LIFE AND BY DIAGNOSIS OF LEUKEMIA OR NEGATIVE FOR LEUKEMIA

F^1 (STOLI/C58) females are from young (Y-) or old (O-) mothers, nursed by young (-Y) or old (-O) STOLI foster nurses. Data from an earlier experiment ([1], p. 473, Table 2) are repeated as indicated for classes Y-Y and O-O.

Days lower limits of classes	Y-Y				Y-O		O-O				O-Y	
			Previous experiment						Previous experiment			
	Leuk.	Neg.	Leuk.	Neg.	Leuk.	Neg.	Leuk.	Neg.	Leuk.	Neg.	Leuk.	Neg.
250	4	2									1	
300	4	2	1	1								
350	2		4	1			3				1	
400	7	1	5		1						1	2
450	13		5	2	1		1				2	1
500	11	3	8			1				1	2	
550	11	3	9	4	3	2	3		2	2	4	
600	11	6	10	1	5	5	3	2	1	3	2	4
650	12	1	7	1	6	2	5	4	5	3	5	6
700	6	1	4	1	5	5	2	2	6	7	8	1
750	8	1	4		5	5	4	8	4	5	7	3
800	2	4	1	1	10	4	10	6	4	5	9	3
850		1	4	1	19	3	5	4	7	6	6	3
900	2				5	1	4	2	13	4	6	1
950	1				4	6	2	5	3	1	2	
1000					4	2	2	4	3			
1050		1			1	2	1		1	1	2	
1100									1			1
1150						1						
Totals	94	26	62	13	69	39	45	37	50	38	58	25
Per cent leukemia	78.3		82.6		63.8		54.8		56.8		69.9	
Mean life (days) from indiv. records	577.5	621.0	596.7	584.8	813.1	809.7	763.3	836.8	844.2	779.6	749.1	721.7
Leukemics+Negatives	587.2± 14.8		594.6± 16.0		811.9± 14.0		796.5± 16.7		816.3± 13.5		740.8± 17.8	

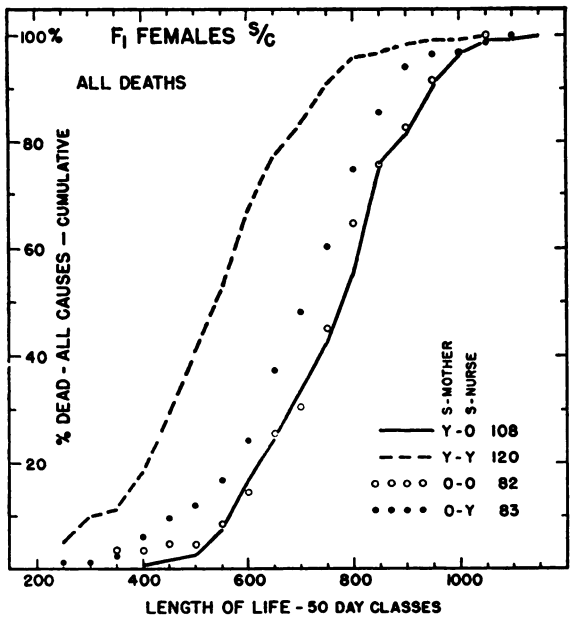

CHART 1.—Length of life: cumulative per cent dead of all mice within each experimental class without regard to diagnosis. Number of mice in each class is indicated. Figures on base line are lower limits of periods.

as leukemic or negative for leukemia and classified in 50-day age groups; as a measure of the reliability of these results, figures from an earlier experiment (1, Table 2) are repeated as indicated. Chart 1, which gives the cumulative per cent dead of all mice within each experimental class without regard to the diagnosis, shows: (*a*) that the distributions of deaths in classes Y-O and O-O are indistinguishable; (*b*) that class Y-Y died sooner, with a difference at the 50 per cent level of about 200 days; (*c*) that class O-Y lived longer than Y-Y but probably not quite so long as Y-O and O-O. Similar curves for leukemics and negatives separately are shown in Chart 2. To increase numbers, especially of negatives, the data from the previous experiment (1) for classes Y-Y and O-O have been included. Although the number of negatives is still small, and the curves are less smooth than those for the leukemics, it seems clear that the leukemics and negatives show essentially the same results.

Incidence of leukemia.—The cumulative incidence of leukemia, in terms of percentage of the total number in the class, is given in Chart 3, again on a base-line of 50-day age classes. The last point for each class indicates the death of the last mouse and the total incidence in that class. The

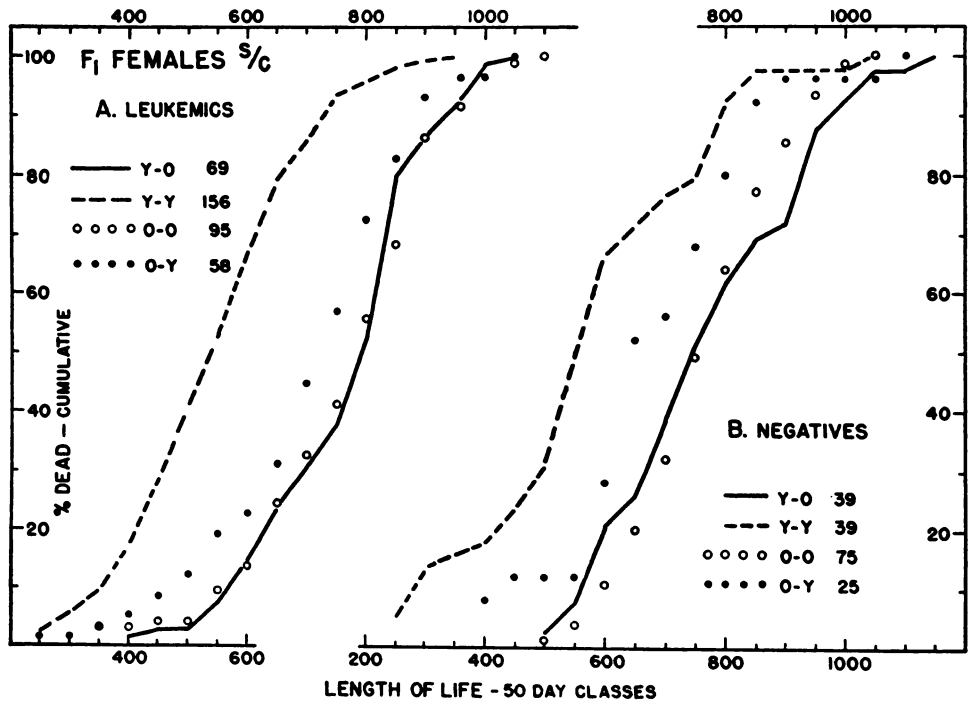

CHART 2.—Length of life: cumulative per cent dead of leukemics (A) and of negatives (B). Numbers of mice for Y-Y and O-O include those of a previous experiment, republished in Table 2.

solid and broken lines (Y-O compared with Y-Y) give the primary result. Class O-O (open circles) shows a close agreement with Y-O until 850 days, when an inconsistently large increment for Y-O brings the final incidence above that for O-O. Class O-Y is omitted (data in Table 2). Data from the previous experiment for Y-Y are shown by dots.

The transmission of the resistance factor by milk from STOLI nurses is confirmed, and its dependence on the age of the nurse is clearly indicated. This factor lengthens life whatever the

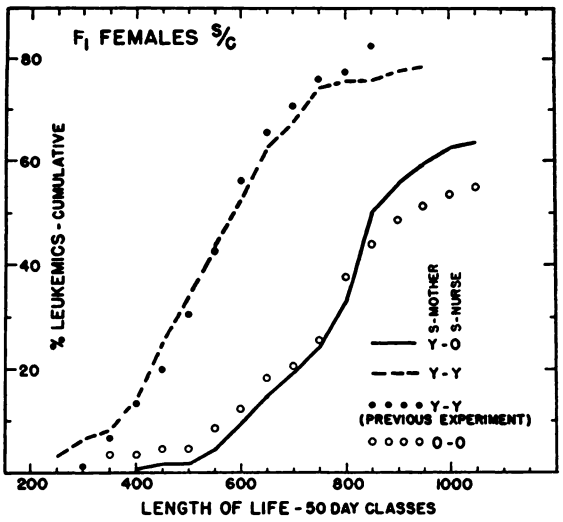

CHART 3.—Cumulative per cent leukemics

final diagnosis, and reduces the incidence of spontaneous leukemia.

It seems probable that the number of positive cases does not give the full measure of the effect of the resistance factor on leukemia. When this factor is present, whether received from the mother before birth or from the foster nurse afterwards, the proportion of difficult, borderline diagnoses is greater. This suggests that the resistance factor may modify the expression of leukemia without entirely suppressing it. Evidence of such partial resistance is given by the drawings of gross autopsies. A rough classification of the wide range of conditions shown in the autopsy pictures for the cases diagnosed as leukemic suggests some positive correlation between the size of lymph nodes and the proportion of leukemics in the different classes. In class Y-Y the relative frequency of cases with the lowest of three grades of generally enlarged nodes equalled that of the highest grade; but in the other classes, O-Y, Y-O, and O-O, the frequency of the lowest grade was, respectively, 2, 3, and 4 times that of the highest. Cases in which enlargement involved chiefly the mesenteric node, or the spleen, show no correlation, and the distributions of individual measurements of leukemic spleens are parallel for each of the four classes. No obvious correlation appears between the type of autopsy pattern and the length of life. Although within each of the four classes the distributions of length of life for each autopsy type are similar, within each autopsy type the distribution of length of life for Y-Y is shifted further toward shorter life than is the case for the three other classes.

Thus, the gross autopsies suggest that the resistance factor has no influence on the location of leukemic infiltrations, either directly or through an influence on longevity, but may have a general restraining influence upon the size of these infiltrations. In this case the incidence of leukemia becomes a conservative measure of the resistance, no matter how differently borderline cases might be diagnosed by different pathologists.

The reduction of the total incidence is unquestionable, although not so great as in previous experiments. Variation in the final incidence from experiment to experiment cannot be considered surprising, since at best about half the deaths were leukemic. Other factors, still unknown, decide the outcome in individual cases. Variation in such factors, as well as the occurrence of partial resistance, would add to the variation due to personal judgment of borderline cases.

SUMMARY

A resistance factor, which lengthens life and reduces the incidence of spontaneous leukemia, is transmissible either *in utero* alone, or through milk alone, by old, but not by young, females of strain STOLI, to their hybrid (F_1) offspring with leukemic (C58) sires.

REFERENCES

1. MACDOWELL, E. C., and TAYLOR, M. J. Mouse Leukemia. XIII. A Maternal Influence That Lowers the Incidence of Spontaneous Cases. Proc. Soc. Exper. Biol. & Med., **68**: 571–77, 1948.